EFFECT OF GRADED INCLUSION OF *PENNISETUM PURPUREUM* GRASS ON GROWTH PERFORMANCE, RUMEN FERMENTATION AND MEAT QUALITY OF FEEDLOT STEERS

By

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DECLARATION AND COPYRIGHT

I, Rabatseta Thandi Phuti, identity number _____, student number _____, hereby declare that this dissertation: **Effect of dietary inclusion of *Pennisetum purpureum* grass on growth performance, rumen fermentation and meat quality of feedlot Sussex red steers,** is submitted in partial fulfilment of the requirement of the degree of Master of Agriculture at the Central University of Technology. This dissertation is my own work and has not been submitted to any degree at any University in fulfilment of the requirement for the attainment of any qualification. Its original design and in execution, and all reference material contained in this dissertation has been duly acknowledged and comply with the Code of academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology.

Signature:
Date: 16 October 2023

Rabatseta T.P.

DEDICATION

First and foremost, I dedicate this work to God Almighty for the strength and His love that never ceased to amaze me throughout the duration of the study. To my parents, Mr Frans and Mrs Rachele Rabatseta, and my siblings (Phillip, Benford, MacDonald, Marius, Witness, Bontle and Mamoloko), you are my inspiration that aspired me to keep going. Thank you for the support you have shown from day one, and believing in me more than I believed in myself.

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LIST OF ACRONYMS AND ABBREVIATION

Abbreviations	Description
%	Percent
°C	Degrees Celsius
a*	Meat redness
ADF	Acid detergent fibre
ADG	Average daily gain
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ARC	Agricultural Research Council
b*	Meat yellowness
BW	Body weight
C*	Chroma
Ca	Calcium
CCW	Cold carcass weight
CIE	Commission Internationale de l`Eclairage
CP	Crude protein
CW	Carcass weight
DAFF	Department of Agriculture Fisheries and Forestry
DE	Digestible energy
DFD	Dark firm dry
DM	Dry matter
DP %	Dressing percentage
EE	Ether extract

FAO	Food and Agricultural Organization
FCR	Feed conversion ratio
FI	Feed intake
FW	Final weights
g	Gram
GDP	Gross domestic product
GE	Gross energy
h*	Hue angle
IW	Initial weights
kg	Kilogram
L	Litre
L*	Meat lightness
LA	Lactic acid
LSD	Least Square Difference
Mb	Myoglobin
N	Number of animals
NDF	Neutral detergent fibre
NRC	National Research Council
OM	Organic matter
P	Phosphorus
PA	Propionic acid
pH_i	Initial pH
pH_u	Ultimate pH
SAS	Statistical Analysis System

SD	Standard Deviation
SE	Standard Error
VFA	Volatile fatty acid
WBSF	Warmer-Bratzler Shear Force
WCW	Warm carcass weight
WHC	Water-holding capacity
Wt	Weight

ABSTRACT

The study was carried out to evaluate the effect of graded dietary inclusion levels of Napier grass (*Pennisetum purpureum*) in steers` fattening diets, on growth performance, rumen fermentation traits, carcass characteristics and meat quality of steers. Representative samples of Napier grass were analysed for proximate (dry matter, crude protein, gross energy, ether extract and ash), fibre fractions (acid detergent fibre, acid detergent lignin and neutral detergent fibre) and mineral profile. Twenty-four 8 months-old male weaners with an average body weight of 176.5±20kg were used. Three experimental diets were formulated wherein Napier grass was included at 0% (Diet 1- control diet), 30% (Diet 2) and 60% (Diet 3) on forage basis to meet the nutrient requirements of growing fattening steers. The steers were allocated to the treatment diets in a completely randomized design. The steers were fed the experimental diets for 120 days during which data was collected following 14 days of adaptation period. Steers were individually weighed at the beginning of the trial and weekly until trial termination. At the end of growth study, the steers were slaughtered and eviscerated. Following evisceration, rumen digesta pH was measured and rumen digesta was collected for volatile fatty acid analysis. Carcass samples were collected and sampled. Dietary inclusion of 60 % Napier grass in steers fattening diet reduced ($P<0.05$) body weight gain, daily feed intake and feed efficiency of the steers. The rumen fermentation dynamics of the steers were similar ($P>0.05$) across the treatment diets. While warm and cold carcass weight, as well as cold muscle temperature of the steers fed diets containing 0 and 30% of Napier grass were higher compared to that containing 60 %; dressing percentage, warm and cold muscle pH of the steers were similar ($P>0.05$) across dietary treatments. The meat physical attributes (drip loss, colour, myoglobin water holding capacity, cooking loss and tenderness) of the steers did not differ ($P>0.05$) among the treatment diets. The 7-days aged meat thaw loss increased ($P<0.05$) with increasing inclusion level of Napier grass. Dietary inclusion of Napier grass at 30% in steers fattening diets yielded meat high in protein and fat. Further research on the fatty acid profile of the meat fat is suggested. Moreover, study to quantify enteric methane production of steers consuming Napier grass-based diets need to be conducted.

Keywords: average daily gains, feed intake, growth, carcass, meat quality, rumen digesta

CHAPTER 1

1.1. Introduction and background

The livestock industry offers great potential for the agricultural sector. Livestock production in South Africa (SA) is a significant contributor to food security and clothing and provides many social and economic attributes to the country (Meissner *et al*., 2013). Presently, livestock production in SA is with almost 14 million cattle, which constitute 1.6 million dairy and 12.4 million beef cattle (Visser *et al*., 2020). According to Lenne & Thomas (2006), approximately 70% of the human population depends on livestock for meat and milk, which represents 20% of the animal protein in human diets. At about 48% of the gross livestock production, beef is the second largest source of income for stock producers, followed by veal (22%), fresh milk (13%), sheep and goat meat (5%), pork (4%), and then small stock fibers (3%) (Cloete *et al*., 2014).

Shortage of pasture, inadequate nutrition and insufficient feed supply are among factors that contributes to the low livestock production in SA (Kunene *et al*., 2002). These factors are propelled by conversion of grazing lands used for natural hay production during dry seasons to croplands and plantations (Berihun *et al*., 2019). While these factors lead to poor livestock growth and fertility (Stroebel, 2004; Mbatha, 2021), they also prolong the feeding period to attain market or slaughter weights, thus low income for the farmers. Most importantly, the demand for good quality beef by consumers is fuelled by the growing focus on the relationship between nutrition and health (Franco *et al*., 2014). This demand thus places an intense pressure on the livestock producers (Thornton, 2010; Meissner *et al*., 2013). In an attempt to improve livestock production, intensive feeding program has to be put in place, wherein farmers fatten animals using adequate feeds that will meet their nutrient requirements, thus slaughter weight in a short period to achieve economic returns. Adequate feeds are necessary to ensure optimal livestock health, productivity and thus production (Jayanegara *et al*., 2016). Intensification of livestock is then highly considered as one phenomena that could relieve the pressure of the increasing demand for animal protein by the growing human population from the SA livestock producers.

Currently the livestock feed industry in SA is focusing on exploring feed resources as potential sources of protein and energy. Scanty attention is steered to sources of forage despite their equal significance in ruminant diets as the main component. In addition to the less attention that is paid to sources of forages, *Eragrostis curvula* (African lovegrass), which is one of the forages that is currently utilised in intensive farming system, is an invasive C_4 perennial grass that is endangering natural ecosystems globally (Roberts *et al*., 2021). There is therefore an urgent need to search for alternative primary forage that can fit in the intensive feeding program, either as a supplement to a regular ration, or as a replacement for part of the ration.

Napier grass (*Pennisetum purpureum*), a C_4 grass species of perennial tropical forage native to Sub-Saharan Africa (Franco *et al*., 2014), is considered to be one of the most important tropical forages due to its high biomass production (Meng *et al*., 2019) and adaptability to a variety of ecosystems (Muktar *et al*., 2019). The growth rate and biomass production of Napier grass surpasses that of other tropical grasses including maize (*Zea mays*), Switchgrass (*Panicum virgatum*), Johnson grass (*Sorghum halepense*), and Sugarcane (*Saccharum officinarum*) (Ra *et al*., 2012). It has the ability to withstand repeated cutting and rapidly regenerate and produce palatable leafy shoots (Lowe *et al*., 2003). Research shows that Napier grass is the most potential fodder grass in animal production systems, with its remarkable characteristics that further includes regrowth ability, high yield per unit area, drought tolerance (Fukagawa & Ishii, 2018) and high water use efficiency (Kabirizi *et al*., 2015). However, this fodder contains a low level of crude protein (CP), being 7.6 g CP/kg DM while ARC (1980) recommended dietary CP levels that ranges from 10 to 12 g CP/kg DM for growing heifers. This suggests that Napier grass should be supplemented with sources of CP to improve its utilization by ruminants. Kariuki *et al*. (1999) supplemented degraded levels of Lucerne to heifers fed Napier grass hay and reported improved animal performance with the supplementation. Further, Muia *et al*. (2000) fed heifers on Napier grass supplemented either with sunflower meal or with poultry litter and reported improved performance with supplementation. Lastly, inclusion of *Desmodium* and sweet potato vine could play an important role in improving animal performance

when Napier grass is fed as the basal diet (Kariuki *et al*., 2001). Research on feeding a total mixed ration (TMR) that includes hay from Napier grass in South Africa is limited. This study aims to formulate TMR with degraded levels of Napier grass hay and feed to Sussex heifers intensively, and measure growth performance, fermentation dynamics, carcass traits and meat quality from the steers.

1.2. Problem statement

The high demand for animal products in SA calls for an increase in livestock production particularly from intensive farming systems. However, utilization of sufficient adequate feed to increase livestock production in intensive farming systems is equally significant. The livestock feed industry in SA solely relies on conventional feedstuffs that are pricy for farmers. The high price of conventional feedstuff such as soyabean meal (SBM), which is a major nutrient resource in livestock feed, is fueled by the importation bill. The country thus rely on imports for the shortfall. There is scarcity of roughages for fattening ruminants during dry seasons and some of the available roughages are deficient in nutrients. The use of pastures such as kikuyu have been utilized, however, kikuyu provides throughout the Mediterranean summer when there are high temperatures and low rainfall (Marais, 2001). During winter and springs, the grass may be dormant but have a very low CP content resulting in shortage of nutrient production (Viljoen *et al*., 2020). Feeding such poor quality feed decreases the growth rate of the microbial population in the rumen, thereby decreasing fermentation and digestibility of the roughage. The key problem to the effective use of kikuyu in livestock feeding is seasonal changes that influence the availability of good quality roughage. While other roughages such as Lucerne, red clover and white clover have adequate quantities of the required nutrients, the nutrients are cleaved because of the anti-nutritional compounds prohibiting nutrient availability and decreasing degradability rate of the degradable matter in the rumen (Aganga & Tshwenyane, 2003). Alternatively, farmers are seeking affordable feed resources that are biomass dense to improve livestock production, and thus meet the demand for animal products.

1.3. Rationale and motivation

Napier grass is a vital roughage resource for the livestock sector, particularly in regions with seasonal fluctuations in feed quantity and quality. Napier grass has a high nutritional value, drought tolerance, and adapts extensively across a wide ecological range (Mogotsi *et al*., 2020). In tropical and subtropical regions of the world, Napier grass is used in feeding systems (Wadi *et al*., 2004; Zailan *et al*., 2016). The work on Napier grass so far has focused on its chemical composition variation based on growth at various heights (Aganga *et al*., 2005; Rambau *et al*., 2016). To date, no research has been conducted in SA on the effects of Napier grass on ruminant growth performance, rumen fermentation, carcass traits and meat quality. Given that the growth of Napier grass thrives in South African climatic conditions (Rambau *et al*., 2016), there is thus a need to characterize, both chemically and *in vivo*, the potential of the grass as a dietary protein source in growing fattening steers with the target being to reduce feed cost and be able to intensify beef production. This study can thus provide information on the nutritional value and inclusion level of Napier grass in the diets of ruminants necessary for farmers during feed formulations to improve productivity of their animals.

