THE EFFECTS OF FERTILISATION WITH BIO-DIGESTER SLURRY AND THE INCLUSION OF CARBOHYDRATE ADDITIVES AT ENSILING ON THE NUTRITIVE VALUE OF NAPIER GRASS (*PENNISETUM PURPUREUM*) SILAGE

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DECLARATION

I, Mashudu Daniel Rambau, hereby declare that this dissertation submitted in fulfilment of the requirements for the degree of Masters of Science in Agriculture (Animal Science) at the Department of Animal Science, School of Agriculture, University of Venda by me is of my own work and investigation, and has not previously been submitted for a degree at this or any other university. Reference material contained in this proposal has been duly acknowledged.

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M.D. Rambau

DEDICATION

This dissertation is dedicated to my parents, Mr. Daniel and Mrs. Salminah Rambau, and my lovely brothers and sisters. I am also dedicating this dissertation to the Lord.

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ABSTRACT

The objective of the study was to determine the effects of fertilisation with bio-digester slurry and the inclusion of carbohydrate additives at ensiling on the fermentation characteristics, chemical composition, ruminal degradability, and *in vitro* digestibility of Napier grass silage. Napier grass planted at the School of Agriculture Experimental Farm, University of Venda in 5 m x 4 m plots replicated three times in a completely randomised design and was irrigated with either bio-digester slurry or no bio-digester slurry (tap water) for a period of 12 weeks. After 12 weeks, the Napier was freshly cut and ensiled for 90 days in 1 litre glass jars in a 2 (Control - tap water and slurry irrigation) x 4 (No additive, molasses, maize meal and brown sugar) factorial arrangement. Fermentation quality and nutritive composition were determined using standard protocols. The dry matter (DM) and crude protein (CP) ruminal degradability was determined *in sacco* by incubating feed samples in nylon bags (external dimension: 6 × 12 cm, pore size of 46 µm) in the rumen in three Bonsmara steers fitted with rumen cannulae for 0, 6, 12, 24, 48, 72, 96 and 120 hours (h). Parameters to describe the dynamics of ruminal degradability of DM and CP were obtained by fitting the data on the exponential equation $P = a + b (1 - e^{-ct})$ using the NEWAY computer program, where "*a*" is the rapidly degradable fraction, "*b*" is the slowly degradable fraction and "*c*" is the outflow rate. The *in vitro* DM and CP degradability of rumen undegradable residue collected after 12, 24 and 48 h incubation was determined by sequential digestion in pepsin (abomasal) and pancreatin (small intestine) solutions. Fertilisation with bio-digester slurry increased (P <0.05) CP content of fresh cut Napier grass pre-ensilage. Bio-digester slurry fertilisation with molasses inclusion improved (P <0.05) the silage DM content which improved (P >0.05) fermentation characteristics with pH of 4.2 and lowest NH_3-N of 13.3 g/kg. Other chemical compositions and fermentation characteristics were not affected (P >0.05) due to fertilisation x additives treatment combinations. No bio-digester slurry fertilisation with maize meal inclusion increased (P <0.01) DM degradability at 0 h incubation. As time progressed to 24 h, no bio-digester slurry fertilisation with no additive included reduced (P <0.01) DM degradability with no difference (P >0.05) on other treatments. Potential DM degradability ($a + b$) of no bio-digester slurry fertilisation with no additive inclusion silage was reduced (P <0.01). The reduction was associated with low levels (P <0.01) of slowly degradable fraction "*b*". *In vitro* DM and CP digestibility were not affected (P >0.05) due to fertilisation x additives treatment combinations. In conclusion, bio-digester slurry application improved the quality of fresh cut Napier grass, with the combination of bio-digester slurry fertilisation and molasses addition yielding the best silage quality.

Keywords: mineral, *in sacco*, dry matter, crude protein, ruminal kinetics, *in vitro* digestibility

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LIST OF ABBREVIATION

a	Soluble Fraction
a + b	Potential Degradability
ADF	Acid Detergent Fibre
ADL	Acid Detergent Lignin
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
B	Boron
b	Insoluble but Potentially Degradable Fraction
c	Outflow Rate of Degradation (h^{-1})
Ca	Calcium
cm	Centimetres
CP	Crude Protein
CRD	Completely Randomised Design
Cu	Copper
DM	Dry Matter
ED	Effective Degradability
FAO	Food and Agriculture Organization
Fe	Iron
g	Grams
g kg^{-1} DM	Grams per Kilogram Dry Matter
GLM	General Linear Model
h	Hour
h^{-1}	Per Hour
ICP-OES	Inductively Coupled Plasm Optical Emission Spectrometry
ILRI	International Livestock Research Institute
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^{-1} DM	Milligrams per Kilogram Dry Matter
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
NDF	Neutral Detergent Fibre
NH_3-N	Ammonia Nitrogen
NPN	Non Protein Nitrogen
NRC	National Research Council
P	Phosphorus
S	Sulphur
SEM	Standard Error Mean
SOP	Standard Operating Procedure
t ha^{-1}	Tons per Hectare
TSP	Three Step Procedure
WSC	Water Soluble Carbohydrates
Zn	Zinc

CHAPTER 1: INTRODUCTION

1.1 Background

Feed supply and quality are major constraints experienced by small-holder livestock farmers during dry seasons in the tropics. Natural pastures rarely provide sufficient year-round feed of reasonable quality to match the nutritional requirements of livestock, and to support satisfactory livestock production and reproduction (Suttie, 2000). As a result, animals go through cycles of weight gain in the wet summer months and weight loss in the remaining dry seasons (Clatworthy, 1998). Forage conservation could mitigate the dry season feed deficit (Reiber *et al.*, 2010). Ensilage is one efficient way of preserving high-moisture green fodder (Cao *et al.*, 2009) which ensures continuous supply of quality fodder and maintain animal productivity throughout the year [Food and Agriculture Organization (FAO), 2009].

Although Napier grass (*Pennisetum purpureum*), also known as Elephant grass, is not commonly grown in Vhembe district, it may be a viable alternative to expensive maize silage production. Napier grass is a perennial tropical grass native to the African grasslands which has been primarily used for grazing. It is highly productive and palatable (Santos *et al.*, 2013), and is widely adapted to different environments. The grass is easily propagated and managed, which makes it preferable for cultivation [International Livestock Research Institute (ILRI), 2013]. The crude protein (CP) content ranges from 60 – 120 g kg^{-1} during the wet season, and declines to less than 50 g kg^{-1} during the dry period (Njoka-Njiru *et al.*, 2006). However, the quality of Napier silage is directly linked to the original fresh cut material (Loures *et al.*, 2003).

Soil fertility is a major problem affecting crop quality and production (Hamie *et al.*, 2009). Farmers need to use fertilisers (inorganic or organic) to add nutrients into the soil for the crop. However, chemical fertilisers are not the most appropriate solution to overcome this constraint due to their cost and they also pose a threat to human health (Weltzein, 1990). Therefore, more emphasis should be based on finding alternatives to chemical fertilisers such as bio-digester slurry, which are less expensive and safer than inorganic sources of nutrients. Tuyishime (2012) reported that most small-scale farmers lack the knowledge and awareness about the use of bio-digester slurry.

Bio-digester slurry is the by-product of gas production generated from biodegradable products under anaerobic conditions (Garg *et al.*, 2005). It is an organic fertiliser which when used properly, can reduce the dependency on chemical fertiliser. It contains 93 % water and 7% dry matter (DM) and is an almost ready-made fertiliser containing macro-and-micro nutrients needed by the plants (Gurung, 1997). Bio-digester slurry is ideal for long-term improvement of soil properties (Garg *et al*., 2005). Therefore, the use of bio-digester slurry on the production of fodder can improve production and nutritional quality as demonstrated where the slurry was used for irrigation in the field crops (Getachew, 2006; Zhou, 2009; Masarirambi *et al*., 2010).

The tropical grasses present low DM contents, high buffering capacity and low fermentable carbohydrates in growth stages in which they present good nutritive values, endangering the conservation through ensilage (Evangelista *et al.,* 2004; Iqbal *et al*., 2005; Nisa *et al.*, 2006). Therefore, inclusion of carbohydrate additives can improve the quality of silage by enriching the fresh material with carbohydrates which are the primary substrate during fermentation. Additives are natural or industrial products of high water soluble carbohydrates (WSC) content which are added to the forage to promote the growth of lactic acid bacteria (LAB) to produce lactic acid (LA) in high quantities enough to ensure good silage (Yitbarek and Tamir, 2014).

1.2 Problem statement

In Vhembe district, ruminants are largely raised on rangeland. The area is characterised by dry winter seasons during which the forage availability and quality cannot match the nutritional requirements of livestock and support satisfactory livestock production and reproduction. As a result, animals go through cycles of weight gain in the wet summer months between November and May and weight loss in the remaining dry season. To overcome this problem and maintain the continuity of feed supply, cultivated fodder or surplus forage can be conserved as silage for use when feed is in short supply. However, soil fertility directly affects silage quality by affecting the crop quality. Addition of bio-digester slurry might be useful to improve soil fertility and quality of forages grown on such soils. In addition, silage quality is also affected by the pattern of fermentation. The low WSC has been reported to be the common problem affecting patterns of Napier grass fermentation (Evangelista *et al.*, 2004; Iqbal *et al*., 2005; Nisa *et al.*, 2006; Bureenok *et al.*, 2012). The application of carbohydrate additives such as molasses, brown sugar and maize meal promotes desirable fermentation (Bureenok *et al.*, 2012; Lubisi, 2014).

1.3 Justification

Feed conservation through ensilage of forage could mitigate the problem of winter feed quality and shortage. Silage making would ensure a steady supply of feed throughout the year (Moran, 2005). This method of fodder conservation is suitable for small scale farmers because it is reliable and repeatable. It uses simple technology, locally sourced equipment and consumables and most importantly, it gives significant returns on investment (Machin, 1999). However, ensilage does not make poor forage good silage but rather preserve maximum nutrients on good quality forage, provided all basic principles of ensiling are followed (Cao *et al*., 2009). Maintaining soil fertility for improvement of crop quality and yield is a topical issue among farmers and agricultural scientists. Therefore, the addition of organic matter such as bio-digester slurry might be useful to improve soil fertility by increasing organic matter content. Yield increase and improved crop quality due to bio-digester slurry application has been reported in many crops (Krishna, 2001; Zhou, 2009; Ding *et al*., 2011; Lubisi, 2014). However, tropical grasses are not easy to ensile as they contain low levels of water-soluble carbohydrates. Thus, it is important to find locally available carbohydrate sources that can be used as fermentable carbohydrate additives by rural farmers to ensure successful ensilage. Therefore, this study will generate information on the effect of irrigation and fertilisation with bio-digester slurry and carbohydrates additives application on ensilability of Napier grass, ruminal degradability and *in vitro* digestibility of Napier grass silage since such information is limited. Such information is important in devising feeding strategies that improves productivity of ruminants. High production of ruminants may improve the economic, nutritional and cultural status of ruminant farmers in communal areas of Limpopo Province, South Africa.

1.4 Objectives

1.4.1 Main objective

The broad objective of the study was to improve the nutritive value of Napier grass (*P. purpureum*) silage through fertilisation of the crop with bio-digester slurry and through the inclusion of carbohydrate additives at ensiling.

1.4.2 Specific objectives

The specific objectives of the study were to determine:

a) The effect of irrigation and fertilisation with bio-digester slurry on fermentation characteristics [pH and water soluble carbohydrates (WSC)] and chemical composition [dry matter (DM), crude protein (CP), non-protein nitrogen (NPN), ash, fat, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL)] of fresh cut Napier grass.
b) The effect of irrigation and fertilisation with bio-digester slurry and the inclusion of carbohydrate additives at ensiling on:
   i. Fermentation characteristics [pH, WSC, ammonia nitrogen (NH_3-N) and lactic acid (LA)] and chemical composition [DM, CP, NPN, ash, fat, NDF, ADF, ADL, Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Sulphur (S), Boron (B), Copper (Cu), Iron (Fe), Manganese (Mn), Molybdenum (Mo) and Zinc (Zn)] of Napier grass silage,
   ii. Dry matter and crude protein ruminal degradability of Napier grass silage using the nylon bag technique, and
   iii. Dry matter and crude protein *in vitro* digestibility of Napier grass silage using the pepsin-pancreatin method.

1.5 Hypotheses

The null hypotheses were that:

a) Irrigation and fertilisation with bio-digester slurry does not affect the fermentation characteristics and chemical composition of fresh cut Napier grass at pre-ensiling.
b) Irrigation and fertilisation with bio-digester slurry and the inclusion of carbohydrate additives at ensiling do not affect the:
   i. Fermentation characteristics and Chemical composition of Napier grass silage,
   ii. Dry matter and crude protein ruminal degradability of Napier grass silage using the nylon bag technique, and
   iii. Dry matter and crude protein *in vitro* digestibility of Napier grass silage using the pepsin-pancreatin method.

CHAPTER 2: LITERATURE REVIEW

2.1 General introduction

Feed scarcity during dry seasons is one of the major constraints in animal production (Reiber *et al*., 2010). Therefore, the management strategies should always be geared towards maintenance of soil fertility and feed conservation. Biogas production has gained popularity in many countries around the world and is considered an efficient renewable energy for rural areas (FAO, 1992). Biogas digesters were introduced by the Water Research Commission in some parts of South Africa (Kotzé, 2014) for production of biogas. The effluent or bio-digester slurry from bio-degradable materials such as cattle manure have shown effectiveness on crops (Krishna, 2001; Zhou, 2009; Ding *et al*., 2011). Bio-digester slurry is one of the potential sources of soil nutrients for small holder rural farmers. It contains substantial amounts of Nitrogen (N), potassium (K) and phosphorus (P) (Zhang, 2003). The use of bio-digester slurry as liquid fertiliser could improve soil fertility and therefore ensure increased high quality fodder production which could be conserved as silage to feed animals during dry season. Silage is the end product produced by controlled fermentation of a crop of high moisture content, achieved by anaerobic condition which then discourage the activities of spoilage microorganism by a rapid drop in pH content of ensiled material (McDonald *et al*., 2011). Hence, when ensiling there is a need to use additives to ensure satisfactory preservation by producing LA which reduces pH content of silage.

2.2 Potential of bio-digester slurry as a soil amendment

Ruminant animal rearing in Vhembe district, Limpopo province, South Africa is traditionally based on natural pastures as feed source for animals, which is a practical and economical way. Poor nutrition during the dry season is a main constraint to livestock since the feed quantity is low and quality of natural grass is extremely poor. Use of fertilisers to enhance quality and yield of cultivated pasture, and commercial concentrates as livestock supplements to improve nutritive value is limited due to inability of farmers to purchase them (Njoka-Njiru *et al*., 2006). However, efficient management of nutrients in livestock manure is key to crop production, especially for smallholder farmers who rarely use commercial fertilisers to maintain soil fertility (Katuromunda, 2010). Application of bio-slurry has been reported (Zhu and Chen, 2002; Yu *et al*., 2010) to improve soil properties such as increased water-holding capacity and diversifications of nutrients for sustainable crop productivity. Therefore, the use of bio-digester slurry as fertiliser can offer a promising win-win situation by improving the nutrient quality of Napier fodder which will again be fed to animals, and at the same time preventing adverse environmental impacts of waste disposal. This then leads to the promotion of integrated management of livestock production in rural areas.

Bio-digesters convert biodegradable materials into combustible gas, known as 'bio-gas'. The biogas is the gas produced during anaerobic digestion (Singh, 2014) which consists of approximately 65 % of methane, 35 % of CO_2 and traces of N_2, H_2, H_2S, O_2 and ammonia (NH_3) (Bhattacharya and Salam, 2002). During anaerobic digestion, about 25 - 30 % of the total DM (total solids content of fresh dung) of animal/human wastes will be converted into a combustible gas and a residue of 70 - 75 % of the total solids content of the fresh dung comes out as bio-digester slurry. Bio-digester slurry can be easily collected and used as a potent organic fertiliser to enhance agricultural productivity (Rui *et al*., 2014).

The composition of bio-digester slurry depends upon several factors. These include the kind of dung (e.g. animal or human), water, breed and age of animals, types of feed and feeding rate. With the right amounts of materials, the composition of the bio-slurry can exist of 93 % water and 7 % of DM, of which 4.5 % is organic matter and 2.5 % inorganic matter (Gurung, 1997). The percentage of Nitrogen, Phosphorus and Potassium content of slurry on wet basis is 0.25, 0.13 and 0.12 while in dry basis it is 3.6, 1.8 and 3.6 respectively (Aminul, 2013). In addition to the major plant nutrients, it also provides micro-nutrients such as Zn, Fe, Mn and Cu that are also essential for plants though required in trace amounts (Aminul, 2013).

