EFFECT OF *SERIPHIUM PLUMUSOM* MEAL INCLUSION IN DIETS ON PRODUCTIVITY, CARCASS CHARACTERISTICS, METHANE PRODUCTION AND EMISSION BY NGUNI STEERS

by

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DECLARATION 1: ORIGINALITY

I, Motswapo Esther Phoko, carried out the research in this thesis under the supervision of Prof Jones Wilfred Ng’ambi and Dr Tlou Julius Tjelele from the University of Limpopo and Agricultural Research Council - Animal Production, respectively. I, therefore, declare that this thesis hereby submitted to the University of Limpopo for the degree of Doctor of Philosophy in Agriculture (Animal Production) represents original work by me and the contents of this work have not been submitted at this or any other university. This is my own work in design and execution, and that all materials contained herein have been duly acknowledged where other people’s work has been used.

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DECLARATION 2: PLAGIARISM

I, Motswapo Esther Phoko, declare that:

i. The research results reported in this thesis, except where otherwise indicated or acknowledged, is my original work;

ii. This thesis has not been submitted in full or in part for any degree or examination to any other university;

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DECLARATION 3: PRESENTATIONS

The four paper chapters and presentations were written and presented by Motswapo Esther Phoko. Prof Jones Wilfred Ng'ambi and Dr Tlou Julius Tjelele contributed comments to all the manuscripts and presentations. A certain level of repetition in some sections (introduction, materials and methods) of different chapters is unavoidable.

Presentation/s

Motswapo Esther Phoko presented the following paper at the national conference:

Phoko M.E., Ng'ambi J. W. and Tjelele T. J. 2022. Feed intake, nutrient digestibility and growth performance of Nguni steers fed diets with different inclusion levels of *Seriphium plumosum* meal. Proceedings of the 57th Congress of the Grassland Society of Southern Africa. Aldam Resort, Free State Province, South Africa.

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ABSTRACT

Rangeland contribute about 70% of the feed that sustain livestock throughout the year. However, due its degradation arising from among others woody plant encroachment, a shift of savanna and grassland biomes to closed woodlands reduces herbaceous productivity and economic benefits of rangelands to livestock. Woody plant encroachment (also called bush encroachment, bush thickening, shrub invasion) is a natural phenomenon characterised by the increase in woody plant density at the expense of the herbaceous layer. Bush thickening predominantly occurs in grasslands, savannas and woodlands and can cause biome shifts from open grasslands and savannas to closed woodlands. The aim of this study was to determine the effect of *Seriphium plumosm* meal inclusion levels in diets on feed intake, growth performance and digestibility, methane emission and meat quality of Nguni steers. The trial was conducted at the Agricultural Research Council – Animal production, feedlot for 111 days (i.e. 21 days of acclimitazation followed by 90 days of actual data collection). Twenty-eight Nguni steers, aged 22-months, with a body weight of 300kg ± 10 kg, were assigned to four treatment diets containing 0, 10, 20 or 30% of *S. plumosum* meal*,* replicated four times with seven steers per replicate in a complete randomized design. The diets were isocaloric and isonitrogenous and formulated to meet nutritional requirements of growing steers. Animal performance (average daily intake, feed conversion rate, growth performance) were measured, while digestibility trial was conducted following the total faecal collection method. Methane emission was measured using a laser methane detector technique and the bacterial population was investigated by DNA profiling using 16S rRNA gene with Illumina MiSeq. The mineral concentration (calcium, phosphorus, sodium, potassium) in rumen fluid were determined by Perkin Elmer Model Optima 7000 DV spectrometer. Carcass (dressing percentage, eye muscle area and back fat thickness) and meat quality traits (meat colour, sarcomere length, myofibril fragmentations lengths, collagen solubility, drip loss, water holding capacity, lipid and protein oxidation) were sampled and analysed following the commercial abattoir practices at the ARC – AP abattoir. A trained panel evaluated the sensory attributes (taste, aroma, flavour and texture properties) while tenderness was determined by using Warner Braztler blade. Data was analysed using General Linear Model (GLM) of SAS version 9.4. *Seriphium plumosm* meal inclusion levels in the diet did not (P>0.05) affect dry matter intake, body weight gains and feed conversion ratio. However, *S. plumosum meal* inclusion in the diet improved (P<0.05) neutral detergent fibre digestibility, carcass dressing percentage and myofibril fragment length of Nguni steers. Digestibility of neutral detergent fibre and carcass dressing percentage significantly improved (P<0.05) at 10% of *S. plumosum* inclusion, whereas, myofibril fragment length improved (P<0.05) at 30% *S. plumosum* meal inclusion in a diet. Inclusion of *S. plumosum* meal in diet significantly (P<0.05) reduced enteric methane emission. The meat sensory attributes were not affected (P>0.05) by the diets. It was concluded that *S. plumosum* meal inclusion levels used in the study reduced methane emission by Nguni steers, and did not cause any adverse effects on animal perfomance. However, further long-term studies are recommended to ascertain these findings.

Keywords: Animal performance, Beef quality, Forage quality and quantity, Greenhouse gases, Woody plant encroachment

TABLE OF CONTENTS

PAGE

DECLARATION 1: ORIGINALITY ... ii
DECLARATION 2: PLAGIARISM ... iii
DECLARATION 3: PRESENTATIONS ... iv
ACKNOWLEDGEMENTS ... v
DEDICATIONS ... vi
ABSTRACT ... vii
TABLE OF CONTENTS ... ix
LIST OF TABLES ... xiii
LIST OF FIGURES ... xiv
CHAPTER 1 ... 1
INTRODUCTION ... 1
  1.1 Background ... 2
  1.2 Problem statement ... 3
  1.3 Motivation ... 4
  1.4 Objectives ... 4
  1.5 Hypotheses ... 5
  1.6 Outline of the thesis and questions addressed ... 5
  1.7 References ... 6
CHAPTER 2 ... 11
LITERATURE REVIEW ... 11
  2.1 Introduction ... 12
  2.2 Description of *Seriphium plumosum* ... 13
  2.3 Distribution of *Seriphium plumosum* ... 14
  2.4 The importance of woody plant species as feed in ruminants ... 15
  2.5 Nutritional value of woody plant species (leaves and twigs) in animal diets ... 16
  2.6 Tannins ... 16
  2.7 Effect of woody plant species as feed on animal performance ... 17
    2.7.1 Feed intake, growth performance and nutrient digestibility ... 17
    2.7.2 Effect of woody plant species on methane production and emission by ruminants ... 19

2.7.3 Effect of CT on carcass and meat quality traits of ruminants ... 23

2.7.4 Effect of woody plant species as animal feed on meat sensory attributes of Nguni steers ... 24

2.8 Summary ... 25

2.9 References ... 26

CHAPTER 3 ... 41

EFFECT OF *SERIPHIUM PLUMOSUM* MEAL INCLUSION IN THE DIETS ON FEED INTAKE, GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY OF NGUNI STEERS ... 41

Abstract ... 42

3.1 Introduction ... 43

3.2 Materials and methods ... 44

3.2.1 Study area ... 44

3.2.2 Harvesting of the study plant ... 44

3.2.3 Animal management, treatments, design and feeding ... 44

3.2.4 Data collection ... 45

3.2.5 Chemical analysis of diets ... 46

3.2.6 Statistical analysis ... 46

3.3 Results ... 47

3.3.1 Nutritional composition of diets ... 47

3.3.2 Effect of *S. plumusom* meal inclusion levels in the diets on feed intake and digestibility of Nguni steers ... 47

3.3.3 Effect of *S. plumosum* meal inclusion levels in the diet on body weight gain, feed conversion ratio and body condition score of Nguni steers ... 48

3.4 Discussion ... 49

3.5 Conclusions ... 51

3.6 References ... 51

CHAPTER 4 ... 55

EFFECT OF INCLUSION LEVELS OF *SERIPHIUM PLUMOSUM* MEAL IN A DIET ON RUMEN MICROBIAL COMPOSITION AND METHANE EMISSION BY NGUNI STEERS ... 55

Abstract ... 56

4.1 Introduction ... 57

4.2 Materials and methods ... 59

4.2.1 Study area 59
4.2.2 Harvesting of the study plant 59
4.2.3 Animal management, treatments, design and feeding 60
4.2.4 Data collection 60
4.2.5 Chemical analysis...62
4.2.6 Statistical analyses 62
4.3 Results...63
4.3.1 Effect of *Seriphium plumosum* meal inclusion levels in the diets on methane emission by Nguni steers...63
4.3.2 Effects of *S. plumosum* meal inclusion levels in the diets on rumen fluid pH and mineral concentrations of Nguni steers 64
4.3.3 Effect of *Seriphium plumosum* meal inclusion levels in the diets on abundance, diversity and composition of rumen microbes in Nguni steers 65
4.4 Discussion...70
4.6 References 74
CHAPTER 5..81
EFFECT OF INCLUSION LEVELS OF *SERIPHIUM PLUMOSUM* MEAL IN A DIET ON CARCASS CHARACTERISTICS AND MEAT QUALITY OF NGUNI STEERS 81
Abstract 82
5.1 Introduction...83
5.2 Materials and methods 84
5.2.1 Study area 84
5.2.2 Harvesting of the study plant 84
5.2.3 Animal management, treatments, design and feeding 84
5.2.4 Data collection 85
5.2.5 Chemical analyses 88
5.2.6 Statistical analyses 88
5.3 Results...89
5.3.1 Effect of *S. plumosum* meal inclusion level in a diet on carcass pH, dressing percentage, eye muscle area and back fat thickness of Nguni steers 89
5.3.4 Effect of *S. plumosum* meal inclusion level in a diet on sarcomere length, myofibril length, collagen solubility, water-holding capacity, drip loss, and lipid and protein oxidation properties of Nguni steer meat 91
5.4 Discussion...92

5.5 Conclusions 96
5.7 References 96
CHAPTER 6..104
EFFECT OF INCLUSION LEVELS OF *SERIPHIUM PLUMOSUM* MEAL IN DIETS ON SENSORY ATTRIBUTES OF NGUNI MEAT..104
Abstract 105
6.1 Introduction..106
6.2 Materials and Methods 107
  6.2.1 Study area 107
  6.2.2 Harvesting of the study plant 107
  6.2.3 Steer management, treatments, design and feeding 107
  6.2.4 Sample preparation 108
  6.2.5 Thaw loss and total cooking loss 108
  6.2.6 Warner-Bratzler shear force 108
  6.2.7 Sensory analyses 108
6.2.8 Statistical analyses..109
6.3 Results..109
  6.3.1 Effect of *S. plumos*um meal inclusion levels in diets on aroma and flavour of Nguni steer meat..109
  6.3.2 Effect of *S. plumosum* meal inclusion levels in diets on cooking loss of Nguni steer meat 110
  6.3.3 Effect of *S. plumosum* meal inclusion levels in diets on shear force values of Nguni steer meat aged 3 or 14 days..111
  6.3.4 Effects of *S. plumosum* meal inclusion level in the diets and aging on Nguni steer meat tenderness, stringiness and chewiness 112
  6.3.5 Effects of *S. plumosum* meal inclusion levels in the diets on Nguni steer meat taste attributes after 3 or 14 days of aging 113
6.4 Discussion..115
6.5 Conclusions 118
6.6 References 119
CHAPTER 7..126
GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS..126
7.1 General discussion..127

7.2 Conclusions 128
7.3 Recommendations and further studies 129
7.4 References 130

LIST OF TABLES

Table 3.1 Proportion of feed ingredients used in the diets 44
Table 3.2 Nutritional composition of experimental diets 46
Table 3.3 Effect of *S. plumosum* meal inclusion levels in the diets on nutrient intake and digestibility by Nguni steers 47
Table 3. 4 Effect of *S. plumosum* inclusion levels in the diets on growth performance of Nguni steers 48
Table 4.1 Effect of *S. plumusom* meal inclusion in the diets on rumen fluid pH and mineral concentration of Nguni steers 64
Table 4.2 Effect of *S. plumosum* meal inclusion in the diets on rumen fluid bacterial abundance at phylum level of Nguni steers 65
Table 4.3 The effect of *S. plumosum* inclusion levels in the diets on Alpha diversity measures of rumen bacterial communities of Nguni steers 67
Table 4.4 Effect of *S. plumosum* meal inclusion in diets on relative abundances of bacterial genera in rumen fluid samples of Nguni steers 68
Table 5.1 Effect of *S. plumosum* meal inclusion levels in diets on carcass traits of Nguni steers 89
Table 5.2 Effect of *S. plumosum* meal inclusion levels in diets on meat colour attributes of Nguni steers 90
Table 5.3 Effect of *Seriphium plumosum* meal inclusion levels in the diets on Nguni steer meat tenderness, moisture and oxidation properties 91
Table 6.1 Effects of *S. plumosum* meal inclusion level in the diets and aging on meat sensory attributes of Nguni steers 110
Table 6.2 Effects of *S. plumosum* meal inclusion levels in the diets and aging on Nguni steer meat tenderness, stringiness and chewiness 113

Table 6.3 Effect of *S. plumosum* meal inclusion levels in the diets and aging on Nguni steer meat taste attributes 114

LIST OF FIGURES

Figure 2.1 A photo of *S. plumosum* at Modimolle farm, Limpopo Province: Photo credit: Motswapo Phoko 13
Figure 2.2 Map showing Provinces encroached by *S. plumosum* (Avenant, 2015) 14
Figure 2.3 Diagrammatic representation of hydrolysable and condensed tannins (Krause *et al*., 2005). 17
Figure 2.4 Factors influencing methane yield (Sejian *et al*., 2012). 20
Figure 2.5 Enteric methane formation in ruminants (Evert *et al*., 2016) 21
Figure 4.1 Variation in methane emission by Nguni steers according to diet. : Treatments were *Seriphium plumosum* meal inclusion levels in the diets of 0 ($T1_0$), 10 ($T2_{10}$), 20 ($T3_{20}$) or 30 ($T4_{30}$) %.. 63
Figure 4.2 The most predominant of the top 10 bacterial phyla that were identified in rumen fluid of Nguni steers.. 65
Figure 4.3 Correlation analyses between relative abundances of bacterial phyla and genera (present at ≥ 0.1% in at least one sample) and methane emission by Nguni steers as influenced by *S. plumosum* meal inclusion levels. 67
Figure 6.1 Effect of *S. plumosum* meal inclusion levels in diets on cooking loss of Nguni steer meat 111
Figure 6.2 Effect of *S. plumosum* meal inclusion levels in diets on shear force values of Nguni steer meat aged 3 or 14 days.. 112

CHAPTER 1

INTRODUCTION

1.1 Background

Rangelands constitute about 50% of the earth's land surface (Metzger, 1999), and they support approximately 30 to 35 % of the world's human population (Anadón *et al*., 2014). In addition, rangelands sustain the majority of the world's livestock production (Safriel *et al*., 2005); however, they are highly variable to annual rainfall (Reynolds *et al*., 2007). The consequences of the paradigm regime shift are significant and persistent in changes of the structure and function of ecosystems and social-ecological systems (SES) (Briggs *et al*., 2018). Woody plant encroachment is an example of a regime shift that occurs in the savanna systems and this phenomenon threatens the livelihoods of pastoralists (Luvuno *et al*., 2022). The occurrence of woody plant encroachment has been documented in many studies (Luvuno *et al*. 2018, Wilcox *et al*., 2018, Liao *et al*., 2020) and the effect has been attributed to rainfall, overgrazing, fire suppression and an increase in global carbon dioxide (CO_2) concentration (Stevens *et al*., 2016; Venter *et al.,* 2018). The two latter factors are cited in other studies as the main drivers of woody plant encroachment, as they directly or indirectly may be causing the widespread proliferation of shrubs and trees (Parr *et al*., (2014). Approximately 80 % of the land in South Africa is exclusively utilized for agricultural activities, of which 11 % thereof is arable and 69 % is suitable for livestock grazing (Neely *et al*., 2009). The rate and extent of woody plant encroachment in South African rangelands is one of the the main constraints in livestock industry (Gxasheka *et al*., 2013). This phenomenon results in a decline grazing capacity of the rangelands and ultimately affect livestock performance particularly for smallholder farmers who rely largely on extensive grazing (Beyene *et al*., 2014).

Woody plant encroachment does not only reduce grass production but also other ecosystem services such as biodiversity, water retention and soil protection from erosion (Gillson and Hoffman, 2007). Thus, efforts to control encroaching woody plants and to limit their impacts is imminent (Moustakas *et al*., 2010, Wigley *et al*., 2010, O'Connor *et al*., 2014, Stevens *et al*., 2016). There have been numerous efforts to manage the increasing rate and extent of woody thickening (Archer and Predick 2014; Smit 2014; Bassett *et al*. 2020), which some of them are unaffordable to smallholder farmers (Nyamukanza and Scogings, 2008). However, the proliferation of woody plant species and their nutritional composition (Papanastasis *et al*., 2008; Mapiye *et al*., 2009a), may present an opportunity to be strategically integrated in animal diet formulation. *Seriphium plumosum* also known as bankrupt bush, contribute significantly to encroachment of grassland biome in South Africa (Snyman, 2009; 2012). However, there is a lack of literature in the use of *S. plumosum* as feed ingredient in ruminants. This is probably because *S. plumosum* is unpalatable to livestock, which may be attributed to plant secondary metabolites. Therefore, the objective of the study was to evaluate the use of *S. plumosum* as feed ingredient in total mixed ratio diet fed to Nguni steers on feed intake, growth performance, digestibility, methane emission and meat quality.

1.2 Problem statement

Livestock production in South Africa is primarily practised under natural rangeland conditions, to meet their daily nutrient requirements (Kirkman and Carvalho, 2003). One of the limiting factors influencing livestock productivity under extensive production systems in South Africa is the inadequate supply of nutritious feeds, especially during the dry season (Gilhausn and Hölzel, 2016). Thus, cattle lose weight and body condition during the dry season in the semi-arid areas of South Africa (Muchenje *et al*., 2008; Mapiye *et al*., 2009a; Yeshaw, and Mossie, 2017). Generally, poor nutritional value of feed in the dry season affects reproduction, growth performance and meat quality of livestock (Muchenje *et al*., 2008), with subsequent substantial economic losses to the farmers (Salem, 2012).

The fluctuating quality and quantity of natural feed resources is exacerbated by encroachment of woody or shrubby plant species in the rangelands. Woody plant encroachment is characterized by an increase in woody plants and/or shrubs, which alter ecosystem services such as forage production for livestock (Ward, 2005; Archer *et al*., 2017). It is for this reason that bush encroachment is one of the major challenges faced by livestock farmers in South Africa. In South Africa, for example, *S. plumosum* converted extensive areas of the grassland, which supports game and livestock population into less productive rangelands (Rutherford and Westfall, 1994; Snyman, 2012). Many woody plants including *S. plumosum* contain plant secondary metabolites, which may reduce feed intake of ruminants (Estell, 2010). However, cattle and goats browse young plants of *S. plumosum* at certain times of the year, especially after fire (Snyman, 2012).

High methane production and emission from ruminant livestock, which results from poorly digestible feeds are major problems adversely affecting the environment (Lassey, 2007). In particular, ruminants produce and emit huge amounts of methane, a gas generated from rumen during microbial fermentation (Supapong et al. 2017). Haque (2018) reported a loss of about 6 % of energy intake from cattle because of methane production and emission. There is some evidence that tannins in the diets of ruminants can decrease methane production and emission (Supapong *et al*., 2017). However, this evidence is not extensive and conclusive in ruminants (Makkar *et al*., 2007).

1.3 Motivation

Livestock production is a key component of smallholder farming systems and it is increasingly viewed as an important pathway to escape poverty. However, the fluctuating quality and quantity of rangelands are limiting livestock productivity among farmers, especially smallholder farmers (Nyambali *et al*., 2022). This is exacerbated by woody plant encroachment, which is considered one of the form of land degradation affecting grasslands and savannas (O'Connor *et al*. 2014). Although many woody plants such as *S. plumosum* have plant secondary metabolites such as condensed tannins; which affect ruminal fermentation by inhibiting ammonia and methane production through their ability to form complexes with dietary protein and carbohydrates (Carulla *et al*., 2005). Their effects on performance of the animal range from beneficial to toxicity, depending on their concentration (Makkar, 2003). There is inconclusive information on the inclusion levels of *S*. *plumosum* meal in ruminant diets for optimal performance and reduction of methane production and emission by ruminant animals (Makkar, 2003; Makkar *et al*., 2007). *Seriphium plumosum* inclusion levels in a diet for optimal performance of ruminants can be explored through better understanding of rumen functions and dynamics. The use of woody plants species as fodder may be essential for closing feed gaps in livestock production and also contribute to the management of woody plant encroachment.

1.4 Objectives

The broad objective of the study was to evaluate the responses in growth performance, methane production and emission, and meat characteristics of Nguni steers fed varying inclusion levels of *Seriphium plumosum* leaf meal in the diets.

The specific objectives of the study were to determine:

i. the effect of *S. plumosum* meal inclusion level in a diet on feed intake, digestibility, feed conversion ratio and growth rate of Nguni steers.
ii. the effect of *S. plumosum* meal inclusion level in a diet on methane production and emission by Nguni steers.
iii. the effect of *S. plumosum* meal inclusion level in a diet on carcass characteristics and meat quality traits of Nguni steers.
iv. the effect of *S. plumosum* meal inclusion level in a diet on meat sensory attributes of Nguni steers.

1.5 Hypotheses

The hypotheses of the study were:

1. *Seriphium plumosum* meal inclusion level in a diet has no effect on feed intake, digestibility, feed conversion ratio and growth rate of Nguni steers.
2. *Seriphium plumosum* meal inclusion level in a diet has no effect on methane production and emission by Nguni steers.
3. *Seriphium plumosum* meal inclusion level in a diet has no effect on carcass characteristics and meat quality traits of Nguni steers.
4. *Seriphium plumosum* meal inclusion level in a diet has no effect on meat sensory attributes of Nguni steers.

1.6 Outline of the thesis and questions addressed

The thesis is comprised of seven chapters. Chapter one introduces the study and gives background on the state of woody encroachment and cattle population in the world and in South Africa. This chapter further covers the problem the study seeks to address, that is potential use of encroaching woody plants as a supplementary feed, specifically focusing on the impact of *S. plumosum* on animal performance.

Chapter two deals with the literature on the description, distribution and the nutritional characterization of *S. plumosum*. More broadly on the effect of *S. plumusom* meal inclusion in diets on productivity, carcass characteristics, methane production and emission by Nguni steers.

Chapter three evaluated the effect of *Seriphium plumosum* meal inclusion in diets on performance of Nguni steers. This chapter covered specifically feed intake, growth performance and nutrient digestibility. In addition, this chapter addressed the following questions: Does *S. plumosum* inclusion in the diet enhance dry matter intake and improve live weight gain Nguni steers? At what level of inclusion in the diet is *S. plumosum* adequate to improve performance?

Chapter four focused on understanding the biology of CH_4 production, and determining the effect of *S. plumosum* meal on CH_4 production and emission by Nguni steers. This chapter sought to address these questions: a) Does inclusion of *S. plumosum* meal in a diet result in lower methane emission by Nguni steers? and, b) Can inclusion of *S. plumosum* meal in a diet reduce the population and abundance of bacteria responsible for CH_4 production by Nguni steers?

Chapter five investigated the effect of *S. plumosum* inclusion in diets on meat quality of Nguni steers. This chapter addressed the following question: Can inclusion of *S. plumosum* meal in diets of Nguni steers produce the same meat quality as those fed lucerne hay without *S. plumosum* meal?

Chapter six addressed the following questions: Does feeding *S. plumosum* meal improve meat sensory attributes of Nguni steers? What level of *S. plumosum* inclusion in the diets is adequate to improve sensory attributes of beef from Nguni steers?

Chapter seven presents conclusions and provides future directions for all results obtained in the experimental chapters. This chapter summarizes and integrates the results obtained from chapre three, four, five and six.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The world cattle population is estimated to be one billion heads in 2022, which is an increase of approximately 996 million from 2021 (Meissner *et al*., 2022). South Africa alone has about 14 million cattle, which constitute 1.6 million dairy cattle and 12.5 million beef cattle (DAFF, 2019). One of the challenges with having so many animals on the land is their negative effect on environment and their contribution to greenhouse gases (Steinfeld *et al*., 2006). For example, livestock and game species utilize about 70 % of agricultural land in South Africa (Meissner *et al*., 2013), which may lead to over utilization of rangelnds. Overexploitation of rangelands leads to land degradation, which threatens the quality and productivity of these ecosystems (Ramoelo *et al*., 2018). Commercial farmers keep 60 % of cattle in South Africa, while smallholder farmers keep 40 % (DAFF, 2019). The production of beef cattle in South Africa depends largely on natural feed resources in the rangelands (Van der Westhuizen *et al.*, 2020). However, due to the poor state of rangeland in the smallholder and communal farmers, cattle productivity is low, and that increases pressure to provide not only more but also different types of feed, which includes among others increased demand for animal-based protein (Henchion *et al*., 2017). The growth of the human population, mainly in developing countries, will increase demand of livestock and livestock products such as meat and milk (Eckard *et al.,* 2010). Thus, the demand for more livestock products has implications for rangeland utilization and thereby leads to an increase in methane production and emission (González *et al*., 2011; Gerber *et al*, 2013).

A deteriorating quality and productivity of grasses is exacerbated by increasing woody plants (Estell *et al*., 2012). Woody plant encroachment is another form of land degradation compromising livestock production and consequently burdens food (e.g. meat, milk, fibre) supply to meet global population demands (Estell *et al*., 2012). Woody plant encroachment is a phenomenon referred to as an increase of trees and shrubs that suppresses herbaceous layers (Wiegand *et al*., 2006; Ayelew and Mulualem, 2018). Livestock production, which is the dominant land use in communal and smallholder farming where woody plant encroachment occurs, is mostly dependent on grass production, which is frequently outcompeted by proliferarion of woody plants (Smit, 2005). The encroachment of woody plant species also increases the amount of bare soil surfaces and consequently increased runoff, which hinders the recovery of herbaceous plants (Eldridge *et al.,* 2011). Woody plant encroachment has persisted for years, which makes it difficult to control (Luvuno *et al*., 2018).

