EFFECTS OF DIFFERENT INCLUSION LEVELS OF MARULA (*SCLEROCARYA BIRREA*) PULP AT ENSILING ON THE NUTRITIVE VALUE OF NAPIER GRASS (*PENNISETUM PURPUREUM*) SILAGE

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A dissertation submitted in fulfillment of the requirements for the

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DECLARATION

I, Makharamedzha Unarine, hereby declare that this dissertation is submitted in fulfillment of the requirements for the Masters of Science in Agriculture degree in the Department of Animal Science, School of Agriculture, University of Venda and has not previously been submitted for a degree at this or any other university, and that in execution it entirely is my own work and that all reference material contained therein has been duly acknowledged.

Student.....

Makharamedzha U.

Date 06/09/2017

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DEDICATION

It is with great sorrow and heavy heart that I fully dedicate this work to my rock, my anchor, my source of strength, my beloved late father, Mr. Makharamedzha Tshitereke "strike" Alfred. This man picked me up when I was at my lowest, he gave me strength when I had none but above all he encouraged me to never give up and only sleep when I'm dead. Though you left rather too quickly, you shall forever live on in my heart and your dedication and encouragement shall push and support me beyond my wildest imagination. I LOVE YOU DAD AND THIS IS FOR YOU.

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ABSTRACT

This study was conducted to determine the effect of Marula (*Sclerocarya birrea*) pulp inclusion on the chemical composition, rumen degradability and *In vitro* digestibility of Napier grass (*Pennisetum purpureum*) silage. Napier grass was planted at the School of Agriculture Experimental Farm, University of Venda in 4 m x 5 m plots replicated three times and irrigated using spray irrigation for 12 weeks. Fresh Napier grass was manually harvested, chopped and ensiled with Marula pulp included at four different levels of 0 % (control), 10 %, 30 % and 50 % of the grass. After 8 weeks, the Napier grass silage samples were analyzed for chemical composition using standard procedures. Dry matter (DM), crude protein (CP) and neutral detergent fibre (NDF) were determined. Rumen degradability profiles were detected at 0, 6, 12, 24, 48, 72, 96 and 120 hours (h) of sample incubation using three Bonsmara steers fitted with rumen cannulae. Rumen degradability constants were determined by fitting degradability data to the exponential equation $p = a + b\ (1\text{-}e^{-ct})$ using NEWAY computer program. *In vitro* DM and CP digestibility of rumen undegraded residues after 12, 24 and 48 h of sample incubation was determined by sequential digestion in pepsin and pancreatin solutions. Fermentation characteristics and chemical composition did not differ ($P>0.05$) with the level of inclusion of Marula pulp. However, the silage was good with 10 % inclusion having the highest CP (97.0 g/Kg) and lowest pH of 3.5. The DM, CP and NDF disappearance of all treatments increased linearly with the duration of ruminal incubation. Fifty percent Marula pulp inclusion increased DM disappearance at 0, 6, 96 ($P<0.01$) and 120 ($P<0.05$) incubation h with no effect ($P>0.05$) on other incubation h. Fifty percent Marula pulp inclusion increased CP disappearance at 6 and 96 ($P<0.05$), 12 and 120 ($P<0.01$) incubation h. However, 50 % Marula pulp inclusion increased NDF disappearance at 24 ($P<0.05$) while no additive treatment outperformed ($P<0.05$) other treatment at 72 incubation hour. The soluble fractions "*a*" ranged from 10.9 – 15.7 % among the evaluated treatments. Potential degradability "*a+b*" of DM showed 30 and 50 % inclusion levels with the highest effective DM degradability ($P<0.01$) as compared to the 0 and 10 %. Marula pulp additive inclusion had no effect ($P>0.05$) on both DM and CP *in vitro* digestibility after 12, 24 and 48 h. In conclusion, fresh Marula pulp additive inclusion improved the nutritive value of Napier grass silage with 10 and 50 % inclusion dominating in terms of quality, chemical composition and rumen DM and CP degradability as compared to the other treatments.

Keywords: Napier, Marula, chemical composition, *in sacco*, *In vitro*, degradability, digestibility.

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ABBREVIATIONS

A	Soluble fraction
a+b	Potential degradability
ADF	Acid detergent fiber
ADL	Acid detergent lignin
ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemists
B	Insoluble but potentially degradable fraction
C	Outflow rate of degradation (h^{-1})
Ca	Calcium
Cm	Centimetres
CP	Crude Protein
CRD	Completely Randomized Design
Cu	Copper
DM	Dry Matter
ED	Effective degradability
FAO	Food and Agriculture Organization
Fe	Iron
G	Grams
g/kg DM	Grams per Kilograms Dry matter
g/kg TN	Grams per Kilograms Total Nitrogen
GLM	General Linear Model

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H	Hour
ICP-OES	Inductively Coupled Plasma- Optical Emission Spectroscopy
IVCPD	*in vitro* crude protein digestibility
IVDMD	*In vitro* dry matter digestibility
K	Potassium
K	Rumen Outflow Rate (h^{-1})
La	Lactic acid
LAB	Lactic Acid Bacteria
M	Metres
Mg	Magnesium
mg/kg DM	Milligrams per Kilograms Dry Matter
Mn	Manganese
N	Number of observations
Na	Sodium
NDF	Neutral Detergent Fiber
NH3-N	Ammonium nitrogen
NPN	Non-protein nitrogen
NSC	Non Structural Carbohydrates
P	Phosphorus
SEM	Standard Error Mean
SOP	Standard Operating Procedure
TSP	Three Step Procedure

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WSC	Water Soluble Carbohydrates
Zn	Zinc

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CHAPTER ONE: INTRODUCTION

1.1 Background of study

Drought, rainfall patterns, global warming, and temperature variations especially in the tropical regions of Southern Africa limit forage supply, livestock production and competitiveness of livestock systems (Suttie, 2000). In most areas with dry seasons, tropical pastures rarely provide sufficient year-round quality feed with adequate nutritional demands and satisfactory support for both livestock production and reproduction (Suttie, 2000). Hence, conservation of forage in the form of silage is one of the best ways to alleviate feed constraints and maintain animal productivity especially during dry periods.

Silage can be described as one of the technologies to be adopted that can be used in feeding of livestock during the long and dry winter seasons when feed is scarce. Ensuring satisfactory supply of highly demanded nutrients for livestock production, silage making may in fact be the only technology that would assist small scale farmers in the semi-arid regions of the tropics (Heinritz, 2011). Obua (2005), reported silage as the most succulent feed produced because of controlled fermentation of fresh forage or other material of high moisture stored under anaerobic conditions. However, for the utilization of these grasses and legumes as livestock fodder supplements, Aganga *et al.,* (2005) reported that under wilting condition, additives are recommended to improve fermentation and the nutritive value of the silages. Tropical forages are low in water soluble carbohydrates (WSC), which are important for ensiling. Addition of sources of WSC to tropical forages have resulted in increased lactic acid production (McDonald, 1981). It is for this reason that Marula pulp is being introduced as a silage additive in the current study.

1.2 Problem statement

Smallholder farmers in Vhembe district practice ruminant livestock farming largely on rangeland. The scarcity of grazing pastures during dry winter seasons hinder satisfactory livestock production due to low nutritive value of grasses. As a result, animals gain weight during wet summer season when feed is abundant and lose weight during the dry winter season when fodder quality and quantity is low. The preservation of quality cultivated pasture or surplus summer forage in form of silage could help mitigate feed scarcity during the dry season.

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Although Mugweni *et al.* (2000); Titterton *et al.* (2002) reported successful (fodder) maize silage making, maize in South Africa (SA) cannot be considered as a reliable crop for silage in the marginal and semi-arid areas due to its susceptibility to moisture stress and also because maize is used as a staple food for humans. However, Napier grass (*Pennisetum purpureum*) is more drought tolerant than maize and has a high yield potential (Titterton and Bareeba, 1999), thereby making it a potential crop for silage making for use during the dry season. Napier grass contains low levels of fermentable carbohydrates and this is reckoned to be one of the causes of low Napier grass silage quality (Iqbal *et al.*, 2005); Meeske, 2005). However, the addition of carbohydrate based additives can be used to counter this problem when ensiling Napier grass as a way of improving fermentation. Carbohydrate additives such as molasses have been used in promoting desirable fermentation in Napier grass silage (Lubisi, 2014). However, the sugar cane industries are only located in sugar cane producing provinces (e.g. Mpumalanga and Kwa-Zulu Natal (KZN) in S.A) which makes accessibility of molasses in the north-eastern parts of Limpopo an impossible endeavor especially for smallholder rural based farmers. Inclusion of readily available and more accessible products as additives such as Marula pulp could improve ensilage and composition of forage such as Napier grass, for optimal use as winter stock feed. Marula pulp is produced from Marula wine brewing in the rural areas of Limpopo. The pulp is currently discarded as waste material that usually causes environmental pollution. Instead, the pulp can be used as an additive during silage making for winter feeding of livestock.

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1.3 Justification

Conservation through ensilage of forage produced during the rainy season should be practiced in regions or areas where lack of availability of fodder is the major constraint to livestock production. This is mainly due to the fact that forage availability for small-holder farmers is more dependent on distribution of seasonal rainfall. As such, forage harvesting and storage during the wet season for use during the dry season stands out as a better solution to alleviate winter and drought induced forage shortages. Moran, (2005) reported that ensiling produces good quality fodder than hay resulting from the short duration required to wilt the feed and when the forage loses nutrients, causing a reduction in feed quality. Hence silage can be used for the maximization of production even in winter when there is little or no forage. Moran (2005) also argued that although the quality of the forage may be slightly lower than its fresh state at about 10-15% lower in good ensiling conditions, it still has better quality than many of the forages available for dry season feeding. Furthermore, forage conservation ensures more feed for

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livestock with the least cost possible so that the small-scale farmers are not forced out of business or experience loss of animals due to insufficient winter feed. As a result, a steady supply of feed throughout the year would be ensured as well as the availability of livestock and livestock products throughout the year.

In silage making, the choice of an additive to be used in the silage should be based on the availability of acquisition of the product, not leaving toxic waste, efficiency in promoting fermentation, increase of the energetic or protein value in relation to silage without additives and whose cost is compatible with the quality provided in the final product (Evangelista and Lima, 1999). On small scale farms, commercial additives, which comprise inoculants and enzymes, may be too costly or unavailable. Therefore, the substrate or nutrient source of the additive can be of most benefit to silage made on small holdings. In this case, Marula pulp is available in abundance to small holder rural farmers at no cost every year and can therefore be used as an additive during silage preservation that can be made available to livestock during dry seasons when feed is scarce. The main challenge facing smallholder farmers in this case, however, is unavailability or lack of information on the chemical composition, degradability and *in vitro* digestibility of Marula pulp as an additive. This is because there is very minimal if not no literature on the use of Marula pulp as an additive in silage making or rather the use of Marula pulp in ruminant nutrition. Therefore, this study will generate new information on the use of Marula pulp as an additive in Napier grass-Marula silage for better utilization by smallholder farmers.

1.4 Objectives

1.4.1 Main objective

The main objective of this study was to evaluate the effect of Marula pulp as an additive to increase the quality of Napier grass silage.

1.4.2 Specific objectives

The specific objectives of the current study are to determine the effect of Marula pulp inclusion in Napier grass silage on:

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i. The chemical composition (pH, Dry Matter (DM), Ash, Organic Matter (OM), Crude Protein (CP), Non-Protein Nitrogen (NPN), Water Soluble Carbohydrates (WSC), Ammonia, Lactic Acid, Acid Detergent Fibre (ADF), Neutral Detergent Fibre (NDF) and Acid Detergent Lignin (ADL)) of Napier grass silage, pre-ensiled Napier grass and Marula pulp.
ii. Dry matter, crude protein and neutral detergent fibre rumen degradability.
iii. *In vitro* dry matter and crude protein post-ruminal digestibility of the silage.

1.5 Hypotheses

The null hypotheses:

i. Marula pulp Inclusion has no effect on chemical composition of Napier grass silage.
ii. Marula pulp Inclusion has no effect on dry matter, crude protein and neutral detergent fibre rumen degradability profiles of Napier grass silage.
iii. Marula pulp Inclusion has no effect on *in vitro*, dry matter and crude protein digestibility of Napier silage.

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CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

Most smallholder rural based farmers' rear livestock for different purposes but mostly for subsistence reasons. These livestock are reared productively during the wet season when feed is in abundance; however, livestock become emaciated during the dry season where there is a drop in fodder production. This then calls for conservation of excess green material during the wet season for use during the dry season. Forages like the veld and Napier grasses can be conserved in the form of hay, silage, foggage, and spared veld, and all these types of stored feed (Arnott, 2005) may be used during the dry season. The one form which supplies more nutrients to livestock and has not been fully exploited by rural smallholder farmers around Vhembe District is silage.

Through silage making, a steady supply of feed throughout the year is ensured since it has proven to be a major problem for smallholder farmers to accumulate enough feed to maintain good milk production in dairy and good body condition in beef cattle especially during drier months (Cerisuelo *et al.*, 2010). It would then be much easier to maintain livestock during dry periods as farmers would not be forced to purchase bales of hay, feed them crop residues or agro-industrial by-products when they could have preserved excess feed during the period of surplus. This is because, well-fed animals tend to maintain good condition throughout the year and become ready for breeding more quickly. In cattle, feed availability therefore guarantees consistency in production levels; fulfillment of the required calf per cow per year phenomenon and consistent milk supply thus allowing farmers to get more money in periods when these are in short supply. (ftp://ftp.fao.org/docrep/fao/011/i0588e/i0588e01.pdf).

2.2 Napier grass

2.2.1 Morphological and ecological aspects of Napier grass

Napier grass also known as Elephant grass (*Penisetum purpureum*) amongst other forage crops holds its importance. This is so because of its vigorous, hardy, high-yielding perennial nature, ease of propagation and management (Orodho, 2006). As such, this grass is now widely recognized as a valuable fodder grass (Chandy, 2012). Napier grass is a tall perennial plant,

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growing to 2 - 4.5 m tall, rarely up to 7.5 m, with leaves 30 -120 cm long and 1-5 cm broad (Orodho, 2006). Yield wise, Napier grass can produce an average of about 12 to 25 tons/ha of dry matter depending on agro-ecological zone and management (Wanyama, 2006). Its cultivation is done in various countries such as Pakistan, Sri Lanka and India (Chandy, 2012).

Napier grass is widely planted and grows throughout the year in tropical and subtropical regions of the world (Yunus *et al.*, 2000). According to Chandy (2012), the grass requires a warm and moist climate. The temperature range suitable for its growth and development is between 15°C to 30°C (Chandy, 2012). However, according to Russell and Webb (1976) and Orodho (2006), the optimal temperatures for growth range between 25 to 40 ºC with high, well-distributed rainfall (more than 1000 mm per annum). Napier grass can however, tolerate a moderate dry season of about 3-4 months because of its deep root system. Chandy, (2012) reported that very cold temperatures, below 15°C, are not suitable for plant growth and frost also kills the growing and dividing cells in the tip region of the shoot (Orodho, 2006).

Napier grass has the ability to grow in a wide range of soils, performing best in fertile and well drained soils (Bogdan, 1977; Orodho, 2006; Wanyama, 2006). Chandy, (2012), reported that Napier grass does not grow well in waterlogged and flood prone areas, however, it is well established in clay, sandy loam and deep, fertile loam soils with good drainage (Skerman and Riveros, 1990; Orodho, 2006; Chandy, 2012). It tolerates a soil pH ranging from 5 to 8 therefore, loamy and sandy soils are very much preferred to heavy soils (Chandy, 2012).