Bio-digester slurry fertiliser is 20 - 30 % more nutritious than commonly used organic fertilisers such as cow-dung, duck and poultry manure, farmyard manure and compost as it is especially produced from biogas plants (Shahariar *et al*., 2013). It is environmentally friendly, with non-toxic or harmful effects, at the same time ensuring minimal dependence on chemical fertilisers. Additionally, nutrients from organic sources are efficient and environmentally friendly (Ahmad *et al*., 2014).

2.3 Effect of bio-digester slurry on forage production

Several authors have reported yield increase and better quality due to bio-digester slurry application in many crops (Daudén and Quilez 2004; Getachew, 2006; Zhou, 2009; Masarirambi *et al*., 2010). However, there is limited information about the effect of bio-digester slurry on fodder. Rahman *et al*. (2008), in Korea, reported that plant height, stem circumference, highest biomass and the CP content of maize (*Zea mays*) fodder were significantly influenced by the increasing rate of cattle bio-digester slurry but the number of leaves of fodder plants, ash, acid detergent fibre (ADF) and neutral detergent fibre (NDF) contents showed no significant differences among treated groups. Similar results were reported by Islam *et al*. (2010) in Bangladesh. Increased fodder dry biomass yields of 17 tons per hectare (t ha^{-1}) and 16 t ha^{-1} were reported by Lubisi (2014) when poultry and cattle bio-digester slurry was applied respectively, compared to the treatment in which no bio-digester slurry was used. Moreover, application of bio-digester slurry as N fertiliser had positive effect on CP of the Napier fodder at late stages of maturity.

2.4 Fodder conservation techniques

Livestock producers are faced with seasonal fluctuations in the quantity and quality of feeds. Feed costs generally account for 70 - 80 % of the total animal production costs and reducing feed costs key for profitable livestock production (Henning, 1998). The reduction in feed cost can be achieved through the use of pasture and/or fodder, which remain the cheapest form of animal feed available. Natural pastures have been reported to rapidly mature in the rainy season, which results in quality deterioration if not harvested (Mbuthia and Gachuiri, 2003). Forage conservation technologies could mitigate dry season feed problems but their adoption in smallholder systems has so far been low (Reiber *et al*., 2010). However, the required pasture and/or fodder species for good animal production should provide high yield of palatable and digestive herbage, containing adequate nutrients (Kisitu, 2010) which varies with species, season and soil fertility.

The main aim of conserving feed is to preserve feed at optimum nutritional value and shift available feed from the present to the future (Kaiser and Evans, 1997). Forages can be conserved as hay or as silage. However, Nyambati *et al*. (2010) reported that Napier grass stems have a diameter ranging from 4.0 - 6.84 cm at eight weeks of re-growth. Hence, the thick stem makes it difficult to be naturally conserved by sun drying to make hay without spoilage and may require high energy and cost to dry. Therefore, ensiling can be considered as a better way of preserving high-moisture forage crops (McDonald *et al*., 2011), provided all basic principles of ensiling are followed (Cao *et al*., 2009).

2.5 Napier grass

2.5.1 Morphological and ecological aspects of Napier grass

Napier grass (*P. purpureum*), also known as Elephant grass, has been valued for its high yield and nutritional quality (Aganga *et al*., 2005; Wijitphan *et al*., 2009; Nyambati *et al*., 2010; ILRI, 2013). The crop is an important forage and pasture grass especially for cattle throughout the tropics. The grass by name, Elephant grass, implies that it is an important forage for elephants in Africa (Cook *et al*., 2005). Napier grass originated from Sub-Saharan tropical Africa (Clayton *et al*., 2013) and was introduced in most tropical and subtropical regions worldwide as forage.

Napier grass is very similar in appearance to sugarcane (*Saccharum officinarum*) but has narrow leaves and taller stems (Department of Agriculture, Forestry and Fisheries (DAFF), 2014). It grows approximately 2 - 4.5 metres (m) tall, rarely up to 7.5 m, with leaves about 30 - 120 centimetres (cm) long and 1 - 5 cm broad (Mdziniso, 2012). It is a summer growing grass (Francis, 2004) that does well in temperature ranging from 25 – 40 °C (FAO, 2015). Duke (1983) reported that Napier grass stops its growth below 15°C and is sensitive to frost. Napier grass grows on range of soil types from poorly drained clay soils to excessively drained sandy soils but its growth is best on rich, moist, well-drained medium-textured soils (Phasha, 2013). The soil reaction should range from pH 4.5 - 8.2 (Center for New Crops and Plant Products, 2002).

2.5.2 Nutrient content of Napier grass

Napier grass is characterized by reasonable amount of CP content, with a range of 60 – 120 g kg^{-1} during the wet season, which declines to less than 50 g kg^{-1} during the dry period (Njoka-Njiru *et al*., 2006). Aganga *et al*. (2005) reported a CP content of 6.67 % for Napier grass harvested at a height of 1.25 m. According to Katuromunda (2010), the nutritive quality and DM yield of Napier grass depended on rainfall and its distribution, soil fertility, ambient temperature and level of management. Lubisi (2014) reported that the bio-digester slurry had no effect ($P > 0.05$) on chemical composition Napier grass as compared to the non-bio-digester slurry irrigated plots. The results from study conducted by Rambau *et al*. (2016) showed that CP decreases with maturity whereby CP was observed to decrease from 158 - 132 g kg^{-1} at week 4 and 12, respectively. Generally, maturation increases the structural carbohydrate fraction resulting in the decrease in digestibility of forage crop (Moore and Jung, 2001). Therefore, conservation is critical while nutritive value of Napier grass is still at its optimum.

2.6 Silage quality

A good quality well preserved silage has a pH value of less than 4.5 (McDonald *et al*., 2011; Bilal, 2009) which is achieved by efficient conversion of WSC to LA production which also lowers the ammonium nitrogen to less than 100 g ammonia-N kg^{-1} total N and butyric acid with a value of less than 10 g kg^{-1} DM (Kung and Shaver, 2001, McDonald *et al*., 2002; Bai *et al*., 2011; Yitbarek and Tamir, 2014). These attributes provide better preservation and more stable silage. The successful use of silage additives has helped in preventing protein degradation during ensilage in order to improve protein utilization by ruminants (Purwin *et al*., 2010). Hence, several studies have shown such good attributes due to carbohydrate additives on silage preservation (Mtengeti *et al*., 2006; Mahala and Khalifa 2007; Arbabi and Ghoorchi, 2008; Bilal, 2009; Nkosi *et al*. 2012a; Lubisi, 2014: Mutavhatsindi, 2015; Wyss and Arrigo, 2015; Kanengoni *et al*., 2016).

Good silages have been reported when molasses was applied at 3 – 5 % (Van Niekerk *et al*., 2007). Rong *et al*. (2013) concluded that the combination of 4 % molasses with 0.4 % urea improved the fermentation of silage. Addition of fermented juice of epiphytic LAB to Napier grass improved the quality of the silage (Bureenok *et al*., 2012). The pH value of the treated silages rapidly decreased, and reached to the lowest value within 7 days of the start of fermentation, as compared to untreated silage. However, study conducted by Bilal (2009) reported that Mot grass silage without additives showed the highest pH and low LA, loss in DM and CP, indicating the poor quality silage.

Although Lubisi (2014) found that molasses, brown sugar and maize have a positive effect on the chemical composition of silage. He recorded the highest CP content of 120 g kg^{-1} DM on silage with maize meal as an additive of Napier grass irrigated with no bio-digester slurry while molasses treated silage had the lowest CP content of 90 g kg^{-1} DM. Increase of CP due to urea addition has also been reported by some researchers (McDonald *et al*., 1991; Hill and Leaver, 1999; Yunus *et al*., 2000).

Zwane *et al*. (2014) reported the DM content of the silages ranged from 225.8 to 261.8 g kg^{-1}, CP content of the Napier grass alone silage was 74 g kg^{-1}, which was improved to 108.5 g kg^{-1} and 113.7 g kg^{-1} by the addition of 50 % Silver-leaf, with 5 % and 3 % molasses respectively. On the other hand, Mdziniso (2012) reported that silage prepared with 5 % molasses had the highest CP content of 46 g kg^{-1} and highest DM content of 195.1 g kg^{-1}. The report agrees with the results of Lubisi (2014) who found high DM content on silage treated with molasses.

Little information has been documented regarding mineral composition of silage with the main focus on Ca and P (Ukanwoko and Igwe, 2012), but Lubisi (2014) study covered many mineral compositions. Lubisi (2014) reported that fertilisation with bio-digester slurry and carbohydrate additives had no effect (P >0.05) on mineral composition of silage. This focus is probably due to the important role played by Ca and P in the normal development and maintenance of the animal organism (Nadia and Ioan, 2013).

2.6.1 Quality of ensilage materials

Ensiling can be considered as an efficient way of preserving high moisture materials, provided all basic principles of ensiling are followed (Cao *et al*., 2009). However, the quality of silage produced is dependent on many factors which include the quality of material used in ensiling, stage of harvest, period and type of fermentation, temperature during fermentation and whether the silage is densely packed and free of air space (Moran, 2005). Ensiling forage materials has been dependent on at least two characteristics of forage materials necessary to ensure a good silage: adequate level of fermentable carbohydrates in a form of WSC (McDonald *et al*., 2011) and a DM content of 250 to 400 g kg^{-1} (Wilkinson, 2005). Production of well-fermented Napier grass silage requires that the fresh forage has a minimum amount of 37 g WSC kg^{-1} DM (Haigh, 1990) and adequate LAB prior to ensiling (Wilkinson, 2005). According to McDonald *et al*. (2011), ensiling forage material with high moisture content can adversely affect fermentation quality of the silage and also lead to high effluent production which drain away silage nutrients. Therefore, a dry matter (DM) content between range of 250 to 400 g kg^{-1} is recommended. In addition, CP content of ensiled materials should be above the critical 75 g kg^{-1} DM (Jusoh *et al*., 2014) essential for rumen function. However, soluble carbohydrates have been reported to be limited (27 g kg^{-1} DM) in fresh cut Napier grass before ensiling (Yahaya *et al*., 2004). To stimulate the fermentation process for the production of silage, a source of soluble carbohydrate such as molasses, maize meal and brown sugar has been used extensively as silage additives to improve the concentrations of WSC and LAB prior to ensiling (Yunus *et al*., 2000; Van Niekerk *et al*., 2007; McDonald *et al*., 2011; Lubisi, 2014).

2.6.2 Fermentation pathways

An efficient silage system for feed production should ensure low levels of nutrient losses and high quality silage (Kohler *et al*., 2013). The process of producing silage is a preservation method referred to as ensilage, the preservation can either be artificially or natural (Moran, 2005). It is based on LA producing bacteria which are facultative anaerobes that ferment sugars (mainly glucose and fructose) to produce an organic acid, called LA (McDonald *et al*., 2011). Anaerobic conditions are needed to reduce the activity of respiratory enzymes in forage material. Such enzymes tend to promote heat buildup and reduce both total DM and the nutritional value of silage if left unchecked (Nkosi, 2010). The anaerobic condition is achieved by a container in which silage is preserved, often called silo (McDonald *et al*., 2011). The residual oxygen between the plant particles is reduced by respiratory activity of the plant material and other aerobic or facultative aerobic micro-organisms like yeasts and enterobacteria (Weinberg and Muck, 1996). Likewise, proteases and carbohydrases of the plant are active at this stage, when pH is still within the usual range for fresh forage between 6.5 and 5.0. The fermentation phase starts when conditions in the silo becomes anaerobic and if fermentation proceeds successfully, the LAB become the major micro-organisms. Thus, homofermentative bacteria such as *Lactobacillus plantarum*, are desirable in the fermentation of silage. These homofermentative LAB are characterized by a faster fermentation rate, reduced proteolysis, higher LA concentrations, lower acetic and butyric acids contents, lower ethanol content, and higher energy and dry matter recovery. On the other hand, heterofermentative bacteria utilize pentoses as substrate for acetic and propionic acids production, which are effective at controlling fungi, at low pH values. The silo pH will be ideally reduced to 4.0 over a period of several days and plant material will be well preserved (McDonald *et al*., 2002). The rate of LA production is an important factor in inhibiting the activities of undesirable organisms and the production of objectionable fermentation products (McDonald *et al*., 2011). These characteristics in combination with anaerobic storage conditions, promote effective fermentation.

2.6.3 Use of additives

Maintaining the original quality of the preserved material is one of the main objectives of silage making (Wilkinson and Daives, 2012). As a result carbohydrate additives are used to direct the fermentation process towards production of LA as the main fermentation product (Nkosi *et al*., 2016). Additives will not make poor quality forage into good silage but they can help make top quality forage into excellent quality silage by enhancing silage fermentation (Kenilworth and Warwickshire, 2012). Additives are often used if there is any doubt as to whether natural fermentation is capable of ensuring satisfactory preservation, or routinely as an insurance against poor preservation (McDonald *et al*., 2011). Different additives have specific different mode of action for preservation of forage materials under anaerobic condition. Silage additives can be classified according to their functions: i) fermentation stimulants, such as bacterial inoculants and enzymes, ii) fermentation inhibitors such as propionic and formic acids and iii) substrate or nutrient sources such as maize meal and molasses.

Fermentation stimulants: Microbial inoculants and enzyme preparations are regarded as natural product. They are safe to handle, noncorrosive to machinery, do not cause environmental problems and their usage has expanded remarkably in the last decades (Yitbarek and Tamir, 2014). Microbial inoculants have proved effective in improving silage fermentation (Kristensen *et al*., 2010; Ferreira *et al*., 2014; Yitbarek and Tamir, 2014). They contain freeze-dried cultures of homofermentative LAB. Successful control of fermentation, using these inoculants, depends upon inoculation rate and the presence of an adequate level of fermentable carbohydrates (McDonald *et al*., 2011). On the other hand, enzymes are proteins that assist in metabolic processes (Kung, 2014) and have been used as silages additives. They degrade the cell walls of plants, thus releasing sugars, which are then available for fermentation by the LAB (McDonald *et al*., 2011). Yitbarek and Tamir (2014) reported improved fermentation by stimulating acid production, lowering pH, and lowering ammonia N as a result of enzymes application.

Fermentation inhibitors: Acids are added to forage at ensiling to cause an immediate drop in pH. Addition of acids may be beyond the resources of smallholders and can be dangerous ('t-Mannetje, 2008). Propionic acid is the short-chain fatty acids with greatest antimycotic activity. It is effective in reducing yeast and moulds which are responsible for aerobic deterioration in silages (Kung, 2014). Propionic acid is difficult to handle because it is corrosive. On the other hand, formic acid has also been reported unsafe in handling, application and is corrosive to equipment (Yitbarek and Tamir, 2014).

Substrate or nutrient sources: This group has been used to sustain nutritional quality and enhance the fermentation process during ensiling. Molasses is a by-product of the sugar beet and sugarcane industries (Kung, 2014). It is one of the earliest silage additives used as a source of sugars (McDonald *et al*., 2011). Water-soluble carbohydrate content of about 70 % DM and 79 % has been reported by McDonald *et al*. (2011) and Kung (2014) respectively. Molasses is one of the widely used WSC additives to stimulate rapid increase or dominance of LAB.

The choice of an additive to be used in the silage should be based on the availability and acquisition of the product; present facility in the management; 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 (Yitbarek and Tamir, 2014). On small scale farms, commercial additives, which comprise inoculants and enzymes, may be too costly or unavailable. Traditionally, it can be useful for local farmers to use locally available nutrient source additives (molasses, maize mail and brown sugar) due to their availability and affordability.