1.4. Objectives

The main objective of this study is to evaluate the chemical composition of Napier grass and determine the effects of graded dietary inclusion levels of Napier grass on growth performance, rumen fermentation characteristics, carcass traits and meat quality of feedlot steers.

The specific objectives will be:

i. To determine the nutritional value of Napier grass.

ii. To determine the effect of graded dietary inclusion levels of Napier grass in based diets on growth performance [daily feed intake (DFI), feed convention ratio (FCR) and average daily gain (ADG)] of Sussex red steers.

iii. To determine the effect of graded dietary inclusion levels of Napier grass in based diets on rumen fermentation kinetics (volatile fatty acids (VFA) and rumen digesta pH) of Sussex red steers.

iv. To determine the effect of graded dietary inclusion levels of Napier grass-based diet on carcass traits and quality of meat from Sussex red steers.

Hypotheses

i. H_1 Due to its high nutritional value, Napier grass can be used as a feed ingredient in steers fattening diets.
ii. H_0 Due to its low nutritional value, Napier grass cannot be used as a feed ingredient in steers fattening diets.
iii. H_1 Graded inclusion levels of Napier grass in fattening diets will improve growth performance of Sussex red steers.
iv. H_0 Graded inclusion levels of Napier grass in fattening diets will impair growth performance of Sussex red steers.
v. H_1 Graded inclusion levels of Napier grass in fattening diets will enhance fermentation dynamics in Sussex red steers.
vi. H_0 Graded inclusion levels of Napier grass in fattening diets will diminish fermentation dynamics (rumen digesta pH and volatile fatty acids) in Sussex red steers
vii. H_1 Graded inclusion levels of Napier grass as a source of fibre in fattening diets will improve carcass traits and quality of meat from Sussex red steers.
viii. H_0 Graded inclusion levels of Napier grass as a source of fibre in fattening diets will deteriorate carcass traits and reduce the quality of meat from Sussex red steers.

CHAPTER 2: LITERATURE REVIEW

2.1. Introduction

This section focuses on the production of Napier grass relative to other fodders and consumption of the grass by livestock. Research outcomes pertaining to the use of Napier grass as a feed ingredient in livestock feed are also reviewed. Dietary inclusion of Napier grass in ruminant diets could have different effects on growth performance (feed intake, feed conversion ratio and weight), fermentation dynamics (rumen digesta pH and microbial activities) of fed animals as well as carcass traits and quality of meat from the fed animals.

The agricultural industry possesses major potential for improving the welfare of rural communities at a global level, with livestock farming as a fundamental component. The importance of livestock farming in the agricultural industry cannot afford to be understated, given its crucial contribution to food security and rural development worldwide (Mirón *et al*., 2023). Moreover, sustainable livestock production is undeniably dependent on the availability of adequate feed. The availability of feed rich in nutrients is the key point of achieving improved livestock production, particularly under extensive livestock production. Livestock farmers (both smallholders and commercial) are often faced with insufficient supply of adequate feed and or unavailability of the latter. Subsequently, they opt to the utilization of low quality feed ingredients, which then becomes an impediment to the production of livestock, thus reduction in livestock products (Lamega *et al*., 2021).

Feed scarcity in quantitative and qualitative aspects is one of the major hindrances to livestock production. The quantity and quality of feed resources available to livestock in sub-Saharan Africa are mainly affected by seasonal fluctuation of rainfall and other environmental factors (Godde *et al*., 2021) such as occurrence of droughts and desertification (Moyo et al., 2019).

For instance, natural pastures provide more than 90% of the livestock feed that are generally poorly managed. Grazing lands used for production of natural hay during dry season are commonly converted to plantations, croplands, industrial estates and human settlements, therefore forcing livestock to graze on overgrazed and marginal lands with poor-quality pasture. Moreover, natural pastures are low in phosphorus, which predisposes cows to impaired muscle function, retained placenta and downer cow syndrome (Molefe & Mwanza, 2020). In addition, the most reliable perennial grass that is used in the South African livestock industry is *Eragrostis curvula* (Weeping lovegrass). *Eragrostis curvula* hay is a popular feed resource in South Africa, with average crude protein content of less than 60g/kg DM, which may require supplementation or treatment with NPN when feeding livestock (Tesfayohannes *et al*., 2013). During winter months, this grass has an insufficient energy value of 4.8 MJ/kg DM and protein content of less than 20g/kg DM to can meet livestock nutrient requirements. Subsequently, palatability and intake are reduced because of the high NDF (90%) coupled with low digestibility (less than 39%) (Adejoro & Hassien, 2017).

Supplementation of *Eragrostis curvula* with concentrates such as soyabean meal (SBM) is ideal to enhance the nutrient value of the grass as forage-based diet. The importance of SBM in diets is based on its high content of crude protein as an indicator of its concentration of both essential and non-essential amino acids (Bajjalieh, 2012). Given its dominant nutritive value, SBM is not fully utilized as a feed resource by farmers due to its high pricing, which is fueled by importation tariffs and competition between humans and animals.

Alternatively, livestock are fed on cultivated crop residues, fodder, concentrate and agro industrial by-products (Rusdy, 2016). However, the availability of crop residues is seasonal and characterized by variations in nutrient content (Malebana *et al*., 2018). The concentrates and agro industrial by-products are scares and costly, accounting to 70% of the overall livestock operational expenses (Makkar, 2018). As a result, livestock are unable to meet their nutrient requirements from the poor quality feed available. The inability of the livestock to meet their nutrient requirements ultimately results in perturbed animal performance coupled with increased production costs and failure to meet the demand for livestock products. An establishment and maintenance of a suitable forage resource base that can accommodate the desired livestock units and meet the increasing market demand for livestock products is imperative.

Forages are necessary components in ruminant' diets, as they provide the dietary fibre needed to optimize rumen function as well as source of energy for metabolic processes. As primary sources of roughages, forages fit into the feeding program either as replacements for portion of the ration, or as supplements to a regular ration (Myer, 2003). As the sole diet, roughages cannot meet the nutrient requirements for high producing animals.

Smallholder farmers have thus used trees and browse species as feasible roughages for centuries, especially during dry season when rangeland vegetation is inert (Salem *et al*., 2004). The incorporation of leguminous trees such as *Sesbania sesban* (Farghaly *et al*., 2022), *Gliiricidia sepium* (Rusdy *et al*., 2019), and *Vachellia* species (Brown *et al*., 2016) into livestock rations has gained popularity in South Africa owing to the ability of these fodders to enhance rumen digestion and control internal parasites, and reduce methane production (Ravhuhali *et al*., 2022). Nonetheless, numerous limitations restrict the use of these forage resources due to either presence of spine and thorns on *Vachellia karoo* for instance, might cause injury to the animals or make it difficult for them to consume the fodder (Brown *et al*., 2016). In addition to causing injuries, these fodders are high in fibre content, anti-nutritive factors and other toxic compounds that negatively affect intake, organic matter digestibility and net energy in roughage resources especially in the woody species (Mapiye *et al*., 2011).

Planted pastures that are not only sources of roughage for livestock, but also important to conserve or enhance natural resources by reducing erosion, restoring soil fertility and degraded land while enhancing biodiversity are worth being researched. Napier grass (*Pennisetum purpureum*) is one such pasture. Napier grass is a tall fast-growing perennial grass indigenous to tropical and subtropical climates; and is currently the most potential fodder grass in animal production systems. This is due to its remarkable characteristics; including high yield, regrowth ability, drought tolerance (Fukagawa & Ishii 2018), high water use efficiency (Kabirizi *et al*., 2015) as well as resistance to a broad spectrum of diseases and pests (Van den Berg & Van Hamburg, 2015). Napier grass is a perennial C4 grass species, which is native to sub-Saharan Africa (Yan *et al*., 2021). It is adapted to grow best in areas with an annual rainfall of between 750 and 1500 mm (Singh *et al*., 2013). This grass is naturalized in areas of South and Central America, tropical parts of Asia, Australia, the Middle East and the Pacific

Islands (Cook *et al*., 2005). As a result, today Napier grass is widely grown in tropical and sub-tropical regions of the world.

Napier grass is considered one of the most vital tropical forages owing to its acceptance by livestock (Muktar *et al*., 2019). The grass is widely used to feed cattle both in cut and carry systems and as pasture. A multipurpose forage can be directly grazed or compacted into silage or rolled as hay. It is also considered a useful feedstuff for pigs due to its high soluble carbohydrate content that is favorable to good fermentation (Ferreira *et al*., 2014). Additionally, due to the high content of fibre and its quality, Napier grass is of interest in developing the well-being of modern pigs. Napier grass has also been used for feeding grass carp and tilapia in Epal (Pandit *et al*., 2004) and Bangladesh (Shrestha and Yadav, 1998). A study by Akah & Onweluzo (2014) reported that young shoots of Napier grass were consumed as cooked vegetable in Nigeria. These various uses offer an indication of the diversity of roles that Napier grass could contribute to poverty alleviating particularly in rural communities. Most importantly, the growth rate and biomass production of Napier grass exceeds that of other tropical grasses including Switchgrass (*Panicum virgatum*), Johnson grass (*Sorghum halepense*), Sugarcane (*Saccharum officinarum*) and maize (*Zea mays*) (Ra *et al*., 2012). The above-mentioned qualities of Napier grass mark it as an attractive option for livestock production system. However, the utilization and adoption of Napier grass as an alternative forage crop for source of fibre has not been effective due to the scanty amount of research and attention afforded to this crop. Although Napier grass has low protein content, it can supply forage source for livestock provided it is supplemented with protein concentrates and legumes in the diets. The chemical composition and effect of the grass provenance to South Africa as a feed ingredient in steers diet on growth performance, microbiota, carcass traits and quality of meat from the steers in not known. Therefore, this study will review previous research on Napier grass grown in other countries as livestock feed ingredient and interrogate its potential as a forage source to complement *Eragrostis curvula* and other commercial forages that are currently used in the feed industry as sources of fibre, thus energy.

2.1.1. Nutrient value

(i) Nutritive value of conventional fibre sources used in livestock feed

Sudarman *et al*. (2019) reported that coffee husk provenance to Indonesia had 9.20, 8.22, 0.58, 31.62, 50.38 and 51.57 % of ash, crude protein, ether extract, crude fibre, nitrogen free extract and total digestible nutrients, respectively. The varieties of rice straw reported by Rahman *et al*. (2010) ranged from 92.21 to 93.05 percent, 3.49 to 5.10 percent, 41.38 to 46.32 percent, 72.16 to 77.57 percent, and 4.3 to 6.97 % for DM, CP, ADF, NDF, and ADL, respectively. The study by Aquino *et al*. (2020) found that the DM content (92 to 96 %) and CP content (3 to 7 %) of rice straw were comparable. These feed sources are essentially low in protein and high in cell walls, neutral detergent fibre (ADF) and acid detergent fibre (ADF), which are made up of the degradable carbohydrate fractions like cellulose, hemicellulose, and starch.

(ii) Nutritive value of non-conventional fibre source used in livestock feed

Tesfaye *et al.* (2016) reported a crude protein content of 13.85 % and metabolizable energy of 10.22 MJ/kg DM when ensiling Napier grass provenance to Ethiopia, which had a better nutritive value compared to natural grass hay with crude protein content of 11.72 % and 7.98 MJ/kg DM of metabolizable energy. A study evaluating the nutritive value of four different cultivars of Napier grass (*Pennisetum purpureum* Schumach) provenance to Tanzania, showed that on average the grass had 9.90, 1.99, 8.83, 64.63, 37,18 and 7.94% of crude protein, ether extract, ash, neutral detergent fibre, acid detergent fibre and metabolization energy (Maleko *et al*., 2019). Napier grass from Kenya showed a crude protein, ash, neutral detergent fibre, acid detergent fibre and acid detergent lignin content of 9.01, 15.83, 60.34, 31.12 and 28.10 % (Kariuki *et al*., 2001). The nutritive value of Napier grass has shown to be influenced by numerous factors including edaphic environment, climate conditions, agronomic practices and genotypes (Negawo *et al*., 2017). Therefore, the knowledge on the suitability of Napier grass for a particular environment is worth generating towards fostering feasible production for mainly utilizing in developing the forage base for livestock production.