Although Mugweni *et al.*, (2000) and Titterton *et al.*, (2002) reported successful (fodder) maize silage making in SA, it cannot be considered as a reliable crop for silage in the marginal and semi-arid areas due to its susceptibility to moisture stress and high cost of production. Napier grass (*P. purpureum*) can therefore, be used as a silage crop because it is more drought tolerant than maize and has a high yield potential (Titterton and Bareeba, 1999).

2.2.2 Nutrient content of Napier grass

Napier grass on average contains 200 g/kg of DM, 70 to 100 g/kg DM of CP, 700 g/kg DM of NDF, 450 g/kg DM of ADF (Gwayumba *et al.*, 2002; Islam *et al.*, 2003). Njoka-Njiru *et al.*, (2006) also reported a CP content with a range of 60 - 120 g/kg during summer which declines to less than 50 g/kg during dry winter seasons.

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Napier grass is a high yielding fodder that gives DM yields ranging from 7.4 %, which was improved to 10.85% and 11.37% by the addition of 50% Silver-leaf, with 5% and 3% molasses respectively surpassing most tropical grasses and in turn gives fodder throughout the year (Skerman and Riveros, 1990; Humphreys, 1994). Napier grass has also become the major livestock feed especially in countries such as Kenya where natural grazing is no longer available due to dense population (Orodho, 2006).

Like other tropical grasses, Napier grass has low content of fermentable carbohydrates with 1.5 to 2% of fresh material which are essential for successful ensilage (Iqbal *et al.*, 2005; Meeske, 2005). As such improvement of fermentable carbohydrate levels, buffering reduction and prevention of proteolysis would be possible through the employment of several practices, and as a result success in production of good quality silage (Titterton and Bareeba, 1999). These practices include mixing legumes with cereal crops, wilting and silage additives in small scale silos. The addition of carbohydrate based additives can be used to counter the problem of low content of fermentable carbohydrates when ensiling Napier grass as a way of improving fermentation and silage preservation by ensuring that lactic acid bacteria dominate the fermentation phase. This is through the fermentation of plant sugars which serve as the primary substrates (fuels) for the lactic acid producing bacteria (Jennings, 2011). Henderson (1993) reported that silage additives have been developed over years for the improvement of nutritive value of silages, furthermore, Meeske (2005) reported that silage additives have also been developed for the reduction of some of the risks during the ensiling process.

2.3 Fodder conservation and silage quality

Silage can be defined as forage, crop residues or agricultural and industrial by-products preserved by acids that are either artificially added or produced by natural preservation, in the absence of air (Moran, 2005). Silage is further defined a method of forage preserved through a stabilized fermentation process with a decrease in pH within a minimum fermentation period (Bilal, 2009). Production of Silage can be achieved by harvesting a forage crop at a high moisture content that is greater than 50 % and subsequently fermenting that crop in a pit, tower, bunker, trench or plastic silos in the absence of air (Jennings, 2011).

Surplus forage harvesting of silage ensures a high quality regrowth succession for grazing that can be used to prepare an area for foggage production (De Figueiredo, 2000). During late

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winter and early spring periods when the pasture is low in protein content, but high in dry matter (DM) concentration, silage could help in supplying quality roughages (Rethman, 1983; Tainton, 2000). As conserved fodder, silage has several advantages over hay. Silage increases animal productivity through the utilization of excess pasture growth, for better management and extended utilization when administered as a routine feed supplement. Hay oxidizes during storage limiting its maintenance quality while silage can be maintained for longer. Thus silage is better as a fodder bank than hay. It does, however, require a good microbial fermentation process to produce good quality silage. For tropical grass species, desirable anaerobic fermentation might not take place to ensure a high level of lactic acid production with a low pH as well as low acetic acid and ammonia nitrogen (NH3-N) concentrations (Catchpoole and Henzell, 1971; Sujatha *et al.,* 1986; Imura *et al.,* 2001).

2.3.1 Quality of ensilage materials

Having become the major forage component in ruminant rations over the past years, ensiling is considered as one of the most important fodder preservation methods used for maintaining animal diets especially during feed scarcity periods. This technique enhances feed availability both in quantity and quality during dry seasons as a result, further strengthening animal production by complementing animal diets with a valuable source of energy and protein (Heinritz *et al.,* 2012).

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During ensiling, energy losses can occur via plant respiration, anaerobic fermentation, aerobic decomposition and loss of effluent, especially during preservation of high moisture materials (van Soest 1994). It is therefore important to follow basic principles of ensiling in order to ensure efficiency in the silage of high moisture forage plants (Kayouli and Lee, 1999; Cao *et al.*, 2009).

The quality of silage produced is dependent on the quality of material used during ensiling, fermentation period and type, temperature during fermentation, dense compaction of silage and air space free (Moran, 2005). Ensiling has however, depended on at least two characteristics of forage materials as a way of ensuring a good silage namely; adequate soluble carbohydrates content in form of WSC (McDonald *et al.*, 2011) of approximately 12–15% (Pereira *et al.,* 2004; Coutinho *et al.*, 2015), low buffering capacity and dry matter (DM) content between 300–350 g/kg (McDonald, 1981) or 250 – 400 g/kg (Wilkinson, 2005) to ensure that quality losses are minimal. Silage nutritional value may be improved by increasing the residual water-soluble

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carbohydrate (WSC) concentration though aerobic stability can be impaired (Conaghan *et al.*, 2012). However, reports show a limited availability of soluble carbohydrates in freshly cut Napier grass before ensiling, as such a source of soluble carbohydrates such as molasses, brown sugar and maize meal have been used as silage additives to stimulate fermentation and improve WSC and lactic acid bacteria (LAB) concentrations before ensiling (Yunus *et al.*, 2000; McDonald *et al.*, 2011; Lubisi, 2014). Furthermore, the CP content of ensiled materials should be critically above 75 g/kg DM (Jusoh *et al.*, 2014) or 80 g/kg DM (Norton, 1994).

2.3.2 Silage additives

Additives are used for the improvement of sugar (e.g. sugarcane molasses, glucose, etc.), dry matter (DM) content (e.g. absorbents, etc.), microbial populations (e.g. Lactic acid bacteria (LAB) inoculants, enzymes) and chemicals (e.g. formic acids, etc.) (Yitbarek and Tamir, 2014). Furthermore, silage additives help improve fermentation and nutritive value of the ensiled material. Moreover, another purpose of silage additives is to control the preservation process in order for the silage to retain as many nutrients present in the original forage as possible when fed. Positive effects on fermentation through cell wall degradation have been reported when formic acid was used, thus making more fermentable substrate available (Rowghani and Zamiri, 2009). Due to the fact that chemical additives are corrosive to equipment and can be dangerous to handle, biological additives are therefore often preferred (Gwayumba, 1997). Biological additives are safe and easy to use, non-corrosive to machinery, do not pollute the environment and are regarded as natural products. They are also used for improving the concentrations of WSC and lactic acid bacteria (LAB) prior to ensiling (McDonald *et al.*, 2011) and have been used to improve the ensiling process and nutrient utilization by ruminants (Muck, 2010).

LAB populations on plant material are naturally often low in number (Kung 2014). However, when the activities of undesirable organisms and objectionable fermentation production are minimized, successful ensilage is achieved (McDonald *et al.,* 2011). Moreover, encouraging the growth of lactic acid bacteria lowers the pH of the silage (Yitbarek and Tamir, 2014). Furthermore, additives are used to enhance silage preservation by encouraging lactic acid bacteria to dominate in the fermentation phase.

Additives will not make poor quality forage into good silage but help make top quality forage into excellent quality silage (Kenilworth and Warwickshire, 2012). During storage useful silage additives decrease heating and molding (Kung, 2014).

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The pH of silage is a key criterion to evaluate silage fermentation (Seglar, 2003). The lower the pH (3.7-4.7), the better preserved and more stable is the silage. Usually accomplishment of low pH is achieved through the fermentation of plant sugars (water soluble carbohydrates) in the crop to lactic acid by lactic acid bacteria, which decrease plant enzyme activity and prevent the proliferation of unfavorable anaerobic microorganisms, especially clostridia and enterobacteria (Yang *et al.*, 2004; Moran, 2005).

The pH (acidity) of the ensiled forage is reduced by these bacteria through lactic acid production and stands as a key factor in the stability and long-term preservation of silage (Jennings, 2011; Moran, 2005). Primary substrate (WSC) availability is necessary to produce lactic acid and can limit excessive or prolonged respiration, thereby decreasing good fermentation potential. An anaerobic condition should be achieved through material compaction; thus squeezing out air as air is said to be silage's biggest enemy (Moran, 2005). Good silage can be produced with chopped material because more air is squeezed out during the compacting process.

2.3.3 The use of other fruit pulps in silage making

Fresh citrus peels have been added with levels up to 50% to the ensilage of Napier grass, improving fermentation quality as determined by low pH values (3.7-4.7) and low butyric acid content and adequate lactic acid production (Faria *et al.,* 1972). Citrus peel may contain 50% WSC in DM but the low DM content (14 - 21%) and intensive initial fermentation lead to high seepage losses, causing a serious pollution problem (Ashbell, 1992).

The DM, WSC and fermentation acids content of elephant grass silage was increased whilst pH was reduced with the addition of 0, 5, 10, 15 or 20% of dried citrus pulp (Faria *et al.,* 1972). Levels up to 30% of dried ground citrus pulp were added to a 75-day regrowth cut of elephant grass, resulting in silages with a corresponding linear increase of DM content, a pH in a range from 3.49 to 3.68 and a linear decrease of ammonia-N (Evangelista *et al.,* 1996).

2.4 Rumen degradability of silages

In vivo study entails the use of markers, *in sacco* requires animals surgically fitted with rumen cannulae and *in vitro* requires the use of ruminal fluid acquired from cannulated animals techniques are the three methods used in estimating the degradability of feeds (Mohamed and

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Chaundry, 2008). However, *in sacco* technique is mostly preferred over the other two mainly because of its accuracy in prediction, simplicity and reliability (Ferret *et al.*, 1997). It is also preferred for the capacity it holds in quantifying degradation rates and pools being degraded in the rumen (Osuji *et al.*, 1993). Quin *et al.*, (1938) was the first to suggest the *in sacco* technique, hence the method has since been used in estimating either forage utilization (van Keuren and Heinemann, 1962) or that of concentrates and high-proteins (Mehrez and Ørskov, 1977). Furthermore, the *in situ* incubation technique has been referred to as a reference method when estimating degradation parameters such as, soluble, insoluble but degradable and undegradable fractions, potential and effective degradability when subjected to suitable non-linear models (Ørskov and McDonald, 1979).

Little research has however been done on ruminal degradability assessments in silages with the inclusion of byproducts of natural grown trees. It is very important for research to focus more on degradation in the rumen because degradation level rate can give ideas of how the quality of an individual feed is reflected. However, digestibility of forage crops may differ due to several factors such as grass species, stage of maturity, temperature, rainfall, light intensity, preservation method and concentration of soluble carbohydrates (Komprda *et al.*, 1996; Aufrere *et al.*, 2003; Kasuya *et al.*, 2008). In addition, other factors may cause differences among experiments and these include factors such as pore size of nylon bags, milling screen sizes, differences in protein sources and fistulated animals used (Canbolat *et al.*, 2005). However, it has also been reported that through the decrease of retention times in the rumen, rumen degradation of proteins can be reduced. Factors known to influence this include the level of feed intake, specific gravity, particle size of diet, concentrate to roughage ratio and rate of rumen digestion (Kamalak *et al.*, 2005).

Lower results for potential DM degradability were observed when rumen degradability of elephant grass silage was assessed and 64.9% dry matter potential degradability was verified (Cabral *et al.*, 2005). The low potential degradability of dry matter reported by these authors can be attributed to the more advanced stage of maturation of elephant grass used (120 days of growth). However, Rambau *et al.*, (2016) reported values of potentially degradable DM and CP fraction that ranged between 85.6 - 93.6 % and 99.7 – 100 % respectively in a study observing Napier grass leaves harvested at three different maturity stages (4, 8 and 12 weeks after sprouting).

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Mott Grass Silage ensiled using cane molasses and crushed corn grains as additives, each at 2, 4 and 6% of forage DM for 30 and 40 days in laboratory silos, ruminal degradability on DM and neutral detergent fiber (NDF) basis were higher than that of Mott grass (Nisa *et al.*, 2004). The results found in a study by Kim *et al.*, (2014) also agrees with higher ruminal DM and NDF degradabilities. The rate of DM and CP degradation in the by-product feed-based silage was higher than that of rice straw and ryegrass straw (Kim *et al.*, 2014). Furthermore, it was discovered that the higher ruminal degradation of DM and NDF of Mott Grass silage than Mott Grass was probably a reflection of fermentation of Mott Grass during ensilage that improved its degradability by improving the availability of easily degradable structural polysaccharides to ruminal microbial population (Kim *et al.*, 2014)

2.5 *In vitro* digestibility of silage

Nutrient digestibility is considered the most essential factor in the evaluation of nutritional feed quality because it represents the amount of nutrients in feed that is accessible to the animal (McDonald *et al.*, 2011). Either *in vivo* or *in vitro* techniques can be used to measure nutrient digestibility of feed. *In vivo* technique requires the use of animals that are fitted with duodenal and ileal cannulas when measuring digestibility. Furthermore, *in vivo* analysis have been observed to be time consuming, labor intensive and costly. As a result, *in vitro* digestibility may be more economical and allow for quality control of processed feeds, even though there is currently no standardized *in vitro* technique for digestibility analysis of ruminant feeds (Schneider and Flatt, 1975).

Very few reports found in previous literature show contradictions towards *in vitro* nutrient digestibility of silages pre-incubated in the rumen. However, Beever *et al.* (1974) and Thomson *et al.* (1981), reported a reduction in the amount of nutrients that entered post ruminal digestion caused by pre-incubation of feeds, in a case referring to *in vitro* simulation of nutrient digestibility in abomasal and intestinal digestion. Zereu *et al.* (2015) silage has improved DM digestibility value when using molasses during of ensilage of vines of four sweet potatoes. Nowak *et al.* (2004) reported a significant increase in value of intestinal digestibility of undegraded protein in formic acid treated silage.

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2.6 Summary of Literature Review

The use of silage has long been an integral component of temperate feeding systems worldwide, to ensure year-round feed supply for high production animals. However, its use in the tropics has been restricted to isolated cases, usually involving higher-return enterprises, and particularly the dairy industry. This study is set to review the potential for use of tropical silage with the use of naturally and locally produced by-products for livestock production, with special reference to the smallholder farming. Furthermore, for successful ensilage additives have been previously used to aid satisfactory preservation or routinely as an insurance against poor preservation (McDonald *et al.*, 2011). Additives help ensure that by the time of feeding, the feed has retained as much nutrients as it is contained in the original fresh forage. However, additives will not make poor quality forage into good silage but help make top quality forage into excellent quality silage (Kenilworth and Warwickshire, 2012). Furthermore, Lubisi (2014) and Ukanwoko and Igwe (2012) reported that additive inclusion didn't differ significantly as compared to no additive silage.

Data in previous literature revealed that additive inclusion enhanced silage DM ruminal degradability of silage because of high solubility and non-fibrous additives in the rumen (Nisa *et al.*, 2005; Kim *et al.*, 2014), hence, others reported that additive inclusion had no effect on silage DM and CP degradability (Nowak *et al.*, 2014). There is not much literature on *in vitro* digestibility of silages, however, molasses inclusion on vines of four sweet potato silages has been reported to improve DM digestibility (Zereu *et al.*, 2015). Therefore, further research needs to be done to evaluate the effect of Marula pulp inclusion on fermentation characteristics, chemical composition, rumen degradability with the use of nylon bag technique and *in vitro* digestibility using modified three step procedure of Napier grass-Marula silage.