2.7 Rumen degradability

Methods for estimating the degradability of feedstuffs include *in vivo* (involve the use of markers), *in sacco* (requires animals that are surgically fitted with rumen cannulae) and *in vitro* (requires the use of rumen fluid obtained from cannulated animals) techniques (Mohamed and Chaudhry, 2008). The *in sacco* technique is preferred due to its simplicity and reliability, accuracy of prediction and for its capacity to quantify degradation rates and pools being degraded in the rumen (Osuji *et al*., 1993) but the surgical process raises ethical and moral issues. The *in sacco* technique was first suggested by Quin *et al*. (1938) and it has since been used to estimate utilisation of either forages or concentrates and high-protein feeds. The ruminal *in situ* incubation technique is considered to be a reference method to estimate degradation parameters, such as soluble, insoluble but degradable and undegradable fractions, potential and effective degradability, when adjusted to suitable nonlinear models (Ørskov and McDonald, 1979). These parameters are used by feeding evaluation models to estimate the nutritive value, nutrient supply and animal performance (Hackmann *et al*., 2010). For example, high rates of degradation of feed implied the high voluntary intake and thus higher performance (Sun *et al*., 2012).

The rate and extent of rumen DM fermentation are very important determinants for the nutrients absorbed by the ruminants (Kamalak *et al*., 2004). Rumen degradability of protein is an important quantitative measure of the nutritional value of feed protein because it determines the supply of ammonia, peptides and branched-chain fatty acids to ruminal microorganisms, and the passage of undegradable proteins to the intestine (Hvelplund and Weisbjerg, 2000). *In sacco* analyses are the most frequently used methods for determination of degradability parameters of DM, organic matter, protein, fibre, minerals and other nutrients of feeds (Harazim *et al*., 2002; Třináctý *et al*., 2003; Čerešňáková *et al*., 2007; Homolka *et al*., 2008; Jančík *et al*., 2009).

In most studies, less attention has been paid to assessment of ruminal degradability of silage. The knowledge of degradation in the rumen is very important because the rate of degradation level can give an idea of how the quality of individual feed is reflected. However, the digestibility of forage crops differs due to grass species and are also influenced by environmental condition, including temperature, light intensity, total rainfall, edaphic factor, fertilisation level, and by method of preservation, concentration of soluble carbohydrates and stage of maturity (Huhtanen *et al*., 2006; Kasuya *et al*., 2008 Jančík *et al*., 2009). Furthermore, Canbolat *et al*. (2005) also reported that factors such as differences in protein sources, pore size of the nylon bags, milling screen sizes and fistulated animals used cause differences among experiments. However, ruminal degradability of silage DM and CP increase with duration of ruminal incubation. This is due to residues remaining in the rumen for longer periods and tend tends to endured more prolonged microbial action.

Nisa *et al*. (2005) reported higher ruminal DM and NDF degradabilities of 59.2 % and 56.0 %, respectively, of Mott grass silage which was ensiled using two additives, cane molasses and crushed corn grains compared to that of Mott grass. The higher ruminal DM and NDF degradabilities agree with the results of Kim *et al.* (2014) who observed higher rate of DM and CP degradation in the by-product feed-based silage compared to that of rice straw and ryegrass straw. However, Nisa *et al*. (2005) further stated that the higher ruminal degradation of DM and NDF of Mott grass silage than that of Mott grass was probably a reflection of fermentation of Mott grass during fermentation that improved its degradability by improving the availability of easily degradable structural polysaccharides to ruminal microbial population.

Inoculation with *Streptococcus bovis* improved the rumen degradability of the nutrients. The soluble fraction degradability in the silages inoculated with *S. bovis* JB1 and HC5, had higher values, 30.77 and 29.97 %, for DM and 31.01 and 36.66 % for CP, respectively (Ferreira *et al*., 2014). Rêgo *et al*. (2011), on the other hand, observed values of potentially degradable CP fraction ranging from 59.0 - 64.7 % for elephant-grass silages harvested at three different maturities (70, 90 and 110 day of regrowth). Rambau *et al.* (2016) reported values of potentially degradable DM and CP fraction ranging from 85.6 – 93.6 % and 99.7 - 100 % respectively for Napier grass leaves harvested at three different maturities (4, 8 and 12 weeks after sprouting). When Cabral *et al*. (2005) assessed ruminal degradation of elephant grass silage, lower results for potential DM degradability were observed and verified 64.9 % DM potential degradability. The low potential degradability of DM can be attributed to the more advanced stage of maturation of Elephant grass used (120 days of growth). Maturation results in dilution of the CP contents of the forage crops by the rapid accumulation of cell wall carbohydrates at the later stages of growth (Van Soest, 1994). Working with corn silages ensiled with application of bacterial and/or enzymatic inoculants, Gimenes *et al*. (2006) found potentially degradable CP fraction ranging from 74.15 % to 78.81 % and fractional rate of CP disappearance between 1.73 - 3.01 %. However, Nowak *et al*., (2004) and Granzin and Dryden (2005) reported that additives inclusion had no effect on silages DM and CP degradability.

2.8 *In vitro* digestibility

Nutrients digestibility in feed samples represents the amount of nutrients in a feed actually accessible to the animal (McDonald *et al.*, 2011) hence, considered as the most essential factor in evaluating the nutritional quality of feed. Nutrients digestibility can be measured using *in vivo* or, alternatively, *in vitro* techniques. *In vivo* analysis involves the use of animals fitted with duodenal and ileal cannulas to measure digestibility. *In vivo* analysis have been reported to be time consuming, costly, labor intensive and the surgical process raises ethical and moral issues. Even though there is currently no standardized *in vitro* technique for digestibility analysis of ruminant feeds, *in vitro* digestibility analysis may be more economical and also allow for quality control of processed feeds (Schneider and Flatt, 1975).

The three-step procedure closely simulates physiological conditions in the animal. This is achieved by estimating the nutrient digestibility in abomasal and intestinal digestion using pepsin and pancreatin, respectively, from ruminal degradability residues (Gargallo *et al*., 2006). Because protein may be degraded in the rumen, less digestible dietary protein reaches the abomasum and subsequently the small intestine. The use of estimates of intestinal digestion in combination with estimates of protein degradation in the rumen may provide estimated values of intestinally absorbable dietary protein derived from individual ingredients (Calsamiglia and Stern, 1995).

Few reports found in the literature contradict the *in vitro* nutrients digestibility of silages pre incubated in the rumen. However, Thomson *et al*. (1981) reported that pre-incubating feeds results in reduced amounts of nutrients which will enter post ruminal digestion, with reference to *in vitro* simulation of nutrient digestibility in abomasal and intestinal digestion. Kaldmäe *et al*. (2009) reported that application of inoculant had no effect on the *in vitro* dry matter digestibility of red clover silages while Zereu *et al*. (2015) reported that using molasses in silage improved the DM digestibility of ensiled vines of four sweet potato. On the other hand, Nowak *et al*. (2004) reported a significant increase of intestinal digestibility of undegraded protein in formic acid treated silage.

2.9 Summary

Bio-digester slurry is widely reported to improve both yield and quality of cash crops (Daudén and Quilez 2004; Getachew, 2006; Zhou, 2009; Masarirambi *et al*., 2010). There is limited information regarding the fertilisation effect of bio-digester slurry on forage crops (Lubisi, 2014). Hence, the proposed use of bio-digester slurry to improving quantity and quality of Napier forage for ensilage can therefore ensure regular supply of feed throughout the year. The evidence suggests that ensiling forages with inclusion of additives may improve the fermentation when the forage cannot ensure optimal natural fermentation (McDonald *et al*., 2011). Generally, the benefit of additives on good quality fresh cut forages grasses such as Napier is equivocal. The efficacy of additives to alter the chemical and fermentation quality, ruminal degradability and digestibility is inconsistent in literature. Therefore, further investigation is necessary to determine the interactive effects of fertilisation with bio-digester slurry and the inclusion of carbohydrate additives at ensiling on fermentation characteristics and chemical composition, ruminal degradability using the nylon bag technique, and *in vitro* digestibility using modified three step procedures of Napier grass silage.

CHAPTER 3:

EFFECT OF FERTILISATION WITH BIO-DIGESTER SLURRY AND THE INCLUSION OF CARBOHYDRATE ADDITIVES DURING ENSILING ON FERMENTATION CHARACTERISTICS AND CHEMICAL COMPOSITION OF NAPIER GRASS SILAGE

3.1 Introduction

The dry season poses a major constraint to ruminant livestock production in the tropical areas due to nutritional stress (Murphy and Colucci, 1999). Cultivation with ensilage of surplus forages during rainy seasons can mitigate this challenge. Ensiling is considered as an efficient process of preserving forage of sufficiently high moisture content (Oude Elferink *et al.*, 2000). Napier silage quality is directly linked to the original material (Loures *et al.*, 2003). To achieve high yields of quality forage, the maintenance of soil fertility is critical. Organic amendments are often applied to soils to increase crop productivity, crop quality, or both (Bresson *et al.*, 2001; Edmeades, 2003). Therefore, the use of organic amendments such as bio-digester slurry to promote soil health in sustainable cropping systems (Timsina and Conner, 2001) needs to be fully exploited. Bio-digester slurry is the by-product of gas production generated from bio-degradable products through an anaerobic degradation (Garg *et al.*, 2005). It contains substantial amounts of N, P and K (Zhang, 2003). Bio-digester slurry has been demonstrated to increase quality and yields in many crops (Krishna, 2001; Gurung, 1997). However, the benefits of application of bio-digester slurry on fodder production and its quality has not been tested in many pasture species.

Previous studies (Itavo *et al.*, 2000; Lallo *et al*., 2003; Pirmohammadi *et al.,* 2006) showed that through proper ensiling, silage can reduce the cost of feeds through ensuring a steady supply of quality feed for ruminants. However, the characteristically high CP content with low fermentable carbohydrates of tropical grasses (Nisa *et al.*, 2006) are attributes which may negatively impact on silage quality. Napier grass is commonly used as a silage crop in tropical climates due to its high quality and yield (Bureenok *et al.*, 2012; Sebolai *et al.*, 2012). However, the low WSC content is of concern. Therefore, the application of carbohydrate additives can be a useful technique to enhance the quality of the silage and ultimately the performance of livestock animals (Tauqir *et al.*, 2009). Readily available, low cost carbohydrate additives such as brown sugar, molasses, and maize meal can be of good benefit to local farmers. Therefore, the study assessed the effect of fertilisation with bio-digester slurry and the inclusion of carbohydrate additives at ensiling on the fermentation characteristics and chemical composition of Napier grass 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). The area receives an annual rainfall of ± 500 mm that falls predominantly in summer. The average maximum and minimum temperatures are 31^{o}C and 18^{o}C, respectively (Tadross *et al.*, 2005). The area is characterised by deep, well drained red clay soils (Soil Classification Working Group, 1991).

3.2.2 Forage management and experimental design

Napier grass was established by ploughing using a tractor, harrowing, marking and demarcating 4 x 5 m plots in a completely randomized design (CRD) with the fertilization treatments replicated 3 times (Figure 3.1). Chemical fertilisers were not used as a positive control because the study was implemented to minimize economic cost of buying inputs such as chemical fertilisers by smallholder farmers. Napier cuttings with three nodes were planted manually, two nodes in the ground and one up at an angle of about 30 to 45° (Tainton, 2000) and spacing of 70 cm inter-and-intra-rows, at a plant population of 48 plants/plot (Figure 3.2). In the second season, the grass was cut 15 cm above the ground (Mtengeti *et al.*, 2006) to allow uniform regrowth for 12 weeks (Plate 3.1 a, b).

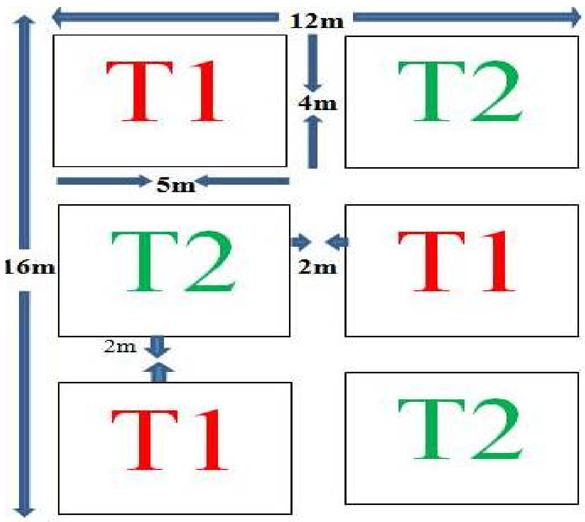

Figure 3.1: Forage production field layout showing fertilisation treatments (**T1 = Slurry + Napier grass,** and **T2 = No slurry + Napier grass**)

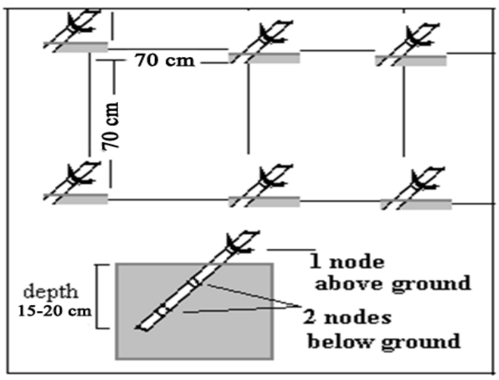

Figure 3.2: Napier grass (48 cuttings per plot) planted at 30-45° to the soil surface (Source: Lubisi, 2014)

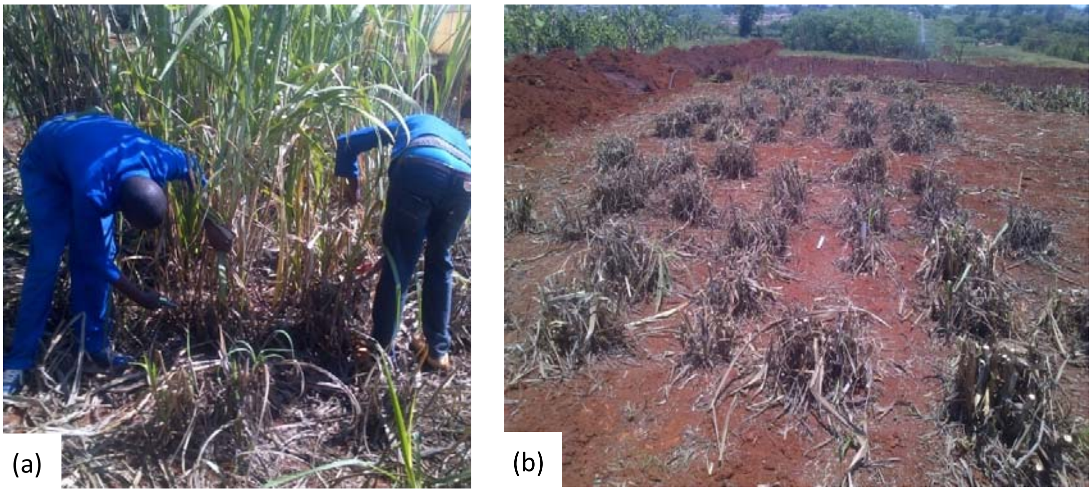

Plate 3.1: (a) Cutting of Napier grass (b) plots after cutting of Napier grass

The trial was conducted from early November 2015 to the end of January 2016. Monthly data for weather (Table 3.1) were obtained from Agricultural Research Council – Institute for Soil, Climate and Water (ARC-ISCW) station situated 20 m away from the experimental site at the University of Venda.

Table 3.1: Weather data during the experimental period

Month	Mean Temperature (°C)	Solar Radiation (MJ m^{-2} d^{-1})	Total Rainfall (mm)	Relative Humidity (%)
November	24.8	22.4	52.8	53.2
December	27.7	22.9	105.7	57.1
January	25.9	20.6	80.5	60.9

The bio-digester (Plate 3.2a) was fed with cattle dung and water in a 1:1 ratio every day for the production of bio-digester slurry. Before irrigation, bio-digester slurry samples were collected in 50 millilitres (mℓ) containers for mineral analysis. Bulk bio-digester slurry collected was then diluted with water at a ratio of 1:1 for easier application from irrigation cans. Fodder was manually irrigated with either bio-digester slurry (Plate 3.2b) or water (Plate 3.2c) weekly using 10 litres (ℓ) watering cans. Irrigation with both bio-digester slurry and water were applied at the rate of 30 tons per hectare (t ha^{-1}) (30 ℓ per $20m^2$) making it 10 ℓ per two rows. Plots were kept weed free for the 12-week experimental period, after which the fodder was harvested.

Plate 3.2: (a) Bio-digester chamber used for the production of bio-digester slurry during study period. Irrigating with bio-digester slurry (b) and water (c)

3.2.3 Determination of nutrients in soil and bio-digester slurry

3.2.3.1 Soil and Slurry sampling

A total number of 24 soil samples from the field plots were collected randomly at the site within 0 to 20 cm soil depth using a soil auger (Plate 3.3a). Samples were mixed to produce a composite sample, air-dried and sieved through a 2 mm sieve for analysis. The bio-digester slurry samples were collected every week before irrigating for 12 weeks.