In South Africa, over half of the grassland biome has been converted into less productive shrub land by *S. plumosum* (Snyman, 2012). *Seriphium plumosum* is highly unpalatable to livestock and the chemical compounds responsible for this are poorly understood (Pule *et al*., 2018). Generally, tannins are contained in many woody plants including *S. plumosum* and when over-ingested (> 55 g CT kg^{-1} DM) by herbivores, they may bind with diverse proteins, thus making them inaccessible to herbivores (Moore *et al*., 2014; Venter *et al*., 2018). Some of the woody plant species are nutritionally superior to grasses at certain times of the year, and their strategic integration as supplementary feed in ruminants may be beneficial in closing the feed gap during drought or dry season (Belayneh and Tessema, 2017). This study explores the integration of *S. plumosum* as feed ingredient in diets on performance, digitibility, methane emission in ruminants.

2.2 Description of *Seriphium plumosum*

Seriphium plumosum, popularly known as bankrupt bush, is aggressive. The plant is further characterised by a small multi-woody stem that grows to an average height of 60 cm and a width of 60 cm (Jordaan and Province, 2009). The species is light green in colour during wet season, which reflects sunlight, and brownish in dry season, and has small leaves that reduce water loss. It is adapted to long and dry winters (Avent, 2015). The plant is aromatic and yields volatile oils that serve as a protective measure against herbivory (Figueiredo *et al*., 2008). *Seriphium plumosum* grows well in lighter solid soils that occur mostly on the foot-slope and mid-slope terrains, but it can also be found on clay soils (Avenant, 2015).

Seriphium plumosum plants are part of the family *Asteraceae* (daisy family) and the name *Seriphium* is derived from *Seriph,* which is a stroke or line of letters: *plumosum* means feathery (Snyman, 2009). The genus *Seriphium* consists of 34 species with *S. plumosum* known as the most aggressive encroacher (Snyman, 2009). This woody dwarf species significantly reduces the grazing capacity by up to 80 % due to its rapid spread in the grasslands (Wepener *et al*., 2008). *Seriphium plumosum* reduces grass productivity, which consequently causes socio-economic challenges, such as loss of productive land for livestock production (Marquart *et al*., 2022). At a young and flowering stage, sheep and goats browse it, although its utilization by cattle is unknown (Snyman, 2010).

Figure 2.1 A photo of *S. plumosum* at Modimolle farm, Limpopo Province: Photo credit: Motswapo Phoko

2.3 Distribution of *Seriphium plumosum*

Seriphium plumosum is indigenous to South Africa, and has highly infested seven provinces of South Africa i.e. North West, Free State, Eastern Cape, Mpumalanga, Gauteng and certain parts of KwaZulu-Natal (Figure 2.2) (Jordaan and Province, 2009). Although, *S. plumosum* originated from South Africa, it has spread to other African countries (Angola, Namibia, Mozambique and Zimbabwe) (Koekemoer, 2001) and is found in Madagascar and the United States of America (Schmidt *et al*., 2002, Snyman, 2009). *Seriphium plumosum* comprises nine species of which five occur in South Africa and the rest in East Africa, Madagascar and the Reunion (Koekemoer, 2001). *Seriphium plumosum* produces thousands of seeds annually, which are distributed by wind and may infest areas that were previously free of *S. plumosum* (Snyman, 2010). The species spreads, germinates and regrows after three to five years. The high rate of *S. plumosum* encroachment in most of the provinces in South Africa poses a serious threat to grassland biomes (Synman, 2012). Overgrazing and lack of controlled burning among other drivers led to higher rate of encroachment by *S. plumosum* (Trollope 1987; Wepener 2007).

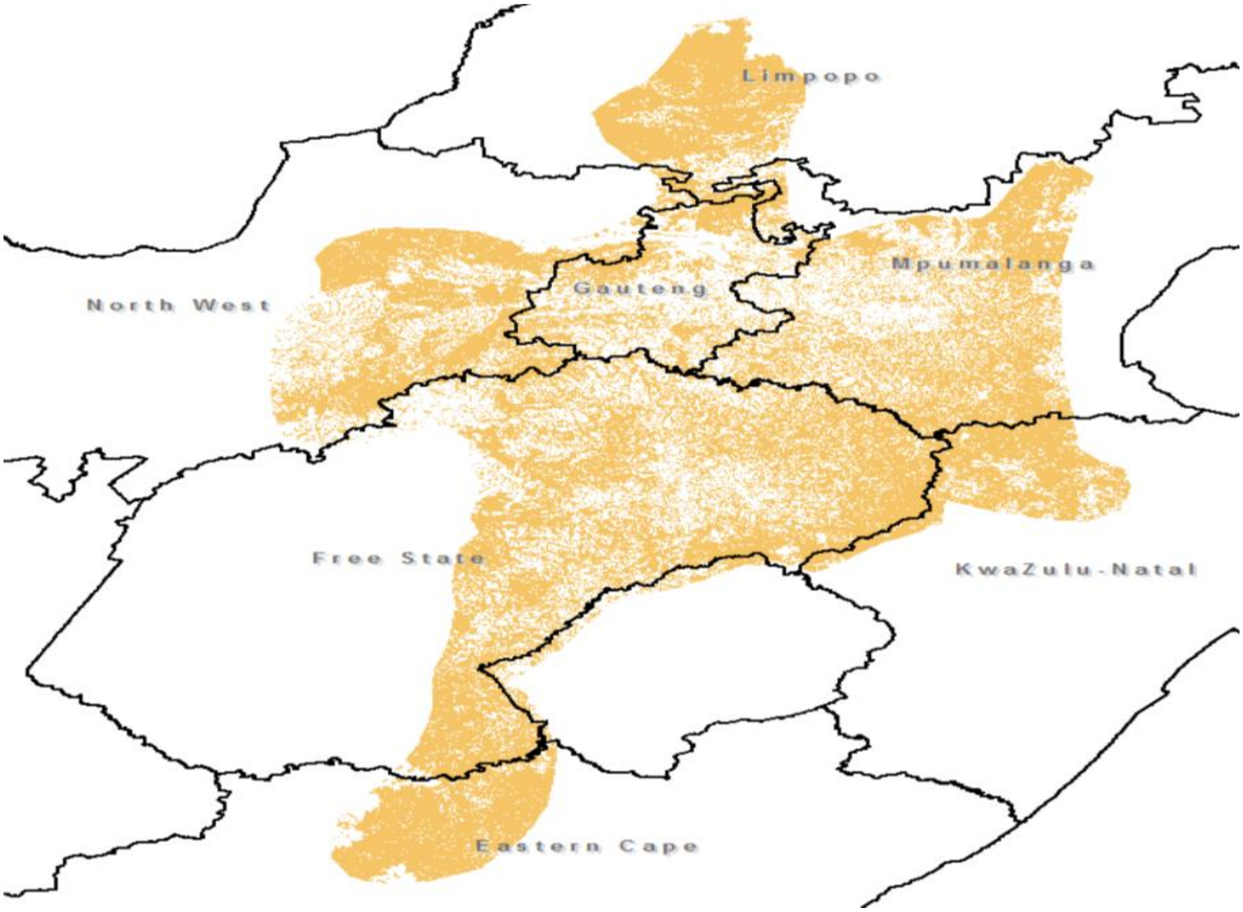


Figure 2.2 Map showing Provinces encroached by *S. plumosum* (Avenant, 2015)

2.4 The importance of woody plant species as feed in ruminants

Nutrient supply particularly that of crude protein is too low in the dry season to meet the cattle nutritional requirements for maintenance (Devendra and Sevilla, 2002; Mapiye *et al*., 2009). This is even worse for the communal and/or smallholder farmers whose rangeland is often degraded (Ndlovu *et al*., 2009). The use of indigenous woody plants, which is often high in crude protein and readily available, may be explored as feed supplement. Woody plants such as *Vachellia* species can be used as substitutes during feed shortages and compensate nutrient deficiencies (Mlambo *et al*., 2015; Gebru *et al*., 2018).

Utilization of woody species as supplementary feed during the dry season when grasses are of poor quality could minimise supplementary feed costs and improve productivity of livestock particularly in developing countries where majority of people depend on livestock for livelihood (Sanon, 2007). Woody species contain PSM such as condensed tannins (CT) which are regarded as anti-nutritional factors due to their ability to bind proteins and carbohydrates in the rumen (Kelln *et al*., 2020). However, at a lower concentration (<5 %) CT in animal diets can reduce bloat risk, increase ruminal undegradable protein, reduce enteric methane production, and benefit anthelmintic activity (Wang *et al*., 2015).

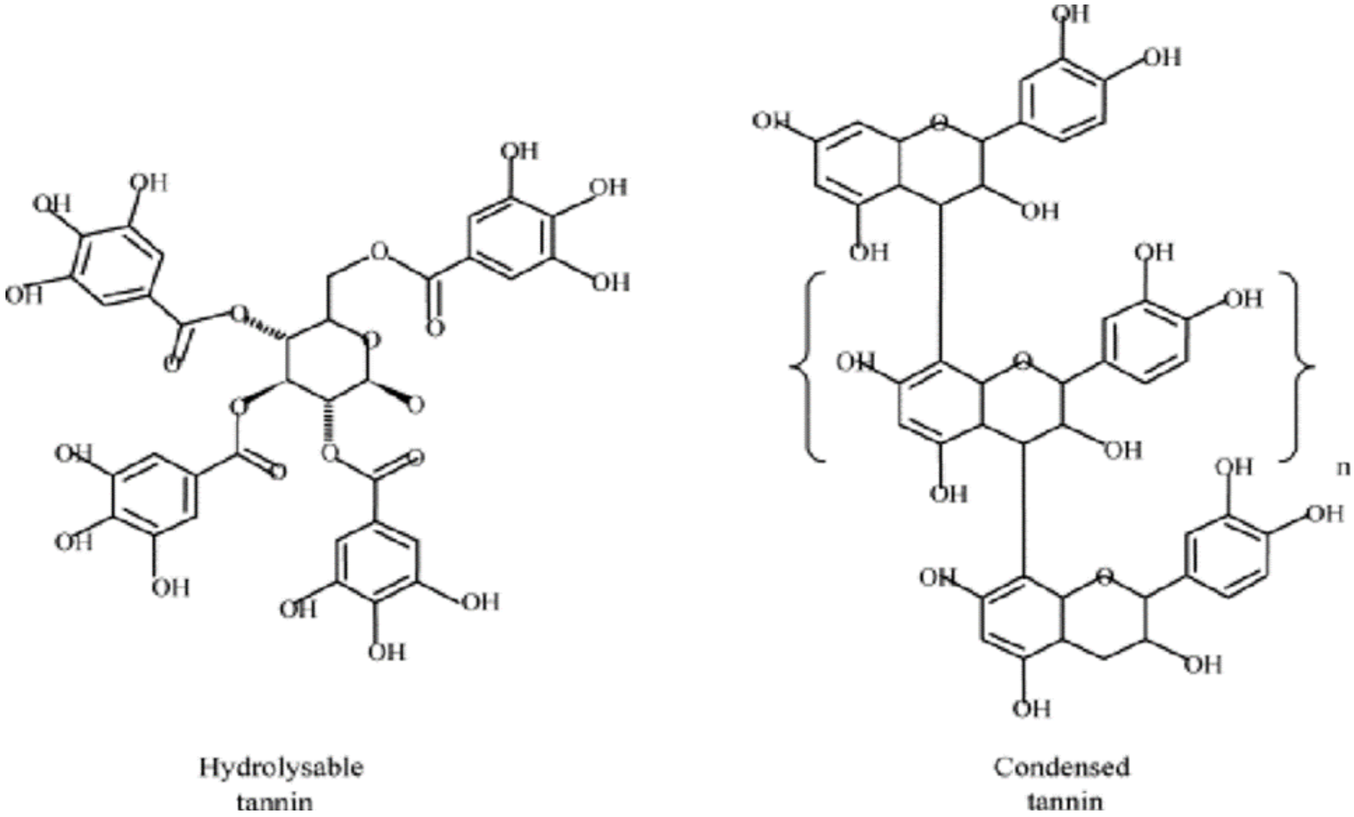
2.5 Nutritional value of woody plant species (leaves and twigs) in animal diets

Woody plant species comprise crucial nutrients that could improve livestock performance (Devine *et al*., 2017). However, their use as feed supplement in livestock is restricted due to a lack of knowledge about their nutritional status (Mudau *et al*., 2021) and ability to formulate diet using encroaching woody plants such as *S. plumosum*. Crude protein (CP) from woody plants can improve low-quality roughages in diets (Mudau *et al*., 2021). Devine *et al*. (2017) reported adequate CP contents of *Atriplex* spp, *A. salamancensis* and *A. patagonica*. *Atriplex salamancensis* species have leaves with an average of 180 g/kg CP. Higher fat contents were reported in leaves of *Nardophyllum bryoides* (280 g/kg) and in the leaves of Ameghinoa *patagonica* (6 %) and *Adesmia salamancensis* (5 %) (Barria *et al*., 2020). Van der Baan *et al*. (2009) reported similar contents of CP and fat. Moleele (1998), reported that *Dichrostachys cinerea* and *Grevia flava* contained high crude protein contents as compared to the grasses as feed for cattle. Aganga *et al*. (2005) and Al Shafei and Naur (2016) reported averages of 32.5 to 150.0 g/kg ash contents of various woody species. Mapiye *et al*. (2001) revealed the potential use of *Acacia karoo* leaves as feed to improve growth rates and digestibility, carcass traits, and beef quality in cattle. The review of nutritive values of woody plant species is useful in the determination of the best species suitable for livestock.

2.6 Tannins

Tannins are secondary plant metabolites that occur naturally in the plants and are distributed across in the bark, leaves, flowers and seeds (Wang *et al*., 2015). Tannins (Figure 2.3) are exclusively spread across plants, especially in woody, shrubs, cereals and medicinal plants (Berard *et al*., 2011). These polyphenol compounds are classified in two categories, thus, hydrolysed tannins (HT) and condensed tannins (CT) which can be distinguished by their molecular structure and size (Min *et al*., 2003) (Figure 2.3). Due to their variation in chemical structure, the content of plant tannins varies among different plant species, growth stages and conditions (Jin *et al*. (2012; Li *et al*., 2014). Condensed tannins are oligomeric or polymeric flavonoids composed of flavane-3-ols, including catechin, epicatechin, gallocatechin, and epigallocatechin, and dominate HT in most plants (McSweeney *et al*., 2001). The HT consist of nuclei with molecular weights ranging from 500 to 3,000 Daltons (Da) (Van Parys *et al*., 2010).

Tannins contain antioxidant properties, which play a significant role in food and medical fields (Tong *et al*., 2021). Tuyen *et a*l. (2017) reported that purple prairie clover (PPC) decreased protein degradation *in vitro* but had minimal effects on overall rumen fermentation. Deng *et al*. (2016) reported that CT from leaves of *F. altissima* possessed antioxidant and antityrosinase activities, suggesting that *F. altissima* might be a good source of proanthocyanidins with biological activities. Various plants synthesise different types of tannins, however, this review will focus on condensed tannins, which are high in *S. plumosum* (Beauchemin *et al*., 2008; Tong *et al*., 2021).



Hydrolysable tannin

Condensed tannin

Figure 2.3 Diagrammatic representation of hydrolysable and condensed tannins (Krause *et al*., 2005).

2.7 Effect of woody plant species as feed on animal performance

2.7.1 Feed intake, growth performance and nutrient digestibility

Palatability of woody plants to ruminants is determined by astringency linked to CT – protein complexes formed from proteins in saliva, therefore, the greater the protein bound by CT, the greater the astringency and the lower the palatability (Naumann *et al*., 2017). High levels of dietary CT (> 5 % tannins in a diet) noticeably depress feed intake and digestibility in ruminants (Frutos *et al*., 2004).

Although higher CT in the diet account for antiherbivory effects in certain animal species, Salminen and Karonen (2011) revealed that their interaction with ruminants suggests that its effect is minimal because forages containing acceptable CT (<5 %) are consumed, and at times preferred over grasses (Waghorn, 2008). Beneficial ruminant response to diet containing less than 5 % CT include amongst others, improved DM intake (DMI), organic matter (OM) digestibility, and weight gain (Mueller-Harvey *et al*., 2019). Beauchemin *et al*. (2007) indicated the positivity CT had on DM and fibre digestion at 0, 1 and 2 % DM of a *quebracho* tannin extracts. It had no effect on DM, energy, acid detergent fibre or neutral detergent fibre digestibilities. Similarly, McMahon *et al*. (1999b) did not observe any effect of CT in *sainfoin* on *in vitro* DM disappearance. Similarities in results among studies prove that the use of woody species with less than 5 % causes no adverse effects on ruminant performance.

Increase in DMI and growth efficiency of cattle fed diets containing <5 % CT has been observed in multiple studies. A study by Rivera-Méndez *et al*. (2017) observed 6.5 % average daily gain (ADG) and DMI, and a 5.5 % increase in gain efficiency with dietary CT supplementation levels of 0, 0.2, 0.4 and 0.6 % on DM basis. Consistent trends of increased DMI of forage diet containing CT <5 % have also been reported in a feeding trial of goats (Puchala *et al*. 2005), lambs (Douglas *et al*. 1995) and cows (Woodward *et al*. 2000). Ng'ambi *et al*. (2022) also report no adverse effect on growth performance of Bapedi sheep supplemented with CT at 0, 30, 40 or 50 g/kg DM diet. Volpi-Lagreca *et al*. (2013) observed enhanced DMI, ADG and gain efficiency in feedlot heifers fed a whole corn diet supplemented with CT. However, Krueger *et al*. (2010) and Mezzomo *et al*. (2011) observed a decline in DMI when feedlot steers were fed diets with either 1.5 % or 0.4 % CT. However, Hervás *et al*. (2003) observed no decline in DMI when lambs were feed diets with 1.5 g of condensed tannins per kg DM. Growth performance responses to supplemental tannins have been generally attributed to enhancements in intestinal metabolizable protein supply (Waghorn 1996; Ben-Salem *et al*., 1999; Min *et al*., 2003). A decline in growth can be attributed to reduced intake and low digestibility of protein by animals (Tanner *et al*., 1990; Addisu, 2016).

The ingestion of CT in ruminant rations at low to moderate levels has proved to increase digestibility of the diets (Jerónimo *et al*., 2016). The main role of CT in a diet is to bind protein and carbohydrates and prevent their degradations in the rumen, regulate the rate of nitrogen release in the rumen and increase the flow of essential amino acids to the small intestines (Makkar, 2003). Digestibility of diets containing CT depends on animal species. For example, goats have shown to adapt to diets containing CTs than sheep and cattle (Aboagye and

Beauchemin, 2019). The higher production of tannin-binding salivary proteins by goats makes them less vulnerable to the negative effects of tannins, particularly at higher concentrations as compared to sheep and cattle (Shimada, 2006). Previous studies showed a significant improvement of crude protein digestibility in beef steers consuming diets containing 1.33 % CT from *Acacia mearnsii* (Koenig *et al*., 2018). However, inclusion levels of *Acacia* mearnsii CT at 0, 5, 10, 15, and 20 g/kg of diet DM did not affect DM, OM, NDF, EE, NFC and TDN digestibility values of steers (Avila *et al*., 2020). In contrast, Ahnert *et al*. (2015) reported a decline in the digestibility of DM, OM, NDF and ADF in steers fed 10, 20, 40 or 60 g of CT from *Quebracho* /kg DM diet. Kozloski *et al*. (2012) observed a reduction in digestibility in sheep fed *A. mearnsii* at the levels of 20, 40 and 60 g/kg DM. It can be recommended that a detailed analysis of CT structure be done to better understand the diverging effects of CT supplementation in ruminant digestion.

2.7.2 Effect of woody plant species on methane production and emission by ruminants

An increase in human population increases the demand for livestock products, which will in turn result in larger livestock populations. This is likely to exacerbate the emissions of CH_4 to the atmosphere (Gerber *et al*., 2013). The concentration of greenhouse gases (GHG) in the atmosphere causes the global temperatures to rise and consequently affect human life, livestock, rangeland ecosystems and many other factors (Moss *et al*., 2000). Carbon dioxide and nitrous oxide are also contributors to GHG. However, CH_4 is the most worrisome gas because of its significant warming potential (Broucek, 2014). Enteric CH_4 contributes about 63 % of total CH_4 emissions (Merino *et al*., 2011; Beauchemin *et al*., 2011). Strategies to mitigate CH_4 emission from livestock must be developed and implemented. Due to their high abundance in forage plants, plant secondary metabolites (PSM) have the potential to reduce CH_4 production and emission by ruminants (Piñeiro-Vázquez *et al*., 2015).

Plant secondary metabolites (PSM) contain a group of structural diverse compounds that originate from primary metabolites (Piasecka *et al*., 2015). Ku-vert *et al*. (2020) reported that PSM from forage plants are capable of modulating rumen microbes, modify their functions, and reduce feed energy loss in ruminants. These groups of plant compounds, further enhance plant growth and developmental processes, innate immunity (Piasecka *et al*., 2015), defence response signalling (Isah, 2019), and response to environmental stresses (Yang *et al*., 2018). In ruminants, PSM act as signals for symbiosis between plants and microbes, and modify microbial communities associated with hosts (Guerrieri *et al*., 2019). Some studies on the use of CT extracts from various woody and shrubby plant species in animal diets have indicated that it may have economical value for livestock producers to mitigate enteric methane emissions (Aboagye and Beauchemin, 2019). Generally, beef production accounts for about 80 % of enteric CH_4 gas production (Broucek, 2014). Methane production and emission in ruminants is influenced by multiple factors, which include amongst others: level of intake, type and quality of feeds, energy consumption, animal size, growth rate, level of production, and environmental temperature (Figure 2.4).

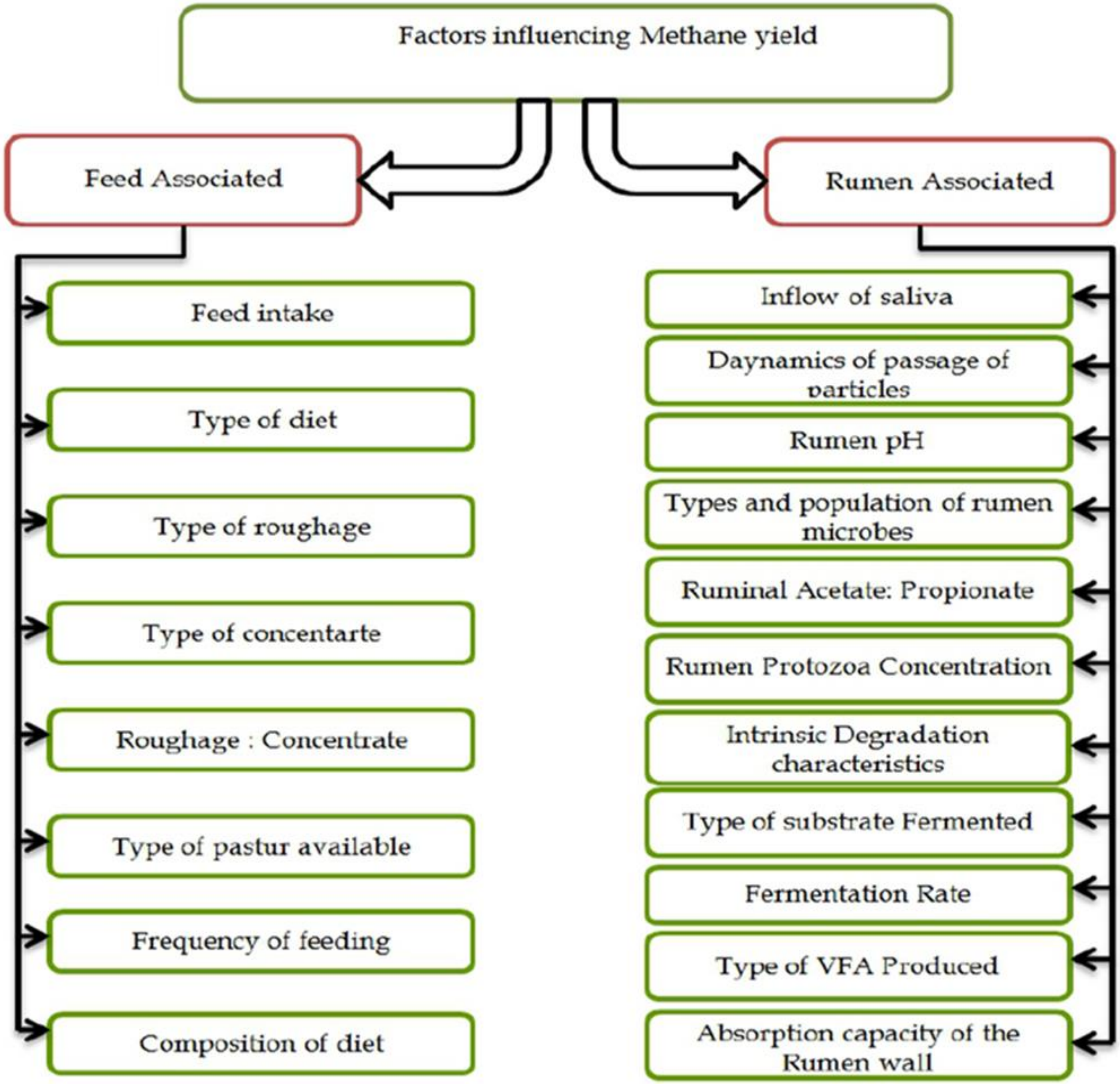


Figure 2.4 Factors influencing methane yield (Sejian *et al*., 2012).

Methane is formed in the rumen by microbial fermentation of hydrolysed carbohydrates, and as a result, energy from the host is lost (Figure 2.4). Emission of enteric CH_4 by ruminants represents an economic loss to the farmer when feed is converted to CH_4 rather than to product output (Martin *et al*., 2010; Aluwong *et al*., 2011). Ruminants ingest feeds, which are then degraded in the digestive tract into small molecules and transferred into the blood flow through the digestive mucosa (Benchaar *et al*., 2001) (Figure 2.5). Studies indicate that increased DMI by ruminants reduce methane production by 7 to 40% (Benchaar *et al*., 2001).