(iii) Effects of conventional fibre sources on livestock growth performance

In a study, evaluating the performance of Madura cattle fed diets containing coffee husk to replace Napier grass as a source of fibre, dry matter intake, average daily gain, feed efficiency and feed digestibility of cattle in each treatment was similar (Sudarman *et al*., 2019). These results point to show that Napier grass is comparable to coffee husk as source of fibre in fattening cattle. Although the crude fibre content in coffee husk is significantly higher than in Napier grass, when the grass was replaced with coffee husk in fattening diets, the growth performance of the cattle did not differ. SBM are the most widely used energy-rich feedstuffs in conventional agriculture. According to Alves *et al*. (2016), replacing SBM with castor bean cake as a protein source in lamb diets resulted in no variations in nutrient intake or average daily gain.

Table 2.1: Comparison of Napier grass with different fodders

Fodder	Nutritive value	Yield	Adaptability	Maintenance	References
Napier grass (*Pennisetum Purpureum*).	CP (6-12%), DE, 11-14 MJ/kg.	High yield 25-60t/ha/yr.	Very adaptable. Grows in wide range of climate.	Low Maintenance.	Ansah *et al*., 2010; Nyambati *et al*., 2010.
Kikuyu (*Pennisetum Clandestinum*).	CP 10-12%.	10-15 t/ha/yr.	Highly adaptable. Tolerate a variety of soil types.	Low Maintenance. Requires regular mowing to maintain it.	Marais, 2001.
Eragrostis Curvula.	High protein and low energy values.	Low yield 4-6 t/ha/yr.	Very adaptable. Grows well in marginal soils.	Low maintenance. Regular moving to control its growth. Regular irrigation during dry season.	Skerman & Riveros, 1990; Roberts *et al*., 2021.

2.2. Effects of Napier grass on growth performance

In beef cattle production system, the growth performance of cattle is of economic importance (Sturaro *et al*., 2005). Growth is the key characteristic of animals and is defined as any change in body size over time (Chilanga, 2020). Growth in terms of weight gain is one of the most important aspects in livestock production. Growth of animals manipulated through energy and protein content in the diet, brings changes in muscle composition (Webb, 2014). According to Christobal-Carballo (2009), the difference in muscle composition determines the nutrient requirement for animal growth, meaning animals with same birth weight but different mature size will have different nutrient requirements. The important criterion about this is discovered in research, whereby live weight gain (LWG), feed intake and feed conversion ratio of an individual animal live weight is dependent on the supply of protein delivered to the tissues (Asizua, 2010).

Primarily growth performance depends on the availability of feed resources in both quantity and quality and the feeding regime employed by the farmer. Growth rate of livestock feeding on tropical grasses and crop residues alone are usually low and their performance is about 10% of the animal genetic potential (Leng, 1990). One major cause that accounts for this challenge is the low nutrient composition derived from digestion in such feed resources. However, improving nutrition could enhance growth and productivity (Gemechu *et al*., 2021). This study discovered that the inclusion of Napier grass has shown to increase dry matter intake, which has been attributed to improve palatability and digestibility (Gemechu *et al*., 2021).

A study by Amata & Okorodudu (2016) observed that rabbits fed with Napier grass gained the highest weight compared to rabbits fed with either *Panicum maximum*, *Myrianthus arboreus* or *Gmelina arborea*. The results observed in the study by Rusdy (2018), the growth performance of animals fed sole Napier grass diet were generally low and the performance improved when *Leucaena* was supplemented. The difference between the inclusions in the diet could be attributed to the higher digestible protein content in *Leucaena*, which might have enhanced the efficiency of rumen microorganism compared to the Napier grass-based diets.

The study of Miegoue *et al*., (2018) obtained Guinea pig weights of 59.27g at birth and 145.10 g at weaning when feeding animals with *Napier grass* alone. The highest average daily gain with *P. maximum* was recorded in females (4.53g) while that of *Napier grass* was recorded in males (4.05g). Kariuki *et al*. (1999) reported the highest daily gain of 0.5 kg/head, and Kaitho & Kariuki (1998*)* reported the highest daily gain of 1.00 kg/head with sole elephant grass harvested at 7 weeks of regrowth in Kenya. The study of Antari *et al*. (2016), reported that Limousin-Ongole bulls performed better than Ongole or Brahman When fed Napier grass ad *libitum*, Limousin-Ongole bulls had the highest average daily live weight gain and the lowest feed conversion rate.

2.3. Milk production from cattle fed forages

The health and performance of dairy cows depend on the intake and metabolisable nutrients from the feeds. Fibre from roughages is also required for maximum intake and rumen health maintenance in dairy production (NRC, 2001). According to Van Soest (1994), roughages are essential for feeding lactating cows, as it is the main source of fibrous carbohydrates. However, the consumption of forages should be controlled in early lactation since cows are in a negative energy balance, this should be done to avoid cows consuming too much forage leaving no room for concentrate consumption, which are necessary to meet energy requirements for production. Feeding dairy cows with high concentrate diets with low quality forages puts cows in higher risks of metabolic disorders like ketosis and sub-acute ruminal acidosis (Zhang *et al*., 2013). Although, the concentrate to forage ratio varies from time to time depending on the stage of lactation as the nutritional needs of the cows' changes.

The study of Tesfaye *et al*. (2016), cows fed with sole Napier grass produced more milk than those fed 25% and 50% of Napier grass, the difference in milk yield among treatments was attributed to the difference in crude protein and energy contents in the diets. Cows fed sole Napier grass produced milk yield of 6.89kg/d, milk fat 4.51% andmilk protein of 3.62% while those fed 25 and 50 % produced 6.31-6.43kg/d, 4.50-4.97% and 3.63 respectively. The crude protein is lower than the minimum dietary requirement for milk production from dairy cows. However, appears to meet the energy requirement of dairy cattle reared by smallholder farmers (Muia *et al*., 2000).

2.4. Beef production under intensive system

Intensive livestock production is dependent on the availability of nutritionally adequate feed ingredients for the formulation of livestock feeds (Malebana *et al*., 2018). The intensive production system ensures that specialists undertake the finishing stages of beef cattle and this is usually done in feedlot, feeding is monitored to produce the required weight in animals in the shortest period. According to Frylinck, (2013), it is estimated that feedlot produce about 75 % of the total beef production in SA. The main factors affecting feedlot profit margins include the buying price of store beef, the price of meat produced, along with the dressing percentage of the carcass, the price of feed consumed by the animal, as well as efficiency of growth achieved (Lima *et al*., 2017). Due to differences in maturity for the different breeds, consideration is needed in determining the slaughter weight of a specific breeding order to prevent carcass classified as overfat and so reducing the value of the carcass.

2.5. Feedlot cattle breeds in South Africa

The South African industry is made up of a range of breeds that are suited for meat production. There are significant differences between different breeds in terms of feedlot performance, as well as their live weight and carcass weight. These differences are presented on Table 2.2.

Table 2.2. South African feedlot cattle classified into maturity type and expected carcass weight. Adapted from Bosman (2002)

Early maturing (Live weight<360kg; carcass weight 180-200kg)	**Intermediate maturing (Live weight 380 -420 kg Carcass weight 210 -230kg)**	**Late maturing (Live weight 420 -450 kg Carcass weight 235 -252 kg)**
Afrikaner	Beef master	South Devon
Sussex	Bonsmara	Simmentaler
Tuli	Brangus	Limousine
Nguni	Drakensbergers	Charolais
SA Angus		Brown swiss
Hereford		Pinzgauer
Brahman		

2.6. Effect of Napier grass on rumen fermentation

Rumen fermentation is a process whereby feed is digested with the aid of microorganisms in the first two stomachs of the ruminant stomach (Marais *et al*., 2007). During fermentation, physical and microbiological activities convert feedstuffs to useful products such as volatile fatty acids, microbial protein and B-vitamin (Saldias, 2014). According to Castillo-Gonzalez *et al*., (2014), ruminal fermentation processes may depend on the environmental conditions and the type of diet, so these factors may influence the osmotic pressure of the rumen (Castillo-Gonzalez *et al*., 2014). Ruminants obtain their nutrients mainly from the products of rumen fermentation (microbial protein, VFA) and in some situations, through dietary by-pass (Leng, 1990).

The study of Kariuki *et al*. (2001), reported pH values of all diets between pH 6.0 and pH 7.0, a range which is considered to be optimum for the activity of cellulolytic microbes and VFA absorption. While the study of Isah *et al*. (2013) reported similar results ranging from 5.77 to 7.14 in goats fed Napier grass supplemented with sweet potato vine or *desmodium*. Total VFA concentrations were generally higher in supplemented diets, indicating that *desmodium* and sweet potato vine had a positive effect on digestion. Ruminal proportions of VFA were not changed but the acetate to propionate ratio was within the expected range for forage diets and remained consistent (Isah *et al*., 2013).

Several studies (Weimer *et al*., 2010; McCann *et al*., 2014), have specified that diversity, density and functions of such volatile fatty acid end products are affected by certain factors (diets, feeding strategy, feed intake and physiological condition of the animal). The type and amount of diet consumed by the host affects the nutritional supply to ruminal microbes and the products synthesized, thereby influencing the nutrient absorbed by the host (Hernandez-Sonabria *et al*., 2012). Karuiki *et al*. (2001) and Ningal (2020) reported that the rumen pH and volatile fatty acids of animals fed *Pennisetum purpureum* grass improved with the changes in supplementation in the diets. In the study of Karuiki *et al*. (2001), the concentrations of total VFAs in supplemented diets were generally higher, indicating that *Desmodium* and sweet potato vine had a beneficial effect on digestion and the pH values of all diets were between pH 6.0 and pH 7.0.

2.7. Effect of Napier grass on carcass quality

The impact of plane of nutrition has been demonstrated to be mainly reflected in the carcass weight and dressing percentage (Nguyen, 2014). Dressing percentage is both a yield and value-determining factor and is therefore an important parameter in assessing performance of animal (Yesihak, 2015). Dressing percentage reflects proportion of a live animal`s weight, which will result in carcass weight. Nguyen (2014), defined carcass weight as the best index to measure meat production. The study of Okoruwa & Okunlowa (2017), showed the lowest sheep carcass weights in diets containing 70% of Napier grass and 30% of concentrated diets while compared to treatment containing different graded levels of cocoa pod husk. In contrast, the study of Purbowati *et al*., (2021) discovered that the carcass characteristics of lamb fed sole Napier grass were similar to lamb fed with different diet containing 50% of Napier grass with by-products while substituting the Napier grass.

2.8. Influence of dietary on meat quality

Meat quality is crucial since it affects consumer acceptance and sustained interest in the product. Depending on the application, meat quality is commonly defined in terms of eating quality or processing quality (Webb *et al*., 2005). In addition, it is a broad term that refers to the measurement of traits that determine whether meat can be consumed fresh or kept for a reasonable period without deterioration. According to Warner *et al*., (2010), for livestock industries to consistently produce high quality meat, there must be an understanding of the factors that cause quality variation, as well as the implementation of management systems to minimize quality variation.

The concept of meat quality is a complex of numerous factors that interact to affect the ultimate quality of meat from conception to consumption (Casey & Webb, 2010). The quality of meat can be explained by its ability to provide all sorts of nutrients to the consumer. Various factors, including the type of diet consumed by the animal, have been observed to affect meat quality parameters such as meat pH, water holding capacity, marbling, meat tenderness and meat colour (Mulaudzi, 2019). All these quality parameters are important in determining consumer preferences and meat purchasing decisions (Rimal, 2005).

Notwithstanding, the tenderness and colour are some factors that determine the quality of the meat, although colour is usually to attract buyers. Notably, meat has physical and chemical components such as meat color, sarcomere length, Warner Bratzler shear force, myofibrillar fragmentation length, water holding capacity, drip loss and meat chemical composition (moisture, protein content, and fat content) (Muchenje *et al*., 2009).