Plate 3.3: (a) Soil sample collection using a soil auger and (b) bio-digester slurry samples

3.2.3.2 Chemical Analysis of Soil and Slurry Samples

Soil texture was determined using the hydrometer method (Bouyoucos, 1962). Slurry samples were dried at 60°C in an oven for 48 hours (h) to determine DM content (AOAC, 1990; method 930.15). Organic carbon was determined using the Walkley and Black method outlined by Nelson and Sommers (1996). The pH of soil and slurry samples was determined in 1:2.5 ratio of soil: deionized water (Liang *et al*., 2014) and 1:5 ratio of slurry: deionized water (Mtengeti *et al.,* 2006) respectively. The minerals in the soil and slurry (P, K, Ca, Mg, S, B, Cu, Fe, Mn, Mo and Zn) were determined using Inductively Coupled Plasm Optical Emission Spectrometry (ICP-OES) following Standard Operating Procedure (SOP) (2005) while the N content was analysed using the Kjeldahl procedure (AOAC, 1990; method 984.13). Table 3.2 shows the mineral composition of the soil and bio-digester slurry.

Table 3.2: Mineral composition of the experimental soil and cattle bio-digester slurry

Component	**Soil**	**Slurry**
Clay %	> 60	nd
Dry matter (g kg^{-1})	nd	12.11
Organic carbon %	3	nd
pH	5.02	8.11
Macro-minerals (g kg^{-1})		
Calcium	0.83	0.18
Magnesium	0.32	0.28
Nitrogen	2.60	0.35
Phosphorus	0.01	0.02
Potassium	0.17	0.88
Sodium	nd	0.20
Micro-minerals (mg kg^{-1})		
Aluminum	nd	8233.00
Copper	21.90	1183.00
Iron	nd	110433.00
Manganese	47.00	4667.00
Zinc	3.20	5200.00

nd: not determined.

3.2.4 Ensiling Napier grass fodder

3.2.4.1 Fresh cut Napier grass sampling

Approximately 1000 g of fresh Napier grass samples were collected randomly from each plot by cutting the grass 15 cm above the ground using a pruning scissors at 12 weeks after regrowth, when the grass was between 1.2 to 1.5 m in height. The grass was chopped to 2 to 4 cm length pieces while packed into brown bags. Approximately 40 g was sampled for pH determination and the remaining samples were immediately weighed and dried for subsequent chemical analysis. The remaining fodder was similarly harvested for ensilage.

3.2.4.2 Preparation for ensiling and experimental design

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 carbohydrate additives. For each fertilization treatment (Napier with and without bio-digester slurry), four carbohydrate additives [no additive (control), molasses, brown sugar and maize meal] were used, each replicated three times (Table 3.3). The experiment was set up as a 2 x 4 factorial arranged in a CRD. In order to be able to evenly spread the molasses on the chopped material, the molasses was thickened under the sun in a container for some time.

Table 3.3: Treatment combinations of Napier grass silage produced

Fertilisation	Carbohydrate additives
No slurry	No additive
No slurry	Molasses
No slurry	Maize meal
No slurry	Brown sugar
Slurry	No additive
Slurry	Molasses
Slurry	Maize meal
Slurry	Brown sugar

3.2.4.3 Ensiling procedure

Approximately 600 g (wet basis) of chopped grass was weighed for ensilage. Additives were spread at 10 % of the total weight after which the forage was mixed thoroughly and ensiled in 1 ℓ Consol anaerobic bottle jars. The grass was compressed using pruning scissor (Plate 3.4a) to squeeze air out of the jars, to promote anaerobiosis. The jars were tightly sealed with lids that were pre-heated in warm water (Plate 3.4b), sellotaped and then stored at room temperature for 90 days.

Plate 3.4: (a) Compacting Napier with pruning scissor and (b) Jars for one fertilisation treatment after tightly sealed

3.2.4.4 Analysis of fresh cut and ensiled Napier grass

Fresh-cut grass and silage samples obtained after 90 days of fermentation were analysed in the Animal Science Nutrition Laboratory, University of Venda, Thohoyandou. A pH meter was used to measure the pH according to Mtengeti *et al.* (2006). Fresh 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 ground through a grinding mill of 1 mm screen size. The WSC content was determined using the Anthrone method (Murphy, 1958). Ash was analyzed by combusting at 550°C overnight (AOAC, 1990; method 942.05). The N content was determined using a Kjeldahl procedure (AOAC, 1990; method 984.13) and the CP was calculated as N x 6.25. Non-protein N (NPN) was determined according to Licitra *et al.* (1996). Fat content was determined using the soxhlet fat extraction method (AOAC, 1990; method 930.15). Forage NDF, ADF and acid detergent lignin (ADL) contents were determined using the technique of Van Soest *et al.* (1991).

In addition, silage was analysed for LA and NH_3-N using protocols described by Faithfull (2002) and AOAC (1990; method; 941.04), respectively. Minerals [P, K, Ca, Mg, S, B, Cu, Fe, Mn, Mo and Zn] were determined using ICP-OES (SOP, 2005).

3.2.5 Ethical consideration

The study was approved by the Ethics Committee of the University of Venda (SARDF/16/ANS/05) and has therefore been performed in accordance with the ethical standards.

3.2.6 Statistical analysis

Analysis of variance (ANOVA) on Fresh cut grass (Model I) and silage quality data (Model II) 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). Where significant differences between the treatment groups was detected, means were separated using the Tukey's test (Tukey, 1953).

$$Y_{ij} = \mu + S_i + \varepsilon_{ij} \qquad \text{Model I}$$

Where, Y_{ij} = the observation - pH, DM, WSC, CP, fat, ash NDF, ADF and ADL;

μ = overall mean common to all observations;

S_i = effect of i^{th} bio-digester slurry, i = 1 or 2; and

ε_{ij} = random residual error.

$$Y_{ijk} = \mu + S_i + C_j + (SC)_{ij} + \varepsilon_{ijk} \qquad \text{Model II}$$

Where, Y_{ijk} = the observation - pH, DM, WSC, CP, NDF, ADF, ADL, Fat, Ash, LA, Ammonia-N, and minerals;

μ = overall mean common to all observations;

S_i = effect of i^{th} bio-digester slurry, i = 1 or 2;

C_j = effect of j^{th} carbohydrates additive, j = 1, 2, 3 or 4;

$(SC)_{ij}$= interaction between i^{th} bio-digester slurry and j^{th} carbohydrates additive; and

ε_{ijk} = random residual error.

3.3 Results

3.3.1 Chemical composition of fresh cut Napier grass

The nutrient composition of fresh-cut, pre-ensiled Napier grass is shown in Table 3.4. Fertilisation with bio-digester slurry increased (*P* <0.05) pH and CP and reduced (*P* <0.05) fat content, with no effect (*P* >0.05) on DM, WSC, Ash, NDF, ADF and ADL.

Table 3.4: Chemical composition (g kg^{-1} DM) of fresh-cut, pre-ensiled Napier grass irrigated with and without bio-digester slurry (N = 3)

Parameters	Fertilisation		SEM	Significance
	No slurry	Slurry		
DM (g kg^{-1})	278.2	270.6	4.48	ns
pH	5.9^{b}	6.0^{a}	0.04	*
(g kg^{-1} DM)				
WSC	54.3	53.6	2.23	ns
CP	105.9^{b}	118.6^{a}	2.96	*
Ash	61.4	66.7	2.69	ns
Fat	23.1^{a}	16.9^{b}	1.15	*
NDF	788.2	790.6	6.41	ns
ADF	562.3	580.6	6.90	ns
ADL	28.4	28.6	13.50	ns

*: *P* <0.05; (ns) not significant: *P* >0.05. ^{ab} Row means with different superscripts differ significantly at *P* <0.05. DM: Dry Matter, WSC: Water Soluble Carbohydrates, CP: Crude Protein, NDF: Neutral Detergent Fibre, ADF: Acid Detergent Fibre, ADL: Acid Detergent Lignin, g kg^{-1}: Grams per Kilogram, g kg^{-1} DM: Grams per Kilogram Dry Matter and SEM: Standard Error Mean.

3.3.2 Fermentation characteristics and chemical composition of Napier grass silages

The effects of fertilisation with bio-digester slurry and carbohydrate additives inclusion on fermentation characteristics of Napier grass silages are presented in Table 3.5. There were no significant (*P* >0.05) treatment effects on silage fermentation characteristics. Fertilisation with bio-digester slurry had no effect on silage fermentation characteristics. Maize meal treated silage had higher (*P* <0.05) NH_3-N content while molasses treated silage had higher (*P* <0.05) WSC. The additives did not affect (*P* >0.05) pH and LA.

Table 3.5: The mean fermentation characteristics (g kg^{-1} DM) of Napier grass silage after 90 days of ensiling

Fertilisation	Additives	N	pH	Fermentative characteristics		
				WSC	LA	NH_3-N
				(g kg^{-1} DM)		(g kg^{-1} TN)
No slurry	No additive	3	4.4	14.0	30.2	45.9
	Molasses	3	4.2	29.4	29.9	14.8
	Maize meal	3	4.3	20.7	21.7	41.4
	Brown sugar	3	4.1	21.0	31.1	22.1
Slurry	No additive	3	4.4	14.8	26.1	37.8
	Molasses	3	4.2	28.3	30.9	13.3
	Maize meal	3	4.5	15.6	16.8	46.4
	Brown sugar	3	3.9	22.2	29.5	15.9
SEM			0.27	3.91	6.66	12.03
Fertilisation						
No slurry		12	4.3	21.3	28.2	31.0
Slurry		12	4.3	20.2	25.8	28.3
SEM			0.14	1.96	3.33	6.02
Additive						
	No additive	6	4.4	14.4^{b}	28.1	41.8^{a}
	Molasses	6	4.2	28.9^{a}	30.4	14.0^{b}
	Maize meal	6	4.5	18.1^{ab}	19.2	43.9^{a}
	Brown sugar	6	4.0	21.6^{ab}	30.3	19.0^{ab}
SEM			0.19	2.77	4.71	8.51
Significance						
	Fertilisation (F)		ns	ns	ns	ns
	Additives (A)		ns	*	ns	*
	F x A		ns	ns	ns	ns

*: *P* <0.05; (ns) not significant: *P* >0.05. ^{ab} Column means with different superscripts differ significantly at *P* <0.05. WSC: Water Soluble Carbohydrates, LA: Lactic Acid, NH_3-N: Ammonium Nitrogen, N: Number of Observations, g kg^{-1} DM: Grams per Kilogram Dry Matter, g kg^{-1} TN: Grams per Kilogram Total Nitrogen and SEM: Standard Error Mean.

The effects of fertilisation with bio-digester slurry and carbohydrate additives inclusion on nutrient compositions of Napier grass silages are presented in Table 3.6. The combination of fertilisation with bio-digester slurry and molasses inclusion increased (*P* <0.05) DM content whereas combination with maize meal inclusion increased (*P* <0.05) the fat content. Fertilisation with bio-digester slurry did not affect (*P* >0.05) the chemical composition of Napier silage. Additives increased (*P* <0.01) silage DM and reduced (*P* <0.01) ADF and NDF, with no effect (*P* >0.05) on CP, NPN and ADL. Maize meal increased (*P* <0.05) fat content while molasses increased (*P* <0.01) the ash content.

The effects of fertilisation with bio-digester slurry and inclusion of carbohydrate additives on the mineral compositions of Napier grass silages are presented in Table 3.7. Fertilisation with bio-digester slurry and treatment with maize and brown sugar reduced (*P* <0.05) Mn content. Fertilisation with bio-digester slurry reduced Mg, Fe (both at *P* <0.05) and Mn (*P* <0.01) but increased (*P* <0.05) P content. Molasses increased (*P* <0.01) silage Ca, Mg, K, and Na content and also reduced (*P* <0.05) Zn content of silage. Furthermore, no additive and molasses included silages had higher Mn content of silage.

Table 3.6: The mean chemical composition (g kg^{-1} DM) of Napier grass silage after 90 days of ensiling

Fertilisation	Additives	N	DM	CP	NPN	Fat	Ash	NDF	ADF	ADL
			(g kg^{-1})	(g kg^{-1} DM)						
No slurry	No additive	3	286.5^{b}	78.5	9.8	17.3^{ab}	81.0	500.1	759.2	73.9
	Molasses	3	342.9^{ab}	100.6	9.3	16.1^{ab}	106.7	368.8	583.0	65.0
	Maize meal	3	346.3^{ab}	92.0	7.9	16.8^{ab}	65.5	372.5	581.2	65.4
	Brown sugar	3	304.4^{b}	89.8	8.2	20.1^{ab}	66.3	418.0	678.5	66.9
Slurry	No additive	3	275.6^{b}	86.7	8.1	22.0^{ab}	70.0	500.0	754.5	66.2
	Molasses	3	397.4^{a}	107.0	8.9	12.9^{b}	105.1	330.4	621.2	45.6
	Maize meal	3	316.1^{b}	90.4	9.0	24.0^{a}	64.4	389.5	607.6	52.6
	Brown sugar	3	336.9^{ab}	94.2	6.0	17.3^{ab}	58.2	398.0	648.1	58.2
SEM			14.89	7.89	1.05	1.93	5.08	31.82	27.30	31.91
Fertilisation										
No slurry		12	320.0	90.2	8.8	17.6	80.0	414.8	650.5	67.8
Slurry		12	331.5	94.6	8.0	19.0	74.4	404.5	657.8	55.6
SEM			7.45	3.95	0.52	0.96	2.54	15.91	13.65	15.96
Additive										
	No additive	6	281.0^{c}	82.6	8.9	19.7^{ab}	75.5^{b}	500.1^{a}	756.9^{a}	70.0
	Molasses	6	370.1^{a}	103.6	9.1	14.5^{b}	105.9^{a}	350.0^{b}	602.1^{b}	55.3
	Maize meal	6	331.2^{ab}	91.2	8.4	20.4^{a}	65.0^{b}	381.0^{b}	594.4^{b}	59.0
	Brown sugar	6	320.7^{bc}	92.0	7.1	18.7^{ab}	62.3^{b}	408.0^{b}	663.3^{b}	62.6
SEM			10.53	5.58	0.74	1.36	3.59	22.50	19.30	22.57
Significance										
	Fertilisation (F)		ns	ns	ns	ns	ns	ns	ns	ns
	Additives (A)		**	ns	ns	*	**	**	**	ns
	F x A		*	ns	ns	*	ns	ns	ns	ns

**: P <0.01; *: P <0.05; (ns) not significant: P >0.05. ^{abc} Column means with different superscripts differ significantly at P <0.05. DM: Dry Matter, CP: Crude Protein, NPN: Non Protein Nitrogen, NDF: Neutral Detergent Fibre, ADF: Acid Detergent Fibre, Acid Detergent Lignin, N: Number of Observations, g kg^{-1}: Grams per Kilogram, g kg^{-1} DM: Grams per Kilogram Dry Matter and SEM: Standard Error Mean.