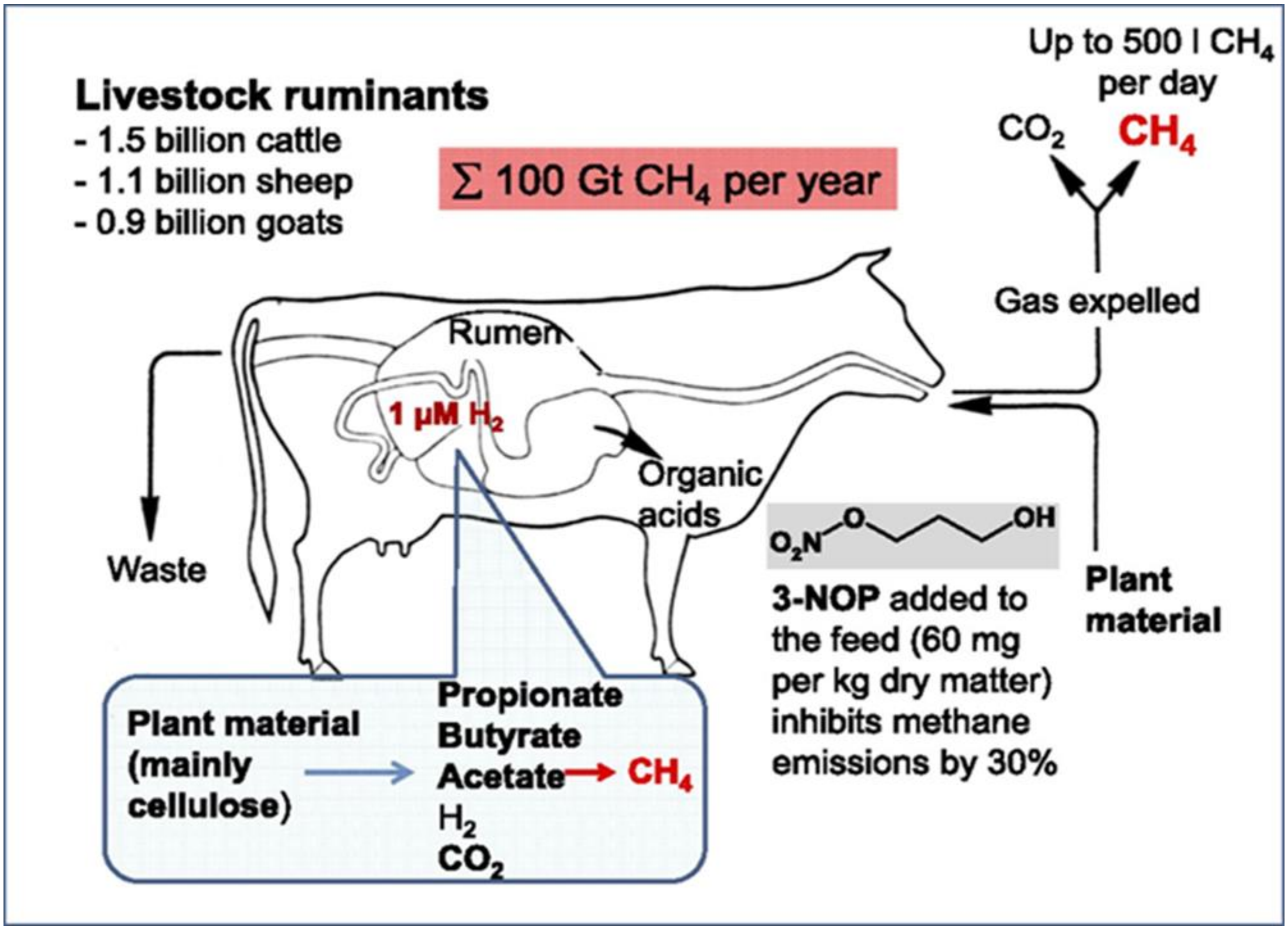


Figure 2.5 Enteric methane formation in ruminants (Evert *et al*., 2016)

Methanogen bacteria are the common bacteria responsible for CH_4 production, and as such, mitigation strategies to reduce CH_4 production are directed at them (Hook *et al*., 2010). The abundance of methanogens in the rumen is largely influenced by diet, particularly fibre during fermentation of feeds (Hook *et al*., 2010). During enteric fermentation, hydrogen is released by other microbes and used by methanogenic archaea, which is methanogens, to convert carbon dioxide to methane (Joblin, 1996). The population of methanogenic species vary depending on the diet and geographical location of the host (Hook *et al*., 2010).

Livestock produce CH_4 into the atmosphere by belching the gas through auxiliary (mouth and nostrils) (Chagunda *et al*., 2009). Ninety-five percent of CH_4 is excreted by eructation, 89 % produced from the hindgut is excreted thorough the breath, whereas emission through the anus accounts for about 11 % (Murray *et al*., 1999). Digestion of ingested feed by microbes leads to the production of volatile fatty acids (VFAs), which in turn are used by the host as source of energy (Broucek, 2014). Therefore, the production and emission of CH_4 by ruminants is a loss of feed energy from the diet and represents inefficient utilization of the feed (Chagunda *et al*., 2009). Plant secondary metabolities, such as CT have the potential to inhibit methanogens thereby reducing hydrogen availability (Tavendale *et al*., 2005). Antimicrobial properties available in CT of woody and shrubby plants have the potential to reduce CH_4 production in ruminants (Molina-Botero *et al*., 2019). Diets containing CT at less than 5 % concentration significantly reduced CH_4 emission in an *in vitro* study by 20 g of methane/kg DM (Jayanegara *et al*., 2015). Other studies attributed a decrease of up to 50 % of CH_4 emission from ruminal fermentation in response to diets containing <5 % CT (Goel *et al*., 2007). Rira *et al*. (2015) also pointed out that the inclusion of *Acacia cyanophylla* at 30 and 60% in diets that had less than 5 % CT concentration reduced CH_4 emission in an *in vitro* study. Woody species *Lespedeza cuneate, Acacia mearnsii and Callinada calothyrsus* containing less than 5 % CT reduced CH_4 emission by 57 % in goats, 13 % in sheep and 24 % in lambs (Puchala *et al*., 2005; Carulla *et al*., 2005; Tiemann *et al*., 2008).

Tannins affect methanogens through various mechanisms including among others: tannins acting directly on methanogens or protozoa that are associated with methanogens, tannins acting on fibrolytic bacteria and decreasing fibre degradation or tannins acting as a hydrogen sink (Díaz Carrasco *et al*., 2017; Carulla *et al*., 2005; Becker *et al*., 2014). The effect of tannins on CH_4 production depends on their molecular weight (Aboagye and Beauchemin, 2019). Evidently, Saminathan *et al*. (2016) showed that archaeal community decreased with decreasing MW of CT, although total methanogens increased. Several authors argued that mechanisms of action of CT on rumen microorganisms affect growth and activity of rumen population (Min *et al*., 2014; Goel *et al*., 2005; McSweeney *et al*., 2001). However, what is partially unknown is that there are certain bacterial populations with the ability to degrade tannins and to develop enzymatic mechanisms to degrade their effects (Goel *et al*., 2007).

2.7.3 Effect of CT on carcass and meat quality traits of ruminants

Beef serves as an important diet for humans, and it has robust implications on human health and also on the economy and culture worldwide (Pighin *et al*., 2016). Beef constitutes a crucial part of dietary source of conjugated linoleic acid (CLA) especially cis-9 and trans-11 isomers and is an important health promoter factor including antitumoral and anticarcinogenic activities (Salter, 2013). Beef has significant nutritional factors like lipids and proteins with high biological value, trace elements and vitamins (Pighin *et al*., 2016). Nutrition plays a significant role on the quality of carcass. Forage diet is barely used to improve beef quality, but it is unclear if it is because of its lower energy supply compared to grain diet (Keller *et al*., 2022). Feeding steers forage diets as compared to concentrate diets producecd acceptible leaner carcasses with larger concentrations of n-3 fatty acids and conjugated linoleic acids (CLA) (Duckett *et al*., 2013). In human health, meat containing n-3 fatty acids reduce cardiovascular morbidity and mortality (Yashodhara *et al*., 2009). The lower fat content in the carcasses of cattle finished on forage at constant carcass gain contrasts results from comparisons of forage diet and high-concentrate diets. High-concentrate diets produce slightly fatter carcasses than forage diets (Robson, 1995).

Meat colour, flavour, tenderness, texture, juiciness, and odour as well as its nutritional properties depend on animal genetics, management practices, post mortem processes that take place during the conversion of muscle into meat, but most importantly, diet is a significant effect on the quality of meat (Hocquette *et al*., 2012). Feeding costs are a major challenge in the economic balance for smallholder farmers, particularly during the dry seasons when most rangeland species are low in nutritive value (Priolo and Vasta, 2007). Numerous studies have been conducted to test the use of woody and shrubby species as supplementary feed in livestock (Silanikove *et al.*, 1994; Ben Salem *et al.*, 1996; Priolo *et al.*, 1998).

The length of time and increased slaughter period affect meat stability due to a reduction and depletion of co-factors of metmyyoglobin (Boles and Pegg, 2010). The final pH of the meat depends on the glycogen reserves in the muscle and the acidification processes that occur during the conversion of the muscle into meat (Oliveira *et al*., 2015). Pérez *et al*. (2021) reported that supplementation of CT from quebracho had no effect on lamb meat pH. The absence of effects on meat pH when tannins are supplemented is consistent with the reports of Biondi *et al*. (2019). Pimentel *et al*. (2021) observed no differences in carcass yields of goats fed on diets with or without CT extracts from *Vachellia mearnsii*.

Pimentel *et al*. (2021) reported an increase in carcasses of goats with increase in the levels of CTs from *Acacia mearnsii* extracts. Souza *et al*. (2018) indicated that feeding CT to ruminants has some economic values. Ng'ambi *et al*. (2022) reported a significant increase in antioxidant activity values of meat from rams fed CT at 30, 40 or 50 g/kg DM diet.

Colour is an important trait that creates the first impression that attracts consumers. Meat colour may vary from deep purplish-red of freshly cut beef to the light grey (Rodriguez *et al*., 2014). This attribute in meat quality can be efficiently managed if the main factors that influence it are understood (Boles and Pegg, 2010). Mapiye *et al*. (2010) indicated a decline in *L** colour of steers supplemeneted with *Acacia karoo* leaf meal. Monsón *et al*. (2005) and Strydom *et al*. (2000b) showed that cattle fed on concentrate diets grow more rapidly before slaughter and as a result have increased rates of protein turnover that lead to intramuscular fat and marbling. Verna *et al.* (1989) reported a significant increase in lightness of the lambs fed diets containing CT. Priolo *et al.* (1998) found that feeding tannins from *Carob pulp* increased *longissimus* muscles in lambs. Zembayashy *et al*. (1999) reported a substantial increase in meat lightness of beef heifers fed on tannins from *Acacia* species. Priolo *et al*. (2001) associated dark colour to increased myoglobin, decreased muscle glycogen, or both, and a reduction in yellow fat due to tanniniferous forage supplementation. Mapiye *et al*. (2010b) attributed higher redness (a*) values in meat from steers supplemented with *A. karroo* compared to those fed on sunflower cake diets without CT. Similar results were obtained in chickens (Du *et al*., 2002), sheep (Luciano *et al*., 2009) and rabbits (Liu *et a*l., 2009). Larraín *et al*. (2007) and Dai and Mumper (2010) associated the role of CT in improving meat redness to the antioxidant nature of condensed tannins.

2.7.4 Effect of woody plant species as animal feed on meat sensory attributes of Nguni steers

To improve beef quality, it is important to analyse consumer attitudes, behaviours, emotion and their perception (Ruiz-Capillas *et al*., 2021). In context of beef, sensory analysis implies the assessment and response of the particularities of samples that are observed and interpreted by the panellists using their senses of sight, smell, taste and touch (Stone *et al*., 2020). Sensory attributes of beef further define the quality of beef from a consumer's perspective as well as the production variables affecting it (Santos *et al*., 2021). The type of diet fed to animals has a significant impact on the sensory attribute of the produced meat (Scollan *et al*., 2006). Beef is the mostly consumed protein worldwide, and consumers are more concerned about its production of safe meat with no undesirable effects on their health

(Andersen, 2005). Beef quality is determined by sensory attributes of the meat and regarded a key driver within the beef category (Steiner, 2014). Tenderness is one of the most important sensory attributes by which consumers' judge meat quality (Stolowski *et al*., 2006). Meat tenderness is strongly affected by post-mortem processes such as aging and cooling (Aberle *et al*., 1981), however, nutritional factors may be considered to optimise beef quality (Sami *et al*., 2006). The volatile components of lipids within meat are the major contributors to meat flavour, and fatty acid composition is correlated with meat flavour (Larick and Turner, 1990). Melton (1983) highlighted that the largest differences in flavour occurred between beef from steers finished directly off forage and beef from steers finished on high concentrate corn diets, but these differences between diets were often confounded with differences in fatness.

2.8 Summary

The economic implications of woody plant encroachment significantly affect pastoral rangeland productivity. Woody plant encroachment leads to reduced herbage DM yield and quality. This leads to poor livestock productivity from such rangelands. Utilization of tanniniferous woody plant species as feed for ruminants would be a good strategy for reducing woody encroachment. Beef animals are economically, nutritionally and culturally important to the people of South Africa. However, ruminants produce a lot of methane in the rumen during microbial fermentation of feeds. High methane production and emission from ruminant livestock are major problems adversely affecting the environment, and hence climate change. Ruminant livestock lose about 6 % of energy intake through methane emission. Tannins in the diets of ruminants can decrease methane production and emission (Vasta *et al*., 2019). Tannins are divided into two major structural classes: condensed and hydrolysable tannins. If large quantities are consumed, both condensed and hydrolysable tannins are harmful to the animal. However, small intakes of condensed tannins may improve productivity of the animal and reduce methane production and emission (Vasta *et al*., 2019). Tannins affect ruminal fermentation by inhibiting ammonia and methane production through their ability to form complexes with dietary protein and carbohydrates. The effects of these on the animal performance range from beneficial to toxic, depending on their concentration and nature. Thus, information on the effect of tannins on ruminant production and methane emission is inconclusive. Therefore, it is important to determine tanniniferous *S. plumosum* supplementation levels for optimal performance and methane reduction in Nguni cattle. Such information will be useful for improving Nguni cattle production and reducing methane production and emission from them.

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CHAPTER 3

EFFECT OF *SERIPHIUM PLUMOSUM* MEAL INCLUSION IN THE DIETS ON FEED INTAKE, GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY OF NGUNI STEERS

Abstract

One of the limiting factors influencing livestock production under extensive production systems in South Africa is inadequate supply of nutritious feeds, especially during the dry season. The fluctuating quality and quantity of natural feed resources is exacerbated by encroachment of plant species, such as *Seriphium plumosum*. This study was conducted to determine the effect of inclusion levels of *S. plumosum* meal in diets on feed intake, digestibility, growth performance and body condition score of Nguni steers. Twenty-eight Nguni steers aged 22 months old with initial body weight of 300 ± 10 kg were were assigned to four treatment diets containing ($T1_0$), 10 ($T2_{10}$), 20 ($T3_{20}$) or 30 ($T4_{30}$) % replicated four times with seven steers per replicate in a complete randomized design. The diets were isocaloric and isonitrogenous. The experiment was conducted over 111 days, with 21 days of adaptation followed by 90 days of actual data collection. *Seriphium plumosum* meal inclusion levels in the diets of 0, 10, 20 or 30 % did not affect ($P>0.05$) diet intake, digestibility, feed conversion ratio, body weight gains and body condition scores of Nguni steers, indicating that inclusion levels used did not have any adverse effects on the steers. Utilization of *S. plumosum* in diets of cattle could be a strategy to manage woody encroachment in semi-arid grasslands.

Keywords: Animal performance, Bush encroachment, Forage quality, Productivity, Nguni cattle

3.1 Introduction

South Africa has a population of about 14 million beef cattle (Mare, 2018). Smallholder farmers keep approximately 47 % cattle in informal production systems and 53 % are kept by their elite counterparts in commercial production systems (Sikhweni *et al*., 2014). Livestock production in South Africa is primarily practised under extensive natural conditions, which does not meet their nutrient requirements (Kirkman and Carvalho, 2003; Meissner *et al*., 2013). This is because, among others, the rangelands that sustain livestock particularly in communal areas are degraded resulting in poor quality forages especially during the dry season (Rochana *et al*., 2016). The fluctuating quality and quantity of natural feed resources are also exacerbated by encroachment of woody and/or shrubby plants. Woody encroachment is characterized by an increase of woody or shrubby plants at the expense of herbaceous layer that alters ecosystem services such as forage production (Archer *et al*., 2017; Ding and Eldridge, 2019).

The proliferation of woody plant encroachment is caused by among others, erratic rainfall, suppression of fire and poor rangeland management such as overgrazing (Eldridge *et al*., 2015; Venter *et al*., 2018). It is for this reason that the rate and extent of woody encroachment are some of the major challenges to farmers, particularly those who rely on natural grazing for livestock production (Venter *et al*., 2018). In South Africa, for example, *Seriphium plumosum,* also known as bankrupt bush, has converted extensive areas of the grassland into less productive rangelands and consequently reducing the grazing capacity of rangelands (Snyman, 2012). *Seriphium plumosum* is an unpalatable indigenous dwarf woody shrub that encroached large parts of grasslands and some savanna areas of southern African rangelands e.g. South Africa, Angola, Namibia, Mozambique and Zimbabwe (Snyman, 2010, Pule 2021). Many woody plants including *S. plumosum* contain plant secondary metabolites, which inhibit feed intake and digestibility in ruminants (Estell, 2010). Nonetheless, cattle and goats browse young plants of *S. plumosum* at certain times of the year (Avenant, 2015), especially after fire and during the dry season.

Numerous studies have not yielded an integrated approach to develop management strategies for reducing woody plant encroachment (Ward, 2005; Brits *et al*., 2008). Supplementing livestock with locally available feed resources rich in nutrients (e.g. protein) may increase dry matter intake, body condition and animal weight gain (du Plessis and Hoffman, 2004; Mapiye *et al*., 2009a). Pule (2021) reported a 7 % crude protein content in

S. plumosum during the wet season after burning, which is within the maintenance requirements for ruminants (NRC, 2000). Therefore, exploring the strategic use of encroaching *S. plumosum* in diet formulation may be essential for improving livestock production and managing woody plant encroachment. The objective of the current study was to evaluate the effects of different dietary inclusion levels of *S. plumosum* meal on feed intake, nutrient intake and growth performance of Nguni steers.

3.2 Materials and methods

3.2.1 Study area

The study was carried out at the Agricultural Research Council - Animal Production (ARC-AP), Gauteng province, South Africa, during the summer season of 2020. The ARC-AP lies at 25° 53'S and 28° 11'E. The mean ambient temperatures in the study area are 15.8 ˚C in winter and 28 ˚C in summer. The dry season occurs between April and October and the mean annual rainfall is 661 mm, which falls between November and March (Kutu and Asiwe, 2010). The Agricultural Research Council, Animal Production Research Ethics Committee (Certificate: APAEC – 2020/16) and the University of Limpopo Animal Research and Ethics Committee (Certificate: AREC/01/2020: PG) approved the animal ethics protocol for the study.

3.2.2 Harvesting of the study plant

Seriphium plumosum forage was harvested at a private farm in Vanderbiljpark, Gauteng province between January and March 2020. The edible materials (leaves and branches) were harvested using a tractor slasher. The harvested *S. plumosum* material was sun-dried for three days before bailing and milling to pass through a 2 cm sieve size. The *S. plumosum* meal was stored in a well-ventilated dry shade pending the feeding trial.

3.2.3 Animal management, treatments, design and feeding

Twenty-eight Nguni steers aged 22 months old with initial body weight of 300 ± 10kg were assigned to four treatment diets containing ($T1_0$), 10 ($T2_{10}$), 20 ($T3_{20}$) or 30 ($T4_{30}$) % of *S. plumosum* replicated four times with seven steers per replicate in a complete randomized design. The Nguni steers on the diets were allowed 21 days to adapt to their respective diets and trained to feed from individual troughs prior to the 90-day supplementary feeding trial. Each steer was individually fed and all experimental steers had free access to clean water and feed. The experimental diets were formulated to meet the nutritional requirements of growing steers (NRC, 2000). The ingredients used to formulate treatments and chemical composition of the diets are shown in Table 3.1.

Table 3.1 Proportion of feed ingredients used in the diets

	Diets[#]			
Ingredients (%)	$T1_0$	$T2_{10}$	$T3_{20}$	$T4_{30}$
Lucerne hay	30.0	20.0	10.0	0.0
Maize meal	55.2	54.6	54.9	55.7
Molasses meal	8.1	8.6	7.9	5.4
Soya bean meal	6.0	6.0	6.2	7.2
Feed grade urea	0.2	0.6	0.5	1.2
Cattle salt	0.5	0.5	0.5	0.5
S. plumosum	0.0	10.0	20.0	30.0
TOTAL	100	100	100	100

[#]: Treatments were dietary *Seriphium plumosum* meal inclusion levels of 0 ($T1_0$), 10 ($T2_{10}$), 20 ($T3_{20}$) or 30 ($T4_{30}$) %.

3.2.4 Data collection

3.2.4.1 Animal feed and growth measurements

The steers were weighed fortnightly with a heavy-duty scale (Cattleway, Johannesburg, South Africa). Average daily gain (ADG) (g/day) was determined as a difference between final and initial body weight divided by the feeding days. Feed conversion ratio was calculated as the proportion of daily dry matter intake to daily weight gain (McDonald and Ho, 2010). The daily feed intake of each steer was calculated as the difference between feed offered and feed refused. Body condition score was visually appraised using a 5-point scale (1—very thin to 5—very fat) fortnightly (McDonald and Ho, 2010).

3.2.4.2 Digestibility trial

The digestibility trial was conducted at the end of the growth trial for five consecutive days using total faecal collection method (McDonald and Ho, 2010). Excreted faeces from each steer were collected from the concrete floor then thoroughly mixed, weighed and recorded. Twenty percent of the daily collected faeces from each steer was collected and frozen at -20˚C pending the analysis. At the end of the faecal collection period, the daily faecal output from each steer were mixed to form one representative sample. Faecal samples were then oven dried at 60°C for 72h. The dried samples were taken to ARC - Analytical Services laboratory for chemical analysis. Nutrient digestibility was done following equations of Mekuriaw and Asmare (2018).

3.2.5 Chemical analysis of diets

Dry matter of feeds and feed refusals were determined by drying the samples in the oven for 72 hours at 60 °C (AOAC, 2010). Dried feed samples were ground and milled to pass through a 1-mm sieve (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA) and stored in labelled airtight bottles for chemical analyses. Crude protein (CP), and ether extract (EE) of the diets were analysed according to AOAC (2010), while acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined according to the technique prescribed by Van Soest *et al*. (1991). Crude fibre (CF), calcium (Ca), phosphorus (P) and CP of feeds and *S. plumosum* were analysed following the procedures of AOAC (2010), whereas 2.3% condensed tannin (CT) was analysed by a method of Hattas *et al*. (2005).

3.2.6 Statistical analysis

Feed intake, nutrient digestibility, growth performance and feed efficiency were analysed using analysis of variance procedures of SAS (2018). Where a significant ($P<0.05$) treatment effect was found, the least significant difference test was used for mean separation. The statistical model was:

$\mathrm{Y}ij = \mu + ti + eij$;

where: $\mathrm{Y}ij$ = an observation from the jth animal that was subjected to the ith treatment; μ = the grand mean that is common to all observations; ti = the ith treatment; and eij = the random error. The performance parameters responses to *S. plumosum* meal inclusion levels in the diet were modelled for linear and quadratic effects using the following quadratic equation (SAS, 2008):

$$\mathrm{Y} = \mathrm{a} + b_1\mathrm{x} + \mathrm{b}_2x^2 + \mathrm{e}$$

Where Y = Feed intake, nutrient digestibility, growth performance and feed efficiency; a = intercept; b_1 and b_2 = coefficients of the quadratic equation; x = *S. plumosum* meal inclusion levels; -b1/2b2 $=\mathrm{x}$ value for optimal response and e is the error.

3.3 Results

3.3.1 Nutritional composition of diets

Results of the nutrient composition of the experimental diets are presented in Table 3.2. The diets were similar (P>0.05) in all the nutrients except that they had different (P<0.05) *Seriphium plumosum* meal inclusion levels.

Table 3.2 Nutritional composition of experimental diets

	Diets$^{\# *}$				***S. plumosum***
Parameters	$T1_{0}$	$T2_{10}$	$T3_{20}$	$T4_{30}$	
Dry matter (%)	90.6±3.51	91.3±4.42	91.0±3.51	91.8±3.60	87.9
Crude protein (%)	12.6±1.62	13.2±1.81	11.9±1.62	11.9±1.73	12.5
Energy (MJ/kg DM)	13.4±0.41	13.2±0.40	13.2±0.37	13.0±0.43	-
Fat (%)	2.7±0.72	2.4±0.51	2.3±0.42	2.3±0.61	2.2
NDF (%)	32.0±1.06	32.0±2.10	32.0±1.51	33.0±1.42	66.1
ADF (%)	26.0±2.45	26.1±3.34	26.2±3.45	26.3±3.46	37.0
Condensed tannins (%)	0.021	0.024	0.030	0.032	0.23

#: Diets were dietary inclusion levels of 0 (T10), 10 (T210), 20 (T320) or 30 (T430) % of Seriphium plumosum meal.

*: Values presented as mean ± standard deviation (SD).