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CHAPTER THREE:

EFFECT OF DIFFERENT INCLUSION LEVELS OF MARULA (*SCLEROCARYA BIRREA*) PULP ON THE CHEMICAL COMPOSITION OF NAPIER (*PENNISETUM PURPUREUM*) GRASS SILAGE

3.1 Introduction

Rural areas in the tropics consider ruminants to be the most important animal resources as they play a predominant role in the sustenance of the livelihoods of impoverished families. Feed resources for ruminant livestock in the tropics are natural pastures which are limited in supply during the dry season. Cultivation of forage with consideration of ensilage of the surplus forage during the rainy season can mitigate this challenge. Oude Elferink *et al.,* (2000) reported ensiling as an efficient process of preserving forage of high moisture content. Through proper ensiling, silage can be used to replace the costs of feeds by ensuring a steady supply of quality feeds for ruminants (Lallo *et al.,* 2003; Pirmohammadi *et al.*, 2006). Napier grass is used commonly as a silage crop in tropical climates due to its high quality and quantity (Bureenok *et al.*, 2012).

Napier grass ensilage with no additive proves to be difficult because of its low water soluble carbohydrates (WSC) content which then results in low lactic acid bacteria (LAB) productivity during fermentation. This phenomenon then probes for the addition/inclusion of highly fermentable carbohydrates such as molasses that are normally used as additives. However, the sugar cane industries are only located in sugar cane producing provinces (e.g. Mpumalanga and Kwa-Zulu Natal (KZN) in S.A). These then makes accessibility of molasses in the north-eastern parts of Limpopo a difficult and expensive endeavor. Inclusion of readily available and more accessible products as additives would be a plus. Therefore, the additive with high WSC properties such as Marula pulp would serve as a better substrate for LAB, ensuring better conditions for desirable fermentation.

In South Africa, Marula trees fall under the endangered tree species and their fruits are consumed by animals as they are. However, most of the fruits are collected by people for Marula wine making. Wine is produced from Marula fruit when they are ripen (yellow in colour). This process involves the squeezing of fruit pulp to release the juice. The fermentation of the juice is done without the use of the additives, making the pulp a good source of fermentation.

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Hence, this fruits are found only seasonally (summer) in abundance of feed. Ensilage, being one of the preservation methods used to preserve fresh feed material, can therefore be a useful method for Marula pulp preservation for use during the winter seasons when feed is rather scarce.

An increase in the production of Marula beer and other Marula products from different commercial companies and rural communities in southern Africa, gives an overabundant supply of the pulp generated from their processing. Even though, the pulp has been informally used as feed for livestock, there is a need to study its chemical and nutritional composition. In literature, only Marula seed cake has been used as a protein source for ruminant livestock, (Mlambo *et al.*, 2011). The rest are considered as waste material which gets discarded and due to abundance of grazeable forage in summer, animals eat very little of these discarded Marula pulp. These constitute as an environmental nuisance through pollution. Therefore, the objective of this study was to determine the effect of Marula pulp inclusion during ensiling of Napier grass on the fermentation characteristics and chemical composition of the silage.

3.2 Materials and methods

3.2.1 Experimental site

The study was conducted at the School of Agriculture Experimental Farm (22°58'32"S, 30°26'45"E; Altitude of 596 m above sea level) and the samples were analyzed in the Animal Science Nutrition Laboratory, University of Venda, Thohoyandou. The daily temperatures at Thohoyandou range from a minimum of 18˚C to a maximum of about 31˚C (Tadross *et al.*, 2005). The area receives an average annual rainfall of ± 500 mm that falls predominantly in summer. The area is characterized by deep well drained red clay (Soil Classification Working Group, 1991).

3.2.2 Napier grass (*Pennisetum purpureum*) production and harvesting

Napier grass was planted at the end of January 2015 in the School of Agriculture farm plots. Land was ploughed and disced, then demarcated into four plots of 4 x 5 m each in a completely randomized design. Napier cuttings with three nodes were planted, with two nodes under the ground and one above ground level at an angle of about 30 - 45° (Tainton, 2000) with a spacing

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of 70 cm inter-and-intra row. Weeding was done immediately when the weeds started to grow during the entire experimental duration. The fodder was irrigated weekly using tap water to maximum field capacity using spray irrigation system. Plots were kept weed free for the experimental duration of 12 weeks and thereafter the fodder was fully harvested, chopped to about 2 to 4 cm and ensiled with or without Marula pulp.

3.2.3 Marula pulp (*Sclerocarya birrea*) collection

Marula fruit were collected from Niani, Mapakoni village (22°27'37.73''S; 30°40'49.51''E), in Vhembe District of Limpopo province, dominated by Mopani bushveld (Acocks, 1953). The vegetation is classified as Mopane Bushveld, which is characterized by annual summer rainfall ranging between 250 and 500 mm with temperatures varying between the extremes of 1.5 and 42.5 °C, and an average of 22 °C (Acocks, 1953).

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3.2.4 Silage making

3.2.4.1 Preparation of Marula pulp before ensilage

Marula pulp was extracted from the fruit manually using a fork during Marula beer making to remove the nut/seed. The juice was used for wine brewing and the waste pulp was stored in a refrigerator at 6ºC. Before ensiling, the pulp was thawed overnight and then ensiled with Napier grass.

3.2.4.2 Napier grass sampling

Approximately 1000 g of freshly cut Napier grass samples were randomly collected from each plot by cutting the grass at 15 cm above the ground using pruning scissors and chopped to 2 to 4 cm length pieces and packed into brown sample bags. The collected fresh materials were immediately weighed, dried and subsequently determined for chemical composition. The remaining fodder from the plots was then fully harvested manually by cutting the grass at 15 cm above the ground using pruning scissors for ensiling when their regrowth were still vegetative at 12 weeks of age.

3.2.4.3 Preparation for ensiling

Before ensiling, the harvested grass was immediately chopped to about 1.2 to 1.27 cm length (Aganga *et al.,* 2005) using pruning scissor and treated with four different inclusion levels of Marula pulp, 0% (no additive), 10 %, 30 % and 50 % Marula pulp inclusion were used and replicated three times. The inclusion was based on fresh weight basis.

3.2.4.4 Experimental design

The experiment was arranged in a completely randomized design (CRD) with four inclusion levels (0%, 10%, 30% and 50%) of Marula pulp serving as factors.

3.2.4.5 Ensiling procedure

Approximately 600 g (wet basis) of chopped grass material was weighed before ensiling in 1 litre (ℓ) glass jars replicated 4 times. The Marula pulp was added at 0 %, 10 %, 30 % and 50 %

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respectively of the total weight of the chopped material. The chemical composition (g/kg DM) of pre-ensiled Napier grass and Marula pulp are shown in Table 3.1 below.

Table 3.1 Chemical composition (g/kg DM) of pre-ensiled Napier grass and Marula pulp

Parameter	Napier grass	Marula pulp
Dry matter (g/kg)	265	16.0
pH	5.20	3.30
(g/kg DM)		
Ash	85.1	91.3
Fat	29.3	33.4
Neutral detergent fibre	840	380
Acid detergent fibre	507	270
Water soluble carbohydrates	40.1	185
Acid detergent lignin	82.8	97.0
Crude protein	94.7	59.0

The weighed chopped grass materials were thoroughly mixed with the Marula pulp and ensiled in 1 ℓ Consol anaerobic glass jars. The chopped materials were then ensiled and compressed using pruning scissors to squeeze out air promoting an anaerobic condition. Immediately after ensiling, the glass jars were tightly sealed using screw on lids that were also fastened with a selotape to prevent entrance of air. The glass jars were then stored at room temperature for 8 weeks at the University of Venda, Animal Nutrition Laboratory.

3.2.4.5 Chemical analysis of Napier grass, Marula pulp and silage

Freshly cut grass samples, Marula pulp and silage samples after 8 weeks of fermentation were analyzed in the Animal Science Nutrition Laboratory, University of Venda, Thohoyandou. The pH of fresh Napier grass, Marula pulp and Napier grass silage samples was measured using a pH meter according to Mtengeti *et al.* (2006). Samples of 40g from each silo were soaked in 200 ml of cool distilled water for 12 hours then filtered and the supernatant used for the determination of the pH. Freshly cut grass and silage samples were dried at 60°C in an oven for 48 hours (h) to determine DM content (AOAC, 1990; method 930.15) and grounded through a grinding mill of 1 mm screen size. WSC content was determined using Anthrone method (Murphy, 1958). Ash was determined by combusting silage samples at 550°C overnight (AOAC, 1990; method 942.05). Nitrogen (N) content was determined using Kjeldahl procedure (AOAC,

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1990; method 984.13) and N was then converted to CP as N x 6.25. Non-protein N (NPN) was determined according to Licitra *et al.* (1996). Neutral detergent fibre (NDF), Acid detergent fibre (NDF) and acid detergent lignin (ADL) contents were determined using the technique of Van Soest *et al.* (1991). Minerals [P, K, Ca, Mg, Cu, Fe, Mn, Na and Zn] were determined using ICP-OES (SOP, 2005). Silage was also analyzed for LA and Ammonium nitrogen (NH_3-N) using protocols described by Faithful (2002) and the AOAC (1990; method 941.04), respectively.

3.2.5 Statistical analysis

Analysis of variance (ANOVA) on silage quality was performed at $P < 0.01$ and $P < 0.05$ according to Steel and Torrie (1980) using General Linear Model (GLM) procedures of MINITAB Statistical package version 17 (Minitab, 2014). Significant differences between the treatments were detected, means were separated using the Tukey's test (Tukey, 1953).

Statistical model for silage nutritional quality was as follows:

$Yij = \mu + L_i + \varepsilon_{ij}$

Where,

Y_{ij} = Observation - pH, DM, WSC, CP, NDF, ADF, ADL, Fat, Ash, LA, Ammonia-N, and minerals;

μ = Overall mean;

L_i = Effect of the i^{th} levels of Marula pulp inclusion, i = 1, 2, 3 or 4;

ε_{ij} = Random error

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3.3 Results

3.3.1 Fermentation characteristics of Napier grass silages

Fermentation characteristics of Napier grass silage after 8 weeks of ensiling are presented in Table 3.2. The different Marula pulp inclusion levels showed no significant effect (P>0.05) on fermentation characteristics. Addition of Marula pulp reduced silage pH as compared to control. LA and WSC, however, decreased numerically with an increase in Marula pulp inclusion.

Table 3.2 Effects of Marula pulp inclusion on pH and fermentation characteristics of Napier grass silage

Additive	pH	NH_3-N	LA	WSC
		g/kg TN	g/kg DM	
Control	4.5[a]	46.9	9.4	19.8
10%	3.5[c]	44.8	8.1	15.1
30%	3.6[c]	63.8	1.4	15.3
50%	3.8[b]	53.8	0.5	13.0
SEM	0.017	13.81	3.98	1.77
Significance	**	ns	ns	ns

**: P<0.01; ns, not significant: P>0.05, WSC, water soluble carbohydrates; NH_3-N, Ammonium-Nitrogen; LA, Lactic acid; SEM: standard error mean; g/kg TN, grams per kilogram total nitrogen

3.3.2 Chemical composition of Napier grass silages

The effect of Marula pulp inclusion on the chemical composition of Napier grass silage after 8 weeks of ensiling, presented in Table 3.3 showed no significant difference in all the treatments (P>0.05). DM (g/kg) showed 30% inclusion recording the highest at 239 g/kg. Although Marula pulp inclusion had no effect on all chemical parameters of Napier grass silage, the highest CP

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content was recorded at 10% inclusion at 97.0 g/kg. NDF also showed an increasing trend with an increase in additive inclusion.

Table 3.3 Effects of Marula pulp inclusion on the chemical composition of Napier grass silage

Additive	DM	ASH	FAT	CP	NPN	NDF	ADF	ADL
	(g/kg)	(g/kg DM)						
Control	190	81.3	28.5	82.3	6.0	810	602	111
10%	216	84.1	24.3	97.0	6.5	835	557	110
30%	239	76.6	23.1	94.9	4.3	839	542	94.6
50%	182	94.6	23.2	90.2	2.70	841	608	146
SEM	13.6	7.77	1.50	18.8	0.95	21.4	34.2	20.7

There were no differences among treatments; DM: dry matter; ADF: acid detergent fibre; NDF: neutral detergent fibre; ADL: acid detergent lignin; CP: crude protein; NPN: Non-Protein Nitrogen; SEM: standard error mean

Table 3.4 Effects of Marula pulp inclusion on mineral composition of Napier grass silage after 8 weeks of ensiling

Additive	Ca	Mg	K	Na	P	Zn	Cu	Mn	Fe
	Macro (g/kg DM)					Micro (mg/kg DM)			
Control	2.6	3.7	22.7	0.3	1.2	36.1	11.0	94.8	608
10%	2.9	3.8	19.6	0.3	1.0	40.8	11.8	117	836
30%	2.5	3.7	16.0	0.2	0.9	33.3	15.1	117	628
50%	3.5	4.3	22.0	0.3	1.2	42.3	29.9	125	536
SEM	0.25	0.23	2.25	0.003	0.19	2.97	4.60	12.2	102

There were no differences among treatments; Ca: Calcium; Mg: Magnesium; K: Potassium; Na: Sodium; P: Phosphorus; Zn: Zinc; Cu: Copper; Mn: Manganese; Fe: Iron; SEM: standard error mean

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3.4 Discussion

3.4.1 Chemical composition of pre-ensiled Marula pulp and Napier grass

At ensiling the DM of a crop has a strong influence on both the rate and extent of silage fermentation (McDonald *et al.*, 2011), hence low DM below 250 g/kg combined with low sugar content increases the chances of clostridial fermentation during ensilage leading to subsequent poor silage acceptance by animals (Fraser *et al.*, 2000). To achieve favorable satisfactory fermentation a DM content that falls within the range 250-400 g/kg in forage is required (Wilkinson, 2005). The DM of Napier grass as observed in the current study agrees with the recommended DM range, Marula pulp was however very low. However, Lubisi (2014) reported a higher dry matter content of 330 g/kg at ensiling for no bio-digester slurry treated Napier grass. The DM differences may be related to stage of grass maturity at harvest (Tyrolova and Vyborna, 2008), furthermore the time of harvest during the day may bring about such differences (Owens *et al.*, 2002) as well as weather variations in the area.

Ash content generally gives a reflection of the presence of minerals in the sample (Nnamani *et al.*, 2009). In the current study, fresh Marula pulp recorded a slightly higher ash content as compared to the fresh Napier grass (Table 3.1). Ash content variations found in different studies most likely reflect soil variation and mineral contents in an area (Islam *et al.*, 2010).

In a study conducted by Mbuthia and Gachuiri (2003), NDF and ADF content for Napier grass varied from 579 to 613 and from 303 to 318 g/kg DM, respectively, in agreement with the range reported by Kariuki *et al.* (1999). However, the results of the current study contradicts with the previous results (Mbuthia and Gachuiri, 2003; Kariuki *et al.*,1999) as Napier grass recorded a much higher NDF content at 839.7 g/kg DM and Marula pulp recorded 380.3 g/kg DM which is slightly lower. ADF content was also higher for Napier grass and slightly lower for Marula pulp at 507.3 and 270.1 g/kg DM, respectively. A study by Ansah *et al.* (2010), who evaluated herbage yield and chemical composition of four varieties of Napier grass harvested at three different days after planting, reported an ADF content ranging from 532.7 to 471.3 g/kg DM agreeing with the current study.