Table 3.6: Effects of fertilisation with bio-digester slurry and carbohydrate additives on macro- (g kg^{-1}) and micro- (mg kg^{-1}) minerals of Napier grass silage after 90 days of ensiling

Fertilisation	Additive	N	Macro-minerals (g kg^{-1})					Micro-minerals (mg kg^{-1})			
			Ca	Mg	K	Na	P	Zn	Cu	Mn	Fe
No slurry	No additive	3	2.9	3.7	17.5	0.3	1.2	39.2	3.6	103.2^{a}	655.0
	Molasses	3	5.4	4.7	29.3	1.0	1.1	29.1	6.4	88.7^{ab}	451.5
	Maize meal	3	2.3	2.8	12.3	0.7	1.1	34.9	5.7	74.1^{b}	564.4
	Brown sugar	3	2.5	3.1	14.8	0.2	1.1	34.6	6.3	83.5^{ab}	430.4
Slurry	No additive	3	2.5	3.0	17.8	0.4	1.4	34.1	6.5	77.4^{b}	416.1
	Molasses	3	5.3	4.6	31.6	0.9	1.3	27.2	6.4	88.8^{ab}	441.3
	Maize meal	3	2.4	2.8	15.5	0.4	1.4	36.8	5.9	76.4^{b}	483.0
	Brown sugar	3	2.2	2.4	13.6	0.4	1.0	29.8	5.0	68.9^{b}	368.7
SEM			0.16	0.23	1.84	0.08	0.09	2.51	0.68	4.20	52.86
Fertilisation Means											
No slurry		12	3.3	3.6^{a}	18.5	0.6	1.1^{b}	34.4	6.2	87.4^{a}	525.3^{a}
Slurry		12	3.1	3.2^{b}	19.6	0.5	1.3^{a}	32.0	5.9	77.9^{b}	427.3^{b}
SEM			0.08	0.12	0.92	0.04	0.05	1.25	0.34	2.10	26.43
Additive Means											
	No additive	6	2.7^{b}	3.3^{b}	17.6^{b}	0.3^{b}	1.3	36.6^{a}	6.4	90.3^{a}	535.6
	Molasses	6	5.4^{a}	4.6^{a}	30.5^{a}	0.9^{a}	1.2	28.2^{b}	6.4	88.8^{a}	446.4
	Maize meal	6	2.4^{b}	2.8^{b}	13.9^{b}	0.5^{b}	1.3	35.9^{a}	5.8	75.2^{b}	523.7
	Brown sugar	6	2.3^{b}	2.8^{b}	14.2^{b}	0.3^{b}	1.0	32.2^{ab}	5.7	76.2^{b}	399.6
SEM			0.11	0.16	1.30	0.06	0.07	1.77	0.48	2.97	37.37
Significance											
	Fertilisation (F)		ns	*	ns	ns	*	ns	ns	**	*
	Additive (A)		**	**	**	**	ns	*	ns	**	ns
	F x A		ns	ns	ns	ns	ns	ns	ns	*	ns

**: *P* <0.01; *: *P* <0.05; (ns) not significant: *P* >0.05. ^{ab} Column means with different superscripts differ significantly at *P* <0.05. Ca: Calcium, Mg: Magnesium, K: Potassium, Na: Sodium, P: Phosphorus, Zn: Zinc, Cu: Copper, Mn: Manganese, Fe: Iron, N: Number of Observations, g kg^{-1} DM: Grams per Kilogram Dry Matter, mg kg^{-1} DM: Milligrams per Kilogram Dry Matter and SEM: Standard Error Mean.

3.4 Discussion

3.4.1 Chemical composition of fresh cut Napier grass at pre-ensiling

The DM content is an ideal characteristic of material for silage preservation due to its strong influence on the rate and extent of the resulting fermentation (McDonald *et al.*, 2011). Wilkinson (2005) recommended that forage with 250 - 400 g kg^{-1} DM content is required for satisfactory fermentation. A DM content below 250 g kg^{-1} at ensiling combined with a low sugar content may also cause high effluent and loss of DM, which increases the chances of a clostridia fermentation, resulting in poor acceptance of the silage by the animals (Fraser *et al.*, 2000; McDonald *et al.*, 2011). In the present study, the observed DM content of fresh cut Napier was lower compared to findings by Lubisi (2014), who reported DM content of 400 and 330 g kg^{-1} for bio-digester slurry and no bio-digester slurry fertilised Napier grass respectively. The differences might be attributed to the stage of maturity at harvest (Tyrolova and Vyborna, 2008) or to the time of harvest during the day (Owens *et al.*, 2002).

The positive effect of fertilisation with bio-digester slurry on pH contradicts the findings of Lubisi (2014), who reported similar pH content of fresh cut Napier grass fertilised with and without bio-digester slurry fertilisation. However, the pH of fresh cut Napier grass materials before ensiling was within the range of 5 and 6 reported by Kung (2001).

Different ranges of WSC have been recommended as essential to achieving a well-preserved silage. In the current study, the concentration of WSC in Napier grass exceeded the recommended range. Haigh (1990) recommended a minimum of 37 g WSC kg^{-1} DM for successful fermentation in a herbage. Lunden-Pettersson and Lindgren (1990), recommended 60 – 70 g kg^{-1} DM of WSC and Jaakkola *et al.* (1991) recommended 60 g kg^{-1} DM. Generally, Napier grass typically contains low WSC (Evangelista *et al.*, 2004; Iqbal *et al.*, 2005; Nisa *et al.*, 2006; Bureenok *et al.*, 2012). The findings of the study are consistent with similar studies on *Pennisetums* grown in tropical environments (Manyawu *et al.*, 2003; Bureenok *et al.*, 2012; Markos and Fulpagare, 2015). Differences in WSC can be attributed to genotype, maturity at harvesting and climatic conditions (Addah *et al.*, 2011).

The positive influence of bio-digester slurry on CP contradicted findings by Lubisi (2014), but was consistent with findings by Beckwith *et al.* (2002). The disparity could be attributed to the dependence of the CP content of Napier grass on soil fertility (Kariuki, 1998), the quality of the bio-digester slurry and other climatic factors.

In the present study, as expected, the slurry treatment had a positive influence on the total ash content in Napier fodder. Similar results were obtained by Rahman *et al.* (2008) and Islam *et al.* (2012). Variations in ash content among different studies likely reflect variations in the soil and slurry mineral contents (Islam *et al.,* 2010). The lack of bio-digester slurry effect on NDF, ADF and ADL content of Napier contradicted previous studies by Islam *et al.* (2010) and Rahman *et al.* (2008), in which the slurry reduced forage NDF and ADF content.

3.4.2 Fermentation characteristics and chemical composition of Napier grass silages

In terms of fertilisation effect, the silage pH range of our study was less than 4.5, which was considered ideal for effective forage preservation (McDonald *et al.*, 1991; Bilal, 2009). The results were consistent with findings by Lubisi (2014) findings, who reported similar reduction of pH of ensiled Napier grass. The low pH of the ensiled silage across all treatments confirmed the availability of WSC and reflected the correct concentration of LAB which were responsible for the fermentation of WSC thus increasing accumulation of LA (McDonald *et al.*, 1991; Seglar, 2003). The pH was characteristic of good silage regardless of carbohydrate additives, with the best effect obtained with the brown sugar treatment. The findings were consistent with terminal silage pH ranges reported in previous studies with additive-treated silages (Ukanwoko and Igwe, 2012; Lubisi, 2014; Wyss and Arrigo, 2015; Nkosi *et al.*, 2016). The positive effect of additives on the silage pH was consistent with findings by Lubisi (2014), who similarly reported reduced pH with molasses, brown sugar and maize meal additives in Napier grass silage.

Concentration of WSC is important since they are regarded as vital substrates for the growth of LAB to enhance efficient fermentation (McDonald *et al.*, 2011). It is not surprising therefore that for both no- bio-digester slurry and bio-digester slurry irrigated Napier grass were successfully ensiled due to adequate WSC at pre-ensiling that exceeded Haigh (1990) minimum recommended concentration. However, fertilisation with bio-digester slurry reduced residuals of WSC content. There is limited information regarding the effect of fertilisation with bio-digester slurry on WSC of silage. However, Wang *et al.* (2009) showed that applying N fertiliser at a rate higher than standard rate reduced the WSC content in timothy silage.

The residual WSC content, as expected, was increased due to additives inclusion with the best effect obtained with the molasses silages. Higher WSC in molasses silage could be beneficial to ruminants, as a result of the positive effect on palatability (Tava *et al.*, 1995). Similar results have been reported in literature (Mutavhatsindi, 2015: Mtengeti *et al.*, 2006). Corroborating the results obtained in this experiment with regards to WSC, Man and Wiktorsson (2002) reported that no-additive silage had nearly the same pH value and LA concentration compared to the silage treated with carbohydrate additives, suggesting that WSC was not the sole substrate for LAB. According to McDonald *et al.* (1991) starch, the main storage carbohydrate in plants could be a substrate after the attack of enzymes in the initial ensiling process, even though the majority of LAB do not attack starch. Hence, LA production was more efficient when WSC instead of starch was fermented (Sibanda *et al.*, 1997).

LA production is essential to obtain high quality silage by preserving silage from spoilage and pathogenic organisms (Holzer *et al.*, 2003). Being the most efficient and strong fermentation acid, it decreases the silage pH value more efficiently than other fermentation products (McDonald *et al.*, 2002) and is a key factor in the stability and long term preservation of silage. In the current study, the reduced LA concentration for bio-digester slurry silage was probably due to reduced residual WSC. The results obtained in this study agree with those of Lubisi (2014) results who observed reduced LA content as a result of bio-digester slurry application. However, negative correlation between LA of silage and DM intake by livestock have been reported by Wright *et al.* (2000). This was because higher concentrations of fermentation acids, LA in particular, represented an important stress factor in ruminal digestion (Doležal *et al.,* 2012), leading to the accumulation of LA which was known as lactic acidosis (Xu and Ding, 2011).

Compared to other fermentation acids (i.e., acetic, propionic, and butyric) in silages, LA is stronger (Khaing *et al.*, 2014) and it prevents the increase of undesirable bacteria. The LA production was not affected by the carbohydrate additives inclusion (Table 3.5). However, a decrease in pH and an increase in concentration of LA due to additives inclusion had been reported previously (Seglar, 2003; Lubisi 2014; Mutavhatsindi, 2015). A range of 60 - 100 g LA kg^{-1} DM was desirable for good quality silage as this retained more DM and energy and preserved the silage well for a long period of time (Bethard, 2006). However, in the current study, LA from maize meal treated silage remained low (19.2 g kg^{-1} DM) compared to molasses treated silage which had the highest (30.4 g kg^{-1} DM) LA content. The LA concentration was in range with some common fodder silages reported (Sibanda *et al.*, 1997; Lubisi, 2014). This was enough evidence that the additives could significantly improve the fermentation quality of silages (Filya *et al.*, 2007).

The ammonia nitrogen (NH_3-N) is an indicator of the fermentative quality of the silage, derived from the degradation of the protein fraction by clostridia (McDonald *et al.*, 1991). Nevertheless, this degradation occurred in Napier ensilage but was inhibited by the rapid decrease in pH as a result of the abundant presence of WSC. In this study, both fertilisation treated silage contained less than 100 g NH_3-N kg^{-1} of total N, which was indicative of well-preserved silage (McDonald *et al.*, 2002). Current findings agreed with the results reported previously by Lubisi (2014) who observed lower than 100 g NH_3–N kg^{-1} in both no slurry and bio-digester slurry treated silage.

Alli *et al.* (1983) recommendations, in agreement with McDonald *et al.* (1991), observed concentrations below 80 g NH_3-N kg^{-1} indicating an excellent quality silage, while on the other hand poor quality silage contained concentrations higher than 150 g kg^{-1}. When comparing the effect of carbohydrate additives, the best effect was obtained with the molasses treatment which recorded lowest NH_3-N content. The traditional method had centered around the reduction of proteolysis by reducing crop pH rapidly, which inhibited protein degradation in silages (McDonald *et al.*, 1991) and resulted in improved efficiency of silage protein utilization and reduced N losses (Charmley, 2001). High NH_3-N in maize meal treated silage might be due to excessive protein breakdown caused by a slow drop in pH or clostridial action (Kung and Shaver, 2001). However, all silages contained less than 100 g NH_3-N kg^{-1} of total N irrespective of treatment, which was indicative of well-preserved silage (McDonald *et al.*, 2002). This could be explained by the fact that reduction in pH of silage, resulted in decreased NH_3-N production. These results were lower compared to the ones reported by Lubisi (2014) who observed NH_3–N content ranging from 4 – 6 g kg^{-1} DM for no additive, molasses, brown sugar and maize meal treated silage.

The silage DM range of our study exceeded 300 g kg^{-1}, which indicated good quality silage regardless of bio-digester slurry treatment. Increased DM content reduces chances of susceptibility to clostridial fermentation (McDonald *et al.*, 2002; Oliveira *et al.,* 2015). In agreement, Lubisi (2014) reported no significant effects of bio-digester slurry on the DM content of Napier grass silage.

Improved DM content of the silage in response to molasses, maize meal and brown sugar was consistent with previous studies (Yunus *et al.,* 2000; Man and Wiktorson, 2001; Hiep *et al.*, 2008;

Lubisi, 2014; Yitbarek and Tamir, 2014). The improvement ensured low effluent, chemical stability and higher DM intake by animals (Tjandraatmadja *et al.*, 1993). However, the superiority of molasses to other carbohydrates treatments confirmed the results of McDonald *et al.* (2002) and Tong (2011). This improvement could be due to homolactic fermentation which decreased DM fermentation losses (Sharp *et al*., 1994). Increased LA production imposes and stabilizes silage at low pH which cellulolytic microbes cannot tolerate (Sebolai *et al.*, 2012; Rusdy, 2015).

In the present study, fermentation decreased the CP content of silages across all treatments, similar to findings by Lubisi (2014). Reduction of CP content in the ensiled forage may be due to hydrolysis of protein, which is accompanied by an increased content of proteolysis products, such as free amino acids and NH_3-N (Duniére *et al.*, 2013). Leibensperger and Pitt (1988) reported that when the pH of the fermented silage is reduced sufficiently to 4.3 or lower, the proteolytic activities are inactivated. Lubisi (2014) observed similar CP content in Napier silage fertilised with and without bio-digester slurry, which confirms present study results.

The effect of carbohydrate additives on CP content in silage were inconsistent, ranging from positive (Mahala and Khalifa, 2007; Arbabi and Ghoorchi, 2008; Nkosi *et al.,* 2012a), negative (Man and Wiktorsson, 2002; Hiep *et al.,* 2008) to no effect (Van Neikerk *et al.,* 2007; Lubisi, 2014). The variation could be attributed to the dependence of the CP content on soil fertility, genotype, climatic or seasonal effects (Mbuthia, 2003). Overall, the observed CP content was above the critical 75 g kg^{-1} DM (Jusoh *et al.*, 2014) essential for rumen function.

Non protein nitrogen is formed due to proteolysis during fermentation as a result of extensive degradation of native plant proteins (Vagnoni *et al.*, 1997). Large quantities of NPN have been reported to affect the efficient utilization of silage by ruminants (Broderick and Kang, 1980). There are no comparable studies on the effects of bio-digester slurry treatment and additives on inhibiting protein degradation during ensiling. In the present study, NPN content of all silages was less than 120 to 150 g kg^{-1} of CP that is associated with clostridial fermentation (Kung, 2010).

Lubisi (2014) findings confirm results of the study on silage ash content. Elevated ash in silage is largely attributed to soil contamination (Seglar, 2003). In addition, loss of nutrients in the form of effluent and gas during ensiling increased the ash content (Pedroso *et al.*, 2005). The greater effect of molasses added silage on ash content were supported by previous studies (Bilal, 2009; Wyss and Arrigo, 2015; Kanengoni *et al.,* 2016), but were contradicted by findings of Lubisi

(2014). Gofen and Khalifa (2007) reported that molasses itself has high mineral content which contributes to the silage ash content when used as a carbohydrate additive.

Improved fat content of the silage in response to maize meal inclusion confirmed data in literature (Nkosi and Meeske, 2010) but contradicted reports (Alikhali *et al.*, 2005; Mokoboki *et al.,* 2016) of reduced fat content due to addition of molasses. The total amount of fat in the silage was lower than National Research Council (NRC) (2007) recommendations which stipulate that fat content in the diet must not exceed 60 to 70 g kg^{-1} DM. Fibre fermentation in the rumen and post ruminal digestibility could be reduced by higher fat content (NRC, 2007).

Fermentation decreased NDF and ADF content of silages in all treatments as the breakdown of the cell wall fractions provides additional substrate for fermentation (Nkosi *et al.*, 2016). Similar findings were reported by Lubisi (2014). The lower NDF and ADF content in additives treated silages is consistent with previous studies on Napier grass (Mtengeti *et al.*, 2006; Bureenok *et al.*, 2012; Lubisi, 2014; Wyss and Arrigo, 2015; Kanengoni *et al.*, 2016), cassava and Gliricidia tops silage (Man and Wiktorsson, 2002), sunflower (Alikhali *et al.*, 2005), sorghum *(*Guney *et al.*, 2007), soybean silage (Tobía *et al.,* 2008) and *Opuntia cladodes* (Mokoboki *et al.*, 2016) silage. This could be explained partially by the additives stimulation effect on the fibre fermentation which thereby reduced the fibre content of silage. The addition of molasses enhanced fermentation, which increased cell wall degradation (Baytok *et al.*, 2005). Due to negative correlation between both NDF and ADF with intake and digestibility, respectively (Minson, 1990), lower contents of both NDF and ADF could result into increases in dry matter intake and digestibility of the silage. However, regardless of the treatments the NDF and ADF contents of all silages were above the minimum of 300 and 190 g kg^{-1} DM respectively, required for healthy rumen of dairy cows (Target, 2002).