2.8.1. Physical components of meat

(i) Meat colour

Meat colour is the most important physical characteristic of meat that consumers use to assess the quality of meat at the retail store, determining the consumer's response, purchasing decision, and perception of meat quality (Muchenje *et al*., 2008; Muchenje *et al*.,2009). The characteristic colour of meat is a function of its pigment content and light scattering properties. The colour is determined by the amount of myoglobin, a protein pigment, present in the muscle, which contribute about 80-90% of pigment (Aberle *et al*., 2012). Myoglobin (Mb) is the haem-protein (contains iron), which is primarily responsible for the colour of meat (Faustman & Suman, 2017). The heme ring contains an iron (Fe) that can exist in a reduced (ferrous) or oxidized state (Ferric).

The colour of meat is defined by the concentration of the iron-based pigment myoglobin and the proportions that exists of its three forms: oxymyoglobin, deoxymyoglobin and metmyoglobin (Muchenje *et al*., 2009), each conferring a different colour to the meat (Castiliego *et al*., 2012). These three forms of myoglobin are formed by oxygenation, reduction and oxidation respectively.

Decreased myoglobin [deoxymyoglobin (Mb)] is characterized by the purple colour of profound muscle resulting from a combination of ferrous heme iron (Fe^{2+}) and vacuumed meat. Oxygenated myoglobin [oxymyoglobin (MbO_2)], which is bright cherry red, commonly referred as “bloom” is considered to imply new meat by the consumer. Oxidized myoglobin [metmyoglobin (MetMb)] is characterized by the grey-brown (Rosenvold & Anderson, 2003; Mancini, 2013). Meat discoloration results from oxidation of both ferrous myoglobin derivatives to ferric iron ($Fe^{2+} \rightarrow Fe^{3+}$).

According to Castiliego *et al*. (2012), the great differences in colour shades, intensity and stability of meat of different origin is the complex synergism of intrinsically factors (species, breed, age, sex, muscle type) and extrinsic conditions (such as diet, housing system, environmental condition, slaughter conditions). The diet that animals are fed can also influence the muscle concentration of myoglobin, and this is most readily observed in veal meat (Faustman & Suman, 2017). According to Mancini (2013), animal diet can affect metabolism, glycogen storage, pH chilling rate, antioxidant accumulation, all of which play a role in meat colour.

Meat colour is objectively defined often in terms of the Hunter colorimetric co-ordinates, L*, a* and b* (Warriss, 2000). L* is the lightness component, indicating the black-whiteness of the meat. Its values range from 0 (all light absorbed) to 100 (all light reflected); a* spans from -60 (green) to +60 (red) and b* spans from -60 (blue) to +60 (yellow) (Young *et al*., 1999). Other parameters may be calculated from these basic three, such as hue angle [tan -1 (b*/a*)], which describes the fundamental colour of a substance; and chroma [(a* 2 +b* 2)], which describes the vividness of the colour. Hunter a* and chroma have been observed to be strongly related to visual colour scores (Eargerman *et al*., 1978).

(ii) Water holding capacity

The moisture content of muscle and meat plays a vital role in muscle of food palatability, functionality, and shelf life as muscle is composed of approximately 75% of water (Braden, 2013). One of the primary quality properties of a new meat is its water holding capacity (WHC), since its impacts consumer acknowledgement and the final weight of the meat. The WHC is one of the important sensory qualities of meat; it determines the juiciness of meat (Muchenje *et al*., 2009). Additionally, and most of the physical properties of meat (such as colour, texture, tenderness) are partially dependent on WHC. It is defined as the ability of meat to hold its own or added water during the application of external forces such as cutting, heating, grinding and pressing, and is implicated in numerous technological processes in which water retention plays a major role (Bowker & Zhuang, 2015). Thereof, the quality of fresh meat depends largely on its WHC, which is very important attribute when making a purchase decision (Modika, 2019). Conversely, there are several ante- mortem and

post-mortem factors contributing ultimately to the WHC of meat. Braden (2013), mention factors such as feed withdrawal, stress and nutrition plane play a significant role in WHC of meat.

(iii) Sarcomere length

The sarcomere is the repeating structural unit of myofibril, and it is also the basic unit in which events of the muscle`s contraction-relaxation cycle occurs. The muscle is composed of a series of many sarcomeres connected to each other; it binds within the muscle fibers (Al-Waeli *et al*., 2022). Sarcomere length is not constant, and its dimensions are dependent upon the state of contraction at the time the muscle is examined and in livestock muscle at rest, a sarcomere length of 2.5 m is typical.

Sarcomere length has some important effect on meat quality. The shorter the sarcomere length, the tougher the meat with low WHC and the longer the sarcomere, more tender the meat (Devine *et al*., 1999). According to Kandeepan *et al*., 2013), stated that sarcomere length decreases with advancing age and increases the toughness of meat.

(iv) Warner-Bratzler shear force (WBSF)

The impact of tenderness on consumer's satisfaction, is one of the important component research has to focus on to measure meat quality. The WBSF is one method that is often used to measure the tenderness of meat. It is defined as the maximum force (expressed as Newtons, N) employed to cleave a cooked subsample of uniform dimensions using cutting plane across the myofibril and a specialized Bratzler Warner blade attached Lloyd analyzer (Modika, 2019). This technique has been used since in 1930, it was designed to assess meat tenderness in examination (Yancey *et al*., 2010; Novakovic & Tomasevic, 2017) being well correlated with consumer tenderness rating. The WBSF measurements give an objective measurement of tenderness (Kandeepan *et a*l., 2013), which is used in research laboratories to evaluate relative differences in tenderness or toughness of meat. According to Chriki *et al*., (2012), low WSBF value is associated with tender meat, whereas a high WBSF value is associated with less tender meat. A lower shear force value indicates that less force is required to shear through the sample and therefore the meat is tender. The threshold value (cut off point for toughness) in beef, for shear force

is considered around 4.5 kg. Values below 4.5 kg (threshold) indicate that consumers could rate it slightly tender or better for the overall tenderness (Duckett, 2001).

(v) Myofibril fragmentation length or index

The amount of myofibrils in the meat that are fragmented by application of mechanical forces determines the texture of the meat product (Kandeepan *et al*., 2013). Myofibrils make up nearly 80% of the volume of the muscle cell; their disruption greatly influences meat tenderness (Zhang *et al*., 2005). More of fragmentation myofibrils, tender will be the product texture. Myofibril fragment length (MFL) refers to the length of the myofibrillar fragments that remain after a defined homogenization procedure (Agbeniga and Webb, 2018). The MFL provides an alternative measure of the extent of the tenderization process, through changes in the fragility of the myofibrils being a good indicator of the extent of proteolysis and is significantly related to meat tenderness (Aroeira *et al*., 2016). Others changes that are correlated with increased tenderness includes breakages within myofibrils themselves, particularly within the I-band. These breakages lead to increased fragility and fragmentation of the myofibrils. The increase in myofibrillar fragmentation is indicative of the amount of tenderization that has taken place in meat (Muchenje *et al*., 2009).

2.8.2. Chemical components of meat

The quality of the meat is dependent upon changes in its chemical components: protein, moisture, fat, and ash (Muchenje *et al*., 2009). Meat is mostly the muscle tissue of an animal, which contains the important quality parameters in the nutrient value of meat. The often-quoted standard composition of normal adult mammalian muscle is 75% water, 19% protein, 2.5% lipids, 2.3% of miscellaneous soluble non-protein substances including inorganic compounds, 1.2% carbohydrates and minute quantities of vitamins (Mpala, 2020). These values may vary considerably with factors such as breed, age, sex, weight, and nutritional history.

2.9. Summary

Due to poor and insufficient nutrition during the dry season, and the growing human population, livestock yield in South Africa is low. The agricultural industry has the ability to improve livestock production by exploring alternative locally accessible feed resources that are rich in nutrient biomass. Napier grass through chemical composition has shown to be high in nutritional value. Although there is limited information of the grass as feed resource for livestock in South Africa.

CHAPTER 3: RESEARCH METHODOLOGY

3.1. Study site

This study was conducted at the Beef Cattle Feedlot of the Agricultural Research Council – Animal Production, Irene campus in Pretoria, Gauteng, South Africa (GPS coordinates: 25º 53' 59.6" S and 28º 12' 51.6" E). The mean rainfall is 700mm per annum, with most of the rain received in summer. The area is characterized by an ambient temperature range of 18 to 29º C during summer and between 5 to 20º C during winter.

3.2. Ethical consideration

Ethical clearance for this study was obtained from Agricultural Research Council Ethics Committee: Animal care and Use (Ref: APIEC 21/13).

3.3. Feed ingredients and diet formulation

The *Pennisetum purpureum* grass was sourced from a private farm in the Vaal area of the Gauteng Province. *Eragrostis curvula* hay was sourced from Agricultural Research Council - Roodeplaat, Gauteng Province. Other feed ingredients such as soyabean, salt, wheat bran, molasses, hominy chop, urea, feedlime and premix were sourced from Obaro at Tshwane in Gauteng Province. *Eragrostis curvula* hay, hominy chop and wheat bran were used during formulation as source of fibre. Molasses meal was used as source of energy, for pelleting and palatability of the diets. Soybean meal and urea were the source of protein. Salt was utilized as a source of mineral. Feed lime was used as the buffering ingredient and energy balance, respectively. The dietary treatments was formulated in a manner that they meet the National Research Council (NRC, 2001) requirements for fattening cattle. The dietary ingredients are presented in Table 3.1.

Table 3.1: Feed ingredients and chemical composition of the dietary treatments on DM basis

Ingredients (% DM)	Diet 1	Diet 2	Diet 3
Pennisetum purpureum grass	0	30.2	60
Maize meal/hominy chop	56.0	42.8	26
Wheaten bran	14.8	14.6	-
Molasses meal	9.9	9.6	11.5
Eragrostis curvula hay	9.9	-	-
Soybean oil cake meal (40%CP)	4.9	-	-
Feed lime	1.5	1.0	1.24
Salt (grade 1 cattle salt)	0.5	0.5	0.5
Feed grade Urea	1.5	0.2	0.2
Vit.Min premix	0.25 1.5	0.2	0.2

% DM = dry matter percentage. Vit.min premix: 6500 IU vitamin A, 1200 IU vitamin D_3, 40 IU vitamin E, 2 mg vitamin K_3 1-5 mg vitamin B_1, 4.5 mg vitamin B_2, 0.03 mg vitamin B_{12}, 2.5 mg vitamin B_6, 25 mg niacin, 12 mg calcium pantothenate, 190.5 mg choline, 0.6 mg folic acid, 0.05 mg biotin, 40 mg manganese, 100 mg zinc, 125 mg copper, 1 mg iodine, 100 mg ferrous and 0.3 mg selenium.
Diet 1, control (no Napier grass); diet 2, 30% Napier grass; diet 3, 60 % Napier grass.

3.4. Experimental animals and management

The Sussex red weaners were sourced from the Free State province. On arrival at the feedlot, animals were ear tagged and weighed individually and quarantined for 14 days. Animals were vaccinated against common feedlot diseases. They were then dewormed, injected with growth stimulants and dipped for external parasites. Twenty-four Sussex red male weaners, aged 8 months old with an average initial live body weight of 176.5±20kg (Mean ± SD) were used for the study. The weaners were allowed to acclimatize to the environment and experimental diets for 14 days before data collection. Following the 14-days acclimatization period, the weaners were grouped into 3 groups of 8 weaners in each group. The animals were housed in individual pens (2.2m^2 per animal), which were designed to meet the welfare standards of the National Society for the Prevention of Cruelty to Animals (ACT 169, 1993). The animals had access to a 60 m^2 gravel-floored rest area. Each pen had its own water and feeding trough to enable the animals to have ad *libitum* access to fresh clean water and feed. Water troughs and feeding pens were cleaned every morning and when necessary to avoid accumulation of waste materials. The steers were randomly allocated to three

dietary treatments. Data was collected for 120 days during which the steers were weighed at the beginning of the trial and continued weekly to determine weight gain. Feed intake was measured by calculating the difference between the quantities of feed offered to steers and feed leftovers. Feed conversion ratio was calculated by feed intake over weight gain.