3.3.2 Effect of *S. plumusom* meal inclusion levels in the diets on feed intake and digestibility of Nguni steers

The effect of *S. plumosum* inclusion levels of 0, 10, 20 or 30 % in the diets on intake and digestibility by Nguni steers are presented in Table 3.3. *Seriphium plumosum* meal inclusion in the diets at 0, 10, 20 or 30 % did not affect (*P*>0.05) DM, CP, energy, acid detergent fibre (ADF), neutral detergent fibre (NDF) and ether extract (EE) intake by Nguni steers. Similarly, *Seriphium plumosum* meal inclusion in the diets had no effect (*P*>0.05) on DM, CP, energy, ADF, NDF and EE digestibility values of Nguni steers.

Table 3.3 Effect of *S. plumosum* meal inclusion levels in the diets on nutrient intake and digestibility by Nguni steers

Parameters[*]	Diet[#]				*P*-value
	T1$_{0}$	T2$_{10}$	T3$_{20}$	T4$_{30}$	
Nutrient intake					
DM (kg/day)	5.6± 1.40	6.2 ± 0.52	6.0 ±1.63	7.1 ±0.73	0.119
CP (kg/day)	0.64±0.101	0.71±0.062	0.69±0.181	0.81±0.283	0.095
Energy (MJ/day)	71.0±10.11	81.8±11.52	79.2±10.31	92.3±12.22	0.081
NDF (kg/day)	1.58±0.29	1.76±0.141	1.70±0.603	2.00±0.204	0.087
ADF (kg/day)	1.11±1.110	1.24±0.101	1.19±0.320	1.41±0.402	0.088
EE (kg/day)	0.15±0.20	0.16±0.103	0.16±0.404	0.19±0.103	0.082
Digestibility (%)					
DM	67.2±7.93	62.3±8.44	57.0±16.62	54.4±8.74	0.199
CP	57.7±12.12	53.4±11.32	50.6±20.21	52.4±9.45	0.827
Energy (%)	60±0.94	60±0.89	59±1.01	59±1.11	0.071
ADF	49.1±10.13	40.2±6.42	36.0±18.73	33.2±13.98	0.186
NDF	77.4±15.80	61.0±13.54	52.5±13.49	50.8±12.22	0.652
EE	65.0±10.03	59.8±10.96	54.9±15.28	46.5±10.97	0.063

[#]: Treatments were *Seriphium plumosum* meal inclusion levels in the diet of 0 (T1$_{0}$), 10 (T2$_{10}$), 20 (T3$_{20}$) or 30 (T4$_{30}$) %.

[*]: Values presented as mean ± standard deviation (SD).

3.3.3 Effect of *S. plumosum* meal inclusion levels in the diet on body weight gain, feed conversion ratio and body condition score of Nguni steers

The effect of *S. plumosum* meal inclusion levels of 0, 10, 20 or 30 % in the diets on feed conversion ratio, body weight and body condition score of Nguni steers are presented in Table 3.4. *Seriphium plumosum* meal inclusion in the diets at 0, 10, 20 or 30 % did not affect (*P*>0.05) body weight, feed conversion ratio and body condition score values of Nguni steers.

Table 3.4 Effect of *S. plumosum* inclusion levels in the diets on growth performance of Nguni steers

	Diets[#]				
Parameters*	$T1_{0}$	$T2_{10}$	$T3_{20}$	$T4_{30}$	*P value*
Initial body weight (kg)	296.1±15.43	301.9±22.32	290.6±22.30	295.0±17.15	0.736
Final body weight (kg)	337.6±38.27	355.3±13.76	341.7±40.56	343.9±26.30	0.713
Daily body weight gain (kg/day)	0.52±0.330	0.58±0.270	0.46±0.550	0.53±0.231	0.936
FCR	11.4± .0.80	10.7±0.20	13.1±0.0.60	13.3±0.20	0.746
Body condition score	2.7±0.26	2.9±0.11	2.9±0.22	2.8±0.12	0.470

[#]: Treatments were *Seriphium plumosum* meal inclusion levels in the diet of 0 ($T1_{0}$), 10 ($T2_{10}$), 20 ($T3_{20}$) or 30 ($T4_{30}$) %.

[*]: Values presented as mean ± standard deviation (SD).

3.4 Discussion

The diets used in the current study were isocaloric and isonitrogenous and the *S. plumosum* inclusion levels in the diets were at 0, 10, 20 or 30 %. The diets met the nutrient requirements for slow-growing Nguni cattle as recommended by McDonald *et al.* (2010) and National Research Council (NRC, 2000). Thus, any observed differences in responses by the steers may be due to *S. plumosum* meal inclusion in the diets. *Seriphium plumosum* meal contained 0.23 % condensed tannins. Condensed tannins bind with diet proteins, carbohydrates and other nutrients, and hence they lower feed intake and digestibility in ruminants when included in large quantities (Makkar, 2003), thus reducing animal performance. However, the diets used in the present study contained a maximum of 0.03 % condensed tannins, far much less than the 2 % recommended by Salami *et al*. (2018) to impact on ruminal microbiome.

High levels of tannins in the diet are associated with their ability to bind with nutrients, resulting in lowering the bioavailability of proteins and carbohydrates in ruminants and hence reduce intake. However, small amounts in the diet improves intake and digestibility (Makkar *et al*., 2007). Tanniniferous *S. plumosum* meal inclusion in diets did not seem to interfere with rumen microbial fermentation and digestion, resulting in similar diet intake and digestibility values of Nguni steers. The results of the present study indicate that *S. plumosum* meal can be included in the diets at 10, 20 or 30% levels without adversely affecting diet digestibilities and intakes of Nguni steers. Koivunen *et al*. (2016) reported no significant differences in diet intake and digestibility values when cattle were fed on diets having diferent levels of tannins. The authors attributed this to relatively low amounts of tannins in the diets. Ng'ambi *et al*. (2022) reported that supplementation levels of 30, 40 or 50 g of purified condensed tannins per kg DM diet did not affect diet intake and digestibility values of Bapedi sheep but reduced methane emission by 51 to 60 %. Mathobela (2018) reported that feeding of different inclusion levels of *Acacia tortilis* leaf meal high in condensed tannins, did not affect organic matter digestibility and feed intake values of Boer goats. Chingala *et al*. (2019) obtained similar intake results when Malawi Zebu steers were supplemented with *Adansonia digitate* and *Vachellia polyacantha* leaf-meals. Similarly, Brown and Ng'ambi (2019) reported that the inclusion of *Vachelia karroo* meal in diets did not affect organic matter, crude protein, acid detergent fibre and neutral detergent fibre intakes of Pedi goats. However, Tiemann *et al*. (2008) included 300 g/kg of DM as foliage of *Calliandra calothyrsus* or *Ficus macrophylla*, high in condensed tannins, in the rations of growing sheep and recorded reductions in organic matter digestibilities of 8.5 and 11.5 %, respectively. There are other reports indicating improvement in intake and digestibility with increase in tanniniferous feeds in the diets (Makkar *et al*., 2007).

Seriphium plumosum meal inclusion levels in the diets of 0, 10, 20 or 30 % did not affect feed conversion ratio, live weight gains and body condition scores of Nguni steers. This indicate that inclusion levels used in the present study did not adversely affect these parameters and that these inclusion levels in a diet can be used without having any adverse effects on the animals. This was expected because *S. plumosum* meal inclusion levels in the diets did not affect digestibility and intake values of Nguni steers. This could be attributed to the relatively high crude protein levels in the diets, which promoted good growth levels of Nguni steers. The similarities in feed intake, digestibility, body weight gain and body condition score of the steers can be attributed to the fact that the diets had similar energy and protein levels. It is also possible that because condensed tannin levels in the diets were low, they did not adversely affect diet intake and digestibility values (Makkar *et al*., 2007). These results are similar to those of Mathobela (2018) who reported that inclusion of *Acacia nilotica* leaf meal in the diet did not affect live weight changes in Boer goats fed an *Avena sativa* hay-based diet. Similarly, Ng'ambi *et al*. (2022) supplemented diets with 0, 30, 40 or 50 g of purified condensed tannins per kg DM and did not have any significant effect on feed intake, digestibility and body weight gain of yearling male Bapedi sheep. The authors indicated that the supplementation levels used in the study did not supply a lot of tannins to adversely affect the parameters, and that the similarities in feed intake, digestibility and body weight gain of the sheep could be attributed to the fact that the diets had similar energy and protein levels.

3.5 Conclusions

Seriphium plumosum meal inclusion levels in the diets of 0, 10, 20 or 30% did not affect diet intake, digestibility, feed conversion ratio, body weight gains and body condition scores of Nguni steers. This indicate that inclusion levels of *S. plumosum* used in the study did not adversely affect these parameters, and that these inclusion levels in a diet can be used without having any adverse effects on the animals. Thus, an encroaching *S. plumosum* has the potential for use as a feed ingredient for ruminants and this may be used as a mitigating strategy for controlling bush encroachment in the semi arid grassland areas of South Africa. However, similar long-term studies are suggested to ascertain the present findings.

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CHAPTER 4

EFFECT OF INCLUSION LEVELS OF *SERIPHIUM PLUMOSUM* MEAL IN A DIET ON RUMEN MICROBIAL COMPOSITION AND METHANE EMISSION BY NGUNI STEERS

Abstract

In ruminants, methane is produced mostly by enteric fermentation where microbes decompose and ferment ingested plant materials, such as celluloses, fibre, starches and sugars. Enteric methane is a by-product of digestive fermentation process expelled by animals through belching or auxiliary. This study evaluated the effect of *Seriphium plumosum* meal inclusion in the diets on methane emission, bacterial profiles and mineral concentrations in rumen fluid of Nguni steers. Twenty-eight Nguni steers aged 22-months and averaging 300 ± 10 kg body weight were assigned to isocaloric and isonitrogenous diets containing 0, 10, 20 or 30 % of *S. plumosum* meal in a complete randomized design. Methane emission was measured using a laser methane detector. Bacterial population was investigated by 16S rRNA gene with Illumina MiSeq. Mineral concentrations were determined by Perkin Elmer Model Optima 7000 DV spectrometer. Inclusion of *S. plumosum* in diets significantly ($P<0.05$) reduced methane emission by Nguni steers. A 13.6 % *S. plumosum* meal inclusion level in the diet was calculated, using quadratic equations, and resulted in optimal CH_4 emission reduction by Nguni steers. Inclusion of *S. plumosum* in diets did not ($P>0.05$) affect rumen fluid pH and mineral concentrations. The *Spirochaetota*, *Actinobacteriota, Bacteroidota, ProteobacteriaSAR324, Verrucomicrobiota, Desulfobacterota, Patescibacteria, Patescibacteria, Verrucomicrobiota and Chloroflexi* were dominant phyla in rumen fluids of Nguni steers, but their relative abundance was not affected (P>0.05) by *S. plumosum* meal inclusion levels in the diets. It was concluded that *S. plumosum* inclusion in the diets reduced methane emission by Nguni steers, and *S. plumosum* may be strategically used as a climate-smart feed resource to reduce methane production and emission by cattle.

Keywords: Bacteria, Methane emission, Nguni cattle, Woody plant species

4.1 Introduction

The livestock sector is responsible for about 14.5% of the global agricultural greenhouse gases (GHG) that contribute to climate change, and ultimately causing increases in land degradation, air and water pollution, and declines in biodiversity (Grossi *et al*., 2019). Ruminants account for 81 % of total methane emissions. Methane (CH_4) is the second largest atmospheric GHG following carbon dioxide; however, it is the most concerning GHG due to its 25 times global warming potential and a 12-year atmospheric lifetime (Hook *et al*., 2010). In South Africa, extensive livestock production is dependent on rangeland species of which the effect of climate change exacerbates their nutritional status, particularly during the dry season (Bekele, 2017). Climate change causes a decline on natural vegetation, which is critical for livestock production, and as such, producers are unable to meet the demand for livestock products (Garnett, 2009). It is, therefore, critical to develop and implement mitigation strategies aimed at reducing CH_4 emissions from the livestock sector and hence limiting the environmental burden while ensuring a sufficient supply of food for a growing world population (Grossi *et al*., 2019).

During degradation of feed in the rumen, ruminants produce 47 % of CH_4 through the process known as enteric fermentation (Haque, 2018). Digestion of feed in the rumen is the result of anaerobic fermentation involving various groups of microbes (bacteria, archaea, protozoa, and fungi). Enteric CH_4 is the single largest source of CH_4 contributed by ruminants and as such, it is a major concern for climate change worldwide (Johnson and Johnson, 1995). Enteric CH_4 is the product of a methanogenesis process that takes place in the rumen where H_2 reduces the CO_2 with the help of methanogenic archaea resulting in CH_4 production (Ellis *et al*., 2008). Methanogens, to be specific, are the only known microorganisms capable of producing CH_4, and as such, their abundance in the rumen makes them of interest when developing strategies aimed at reducing methane production and emission by ruminant animals (Hook *et al*., 2010). The production of enteric CH_4 is associated with a loss of about 2 to 12 % gross energy intake (Johnson and Johnson, 1995), which animals could efficiently utilise for growth, production of animal by-products, reproduction and so forth (Hook *et al*., 2010).

Dietary manipulation is the commonly practised approach that has shown the potential to reduce CH_4 production by 40 % (Mosier *et al*., 1998). This approach is regarded as the most effective and most convenient way to reduce methane production and emissions, and in turn retain energy loss in the animal and increase nitrogen utilization efficiency (Li *et al*., 2022). The diet is a predominant factor affecting the microbial community composition in the rumen and the rumen environment of the host animal (Henderson *et al*., 2015). Thus, when tanniniferous feeds are included in the diet, they alter the availability of nutrients and metabolites and/or inhibit ruminal microbial metabolism of bacteria, protozoa, fungi and archaea populations (Vasta *et al*., 2019). The high molecular weight and polyphenolic nature of tannins result in the formation of complexes with microbial enzymes or cell walls. Thus, the exerted activity may cause the inhibition of cellulolytic or proteolytic bacteria or methanogens (Mannelli *et al*., 2019). The mode of action of tannins is strictly dependant on their chemical structure as well as the bacteria species (Vasta *et al*., 2019).

Condensed tannins (CT) have a stronger binding ability with nutrients than hydrolysed tannins, mainly due to their higher grade of polymerization, which makes their degradation in the rumen environment more difficult (Jayanegara *et al*., 2015). The CT were observed to directly inhibit some ruminal gram-positive specialized fibrolytic bacteria (*Fibrobacter succinogenes, Ruminococcus albus, Ruminococcus flavefaciens, Butyrivrio proteoclasticus*) in an *in vivo* study with fistulated ewes (Costa *et al*., 2018). *Fibrobacter succinogenes* and total methanogen population inhibition of up to 36 % have been reported *in vitro* (Jayanegara *et al*., 2015). Salami *et al*. (2018), included 4 % of *Mimosa pudica* in the diets of lambs and did not observe a difference in absolute abundance of bacteria and fungi, while methanogens abundance decreased by 12 %. In an *in vitro* study, chestnut condensed tannins reduced methane production by 12.5 %, while acetate production increased (Cappucci *et a*l., 2021). A dosage above 20 g CT per kg DM diet has been reported by Jayanegara *et al*. (2012) and Salami *et al*. (2018) to affect ruminal microbiome.

The use of woody plant species is now being introduced as supplementary feed for livestock. When harvested at an early stage, woody plant species contain fermentable carbohydrates and less acid detergent fibre (ADF), and as such, they present an opportunity to reduce CH_4 production without compromising livestock productivity. It is also being reported that feeding *Calpurnia aurea* to goats reduced enteric CH_4 emission by 5 %. Generally, woody plant species contain plant secondary metabolites, such as condensed tannins, phenols, etc. that play important roles in reducing CH_4 production and emission in ruminants (Terranova *et al*., 2020). This suggests that the use of woody plant species as supplementary feed for ruminants could be a plausible strategy for reducing CH_4 emission and improving productivity of ruminant animals (Jerónimo *et al*., 2016).

Seriphium plumosum is an indigenous South African woody plant species that has slowly occupied the significant portions of grassland meant to sustain livestock production. This invasive species contributes highly to woody plant encroachment, which consequently becomes a huge challenge for livestock farmers who rely on grasses for their livestock production. Nonetheless, *S. plumosum* contains plant secondary metabolites that act against rumen microbial activities and reducing methane production and emission (Ku-Vera *et al*., 2020). Additionally, *S. plumosum* contains relatively high amounts of protein, which would be very useful during the dry season when grass has low nutritive values. This creates an opportunity for *S. plumusom* to be used as a feed ingredient when formulating ruminant diets for optimal productivity, particularly during the dry season. Mitigation strategies aimed at reducing the emission intensity of CH_4 are required to meet the increasing demand for livestock products (Waghorn and Hegarty, 2011). Therefore, the aim of the current study was to determine the effect of inclusion levels of *S. plumosum* meal in a diet on rumen microbial composition and methane emission by Nguni steers.

4.2 Materials and methods

4.2.1 Study area

The study was carried out at the Agricultural Research Council - Animal Production (ARC-AP), Gauteng province, South Africa, during the summer season of 2020. The ARC-AP lies at 25° 53’S and 28° 11’E. The mean ambient temperatures around the study area are 15.8 ^{0}C in winter and 28 ˚C in summer (https://en.climate-data.org). The dry season occurs between April and October and the mean annual rainfall is 661 mm, which falls between November and March (Kutu and Asiwe, 2010). The Agricultural Research Council, Animal Production Research Ethics Committee (Certificate: APAEC – 2020/16) and the University of Limpopo Animal Research and Ethics Committee (Certificate: AREC/01/2020: PG) approved the animal ethics protocol for this study.

4.2.2 Harvesting of the study plant

Seriphium plumosum forage was harvested at a private farm in Vanderbiljpark, Gauteng province between January and March 2020. The edible materials, leaves and branches, were harvested using a tractor slasher. The harvested *S. plumosum* material was sun-dried for 3 days before bailing and milling to pass through a 2 cm sieve size. The *S. plumosum* milled material was stored in a well-ventilated dry shade pending the feeding trial.

4.2.3 Animal management, treatments, design and feeding

Twenty-eight Nguni steers aged 22 months old with initial body weight of 300 ± 10 kg were assigned to each of the four dietary treatments, which were *S. plumosum* meal inclusion levels in the diet of 0 ($T1_0$), 10 ($T2_{10}$), 20 ($T3_{20}$) or 30 ($T4_{30}$) % (for ingredients used in the diets refer to Table 3.1, Chapter 3). Each treatment had seven replications and one animal per replicate in a complete randomized design. The Nguni steers were allowed 21 days to adapt to their respective diets, and acclimatized to feed from individual troughs prior to the 90-day collection period. Each steer was individually fed and all experimental animals had free access to clean water and feed. The experimental diets were formulated to meet the nutritional requirements of growing steers (NRC, 2000). The daily feed intake of each steer was determined as the difference between feed offered and refusal.

4.2.4 Data collection

4.2.4.1 Measurements of CH4 emission using a laser methane detector

Daily CH_4 emissions were measured for 10 consecutive days from individually penned steers using the laser methane detector (LMD) (Crowcon Detection Instruments Ltd., Tokyo, Japan). The emitted gas was measured by directing the LMD laser beam to the auxiliary of Nguni steers at an approximately 1-meter distance between the animal and LMD operator (Chagunda *et al*., 2013; Ricci *et al*., 2014). This distance used enabled the operator to measure the CH_4 emission from animals without interfering with their activities (Chagunda *et al*., 2009). Measurements were repeated five times with 1-min halts in-between; and they were taken two hours after the morning feed when animals were ruminating (Roessler *et al*., 2018). The one-minute interval between five minutes' measurements was done to include different stages of the tidal respiratory cycle during measurements (Grobler *et al*., 2014). Methane emission values were recorded manually and an average of thirty-repeated measurements represented a single value of daily CH_4 emission from each steer. Enteric CH_4 emission is normally reported per day or per unit of product produced; however, in the current study, the output was expressed in parts per million meter (ppm-m/d) (Chagunda *et al*., 2009).

4.2.4.2 Analysis of rumen fluid minerals (phosphorus, calcium, sodium and potassium)

The rumen fluid samples were taken from 24 Nguni steers using a stomach tube and then put into honey jar bottles. The pH of rumen fluid was measured immediately after sampling using digital pH meter (781 pH/Ion Meter pH meter by Metrohm).

Samples were frozen at 80 ˚C and stored for later analysis. Determination of minerals was performed using a Perkin Elmer Model Optima 7000 DV spectrometer (Perkin Elmer, USA) while 2-point background correction and 3 replicates were used to measure the analytical signal with the processing mode being the peak area (Abdelrahman *et al*., 2022). A 45 mL aqueous sample was extracted in an acid solution with PerkinElmer Titan MPS and transferred into the digestion vessel to analyse concentrations of phosphorus (P), calcium (Ca), sodium (Na) and potassium (K) in defrost rumen fluid. Emission intensities were obtained for the most sensitive lines free of spectral interference. Calibration standards were prepared by diluting the stock multi-elemental standard solution (1000 mg L^{-1}) in 0.5 % (v/v) nitric acid. Calibration curves for all elements were in the range of 1.0 ng mL^{-1} to 1.0 µg mL^{-1} (1–1000 ppb). A 5.0 mL of liquid nitrogen was added, and the mixture was allowed to react for a period of 20 minutes prior to heating in the microwave oven. Digestion vessel was then carefully opened in a fume hood.

4.2.4.3 Deoxyribonucleic acid (DNA) extraction in rumen fluid for bacterial analysis

The V3-V4 region of the bacterial 16S rRNA gene was amplified using the universal primer set 338F (5’-ACTCCTRCGGGAGGCAGCAG-3’) and 806R (5’-GGACTACCVGGGTATCTAAT-3’) (Mao *et al*., 2015). Bacterial DNA was extracted from rumen fluid samples in triplicate using 300-µL sample per replicate and the FastDNAR Spin kit for soil (MP Biomedicals, LLC). The extraction step was performed in accordance with the manufacturer’s protocol except for an additional purification step to remove polymerase chain reaction (PCR) was performed to amplify the V3–V4 regions of the 16S ribosomal ribonucleic acid (rRNA) gene using universal primers 338F 5′-barcode-ACTCCTACGGGAGGCAG CAG-3′ and 806R 5′- barcode-GGACTACHVGGGTW TCTAAT-3′ (Dennis *et al*., 2013). In brief, samples were washed and re-suspended with a humic acid wash solution, which contained sodium phosphate buffer, MT buffer (provided with the kit) and 5.5 M guanidine thiocyanate. The samples were transferred to SPIN filter, following settling of the binding matrix. In the final step, DNA was eluted by adding 50 µL DNase/Pyrogen-Free water (provided with the kit). DNA concentration was quantified using a Qubit fluorometer (Invitrogen Life Technologies), with a range between 45.7 and 148 ng/µL.

4.2.4.3 16S rRNA sequencing analysis for bacterial population in rumen fluid

Sequence analysis was performed as described in Müller *et al*. (2016). In brief, sequences were quality trimmed and trimmed pair end reads were further processed using the QIIME software package, version 1.8 (Caporaso *et al*., 2010). Sequence data were grouped into operational taxonomic units (OTUs) sharing 97 % sequence similarity using an open reference OTU picking strategy. The most abundant sequence in each OTU was selected as representative sequences, and further aligned against the Green-genes core set using PyNAST software (Caporaso *et al*., 2010). Taxonomy was assigned to each OTU using the Ribosomal Database Project (RDP) classifier with a minimum confidence threshold of 80 % (Wang *et al*., 2007). The OTU tables were subsampled (according to the sample containing the smallest set of sequences) to equalize sampling depth. For further univariate analysis of bacteria, a threshold level at OTUs containing more than 5786 reads (0.1% of total reads) was used. One period for one cow was removed in QIIME because of comparatively fewer reads content in all three replicates (only 0.05 % of total reads compared with the other samples).

4.2.5 Chemical analysis

Dry matter of feeds and feed refusals were determined by drying the samples in the oven for 72 hours at 60 °C (AOAC, 2010). Dried feed samples were ground and milled through a 1-mm sieve (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA) and stored in labelled airtight bottles for chemical analyses. Crude protein (CP), and ether extract (EE) in total mixed ration diets were analysed according to AOAC (2010), while acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined according to the technique prescribed by Van Soest *et al*. (1991). Crude fibre (CF), calcium (Ca), phosphorus (P) and CP of feeds and *S. plumosum* were analysed following the procedures of AOAC (2010), whereas condensed tannins (CT) were analysed by a method of Hattas *et al*. (2005).

4.2.6 Statistical analyses

Data on methane emission, mineral concentrations and the relative abundances of communities at the phyla and genus levels and the alpha diversity indices were assessed by analysis of variance using general linear model of SAS (2008). The dependent variables were CH_4 emission concentration, mineral concentrations and relative abundance of bacterial population at different levels. Least significant differences test was used to test the difference between means, taking P<0.05 as the level of significance. The model $\mathrm{y_{ij}} = \mu + \mathrm{T_i} + \mathrm{e_{ij}}$ was applied, where $\mathrm{y_{ij}} =$ response variables; $\mu =$ population mean; $\mathrm{T_i} =$ fixed effect of the $\mathrm{i^{th}}$ treatment level (i = 0, 10, 20 and 30 g/kg DM) and $\mathrm{e_{ij}} =$ random error. The responses in CH_4 emission and bacterial population to *S. plumosum* meal inclusion levels in the diet were modelled for linear and quadratic effects using the following quadratic equation (SAS, 2008):

$$Y = a + b_1 x + b_2 x^2 + e$$

Where Y = CH_4 emission concentration and bacterial population abundance. ; a = intercept; b_1 and b_2 = coefficients of the quadratic equation; x = *S. plumosum* meal inclusion levels; $-b_1/2b_2 = x$ value for optimal response and e is the error.