WSC are regarded as essential substrates for the growth of LAB in order to enhance efficient fermentation (McDonald *et al.*, 2011). At least 37 g/kg DM of WSC is said to be the minimum concentration required for successful fermentation without the use of inoculants in a herbage

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that contains a DM of 230 g/kg (Haigh, 1990). However, this contradicts with the findings of Jaakkola *et al.* (1991) who recommended a slightly higher minimum WSC content at 60 g/kg DM and Lunden-Petterson and Lindgren (1990) also recommended a minimum of 60 – 70 g/kg DM. In the current study, Napier grass recorded a WSC content that is lower than the recommended 60 g/kg DM hence, Marula pulp itself before ensilage showed a much higher WSC content, therefore making it more suitable to use as an additive as it made a good substrate for LAB. The lower WSC value of Napier in this study agrees with the studies conducted by Yokota *et al.* (1991); Iqbal *et al.* (2005); Nisa *et al.* (2006) and Bureenok *et al.* (2012) who reported that Napier grass typically contains low WSC.

ADL content for Napier grass and Marula pulp was recorded at 82.8 g/kg DM and 97.0 g/kg DM, respectively. The ADL content of 89.0 g/kg DM reported by Ansah *et al.* (2010), agrees with that of the present study. In a study conducted by Mbuthia and Gachuiri, (2003), crude protein (CP) content of Napier grass before ensiling ranged from 95-107 g kg DM at 8 weeks. This result was slightly lower than the 11.7% content reported by Kariuki *et al.* (1999) at the same age, which may be because the CP content of Napier is highly dependent on soil fertility (Kariuki, 1998). The present study results of CP in freshly cut Napier grass is similar to that of Mbuthia and Gachuiri, (2003). Napier CP has no influence on Marula pulp CP content.

The pH content of freshly cut Napier grass materials before ensiling falls within a range of 5 and 6 depending on the crop (Kung, 2001). Marula pulp showed a relatively lower pH while Napier grass was well within range.

3.4.2 Fermentation characteristics of Napier grass silage

One of the most important factors affecting silage quality is the rate at which pH decreases in plant materials that are under preservation. Generally, a pH ranging between 3.7 and 4.2 is considered beneficial for silage preservation (Kung and Shaver, 2001) and the pH of the present study ranged between 3.5 and 3.8 for the treated silages indicating well-preserved silages, while it was 4.9 in the control. The addition of Marula pulp reduced silage pH which was consistent with other reports (Weinberg *et al.*, 1993 and Nkosi *et al.*, 2012). Low pH in ensiled silages given all treatments confirmed WSC availability reflecting the correct LAB concentration which are responsible for WSC fermentation as a result increasing LA accumulation (McDonald *et al.*, 1991)

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In silage, the protein degradation is reflected by NH_3-N content (Wilkinson, 2005) and well preserved silages should contain less than 100 g/kg NH_3-N/TN (McDonald *et al.*, 2002). In the current study, the concentration of NH_3-N was less than this threshold NH_3-N/TN (Table 3.2) indicating well-preserved silages. Marula pulp inclusion reduced silage NH_3-N and pH compared to the control, indicating that less proteolysis has occurred in these silages. These findings are consistent with other studies (Driehuis *et al.*, (1999); Filya, 2003) who did not observe any difference in NH_3-N concentration between the silage treatments used in their studies. Lactic acid showed a reducing trend as the level of inclusion increased with no significance observed. The reduction in LA concentration was probably caused by the reduction in residual WSC.

WSC content of both Napier grass and Marula pulp before ensilage were 40 g/kg DM and 184.9 g/kg DM respectively, and were then reduced to < 20 g/kg DM after ensilage. It can therefore be explained that WSC were consumed by the LAB to produce LA and reduce silage pH (McDonald *et al.*, 1991). The different inclusion level treatments of Marula pulp to Napier grass reduced silage WSC content much more as compared to the control treatment. It can therefore be explained that WSC decreased during ensiling as it supplied energy to the microbes during the fermentation process. This suggests that LAB was high in the Marula added silages as indicated by reduced terminal/final sugar content. Furthermore, this implies that a lot of sugar was consumed by LAB, reducing the silage pH. However, LA was not increased by Marula pulp addition.

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3.4.3 Chemical composition of Napier grass silage

According to Islam *et al.* (2009) silage DM is an important silage quality indicator. McDonald *et al.* (2011) highlighted that low DM content increases nutrient loss with effluent juice. The current study indicates that DM contents at pre-ensiling in Napier grass (Table 3.1) was ideal for fermentation however, Marula pulp inclusion may have contributed to effluent juice losses as DM decreased (Table 3.3) after fermentation period. In addition, a comparison between DM contents of silages, 10 and 30 % performed much better than 0 and 50 % which were both below 200 g/kg.

Increasing levels of Marula pulp inclusion also influenced the crude protein (CP) content of the silage, which showed an increasing trend at all inclusion levels of Marula pulp with 97.0 g/kg DM

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at 10 % inclusion recorded as the maximum CP value of the given treatments although they were all not significant. Nkosi *et al.* (2012a), indicated an increased effect on CP content in terms of carbohydrate additive inclusion. However, Lubisi *et al.* (2014) reported a decreased effect in CP. According to Mbuthia, (2003), variations in CP content in different conducted studies could be explained in consideration with the fact that CP of Napier grass is highly dependent on fodder genetics, climate, seasonality and soil fertility. Hence, silages in the present study recorded a CP content that was higher than the critical values essential for rumen function, reported to be either 80 g/kg DM (Norton, 1994) or 75 g/kg DM (Jusoh *et al.*, 2014).

Proteolysis occurring during fermentation forms NPN because of extensive degradation of native plant proteins (Vagnoni *et al.*, 1997). Kung (2010), reported NPN values ranging between 120 to 150 g/kg of CP, that are usually said to relate to clostridial fermentation leading to low silage quality. The present study however, despite the use of additives, a very low NPN content was recorded ranging between 2.70 to 6.5 g/kg DM which is much lower but showed no significant difference.

Crude fat content decreased with an increase in Marula pulp inclusion, it was however not significant at, agreeing with Alikhali *et al.* (2005) and Mokoboki *et al.* (2016) who reported a reduced fat content due to molasses addition. The fat content of the current study is lower than the recommended maximum level 60 to 70 g/kg DM (National Research Council, 2007).

Fibre plays an important role in ruminant nutrition. This is because minimum fibre concentrations are required to aid in chewing and rumen motility (Nussio *et al.,* 2011). Both the NDF and ADF contents in the Napier grass silage were represented by mean values ranging between 809.6 and 840.6 for NDF and a range between 541.9 and 607.7 for ADF (Table 3.3). One can observe that ADF in all the silages was affected by the increasing levels of Marula pulp, because it showed fluctuations in values as inclusion levels increased. Hence, NDF increased with an increase in Marula pulp inclusion though there was no significant difference. According to Van Soest (1965), NDF levels equal or superior to 70% limit feed intake by the physical effect of rumen filling. However, both the NDF and ADF contents of all the silages were above the minimum 300 and 190 g/kg DM requirement for healthy rumen function in dairy cows (Target, 2002). In addition, ADL showed a reducing trend from 0 to 30% inclusion and increased sharply at 50 % to a maximum of 146.1 g/kg DM but it was also not significant. The decreased results in ADL of the present study are in agreement with Zereu *et al.* (2015), Pinho *et al.* (2004)

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and Baytok *et al.* (2005) who reported reduced effect due to the inclusion of additives during ensiling.

Mirzaei (2012) reported mineral ranges required in ruminants as follows; Ca (2-6), Mg (1.2-2.1), k (8), Na (1-4), P (1.9), Zn (12-20), Cu (8-14), Mn (40) and Fe (30-50). The level of potassium (K) in all the Napier grass silages was much higher than 8 g/kg DM recommended for grazing animals (Underwood, 1981; Mirzaei 2012) with a range between 16.0 g/kg – 22.7 g/kg DM. It has, however, been suggested that some ruminants with high producing, under stress such as heat stress, may require K levels above 10 g/kg DM. Similar K concentrations were also reported (Prabowo *et al.,* 1990, Tiffany *et al.*, 2001). The high levels of K can be explained by how environmental factors play an important role in potassium metabolism in plants. Potassium is naturally high in lush grass during the early grass-growing season especially when environmental conditions like high moisture and heat favor rapid grass growth (Khan *et al.,* 2005). Potassium was however not significant.

The sodium (Na) requirement for ruminants is often debated in literature concerning the dietary concentration demand of animals. An adequate range from 1 - 4 g/kg DM has been recommended (Underwood, 1981; Mirzaei 2012). It has also however been suggested that a sodium intake of 0.87 g/kg DM is required to maintain an adequate Na and K ratio in the parotid saliva of ewe's. The content of Na in the ration is important in determining the adequacy of the minerals. The current study shows a very low Na content ranging between 0.2 – 0.3 g/kg DM which is much lower than the adequate 1- 4 g/kg/DM range at the same time showing no significant difference. The silage samples examined in the current study proved to be very poor sources of Na, such that routine supplementation would likely be necessary. These values were deficient because of being lower than the critical values (requirement of ruminants) (Khan *et al.,* 2007, NRC, 1983, Sial, 1991, Tudsri and Kaewkunya, 2002). This widespread deficiency in forage Na is corroborated with some earlier findings that the most prevalent mineral deficiencies for grazing animals in the world were Na (Khan *et al.,* NRC, 1983, Sial, 1991, Tudsri and Kaewkunya, 2002). In addition, deficiency of this element has been reported in many developing countries (Areghoere, 2002).

Forage calcium (Ca) concentrations of 2 - 6 g/kg, with higher requirements for lactation have been variously recommended for cattle and sheep (McDowell *et al.* 1993; Khan *et al.*, 2006; Mirzaei, 2012). Variations in the levels of Ca between findings in this study with values reported

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in the literature Mean forage Ca concentrations were adequate and sufficiently well within the requirement range of ruminants with the control (0% inclusion) at 2.6 g/kg MD, 10% at 2.9 g/kg DM, 30% at 2.5 g/kg DM and 50% a bit higher at 3.5 g/kg DM. Though Ca levels were not significant, the silages had adequate calcium recommended for growth, hence Forage Ca requirements of ruminants is reported to be a subject of considerable debate as the requirement may be influenced by animal type, level of production, age and weight (Mirzaei, 2012, Reuter and Robinson, 1997).

The Napier grass silages had slightly higher levels of Mg than the recommended requirement, ranging between 3.7 – 4.3 g/kg DM but showing no significance. These silages do however meet the theoretical requirements of Mg for beef cattle and lactating cows (1.2 - 2.1 g/ kg DM) (Khan *et al.*, 2007; Mirzaei 2012). It is also reported that the dietary Mg availability to stock is markedly affected by other dietary components, especially K. High dietary levels of K and N are said to inhibit Mg absorption from the rumen. Hence, Ca and soluble carbohydrates may respectively increase and decrease dietary Mg requirements of livestock, whereas raised dietary P levels appears to lower the requirements for both Ca and Mg (Judson and Mcfarlane, 1998; Mirzaei, 2012).

The iron (Fe) content in all the Napier grass silage samples was above the critical levels of 50 mg/kg proposed as adequate for grazing animals (McDowell 1985; Khan *et al.*, 2005; Mirzaei 2012). However, the proposed variations in the contents of Fe among these silages could be partly explained by forage species' differences and the influence of the level of Fe variations in the soil and climatic conditions of the study sites even though they are not significant. All the inclusion level silages had higher levels of Fe than the critical content of Fe in animal tissues (30 - 50 mg/kg DM). However, Fe bioavailability in ruminants would depend on feed mixture fed together and form of Fe in these feeds. Silage Fe levels during the study were however sufficient for the requirements of ruminants for optimal performance. These levels of silage Fe in the present study may support the reports of various researches (Tejada *et al.,* 1987; Prabowo *et al.*, 1990; Cuesta *et al.*, 1993; Velasquez-Pereira *et al.,* 1997). The generally high forage Fe found in this study is an agreement with the higher forage Fe value of 650 mg/kg reported in other related studies (Khan, 2003).

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High levels of manganese in forage can retard the growth of livestock. The feasibility of breeding for lower manganese concentration in shoots of various forages was examined growing in acid, manganiferous soil. Sampling leaves of a particular physiological age in a field-growing breeding population provided an adequate indicator of manganese concentrations in whole shoots. Silage Mn levels in the current study were found to be much higher than 40 mg/kg DM, the critical level of Mn and found to be sufficiently higher to meet the requirements of ruminants (Tejada *et al.*, 1987; Prabowo *et al.,* 1990; Velasquez-Pereira *et al.*, 1997; Khan *et al.*, 2007; Mirzaei 2012). It has however been reported that low soil Co level would also be a possible explanation of high level of Mn in forages just like in the current study with Mn levels varying between 94.8 -125.6 mg/kg DM, as these elements antagonize in the soil though showing no significant difference (Santana and McDowell, 1994; Khan *et al.*, 2006; Khan *et al.*, 2007).

In a study conducted by the ARC in 1980 and Mirzaei (2012), it was reported that 30 mg/kg DM is the critical level of dietary Zn, although it has been recommended that concentrations ranging between 12 - 20 mg/kg DM are adequate for growing ruminants. In the current study Zn concentrations ranged between 33.3 mg/kg DM – 42.3 mg/kg DM which were slightly higher than the reported critical level. However, forage remains/residues may thus constitute a marginal supply of Zn but the necessity for supplementary Zn needs to be kept under review particularly for goats and sheep in addition to cattle, which require some 35 mg/kg diet (McDowell *et al.*, 1978). This requirement falls well within range in the current study with the control at 36.1 mg/kg DM that is slightly higher and 30% inclusion performing just a little lower at 33.3 mg/kg DM though it showed no significant difference.

It is suggested that the dietary requirement of ruminants for Cu ranges from 8 to 14 mg/kg DM (NRC 1986; Khan *et al.* 2006; Mirzaei 2012). Hence, the current study shows the control and 10% inclusion level falling well within the required range at 11.0 mg/kg DM and 11.8 mg/kg DM respectively. However, 30% inclusion level shows a slightly higher concentration at 15.1 mg/kg DM and 50% showing a much higher concentration at 29.9 mg/kg DM. Copper concentrations of the silages are however not significant with an increase in concentrations as the inclusion levels increase. This situation may be further explained by high levels of dietary Fe. It has also been found that dietary concentrations exceeding 1 g Fe/kg can profoundly reduce the availability of ingested Cu; relatively slight and doubtless common levels of dietary soil contamination can produce Fe concentrations of this order, necessitating the serious consideration of providing

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supplementary Cu in grazing livestock. Hence, Forage Cu content also declines with forage maturity, and is higher in leaf vs. stem fractions. (Rojas *et al.*, 1993; Khan *et al.*, 2007).

3.5 Conclusion

The Inclusion of Marula pulp at different levels during the ensilage of Napier grass improves the fermentation process, promoting good lactic acid contents and reducing the pH, dry matter loss and ammoniacal nitrogen. The nutritional quality of the Napier grass silages improves with the inclusion of Marula pulp, increasing the dry matter and crude protein contents without, however, diminishing the acid and neutral detergent fiber contents. However, the silages were good with 10 % inclusion having the highest CP (97.0 g/Kg) and lowest pH of 3.5. It can therefore be concluded that the addition of 10% Marula pulp has the best potential to be an additive in silage making as compared to the other treatments.