3.5. Chemical analysis

3.5.1. Proximate analysis

The dry matter (DM) of the experimental diets was determined by drying the feed samples using an oven 60°C (AOAC, 2005) using method 935.29. After drying, the samples ware sieved through a 1-mm screen (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA) to analyse crude protein (CP), organic matter (OM) and ether extract (EE). The CP, OM and EE were determined according to AOAC (2005) methods, 990.03, 945.16 and 920.39, respectively. Gross energy (GE) was determined using an MC-1000 Modular Calorimeter (Energy Instrumentation, Centurion, South Africa) equipped with a PC and MC1000 software.

3.5.2. Fibre analysis

The neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) content of the feed samples were determined according to methods described by Van Soest *et al* (1991). Briefly, the Tecator Fiber System was used to determine the aNDF with a heat-stable alpha-amylase (Fibertec System, Tecator, Hoganas, Sweden). About 1g of each feed sample was refluxed in 100ml of neutral detergent solution (NDS) for 60 minutes at 100°C before being filtered and rinsed. The dry residues were then cooled using oven at 100°C overnight, cooled and weighed. The process for determining the ADF content was similar to that used to determine the aNDF content, with an exception that the acid detergent solution (ADS) was used for the extraction rather than NDS. The residue was rinsed, dried in an oven at 100°C overnight, cooled and then weighed. The ADL was determined using the same procedure used for the aNDF content determination, except that after rinsing each sample residue, 100ml of 72% of concentrated sulphuric acid was added to the

residue, which was then heated at 700˚C for three hours to determine the ADL content. The sulphuric acid was removed from the residue by boiling, followed by a hot water wash, a 12-hour drying period at 100°C in an oven, then cooling, and weighing.

3.2.4. Mineral assay

For calcium analysis, each feed sample was prepared using the method of AOAC 953.13 (2000). While sample preparation for phosphorus was carried out using the AOAC 968.08 (2000) method. Calcium concentration was determined using atomic absorption spectrophotometry according to the method described by Giron (1973). Method by AOAC 965.17 (2000) was used to determine the phosphorus concentration of each feed sample.

3.6. Data collection

3.6.1. Growth measurements

Each steer was weighed at the beginning of the trial and each week until the end of the trial to determine the initial body weight, calculation of average daily gain (ADG) and final body weight. The experimental diets allocated were equivalent to 3 % of the animals' live bodyweight (LBW) per day on a dry matter basis (Formula = LBW x 0.03). Feed were offered daily *ad libitum* and feed refusals from the previous day were recorded every morning to determine feed intake (FI). Feed conversion ratio (FCR) as calculated from body weight gain (BDG) and feed intake (FI) as described by Arthur & Herd (2008). At the end of the growth study, animals were transported to the abattoir for slaughtering.

3.6.2. Slaughter, carcass processing and meat sample measurements

After the completion of the growth study, the animals were transported from the feedlot to the abattoir within the ARC-AP, Irene campus, a distance of approximately 4 km. The animals were allowed to rest in lairage with free access to fresh clean water and fasted for 24 hours after which they were humanely slaughtered. The slaughter was conducted in a Grade D, low throughput experimental abattoir (ARC- AP, Irene

Campus, Gauteng Province) in accordance with standard South African protocols as outlined by Hoffman *et al*. (2003).

The steers were stunned on the occipital region of the head using electrical stunner (KZ-3 electric stunner, Kentmaster, Ohama, Nebraska, USA) set at 220V and 1.8A with a current flow of 6s. Following the stunning, each steer was exsanguinated by cutting the jugular vein, carotid arteries and esophagus using a sharp knife. The carcasses were hoisted by the hindleg and allowed to bleed for 8 min on the bleeding rail. Following the bleeding, each carcass was electrically stimulated using Tender pulse high voltage stimulation power supply (Tender-pulse, A.I.S Enterprise, Brisbane, Australia) 1 min for at 810volts. Immediately thereafter, each carcass was hanged on rolling hooks and then skinned. The kidney, liver, lung and heart were eviscerated while the head, skin, feet, and genital scrotum were removed from each carcass.

Following evisceration, the rumen was opened carefully using a knife and the pH of rumen digesta content was determined *in situ* using a 2-point calibrated portable Cyberscan pH 310 digital pH meter (Eutech Instruments, Thermo Fisher Scientific, Vernon Hills, USA). Following the pH measurement, rumen digesta was squeezed by hand to extract rumen fluid. The extracted rumen fluid from each steer was poured in a 100 ml bottle to which 4.4 ml of 25 % phosphoric acid was added to preserve the extract and stored at -20° C pending analysis for volatile fatty acid content of the fluid.

3.6.2.1. Determination of rumen digesta pH and rumen fluid collection

Figure 3.1: Measuring rumen digesta pH and collection of rumen fluid
(Picture **A** Measuring rumen digesta pH using portable Cyberscan pH, picture **B** collection of rumen fluid and picture **C** storing rumen fluid in bottles)

Each carcass was then weighed to determine warm carcass weight (WCW) using an overhead scale (DIGI DS160 scale, Toronto, Canada). Warm carcass temperature and initial pH (pH_i) were measured with a portable pH meter (EUTECH Instruments, Thermo Fisher Scientific Inc. Singapore) on each of the carcass's *longissimus thoracic* muscle (eye muscle) between the third and the fourth rib, 60 mm from the midline. Dressing percentage (DP) of each carcass was calculated as described by Bonvillani *et al*. (2010) as follows:

Dressing percentage = $\frac{W1}{W2} X\ 100$

Where W_1 = weight of each warm carcass and W_2 =slaughter weight

Following measuring the temperature and pH, each carcass was stored in a cold room at 4°C for 24 h, after which cold carcass weight (CCW) of each carcass was measured using overhead scale (DIGI DS160 industrial scale, Toronto, Canada). After determination of cold carcass weight, cold carcass temperature and ultimate pH (pH_u) were measured on the *longissimus thoracis* muscle between the 4^{th} and 5^{th} lumbar vertebra of each carcass. The readings were taken by inserting a portable pH meter Cyberscan pH 300 digital pH meter (Eutech Instruments, Thermo Fisher Scientific, Vernon Hills, USA) fitted with a specially designed meat electrode into the sample to a depth of 1.5cm. The readings were taken at 15 min, and 1, 3, 6 and 24 hrs post mortem. Meat sample (100 g) for other analysis were taken from the *m. longissimus thoracis* at 24 hours post mortem. Each carcass was then chilled at 4° C for 12 hrs prior to determination of subcutaneous fat thickness.

3.6.2.2. Determination of volatile fatty acid content in rumen fluid

Using a gas chromatograph, the volatile fatty acid (VFA) concentration of the rumen fluid was measured in accordance with Firkins *et al*., 1990 methodology. In summary, each sample was allowed to defrost to room temperature. Then, 2.5 ml of distilled water was added to 2 ml of each rumen liquor sample. The mixture was then

centrifuged at 10,000 × g for 15 minutes at 10°C using a Beckman centrifuge (Avanti JE, Beckman Coulte, Inc, CA, Fullerton, USA). To remove the precipitation, 1 ml of the supernatant was combined with 3 ml of distilled water and 1 ml of the internal standard (0.5 g of 3-methyl-n-valeric acid in 1 L of 0.15 mol/L oxalic acid), and then centrifuged at 1400 × g for four minutes. The mixture was then filtered into a chromatographic vial using a Whatman 0.45 µm poly-ethersulphone membrane filter (Sigma-Aldrich, Dorset, UK). One microliter of the filtrate was injected into the apparatus. A Varian gas chromatograph (Varian CP 3800, Massachusetts, USA) fitted with a 25 m × 0.53 mm i.d. megabore column (coating CP-Wax 58 (FFAP) – CB (no. CP7614) (Varian, Middelburg, Netherlands) was used to measure the VFA concentration of each sample.

3.6.3. Subcutaneous fat thickness determination

Thirty-six hours post slaughter, the thickness of the subcutaneous fat of each carcass was determined using a Vernier caliper (150 mm Electronic Digital caliper, Manufacturer, Region). The thickness was measured by placing the jaws of the caliper on the dorsal fat between the 11th and 12th ribs under the skin (without hide), which was separated from the skin by a small knife. The jaws of the caliper were inserted to measure the fat thickness according to Swatland (1984).

3.6.4. Rib eye area determination

The rib eye area was measured after transversal cut of the carcass at the 12-13th rib to expose the *longissimus dorsi* muscle. Transparent paper was used to trace the surrounding of the muscle, then taken to an electronic table to have enclosed area measured by means of video image analysis using a CC12 video camera (Olympus, Tokyo, Japan) as well as image processing and calculations using Analysis Life Science software package (Soft Imaging Systems GmBHu, Münster, Germany).

3.6.5. Meat physical attributes

3.6.5.1. Water holding capacity

Water holding capacity (WHC) was determined by calculating the ratio of meat and

liquid areas after pressing a 400 to 600 mg meat sample from *Longissimus thoracis* on a filter paper (Whatman 4) sandwiched between two Perspex plates, and pressed at a constant pressure of 300 psi for 60 seconds. The meat borders and fluid expressed during pressing were marked out and their areas were measured using a video image analyzer (Soft Imaging System, Olympus, Japan) with a CC12 video camera (Olympus, Tokyo, Japan) and image processor according to the method described by Irie *et al.* (1996). The WHC was then calculated using Analysis Life Science software package (Soft Imaging Systems GmBHu, Münster, Germany).

3.6.5.2. Meat colour determination

Figure 3.2: Meat colour measurement (Schilling *et al*., 2005)

Meat colour was measured with a Chroma Meter CR-200 colorimeter (Minolta Co., Ltd., Osaka Japan) according to Schilling *et al*. (2005). White point calibration was performed 20 min before the colour reading. Reflectance was measured from 400 to 740 nm in increments of 10 nm. The meat samples (40 g) in a tray from the *longissimus dorsi* muscle was used to measure meat colour. Vacuum bags were removed from the samples before being placed on a polystyrene tray and kept in a dark cold room at 3°C to bloom for 60 min. After 60 min of blooming, the samples were blotted with a paper towel before readings were taken at different locations on the surface of each sample. The CIE colour convention was followed where the three fundamental outputs are L*, a*, and b*. Lightness (L*) on a scale of 0 (all light absorbed) to 100 (all light reflected);

redness (a*) spans from + 60 (red) to – 60 (green) and yellowness (b*) values spans from + 60 (yellow) to – 60 (blue) (AMSA, 1991). Chroma (C*) will be calculated as $C* = \sqrt{a*} + b*$ and hue angle (h*), defined as tan-1 (b*/a*) (Young *et al*. (1999) using values obtained from the colour measurements. Myoglobin fractions of the meat samples were measured on meat samples that were used for colour determination, using a portable spectrophotometer (CM-700/CM-600d, Model, Konica Minolta Sensing, Europe B.V) with a large-size colour LCD screen for settings, items and data display. The proportions of different myoglobin redox forms [metmyoglobin (MetMb), deoxymyoglobin (DeoxyMb) and oxymyoglobin (OxyMb)] were calculated. Each sample results was averaged and converted into reflectance attenuance (A= Log10 1/R), at isobestic point 572, 525, and 473 nm [(analogous to calculation of absorbance from transmittance), as described by Mancini *et al*. (2003). Each sample's reflectance percentage was translated into K/S values using the following formula: K/S = (1 − R)2/ (2R), where R is the decimal representation of the reflectance percentage (%), K is the absorption coefficient, and S is the scattering coefficient. Following Krzywicki's (1979) instructions, the K/S values were used to calculate the percentages of metmyoglobin (MMb) and deoxymyoglobin (DMb), while Mancini *et al*. (2003)'s method of obtaining the oxymyoglobin (OMb) percentage was achieved by using the equation %OMb = 100 − (%MMb + %DMb).