4.3 Results

4.3.1 Effect of *Seriphium plumosum* meal inclusion levels in the diets on methane emission by Nguni steers

Results of CH_4 emission by Nguni steers fed diets with different inclusion levels of *S. plumosum* meal are presented in Figure 4.1. Inclusion of *S. plumosum* meal in the diets affected ($P<0.05$) CH_4 emission by Nguni steers. Steers fed a diet having 30 % of *S. plumusom* meal had lower ($P<0.05$) CH_4 emission values than those fed diets having 10 or 20 % of *S. plumosum* meal. Similarly, steers fed a diet having 0 % of *S. plumusom* meal had lower ($P<0.05$) CH_4 emission values than those fed a diet having 10 % of *S. plumosum* meal. However, steers fed diets having 0 or 30 % of *S. plumusom* meal had similar ($P>0.05$) CH_4 emission values. Steers fed diets having 0 or 20 % of *S. plumusom* meal had similar ($P>0.05$) CH_4 emission values. Similarly, steers fed diets having 10 or 20 % of *S. plumusom* meal had the same ($P>0.05$) CH_4 emission values. A 13.6% *S. plumosum* meal inclusion level in the diet was calculated, using quadratic equations, to result in optimal CH_4 emission reduction by Nguni steers ($Y = 104.27 + -1.9646X + 0.06532X^2$).

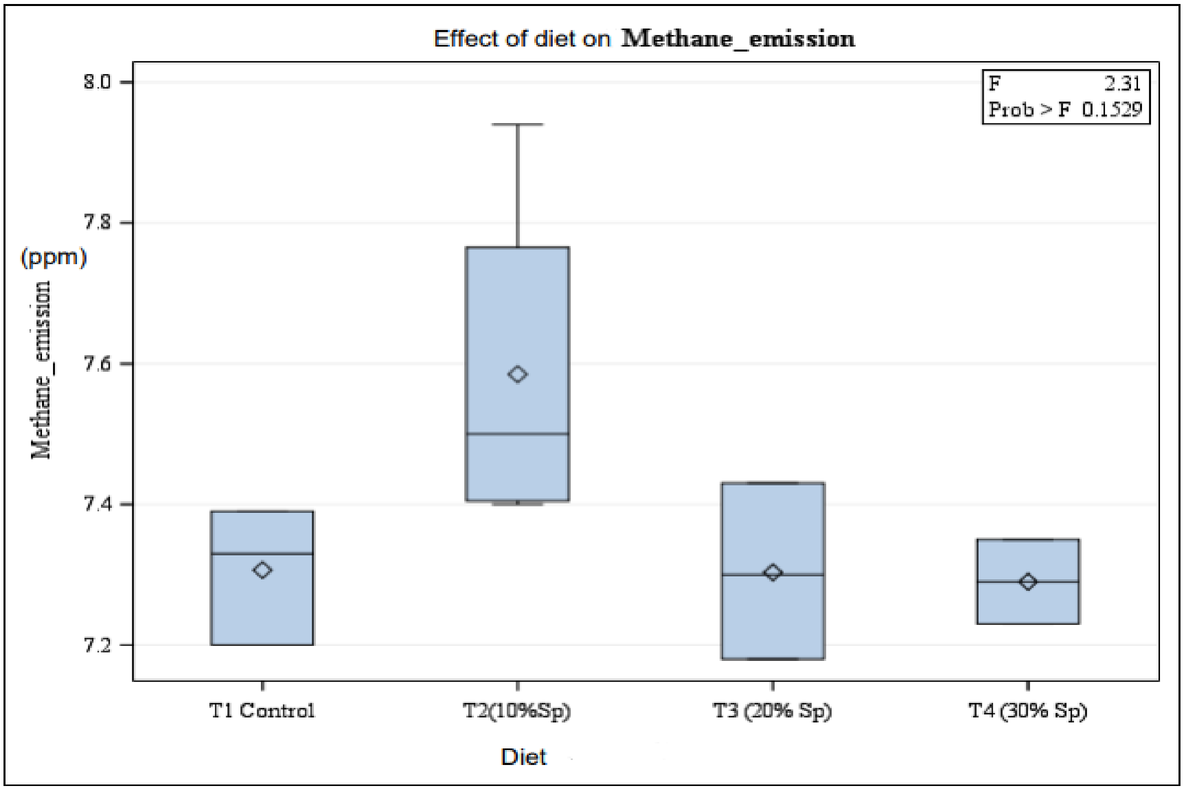

Figure 4.1 Variation in methane emission by Nguni steers according to diet. : Treatments were *Seriphium plumosum* meal inclusion levels in the diets of 0 ($T1_0$), 10 ($T2_{10}$), 20 ($T3_{20}$) or 30 ($T4_{30}$) %. : The horizontal line within each box represents the mean of all the daily methane emission measurements in units of parts per million meter (ppm) methane per kg DM intake. The two segments in the box represent the second and third quartiles, while the lines describe the first and fourth quartiles. Thus, the means were 104.93, 89.19, 93.07 and 103. 45-ppm methane/kg DM intake for $T1_0$, $T2_{10}$, $T3_{20}$ and $T4_{30}$, respectively.

4.3.2 Effects of *S. plumosum* meal inclusion levels in the diets on rumen fluid pH and mineral concentrations of Nguni steers

The results of the effects of *S. plumosum* meal inclusion levels in the diets at 0, 10, 20 or 30 % on pH and sodium (Na), calcium (Ca), potassium (K) and phosphorus (P) mineral concentrations in rumen fluid of Nguni steers are presented in Table 4.1. *Seriphium plumosum* inclusion levels in the diets did not affect (P>0.05) rumen fluid pH values and mineral concentrations.

Table 4.1 Effect of *S. plumusom* meal inclusion in the diets on rumen fluid pH and mineral concentration of Nguni steers

	Diets[#]				
Parameters[*]	$T1_{0}$	$T2_{10}$	$T3_{20}$	$T4_{30}$	*P* value
pH	7.34 ± 0.100	7.58 ±0.200	7.38 ± 0.100	7.32 ± 0.700	0.1257
Sodium (µg/ml)	115±45.1	127±42.7	103 ± 28.9	117 ± 35.9	0.7509
Calcium (µg/ml)	39.2±13.4	51.1± 30.6	51± 32.9	35 ± 18.5	0.6044
Potassium (µg/ml)	66± 43.8	47 ± 22.6	38 ± 12.1	39 ± 16.6	0.2521
Phosphorus (µg/ml)	48 ± 16.9	51 ± 19.1	38 ± 14.5	36 ± 15.3	0.3875

[#]: Treatments were *Seriphium plumosum* meal inclusion levels in the diet of 0 ($T1_{0}$), 10 ($T2_{10}$), 20 ($T3_{20}$) or 30 ($T4_{30}$) %

[*]: Values presented as mean ± standard deviation (SD)

4.3.3 Effect of *Seriphium plumosum* meal inclusion levels in the diets on abundance, diversity and composition of rumen microbes in Nguni steers

A total of 31 phyla, and 260 genera were identified in Nguni steer rumen microbiome. The most abundant 10 phyla and 26 genera are presented in Figure 4.2 and Table 4.4, respectively. Table 4.2 shows the abundancy of 31 bacteria that were detected at the phylum level. Among these 31, 10 phyla with a relative abundance of above 1% at least in one of the treatment diets were identified as the dominant phyla (Figure 4.3). *Seriphium plumosum* meal inclusion levels in the diets did not have effect (P>0.05) on Nguni steer rumen fluid bacterial abundance at phylum level.

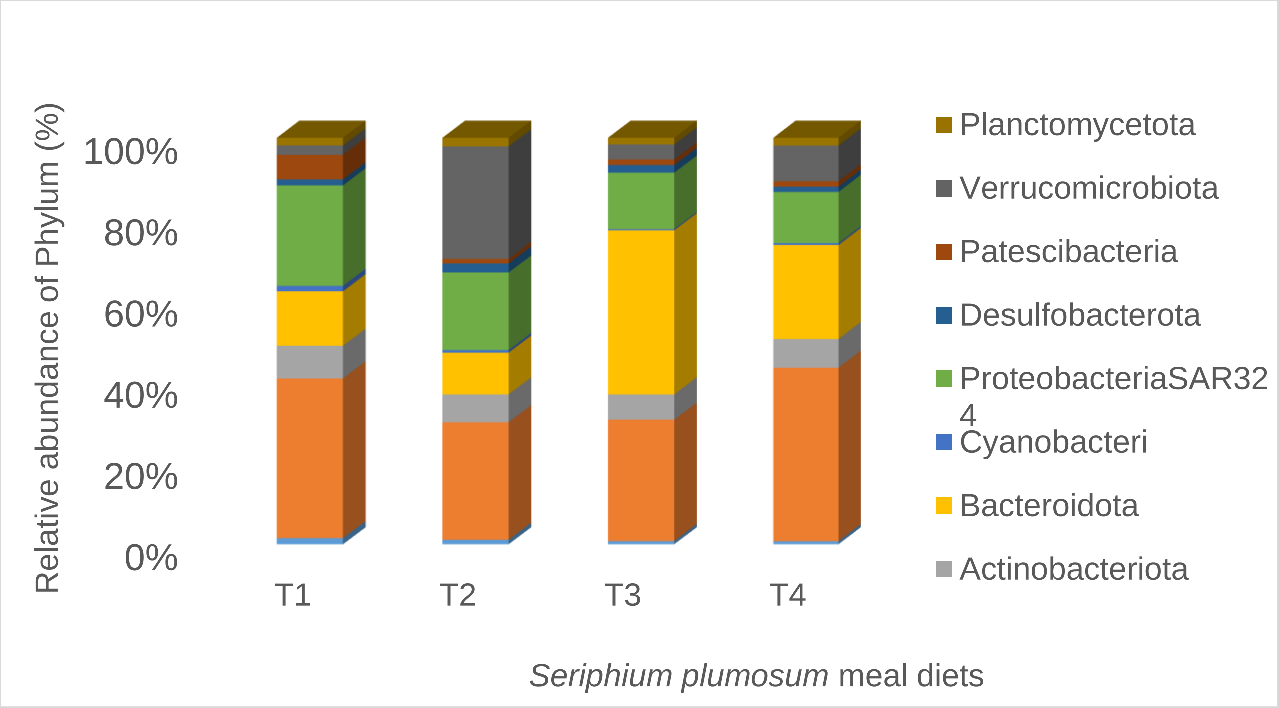

Figure 4.2 The most predominant of the top 10 bacterial phyla that were identified in rumen fluid of Nguni steers. Treatments were *Seriphium plumosum* meal inclusion levels in the diet of 0 ($T1_{0}$), 10 ($T2_{10}$), 20 ($T3_{20}$) or 30 ($T4_{30}$) %.

Table 4.2 Effect of *S. plumosum* meal inclusion in the diets on rumen fluid bacterial abundance at phylum level of Nguni steers

Bacteria[*]	**Diets**[#]				***P***
	T1	**T2**	**T3**	**T4**	**value**
Acidobacteria	0.62±0.100	0.54±0.200	0.39±0.200	0.39±0.100	0.104
Armatimonadota	0.25±0.100	0.35±0.100	0.21±0.100	0.19±0.700	0.104
Chloroflexi	1.3±0.40	0.99±0.30	0.7±0.30	0.7±0.20	0.226
Euryarchaeota	0.42±0.270	0.29±0.180	0.34±0.201	0.23±0.101	0.063
Fibrobacterot	0.37±0.901	0.3±0.101	0.27±0.101	0.22±0.101	0.191
Synergistota	0.78±0.200	0.40±0.500	0.37±0.200	0.29±0.330	0.064
Thermoplasmatota	0.46±0.400	0.33±0.400	0.28±0.100	0.16±0.200	0.062
Campylobacterot	0.22±0.101	0.18±0.801	0.13±0.070	0.11±0.060	0.062
Acidobacteriota	0.62±0.140	0.54±0.210	0.39±0.220	0.39±0.160	0.494
Abditibacteriota	0.11±0.070	0.09±0.030	0.06±0.030	0.05±0.020	0.826
Firmicutes	34±18.40	26±8.40	27±14.10	40±13.30	0.365
Nitrospirota	0.08±0.200	0.09±0.300	0.07±0.4	0.05±0.200	0.835
Planctomycetota	1.6±0.40	1.9±0.80	1.5±0.40	1.8±1.10	0.089

Spirochaetota	0.67±0.301	1.05±0.901	0.81±0.601	0.68±0.401	0.071
Synergistota	0.37±0.201	0.78±0.501	0.41±0.200	0.29±0.100	0.060
Thermoplasmatota	0.46±0.400	0.33±0.400	0.16±0.101	0.28±0.201	0.067
Actinobacteriota	6.9±1.90	6.2±2.04	5.6±2.59	6.6±3.30	0.065
Bacteroidota	11.6±6.30	9.3±3.40	36.3±26.60	21.6±17.30	0.070
Bdellovibrionot	0.37±0.101	0.65±0.301	1.12±1.201	0.29±0.101	0.061
Cyanobacteri	1.14±0.800	0.57±0.400	0.27±0.100	0.43±0.200	0.492
Elusimicrobiota	0.32±0.040	0.36±0.100	0.30±0.200	0.39±0.100	0.061
ProteobacteriaSAR324	21.3±9.90	17.3±6.80	12.5±6.70	11.8±3.80	0.061
Desulfobacterota	1.3±0.300	2.0±1.40	1.7±0.90	1.2±0.40	0.080
Fusobacteriota	0.6±0.40	0.8±0.90	0.3±0.20	0.3±0.10	0.267
Gemmatimonadota	0.09±0.020	0.08±0.030	0.06±0.301	0.06±0.301	0.965
Halobacterota	0.08±0.020	0.09±0.030	0.06±0.300	0.05±0.200	0.851
Myxococcota	0.74±0.201	0.77±0.301	0.45±0.201	0.40±0.101	0.180
Patescibacteria	5.2±13.90	1.0±0.30	1.2±0.90	1.3±1.00	0.061
cladeMarinegroup	0.45±1.201	0.45±0.401	2.84±6.101	0.20±0.310	0.060
Verrucomicrobiota	2.0±0.50	25.0±25.30	3.3±2.30	8.2±12.40	0.924
WPS_2	0.06±0.010	0.52±0.600	0.59±0.600	0.31±0.400	0.839

$^{\#:}$ Treatments were *S. plumosum* inclusion levels in the diet of 0% (T1), 10% (T2), 20 (T3)or 30 (T4) %

: Values presented as mean ± standard deviation (SD) and relative abundance is given as a percentage

Methane emission correlated (98.98%) positively with the relative abundance of the genus *Firmicutes, Actinobacteriota, ProteobacteriaSAR324 and Verrucomicrobiota* (Figure 4.4), whereas correlation with *Bacteroidota* was negative (Figure 4.3). *Paracoccus* was highly correlated with methane production at genus level.

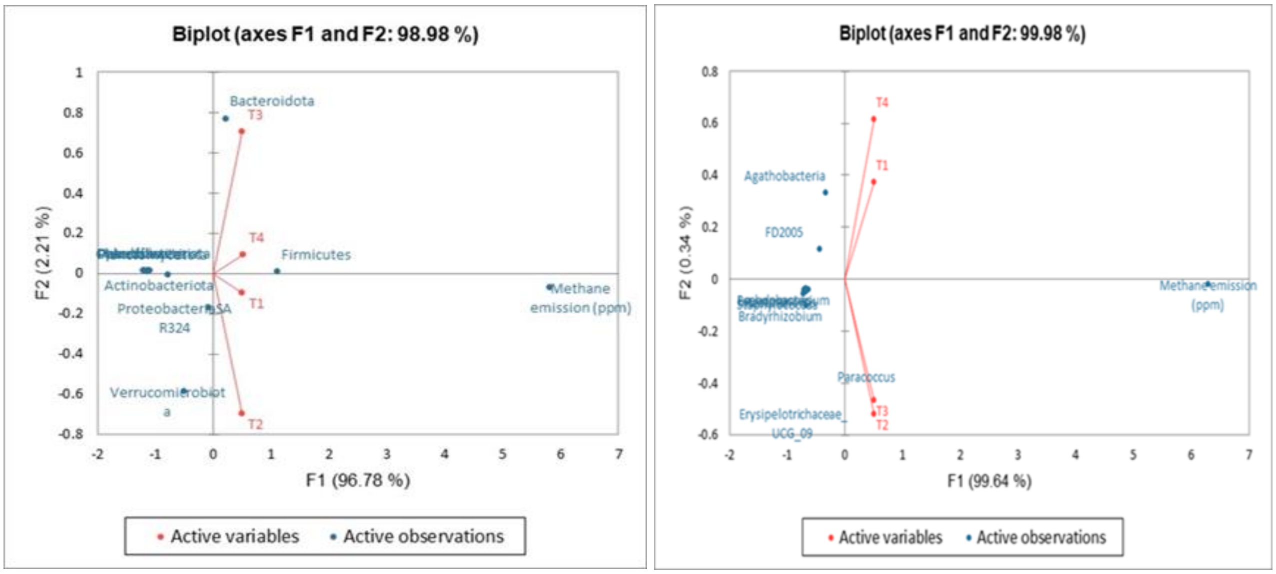

Figure 4.3 Dominant *Phyla* bacteria Figure 4.4 Dominant *Genera* bacteria

Figure 4.3 Correlation analyses between relative abundances of bacterial phyla and genera (present at ≥ 0.1 % in at least one sample) and methane emission by Nguni steers as influenced by *S. plumosum* meal inclusion levels.

Alpha diversity analysis was measured using six indices (Observe, Chaol 1, ACE, Shannon, Simpson and Fisher) and the results are presented in Table 4.3. Inclusion of *S. plumosum* meal in the diets had no effect (P>0.05) on alpha diversity of species.

Table 4.3 Effect of *S. plumosum* inclusion levels in the diets on Alpha diversity measures of rumen bacterial communities of Nguni steers

	Diets[#]				
Bacteria[*]	T1	T2	T3	T4	*P* value
Observe	524±15.4	523±12.9	534±12.04	537±9.364	0.066
Chao1	5.83±2.600	3.76±0.000	3.76 ±0.000	3.67±0.300	0.061
ACE	7.9±1.30	7.4±1.10	7.8±1.80	8.8±1.10	0.093
Shannon	4.8±0.60	4.8±0.91	4.2±1.40	4.5±0.70	0.061
Simpson	0.91±0.080	0.92±0.101	0.83±0.200	0.90±0.070	0.062
Fisher	151±18.8	154±28.7	135±21.5	134±16.1	0.078

[#]: Treatments were *S. plumosum* inclusion levels in the diets of 0 (T1), 10 (T2), 20 (T3) or 30% (T4)

[*] : Values presented as mean ± standard deviation (SD) and relative abundance is given as a percentage

At the genus level, 260 classifiable genera were detected in all samples. In total, 26 of these genera showed a relative abundance of >1% in all samples across the treatment groups, and their relative abundance results are presented in Table 4.4. *Seriphium plumosum* meal inclusion levels in the diets did not have effect (P>0.05) on relative abundance of the majority of genera except (P<0.05) for *Intestinimonas*, *NED5E9*, *Quinella* and *Paracoccus*. The numbers for *NED5E9*, *Quinella* and *Paracoccus* tended to increase (P<0.05) with increased *S. plumosum* meal inclusion level in the diets. However, no particular trend was observed with *Intestinimonas*.

Table 4.4 Effect of *S. plumosum* meal inclusion in diets on relative abundances of bacterial genera in rumen fluid samples of Nguni steers

Genus bacteria*	Diets#				*P* value
	T1	T2	T3	T4	
Agathobacteria	10.5±18.10	0.3±0.50	0.1±0.06	12.7±21.40	0.063
Bradyrhizobium	1.0±1.30	1.3 ±1.20	1.5±1.7	0.2±0.30	0.070
Clostridium_sensu_stricto_1	0.04±0.060	2.01±3.100	0.25±0.210	0.18±0.1001	0.080
Coriobacteriaceae_UCG_002	0.24±0.200	1.91±2.800	0.76±0.800	0.76±1.400	0.061
DEV114	0.08±0.100	7.31±6.300	4.33±4.800	0.80±1.400	0.062
Erysipelotrichaceae_UCG_09	2.74±2.400	0.40±0.600	0.50±0.600	0.46±0.600	0.070
Eubacterium	0.26±0.201	0.17±0.040	0.19±0.100	0.10±0.030	0.061
FD2005	7.02±6.200	0.13±6.800	3.09±5.200	7.02±7.901	0.070
Intestinimonas	1.94^{a}±0.150	0.47^{b}±0.100	0.72^{ab}±1.200	0.62^{ab}±0.900	0.0001
Lachnobacterium	2.46±2.900	0.13±5.900	2.22±3.800	1.74±1.900	0.062
Ligilactobacillus	0.20±0.900	0.43±0.700	3.51±3.100	0.49±0.600	0.062
NED5E9	0.12^{b}±0.200	0.70^{a}±0.100	0.93^{ab}±0.800	1.70^{a}±1.300	0.001
Paeniclostridium	0.16±0.100	0.25±0.500	0.16±0.200	1.07±1.100	0.061
Pantoea	1.62±2.500	0.25±0.200	0.16±0.080	0.16±0.100	0.063
Prevotella_7	0.16±0.200	0.12±0.200	1.30±1.100	1.47±1.600	0.062
Pseudomonas	1.95±2.900	0.28±0.300	0.15±0.090	0.31±0.400	0.209
Quinella	0.39^{c}±0.180	1.90^{b}±0.190	3.53^{a}±0.210	1.98^{b}±0.220	0.001

Ruminobacter	0.29±0.300	1.27±3.060	0.36±1.070	1.19±2.100	0.454
Staphylococcus	1.14±1.800	0.34±0.300	0.16±0.800	0.13±0.100	0.064
Succinimonas	0.87±1.201	0.21±0.701	1.25±2.301	1.82±3.800	0.410
Succinivibrionaceae _UCG_001	0.55±0.900	0.17±0.040	1.40±2.300	0.10±0.030	0.062
Paracoccus	1.78^{a}±0.100	0.29^{b}±0.040	0.12^{b}±0.511	0.18^{b}±0.501	0.001
Roseicitreum	0.19±0.101	0.17±0.040	0.16±0.080	0.10±0.030	0.064

[#]: Treatments were *S. plumosum* inclusion levels in the diets of 0% (T1), 10% (T2), 20 (T3) or 30 (T4) %

[*]: Values presented as mean ± standard deviation (SD) and relative abundance is given as a percentage

[a, b, c]: Means with different superscripts in the same row indicate significant differences between treatments (P<0.05)

4.4 Discussion

The nutritional effects of tannins are associated with their ability to bind with nutrients resulting in an overall effect of lowering the bioavailability of nutrients like proteins and carbohydrates in the rumen and hence reducing methane production and emission by ruminant animals (Makkar *et al*., 2007). Indeed, replacing lucerne hay with *S. plumosum* meal affected methane emission in Nguni steers. The reductions in methane emission ranged from 1.41 to 15.0 %. Higher *S. plumosum* meal inclusion levels in the diet tended to have less effect on methane reduction. A 13.6 % *S. plumosum* meal inclusion level in the diet was calculated, using quadratic equations, to result in optimal CH_4 emission reduction by Nguni steers. *Seriphium plumosum* meal contained reasonable amounts of condensed tannins (CT). These tannins reduce methane production and emission through formation of complexes with dietary proteins and carbohydrates in the rumen (Currulla *et al.,* 2005). Thus, methanogenesis is reduced (Tedeschi *et al.,* 2011). The present finding that tannin levels in a diet reduced methane emission in Nguni steers is similar to the findings of Jayanegara *et al.* (2011) who reported that a reduction in methanogenesis was realized when goats were fed diets high in condensed tannins. The authors indicated that this was due to the combined effects of reduced archaea/protozoa fibre digestibility. Curulla *et al.* (2005) suggested that inhibition of methanogens by CT was primarily the result of suppressed fibre degradation that limits H_2 derived from synthesis of acetate. Depressed fibre degradation could be due to a reduced number of cellulolytic bacteria (McSweeney *et* *al.,* 2001), formation of tannin–cellulose complexes (Makkar *et al.,* 1995), and/or impaired bacterial adhesion to substrate and fibrolytic activity of rumen microbes (Bento *et al.,* 2005a). Ng'ambi *et al*. (2022) reported that supplementation levels of 30, 40 or 50 g of purified condensed tannins per kg DM diet reduced methane emission by 51 to 60 %, and increased sheep meat antioxidant activity values. A reduction in the methane emission values observed in the current study are similar to the findings of various researchers (Grainger *et al.,* 2009; Jayanegara *et al*., 2009; Animut *et al*., 2008; Min *et al.,* 2005; Woodward, 2002) who noted a decrease in CH_4 production due to supplementation of condensed tannins or feeds high in condensed tannins. Goel *et al* (2008) reported a reduction of 19.9% in CH_4 production when 150 mg of *Carduus pynochepalus* was added to the diets of ruminants. Tiemann *et al*. (2008) included 300 g/kg of DM as foliage of *Calliandra calothyrsus* or *Ficus macrophylla* (high in CT) in the rations of growing sheep and recorded reductions of 21 and 17.4% in the production of enteric CH_4, respectively. The authors indicated that the reduction could have been caused by the decrease in organic matter digestibility of 8.3 and 11.5%, respectively. Condensed tannins from *Lotus* forage have been reported to reduce methane production by about 15% in sheep and dairy cows (Woodward and Waghorn 2006). However, Sliwinski *et al.* (2002) observed that increasing condensed tannins in the diet did not have any effect on methanogenesis in sheep. Mathobela (2018), also, indicated that feeding of different inclusion levels of *Acacia tortilis* leaf meal, high in tannin contents, had no anti-methanogenic effects on Boer goats. Beauchemin *et al.* (2007) did not find any effects on CH_4 production when Quebracho tannin extracts of 10 - 20 g/kg dry matter feed was fed to beef cattle for 28 days. The variation in terms of CH_4 emission values obtained by different studies could be attributed to doses, types and sources of tannins, and types of diets used in the experiments (Beauchemin *et al*., 2009; Beauchemin *et al.,* 2007).