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CHAPTER FOUR:

EFFECT OF MARULA PULP ON DRY MATTER, CRUDE PROTEIN AND NEUTRAL DETERGENT FIBRE RUMEN DEGRADABILITY OF NAPIER GRASS SILAGE

4.1 Introduction

The waste products from Marula processing have the potential to be included in animal diets. The use of fruit residues in animal feed can be beneficial to animal diet in South Africa. As a result, this therefore ensures greater chances of increasing feed availability and at the same time reduce environmental pollution caused by discarding these by-products (Cruz *et al.*, 2013).

In situ techniques can be used in the evaluation of feed quality, the amount of degraded feed and the rate at which feed is being degraded that accounts for the variety of digestive events that occur in an animal (Santos *et al.*, 2012). In addition, Jobim *et al.,* (2012), reported that the knowledge of rumen kinetics generates important information about digestive processes best describing the nutritional value in order for food nutritionists to provide the best care when formulating and balancing animal rations.

The ruminal *in situ* incubation technique is a reference method to estimate degradation parameters, such as soluble, insoluble but degradable, undegradable fractions, outflow rate and potential degradability. Parameters are quantified by the application of the equation, $P= a + b (1 - e^{-ct})$. In addition, the method can also be used to determine effective degradability when adjusted to suitable nonlinear models (Ørskov and McDonald, 1979). The characterization of the fractions that constitute carbohydrates of food obtained in tropical conditions and the evaluation of its degradation kinetics pattern are valuable tools in diet formulation because they aim to maximize ruminal microbial growth and consequently result in a better prediction of animal performance (Dantas *et al.*, 2014; Negrão *et al.*, 2014).

There is no information regarding the degradation kinetics of silage with different inclusion levels of Marula pulp in Napier grass silage. The objective of this experiment was to evaluate the effect of Marula pulp inclusion in Napier grass silage on dry matter (DM), crude protein (CP) and neutral detergent fibre (NDF) rumen degradability profiles.

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4.2 Materials and method

4.2.1 Experimental site

The study was conducted at the University of Venda, School of Agriculture Experimental Farm as described in Chapter 3, subsection 3.2.1.

4.2.2 Ethical consideration

Approval from the Ethics Committee of the University of Venda was sought and granted before the research trials with animals begun, **Project no: SARDF/16/ANS/07/2208**. In this case, the caring of animals according to the guidelines of the animal's ethics committee of the University of Venda were duly adhered to.

4.2.3 Animals and management

Three mature Bonsmara steers (weighing approx. 420 kg), with surgically fitted rumen cannulae of 10 cm internal diameter (ANKOM-flexible cattle purchased from Bar Diamond Inc.) were used to determine the degradability profiles of DM, CP and NDF of Napier grass-Marula silage. The animals were housed in individual pens and fed commercial complete cattle finisher diet (Table 4.1) *ad libitum*, starting 21 days prior to the commencement of the incubation of bags. Clean drinking water was always available in drinking troughs.

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Table 4.1: Chemical composition of commercial complete cattle finisher diet that was used in the study

Composition	g/kg
Protein (min)	120
Calcium (max)	8
Phosphorus (min)	3
Moisture (max)	120
Fibre (max)	200
Fat (min):	25
Urea (max):	1.25
% Derived from Urea	29.9%
	mg/kg
Monensin NA	30
Zinc Bacitracin	50

*Supplied by Driehoek feeds (Vaal water, Waterberg, Limpopo, South Africa)

4.2.4 Napier grass silage and sample preparation

Napier grass samples used in current experiment were prepared as described in Chapter 3. Before rumen incubation, representative samples from each silage treatment (0% Marula pulp, 10% Marula pulp, 30% Marula pulp and 50% Marula pulp) were oven dried at 60 ºC for 48 h (AOAC, 1990; method 930.15) and ground to pass a 1 mm screen.

4.2.5 Experimental design

The experiment was arranged in a completely randomized design (CRD) with four inclusion levels (0%, 10%, 30% and 50%) of Marula pulp serving as factors.

4.2.6 Determination of rumen degradability

Duplicate bags per silage treatment sample were incubated simultaneously in the ventral rumen of three Bonsmara steers. Nylon bags (external dimension: 6 x 12 cm, 46-µm pore size) containing approximately 5.0 ± 0.5 g of Napier grass silage were incubated in the rumen of each

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animal. The nylon bags were attached, using plastic bands, to flexible vinyl plastic tubes which is resistant to rumen micro-organism, approximately 40 cm long and of 6 mm outer diameter. The flexible vinyl plastic tubes were tied with 10 cm ropes and then secured to a rubber stopper. Nylon bags were inserted into the rumen and withdrawn in a subsequently at: 6, 12, 24, 48, 72, 96 and 120 h of incubation (Osuji *et al.*, 1993). All incubations began at 07:00, an hour before the morning feeding. After recovery from the rumen, the bags were washed using running tap water until the water was clear. The same procedure was applied to a series of bags to obtain the 0 hour value.

4.2.7 Chemical analysis

The washed bags were dried in a force air oven at 60 ºC for 48 h (AOAC, 1990; method 930.15), desiccated for 30 minutes and then weighed to determine DM content. The final residues in all bags were composited by silage treatment, incubation hour and steers and subsequently ground through a 1 mm sieve and analysed in duplicates. The residues were analysed for Nitrogen (N) content using the Kjeldahl procedure (AOAC, 1990; method 984.13) and N was converted to CP as N x 6.25. NDF contents were determined using the technique of Van Soest *et al.* (1991).

4.2.8 Mathematical calculations

Degradability was calculated as the difference between the nitrogen initially present in the bag and that present after incubation, stated as a proportion of the initial nitrogen (Ørskov and McDonald, 1979). The DM, CP and NDF degradability characteristics were then calculated according to the model of Ørskov and McDonald, (1979):

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

Where: P = the DM, CP and NDF disappearance at time t

a is the soluble fraction;

b is the insoluble but potentially rumen degradable fraction and

c is the rate of passage through the rumen

Potential degradability (PD) was estimated as (*a* + *b*) and effective degradability was calculated at rumen fractional outflow rates (Kp) of 0.02, 0.05 and 0.08 per hour representing low, medium

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and high feeding amounts and typical rates of ruminal solid outflow for forages according to Ørskov and McDonald (1979):

ED = a + bc/(k+c)

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

4.2.9 Statistical analysis

Analysis of variance (ANOVA) on silage degradability data was performed at $P < 0.01$ and $P < 0.05$ according to Steel and Torrie (1980) using General Linear Model (GLM) procedures of MINITAB Statistical package version 17 (Minitab, 2014). Significant differences between the treatments were detected, means were separated using the Tukey's test (Tukey, 1953).

Statistical model for silage degradability data was as follows:

$Yij = \mu + A_i + L_j + \varepsilon_{ijk}$

Where,

Y_{ijk} = Observation - ruminal degradability of DM, CP and NDF, ruminal kinetics;

μ = Overall mean;

A_i = fixed animal effect, i = 1, 2 or 3;

L_j = Effect of the j^{th} levels of Marula pulp inclusion, j = 1, 2, 3 or 4;

ε_{ijk} = Random error

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4.3 Results

4.3.1 *In sacco* dry matter, crude protein and neutral detergent fibre degradability

In sacco DM disappearance (% DM) of Napier grass silage is presented graphically in Figure 4.1 below. Fifty percent Marula pulp inclusion increased DM disappearance at 0, 6, 96 (all at $P<0.01$) and 120 ($P<0.05$) incubation hours with no effect on 12, 24, 48 and 72 incubation hours.

Figure 4.2 show a linear expression of CP disappearance with an increase in incubation time. All silages showed a similar ($P>0.05$) increasing trend for CP degradability at 0, 24, 48 and 72 incubation hour. However, 50 % Marula pulp inclusion had great CP disappearance at 6 and 96 ($P<0.05$), 12 and 120 ($P<0.01$) incubation hours than the other treatments.

Figure 4.3 below shows the *in sacco* NDF disappearance trend. Marula pulp inclusion had no effect ($P>0.05$) on NDF disappearance at 0, 6, 12, 48, 96 and 120 incubation hours. However, 50 % Marula pulp inclusion increased NDF disappearance at 24 ($P<0.05$) while no additive treatment was greater ($P<0.05$) than other treatment at 72 incubation hour.

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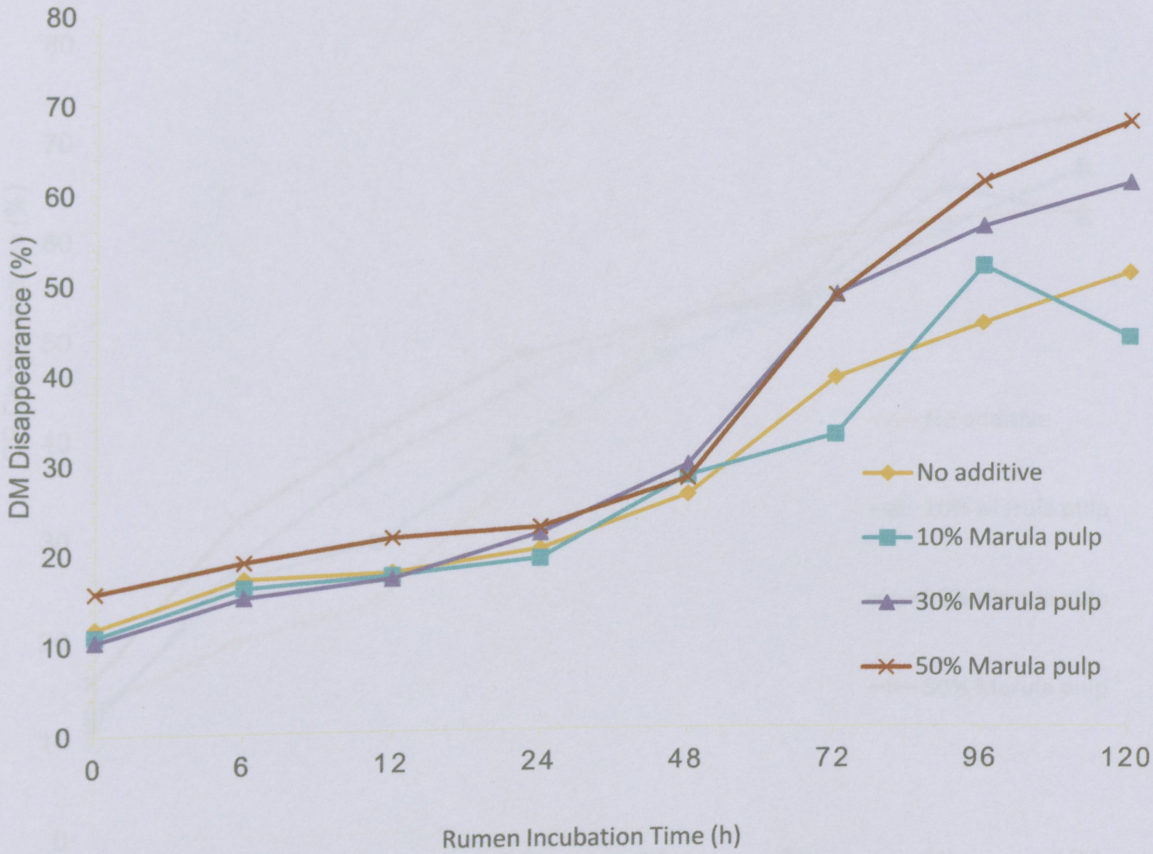


Figure 4.1. Effect of Marula pulp inclusion on *in sacco* dry matter disappearance (%) of Napier grass silage after 8 weeks of ensiling

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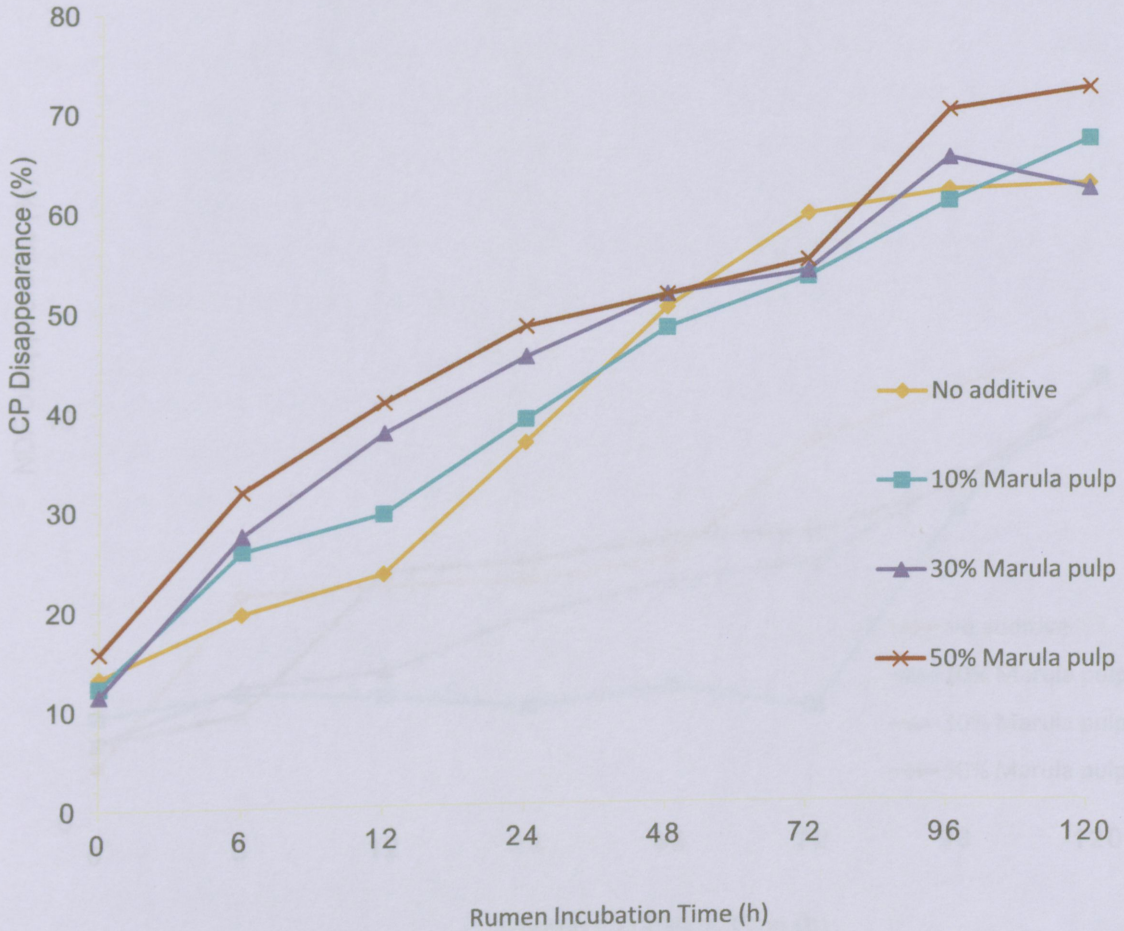


Figure 4.2 Effect of Marula pulp inclusion on *in sacco* Crude protein disappearance (%) of Napier grass silage after 8 weeks of ensiling