3.6.5.3. Drip loss determination

Drip loss was determined by weighing the packaged meat samples with their labels using Radwag Wagi Electroniczne (Model, PS 750/C/2 Lasec, South Africa). The drip loss of approximately 27.5 g cube of meat was hanged using an opened paperclip from a small screw hook screwed into the lid of the screw cap of a 200 ml honey bottle. The honey bottles with the sample hanging from the lid (not touching the sides of the bottles) were kept in a chiller for 48 hours at 4°C degrees. After chilling, each cube was blotted dry with a paper towel and the weight determined using the Radwag Wagi Electroniczne. Drip loss was calculated as the difference between the initial and final weight of the strip and expressed as a percentage of initial weight using the following equation:

Drip loss percentage (%) = $W_1 – W_2 / W_1 \times 100$;

Whereby W_1 = initial weight of the sample and W_2 = final weight of sample.

3.6.6. Moisture characteristics

3.6.6.1. Thaw loss determination

For thaw loss, meat samples were weighed and stored in a freezer at -20°C for 7 days. Following the freezing of the two 60-mm meat samples from *m. longissimus thoracis*, the initial weights were measured using a digital balance scale (Model GM- 501, Lutron Electronic, Coopersburg, USA). The meat samples were thawed at 4 °C for 24 hours before the final weights were measured on the digital balance scale. Thaw loss was calculated using the equation of (Jama *et al*., 2008); thaw loss percentage (%) = $W_1 – W_2 / W_1 \times 100$.

Where W_1 is the weight of frozen the sample and W_2 is the weight of thawed the sample.

3.6.6.2. Cooking loss determination

Immediately after evaluation of thaw loss, each thawed meat sample was broiled in an oven (Model H217, Miele & Cie, Gutersloh, Germany) using direct radiant heat according to AMSA (2016). The electric oven was set on “fan heat” at 160°C for 10 min prior to broiling of the meat samples. The meat samples were put on top of a rack that was on a pan and placed inside the preheated oven. The steak was broiled until an internal temperature of 50°C was reached. After broiling, the final weight of each sample was determined. Cooking loss was calculated according to Hwang & Thompson. (2001) using the equation:

Cooking loss percentage (%) = $W_1 – W_2 / W_1 \times 100$;

where W_1 is weight before cooking* and W_2 is the weight after cooking.

3.6.7. Meat tenderness

3.6.7.1. Warner-Bratzler shear force (WBSF) determination

Broiled meat samples that were used to determine the cooking loss were used to measure WBSF. Each meat sample was cooled down in an air- conditioned room for 2-3 hours to reach an internal temperature of 16°C. Six round cylindrical cores (12.5-mm diameter) were cored on each meat sample, parallel to the orientation of the muscular fibers using coring device. Each core of 12.7 mm diameter was sheared once across its length using Warner-Bratzler shear force device, mounted on a Universal Instron apparatus (Model 4301, Instron Ltd, Buckinghamshire, England). Each core was sheared at a crosshead speed of 200 mm/min. A mean value of the maximum force required to shear each set of cores in kg was used as shear force value (Humling *et al*. 2008).

3.6.7.2. Sarcomere length (SL) determination

Meat sample was prepared according to Hegarty & Naude (1970). Briefly, meat samples excised from the *longissimus thoracis* (LT) muscle (24-hour post-mortem) weighing approximately 5 g were homogenized in approximately 15 ml distilled water (Dreyer *et al*., 1979) using an Ultra-Turrax blender (Description) at low speed until all individual fibers were separated. A few drops of the homogenate were mounted on a slide and covered with a cover slip. The slides were viewed under an Olympus B340 microscope system attached to CC12 video camera (Olympus, Tokyo, Japan) at 31 000 × magnification. Twenty-five sarcomeres were measured per sample and their mean was used for statistical analysis. Analysis Life Science software package (Soft Imaging systems Gmbh, Munster, Germany) was used to process the sarcomere length data.

3.6.7.3. Myofibril fragmentation length (MFL) determination

Myofibrils were extracted according to Culler *et al*. (1978) method. In summary, connective tissue and fat were removed from each 50g frozen (20°C) sample after it had been thawed at room temperature. Each trimmed sample was divided into three-gram pieces. Using a Bühler HO4 homogeniser (Type H0-4 NR 3012, Labortechnik Admund Bühler, Wehingen, Germany), each of the 3g sample pieces was placed into

a 50ml Bühler round-bottom glass that contained 30ml of 0.02M potassium phosphate extraction buffer (made up of 100mM potassium chloride, 1mM magnesium chloride, 1mM ethylenediaminetetraacetic acid, and 1mM sodium azide); and was homogenized for 30 seconds. Following homogenization, a Hermle centrifuge (Hermle Labortechnik GmbH Z 36 HK, Wehingen, Germany) was used to centrifuge each mixture for 10 minutes at 2987 × g at 4°C. After discarding the supernatant, the pellet was resuspended in 30 ml of extraction buffer and centrifuged once more for 10 min at 4°C and 2987 × g. After discarding the supernatant, the pellet was suspended in 10 ml of extraction buffer and centrifuged for 10 min at 4°C at 2987 × g for the third time. The suspension was immediately centrifuged and then vacuum-filtered through a 1000µm polyethylene strainer (Krackeler Scientific, Alany, New York). After that, a 250µm polyethylene strainer (Krackeler Scientific, Alany, New York) was used to filter the filtrate under vacuum. A 50µL drop of the filtrate was placed on a microscopic slide, covered with a coverslip, and examined under an Olympus BX40 system microscope (Olympus, Tokyo, Japan) at a 400 x magnification in order to determine the meat's MFL. The average length of 100 single myofibril fragments per sub-sample was used to calculate the MFL.

3.6.8. Meat chemical composition

The 50 g meat sample from *Longissimus thoracis* of each carcass was dried and analysed for dry matter, organic matter, protein and fat according to AOAC (2005) methods, 935.29, 990.03, 945.16 and 920.39, respectively.

3.7. Statistical analysis

Data was analysed in a completely randomized design using Genstat statistical software (Genstat, 2000) and subjected to a one-way analysis of variance (ANOVA). Differences among means were separated using Fisher's Least Significant Difference (LSD) test. The level of significance was declared at 5% using Fisher`s (least significant difference) LSD test. The data were fitted in the model:

$$Y_{ij}=\mu+T_i+\varepsilon_{ij}$$

Where Y_{ij} is the individual observation of the i^{th} dietary treatment and the j^{th} replicate, μ is the overall mean, T_i is the fixed effect of the i^{th} dietary treatment (i=1,2,3), ε_{ij} is the random residual error.

CHAPTER 4: RESULTS

Data on the nutritive value of the Napier grass and *E. curvula* hay used is shown in Table 4.1. The proximate analysis of the treatment diets is shown in Table 4.2. Increasing Napier grass inclusion reduced (P<0.05) the daily feed intake, which subsequently reduced (P<0.05) daily gains and final body weight of the steers (Table 4.3). In addition, steers fed the diet that contained 60 % Napier grass had the highest feed conversion ratio compared to the steers in other diets.

Table 4.1: Chemical composition of Napier grass and *Eragrostis curvula* hay (n=3)

Parameter	**Napier grass**	***Eragrostis curvula* grass**
Proximate (%DM)		
DM	93.18	93.7
OM	93.30	93.01
CP	12.00	4.5
Ether Extract	2.22	-
Fibre fraction (%DM)		
NDF	62.65	78.9
ADF	36.38	43.2
ADL	4.37	7.0

DM= dry matter, OM = organic matter, CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin.

Table 4.2. Chemical composition (%) of experimental diets Napier grass (*Pennisetum purpureum*) (n=3)

	Diet					
Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**LSD**	**SE**	**P**
Proximate (%DM)						
Dry matter	88.61[a]	82.29[c]	86.85[b]	0.296	0.14	<.001
Crude protein	7.51[c]	11.75[a]	10.93[b]	0.54	0.28	<.001
Ash	7.49[b]	4.15[c]	13.74[a]	0.673	0.337	<.001
Fat	2.19[b]	2.52[a]	1.31[c]	0.11	0.06	<.001
Gross energy (MJ g/kg)	14.99[a]	15.26[a]	13.82[b]	0.622	0.203	0.010
Fibre fraction (%DM)						
Neutral detergent fibre	46.44[a]	30.30[b]	46.37[a]	4.654	2.330	<.001
Acid detergent fibre	26.82[a]	15.28[c]	23.19[b]	0.810	0.406	<.001
Acid detergent lignin	15.67[a]	8.05[c]	11.15[b]	1.040	0.521	<.001
Macro-minerals						
Calcium	11.37[c]	17.78[a]	13.82[b]	0.55	0.17	<.001
Phosphorus	7.59[b]	11.64[a]	10.89[a]	1.056	0.332	0.002

[a,b,c] Means with different superscripts within a row differ significantly at P<0.05. Diet 1, control (no Napier grass); diet 2, 30% Napier grass; diet 3, 60 % Napier grass LSD, least significant difference, SE, standard error, P, probability value.

Table 4.3: Effect of dietary inclusion level of Napier grass (*P. clandestinum purpureum)* on growth performance of Sussex red steers (n=8)

	Diet					
Parameters	**Diet 1**	**Diet 2**	**Diet 3**	**LSD**	**SE**	**P**
Initial body weight (kg)	182.4	184.2	188.3	7.73	6.89	0.290
Final body weight (kg)	399.6[a]	379.6[a]	294.16[b]	22.47	20.01	<.001
ADG (g/day)	1.80[a]	1.72[b]	0.53[c]	1.18	0.16	<.001
DFI (g/day)	6.78[a]	6.33[a]	4.47[b]	0.670	0.596	<0.001
Feed conversion ratio (g/g)	3.78[c]	5.21[b]	8.69[a]	0.996	0.887	<.001

[a,b,c] Means with different superscripts within a row differ significantly at P<0.05.. Diet 1, control (no Napier grass); diet 2, 30% Napier grass; diet 3, 60 % Napier grass.

ADG, average daily gain, DFI, daily feed intake, LSD, least significant difference, SE, standard error, P, probability value.

The results for rumen fermentation of steers fed different inclusion levels of Napier grass are shown on Table 4.4. The rumen digesta pH of the steers were similar (P>0.05) across dietary treatments. There was no significant difference (P>0.05) in the rumen fluid volatile fatty acid content of the steers among dietary treatments.

Increasing dietary inclusion levels of Napier grass reduced (P<0.05) the warm and cold carcass weight, and warm and cold muscle temperature of the steers (Table 4.5). However, the dressing percentages of the carcasses as well as the warm and cold muscle pH were not affected by the dietary treatments. Dietary inclusion of Napier grass in fattening diets did not affect (P>0.05) the drip loss, colour and myoglobin redox reaction of the meat from steers (Table 4.6). Thaw losses for meat aged for days 1 and 7 increased with increasing dietary inclusion of Napier grass in steers fattening diets, while cooking loss, water holding capacity and warner Bratzler shear force of meat from steers were similar (P>0.05) across dietary treatments (Table 4.7). The results for the sarcomere length and myofibrillar fragmentation length are shown on Table 4.8. Sarcomere and myofibrillar fragmentation lengths were not (P>0.05) affected by the dietary treatments. Increasing the dietary inclusion level of Napier grass in the steers fattening diet to 60 % reduced (P<0.05) the DM and OM of the meat. The CP and fat content of the meat from steers fed diet with 60 % of Napier grass was lowest (P>0.05) compared to their counterparts (Table 4.9).