The replacement levels of lucerne hay with tanniniferous *Seriphium plumosum* meal used in the present study did not affect rumen fluid pH values and mineral concentrations. These results suggest the potential use of *Seriphium plumosum* meal inclusion levels in future studies of cattle. In addition to the ability to bind to and precipitate proteins and carbohydrates in the rumen, condensed tannins and other polyphenols also efficiently bind to Al, Ca, Co, Cu, Fe, Mg, Mn, P, and Zn. The minerals bound by CT in the rumen become available in the small intestines. However, it is generally accepted that these complexes are stable over a wide pH range and throughout the entire gastrointestinal tract (Scalbert, 1991). As research in CT and ruminant interactions progresses, it is suggested that further studies be conducted to measure the implications of CT on mineral balance and overall performance to ensure the safety of long-term CT supplementation.

Rumen bacterial communities play a major role in the microbial production and health status of ruminant animals (Schloss *et al*., 2011). The rumen microbiota, which develops with the host since birth, affects the regulation of feed degradation in the rumen (O'Hara *et al*., 2018; Jami and Mizrahi, 2012). Of the 260 detected genera above the threshold of 0.1 % relative abundance, 27 representing 10 % of the entire bacteria population observed in the present study were above the threshold of 0.1 % relative abundance from individual animals across all the four treatments. The *Spirochaetota*, *Actinobacteriota, Bacteroidota, ProteobacteriaSAR324, Verrucomicrobiota, Desulfobacterota, Patescibacteria, Patescibacteria, Verrucomicrobiota and Chloroflexi* were dominant phyla in rumen fluid of Nguni steers in the current study, but their relative abundance was not affected by *Seriphium plumosum* meal inclusion levels the diets. Thus, in the present study *S. plumosum* meal inclusion levels used in the diets did not influence the abundance of microbes in the rumen of Nguni steers. It is possible that lower dosages than the 20 g of CT per kg DM of feed used in the present study were the reasons for having no impact on the Nguni steer ruminal microbiome (Salami *et al*., 2018; Fernando *et al*., 2010). This is contrary to the observation by Costa *et al*. (2018) that CT inhibited some ruminal gram-positive specialized fibrolytic bacteria (*Fibrobacter succinogenes, Ruminococcus albus, Ruminococcus flavefaciens, Butyrivrio proteoclasticus*) in an *in vivo* study with fistulated ewes. *Fibrobacter succinogenes* and total methanogen population inhibition of up to 36% have been reported *in vitro* (Jayanegara *et al*., 2015). However, Salami *et al*. (2018) included 4 % of *Mimosa pudica* in the diets of lambs and did not observe a difference in absolute abundance of bacteria and fungi, while methanogen abundance decreased by 12 %. In a recent *in vitro* study, chestnut hydrolysable tannins reduced methane production by 12.5 %, while acetate production increased (Cappucci *et al*., 2021). Salami *et al*. (2018) reported a dosage above 20 g of CT per kg DM of feed to impact on the ruminal microbiome.

Microbial population diversity is closely related to the rumen ecosystem, and analysis of the population diversity in the rumen (Konopka, 2009). Alpha diversity measures were used to test for differences in richness and diversity among the rumen fluid of alpha bacteria in Nguni steers. Alpha diversity analysis was measured using six indices (Observe, Chaol 1, ACE, Shannon, Simpson and Fisher) and the results indicated that inclusion of *S. plumosum* meal in the diet had no effect on alpha diversity of species. Similarly, *Seriphium plumosum* meal inclusion in the diets did not have effect on relative abundance of the majority of genera except for *Intestinimonas*, *NED5E9*, *Quinella* and *Paracoccus*. The numbers for *NED5E9*, *Quinella* and *Paracoccus* tended to increase with increased *S. plumosum* meal inclusion levels in the diets, possibly indicating why there was a decrease in methane emission with increase in *S. plumosum* inclusion levels in the diets. Condensed tannins bind with carbohydrates and proteins, thus supporting microbes that promote propionic acid production, resulting in reductions of methane production and emission by ruminant animals (Makkar *et al*., 2007). Fernando *et al*. (2010) demonstrated that feeding tanniniferous forage diets to beef cattle increased *Proteobacteria* and *Firmicutes* which promote propionic acid production, resulting in reduced methane production and emission.

4.5 Conclusions

Replacing Lucerne hay with *S. plumosum* meal reduced methane emission by Nguni steers. The reduction in methane emission ranged from 1.41 to 15.0 %. A 13.6 % *S. plumosum* meal inclusion levels in the diet was calculated, using quadratic equations, to result in optimal CH_4 emission reduction by Nguni steers. This could be attributed to the fact that condensed tannins found in *S. plumosum* meal have high binding capacity for dietary proteins and carbohydrates, thus reducing the degradability of proteins and fermentation of carbohydrates in the rumen leading to low methane production and emission. It is concluded that a tanniniferous *S. plumosum* meal, provided at the levels of 10, 20 or 30% of the diet, may be used to replace lucerne hay in Nguni fattening diets without apparent adverse effects on the animals. The replacement levels of lucerne hay with tanniniferous *Seriphium plumosum* meal used in the present study did not affect Nguni steer rumen fluid pH, mineral concentration values and microbial population, possibly indicating that the inclusion levels used did not cause any adverse effects in the rumen. It is recommended that further similar studies be conducted to ascertain the present findings.

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CHAPTER 5

EFFECT OF INCLUSION LEVELS OF *SERIPHIUM PLUMOSUM* MEAL IN A DIET ON CARCASS CHARACTERISTICS AND MEAT QUALITY OF NGUNI STEERS

Abstract

The use of encroaching woody plants such as *Seriphium plumosum* for livestock feeding is gaining importance. The objective of this study was to evaluate the effects of *S. plumosum* meal inclusion level in a diet on meat characteristics and quality of Nguni steers fed a diet based on lucerne hay and maize meal. Twenty-eight Nguni steers aged 22 months with an initial mean body weight of 300 ± 10 kg were randomly assigned to isocaloric and isonitrogenous diets containing 0, 10, 20 or 30 % of *S. plumosum* meal in a complete randomized design, each treatment having seven replications and one steer per replicate. The experiment lasted for 111 days (21 days for adaptation and 90 days for the actual feeding trial). The steers were slaughtered at 25 months of age and carcasses were evaluated for carcass traits, meat tenderness traits (sarcomere length, myofibril length and collagen solubility), meat colour, drip loss, water holding capacity, and lipid and protein oxidation properties. *Seriphium plumosum* meal inclusion levels in the diets did not affect ($P>0.05$) meat pH and temperature values, carcass weight, fat code, confirmation score, eye muscle area and back fat thickness. However, *S. plumosum* meal inclusion levels in the diets affected ($P<0.05$) dressing percentage of Nguni steers. Steers fed a diet having 10 % of *S. plumusom* meal had higher ($P<0.05$) dressing percentage values than those fed diets having 20% of *S. plumosum* meal. *Seriphium plumosum* meal inclusion level in a diet did not affect ($P>0.05$) sarcomere length (SL), collagen solubility (CS). However, *S. plumosum* meal inclusion levels in diets affected ($P<0.05$) meat myofibril length of Nguni steers. Nguni steers fed a diet having 20% of *S. plumusom* meal had lower ($P<0.05$) meat myofibril length values than those fed diets containing no *S. plumosum* meal. A 16.11% *S. plumusom* meal inclusion level in the diet was calculated, with the use of quadratic equations, to result in optimal Nguni steer meat myofibril lengths ($r^2 = 0.866$. *Seriphium plumosum* meal inclusion level in a diet had no effect ($P>0.05$) on meat colour attributes. Similarly, water-holding capacity (WHC), drip loss, and lipid and protein oxidation properties of Nguni steer meat were not affected by inclusion of *S. plumosum* meal in diets. It was concluded that *S. plumosum meal* inclusion levels at 10, 20 or 30 % in the diets did not adversely affect Nguni steer carcass traits, meat tenderness traits, meat colour, water-holding capacity , and lipid and protein oxidation properties. This is an indication that *S. plumosum* meal can be included in Nguni cattle diets without causing any adverse effects on meat characteristics and quality parameters. However, long-term studies are recommended to ascertain the present findings.

Keywords: Beef quality, Carcass characteristics, Nguni steers, *Seriphium plumosum* meal

5.1 Introduction

Beef is a high-quality source of nutrients that provide more than 25 % dietary intake of protein in humans (Mwangi *et al*., 2019). As human population increases worldwide, the demand for beef consumption is also projected to increase by 8 and 21% by 2027 in the developed and developing countries, respectively (FAO, 2007; Mwangi *et al*. 2019). The indigenous Nguni cattle breed is characterized by a small to medium frame (200–400 kg), hardiness and adapts to subtropical conditions (Strydom, 2008). Moreover, the ability of Nguni cattle reared on woody plants fodder such as *Vachellia* species as source of feed has shown the potential for producing safe and health-promoting natural meat (Mapiye *et al*., 2007).

Livestock browse some species at certain periods of the year, and as such, an increase in woody and shrubby plants causes reduction in herbage production and consequently exacerbates the rate of bush encroachment (Kelly, 1977). Bush encroachment is the main challenge for livestock in South Africa as it alters natural grazing, and therefore it is important to develop ecological control measures (Snyman, 2012). The major constraints associated with finishing livestock on rangeland-based systems, particularly during the dry season, is the limited protein intake of less than 50 g/kg DM feed (Mapiye *et al*., 2009b) which has been reported to be far below the recommended level of between 120 g/kg and 150 g/kg to maintain beef cattle (NRC, 2000). Moreover, finishing cattle on rangeland has been associated with toughening of the beef, decreased beef colour and flavour, which are unacceptable by consumers (Hedrick *et al*., 1983).

The integration of fodder from woody plants species as feed ingredients to boost beef quality could overcome both feed shortage during the dry season and reduce bush encroachment (Mapiye *et al*., 2009a). Furthermore, utilization of such fodder as part of animal diets could provide cost-effective alternatives to finishing diets, particularly under intensive feeding where production of beef is associated with high costs (Mapiye *et al*., 2009b). *Seriphium plumosum*, commonly known as Bankrupt bush, is amongst the dominating woody plants species that exacerbate the problem of bush-encroachment in the semi-arid areas of South Africa (Snyman, 2012). Nonetheless, *S. plumosum* contains relatively high amounts of protein that can improve ruminant productivity (Pule, 2021). Previous studies demonstrated that feeding woody plants fodder results in positive responses in animal performance

(Mapiye *et al*., 2009). However, data on *S. plumosum* inclusion levels for optimal meat characteristics and quality of Nguni steers is not available. This study, therefore, focused on the effect of inclusion levels of *S. plumosum* meal in a diet on carcass traits and meat characteristics and quality of Nguni steers fed a diet based on lucerne hay and maize meal. Such information is useful when formatting strategies for improving Nguni cattle production and reducing bush encroachment in South Africa.

5.2 Materials and methods

5.2.1 Study area

The study was carried out at the Agricultural Research Council - Animal Production (ARC-AP), Gauteng province, South Africa, during the summer season of 2020. The ARC-AP lies at latitude E 25° 53' S and longitude 28° 11' E. The mean ambient temperatures around the study area are 15.8 °C in winter and 28 °C in summer (https://en.climate-data.org). The dry season occurs between April and October and the mean annual rainfall is 661 mm, which falls between November and March (Kutu and Asiwe, 2010). The Agricultural Research Council, Animal Production Research Ethics Committee (Certificate: APAEC – 2020/16) and the University of Limpopo Animal Research and Ethics Committee (Certificate: AREC/01/2020: PG) approved the animal ethics protocol for this study.

5.2.2 Harvesting of the study plant

Seriphium plumosum forage was harvested at a private farm in Vanderbiljpark, Gauteng province between January and March 2020. The edible materials, leaves and branches, were harvested using a tractor slasher. The harvested *S. plumosum* material was sun-dried for three days before bailing and milled to pass through a 2 cm sieve size. The *S. plumosum* meal was stored in a well-ventilated dry shade pending the feeding trial.

5.2.3 Animal management, treatments, design and feeding

Twenty-eight Nguni steers aged 22 months old with initial body weight of 300 ± 10 kg were assigned to each of the four dietary treatments, which were *Seriphium plumosum* meal inclusion levels in the diet of 0 ($T1_0$), 10 ($T2_{10}$), 20 ($T3_{20}$) or 30 ($T4_{30}$) % (for ingredients used in the diets refer to Table 3.1, Chapter 3). Each treatment had seven replications, and one animal per replicate in a complete randomized design. The Nguni steers on the diets were allowed 21 days to adapt to their respective diets and trained to feed from individual troughs prior to the 90-day collection period. Each steer was individually fed and all experimental animals had free access to clean water and feed. The experimental diets were formulated to meet the nutritional requirements of growing steers (NRC, 2000).

5.2.4 Data collection

5.2.4.1 Slaughter procedures and carcass measurements

On Day 111 of the experiment, steers were weighed and transported to ARC, AP abattoir, where they were deprived of feed except water, for approximately 24 hours. All steers were slaughtered in December 2020 at 25 months of age, following the commercial abattoir practices at the ARC registered abattoir (Muchenje *et al*., 2008).

5.2.4.2 Carcass pH and dressing percentage

The carcasses were dressed, inspected and split into left and right halves. At approximately 60-min post-mortem, pH and temperature of the carcasses were recorded on the *M. longissimus dorsi* (LD) of the left half of the carcasses using a pH digital meter (Sentron, Model 1001). The pH and temperature were recorded again at 3, 6 and 24 hrs post-mortem at the same anatomical position as for the 60-min post-mortem pH. Dressing percentage was calculated by dividing the warm carcass weight by the final live body weight (Greenwood, 2020).

5.2.4.3 Back fat thickness and eye muscle area

Back fat thickness was measured on *Longissimus dorsi* muscle 5 cm laterally from the mid-line cut between the 12th and 13th rib. Back fat thickness was also measured at nine other positions, four positions posterior and five positions anterior to the last rib, each at 5-cm intervals on the mid-line and 2.5 and 5.0 cm off the mid-line. The eye muscle area was measured by tracing the *M. longissimus thoracis* eye muscle on an animal between the 10th and 11th thoracic vertebrae (McGilchrist *et al*., 2012), and as advised by the manufacturer (VIA, Kontron, Germany).

5.2.4.4 Meat colour measurements

Meat colour was measured with a Minolta meter (Model CR200, Osaka, Japan) on fresh samples 1-day post-mortem. Two freshly cut steaks of 15 mm thickness each of the *M. longissimus dorsi* (LD) were allowed to bloom for 60 min at 4±2 °C before recording. Three recordings were performed on each steak. The steak colours were recorded as three components: luminance or lightness, L* (dark to light); and two chromatic components: a* (green to red); and b* (blue to yellow) values (CIE, 1986). The following calculations were done to determine the physiological attribute of chroma (intensity of the red colour) also known as saturation index (S)=(a*2+b*2)1/2) (MacDougall, 1977) and hue angle (discolouration) = tan−1 (b*/a*) (Young *et al*., 1999). Mean values were used for statistical analysis.

5.2.4.5 Sarcomere lengths

Sarcomere lengths (SL) of fresh LD samples (1-day postmortem), were prepared according to Hegarty and Naudé (1970). Fifty sarcomeres per sample were measured by means of VIA using an Olympus B340 system microscope at a 31,000 magnification equipped with a CC12 video camera (Olympus, Tokyo, Japan). AnalySIS Life Science software package (Soft Imaging Systems Gmbh, Münster, Germany) was used to process and quantify measurements.

5.2.4.6 Myofibril fragmentation length

Myofibril fragmentation length (MFL) was determined by the method of Culler *et al*. (1978) with some modifications by Heinze *et al*. (1994). Meat samples of about 3 g slices aged at 3 and 14 days were cut from a frozen muscle with a knife, and any visible fat and connective tissues were removed. The methods involved the extraction of the myofibres in potassium phosphate buffer (Heinze and Bruggemann, 1994; Culler *et al*., 1978). The MFL was determined as the average length of the first 50 myofibrils by means of a video image analyser (Olympus BX40 system microscope at a 1000X magnification). One hundred myofibrillar fragments per sample were examined and measured. The mean values represented the MFL for each sample (Frylinck *et al*., 2013).

5.2.4.7 Collagen solubility

Collagen solubility (CS) was determined by the method described by Kristensen *et al*. (2002). Briefly, a sub-sample of approximately 300 mg was taken from lyophilised and powdered meat samples, mixed with 12 mL 6 M HCl and hydrolysed for 24 h at 110 °C in an oil bath (Bravetti Profesional). The hydrolyzates were allowed to equilibrate to room temperature, neutralized with an equal volume of 6 M NaOH, filtered through filter paper Whatman N° 1, and diluted 10 times with distilled water. The hydroxyproline concentrations of the diluted samples were determined using the rapid procedure described by Bergman and Loxley (1963). The concentration of hydroxyproline was determined according to the NMKL method described by Kolar (1990). The amount of heat-soluble collagen was calculated from the hydroxyproline concentration using a conversion factor of 7.14. Total collagen content was then calculated from the sum of the hydroxyproline concentration in the pellet (insoluble collagen) and in the supernatant (soluble collagen) and expressed in mg per gram wet tissue (Kolar, 1990). The collagen in the supernatant was expressed as the percentage of the total collagen content.

5.2.4.8 Drip loss and water holding capacity

Drip loss was measured as described by Strydom *et al*. (2009) using 50 g of fresh LD (1-day postmortem) sliced into samples of 10×10×20 mm that were suspended on a pin inside a sample bottle (200 ml). Duplicate samples were stored for 3 days at 4 ± 2 °C. Drip loss percentage was calculated as the weight of drip divided by the raw weight before drip. Drip loss = [drip weight/raw weight before drip] × 100. Water holding capacity (WHC) was determined by calculating the ratio of meat area and liquid area after pressing a 400 to 600 mg meat sample on a filter paper (Whatman 4) sandwiched between two perspex plates, and pressed at constant pressure for 5 min as described by Grau and Hamm (1953). The areas were measured by means of a video image analysis (VIA) (Soft Imaging System, Olympus, Japan) described by Irie *et al*. (1996) and WHC was expressed as the area of the meat divided by the area of the moisture.

5.2.4.9 Lipid and protein oxidation

The lipid oxidation in the cube roll slices was assessed by the thiobarbituric acid reactive substances (TBARS) procedure (Witte *et al*. 1970) expressed in mg of malondialdehyde (MDA) per kg of muscle. Samples for TBARS analysis (30 g) were collected and frozen at 0, 1, 3 and 10 days of display. Protein oxidation (Thiols) was measured as the amount of free thiol groups in proteins determined as µM thiol per mg protein (high values indicate low oxidation). The amounts of free thiol groups were determined by the method of Lund *et al*. (2008). The supernatants were diluted to a concentration of 1.5 mg/mL with 5% SDS in 0.10 M Tris-HCL buffer. Then the assay was prepared by mixing 0.50 mL diluted filtrate, 2.0 ml Tris-HCL buffer (0.10 M, pH 8.0) and 0.5 ml of 10 mM DTNB (5,5′-Dithiobis (2-nitrobenzoic acid)) in 0.10 M Tris-HCL buffer. After 30 minutes, the absorbance of the samples was measured at 412 nm against an aqueous reference solution of 0.50 ml 5% SDS in 0.10 M Tris-HCL buffer, 2.0 ml Tris-HCL buffer. The amount of free thiol groups was expressed as μM thiol per mg protein.

5.2.5 Chemical analyses

Dry matter of feeds, feed refusals and faecal material was determined by drying the samples in the oven for 72 hours at 60 °C (AOAC, 2010). Dried feed samples were ground and milled through a 1-mm sieve (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA) and stored in labelled airtight bottles for chemical analyses. Crude protein (CP), and ether extract (EE) in total mixed diets were analysed according to AOAC (2010), while acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined according to the technique prescribed by Van Soest *et al*. (1991). Calcium (Ca), phosphorus (P) and CP of feeds and *Seriphium plumosum* were analysed following the procedures of AOAC (2010), whereas condensed tannins (CT) were analysed by a method of Hattas *et al*. (2005).

5.2.6 Statistical analyses

Data on carcass pH, dressing percentage, eye muscle area, back fat thickness, meat colour, sarcomere length, myofibril fragmentation lengths, collagen solubility, drip loss, water holding capacity, lipid and protein oxidation were subjected to analysis of variance using SAS version 9.3.1 software program (SAS, 2008), with *S. plumosum* meal inclusion rates as the main effects. Means were separated using the least significant difference (P<0.05) for variables when *P*-values declared a significant difference. The model $\mathrm{Y_{ij}} = \mu + \mathrm{T_i} + \mathrm{e_{ij}}$ was applied, where $\mathrm{Y_{ij}} =$ response variables; $\mu =$ population mean; $\mathrm{T_i} =$ fixed effect of the $\mathrm{i^{th}}$ treatment level (i = 0, 100, 200 and 300 g/kg DM) and $\mathrm{e_{ij}}$ = random error. The responses in carcass characteristics and meat quality traits to *S. plumosum* meal inclusion levels in the diet were modelled for linear and quadratic effects using the following quadratic equation (SAS, 2008):

$$\mathrm{Y} = \mathrm{a} + b_1\mathrm{x} + \mathrm{b}_2 x^2 + \mathrm{e}$$

Where Y = back fat thickness, dressing percentage, eye muscle area, meat colour, drip loss, WHC, sarcomere length, myofibril fragment length, collagen solubility, lipid and protein oxidation; a = intercept; $\mathrm{b_1}$ and $\mathrm{b_2}$ = coefficients of the quadratic equation; x = *S. plumosum* meal inclusion levels; $-\mathrm{b_1}/2\mathrm{b_2} = \mathrm{x}$ value for optimal response and e is the error.

5.3 Results

5.3.1 Effect of *S. plumosum* meal inclusion level in a diet on carcass pH, dressing percentage, eye muscle area and back fat thickness of Nguni steers

The effects of *S. plumosum* meal inclusion levels in diets on carcass pH, dressing percentage, eye muscle area and back fat thickness of Nguni steers are presented in Table 5.1. *Seriphium plumosum* meal inclusion levels in the diets did not affect (P>0.05) meat pH and temperature values, carcass weight, fat code, confirmation score, eye muscle area and back fat thickness. However, *S. plumosum* meal inclusion levels in diets affected (P<0.05) dressing percentage of Nguni steers. Steers fed a diet having 10 % of *S. plumusom* meal had higher (P<0.05) dressing percentage values than those fed diets having 20 % of *S. plumosum* meal. However, steers fed diets having 0, 10 or 30 % of *S. plumusom* meal had similar (P>0.05) dressing percentage values. Similarly, steers fed diets having 0, 20 or 30 % of *S. plumusom* meal had the same (*P*>0.05) dressing percentage values.

Table 5.1 Effect of *S. plumosum* meal inclusion levels in diets on carcss traits of Nguni steers

	Diets$^{\#}$				
Parameters*	$T1_{0}$	$T2_{10}$	$T3_{20}$	$T4_{30}$	*P* value
Warm carcass weight (kg)	191±27.3	203±4.75	190±29.9	190±13.9	0.6271
Cold carcass weight (kg)	188±27.4	199±5.06	186±30.9	186±14.8	0.6516
Dressing percentage (%)	60^{ab}±4.13	63^{a}±2.34	57^{b}±4.36	60^{ab}±3.02	0.0255
pH (1hr)	6.5±0.24	6.6.0±0.20	6.6±0.20	6.67±0.22	0.0585
pH (24hr)	5.6±0.49	5.56±0.56	5.78±0.34	5.86±0.51	0.3495
Temperature (1hr)	37.1±1.19	36.9±1.68	36.7±2.21	35.9±2.41	0.5153
Temperature (24hrs)	4.3±1.90	4.2±1.38	4.6±1.37	4.64±0.93	0.5366
Fat code	2.3±2.1	2.3±2.1	2.14±2.0	2.4.4±1.5	0.0831

Confirmation score (%)	2.85±0.3	3±0.00	2.85±0.37	2.85±0.37	0.8013
Backfat thickness (cm)	3.1±1.31	3.7±1.22	3.0±1.34	3.3±1.14	0.7449
Eye muscle area (mm2)	6152±779.8	6396±482.0	6002±649.9	5912±595.0	0.6737

$^{\#}$: Diets were *Seriphium plumosum* meal inclusion levels in the diets of 0 ($T1_{0}$), 10 ($T2_{10}$), 20 ($T3_{20}$) or 30 ($T4_{30}$) %

$^{a, b}$: Values with different superscripts in the same row indicate significant differences between treatments (P<0.05)

*: Values presented as mean ± standard deviation (SD)

The effects of *S. plumosum* meal inclusion level in a diet on meat colour attributes of Nguni steers are presented in Table 5.2. *Seriphium plumosum* meal inclusion level in a diet did not affect (P >0.05) lightness, redness, yellowness, chroma and hue angle of Nguni steer meat.

Table 5.1 Effect of *S. plumosum* meal inclusion levels in diets on meat colour attributes of Nguni steers

Meat colour traits*	Diets$^{\#}$				*P* value
	$T1_{0}$	$T2_{10}$	$T3_{20}$	$T4_{30}$	
Lightness, *L**	29.6 ± 4.42	27.7 ± 4.36	27.4 ± 4.7	27.8 ±4.02	0.7837
Redness, *a**	9.9 ± 3.16	9.8 ± 2.36	9.5 ± 1.74	9.3 ±2.19	0.9684
Yellowness, *b**	9.6±2.98	9.8 ± 2.29	9.0 ± 2.10	9.3±2.05	0.9362
Chroma, *C**	13.8 ±4.30	13.8±3.25	13.1 ± 2.60	13.2 ± 2.93	0.9615
Hue angle, *H**	44.1±2.17	45.1±2.28	43.2 ± 3.44	45.1 ± 2.89	0.5270

$^{\#}$: Diets were *Seriphium plumosum* meal inclusion levels in the diets of 0 ($T1_{0}$), 10 ($T2_{10}$), 20 ($T3_{20}$) or 30 ($T4_{30}$) %

*: Values presented as mean ± standard deviation (SD)

5.3.4 Effect of *S. plumosum* meal inclusion level in a diet on sarcomere length, myofibril length, collagen solubility, water-holding capacity, drip loss, and lipid and protein oxidation properties of Nguni steer meat

The results of the effect of *S. plumosum* meal inclusion level in a diet on sarcomere length (SL), myofibril length (MFL), collagen solubility (CS), water-holding capacity (WHC), drip loss, and lipid and protein oxidation properties of Nguni steer meat are presented in Table 5.3. *Seriphium plumosum* meal inclusion level in a diet did not affect ($P>0.05$) sarcomere length (SL), collagen solubility (CS), water-holding capacity (WHC), drip loss, and lipid and protein oxidation properties of Nguni meat. However, *S. plumosum* meal inclusion levels in diets affected ($P<0.05$) meat myofibril length of Nguni steers. Nguni steers fed a diet having 20% of *S. plumusom* meal had lower ($P<0.05$) meat myofibril length values than those fed diets containing no *S. plumosum* meal. However, steers fed diets having 0, 10 or 30 % of *S. plumusom* meal had similar ($P>0.05$) meat myofibril length values. Similarly, Nguni steers fed diets having 10, 20 or 30 % of *S. plumusom* meal had the same ($P>0.05$) meat myofibril length values. A 16.11 % *S. plumusom* meal inclusion level in the diet was calculated, with the use of quadratic equations, to result in optimal Nguni steer meat myofibril lengths ($Y = 27.923 + 0.2897X + 0.00865X^2$; $r^2 = 0.866$).