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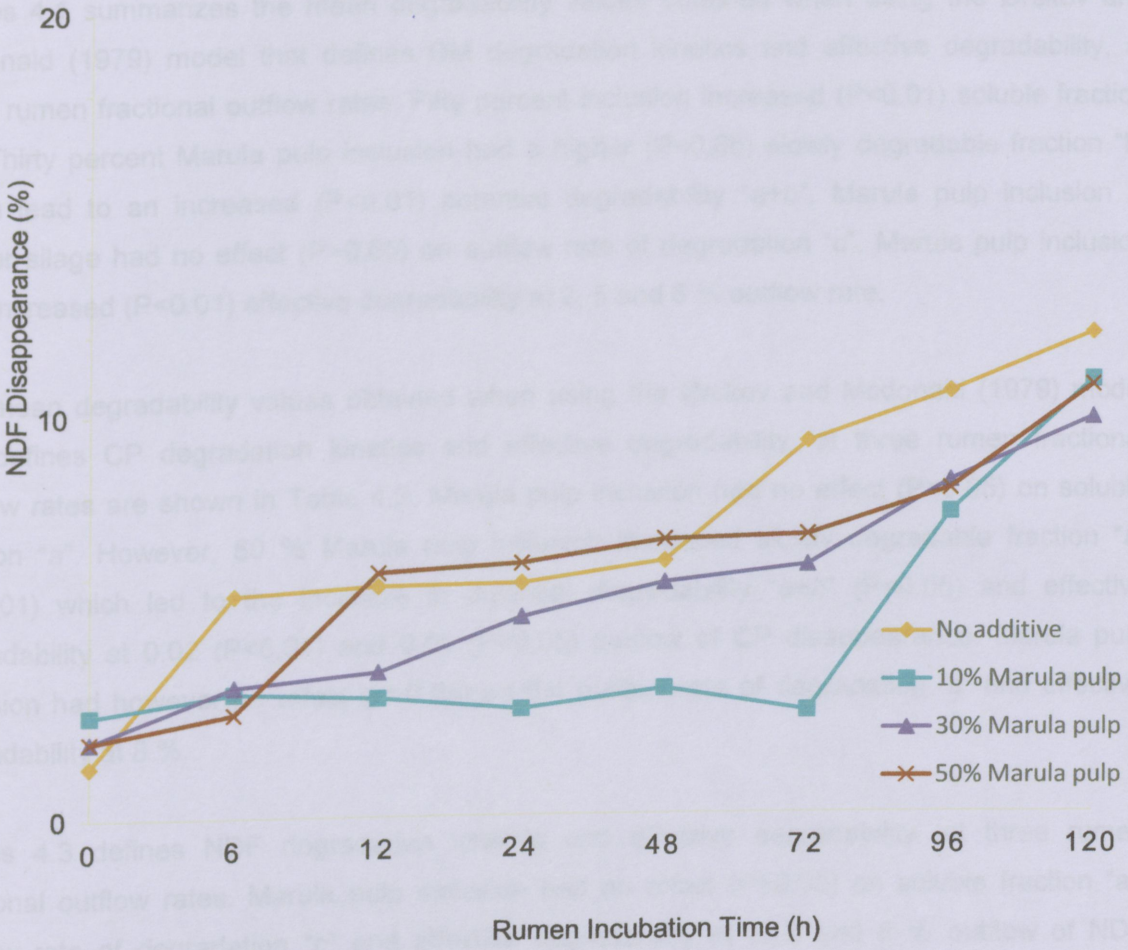


Figure 4.3 Effect of Marula pulp inclusion on *in sacco* NDF disappearance (%) of Napier grass silage after 8 weeks of ensiling

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4.3.2 Dry matter, crude protein and neutral detergent fibre degradability kinetics

Tables 4.1 summarizes the mean degradability values obtained when using the Ørskov and Mcdonald (1979) model that defines DM degradation kinetics and effective degradability, at three rumen fractional outflow rates. Fifty percent inclusion increased (P<0.01) soluble fraction "a". Thirty percent Marula pulp inclusion had a higher (P<0.05) slowly degradable fraction "b" which lead to an increased (P<0.01) potential degradability "a+b". Marula pulp inclusion in Napier silage had no effect (P>0.05) on outflow rate of degradation "c". Marula pulp inclusion also increased (P<0.01) effective degradability at 2, 5 and 8 % outflow rate.

The mean degradability values obtained when using the Ørskov and Mcdonald (1979) model that defines CP degradation kinetics and effective degradability, at three rumen fractional outflow rates are shown in Table 4.2. Marula pulp inclusion had no effect (P>0.05) on soluble fraction "*a*". However, 50 % Marula pulp inclusion increased slowly degradable fraction "*b*" (P<0.01) which led to the increase in potential degradability "*a+b*" (P<0.05) and effective degradability at 0.02 (P<0.01) and 0.05 (P<0.05) outflow of CP disappearance. Marula pulp inclusion had however no effect (P>0.05) on the outflow rate of degradation "c" and effective degradability at 8 %.

Tables 4.3 defines NDF degradation kinetics and effective degradability, at three rumen fractional outflow rates. Marula pulp inclusion had no effect (P>0.05) on soluble fraction "a", outflow rate of degradation "c" and effective degradability at 2, 5 and 8 % outflow of NDF disappearance. However, 10 % Marula pulp inclusion had higher (P<0.01) slowly degradable fraction "b" which also led to an increased (P<0.01) potential degradability "a+b".

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Table 4.2 Degradability constants and effective degradability at three outflow rates for DM disappearance of Napier grass silage after 8 weeks of ensilage

Additive	Degradability constants				ED (%) at different outflow rates		
	a	*b*	*c*	*a+b*	*K* = 0.02	*K* = 0.05	*K* = 0.08
Control	11.9^{b}	82.2^{ab}	0.003	94.1^{ab}	28.1^{bc}	18.7^{b}	16.1^{b}
10%	10.9^{b}	65.6^{b}	0.007	76.5^{c}	26.6^{c}	18.0^{b}	15.4^{b}
30%	12.1^{b}	87.9^{a}	0.002	100.0^{a}	31.2^{b}	19.7^{b}	16.6^{b}
50%	15.7^{a}	84.2^{ab}	0.002	100.0^{a}	35.9^{a}	22.8^{a}	19.6^{a}
SEM	0.71	5.20	0.001	4.95	1.11	0.73	0.62
Significance	**	*	ns	**	**	**	**

**: P <0.01; *: P <0.05; (ns) non-significant: P >0.05. ^{ab} Column means with different superscripts differ significantly at P <0.05. *a*: soluble fraction (%), *b*: insoluble but potentially degradable fraction (%), *a+b*: Potential degradability (%), *c*: outflow rate of degradation (h^{-1}), ED: effective degradability, *k*: rumen outflow rate (h^{-1}) and SEM: Standard Error Mean

Table 4.3 Degradability constants and effective degradability at three outflow rates for CP disappearance of Napier grass silage after 8 weeks of ensilage

Additive	Degradability constants				ED (%) at different outflow rates		
	a	*b*	*c*	*a+b*	*K* = 0.02	*K* = 0.05	*K* = 0.08
Control	15.5	50.8^{b}	0.059	66.3^{b}	41.4^{a}	31.2^{a}	26.3
10%	12.3	67.2^{ab}	0.013	79.6^{ab}	34.4^{ab}	23.9^{b}	20.2
30%	11.5	55.0^{b}	0.111	66.5^{b}	40.2^{ab}	31.0^{a}	26.3
50%	15.8	81.2^{a}	0.005	92.9^{a}	33.2^{b}	23.8^{b}	21.0
SEM	2.01	6.35	0.028	7.03	1.85	2.05	1.93
Significance	ns	**	ns	*	**	*	ns

**: P <0.01; *: P <0.05; (ns) non-significant: P >0.05. ^{ab} Column means with different superscripts differ significantly at P <0.05. *a*: soluble fraction (%), *b*: insoluble but potentially degradable fraction (%), *a+b*: Potential degradability (%), *c*: outflow rate of degradation (h^{-1}), ED: effective degradability, *k*: rumen outflow rate (h^{-1}) and SEM: Standard Error Mean

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Table 4.4 Degradability constants and effective degradability at three outflow rates for NDF disappearance of Napier grass silage after 8 weeks of ensilage

Additive	Degradability constants				ED (%) at different outflow rates		
	a	*b*	*c*	*a+b*	*K* = 0.02	*K* = 0.05	*K* = 0.08
Control	1.3^{b}	71.2^{a}	0.001	72.5^{ab}	4.3	2.5	2.1
10%	2.6^{a}	97.4^{ab}	0.001	100.0^{a}	4.6	3.0	2.7
30%	1.9^{b}	36.3^{bc}	0.319	38.2^{bc}	2.7	2.7	2.6
50%	1.9^{b}	33.5^{c}	0.002	35.4^{c}	4.4	2.9	2.6
SEM	1.14	8.77	0.159	9.50	1.13	1.07	1.06
Significance	**	**	ns	**	ns	ns	ns

**: P <0.01; *: P <0.05; (ns) non-significant: P >0.05. ^{ab} Column means with different superscripts differ significantly at P <0.05. *a*: soluble fraction (%), *b*: insoluble but potentially degradable fraction (%), *a+b*: Potential degradability (%), *c*: outflow rate of degradation (h^{-1}), ED: effective degradability, *k*: rumen outflow rate (h^{-1}), N: Number of Observations and SEM: Standard Error Mean

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4.4 Discussion

4.4.1 *In sacco* dry matter, crude protein and neutral detergent fibre degradability

The disappearance of DM contents increased linearly with the duration of ruminal incubation (Figure 4.1). This was expected, because the longer the residue remains in the rumen the more prolonged microbial action is endured. According to Uchoa *et al.* (2008), the disappearance of the DM in the by-product begins after 3 hours, because fruits rich in sugars (glucose and fructose) have a high fermentation potential. Regarding the effect of incubation period, all silages displayed increasing values for DM disappearance until 96 h with 50 % additive inclusion performing best at 60.76 %. During the time interval 0 to 24 h, the silages with Marula pulp inclusion and the silage made exclusively with Napier grass showed a similar leveled trend in DM disappearance values. However, during the time interval of 24 to 96 h of incubation, the DM disappearance values were higher in the silages with Marula pulp inclusion as compared to the exclusive Napier grass silage. This may be because high solubility and digestibility of carbohydrate additives in the rumen increased DM disappearance of the silage (Gomes *et al.*, 2015) by breaking down cell wall bonds during fermentation of Napier silage resulting in improvement of DM degradability (Fazaeli *et al.*, 2004 and Nasehi *et al.*, 2014).

Unlike the results recorded in the present study, in which greater DM disappearance values were found in the silages with an increase in Marula pulp additive inclusion levels, Rogério (2005) observed that only at the incubation time of 6 h did the pineapple byproduct show higher DM disappearance values (34.64 % DM) than Napier grass (30.27 % DM), stabilizing at 12 h and 24 h of incubation, while the elephant grass displayed increasing values until 96 h. However, at 96 h, both feeds showed the highest DM disappearance rate (59.28 and 68.79 % DM, respectively). According to the author, the response was related to the nutritional characteristics of the evaluated elephant grass compared with that of pineapple byproduct. Nevertheless, in the present study, Marula pulp inclusion silages showed a stabilized increasing effect up to 24 h, picking at 48 h with 10, 30 and 50 % showing higher DM disappearance values (28.11, 29.36 and 27.82 % respectively) as compared to Napier grass silage with no additive inclusion (0 %) with a slightly lower value of 26.01% DM disappearance. Hence, at 120 h, all silages (0, 10, 30 and 50) showed the highest dry matter disappearance rate (50.66, 43.43, 50.79 and 67.57 % respectively).

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In determining CP disappearance, it was found that both incubation time and levels of Marula pulp additive inclusion to the ensilage of Napier grass interfered with CP disappearance, with increasing values for all levels of addition of the byproduct.

The influence of the level of inclusion of the byproduct in the disappearance of CP, from 0 to 24 h incubation time, the silages containing Marula pulp showed a higher disappearance value than the silage made with elephant grass only. At 96 h, the exclusive Napier grass silage showed a similar CP disappearance value to that observed in the silage with 10 % Marula pulp inclusion. The higher CP disappearance values (Figure 4.2) in the silages containing 10, 30 and 50 % Marula pulp inclusion as compared to the exclusive Napier grass silage may be related to the increase in the CP contents of the silages in which the byproduct was included. Habib *et al.* (2013) stated that the rumen CP degradation kinetics of high protein byproducts are strongly affected by intrinsic variation in feedstuffs, e.g., plant cultivars, growing conditions, and processing methods, and extrinsic factors, such as bag pore size and substrate particle size.

In an evaluation carried out using the pineapple byproduct, Rogério (2005) indicated that the disappearance of CP from the byproduct increased to 13.58 % from 6 to 12 h of incubation, showing lowest degradation at 24 h, and then increased again to 14.82 % at 48 h, with the greatest disappearance observed at 96 h (73.11 %). In comparison with Napier grass, the author noted that the CP disappearance values always increased, and were higher than that of the pineapple byproduct. These results differ from the response observed in the current study, in which higher CP disappearance rates were found in the silage with Marula pulp additive inclusion where 10, 30 and 50 % inclusion increased to 29. 40, 37.51 and 40.60 % respectively from 6 to 12 h as compared to the no additive inclusion (0 %) silage which increased to only 25.31 %. The current study showed a higher CP content with increased Marula pulp inclusion.

The silages showed slow degradation at 48 h, increasing again at 72 h, recording the greatest CP disappearance at 120 h with 0 % inclusion at 62.28%, 10 % inclusion at 66.79 % and 50 % at the highest at 72.02 % CP disappearance. Hence, 30 % inclusion recorded the highest disappearance at 96 h (64.9 %) decreasing to 61.75 % at 120 h. However, the CP content of all the Napier silages was above the minimum CP level of 7.5 % required for adequate rumen function in ruminants (Van Soest, 1994). It was also above the minimum CP content as stated in Tessema and Baars (2004) required for lactation and growth of cattle which is 15.0 % (Norton,

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1981) suggesting that the Napier-Marula pulp mixtures were almost equal, and/or slightly above, the correct recommendations.

The disappearance of NDF increased linearly with the duration of ruminal incubation especially the 30 % Marula pulp additive inclusion curve from 0 to 120 h, recording its highest value at 120 h (9.84 %) as compared to the other silages (Figure 4.3). About the effect of the addition of Marula pulp inclusion on the disappearance of NDF at each incubation time, it was observed that the exclusive elephant grass silage had higher values than the others (Figure 4.3). The no additive inclusion curve increased to 5.52% from 0 to 6 h, stabilizing at slow degradation from 6 to 48 h, increasing once again from 48 to 120 h and recording the highest NDF disappearance at 120 h (11.96 %).