In the present study, ADL content was increased by ensilage, which can be explained by the depletion of fermentable organic matter, as supported by the increased ADL across all carbohydrate additives. In agreement with present findings, Bai *et al.* (2011) reported higher ADL content in no additive silages compared to those of additives treated as a result of fermentation. Contradictory to this observation Pinho *et al.* (2004), Baytok *et al.* (2005) and Zereu *et al.* (2015) reported reduced effect due to additives inclusion during ensiling.

The NRC (1976) recommended 3.0 g kg^{-1} as the critical level of Ca in the diet of ruminants. In terms of fertilisation treatment, concentrations of Ca in silages were in an acceptable range. However, higher Ca in molasses silage was consistent with its Ca content, but no additive, maize meal and brown sugar treatments were not in NRC (1976) acceptable range. The present study results were in agreement with data reported by Amodu *et al.* (2005) and Lubisi (2014) of 2.5 to 6.0 g kg^{-1} Ca content of both Lablab, Pearl millet and Napier grass silage.

Lubisi (2014) reported an average of 30 g kg^{-1} Mg content for both slurry and no slurry treated silage which was higher than 3.6 and 3.2 g kg^{-1} for no slurry and slurry treated silage respectively, as observed in this study. Furthermore, molasses silage had higher (P <0.01) Mg content compared to no additive, maize meal and brown sugar treated silage. However, the results of the present study are in line with findings in literature whereby Lubisi (2014) reported Mg content ranging from 2.0 to 5.0 g kg^{-1} on grass silage treated with molasses, brown sugar, maize meal and no additive. Although early lactation ruminants require 2 g kg^{-1} (Gill *et al.*, 2004), all silages would supply adequate Mg to meet the Mg requirements of ruminants.

A higher content of K found in bio-digester slurry treated silage might be due to application of bio-digester slurry (Table 3.2). While molasses inclusion outperformed other treatments, implying that molasses might be a good source of minerals. The present results contradicts those of Lubisi (2014), who reported no significant effect due to carbohydrate additives. Hence, 8 g kg^{-1} was a recommended critical requirement level for grazing animals (Underwood, 1981). High producing ruminants, under stress such as heat stress, may require K levels above 10 g kg^{-1}. Therefore, the results suggested that ruminants would need K supplementation.

The low content of Na in bio-digester slurry (Table 3.2) did not have a positive effect on Na content of silage. On the other hand, molasses additive treatment continued to outdo other additives treatments in terms of Na content. However, the results contradicted with those of Lubisi (2014) who reported significantly higher Na content in maize meal silage. Furthermore, regardless of treatment Na concentration was lower than Underwood (1981) and Gill *et al.* (2004) recommended a range of 1 - 4 g kg^{-1} for growing and finishing ruminants. To support current findings, natural forages with deficiency of this element had already been reported in other regions of the world (Aregheore, 2002).

It is not surprising that the lower content of P in silage did not differ due to fertilisation since both experimental soil and slurry used (Table 3.2) contained low content of P. Carbohydrate additives diluted the P content of silage. Contrastingly, Lubisi (2014) observed no significant effects due to additives. P concentration was lower than recommended (1.9 g kg^{-1}) for beef cattle as observed by Gill *et al.* (2004), implying that, P supplementation was required.

Even though bio-digester slurry had higher total zinc content (Table 3.2) the bio-availability forms of Zn might have caused reduction in Zn content of silage (Fuentes *et al.*, 2004). Carbohydrate additives reduced the Zn content of silage. The results were in agreement with findings of Lubisi (2014) study. Zinc concentration for all treatments were above the recommended ranges of 12 - 20 mg kg^{-1}, adequate for growing ruminants (Gill *et al.*, 2004).

It is commonly suggested that the dietary requirement of ruminants for Cu ranges from 8 to 14 mg kg^{-1} (Gill *et al.*, 2004; Khan *et al.*, 2006). Hence, Cu content for all silage treatments were lower than Cu recommended rates. Similar results have been reported by Lubisi (2014).

The high content of Mn in bio-digester slurry (Table 3.2) caused significant reduction in Fe content of silage. Carbohydrate additives reduced the Mn content of silage. The present results contradicted results reported by Lubisi (2014) who observed no significant effect due to bio-digester slurry fertilisation and significant reduction in Fe content due to carbohydrate additives. Manganese concentrations of the present study for all treatments were above the recommended levels of 20 mg kg^{-1} for growing and finishing cattle and 40 mg kg^{-1}, the critical level of dietary Mn (Gill *et al.*, 2004).

The high content of Fe in bio-digester slurry (Table 3.2) caused significant reduction in Fe content of silage. Carbohydrate additives reduced the Fe content of silage. The present results contradicted results reported by Lubisi (2014) who observed no significant effect due to bio-digester slurry fertilisation and significant reduction in Fe content due to carbohydrate additives. Iron concentration were above the levels of 50 mg kg^{-1} proposed as adequate for grazing animals (Gill *et al.*, 2004; Khan *et al.*, 2005). In nature, red clay soils used in the present study are known to contain high Fe content.

3.5 Conclusion

Fertilisation with bio-digester slurry improved the CP content of fresh cut Napier grass. Furthermore, fertilising with bio-digester slurry and molasses inclusion at ensiling improved the quality and fermentation characteristics of Napier grass silage. Therefore, ensiled Napier grass can be a substitute for natural pasture during winter.

CHAPTER 4:

EFFECT OF FERTILISATION WITH BIO-DIGESTER SLURRY AND THE INCLUSION OF CARBOHYDRATE ADDITIVES AT ENSILING ON DRY MATTER AND CRUDE PROTEIN RUMINAL DEGRADABILITY OF NAPIER GRASS SILAGE USING THE NYLON BAG TECHNIQUE

4.1 Introduction

Digestion in the rumen involves a sequential attack by ruminal microorganisms on feed (Cheng *et al.*, 1991). Feed protein is either rumen-degradable or rumen-undegradable. The rate and extent of degradation in the rumen is very important as this determines the supply of dietary nutrients both to the rumen microbes and to host animal body tissues (Mohamed and Chaudhry, 2008). The *in sacco* technique has been used to rank feeds according to the rate and extent of degradation of dry matter, organic matter, nitrogen, neutral detergent fibre and acid detergent fibre (Harazim *et al.*, 2002; Třináctý *et al.*, 2003; Čerešňáková *et al.*, 2007; Homolka *et al.*, 2008; Jančík *et al.*, 2009; Rambau *et al.*, 2016). The method is widely applied due to its simplicity, reliability and accuracy of prediction (Osuji *et al.*, 1993). The method is considered to be a reference method to estimate important degradation parameters such as the soluble "*a*", the insoluble but degradable and undegradable "*b*" fractions, outflow rate "*c*", potential "*a + b*" and effective degradability (ED). Parameters are conveniently quantified by applying the equation, P= a + b $(1 - e^{-ct})$ (Ørskov and McDonald, 1979). These parameters are applied in feeding evaluation models to estimate the nutritive value, nutrient supply, predict feed intake and growth rate (Hackmann *et al.*, 2010). For example, high rates of degradation have imply to result in high voluntary intake and thus higher performance (Sun *et al.*, 2012).

The potential of silage in ruminant animal nutrition has long been recognized (Manyawu *et al.*, 2003; Bureenok *et al.*, 2012; Ukanwoko and Igwe, 2012; Lubisi, 2014; Markos and Fulpagare, 2015; Nkosi *et al.*, 2016). However, very little is known about the ruminal degradability. Carbohydrate additives might improve the quality of silage protein by reducing the rumen degradable protein, hence, increasing the intestinal supply of rumen undegradable protein (Nowak *et al.*, 2004). The objective of this study was to evaluate the effect of fertilisation with bio-digester slurry and inclusion of carbohydrate additives at ensiling on the dry matter (DM) and crude protein (CP) ruminal degradability of Napier grass silage using the nylon bags technique.

4.2 Materials and methods

4.2.1 Experimental site

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

4.2.2 Napier grass silage

Samples used in current experiment were prepared as described in Chapter 3.

4.2.3 Experimental design

Two factors were considered. These were fertilisation with bio-digester slurry and carbohydrate additives inclusion. The first factor involved two fertilisation levels; No bio-digester slurry (control-water irrigation) and bio-digester slurry irrigation (30 t ha^{-1}). The second factor was at four treatment levels; no additive (control), molasses, brown sugar and maize meal included at 10 % of fresh materials weighed. Accordingly, the experiment was set up as a 2 x 4 factorial arranged in a CRD, with three replications in each treatment. Thus, there were 8 treatment combinations.

4.2.4 Sample preparation

Representative silage for each fertilization treatment (Napier with and without bio-digester slurry) treated with four carbohydrate additives [no additive (control), molasses, brown sugar and maize meal] were oven dried at 60 ºC for 48 h (AOAC, 1990; method 930.15) and ground to pass a grinding mill of 1 mm screen size before incubation in the rumen.

4.2.5 Ethical consideration

The study was approved by the Ethics Committee of the University of Venda as described in Chapter 3, subsection 3.2.5.

4.2.6 Animal housing and feeding

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

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

Composition	**g kg^{-1}**
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^{-1}
Monensin NA	30
Zinc Bacitracin	50

*Supplied by Driehoek feeds (Vaalwater, Waterberg, Limpopo, South Africa). g kg^{-1}: Grams per Kilogram, mg kg^{-1}: Milligrams per Kilogram, %: Percentage, min: Minimum and max: Maximum

4.2.7 Degradability study

The nylon bag technique of Ørskov and McDonald (1979) was used. Representative silage samples of approximately 5 g each were weighed in well-labeled nylon bags (external dimension: 6 × 12 cm, pore size of 46 μm). The sample bags were duplicated per animal and incubation period in the rumen giving a total of 384 samples. According to Osuji *et al.* (1993), 40 – 60 bags can be incubated at the same time in the rumen of cattle. Hence, for present study, bags for 3 incubation hours (about 48 bags per steer) were incubated for the first time followed by last 4 incubation hours (about 64 bags per steer). 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 (Plate 4.1a). 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 (Plate 4.1b) of three Bonsmara steers and withdrawn subsequently at: 6, 12, 24, 48, 72, 96 and 120 incubation h for forages (Osuji *et al.*, 1993).

The bags were inserted in the rumen at 06.00 h before the morning feeding time. Immediately at the end of each incubation time, the bags were removed from the rumen, immediately washed gently under low running tap water while rubbing gently between thumb and finger, till the water was clear and rinsed with deionized water. The zero hour (control) bags were washed without incubation in the rumen.

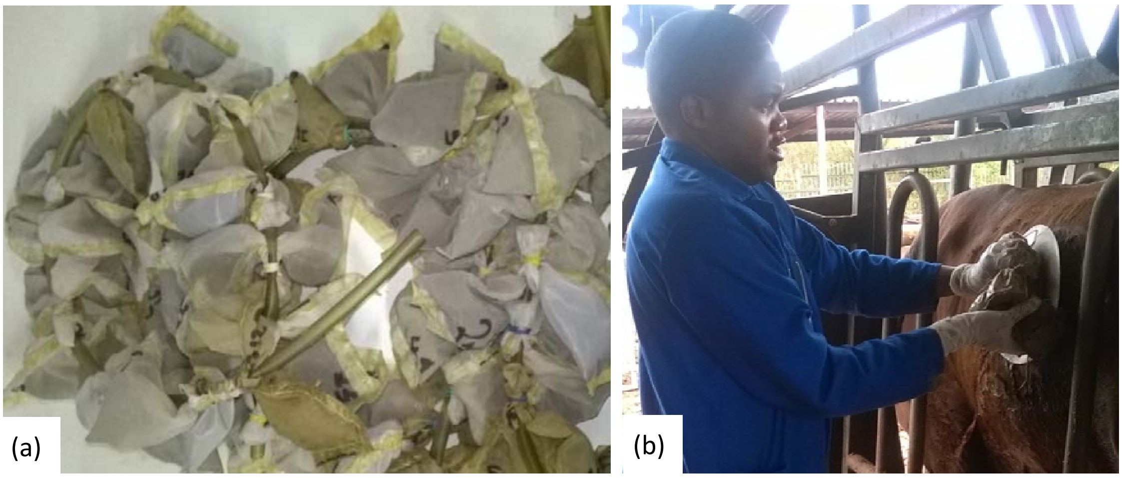

Plate 4.1: (a) Nylon bags attached to flexible vinyl plastic tubes and (b) Inserting nylon bags for incubation in the rumen

4.2.8 Chemical analyses

The washed bags were dried in a forced air oven at 60°C 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 the silage treatment, incubation hour and steers and subsequently ground through a 1 mm sieve and analysed in duplicates. The residues were then 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.

4.2.9 Mathematical analyses

The nutrient degradation was calculated by the difference between the amount in control sample and degraded residues, expressed as a percentage. The degradation profiles of DM and protein with time for each sample was described using the mathematical model of Ørskov and McDonald (1979):

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

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

a = the zero time intercept;

b = the slowly degradable fraction; and

c = the rate of degradation.

Potential degradability (PD) was estimated as (*a* + *b*). Effective degradability (ED) was calculated using rumen fractional outflow rates (k) of 0.02, 0.05 and 0.08 per h according to Ørskov and McDonald (1979):

$$ED = a + \frac{bc}{(k + c)}$$

The degradation constants were estimated using the Neway "Fitcurve" Excel software version 6 (Chen, 1995).

4.2.10 Statistical analysis

Analysis of variance (ANOVA) on silage degradability data (Model I) was performed at *P* <0.01 and *P* <0.05 according to Steel and Torrie (1980) using GLM procedures of Minitab Statistical package version 17 (Minitab, 2014). Where significant differences between the treatment groups was detected, means were separated using the Tukey's test (Tukey, 1953).

$Y_{ijkl} = \mu + A_i + S_j + C_k + (SC)_{jk} + \varepsilon_{ijkl}$ Model I

Where Y_{ijkl} = the observation, ruminal degradability of DM and N, ruminal kinetics;

μ = overall mean common to all observations;

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

S_j = effect of j^{th} bio-digester slurry, j = 1 or 2;

C_k = effect of k^{th} carbohydrate additive, k = 1, 2, 3 or 4;

$(SC)_{jk}$ = interaction between j^{th} bio-digester slurry and k^{th} carbohydrates additive; and

ε_{ijkl} = random residual error.

4.3 Results

4.3.1 *In sacco* dry matter and crude protein degradability

In sacco DM disappearance (%) of Napier grass silage is presented in Figure 4.1. No bio-digester slurry fertilisation with maize meal inclusion had greater (*P* <0.01) DM degradability at 0 h incubation. The control (i.e. no treatment) had lowest (*P* <0.01) DM degradability after 24 h incubation while no bio-digester slurry fertilisation with molasses inclusion increased (*P* <0.01) DM degradability at 96 incubation h. On the other hand, no bio-digester slurry fertilisation with molasses inclusion and bio-digester slurry fertilisation with molasses inclusion increased (*P* <0.05) DM degradability at 120 incubation h.