Table 5.2 Effect of *S. plumosum* meal inclusion levels in the diets on Nguni steer meat tenderness, moisture and oxidation properties

	Diets$^{\#}$				
Parameters*	$T1_0$	$T2_{10}$	$T3_{20}$	$T4_{30}$	*P value*
Meat tenderness properties					
Sarcomere length (µm)	2.0±0.08	2±0.1	2±0.1	2 ±0.1	0.6278
MFL (µm)	28^{a} ±5.1	26^{ba} ±3.6	25^{b} ±3.3	27^{ba} ±4.4	0.0067
Collagen solubility (%)	9.7±3.12	13.8±5.83	10.2± 3.63	15.8± 5.15	0.0630
Moisture and oxidation properties					
Water-holding capacity (%)	0.4± 0.60	0.4 ± 0.61	0.4 ± 0.05	0.4 ± 0.03	0.9332
Drip loss (%)	1.1 ± 0.01	1.1 ± 0.45	1.3 ± 0.41	1.2 ± 0.45	0.5317
TBARS (µM/mg)	0.1 ±0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.6482
Thiol (µM/mg)	66.8± 7.15	65.9 ± 8.11	68.4 ± 5.86	64.8 ± 8.98	0.6788

[#]: Diets were *S. plumosum* inclusion levels in the diets of 0% (T1), 10% (T2), 20 (T3) or 30 (T4) %

[a, b]: Means with different superscripts in the same row indicate significant differences between treatments ($P<0.05$)

[*]: Values presented as mean ± standard deviation (SD)

* MFL: myofibril fragment length; TBARS: thiobarbituric acid reactive substances.

5.4 Discussion

Seriphium plumosum meal inclusion level in the diets affected dressing percentage of Nguni steers. Steers fed a diet having 10 % of *S. plumusom* meal had the highest dressing percentage of 63 % while those on 20 % *S*. *plumosum* meal inclusion level had the lowest dressing percentage of 57 %. However, dressing percentages of Nguni steers in the present study were within a minimum threshold of 57% which is acceptable for beef cattle (Avilés *et al*., 2015), possibly indicating that lucerne hay can be replaced by *Seriphium plumosum* meal in a diet without causing any adverse effect on the dressing percentage of Nguni steers. Sami *et al*. (2004) reported similar results in a study that compared dressing percentages of young bulls fed on forage and grain diets. Egea *et al*. (2014) reported dressing percentages of cattle ranging from 61.5 to 62.2 %. In contrast, Modika *et al*. (2019) reported lower values of dressing percentage in Nguni steers than the ones reported in the present study. It is difficult to compare results from different experiments because of differences in the diets.

Seriphium plumosum meal inclusion in the diet did not affect meat pH values at 1 and 24 hrs post-slaughter. The meat pH values obtained in the present study are within the acceptable range for beef cattle (Sami *et al*., 2004). Sami *et al*. (2004) reported similar meat pH values for Simmental bulls fed forage diets. However, other authors (Kannan *et al*., 2002) found that diets, particularly those having condensed tannins affected meat pH values of beef cattle. Diet type and pre-slaughter activities (physical exhaustion and ability of animals to accumulate adequate lactic acid production) affect meat pH values of beef cattle (Kannan *et al*., 2002).

Replacement of lucerne hay with *S. plumosum* meal in a diet had no effect on back fat thickness of Nguni steers, possibly indicating that lucerne hay can be replaced by *S. plumosum* meal in a diet without causing any adverse effects on meat parameters of the steers. *Seriphium plumosum* meal inclusion level in the diets did not affect back fat thickness of Nguni steers. However, other authors (Lee *et al*., 2003) observed that diets affected back fat thickness of beef cattle. The back fat thickness values obtained in the current study are similar to those observed in cattle by Santos *et al*. (2003); however, they are lower than those reported by Lee *et al*. (2003) cattle forage diets. Back fat thickness is associated with the level of visual and intramuscular fats on the carcass (Stolowsk *et al*., 2006).

Seriphium plumosum meal inclusion level in the diets did not affect Nguni steer meat lightness, redness, yellowness, chroma and hue angle, possibly indicating that *Seriphium plumosum* meal can replace lucerne hay in a diet without causing any adverse effects on meat colour of the steers. The *L** values ranged from 27 to 29. These low *L** values found in the current study were associated with darker meat and were below the range of 35 to 38 associated beef cattle (He *et al.,* 2018). The findings of the current study are similar to those of Zorzi *et al.* (2013) who reported low *L** values in a study of beef steers fed a forage diet. Furthermore, low *L** values in the current study are consistent with the findings of Bennett *et al*. (1995) who reported darker meat for carcasses of forage-fed cattle compared to those of cattle fed concentrate diets. In contrast, Ornaghi *et al*. (2020) reported higher *L** values of 37 to 40 in a study of Nguni, Bonsmara and Angus steers fed a diet with inclusion of natural additives. A study by Frylinck *et al*. (2013) showed higher values of lightness (35 - 39) on the steaks in an investigation sought to compare intensive grain fed steers and pasture fed steers. Lower *L** values of the meat are a reflection of the darker meat. Colour of the meat is influenced by several factors, such as age, breed, diet, and sex (Guerrero *et al*., 2018; Baublits *et al*., 2004). Lower *L** colour values in the current study can be due to the non-castrated steers used (Zorzi et al., 2013) and the Nguni breed (Muchenje *et al*., 2008). The low *L** values recorded in the current study can also be attributed to high amount of catecholamine realised by Nguni steers (O'Neill *et al*., 2006). Catecholamine leads to a decrease in post-mortem lactic acid production and increases meat pH values, and consequently reduces the L* colour of the meat (Mounier *et al*., 2006).

The redness (*a**) values that represent the red colour intensity of beef steak were below the acceptable range of 18 to 22 associated with beef cattle (He *et al*., 2018). The low levels of redness colour of the steaks may be attributed to increased myoglobin concentration and decreased muscle glycogen of steers pre-slaughter (Baublits *et al*., 2004). The redness values in the present study were lower than 14.55 and 15.76 values reported by Zorzi *et al*. (2013) and Fernandes *et al*. (2008), respectively. Similarly, Ornaghi *et al*. (2020) reported higher redness values of 14.91 in a study sought to improve meat colour of beef steers through natural feed additives.

The b^* values indicate a degree of yellow appearance. There were no differences between treatments in meat yellowness colour. However, the values are on the lower side for beef cattle (Page *et al*., 2001; O'Neill *et al*., 2006). This could be due to the fact that some breeds like the Nguni cattle naturally produce darker meat (O'Neill *et al*., 2006). The steaks across all treatment diets had similar values of chroma and hue. The ranges of values are similar to those observed by Priolo *et al*. (2001) who pointed out that muscles from forage-fed cattle tend to be darker than the grain fed ones.

Seriphium plumosum meal inclusion level in the diets did not affect Nguni steer meat sarcomere length (SL), collagen solubility (CS), water-holding capacity (WHC), drip loss, and lipid and protein oxidation, possibly indicating that *S. plumosum* meal can replace lucerne hay in a diet without causing any adverse effects on steer meat sarcomere length, collagen solubility, WHC, drip loss, and lipid and protein oxidation. The present meat sarcomere length values ranged from 1.96 to 1.99μm, possibly indicating that the meat was tender. The meat with sarcomere length values ranging from 1.7–2.0μm is regarded as moderately tender (Marsh and Leet, 1966). Other authors (Muir *et al.,* 2000) obtained similar to the present meat sarcomere lengths when beef steers were either supplemented with forage or not. However, higher meat sarcomere lengths have been reported for beef steers (Silva *et al*., 2019) and lambs (Cohen-Zinder *et al.*, 2017). Longer sarcomere length (SL) values in beef cattle are associated with more tender meat (Devine *et al*., 1999). In contrast, Frylinck *et al*. (2013) observed a lower meat SL value of 1.66μm in beef steers fed forage or grain diets. In the present study, Nguni steer meat collagen solubility values ranged from 9.78 to 15.75 %. These values were not affected by the replacement of lucerne hay in the diet with *S. plumosum* meal, possibly indicating that *S. plumosum* meal can replace lucerne hay in a diet without causing any adverse effects on Nguni steer meat collagen solubility. These results are similar to those of Mapiye *et al*. (2008) observed in Nguni cattle. Modzelewska-Kapituła and Nogalski (2016) reported similar results in a study sought to investigate the effect of intensive feeding on young bulls. Cox *et al*. (2006) noted no influence of diet on loin collagen content of beef steers fed forage or grain diets. Similarly, Serrano *et al*. (2007) observed no effect on total collagen content of longissimus thoracic muscle of young bulls fed hay or concentrates. However, it is difficult to compare results from different experiments because of differences in the diets.

Replacing lucerne hay in the diets with *S. plumosum* meal inclusion did not affect water holding capacity (WHC) and drip loss of Nguni steer meat, possibly indicating that *Seriphium plumosum* meal can replace lucerne hay in a diet without causing any adverse effects on Nguni steer meat WHC and drip loss. Santos- Silva *et al*. (2003) reported similar meat WHC values of beef steers fed forage diets. Similarly, Frylinck *et al.* (2013) reported the same meat WHC values from beef steers consuming forage diets under intensive feeding. Lower meat WHC values are usually associated with faster pH decline (Offer and Knight, 1988). Higher meat WHC values are associated with consumption of tanniniferous diets (Huff-Lonergan and Lonergan, 2005). Inclusion of *S. plumosum* meal in the diets did not affect meat drip loss values. The meat drip loss values ranged from 1.1 to 1.3%. Frylinck *et al*. (2013) reported higher drip loss values, ranging from 1.7 to 2.2% in a study that sought to compare grain and forage-fed beef steers. Higher meat drip losses are usually associated with higher myofibrillar protein density (Bertram *et al*., 2002; Rosenvold and Andersen, 2003 Bertram *et al*., 2004).

Inclusion of *S. plumosum* meal in the diets did not affect thiobarbituric acid reactive substances (TBARS) and protein oxidation of Nguni steer meat values, possibly indicating that *S. plumosum* meal can replace lucerne meal in a diet without causing any adverse effects on Nguni meat lipid and protein oxidation values. The lipid oxidation values in the current study ranged from 0.04 – 0.05 µM/mg, and were within a recommended threshold for beef cattle meat (Huang and Ahn, 2019). Formanek *et al*. (1998) reported values ranging from 0.9 to 10.4 µM/mg. It is possible that the low lipid and protein oxidation values observed in the present study were due to tannins found in *S. plumosum* meal. Tannins from tanniniferous plants are reported to reduce lipid and protein oxidation, increase oxidative stability and improve meat quality characteristics (Wapi *et al.,* 2013; Ng'ambu *et al.,* 2013). However, results of the present study are similar to those of Muchenje *et al*. (2008) who supplemented Nguni steers with *Acacia Karoo* leaves containing high amounts of tannins. Inclusion of *S. plumosum* in diets did not affect protein oxidation of Nguni steer meat. These values are similar to those of Muchenje *et al*. (2008) who reported no differences when *Acacia karoo* meal was added to the diets fed to Nguni steers. However, the present lipid oxidation values are higher than those of Modika *et al*. (2019).

Nguni steer meat myofibril length values obtained in the present study ranged from 25.10 to 27.76µm. *Seriphium plumosum* meal inclusion level in the diet affected Nguni steer meat myofibril lengths. Nguni steers fed a diet having 20% of *S. plumusom* meal had lower meat myofibril length values than those fed diets containing no *S. plumosum* meal. The present findings are similar to those observed by Silva *et al.* (2019), but below the values reported by Agbeniga and Webb (2018). A 16.11% *S. plumusom* meal inclusion level in the diet was calculated, with the use of quadratic equations, to result in optimal Nguni steer meat myofibril lengths.

5.5 Conclusions

Seriphium plumosum meal inclusion levels of 10, 20 or 30 % in the diets did not negatively affect pH, weight, dressing percentage, fat code, confirmation score, eye muscle area and back fat thickness, meat colour, tenderness, moisture and oxidation properties. This is an indication that *S. plumosum* meal can be included in Nguni cattle diets without causing any adverse effects on meat quality parameters. However, further studies are suggested to ascertain these findings and explore the effect of *S. plumosum* inclusion in diets on Nguni meat shelf-life or stability during storage, that is, how long the meat remains stable and maintains normal quality after a specific period of storage. It is further suggested that studies on the use of *S. plumosum* meal as livestock feed be conducted to explore its effects on the presence of anti-oxidants in the meat of the animals. Anti-oxidant balance in the meat prolongs oxidative stability of muscle tissues.

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CHAPTER 6

EFFECT OF INCLUSION LEVELS OF *SERIPHIUM PLUMOSUM* MEAL IN DIETS ON SENSORY ATTRIBUTES OF NGUNI MEAT

Abstract

Seriphium plumosum, an indigenous shrub to South Africa, has slowly colonised huge portions of the grasslands biome, which is critical for livestock production. While mechanical chopping, fire and chemical control can temporarily help control encroaching shrubby plant, the problem is still escalating. An alternative sustainable approach may be be to incorporate *S. plumusom* as part of animal feed. The objective of this study was to determine the effect of *S. plumosum* inclusion levels of 0, 10, 20 or 30 % in diets on sensory attributes of Nguni steer meat aged at 3 or 14 days. A trained panel evaluated the sensory attributes (taste, aroma, flavour and texture properties) while shear force was determined using a Warner Braztler blade. Results indicated that *S. plumosum* inclusion levels at 0, 10, 20 or 30 % in diets and aging did not affect (P>0.05) Nguni meat sensory attributes, cooking loss, shear force values, tenderness, stringiness and chewiness. Similarly, *S*. *plumosum* meal inclusion levels in the diets did not affect (P>0.05) Nguni steer meat salty, beef aftertaste or bitter tastes after three days of aging, and Nguni steer meat beef aftertaste, bloody aftertaste or salty taste values after 14 days of aging. However, *S*. *plumosum* meal inclusion levels in the diets affected (P<0.05) Nguni steer meat bloody aftertaste and bitter taste values after 3 and 14 days of aging, respectively. It is concluded that *S*. *plumosum* meal can be included in Nguni steer diets at 0, 10, 20 or 30 % without adverse effects on meat quality and sensory attributes. This would be plausible way of controlling woody plant encroachment, specifically *S. plumosum* as fodder for livestock. However, it is suggested that longer studies on the use of *S. plumosum* meal as livestock feed be conducted to ascertain the present findings.

Key words: Nguni beef, Sensory attributes, Woody plant species

6.1 Introduction

Approximately 29 % of South Africa is grassland (DAFF, 2015). Over 40% of this biome is transformed due to woody plant encroachment, urbanization and mining, exacerbating biodiversity losses of this important ecosystem (Van Wilgen *et al*., 2008). In addition to these losses, lack of conventional grazing proliferation of *S. plumosum*, a fynbos shrub (Snyman, 2009). *Seriphium plumosum*, also known as slangbos or bankrupt bush is indigenous to South Africa (Snyman, 2009). It is estimated to have caused loss of nearly 11 million hectares of rangeland dedicated for livestock production, thereby impairing national food security (DAFF, 2015). *Seriphium plumosum* suppresses grass productivity due to its shading effect, change in soil moisture and allelopathic properties that inhibit seed germination and growth of other species (Snyman, 2009).

An increased livestock production escalates global demand for animal feed ingredients which are already limited especially for water scarce countries like South Africa (Mottet *et al*., 2017; Tayengwa *et al.*, 2020). Indigenous and adaptable cattle breeds, such as the Nguni, have the potential to produce acceptable meat quality that meets consumer expectations (Muchenje *et al*., 2008). In support of the use of Nguni cattle for meat production, particularly by smallholder farmers, Muchenje *et al*. (2008b) and Marufu *et al*. (2011) reported that Nguni steers performed superior to Bonsmara when compared for meat quality, and they were resistant to harsh environments during rearing, and had less stress responsive at slaughter than the Bonsmara.

Meat is an important source of nutrients in human diets, and its consumption is mainly driven by sensory traits (Framer, 1994). Hoffman *et al.* (2007) stated that a number of beef sensory traits including aroma, juiciness and flavour are important beef sensory attributes. The most crucial sensory traits consumers consider when deciding on the meat palatability is the taste and tenderness (Strydom *et al*. 2000). Sensory analysis is an important tool to evaluate attributes that cannot be properly measured by objective methods, such as the aroma, flavour and texture (tenderness and juiciness), as human perception is more complete (Nassu, 2017). The current study investigated whether the inclusion of *S. plumusom* in diets affect cooking texture and overall meat taste, aroma, flavour, and texture properties of Nguni beef.

6.2 Materials and Methods

6.2.1 Study area

The study was carried out at the Agricultural Research Council - Animal Production (ARC-AP), Gauteng province, South Africa, during the summer season of 2020. The ARC-AP lies at latitude E 25° 53' S and longitude 28° 11' E. The mean ambient temperatures around the study area are 15.8 °C in winter and 28 °C in summer (https://en.climate-data.org). The dry season occurs between April and October and the mean annual rainfall is 661 mm, which falls between November and March (Kutu and Asiwe, 2010). The Agricultural Research Council, Animal Production Research Ethics Committee (Certificate: APAEC – 2020/16) and the University of Limpopo Animal Research and Ethics Committee (Certificate: AREC/01/2020: PG) approved the animal ethics protocol for this study.

6.2.2 Harvesting of the study plant

Seriphium plumosum forage was harvested at a private farm in Vanderbiljpark, Gauteng province between January and March 2020. The edible materials, leaves and branches, were harvested using a tractor slasher. The harvested *S. plumosum* material was sun-dried for three days before bailing and milled to pass through a 2 cm sieve size. The *S. plumosum* leaves were stored in a well-ventilated dry shade pending the feeding trial.

6.2.3 Steer management, treatments, design and feeding

Twenty-eight Nguni steers with a mean body weight of 300 ± 10 kg were procured from ARC Loskop Experimental farm, Limpopo province at 10 months of age. All animal care procedures conducted followed protocols approved by both the ARC Animal Ethics Committee and the University of Limpopo Animal Research and Ethics Committee. The study adhered to the guidelines of the South African National Standard (SANS 10386:2008) regarding the care and use of animals for experimental and scientific purposes. The steers were ear-tagged for easy identification and acclimatized for 21 days before the actual feeding trial which lasted 90 days (September to November 2020). Nguni steers were assigned into groups of seven using a completely randomized design, to make four feed treatment groups. The treatments were *S. plumosum* inclusion levels in the diets of 0 (T1), 10 (T2), 20 (T3) or 30 (T4) % (For feed ingredients in the diet, refer to Table 3.1, Chapter 3). A two (aging periods of 3 or 14 days) X 4 (diets of 0, 10, 20 or 30% *S. plumosum* inclusion levels) factorial arrangement in a completely randomized design was used for some meat quality attributes. Water and feed were made available *ad libitum*. Feeding took place daily at 0900 h and was monitored throughout the day to ensure that animals had sufficient food all the time. Animal slaughter and dressing were performed following standard commercial procedures at the registered and accredited ARC-AP Abattoir.

6.2.4 Sample preparation

All sample preparation and evaluation were conducted at the Animal Production of the Agricultural Research Council in Irene. Short loin primal cuts (*M. longissimus dorsi*) were deboned, cut into 30 mm thick steaks, vacuum packed, aged at 4°C for 3 and 14 days, respectively and then kept frozen at -20°C until further analyses. From each treatment, three vacuum packed 30 mm loin steaks per aging period were selected and dedicated for sensory evaluation (2 steaks) and Warner Bratzler shear force (1 steak).

6.2.5 Thaw loss and total cooking loss

Frozen (-20 °C) steaks were thawed for 24 h at 4 °C before oven broiling at 200 °C (Mielé, model H217, Mielé & Cie, Gütersloh, Germany) to an internal temperature of 70°C (AMSA, 2015). Both thaw and total cooking losses were determined on the steaks used for Warner-Bratzler shear force and sensory analysis. Thaw loss was expressed as percentage of leakage during thawing over the weight of the raw steak. Total cooking loss was calculated as the percentage difference in weight of the cooked and raw meat.

6.2.6 Warner-Bratzler shear force

Shear force were performed on six 12.5 mm (diameter) cores removed from cooked samples, parallel to the muscle fibers, after cooling them down to room temperature (18 °C). Cores were each sheared once through the centre, perpendicular to muscle fibre direction, by a Warner-Bratzler shear device, mounted on an Instron Universal Testing Machine (Model 4301, Instron Ltd, Buckinghamshire, England; 200 mm/min crosshead speed).

6.2.7 Sensory analyses

Sensory evaluation attributes used included tenderness, juiciness and various flavour overtones. Control samples were from beef fed on normal feed meal without S*. plumosum* inclusion. Broiled steaks were each given a random three-digit code as identifier. A nine member trained taste panel, selected based on reliability and validity of sensory results, rated the samples using eight different categories: beef aroma, impression of juiciness, muscle fibre and overall tenderness, overall beef flavour intensity, metallic/tin-like/bloody, chemical (salty), and sour. Panellists' scores of 1 represent extreme blandness, dryness or toughness and a score of 10 represents extreme intensities of aroma, flavour attributes or tenderness (Muchenje *et al*., 2008). Evaluations were performed in individual sensory booths and under red light conditions, to mask any differences in colour. A William Latin square design was used to determine the order of evaluation for each assessor. The analyses were conducted over eight days (training included). Seven tasting sessions were conducted per day, with samples from all treatments (*S. plumosum* meal inclusion levels x two aging times) being randomly assigned to the sessions.

6.2.8 Statistical analyses

Beef treatments were evaluated (four feed types that had under gone two ageing periods). Nine panellists evaluated each of the samples seven times for consistency. The data were then analysed using SAS Software (Cary, USA) considering the effects of panellists as random and ageing (3 and 14 days) as fixed effects. Multivariate analysis was used to describe the relationships between sensory properties and to identify differences between feed treatments. Significant differences ($P<0.05$) between sensory attributes were evaluated by a Fischer's LSD test. A Principal Component Analysis was performed on the correlation matrix of the sample means with the XLSTAT Software (USA). Data was standardised to allow the attributes to contribute in the analysis in the same way.

6.3 Results

6.3.1 Effect of *S. plumos*um meal inclusion levels in diets on aroma and flavour of Nguni steer meat

The results of the effect of *S. plumosum* meal inclusion levels in diets and aging on Nguni meat sensory attributes are presented in Table 6.1. *Seriphium plumosum* meal inclusion levels in the diets and aging did not affect ($P>0.05$) Nguni meat sensory attributes.

Table 6.1 Effects of *S. plumosum* meal inclusion level in the diets and aging on meat sensory attributes of Nguni steers

***S. plumosum* Inclusion[#] [*]**	**Beefy Aroma**	**Bloody Flavour**	**Astringency Flavour**
Aging (3 days)			
0 %	4.53 ± 1.7	1.93.± 1.8	0.97 ± 1.2
10 %	4.77 ± 1.7	1.76± 1.9	0.72 ± 1.1
20 %	4.64 ± 1.6	1.50 ± 1.6	0.94± 1.2
30 %	4.58± 1.5	1.47± 1.6	0.89 ± 1.3
Aging (14 days)			
0 %	4.66± 1.7	1.97± 1.9	0.88± 0.1
10 %	4.78± 1.7	1.84±1.9	0.85± 0.2
20 %	4.83± 1.6	1.75± 1.8	0.81±0.2
30 %	4.64± 1.6	1.82± 1.8	0.70± 0.2
Aging periods			
3 days	4.63±1.7	1.67±1.7	0.88±1.2
14 days	4.83±1.6	1.95±1.9	0.87±1.2
Probabilities			
S. plumosum Inclusion	0.7619	0.6495	0.7968
Aging period	0.2029	0.0846	0.9268
S. plumosum inclusion X aging period	0.8483	0.7736	0.9058

[#]: Diets were *S. plumosum* inclusion levels in the diets of 0 (T_1), 10 (T_2), 20 (T_3) or 30 (T_4) %

[*]: Values presented as mean ± standard deviation (SD)

6.3.2 Effect of *S. plumosum* meal inclusion levels in diets on cooking loss of Nguni steer meat

Raw and post-cooked weights were used to compute cooking loss percentage (Figure 6.1). *Seriphium plumosum* meal inclusion levels in the diets did not affect (P>0.05) cooking loss of Nguni steer meat.