Taking a look at Marula pulp addition on the disappearance of NDF, it is likely that the better nutritive value provided by the increased CP content benefited the disappearance of the fibrous portion of these silages as compared with the exclusive Napier grass silage (Figure 4.3). According to Krause *et al.* (2013), there is a cooperation between microorganisms in the rumen, for example, proteolytic and cellulolytic bacteria, and this synergism might have taken place in the silage with the highest Marula pulp inclusion content (50 %). A 10 % Marula pulp additive inclusion showed a rather alarming decrease from 0 to 72 h, increasing drastically from 72 to 120 h, recording its highest NDF disappearance at 120 h (10.78 %). However, 50 % additive inclusion increased to 6.06 % from 6 to 12 h, showing slow degradation from 12 to 72 h and increasing again from 72 to 120 h, recording its highest NDF disappearance at 120 h (10.65 %). In a previous study Rogério (2005) observed that the pineapple byproduct took a longer time to show differences in the degradation of NDF, as a function of the incubation times, and at 48 h and 96 h (35.75 and 47.55 %), the disappearance values were lower than those found for the Napier grass (46.60 and 61.63 %). Studying the addition of annatto byproduct in Napier grass silage, Rêgo *et al.* (2010) found that the disappearance of NDF at 96 h of incubation increased up to 16% of addition. Hence, this differs with the current study as the highest disappearance of NDF at 120 h was found to be lower than that of the previous study at 11.96 %. This can be explained in that the addition of non-fibrous sources in this case Marula pulp might have diluted the proportion of these fibre as a result enhancing fermentation and subsequently increasing cell wall degradation (Baytok *et al.*, 2005)

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4.4.2 Dry matter, crude protein and neutral detergent fibre degradability kinetics

For rumen degradation parameters of DM, Table 4.1, shows an effect of Marula pulp additive inclusion as levels of addition increased, this led to an upward trend in the readily degradable fraction "a" and in the degradation rate of fraction "b" or slowly degradable fraction. The fraction "a" value for the silages containing elephant grass exclusively was found to be 11.9 % and those with Marula pulp addition (10, 30 and 50 %) were 10.9, 12.1 and 15.7 %, respectively, however showing highly significant difference. The degradation rates of fraction "b" however, showed significant difference with silages of Napier grass only recorded at 82.2 % and those with Marula pulp inclusion (10, 30 and 50 %) were 65.6, 87.9 and 84.2 %, respectively.

Manoel *et al.* (2003) evaluated the degradability of DM from fruit pulp waste, and observed that in pineapple byproducts 20.80 % for fraction "a", the degradation rate of 0.05 h^{-1}, and effective degradability (ED) values of 49.20 % for the rate of 0.05 h^{-1}. Casali *et al.* (2004), on the other hand, evaluated the degradability of Napier grass silages and found fraction "a" values of 14.73 %, and fraction "c" of 0.0274 h^{-1}, and ED values of 32.37 % for the passage rate of 0.05 h^{-1}, which are close values to those obtained in the exclusive Napier grass silages from the present study recorded at 15.7 % for fraction "a" and fraction "c" of 0.002 h^{-1}, and ED value of 35.9 % at 2% outflow rate for 50 % Marula pulp additive inclusion silage. Working with silage of pineapple byproduct, Lallo *et al.* (2003) found mean values of 28.80 and 49.40 % DM, respectively, for parameters "a" and "b", and 0.042 h^{-1} for the degradation rate of "c" of DM.

Marula pulp inclusion effect on PD (a+b) of DM, 30 and 50 % inclusion levels showed the highest effective DM degradability as compared to that of 0 and 10 %. This concept can be related to the inclusion of Marula pulp additive with non-fibrous properties which probably increased the slowly degradable fraction "b". The present study recorded the highest PD of DM at 100.00% for both 30 and 50 % inclusion levels.

The variability in the values of "a", "b", and "c" of CP (Table 4.2) of the silages can be attributed to the characteristics of the ensiled material (chemical composition) and to the fermentation quality of the forage in the silo. However no information regarding the evaluation of the nutritional quality of Marula pulp and the effects of its addition to Napier grass silage on the degradability of CP was found in previous literature. Hence, the present study shows that readily degradable fraction "a" of 50 % Marula pulp inclusion was superior to the other silages at 15.8 %. In terms of Marula pulp additive inclusion effect, slowly degradable fraction "b" was

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increased due to Marula pulp inclusion contradicting with Gomes *et al.* (2015) who reported a reduced trend in fraction "b" with an increase in glycerin in silage. The present study however, showed an increasing trend in fraction "b" with 50 % inclusion recorded the highest value at 81.2 % which suggests a good source of degradable rumen protein. Although this value falls a little out of range as compared to the results reported by Kaufmann (1979), where an average ruminal CP degradability of feed is said to fall between 60 to 80 %, 10 % Marula inclusion in this study however falls well within this range at 67.2 % for fraction "b".

The outflow rate "c" in the present study was not affected by additive inclusion and similarly responded to all treatments. The PD of CP in this study was positively affected by Marula pulp additive inclusion in silages. The highest PD of CP was observed in 50 % Marula inclusion at 92.9 %. This can be accredited to its high slowly degradable fraction "b" (Table 4.2). An increase in PD of CP may however, indicate that silages treated with Marula pulp additive inclusion experience a decrease of CP quantity that enters the small intestine (Goes *et al.*, 2012). ED of the present study increased ranging between 33.2 to 41.4 % at 2 % outflow rate, 23.8 to 31.2 % at 5 % outflow rate, hence additive inclusion had absolutely no effect on ED at 8 % outflow rate of CP (Table 4.2).

The silages with Marula pulp inclusion had a highly significant effect on the soluble fraction "a" of NDF, and displaying higher values than the silage with Napier grass exclusively (Table 4.3). The values found in the silages with addition of Marula pulp additive inclusion (1.3 to 2.6 %) can be considered suitable, since the fibrous fraction is mostly insoluble. This fact can be explained by the greater passage of the material through the pores of the bags during washing. Campos *et al.* (2006) reported values of slowly degradable NDF fraction "b" of 63.4% for corn silage, 61.7%, for Napier grass silage and 51.4% for sorghum silage. These authors also reported values of fractional rate of NDF disappearance of 3.08%/h for corn silage, 3.10%/h for Napier grass silage and 2.53%/h for sorghum silage.

Campos *et al.* (2003), reported NDF effective degradability calculated for fractional rate of particulate passage of 2%/h ranged from 30.3 to 39.1% for the silages of different sorghum hybrids. Working with Napier grass silages ensiled with different regrowth ages, Rêgo *et al.* (2011) observed potentially degradable NDF fraction ranging from 46.0 to 58.5%. In the present study, the potentially degradable NDF fraction ranged from 35.4 to 100%, with fractional rate of NDF disappearance ranging between 0.001 and 0.319 %/h. The NDF effective degradability

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values were between 2.7 to 4.6 at 2 % outflow rate, 2.5 to 3.0 % at 5 % outflow rate and also 2.1 to 2.7 % at 8 % outflow rate, respectively for all silages. These results show a good potential degradation of the fibre fraction from Napier grass silages.

4.5 Conclusion

Marula pulp additive inclusion helped improve the DM and CP, PD (potential degradability) and ED of the silage at all inclusion levels. Furthermore, the inclusion had no significant effect on NDF degradability as compared to no additive silage. However, 50 % inclusion level performed at a superior level with regard to the amount of soluble fraction (a) as compared to other treatments. It can therefore be concluded that 50 % inclusion is a better option to use as an additive when ensiling as compared to the other three treatments given the degradability results.

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CHAPTER FIVE:

EFFECT OF MARULA PULP INCLUSION ON *IN VITRO* DRY MATTER AND CRUDE PROTEIN DIGESTIBILITY OF NAPIER GRASS SILAGE

5.1 Introduction

The rate and extent of ruminal protein degradation is very crucial because it determines nitrogen availability to microorganisms and amino acids in the small intestine to the host animals (Promkot *et al.*, 2007). The protein consumed by the animal should be partly degradable in the rumen, as peptides, amino acids and NH_3-N derived from proteolysis and be used in microbial protein synthesis and to improve rumen ecology. It is, therefore, very important to determine the degradability and digestion of different feed ingredients which are grown and used in different locations (Promkot *et al.*, 2007).

Although many techniques have been used to improve the nutritive value of forages and appreciable results obtained, there is still no information on the utilization of Marula pulp as additives during fermentation of tropical forages. Digestibility experiments that have been conducted with grasses and silage show considerable value in the estimation of their nutritive value to the ruminant, but these are tedious experiments that require large quantities of forage. It is in this regard that rapid techniques such as *in vitro* and *in situ* or *in sacco* attracted wide recognition. These techniques satisfactorily provide a rapid guide for measuring *in situ* nutrient disappearance as well as offer quantitative estimates of degradable feed available for rumen microbes and un-degradable feed which may be available for post rumen microbial-enzyme digestion (Yahaya *et al.*, 2004).

However, it must be remembered that every technique given its advantages also has limitations. There are three important limitations when conducting this *in vitro* technique. Firstly, since the sample was confined within the bag and it was therefore not exposed to any breakdown due to chewing and rumination. Secondly, feed would normally be able to leave the rumen once broken down to a suitable particle size. Thirdly, it must be remembered that, strictly speaking, what was measured was the material broken down to a size small enough to leave the bag and not necessarily a complete degradation to simple chemical compounds. It is in this regard that the results of this study must therefore be treated with due caution, and, in general, be used as qualitative indicators of general principles (Ørskov *et al.*, 1980).

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This laboratory study was therefore conducted to determine the *in vitro* dry matter and crude protein digestibility of Napier grass (*Pennisetum purpereum*) without additives (control) and with the inclusion of additives of Marula pulp (Marula processing by products).

5.2 Materials and method

5.2.1 Experimental site

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

5.2.2 Sample preparation

Representative samples from each silage treatment (0% Marula pulp, 10% Marula pulp, 30% Marula pulp and 50% Marula pulp) of Napier silage were dried and ground to pass a 2 mm screen. Eight samples of approximately 5 g from each silage treatment were used as prepared in Chapter 3.

5.2.3 Ethical consideration

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

5.2.4 Experimental design

The experiment was arranged in a completely randomized design (CRD) with four inclusion levels (0%, 10%, 30% and 50%) of Marula pulp serving as factors.

5.2.5 *In vitro* three-step digestibility

Approximately 5 g of the ground silage samples was weighed and put into well-labeled nylon bags (6 × 12 cm, pore size of 46 µm). The nylon bags were attached, using elastic bands, to flexible vinyl plastic tubes, approximately 40 cm long and of 6 mm outer diameter. The flexible vinyl plastic tubes were tied with 10 cm ropes and then secured to a rubber stopper. Triplicate

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bags per silage treatment per incubation time per animal were inserted into the rumen and withdrawn in subsequently at: 12, 24 and 48 incubation h. The bags were inserted in the rumen at 06:00 before the morning feeding time. After each incubation time, the bags were removed from the rumen, washed gently under low running tap water without squeezing, till runoff was clear, then finally washed with deionized water and dried at 60 °C for 48 hours. Rumen un-degradable residuals (RURs) were removed from the bags by manually removing the residues. The RURs were composited by silage treatment, incubation hours and the steers and subsequently ground through a 1 mm sieve.

For the following pepsin + pancreatin digestion trial, a total of 3 bags containing 1 g of RURs of each silage sample were introduced into incubation bottle which contained 2 ℓ of a 0.1 N HCl solution adjusted to pH 1.9 with 1 g ℓ^{-1} of pepsin (P-7000; Sigma), and were incubated for 1 hour with constant horizontal movement at 39°C. After incubation, the bags were rinsed with tap water till runoff was clear, then finally rinsed with deionized water and introduced into the incubation bottles containing 2 ℓ of a pancreatin solution (0.5 mol ℓ^{-1} KH_2PO_4 buffer adjusted to pH 7.75, containing 50 mg kg^{-1} of thymol to prevent bacterial growth and 3 g ℓ^{-1} of pancreatin (P-7545; Sigma). Bags were incubated for 24 hours with constant horizontal movement at 39°C. After incubation, bags were rinsed with tap water until the runoff was clear, then finally rinsed with deionized water and dried at 60 °C for 48 hours. The final residues in all bags were composited by the silage treatment, incubation hours and steers and subsequently ground through a 1 mm sieve. The residues were analyzed for DM and CP contents by the methods as follows;

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5.2.6 Chemical Analysis

Samples were dried at 60°C in an oven for 48 hours (h) to determine DM content (AOAC, 2005) and grounded through a grinding mill of 1 mm screen size. N content was analyzed using Kjeldahl procedure (AOAC, 2005) as described in Chapter 3.

5.2.7 Statistical analysis

Analysis of variance (ANOVA) on silage *in vitro* digestibility data was performed at $P < 0.01$ and $P < 0.05$ according to Steel and Torrie (1980) using General Linear Model (GLM) procedures of

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MINITAB Statistical package version 17 (Minitab, 2014). Significant differences between the treatments were detected, means were separated using the Tukey's test (Tukey, 1953).

Statistical model for ***in vitro*** **digestibility data** was as follows:

$$Yij = \mu + L_i + \varepsilon_{ij}$$

Where,

Y_{ij} = Observation – *In vitro* DM and CP digestibility;

μ = Overall mean;

L_i = Effect of the i^{th} levels of Marula pulp inclusion, i = 1, 2, 3 or 4;

ε_{ij} = Random error

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5.3 Results

5.3.1 *In vitro* dry matter digestibility

Data of ruminal degradability and *In vitro* digestbility of DM of Napier grass silage with the use of nylon bags are presented in Table 5.1 below. Marula pulp inclusion had no effect (P>0.05) on DM degradability at 12, 24 and 48 h of rumen incubation and also *in vitro* DM digestibility after 12, 24 and 48 h. As incubation time is prolonged DM degradability increases for all the silages (P>0.05) but has no significant difference on silage treatments, however as *in vitro* digestibility decreases with increase in incubation time.

Table 5.1 Effect of Marula pulp inclusion on *In vitro* DM (g/kg) digestibility of Napier grass silage rumen residues

Additives	N	Rumen incubation time (h)			*In vitro* dry matter digestibility		
		12	24	48	$IVDMD_{12}$	$IVDMD_{24}$	$IVDMD_{48}$
Control	9	187	222	261	119	90.2	93.7
10%	9	186	205	278	137	143	123
30%	9	193	214	271	131	124	79.4
50%		223	244	289	151	138	126
SEM		11.8	11.6	16.2	28.5	24.2	20.6

There were no significant differences among treatments. N: Number of observations, $IVDMD_{12}$: *In vitro* dry matter digestibility after 12 hours of rumen incubation, $IVDMD_{24}$: *In vitro* dry matter digestibility after 24 hours of rumen incubation, $IVDMD_{48}$: *In vitro* dry matter digestibility after 48 hours of rumen incubation and SEM: Standard Error Mean.

5.4.2 *In vitro* crude protein digestibility

Data of ruminal degradability and *in vitro* digestibility of CP of Napier grass silage with the use of nylon bags are presented in Table 5.2 below. Marula pulp inclusion had no effect (P>0.05) on CP degradability at 12, 24 and 48 h of rumen incubation and *in vitro* CP digestibility after 12, 24 and 48 h. As incubation time is prolonged CP degradability increases for all the silages (P>0.05) but had no significant difference on silage treatments. However, CP *in vitro* digestibility decreases with increase in incubation time. Both DM and CP *in vitro* digestibility depicted the same trend.

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Table 5.2 Effect of Marula pulp inclusion on *In vitro* CP (g/kg) digestibility of Napier grass silage rumen residues

Additives	N	Rumen incubation time (h)			*In vitro* crude protein digestibility		
		12	24	48	$IVCPD_{12}$	$IVCPD_{24}$	$IVCPD_{48}$
Control	6	160	189	236	108	90.8	94.4
10%	6	164	186	243	149	133	125
30%	6	132	193	240	128	124	76.1
50%	6	175	207	256	145	130	117
SEM		22.1	32.2	31.8	22.6	25.6	17.6

There were no significant differences among treatments. [abc] Column means with different superscripts differ significantly at $P < 0.05$. N: Number of observations, $IVCPD_{12}$: *In vitro* crude protein digestibility after 12 hours of rumen incubation, $IVDMD_{24}$: *In vitro* crude protein digestibility after 24 hours of rumen incubation, $IVCPD_{48}$: *In vitro* crude protein digestibility after 48 hours of rumen incubation and SEM: Standard Error Mean.