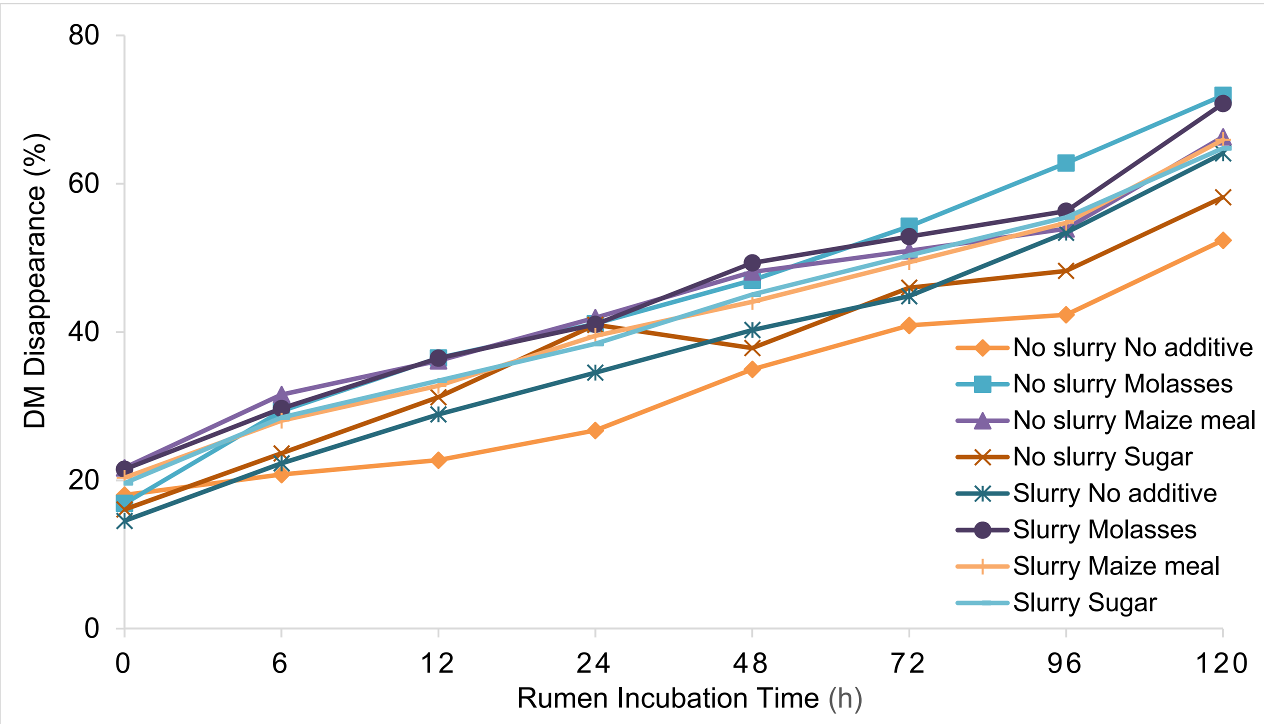

Figure 4.1: *In sacco* dry matter disappearance of Napier grass silage after 90 days of ensiling

Fertilisation treatments had similar (*P* >0.05) DM disappearance for Napier grass silage at 0, 6, 12, 24, 48 and 72 incubation hours, while DM disappearance was increased at 96 (*P* <0.05) and 120 (*P* <0.01) incubation h due to fertilisation with bio-digester slurry as illustrated in Appendix 9. However, carbohydrate additives inclusion increased (*P* <0.01) DM disappearance at 0, 6, 12, 24, 48, 72, 96 and 120 h incubation as illustrated in Appendix 10.

In sacco CP disappearance (% DM) of Napier grass silage is presented in Figure 4.2. Fertilisation with bio-digester slurry and carbohydrate additives interaction had no effect (*P* >0.05) on CP disappearance at 0, 6, 12, 24, 48, 72, 96 and 120 incubation h.

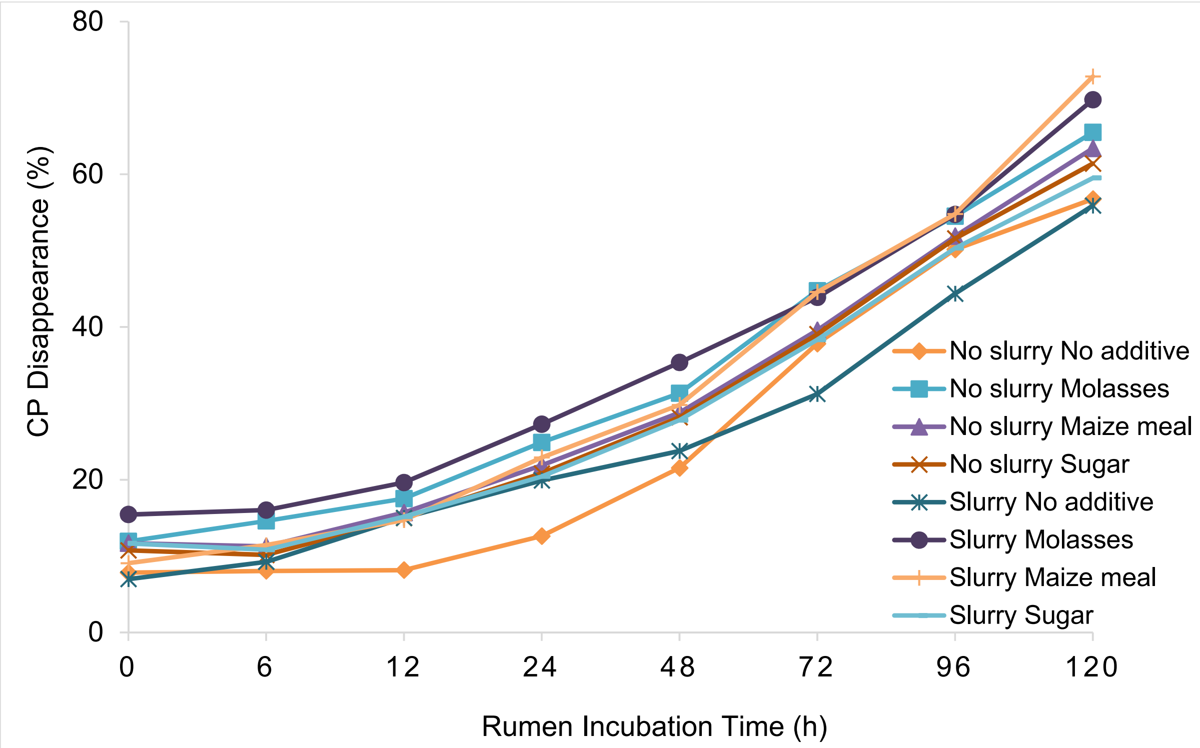

Figure 4.2: *In sacco* crude protein disappearance of Napier grass silage after 90 days of ensiling

Fertilisation treatments had similar (*P* >0.05) CP disappearance for Napier grass silage at 0, 6, 12, 24, 48, 72, 96 and 120 h incubation as illustrated in Appendix 11. Carbohydrate additives increased CP disappearance at 48 (*P* <0.05) and 120 (*P* <0.01) incubation hour, but had no effect (*P* >0.05) on disappearance at 0, 6, 12, 24, 72 and 96 h incubation as illustrated in Appendix 12.

4.3.2 Dry matter and crude protein degradability kinetics

Mean degradability parameter values obtained by fitting the model of Ørskov and McDonald (1979), defining the kinetics of DM degradation and effective degradability at three rumen fractional outflow rates, are presented in Table 4.2. No bio-digester slurry with maize meal inclusion increased (*P* <0.01) soluble fraction "*a*" but the group only differ compared to no bio-digester slurry with brown sugar included and bio-digester slurry with no additive inclusion treatments. On the other hand, no bio-digester slurry with no additive inclusion reduced (*P* <0.01) slowly degradable fraction "*b*" which lead to reduced (*P* <0.01) potential degradability "*a + b"* of Napier grass silage. However, fertilisation with bio-digester slurry and carbohydrate additives interaction had no effect (*P* >0.05) on outflow rate of degradation "*c*" and effective degradability of Napier grass silage at 2, 5 and 8 % outflow rate. Fertilisation with bio-digester slurry increased (*P* <0.05) soluble fraction "*a"*, slowly degradable fraction "*b"* and potential degradability "*a + b"*, and effective degradability at 2, 5 and 8 % outflow rate but had no effect (*P* >0.05) on outflow rate of degradation "*c"*. Lastly, carbohydrate additives increased (*P* <0.01) soluble fraction "*a"*, slowly degradable fraction "*b"* and potential degradability "*a + b"*, and effective degradability at 2, 5 and 8 % outflow rate but had no effect (*P* >0.05) on outflow rate of degradation "*c"*.

Mean degradability parameter values obtained by fitting the model of Ørskov and McDonald (1979), defining the kinetics of CP degradation and effective degradability at three rumen fractional outflow rates, are presented in Table 4.3. Bio-digester slurry fertilisation and carbohydrate additives inclusion interaction had no effect (*P* >0.05) on soluble fraction "*a"*, slowly degradable fraction "*b",* potential degradability "*a + b"*, outflow rate of degradation "*c*" and effective degradability at 2, 5 and 8 % outflow of CP disappearance. Fertilisation with or without bio-digester slurry had similar (*P* >0.05) soluble fraction "*a*", slowly degradable fraction "*b",* potential degradability "*a + b"*, outflow rate of degradation "*c*" and effective degradability at 2, 5 and 8 % outflow of CP disappearance. However, carbohydrate additives inclusion increased (*P* <0.05) potential degradability "*a + b"* but had no effect (*P* >0.05) on soluble fraction "*a"*, slowly degradable fraction "*b",* outflow rate of degradation "*c*" and effective degradability at 2, 5 and 8 % outflow of CP disappearance.

Table 4.2: Degradability constants and calculated effective degradability at three passage rates for dry matter disappearance of Napier grass silage after 90 days of ensiling

Fertilisation	Additives	N	Degradability constants (%)				ED (%) at different outflow rates		
			a	*b*	*c*	*a* + *b*	*K* = 0.02	*K* = 0.05	*K* = 0.08
No slurry	No additive	6	18.0^{abc}	55.2^{c}	0.005	73.2^{d}	55.4	44.2	38.1
	Molasses	6	16.9^{abc}	80.3^{a}	0.007	97.2^{a}	68.3	52.9	44.6
	Maize meal	6	21.7^{a}	74.4^{ab}	0.008	96.1^{ab}	69.1	54.9	47.3
	Brown sugar	6	16.1^{bc}	65.7^{b}	0.016	81.8^{cd}	57.4	44.9	38.2
Slurry	No additive	6	14.5^{c}	71.9^{ab}	0.008	86.4^{bc}	60.3	46.6	39.2
	Molasses	6	21.5^{a}	74.1^{ab}	0.008	95.6^{ab}	72.1	56.9	48.7
	Maize meal	6	20.4^{ab}	73.5^{ab}	0.004	93.9^{ab}	67.5	53.3	45.7
	Brown sugar	6	19.6^{abc}	73.0^{ab}	0.010	92.6^{ab}	65.9	52.0	44.5
SEM			1.10	2.28	0.0047	2.30	2.21	1.81	1.61
Fertilisation									
No slurry		24	18.2	68.9^{b}	0.009	87.1^{b}	62.5^{b}	49.2^{b}	42.1^{b}
Slurry		24	19.0	73.1^{a}	0.008	92.1^{a}	66.4^{a}	52.2^{a}	44.5^{a}
SEM			0.55	1.14	0.0023	1.15	1.10	0.90	0.80
Additive									
	No additive	12	16.3^{b}	63.5^{c}	0.007	79.8^{c}	54.9^{b}	45.4^{b}	38.7^{b}
	Molasses	12	19.2^{ab}	77.2^{a}	0.008	96.4^{a}	70.2^{a}	54.9^{a}	46.6^{a}
	Maize meal	12	21.0^{a}	74.0^{ab}	0.006	95.0^{a}	68.3^{a}	54.1^{a}	47.3^{a}
	Brown sugar	12	17.8^{ab}	69.4^{bc}	0.013	87.2^{b}	61.6^{b}	48.5^{b}	41.4^{b}
SEM			0.78	1.61	0.0033	1.62	1.56	1.28	1.14
Significance									
	Fertilisation (F)		ns	*	ns	**	*	*	*
	Additives (A)		**	**	ns	**	**	**	**
	F x A		**	**	ns	**	ns	ns	ns

**: P <0.01; *: P <0.05; (ns) non-significant: P >0.05. ^{abcd} 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, %: Percentage and SEM: Standard Error Mean.

Table 4.3: Degradability constants and calculated effective degradability at three passage rates for crude protein disappearance of Napier grass silage after 90 days of ensiling

Fertilisation	Additives	N	Degradability constants (%)				ED (%) at different outflow rates		
			a	*b*	*c*	*a* + *b*	*K* = 0.02	*K* = 0.05	*K* = 0.08
No slurry	No additive	6	7.8	56.9	0.007	64.8	45.2	33.3	26.9
	Molasses	6	11.9	68.2	0.002	80.1	56.7	42.8	35.3
	Maize meal	6	11.7	63.0	0.002	74.7	55.1	41.6	34.3
	Brown Sugar	6	10.7	60.8	0.002	71.6	52.3	39.6	32.6
Slurry	No additive	6	7.0	58.1	0.001	65.1	46.9	34.9	28.5
	Molasses	6	15.5	70.3	0.001	85.8	64.7	49.8	41.7
	Maize meal	6	9.1	50.8	0.001	84.2	60.5	44.9	36.5
	Brown Sugar	6	11.7	58.7	0.002	70.3	52.1	39.5	32.8
SEM			4.79	9.33	0.0023	6.06	5.07	4.73	4.56
Fertilisation									
No slurry		24	10.5	62.2	0.003	72.8	52.5	39.3	32.3
Slurry		24	10.8	59.5	0.002	76.4	56.1	42.3	34.8
SEM			2.39	4.66	0.0012	3.03	2.54	2.36	2.28
Additive									
	No additive	12	7.4	57.5	0.004	64.9^{b}	46.1	34.1	27.7
	Molasses	12	13.7	69.2	0.002	82.9^{a}	60.7	46.3	38.5
	Maize meal	12	10.4	56.9	0.002	79.4^{ab}	57.8	43.3	35.4
	Brown Sugar	12	11.2	59.8	0.002	71.0^{ab}	52.5	39.6	32.7
SEM			3.39	6.60	0.0017	4.29	3.59	3.34	3.23
Significance									
	Fertilisation (F)		ns	ns	ns	ns	ns	ns	ns
	Additives (A)		ns	ns	ns	*	ns	ns	ns
	F x A		ns	ns	ns	ns	ns	ns	ns

*: 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, %: Percentage and SEM: Standard Error Mean.

4.4 Discussion

4.4.1 In sacco dry matter and crude protein degradability

The DM degradability of feeds is one of key variables for evaluating nutritive value in feeding systems (Gül *et al.*, 2008). Fertilisation with bio-digester slurry improved the DM degradability of silage, which suggests high voluntary intake of silage and thus higher performance (Sun *et al.*, 2012). Nevertheless, information regarding the effect of fertilisers on *in sacco* DM degradability of forage grasses is lacking.

The high solubility and digestibility of carbohydrate additives in the rumen increased DM disappearance of silage (Gomes *et al.*, 2015). Furthermore, carbohydrate additives might have broken down cell wall bonds during the fermentation of Napier grass silage (Fazaeli *et al.*, 2004; Nasehi *et al.,* 2014) leading to improvement in the DM degradability. Lower values for no additive than those with carbohydrate additives included, likely to affect the efficiency of microbial protein synthesis (Beever and Cottril, 1994), could be attributed to lower levels of rumen degradable protein or higher NDF contained in the no additive silage and/or the presence of high ADL content (Table 3.5). Oliveira *et al.*, (2013) reported that lignin acts as a barrier limiting the activities of digestive enzymes produced by the microorganisms of the rumen and therefore reducing the degradability and digestibility. Researchers reported an effect of higher ruminal DM degradability of grass silage at 8, 16, 24 and 48 hours of incubation due to carbohydrate additives inclusion (Gallop *et al.*, 2005; Shellito *et al.,* 2006; Sahoo and Walli, 2008; Gül *et al.*, 2008; Kaya *et al.*, 2009), while others (Granzin and Dryden, 2005) have reported that additives inclusion had no effect on silages DM degradability. Hence, the difference in the rate of disappearance between Napier grass silages with and without carbohydrate additives can be an important factor to influence animal consumption, improving the rate of passage and feed utilization by ruminant animals (Gomes *et al.*, 2015) as observed in the present study. The disappearance of the DM contents in the silages by the end of 48 incubation hour, generally considered equivalent to digestibility (Ehargava and Ørskov, 1987) and being the mean retention time of fibrous feeds in ruminants (Kimambo and Muya, 1991), revealed that Napier grass silage had less than 50% DM loss for all silage treatments.

All silages were above the minimum 75 g kg^{-1} CP required for the occurrence of a normal rumen function (Jusoh *et al.*, 2014) by maintaining the microbial fermentation activities that occur in the rumen (Silva *et al.*, 2014). In the present study, bio-digester slurry fertilisation had no effect on the CP degradability of silage. Contrastingly, the linear enhancement of CP degradability with more N fertiliser has been reported by Van Vuuren *et al.* (1991) and Sarwar *et al.* (1999), due to the improvement in the fermentation environment by providing deficient nutrients for ruminal microbes. However, information regarding the effect of fertilisers on *in sacco* CP degradability of forage grasses is lacking.