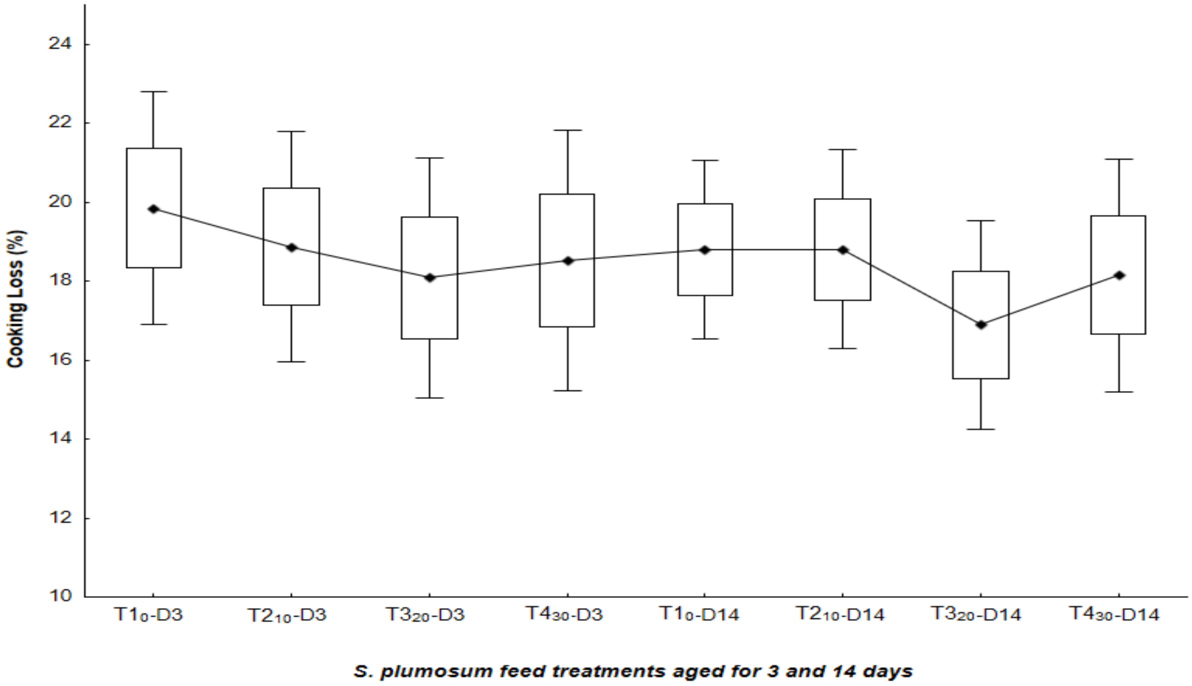

Figure 6.1 Effect of *S. plumosum* meal inclusion levels in diets on cooking loss of Nguni steer meat

: Diets were *S. plumosum* inclusion levels in the diets of 0 (T$_1$), 10 (T$_2$), 20 (T$_3$) or 30 (T$_4$) %

: Values presented as mean ± standard deviation

6.3.3 Effect of *S. plumosum* meal inclusion levels in diets on shear force values of Nguni steer meat aged 3 or 14 days

The results of the effect of *S. plumosum* meal inclusion levels in diets onshear force values of Nguni steer meat are presented in Figure 6.2. *Seriphium plumosum* meal inclusion levels in the diets did not affect (P>0.05) shear force values of Nguni steer meat aged 3 or 14 days.

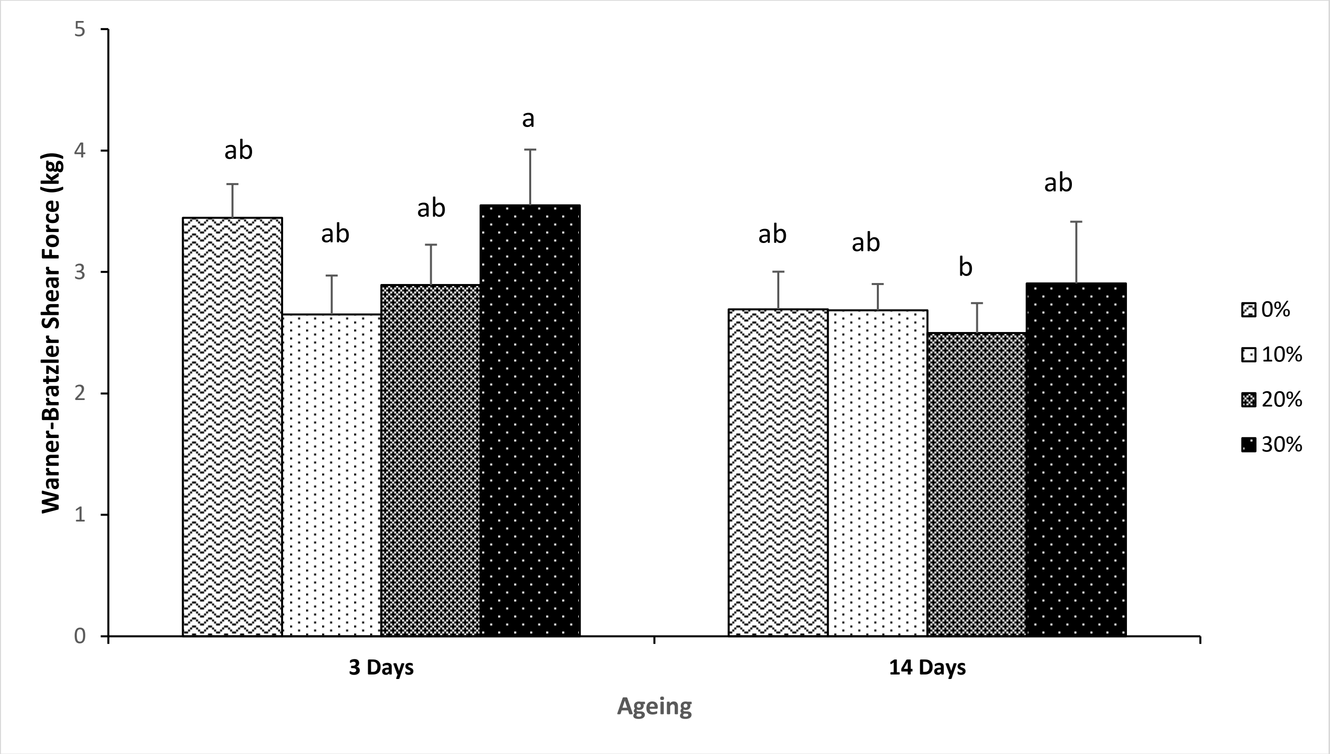

Figure 6.2 Effect of *S. plumosum* meal inclusion levels in diets on shear force values of Nguni steer meat aged 3 or 14 days

: Diets were *S. plumosum* inclusion levels in the diets of 0 (T_1), 10 (T_2), 20 (T_3) or 30 (T_4) %

: Values presented as mean ± standard deviation

6.3.4 Effects of *S. plumosum* meal inclusion level in the diets and aging on Nguni steer meat tenderness, stringiness and chewiness

The results of the effect of *S. plumosum* meal inclusion levels in diets and aging on Nguni steer meat tenderness, stringiness and chewiness are presented in Table 6.2. *Seriphium plumosum* meal inclusion levels in the diets and aging did not affect (P>0.05) Nguni steer meat tenderness, stringiness and chewiness.

Table 6.2 Effects of *S. plumosum* meal inclusion levels in the diets and aging on Nguni steer meat tenderness, stringiness and chewiness

***S. plumosum* Inclusion**[#] [*]	**Tenderness**	**Stringy**	**Chewy**
Aging (3 days)			
0 %	4.0 ± 1.70	2.3± 2.10	2.5 ± 2.41
10 %	4.5 ± 1.90	1.6± 1.81	2.0 ± 2.01
20 %	5.0 ± 1.90	1.4± 1.60	1.9± 2.01
30 %	3.9± 2.00	2.4± 2.41	3.3 ± 2.60
Aging (14 days)			
0 %	4.3± 1.80	2.0± 2.00	2.3± 2.30
10 %	4.6± 1.90	1.6±1.80	1.9± 2.10
20 %	4.0± 1.80	1.4± 1.80	1.9±2.00
30 %	4.2± 2.10	1.2± 2.30	2.8± 2.51
Aging periods			
3 days	4.7±2.00	1.9±2.10	2.5±2.10
14 days	4.3±2.00	1.6±2.00	2.0±2.30
Probabilities			
S. plumosum Inclusion	0.0625	0.0685	0.0779
Aging period	0.0681	0.0975	0.0624
S. plumosum Inclusion X aging period	0.5362	0.4245	0.3307

[#]: Diets were *S. plumosum* inclusion levels in the diets of 0 (T_1), 10 (T_2), 20 (T_3) or 30 (T_4) %

*: Values presented as mean ± standard deviation

6.3.5 Effects of *S. plumosum* meal inclusion levels in the diets on Nguni steer meat taste attributes after 3 or 14 days of aging

The results of the effects of *S. plumosum* meal inclusion levels in the diets on Nguni steer meat taste attributes after 3 or 14 days of aging are presented in Table 6.3. *Seriphium plumosum* meal inclusion levels in the diets did not affect (P>0.05) Nguni steer meat salty, beef aftertaste or bitter tastes after 3 days of aging. However, *Seriphium plumosum* meal inclusion levels in the diets affected (P<0.05) Nguni steer meat bloody aftertaste values after 3 days of aging. Nguni steers fed a diet having 0% of *S. plumusom* meal produced meat with higher (P<0.05) bloody aftertaste values after 3 days of aging than the meat from steers fed diets having 20 or 30% of *S. plumosum* meal. Similarly, Nguni steers fed a diet containing 20% of *S. plumusom* meal produced meat with higher (P<0.05) bloody aftertaste values after 3 days of aging than the meat produced by steers fed diets having 30 % of *S. plumosum* meal. However, Nguni steers fed diets having 0 or 10 % of *S. plumusom* meal produced meat with similar (P>0.05) bloody aftertaste values after 3 days of aging. Similarly, steers fed diets having 10 or 20 % of *S. plumusom* meal produced meat with the same (*P*>0.05) bloody aftertaste values after 3 days of aging.

Seriphium plumosum meal inclusion levels in the diets did not affect (P>0.05) Nguni steer meat beef aftertaste, bloody aftertaste or salty taste values after 14 days of aging (Table 6.3). However, *S. plumosum* meal inclusion levels in the diets affected (P<0.05) Nguni steer meat bitter taste values after 14 days of aging. Nguni steers fed a diet having 20 % of *S. plumusom* meal produced meat with higher (P<0.05) bitter taste values after 14 days of aging than the meat from steers fed diets having 0, 10 or 30 % of *S. plumosum* meal. However, Nguni steers fed diets having 0, 10 or 30 % of *S. plumusom* meal produced meat with similar (P>0.05) bitter taste values after 14 days of aging.

Table 6.3 Effect of *S. plumosum* meal inclusion levels in the diets and aging on Nguni steer meat taste attributes

Treatments$^{\#}$ *	**Ageing (Days)**	**Salty**	**Bitter**	**Beef aftertaste**	**Bloody Aftertaste**
0 %	3	8.4 ± 1.26	5.2 ± 0.73	3.8 ± 0.18	1.8^{a} ± 0.23
10 %	3	7.7 ± 0.85	6.2 ± 0.69	4.1 ± 0.10	1.6^{ab} ± 0.14
20 %	3	6.5 ± 0.65	6.6 ± 1.98	4.1 ± 0.16	1.4^{b} ± 0.15
30 %	3	7.1 ± 1.26	5.7 ± 0.67	4.1 ± 0.12	1.1^{c} ± 0.14
0 %	14	8.1 ± 0.92	5.4^{b} ± 1.19	4.3 ± 0.10	1.7 ± 0.17
10 %	14	7.7 ± 0.93	5.9^{b} ± 0.57	4.3 ± 0.08	1.7 ± 0.10

20 %	14	8.3 ± 1.04	9.1^{a} ± 1.78	4.1 ± 0.20	1.9 ± 0.19
30 %	14	8.5 ± 1.03	5.2^{b} ± 1.04	41 ± 0.16	1.9 ± 0.19

[#]: Diets were *S. plumosum* inclusion levels in the diets of 0 (T_1), 10 (T_2), 20 (T_3) or 30 (T_4) %

[a, b]: Means with different superscripts in the same column indicate significant differences between treatments ($P<0.05$)

*: Values presented as mean ± standard deviation

6.4 Discussion

The flavour of meat mainly comprises of the taste and aroma, though other senses such as astringency, mouth feel, and juiciness are also considered (Dashdorj *et al*., 2015). Flavour and aroma can be difficult to differentiate because of their similarities when consumed during judging of sensory traits (Campos *et al*., 2017). In the current study, *S. plumos*um meal inclusion levels in diets and on meat aging did not affect flavour and aroma of Nguni steer meat. The results possibly indicate that *S. plumos*um meal inclusion levels in diets in this study did not have any adverse effect on meat flavour and aroma and that *S. plumosum* meal has the potential of being used in beef production. These findings are similar to those of Duckett *et al*. (2013) when cattle were fed forages and concentrates. Schreurs *et al*. (2008) reported similar findings where sheep fed lucerne had unacceptable aroma than meat from grass-fed sheep. Frank *et al*. (2016), Ferrão *et al*. (2009) and Cox *et al*. (2006) stated that diets high in forages might change the fat composition and consequently the meat flavour. According to Elmore *et al*. (2005), some types of forage can be responsible for unpleasant flavour and aroma in meat and lead to low acceptability by consumers. However, Oltjen *et al*. (1971) reported higher meat flavour values from cattle fed a 100% alfalfa hay diet compared to the meat from cattle fed a high-concentrate diet.

Cooking loss of meat is an important meat sensory trait commonly used to assess water-holding capacity of meat (Li *et al*. 2013). Consumer perception on meat cooking loss is of importance as it determines meat taste (Ngambu *et al*., 2013). In the current study, diet did not affect cooking loss of Nguni steer meat. Similarly, Jiang *et al*. (2010) reported no significant interaction between forage diet and beef heifer steaks unaged or aged for 14 days. Whereas, Bruce *et al*. (2004) and Hedrick *et al*. (1983) reported higher cooking losses on concentrate supplemented beef when compared with forage-fed cattle. The cooking loss percentages in the current study ranged between 14 and 23 % and they are lower than the values reported by Ngambu *et al*. (2013) in a study where they compared the cooking losses of meat from Nguni, Angus and Bonsmara cattle. Razminowicz *et al*. (2006) reported cooking loss values of around 30% in steaks of beef steers consuming pasture-fed diet. Jeremiah and Gibson (2003) reported an average cooking loss of 22.5 %, which is similar to the present findings. According to Hertog-Meischke *et al*. (1998) ageing of meat significantly affect cooking loss percentage, because enzymatic reactions by endogenous enzymes such as collagenase produced by bacteria within beef or by ionic solubilisation progress at faster rates with ageing. Findings of the present study contradict such reports as ageing of meat at 3 or 14 days had no effect on meat cooking loss. However, reported differences in meat cooking losses between authors can be attributed to factors such as differences in ageing, cooking method applied, cooking temperatures, duration of cooking temperatures, pH and marbling fat (Hedrick *et al*., 1993; Bertram *et al*., 2004; Barbera and Tassone, 2006; Jama *et al*., 2008).

Texture is one of the most important sensory qualities associated with consumers' satisfaction and ageing the meat improves beef quality and meat tenderness over time (Fabre et al., 2018). The Warner braztler shear force values provide a relevant measure for the classification of meat based on their sensory characteristics. In the current study, *S. plumosum* meal inclusion levels in the diets did not affect shear force values of Nguni steer meat aged 3 or 14 days. The shear force values in the present study ranged from 2.5 to 3.5 kg and 2.5 to 3.0 kg for the meat aged 3 or 14 days, respectively. According to the benchmarks of Boleman *et al.* (1997), the loin samples tested in present study were mostly tender. In agreement with findings of the present study, Duckett *et al*. (2013) reported no differences in meat textures from forage and grain-fed beef steers. The present shear force values are better (lower) than those reported by Dikeman *et al*. (2005) for longissimus values of beef steers aged from 2 to 14 days' post-mortem and cooked to an internal temperature of 18^0C. Similarly, the values of the present study are better than the values obtained by Rhee *et al*. (2004) in a study sought to determine various beef muscles. Morgan *et al*. (1991) reported threshold values of 4.6 and 3.9 kg for "retail" and "food service" beef, respectively, which are slightly higher (worse) than the values in the current study. However, a shear force of 4.6 kg can be ascribed to a steak rated as "slightly tender" by consumers

(Shackelford *et al.*, 1991). Some previous studies showed that the texture of beef steers reared in grazing systems could be more tender than that of the beef from concentrate-fed animals (Bruce *et a*l., 2004; Realini *et al*., 2004). Discrepancies in such findings can be attributed to ultimate carcass pH and temperature decline post-mortem (Resconi *et al*., 2010; Purchas *et al*., 1999).

Numerous reviews have been done on beef tenderness, and it was concluded that tenderness is the most important factor in determining consumer satisfaction (Brooks *et al*., 2000; Koohmaraie *et al*., 2003; Chambaz *et al*. 2003; Hanzelkoá *et al*. 2011). According to Scollan *et al.* (2003), aging is the main method that improves tenderness of the meat, and the maximum aging period to achieve optimal meat tenderness in beef cattle is 14 days. Proteins that are involved in scaffolding of the muscle structure degrade, and hence the more tenderized meat becomes with aging (Koohmaraie *et al*., 2005). In the current study, *S. plumosum* meal inclusion levels in the diets and aging at 3 or 14 days did not affect Nguni steer meat tenderness, stringiness and chewiness. This is an indication that *S. plumosum* meal can be included in cattle diets without causing any adverse effects on meat tenderness, stringiness and chewiness. It is known that dietary condensed tannins like those found in *S. plumosum* meal can prolong shelf-life of the meat from animals consuming it (Makkar *et al*., 2007). Tannins are considered as one of natural antioxidants found in tanniniferous plants (Wapi *et al.,* 2013). It is, therefore, suggested that further studies be conducted to determine the effects condensed tannins, as antoxidants, on meat meat tenderness, stringiness and chewiness.

Seriphium plumosum meal inclusion levels in the diets did not affect Nguni steer meat salty, beef aftertaste or bitter tastes after 3 days of aging, possibly an indication that *S. plumosum* meal can be included in cattle diets without having any adverse effects on meat salty, beef aftertaste or bitter taste values. However, *S. plumosum* meal inclusion levels in the diets affected Nguni steer meat bloody aftertaste values after 3 days of aging. The meat bloody aftertaste values after 3 days of aging tended to decrease with increase in *S. plumosum* meal inclusion levels in the diets. This could have resulted from the presence of condensed tannins in *S. plumosum* as they exhibit bitter taste, thus, the corresponding taste receptor cells serve as warning sensors (Behrens and Meyerhof, 2006).

It is not clear why this is so, and further studies are suggested to determine the causes. *Seriphium plumosum* meal inclusion levels in the diets did not affect Nguni steer meat beef aftertaste, bloody aftertaste or salty taste values after 14 days of aging, possibly indicating that *S*. *plumosum* meal can be used in cattle diets without any adverse effect on meat beef aftertaste, bloody aftertaste or salty taste values. However, *S. plumosum* meal inclusion levels in the diets affected Nguni steer meat bitter taste values after 14 days of aging, possibly indicating the effect of of antioxidant activies found in meat of animals on diets high in condensed tannins (Makkar *et al*., 2007). Kerth *et al.* (2018) observed that the addition of any of *Prosopis mesquite*, an encroaching woody plant, into Rambouillet wether lamb diets did not negatively affect any carcass traits and sensory attributes. Mandell *et al*. (1998) reported similar findings in a study of the effect of forage and grain diet on sensory traits of Limousin cross steers.

6.5 Conclusions

Generally, *S. plumosum* meal inclusion levels in the diets did not affect Nguni steer meat quality and sensory attributes, possibly an indication that *S*. *plumosum* meal can be included in cattle diets without having any adverse effects on meat quality and sensory attributes. However, further long-term studies should be conducted to ascertain the present findings.

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CHAPTER 7

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 General discussion

Woody plant encroachment threatens biome shifts from open savanna in semi arid areas to closed woodland, and consequently alters the function and biodiversity leading to reduced rangeland productivity (Eldridge *et al*., 2011). Moreover, this phenomenon is considered a threat to rangeland degradation because it suppresses productivity of herbaceous layer (Lesoli *et al*., 2013). Several methods including fire, tree thinning, chemical treatment have been explored to reverse woody plant encroachment (Trollope, 1996; Higgins *et al*., 2007; Smit *et al*., 2010), however with little success. Fodder from woody plants have the potential to be used as animal feed, although their plant secondary metabolites are known to adversely affect feed intake, reduce productivity, and may cause toxicity if consumed beyond threshold (Rogosic *et al*., 2008). Previous studies reported that domestic and wildlife animals have the ability to adapt and tolerate the ingestion of plant secondary compounds found in woody plants, especially when they are present at lower concentrations (Lamy *et al*., 2011). *Seriphium plumosum* is one of the encroaching species that have invaded the grassland biome in South Africa (Snyman, 2009). Despite infesting most parts of South African rangelands, and other African countries, there is a lack of or limited information on its integration as an animal feed. The aim of this study was to determine *S. plumosum* meal inclusion levels for optimal animal performance and methane reduction by Nguni steers.

Seriphium plumosum meal inclusion in the diets did not significantly affect feed intake, digestibility, FCR and growth performance of Nguni steers, which may be an indication that the diets provided adequate nutrients for maintenance and production. Thus, an encroaching *S. plumosum* has the potential for use as a feed ingredient for ruminant animals, and may contribute to management strategies aimed at controlling its invasion.

Inclusion of *S. plumosum* meal in the diets reduced CH_4 emission by Nguni steers. The reduction in CH_4 emission ranged from 1.41 to 15.0 %. This finding demonstrates its potential as a feed to reduce CH_4 production and emission by ruminant animals. A 13.6% *S. plumosum* meal inclusion level in the diet was calculated, using quadratic equations, to result in optimal CH_4 emission reduction by Nguni steers. Condensed tannins reduce methane production and emission through formation of complexes with dietary proteins and carbohydrates in the rumen (Currulla *et al.,* 2005). Thus, methanogenesis is reduced (Makkar *et al*., 2007). Enteric CH_4 is the product of a methanogenesis process that takes place in the rumen where H_2 reduces the CO_2 with the help of methanogenic archaea, resulting in CH_4 production (Ellis *et al*., 2008; Ng'ambi *et al*., 2022). Methanogens are the only known microorganisms capable of producing CH_4 (Hook *et al*., 2010), and as such, their reduction in the rumen of Nguni steers supports the hypothesis that inclusion of *S. plumosum* meal in the diets reduces CH_4 production and emission by ruminants. These results suggest potential use of *S. plumosum* meal in cattle diets for reduced CH_4 emission. However, further long-term studies should be conducted to ascertain the present findings.

Another objective of the study was to determine the effect of inclusion levels of *S. plumosum* meal in diets on carcass and meat quality. *Seriphium plumosum* meal inclusion in the diets did not affect hot carcass weight, carcass fat and meat quality of Nguni steers. Nguni steer meat pH and temperature were also similar across the treatments. Generally, replacing lucerne with *S. plumusom* meal in the diets did not significantly affect the sensory attributes of beef from Nguni steers. This implies that the inclusion of *S. plumosum* meal in the diet has a great potential for use as feed for beef cattle without compromising meat quality.

7.2 Conclusions

This study demonstrated that feed efficiency and meat quality from Nguni steers fed diets up to 30 % of *S. plumosum* meal inclusion were not adversely affected. The growth performance, feed conversion ratio and digestibility of Nguni steers were not influenced adversely by the inclusion of *S. plumosum* meal in the diets. A number of microbial populations responsible for enteric methane production and emission were significantly reduced by inclusion of *S. plumosum* meal in the diets of Nguni steers. Information from this study is helpful in understanding the key role of ruminal microbiomes and their relationship with methane production and, therefore, will form the basis of valuable and eco-friendly methane mitigation methods while improving animal productivity. A 13.6 % *S. plumosum* meal inclusion level in the diet was calculated, using quadratic equations, to result in optimal CH_4 emission reduction by Nguni steers. This is very valuable but further long-term studies are suggested to ascertain this finding. The carcass, meat quality and sensory attributes of Nguni steers fed diets with up to 30 % *S. plumosum* meal inclusion levels were, generally, similar to those of steers fed diets without any *S. plumosum* meal inclusion. These results suggest that *S. plumosum* meal can replace up to 30 % of lucerne hay in the diet without causing any adverse effects on Nguni steer meat quality. The utilization of S*. plumosum* species in diet formulation for Nguni steers demonstrated its efficiency for use as animal feed, and as such, it be recommended for use as feed particularly during the dry season when there is feed scarcity or the feed available is of poor nutritional value. The use of *S. plumosum* as animal feed can also serve as a climate smart and resilient agricultural strategy to reduce the contribution of enteric cattle CH_4 emission to green house gases.

7.3 Recommendations and further studies

Results from this study demonstrated that the use of *S. plumosum* as a feed ingredient in ruminants could contribute to the control measures aimed at reducing woody plant encroachment in semi arid regions of South Africa. Research on the use of *S. plumosum* in cattle diets is at an early stage and warrants further investigation. Edible material (i.e. leaves and twigs) of *S. plumosum* contained 12.5 % crude protein and <5 % CT, therefore the meal could improve diets for ruminants during the dry season. *Seriphium plumosum* contains CT, and as such, it could be used to reduce CH_4 production and emission in ruminants without any adverse effects on the animal is of great interest. However, further long-term studies should be conducted to ascertain the present findings. Therefore, the following studies are recommended:

a. The effect of *S. plumosum* meal inclusion in diets on performance of different ruminant animals.
b. The effect of *S. plumosum* meal as a supplementary feed on performance of extensive grazing animals.
c. There is a need to further explore the use of *S. plumosum* as climate smart agricultural strategy to reduce CH_4 production and emission by ruminant animals, and hence mitigate against climate change.
d. Research on the economic use of *S. plumosum* as supplementary feed for ruminants is required. This may help in developing cost-effective strategies to reduce production costs during the dry season.
e. Further studies should evaluate the effect of *S. plumosum* meal on antioxidant metabolites in meat and relate their levels to meat oxidative shelf-life.

The insight drawn from the proposed research may contribute to managing *S. plumosum* encroachment, while reducing methane production and emission by ruminant animals.

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