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5.4 Discussion

5.4.1 *In vitro* dry matter digestibility

There is no information on the effect of Marula pulp inclusion on *in vitro* dry matter digestibility of Napier grass-Marula silage. According to van Soest, (1994), positive correlation exists between IVDMD and feed intake, as such Napier grass silage digestibility improvements on residues after 48 h of incubation might suggest increased feed intake. However, higher potential degradabilities "a+b" for DM ruminal degradability of the silages (Table 4.1) in the present study might have been the major cause of reduction on *in vitro* DM digestibility

IVDMD values were low ranging between 118.8 – 151.0, 90.23 – 142.6, 93.7- 126.3 g/kg for residues after 12, 24 and 48 h of rumen incubation showing no significant differences at all, hence confirming Kaldmäe *et al.* (2009), who reported that inoculants into silage had no effect on IVDMD of red clover silages. Since all the silages in the present study were much lower than 650 g/kg minimum requirement for good nutritive value indication in a feed, this then implies a decreased feed intake for all silages.

5.4.2 *In vitro* crude protein digestibility

Beever *et al.* (1974) and Thomson *et al.* (1981) reported previously that CP degradability reduction when formaldehyde is used in treatment of silages resulted in an increase in protein quantity entering the small intestine. Hence, the current findings of this study are in contradiction to the findings by *Nowak et al.* (2004), who reported a significant increase of intestinal digestibility of undegradable protein using silage treated with formic acid. The current study however, shows that Marula pulp additive inclusion had no effect on IVCPD of Napier grass-Marula silage. There is also no available information on IVCPD of Marula pulp additive inclusion on Napier grass-Marula silage in previous conducted studies.

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5.5 Conclusion

Though Marula pulp additive inclusion showed no effect on IVCPD in the current experiment, its addition contributed to an increase in the DM content including rumen degradability of Napier grass silage, which provided a suitable DM in the ensiled material and consequently improved the silage. It can therefore, be concluded that since ruminal degradability plays a very crucial role on *in vitro* digestibility, the higher the rumen degradability the lower the post ruminal digestibility will be and the lower the rumen degradability the higher the post ruminal digestibility given the current findings.

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CHAPTER SIX: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General discussion

The specific objectives of the current studies were to determine the effect of Marula pulp inclusion at 0, 10, 30 and 50 % in Napier silage on the chemical composition (pH, Dry matter, Ash, Organic matter, Crude Protein, Non-Protein Nitrogen, Water Soluble Carbohydrates, Ammonia, Lactic Acid, Acid Detergent Fibre, Neutral Detergent Fibre and Acid Detergent Lignin), dry matter, crude protein and neutral detergent fibre rumen degradability profiles and *in vitro* dry matter and crude protein digestibility. Marula pulp additive was however, included during ensiling with the aim of stimulating LA production during fermentation which then would result in pH reduction thus improving silage quality (McDonald *et al.*, 1991). According to Lubisi, (2014) and Mutavhatsindi, (2015), it was generally reported that the use of additives in silage making has a positive effect on silage fermentation as it increases LA production which is the preferred acid in silage pH reduction.

After 8 weeks of ensilage, results in the current study indicated that Marula pulp inclusion improved DM content of Napier grass-Marula silage. This can be explained about WSC availability attributed to high levels of residual WSC which is utilized by LAB in directing the fermentation process towards LA production as the main fermentation product (Seglar, 2003; McDonald *et al.*, 2011) subsequently causing a decline in pH.

Longer incubation time (48 h) resulted in higher DM rumen degradability. Mgheni *et al.* (1993) suggested that a longer rumen incubation time is necessary to obtain maximum utilization of protein in tropical feeds. This suggestion was based on the observation of increased intestinal digestibility in mobile bags of the proteins in *Leucaena* leaves from 490 g/kg CP DM after 8 h to 930 g/kg CP DM after 96 h of preincubation in the rumen (Mgheni *et al.* 1994). Longer residence time in the rumen allows rumen microbes to attach and degrade the fibre that may otherwise be left undegraded. The value of protein is said to be based on the proportions of protein that will be degraded in the rumen and that which will escape rumen fermentation and be digested post-ruminally (Aganga and Tshwenyane, 2003). Hence, microbial protein synthesis is driven by this rumen degradable protein.

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DM contents disappearance of feed by the end of 48hours of incubation is generally considered to be equivalent to digestibility (Bhargava and Ørskov, 1987). This information provides an insight into the level of rumen undegradable DM post 96 hours. This is because the zero hour degradability could be misleading as some of the samples could have leaked during the washing of the bags instead of being degraded. This study however showed that the prepared silages DM disappearance values for 48hours were in contradiction with the recommended 40-50%(Preston, 1989) as the values were slightly lower.

Soluble DM contents of the silage however indicate the potential of them being good sources of more nutrients for microbial growth (Djouvinov and Todorov, 1994), since Clark *et al.* (1992), Gomes *et al.* (1994) and Masama *et al.* (1997) reported a strong positive relationship between DM intake and microbial growth. The effective degradability of CP followed a similar trend to that of dry matter although with lower values as previously reported by (Mgheni *et al.,* 1996). The disappearance of the CP in all the silages increased with increasing incubation time.

The increase in the degradability of soluble Fraction "a" can be attributed to a greater solubility of the product when isolated as compared with the grass, contributing to a higher soluble fraction of silage where the highest concentration of Marula pulp was added. A higher immediately soluble fraction shows that it has the potential for use as energy for microbial growth. Hence, the present study shows that readily degradable fraction "a" for CP of 50 % Marula pulp inclusion was superior to the other silages at 15.8 %. The silages with Marula pulp additive inclusion also had similar (P>0.05) values for the soluble fraction "a" of NDF, and displayed higher values than the silage with elephant grass exclusively (Table 4.3).

Marula pulp additive inclusion improved DM degradability at 24 and 48 h incubation. Pepsin-pancreatin digestion of DM on residues after 48 h of ruminal incubation was lower (P>0.05) in all treatments probably due to their higher potential rumen degradability (Table 5.1) showing no significant effect on IVDMD which agrees with Keskin *et al.* (2005) who also did not observe any effects from additives on IVDMD.

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6.2 General conclusion

The Inclusion of Marula pulp at different levels during the ensilage of Napier grass improves the fermentation process, promoting good lactic acid contents and reducing the pH, dry matter loss and ammoniacal nitrogen. The nutritional quality of the Napier grass-Marula silages improves with the inclusion of Marula pulp, increasing the dry matter and crude protein contents without, however, diminishing the acid and neutral detergent fiber contents. All silages were good with 10 % inclusion having the highest CP (97.0 g/Kg) and lowest pH of 3.5. Marula pulp additive inclusion helped improve the DM and CP PD and ED of the silage at all inclusion levels but however, the inclusion had no significant effect on NDF degradability as compared to no additive silage. However, 50 % inclusion level performed at a superior level as compared to other treatments in rumen degradability studies. Based on chemical analysis the addition of 10% Marula pulp has the best potential to be an additive in silage making as compared to the other treatments, hence 50 % inclusion is a better option to use as an additive when ensiling as compared to the other three treatments given the degradability results. It can therefore, be concluded that Marula pulp additive inclusive silages all showed good potential as silages and hence can be used during ensilage of feed for ruminants to use particularly during the dry season when feed is scarce and low in quality.

6.3 Recommendations

Based on the results obtained in this study, a strong recommendation is therefore given that ensilage with the use of naturally accessible and locally available Marula pulp be practiced by smallholder farmers as a substitute for natural less nutritive winter pasture and can be equally considered when formulating strategies for feeding ruminants. Hence,

- Marula pulp is a good additive in silage making performing best at 10 and 50 % inclusion level.
- More research still needs to be conducted on how best to preserve fresh Marula pulp for use throughout the year without spoilage.
- More rumen degradability and digestibility studies should be conducted to shed more light on the actual animal performance during feeding.
- More research should also be conducted on the comparison of animal performance between freshly ensiled and dried Marula pulp chemical composition wise.

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- And finally there is a need to encourage smallholder farmers to adopt the practice of ensiling cultivated fodder with natural additive inclusion for use during dry winter seasons.

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APPENDICES

Appendix 1: Analysis of variance (Adjusted mean of squares, error, F-value and P-value) for fermentation characteristics of Napier grass silage after 8 weeks of ensilage

Source	df	pH	NH_3-N	La	WSC
			g/kg TN	g/kg	
Inclusion level	3	0.5987**	2284.3	61.48	24.620
Error	8	0.001	625.5	47.61	9.358
F-value		688.20	3.65	1.29	2.63
P-value		0.000	0.063	0.342	0.122

df, degrees of freedom; WSC, water soluble carbohydrates; NH3-N, Ammonium-Nitrogen; La, Lactic acid; g/kg TN, grams per kilogram total Nitrogen

Appendix 2: Analysis of variance (Adjusted mean of squares, error, F-value and P-value) for chemical composition of Napier grass silage after 8 weeks ensilage

Source	df	DM	ASH	FAT	CP	NPN	NDF	ADF	ADL
		(g/kg)	(g/kg DM)						
Inclusion level	3	2030.1	175.1	19.212	572	8.955	626	3192	1421
Error	8	553.0	180.9	6.735	1059	2.733	1369	3507	1288
F-value		3.67	0.97	2.85	0.54	3.28	0.46	0.91	1.10
P-value		0.063	0.454	0.105	0.668	0.080	0.719	0.478	0.403

df, degrees of freedom; DM, dry matter; ADF, acid detergent fibre; NDF, neutral detergent fibre;; ADL, acid detergent lignin; CP, crude protein; NPN, Non-Protein Nitrogen.

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Appendix 3: Analysis of variance (Adjusted mean of squares, error, F-value and P-value) for mineral composition of Napier grass silage after 8 weeks of ensilage

Source	df	Ca	Mg	K	Na	P	Zn	Cu	Mn	Fe
		Macro (g/kg DM)					Micro (mg/kg DM)			
Inclusion level	3	0.5835	0.2508	28.75	0.00956	0.0527	5158	23409	52173	4981623
Error	8	0.1900	0.1644	15.16	0.00301	0.1054	2643	6356	44252	3139402
F-value		3.07	1.53	1.90	3.18	0.50	1.95	3.68	1.18	1.59
P-value		0.091	0.281	0.209	0.0085	0.693	0.200	0.062	0.377	0.267

df, degrees of freedom; Ca, Calcium; Mg, Magnesium; K, Potassium; Na, Sodium; P, Phosphorus; Zn, Zinc; Cu, Copper; Mn, Manganese; Fe, Iron.

Appendix 4: Analysis of variance (Adjusted mean of squares, error, F-value and P-value) for rumen degradability constants and ED of DM on Napier grass silage

Source	df	Degradability constants				ED (%) at different outflow rates		
		a	*b*	*c*	*a+b*	*K* = 0.02	*K* = 0.05	*K* = 0.08
Animal		0.578	331.8	0.0000291	324.5	22.051*	9.484*	5.572
Inclusion level	3	27.412**	584.9*	0.0000294*	739.6**	101.62**	27.095**	20.076**
Error	8	3.321	143.1	0.0000089	127.0	5.729	2.504	1.942
F-value		9.00	3.61	2.70	5.04	13.81	8.46	8.71
P-value		0.001	0.031	0.073	0.009	0.000	0.001	0.001

**: P <0.01; *: P <0.05; (ns) non-significant: P >0.05. df, degrees of freedom; *a*: soluble fraction (%), *b*: insoluble but potentially degradable fraction (%), *a+b*: Potential degradability (%), *c*: outflow rate of degradation (h^{-1}), ED: effective degradability, *k*: rumen outflow rate (h^{-1}), df: degree of freedom

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Appendix 5: Analysis of variance (Adjusted mean of squares, error, F-value and P-value) for rumen degradability constants and ED of CP on Napier grass silage

Source	df	Degradability constants				ED (%) at different outflow rates		
		a	*b*	*c*	*a+b*	*K* = 0.02	*K* = 0.05	*K* = 0.08
Animal		158.22**	196.6	0.0148*	434.0	41.75	31.90	43.88
Inclusion level	3	28.66	1122.0**	0.0142*	965.9*	100.25**	105.20*	64.26*
Error	8	9.453	247.1	0.00357	296.1	18.14	24.41	20.04
F-value		1.18	4.64	3.01	3.26	4.89	4.18	2.87
P-value		0.343	0.013	0.054	0.043	0.010	0.019	0.662

**: P <0.01; *: P <0.05. df, degrees of freedom; *a*: soluble fraction (%), *b*: insoluble but potentially degradable fraction (%), *a+b*: Potential degradability (%), *c*: outflow rate of degradation (h^{-1}), ED: effective degradability, *k*: rumen outflow rate (h^{-1}), df: degree of freedom

Appendix 6: Analysis of variance (Adjusted mean of squares, error, F-value and P-value) for rumen degradability constants and ED of NDF on Napier grass silage

Source	df	Degradability constants				ED (%) at different outflow rates		
		a	*b*	*c*	*a+b*	*K* = 0.02	*K* = 0.05	*K* = 0.08
Animal		15.4308**	413.1	0.07638	580.0	9.530*	10.434**	11.238**
Inclusion level	3	0.803**	2790.6**	0.07564	2827.9**	2.441	0.154	0.237
Error	8	0.0475	169.9	0.07623	167.4	1.903	1.118	0.775
F-value		0.21	12.10	0.99	10.45	0.64	0.04	0.07
P-value		0.889	0.002	0.444	0.004	0.610	0.987	0.974

**: P <0.01; *: P <0.05. *a*: soluble fraction (%), *b*: insoluble but potentially degradable fraction (%), *a+b*: Potential degradability (%), *c*: outflow rate of degradation (h^{-1}), ED: effective degradability, *k*: rumen outflow rate (h^{-1}), df: degree of freedom

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Appendix 7: Analysis of variance (Adjusted mean of squares, error, F-value and P-value) for *in vitro* DM rumen digestibility of Napier grass silage

Source	df	Rumen incubation time (h)			*In vitro* dry matter digestibility		
		12	24	48	$IVDMD_{12}$	$IVDMD_{24}$	$IVDMD_{48}$
Inclusion level	3	2713	2516	1262	1616	5006	4669
Error	8	1257	1212	2374	7307	5263	3833
F-value		2.16	2.07	0.53	0.22	0.95	1.22
P-value		0.112	0.123	0.664	0.881	0.428	0.319

df: degree of freedom, $IVDMD_{12}$: *In vitro* dry matter digestibility after 12 hours of rumen incubation, $IVDMD_{24}$: *In vitro* dry matter digestibility after 24 hours of rumen incubation and $IVDMD_{48}$: *In vitro* dry matter digestibility after 48 hours of rumen incubation.

Appendix 8: Analysis of variance (Adjusted mean of squares, error, F-value and P-value) for *in vitro* CP rumen digestibility of Napier grass silage

Source	df	Rumen incubation time (h)			*In vitro* crude protein digestibility		
		12	24	48	$IVCPD_{12}$	$IVCPD_{24}$	$IVCPD_{48}$
Inclusion level	3	2066	524	471	2017	2272	2966
Error	8	2917	6224	6060	3051	3917	1866
F-value		0.71	0.08	0.08	0.66	0.58	1.59
P-value		0.558	0.968	0.971	0.586	0.635	0.223

df: degree of freedom, $IVCPD_{12}$: *In vitro* crude protein digestibility after 12 hours of rumen incubation, $IVDMD_{24}$: *In vitro* crude protein digestibility after 24 hours of rumen incubation and $IVCPD_{48}$: *In vitro* crude protein digestibility after 48 hours of rumen incubation.

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