Table 4.4: Effect of graded dietary inclusion level of Napier grass (*Pennisetum purpureum*) on rumen fermentation dynamics of Sussex red steers (n=8)

	Diet					
Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**LSD**	**SE**	**P**
Rumen digesta pH	6.7	6.6	6.6	0.31	0.28	0.980
Volatile fatty acid (g/L)						
Acetic acid	42.4	48.0	40.1	17.32	15.36	0.616
Butyric acid	2.98	4.06	3.50	1.288	1.142	0.237
Propionic acid	8.49	13.20	8.41	7.71	6.83	0.347
Valeric acid	0.50	0.79	0.48	0.44	0.39	0.283
Isobutyric acid	1.30^{b}	1.73^{a}	1.51^{ab}	0.38	0.34	0.088
Isovaleric acid	2.05	2.75	2.38	0.830	0.736	0.239

^{a,b} Means with different superscript within a row differ significantly (P<0.05). LSD, least significant difference, SE, standard error, P, probability value.

Diet 1, control (no Napier grass); diet 2, 30% Napier grass; diet 3, 60 % Napier grass.

Table 4.5: Effect of graded inclusion level of Napier grass (*Pennisetum purpureum*) on carcass characteristics, pH and temperature of muscle from Sussex red steers (n=8)

	Diet					
Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**LSD**	**SE**	**P**
Warm carcass weight (kg)	218.0[a]	204.4[a]	160.7[b]	15.02	13.37	<.001
Cold carcass weight (kg)	213.4[a]	199.7a[a]	158.2[b]	14.46	12.88	<.001
Dressing percentage (%)	54.70	53.81	54.55	2.260	2.013	0.682
Warm muscle pHi	6.00[ab]	6.04[a]	5.95[b]	0.08	0.07	0.077
Cold muscle pH_u	5.58[a]	5.60[ab]	5.51[b]	0.08	0.07	0.081
Warm muscle temperature (°C)	39.46[a]	38.19[b]	37.69[b]	0.974	0.868	0.004
Cold muscle temperature (°C)	11.51[a]	12.60[a]	5.34[b]	3.653	3.253	<.001

[a,b,] Means with different superscripts within a row are significantly different (P <0.05). pHi – initial pH, pHu – ultimate pH. LSD, least significant difference, SE, standard error, P, Probability value. Diet 1, control (no Napier grass); diet 2, 30% Napier grass; diet 3, 60 % Napier grass.

Table 4.6: Effect of graded inclusion level of Napier grass (*P. clandestinum purpureum*) on drip loss, colour and myoglobin redox reaction from Sussex red steers (n=8)

	Diet					
Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**LSD**	**SE**	**P**
Drip loss (%)	1.84	2.04	1.83	1.064	0.948	0.899
L*(D_{65})	32.24	30.49	34.74	5.018	4.468	0.230
a* (D_{65})	9.75	8.11	8.35	2.066	1.839	0.224
b* (D_{65})	11.44	9.57	10.39	2.590	2.306	0.334
C* (D_{65})	15.05	12.57	13.38	3.259	2.902	0.289
H* (D_{65})	49.16	49.50	51.08	2.901	2.583	0.361
Myoglobin redox reaction (%)						
Metmyoglobin	2.71	2.71	2.57	2.316	2.063	0.989
Deoxymyoglobin	60.3	71.3	70.6	13.66	12.16	0.195
Oxymyoglobin	37.1	25.4	27.0	13.90	12.38	0.186

LSD, least significant difference, SE, standard error, P, probability value. Diet 1, control (no Napier grass); diet 2, 30% Napier grass; diet 3, 60 % Napier grass.

Table 4.7: Water-holding capacity, WBSF, Cooking and thaw losses of meat from Sussex red steers fed dietary inclusion levels of Napier grass *(Pennisetum purpureum)* (n=8)

	Diet					
Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**LSD**	**SE**	**P**
Water-holding capacity	0.31	0.37	0.32	0.07	0.06	0.208
1-day aged meat						
Thaw loss (%)	3.41^{ab}	1.83^{b}	4.91^{a}	2.755	2.453	0.090
Cooking loss (%)	19.58	19.74	22.31	5.075	4.519	0.464
WBSF (kg)	3.94	3.61	4.19	0.989	0.881	0.477
7 day aged meat						
Thaw loss (%)	2.61^{b}	1.31^{b}	5.76^{a}	1.930	1.719	<.001
Cooking loss (%)	21.65	18.45	20.98	4.095	3.646	0.249
WBSF (kg)	3.28	3.02	3.32	0.811	0.722	0.697

^{a,b} Means with different superscripts within a row differ significantly (P<0.05). LSD, least significant difference, SE, standard error, P, probability value.

Diet 1, control (no Napier grass); diet 2, 30% Napier grass; diet 3, 60 % Napier grass.

Table 4.8: Effect of graded inclusion level of Napier grass (*Pennisetum purpureum)* on sarcomere and myofibrillar fragmentation lengths from carcasses of Sussex red steers (n=8)

	Diet					
Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**LSD**	**SE**	**P**
Sarcomere length (µm)	1.93	2.02	2.00	0.09	0.08	0.135
Myofibrillar length fragmentation						
MFL 1 dpm (µm)	33.7	31.6	39.6	8.67	7.72	0.162
MFL 7 dpm (µm)	25.83	23.86	27.34	4.616	4.110	0.307

MFL, myofibrillar fragmentation length, dpm, days post-mortem, LSD, least significant difference, SE, standard error, P, probability value. Diet 1, control (no Napier grass), diet 2, 30% Napier grass; diet 3, 60 % Napier grass.

Table 4.9: Effect of graded dietary inclusion level of Napier grass (*Pennisetum purpureum*) on the proximate composition of meat from carcass of Sussex red steers (n=8)

	Diet					
Parameters (%DM)	**Diet 1**	**Diet 2**	**Diet 3**	**LSD**	**SE**	**P**
Dry matter	32.86[a]	31.05[b]	27.78[c]	1.505	2.437	<.001
Organic Matter	31.84[a]	30.05[b]	26.81[c]	1.549	2.509	<.001
Protein	22.92[a]	22.18[a]	20.47[b]	1.383	2.241	0.003
Fat	8.97[a]	7.46[a]	5.51[b]	1.701	2.756	<.001

[a, b, c] Means with different superscripts within a row differ significantly at (P<0.05). LSD, least significant difference, SE, standard error, P, probability value.
Diet 1, control (no Napier grass); diet 2, 30% Napier grass; diet 3, 60 % Napier grass.

CHAPTER 5: DISCUSSION

5.1. Chemical composition of experimental diets

The nutrient content in feed is a major factor that determines feed quality and animal productivity (McDonald *et al*., 2010). The DM content of the diets in this study ranged from 82.29 to 88.61%, which is considered the minimum requirement for rumen microbial activity (Minson & Milford, 1967). The crude protein content in a forage or feed is one of the most important nutrient components that promote animal performance, and low levels might negatively affect animal performance. According to Makesha *et al*. (2002), feed resources that contain less than 7 % CP do not support optimum rumen fermentation and may result in depressed feed intake. The Napier grass of the present study contains CP content of 12 % (Table 4.1.), which is adequate for supporting ruminal functioning. In general, Napier grass has a mean CP of 7.6 %, which is lower than the recommended CP levels of 10 to 12 % for growing steers (ARC, 1980). The CP content of Napier grass used in the current study was higher than that reported by Kariuki *et al*., (2001) and Rahman *et al*., (2015), and lower than that reported by Reddy *et al*., (2014) and Mutimura *et al*., (2018).

Dietary fibre is the non-digestible factor in the diet. Dietary fibre is defined as a non-starch polysaccharide and a sum of lignin that is not digested by endogenous secretion of the gastrointestinal tract (Lindberg, 2014). Dietary fibre is one of the most important nutrients in the diet of ruminant due to its role in maintaining rumen function and ruminant health (Van Soest *et al*., 1991). According to Van Soest (1994), fibre fractions such as NDF and ADF are the major determinants of overall forage quality. The *Eragrostis curvula* grass in the present study had higher fibre fractions than Napier grass (Table 4.1), which resulted in higher fibre content in the control treatment.

Dietary energy is often the first item to consider when formulating animal feed, as it is required for metabolism, physiological functions, maintenance, growth, tissue turnover, and production of heat in the animal body (Wu *et al*., 2020). It is vital for fattening animals in order to facilitate the deposition of intramuscular fat that would

enhance product quality and taste (Nguyen *et al*., 2021). Treatment diet with an inclusion of 30 % Napier grass had the highest gross energy than other diets. This might be related to the high fat content of the diet since dietary fat is related to the dietary energy. The fat content of commercial beef cattle feed is typically in the range of 2 to 5 % and fat content more than 6 % in the diet may cause digestive disturbance, diarrhea, and reduce feed intake (Gautama *et al*., 2016). All experimental diets in the current study had fat content of less than 5 %, which is considered good for ruminant animals.

5.2. Animal Growth performance

5.2.1 Feed intake

Level of feed intake can have a great effect on livestock production. Steers fed diets with 60 % inclusion of Napier grass had a lower daily feed intake (DFI) than steers fed other diets. Similarly, Rusdy *et al*., (2019), Mastika, (2003) and Nurhayu *et al*., (2021) reported a reduced feed intake in cattle fed 70% and 60% of Napier grass in the diet. The reduced feed intake from steers that were fed diet containing 60 % Napier grass could be due to the lower energy content in the diet compared to the other diets as explained by Wu et al. (2020).

5.2.2 Growth performance

Feed conversion ratio (FCR) is the amount of feed consumed divided by body weight gain per unit of time (Beauchemin *et al*., 1997). The FCR or feed efficiency is more efficient if the amount of feed consumed is less but results in a high or the same body weight gain (Nurhayu *et al*., 2021). A decrease in the quantity of feed needed by an animal to reach a target weight may increase farm`s profitability (Schilling, 2005), and good quality feed will result in high body weight gain and low feed efficiency values (Nurhayu *et al*., 2021). Results in this study show that steers fed diets with 60 % Napier grass had higher FCR and lower average daily gain than those that were fed diets with 0 or 30 % Napier grass. The average daily gain of steers in this study followed an opposite trend to the FCR. The results are confirmation that less amount of feed was

required for steers fed diets with 0 and 30 % of Napier to gain more weight due to the diets' high nutrient biomass. Hence, as the efficiency at which the nutrients are being utilized by the steers lowers, body weight gain thus growth is increases. Moreover, the FCR (5.21 kg/kg) of steers that were fed 30 % of Napier grass was comparable to that of steers reported by Mastika (2003), who obtained 5.08 kg/kg when feeding diet that contained 40 % Napier grass. The daily feed intake and final body weight of steers fed diet with 30 % of Napier grass were higher than that of steers fed the 60 % Napier grass diet, but comparable to that of those that were fed the control diet. Although the ADG of steers fed 60 % of Napier grass was low, it was rather high (0.17 kg/day), compared to the ADG of steers fed 60 % Napier grass recorded by Marsetyo *et al*. (2012). The ADG of the steers however, reduced to 0.31 kg/day when the 60 % Napier grass was supplemented with *Gliiricidia* (Marsetyo *et al.,* 2012). Antari *et al.* (2016) also reported low ADG of 0.18 kg, 0.26 kg and 0.11 kg/day on Ongole, Limousin-Ongole and Brahman, respectively, when fed sole Napier grass. Results in this study indicate that inclusion of Napier grass in fattening diets at 30 % did not perturb growth performance traits of the steers.

5.3. Rumen fermentation characteristics

Ruminal pH is influenced by the amount of fibre in the diet as well as the balance between the production of fermentation acids and the secretion of buffers (Krause *et al.,* 2002). The high pH in rumen digesta encourages the growth of cellulolytic microorganisms and the synthesis of acetate as a major product of fermentation with low proportions of propionate and butyrate. Rumen pH below 5.6 is regarded as a threshold for rumen acidosis (Nagaraja & Titgemeyer, 2007). The pH of the rumen digesta of steers in this study was similar across the treatment diets. The pH was between 6.6 and 6.7, which is considered optimum for the quality of cellulolytic microbes and VFA absorption (Karuiki *et al*., 2001). Fibre digesting bacteria, better known as the cellulolytic bacteria, functions best at a pH of 6.2 – 6.8. Results in the current study show that inclusion of Napier grass in steers fattening diets did not affect the rumen pH negatively but enhanced the fiber-digesting bacteria. Our results are in concurrent with those of Kariuki *et al*. (2001) who reported rumen digesta pH that ranged from 6.6 to 6.8 in steers fed Napier grass with different inclusion levels (0%,10%, 20% and 30%) of *Desmodium*.

Volatile fatty acids (VFA) are an innate energy source for ruminants that contribute approximately 70-80 % to energy requirements (Beckett *et al*., 2020). The type of forage and species, and quantity of rumen bacteria affect the concentration of acetic, propionic and butyric acids that are generated in the rumen. They account for 95 % of the total VFA produced in the rumen and provide up to 75 % of metabolic energy (ME) for ruminants (France & Dijkstra, 2005). In the current study, the volatile fatty acids (VFA's) concentration in rumen fluid from steers were similar across the treatment diets. These results indicate that inclusion of Napier grass in fattening diet up to 60 % did not alter the fermentation of the diets' ingredients.

5.4. Carcass quality

Feed is among critical factors that affect the quality of carcass (Drake 2004). Results in this study indicate that dietary inclusion of Napier grass in steers fattening diets up to 60 % resulted in reduced warm and cold carcass weight. The dressing percentage of the steers in this study was similar across the treatment's diets. The dressing percentage of the carcasses was not affected by the treatment diets. This was not expected due to the low final body weight of steers fed fattening diets with 60 % of Napier grass. The dressing percentage of carcass reflects the proportions of a live animal's weight, expressed as a percentage (Eikelenboom *et al*., 2004). Coyne *et al*. (2019) stated that a heavy live animal with a heavy carcass could have the same DP as a light animal with light carcass, which can be attributed to factors such as live weight, time-off feed and water, and pre-slaughter fasting and stress. These results are comparable to the DP of 55 % recorded in Boran cattle by Mummed & Webb (2019). Hattakum *et al.* (2019) obtained similar results when feeding steers on Napier grass and pineapple silage.

Carcasses of steers fed fattening diets containing 0 and 30 % of Napier grass had higher warm and cold pH (initial and ultimate), as well as cold temperature compared to carcasses of steers fed the diets with 60 % of Napier grass. However, steers fed the control diet had higher warm carcass temperature compared to steers fed diets with 30 and 60 % of Napier grass. The muscle pH in living animals is normally around 7.0 and a post-mortem pH decline occurs when the muscle pH reaches 5.4 due to

accumulation of lactic acid produced during anaerobic glycolysis from glycogen (Chilanga, 2020). According to Kumar *et al*., (2010), ultimate pH higher than 5.6 indicates susceptibility of the meat to microbial attack, which negatively affects shelf life. The ultimate pH of carcass from steers among the dietary treatments in the present study ranged between 5.1 and 5.6 indicating that the carcasses in this study will not be affected by microbial attacks negatively. Moreover, the ultimate pH of the carcasses did not exceed the 5.8 that was reported by Pophiwa *et al*. (2017) that any pH value exceeding 5.8 is deems dark, firm and dry (DFD). The pH values were in fact closer to the 5.8 recommended by Tejeda *et al*. (2008) for good quality meat.

Temperature in muscle is associated with increased myosin and sarcoplasmic protein denaturation, both of which contribute to shrinkage of myofilament lattice spacing (Hughes *et al*., 2018). Variations in the pH of muscle post-mortem and a decline in temperature could account for the changes in meat products' technological and sensory qualities (Hwang & Thompson, 2001). The rate at which pH and temperature decline in carcasses affects how calpain binds to calpastatin and how calpains interact with myofibrillar proteins to initiate the tenderization process (Morton *et al*. 1999). Good quality, tender, and juicy meat is indicated by a normal decline in temperature and pH. Results in this study show that inclusion of Napier grass at 0% and 30 % in fattening diets yielded better carcass traits from steers compared to inclusion at 60 %.

5.5. Meat physical attributes

5.5.1 Drip loss

Drip loss refers to inability of fresh meat to retain its natural juices in the muscle and muscle fibres (Neethling *et al*., 2017). Meat drip loss is of high importance to meat quality because beef with high drip loss has been found to be unappealing, which can turn consumers away and lead to a drop in meat sales (Jama *et al*., 2008). According to Murray (2001), a drip loss that ranges from 2 to 5 % is considered to be normal, while values below or above could be a sign of meat deterioration. The drip loss percentage of the meat in the present study was within the indicated range. The drip loss percentage of the meat from steers fed diets with Napier grass was similar to that from steers fed the control diet. This is an indication that supplementation of the grass in the fattening diets did not deteriorate the retaining capacity of the meat.

5.5.2 Meat colour

Fresh meat colour is defined by the total amount of myoglobin (oxymyoglobin, metmyoglobin and deoxymyoglobin), which is associated with the muscle oxidative capacity (Lebret *et al*., 2015). Meat colour is determined by the content of myoglobin and its derivatives as well as the meat pH (Sebsibe, 2008). Moreover, meat myoglobin oxidation is accelerated with increased temperature (Lebret *et al*., 2015). However, results in this study show that although the ultimate pH and temperature of the carcasses were affected by the dietary treatments, meat colour and myoglobin were not affected by the dietary treatments. The L* values were below recommended range of L* (33.2 - 41) (Muchenje *et al*., 2009b). The dark colour stability may be attributed to myoglobin denaturation from high cold carcass temperature (Sammel *et al*., 2002) observed on control and 30% diets.

5.6. Warner Bratzler shear force, water-holding capacity and moisture characteristics.

Juiciness of meat is the moisture sensation of the cooked product, and is closely linked to WHC, thawing loss and cooking loss (Pophiwa *et al*., 2017). Thawing loss refers to the loss of fluid in beef resulting from the formation of exudates following freezing and thawing (Jama *et al*., 2008). In the present study, the thaw loss of meat aged for 7 days increased with 60 % inclusion of Napier grass in steers fattening diets. The increase in thaw loss could be attributed to the progressive denaturation and proteolysis of the myofibrillar and sarcoplasmic proteins present in meat (Lawrie & Ledward, 2006) brought about by the high inclusion level of Napier in the diets.

The water holding capacity (WHC) in the present study was not affected by dietary treatments. However, it ranged from 0.31 to 0.37, which was lower than the normal range of 0.37 and 0.73 reported by Muchenje *et al*. (2009) except for WHC of meat from steers fed diets supplemented with 60 % Napier grass that was 0.37 %. Meat with high WHC value yield more muscles, tenderer, firmer and superior in forming emulsions than meat with low WHC (Pearce *et al*., 2011). Although meat with low WHC is associated with higher drip loss and cooking losses hence less juiciness and tenderness, the WHC of meat in this study was low, but had normal drip loss. The

result of the present study concurs with that of Mosimanyana (2016), but lower than the 0.40 to 0.44 reported by Modika (2019) on cattle fed grain.

Cooking loss refers to the percentage of water that is lost during cooking. In this study, the cooking loss of meat aged for 1 and 7 days was in the range of 18.45 and 21.65 %, which is within the normal range (13.1 – 34.5) for beef muscles (Kadim *et al*., 2006; Lee *et al*., 2008; Muchenje *et al*., 2009). Jama *et al*. (2008) reported that an increase in cooking loss deteriorates the nutrients in meat during cooking. Our results show that inclusion of fattening diets with Napier grass did not deteriorate the cooking loss of the meat from steers.

The term "thawing loss" describes the fluid loss that happens as a result of exudates forming after freezing and thawing of the meat (Jama *et al*., 2008). Thawing is known to increase the amount of water lost in meat, most likely because of changes to the structure of the muscle fibres and/or denaturation of proteins (Leygonie *et al*., 2012). Meat (7 days aged) from steers fed diet with 60 % of Napier grass had higher thaw loss compared to that from steers fed other treatment diets. An increase in meat thaw loss with an increase in Napier grass inclusion level indicate the formation of ice crystals during frozen storage, which will cause cell integrity to be destroyed and the amount of water in extracellular space to increase (Wang *et al*., 2022).

Meat tenderness is one of the main attributes that consumers consider when buying meat. Meat tenderization is a complex process that involves the degradation of collagen during post-mortem, with reduction in the diameter of myofibril bundles and changes in sarcomere length during rigor mortis (Koohmaraie & Geesink, 2006). Warner Bratzler shear force (WBSF) is a scientific tool for quantifying meat tenderness. According to Miller *et al*. (2001), a steak is guaranteed to be tender from consumer acceptability standpoint if it has ≤ 3.0 kg of shear force. Results in this study show that WBSF of the meat from steers was similar across the treatment diets. The WBSF of 1-day aged meat ranged from 3.61 to 4.19 kg, while that of meat aged for 7 days was between 3.02 and 3.32 kg, which is considered tender. The tenderness observed in this study for dietary treatment could have been influenced by rigor resolution due to enzymatic breakdown of collagen holding meat fibres together when ageing (Warris, 2000).

5.7 Muscle sarcomere length and myofibril fragment length

The sarcomere length (SL) recorded in the present study was longer than 1.8 µm, indicating more relaxed muscles and therefore relaxed animals (Muchenje *et al*., 2009). Longer SL is advantageous as it indicates towards a relaxed myosin-actomyosin interaction and therefore more tender meat than those with shorter sarcomeres (Modika, 2019). The similarities (with a range of 1.93 and 2.02 µm) in SL of meat from steers across the dietary treatments in this study indicates that the meat from steers among the treatments were tender.

Myofibrillar contribution to meat tenderness depends on the extent of shortening during rigor development and proteolysis during conditioning (Warris 2000). Considering the different treatments, there were similarities on the myofibril fragment lengths (MFL) on day 1 and 7 of aging. Three factors primarily influence meat tenderness: the quantity and characteristics of connective tissue; the degree of muscle contraction at the end of rigor mortis; and the degree of structural deterioration during post-mortem aging (Rhee *et al*., 2004). The calpain proteolytic enzyme can influence the MFL, as a component of tenderness during aging of carcass post-mortem (Muchenje *et al*., 2008). The MFL meat from steers across dietary treatments were shorter following ageing. According to research, shorter MFLs indicate more aging and consequently tenderization as well as a higher level of proteolysis (van Wyngaard *et al*., 2023). The findings in this study suggest that dietary inclusion of Napier grass did not affect the MFL negatively.

5.8. Meat proximate composition

The quality of meat can be assessed by evaluating the meat proximate composition (Geletu *et al*., 2021). The proximate composition of meat and their ratio are, thus, important for the health of consumers (Webb & O`Neil, 2008). The proximate results of meat in this study show that meat from steers fed the control diet had higher dry matter and organic matter than meat from those fed the other treatments. Inclusion of Napier grass at 0 and 30 % in steers fattening diets resulted in meat with high protein and fat content. The protein content of meat in this study was within the recommended range of 15 and 23% (Gigli *et al*., 2006; Duckett *et al*., 2013). Reduction in meat fat content was expected from the 60 % Napier grass diet due to an increase in the fibre content of the diet. Inclusion of Napier grass in steers fattening diets above 30 %

reduced the DM, thus increased the moisture content and reduced the fat content of the meat. However, the inclusion of Napier grass below 60 % increased the protein content. Inclusion of Napier grass in diets did not reduce the quality of meat from steers.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

This study assessed the use of Napier grass as a forage total mixed ration for growing beef steers. The study showed that Napier grass can be incorporated in the diet at not more than 30 %. When the inclusion level of Napier grass exceeds 30 %, the growth performance of steers, carcass and meet quality were reduced. The most noticeable effect on increasing (i.e. > 30 %) Napier grass inclusion in the diet was the reduction of fat in the meat, which is something important for consumers.

6.2. Recommendations

i. It is recommended that Napier grass can be used as forage for beef steers, but should not exceed 30 % inclusion level.
ii. Factors that influence carcass and meat quality characteristics such as electrical stimulation and freezing should be taken into consideration in future research.
iii. Lastly, future research should examine strategically the effect of dietary inclusion level of Napier grass on gas emissions from ruminants.

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