Carbohydrate additives treatments did not protect silage CP from ruminal degradation. The high rate of CP disappearance from the molasses treated Napier grass silage (Table 4.3) could favor high concentration of NH_3 in the rumen. Thomson *et al.* (1981) reported reduced CP degradability and an increase in the quantity of protein entering the small intestine when silage was treated with formaldehyde. Of all the examined protein residues, the CP of molasses treated silage was highest (P <0.05) at 48 incubation hour while that of maize meal treated silage was highest (P <0.01) at 120 incubation hour. Contrary to the observation, Polan *et al.*, (1998) and Nowak *et al.*, (2004) reported no significant effects of additives inclusion in silage on rumen CP degradation when measured by the *in sacco* method. Lower CP degradability in no additive silage could be due to high lignin content attributed in the silage (Table 3.5) because lignin acts as a mechanical barrier inhibiting microbial action (Van Soest, 1994), thus, rendering nutrient compounds unavailable during digestion (McDonald *et al.*, 2002). The inconsistency in literature data and present study results could be a reflection of variations in plant growing conditions (Table 3.1), such as season of the year, plant species, soil type, plant parts and stage of maturity (Norton and Waterfall, 2000; Kasuya *et al.*, 2008) and anti-nutritional factors such as lignin and tannins.

4.4.2 Dry matter and crude protein degradability kinetics

The fraction "*a*" of feed represents the soluble fractions that are rapidly and completely degraded in the rumen. Bio-digester slurry fertilisation had no effect on the readily degradable fraction "*a*" of DM of silage. Nevertheless, molasses, maize meal and brown sugar treated silage had a higher residual WSC content, giving them greater fraction "*a*" of DM when compared to no additive silage. In agreement, a study by Henriques *et al.* (2004), reported the superiority of fraction "*a*" of DM on Napier grass silage inoculated with microbial additive while Nowak *et al.*, (2004) reported no significance differences observed between silage treatments regarding any of the degradation parameters for the DM of round bale silage.

The information on slowly degradable fraction “*b*” disclosed that there was a big gap between the bio-digester slurry treated silage and no bio-digester treated silages, and also carbohydrate additives treated silages and no carbohydrate additives treated silages in DM degradability.

Similar outflow rate “*c*” fraction for all treatments can result in similar DM intake through similar passage rate in the rumen. On the other hand, the “*c*” of all silages obtained in this experiment were lower than those reported by Nowak *et al.* (2004).

The higher PD (*a* + *b*) of bio-digester slurry treated silage than no slurry, may be related to the growth enhancement of forage cell wall degrading microorganisms in the ensiled forage which thereby increased the DM soluble fraction (Asgharzadeh *et al.*, 2014). In terms of carbohydrate additives, the molasses treated silage outperformed other treatment with the highest PD of DM of 96.38%, which reflected the higher effective DM degradability. This was probably related to the inclusion of non-fibrous carbohydrate additives which increased the slowly degradable fraction “*b*”. Present results for all treatments were higher than potential degradability of 64.9% of DM degradability of Napier grass silage observed by Cabral *et al.* (2005).

Effective degradability of any diet can be considered as the energy digested in the rumen. The ED of bio-digester slurry treated silage was superior at all outflow rates. On the other hand, molasses treated silage was superior in ED of DM at 0.02 and 0.05 outflow rate, while maize meal treated silage was superior at 0.08 outflow rate, indicating the ability of the additives in enhancing rumen degradability of Napier grass silage. Aroeira *et al.*, (1996) reported that the ingestion of feed with increased degradability of DM and CP provided greater energy to microorganisms.

The CP disappearance of readily degradable fraction “*a*” characterizes the readily soluble sugars and nitrogen (N) compounds remaining after the fermentation in the silo (Tonani *et al.*, 2001). The readily degradable fraction “*a*” of bio-digester slurry treated silage was superior. However, the insignificant higher (13.70 %) soluble fraction in molasses silage in our study suggests that this would stimulate greater microbial growth in the rumen than the other treatments. The “*a*” fractions of CP were lower than values reported by Nowak *et al*. (2004) for round bale silage.

The slowly degradable fraction “*b*” was also increased due to carbohydrate additives inclusion in the silage which contradict the results of Gomes *et al.* (2015) who observed reduced fraction “*b*” as the level of glycerin increased in the silage. The residue of molasses treated silage showed a high value (69.23%) for the fraction "*b*", suggesting a source of rumen degradable protein. Kaufmann (1979), however, reported that the average ruminal CP degradability of feed is 60 to 80 %.

The passage rate limits the ruminal degradability of CP of a particular feed (Krizsan *et al.*, 2010). In the present study, the outflow rate "*c*" similarly (*P* >0.05) responded to all the treatments. On the other hand, the "*c*" of all silages obtained in this experiment was lower than that reported by Nowak *et al.* (2004).

In the present study, the PD similarly (*P* >0.05) responded to fertilisation with bio-digester slurry treatment while Van Vuuren *et al.* (1991) reported that the degradation rate of the PD of N fraction increased with increasing application of N fertiliser application rate. In terms of carbohydrate additives, the PD of CP was not affected by carbohydrate additives inclusion in silages, which reflected similar effective DM degradability in all treated silages. The greatest PD of CP was observed in the molasses residue (82.93 %). This could be attributed to its high fraction *b* (Table 4.3) and the low concentration of the lignified fraction (Table 3.5). The carbohydrate additives treated silages had relatively high CP content ranging from 70.95 % for the brown sugar to 82.93% for the molasses treated silage residues. High levels of CP associated with high ED of CP could be indicative of synchronisation between the fermentation of protein and carbohydrates for maximum microbial synthesis (Goes *et al.*, 2012). The increase in PD of CP symbolized a decrease in the quantity of CP entering the small intestine when silage was treated with carbohydrate additives. Volden *et al.* (2002), however, reported that the grass silage N containing compounds was composed of 24 – 80 % of soluble N fraction.

Data on ED of protein in the rumen gave an estimate of N supply to microbes for grazing ruminants (Bowen *et al.*, 2008). The ED of bio-digester slurry treated silage was similar at all outflow rates indicating that fertiliser application was not an issue. However, Vik-Mo (1989) reported that forages with higher CP content tend to show a significantly higher ED which contradicted the bio-digester slurry fertilised silage of present study. Crude protein ED of additives treated silage performed better than no additive treated silage at 2, 5 and 8 % outflow rate even though no statistical difference were observed (Table 4.3). Hence, it could be said that the CP of no additive treated silage took longer to be degraded than the molasses, brown sugar and maize meal treated silages. The improvement was possibly due to presence of carbohydrate additives.

4.5 Conclusion

The fertilization with bio-digester slurry and the inclusion of carbohydrate additives during the ensiling process independently increased in the rates of *in sacco* DM and CP. Combination of control fertilization treatment with molasses inclusion improved the DM PD of silage. Moreover, the advantageous use of above treatment combinations during the ensiling process also improved the effective degradability of Napier grass silage.

CHAPTER 5:

EFFECT OF FERTILISATION WITH BIO-DIGESTER SLURRY AND THE INCLUSION OF CARBOHYDRATE ADDITIVES DURING ENSILING ON *IN VITRO* DRY MATTER AND CRUDE PROTEIN DIGESTIBILITY OF NAPIER GRASS SILAGE USING THE PEPSIN-PANCREATIN METHOD

5.1 Introduction

Ensiling forages is essential to provide winter feed for farm livestock. Numerous previous studies focused on the effect of additives, legumes forage and/or absorbent inclusion on fermentation characteristics, chemical composition and aerobic stability (Mtengeti *et al.*, 2006; Mahala and Khalifa 2007; Arbabi and Ghoorchi 2008; Bilal, 2009; Nkosi *et al.* 2012a; Lubisi, 2014: Mutavhatsindi, 2015; Wyss and Arrigo, 2015; Kanengoni *et al.*, 2016). However, few such studies measure the digestibility of nutrients, which is important to predict the feeding value of the silage (O'Shea *et al.*, 1972), and therefore, to determine the need for supplementation.

It is possible to measure the digestibility of large numbers of forage samples using the *in vitro* digestibility techniques suggested by Tilley and Terry (1963). A modified three step procedure (TSP), by Gargallo *et al.* (2006) mimics post ruminal digestive processes by sequential digestion in pepsin (abomasal digestion) and pancreatin (small intestine digestion). The TSP is fast, more affordable, and equally effective (Pires *et al.*, 2006) for measuring the percentage of proteins which are hydrolyzed by such enzymes compared to *in vivo* assays (Hur *et al.*, 2011).

The study employed the TSP to evaluate the effect of fertilisation with bio-digester slurry and inclusion of carbohydrate additives at ensiling on post ruminal dry matter (IVDMD) and protein (IVCPD) digestibility of from Napier grass silage.

5.2 Materials and methods

5.2.1 Experimental site

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

5.2.2 Napier grass Silage

Samples used in current experiment were prepared as described in Chapter 3.

5.2.3 Experimental design

Two factors were considered. These were fertilisation with bio-digester slurry and carbohydrate additives inclusion. The first factor involved two fertilisation levels; No bio-digester slurry (control-water irrigation) and bio-digester slurry irrigation (30 t ha^{-1}). The second factor was at four treatment levels; no additive (control), molasses, brown sugar and maize meal included at 10% of fresh materials weighed. Accordingly, the experiment was set up as a 2 x 4 factorial arranged in a CRD, with three replications in each treatment. Thus, there were 8 treatment combinations.

5.2.4 Sample preparation

Representative silage for each fertilization treatment (Napier with and without bio-digester slurry) treated with four carbohydrate additives [no additive (control), molasses, brown sugar and maize meal] were oven dried at 60 ºC for 48 h (AOAC, 1990; method 930.15) and ground to pass a grinding mill of 1 mm screen size before incubation in the rumen.

5.2.5 Ethical consideration

The study was approved by the Ethics Committee of the University of Venda as described in chapter 3, subsection 3.2.5.

5.2.6 *In vitro* three-step procedure

Intestinal DM and CP digestibility were estimated using the modified TSP outlined by Gargallo *et al.* (2006). 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 plastic bands, to flexible vinyl plastic tubes, approximately 40 cm long and of 6 mm outer diameter. The flexible vinyl plastic tubes were tired with 10 cm ropes and then secured to a rubber stopper. Triplicate bags per silage treatment per incubation time per animal were inserted into the rumen and withdrawn in a subsequently at: 12, 24 and 48 incubation h. The bags were inserted in the rumen of Bonsmara steers 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 (Plate 5.1a), till runoff was clear, then finally washed with deionized water and dried at 60 °C for 48 hours. Rumen undegradable residuals (RURs) were removed from the bags by manually removing the residues. The RURs were composited by silage treatment, incubation hour 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 bottles 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 (Plate 5.1b). 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 residues were analysed by the methods as follows.

5.2.7 Chemical analysis

RURs and *in vitro* undegradable residues were dried in a forced air oven at 60°C for 48 h (AOAC, 1990; method 930.15) to determine DM content. The final residues in all bags were composited by the silage treatment, incubation hour and steers and subsequently ground through a 1 mm sieve and analysed in duplicates. The residues were then analysed for N content using the Kjeldahl procedure (AOAC, 1990; method 984.13) and N was converted to CP as N x 6.25.

Plate 5.1: (a) Washing samples after withdrawn from the rumen and (b) *in vitro* incubation of rumen residual samples

5.2.8 Statistical analysis

Analysis of variance (ANOVA) on silage digestibility data (Model I) was performed at *P* <0.01 and *P* <0.05 according to Steel and Torrie (1980) using GLM procedures of Minitab Statistical package version 17 (Minitab, 2014). Where significant differences between the treatment groups was detected, means were separated using the Tukey's test (Tukey, 1953).

$$Y_{ijkl} = \mu + S_j + C_k + (SC)_{jk} + \varepsilon_{ijkl} \qquad \text{Model I}$$

Where Y_{ijkl} = the observation, *in vitro* digestibility of DM and N ruminal incubation residues;

μ = overall mean common to all observations;

S_i = effect of i^{th} bio-digester slurry, i = 1 or 2;

C_j = effect of j^{th} carbohydrates additive, j = 1, 2, 3 or 4;

$(SC)_{ij}$= interaction between i^{th} bio-digester slurry and j^{th} carbohydrates additive; and

ε_{ijk} = random residual error.

5.3 Results

5.3.1 *In vitro* dry matter digestibility

The ruminal degradability and *in vitro* digestibility data of DM of Napier grass silage from the nylon bags are summarised in Table 5.1. No bio-digester slurry and maize meal inclusion treatment increased (*P* <0.01) DM degradability at 24 incubation h but did not differ (*P* >0.05) from silage without bio-digester slurry and added brown sugar, and also with bio-digester slurry and included with molasses, maize meal and brown sugar. As incubation time progressed to 48 h, fertilisation with bio-digester slurry and molasses inclusion increased (*P* <0.05) DM disappearance but did not differ (*P* >0.05) from silage not fertilised with bio-digester slurry and added molasses and maize meal, and also silage fertilised with bio-digester slurry and included with maize meal and brown sugar. However, fertilisation with bio-digester slurry and carbohydrate additives interaction had no effect (*P* >0.05) on 12 incubation h, and *in vitro* digestibility after 12, 24 and 48 hour of incubation. Fertilisation with bio-digester slurry reduced (*P* <0.05) DM digestibility after 48 hours of rumen incubation with no effect (*P* >0.05) on DM degradability at 12, 24 and 48 h of rumen incubation and *in vitro* digestibility after 12 and 24 h of incubation. Carbohydrate additives increased (*P* <0.01) DM disappearance at 12, 24 and 48 rumen incubation h but didn't not influence *in vitro* DM digestibility after 12, 24 and 48 h of rumen incubation.

5.3.2 *In vitro* crude protein digestibility

The ruminal and *in vitro* digestibility of CP of Napier grass silage from the nylon bags is summarised in Table 5.2. Fertilisation with bio-digester slurry and carbohydrate additives interaction 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 hour of incubation. Fertilisation with bio-digester slurry 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 of incubation. Carbohydrate additives increased (*P* <0.01) CP disappearance at 12, 24 and 48 rumen incubation h and reduced (*P* <0.01) *in vitro* CP digestibility after 12 h of rumen incubation but did not have an effect (*P* >0.05) on *in vitro* CP digestibility after 24 and 48 h of rumen incubation.

Table 5.1: Dry matter disappearance (g kg^{-1}) after 12, 24 or 48 hour incubation in the rumen and subsequent *in vitro* digestibility of Napier grass silage after 90 days of ensiling

Fertilisation	Additives	N	Rumen incubation time (h)			*In vitro* dry matter digestibility		
			12	24	48	$IVDMD_{12}$	$IVDMD_{24}$	$IVDMD_{48}$
No slurry	No additive	9	229.6	270.4^{c}	350.6^{d}	250.4	186.3	223.8
	Molasses	9	398.8	407.4^{ab}	495.5^{ab}	158.0	141.6	170.4
	Maize meal	9	330.1	422.0^{a}	484.6^{ab}	139.6	163.4	173.1
	Brown sugar	9	330.4	417.1^{a}	376.8^{cd}	161.8	175.2	227.0
Slurry	No additive	9	201.2	349.2^{b}	409.1^{bcd}	228.3	184.3	223.9
	Molasses	9	411.2	415.7^{a}	498.1^{a}	141.0	159.6	157.2
	Maize meal	9	407.6	388.1^{ab}	438.0^{abcd}	110.0	147.2	110.1
	Brown sugar	9	346.0	376.9^{ab}	446.6^{abc}	119.9	163.4	119.9
SEM			43.31	14.58	19.85	43.93	14.46	31.81
Fertilisation Means								
No slurry		36	322.2	379.2	426.9	177.5	166.6	198.6^{a}
Slurry		36	341.5	382.5	447.9	149.8	163.6	152.7^{b}
SEM			21.66	7.29	9.92	21.96	10.22	15.91
Additive Means								
	No additive	18	215.4^{b}	309.8^{b}	379.9^{c}	239.4	185.3	223.8
	Molasses	18	405.0^{a}	411.6^{a}	496.8^{a}	149.5	150.6	163.8
	Maize meal	18	368.8^{a}	405.1^{a}	461.3^{ab}	124.8	155.3	173.4
	Brown sugar	18	338.2^{a}	397.0^{a}	411.7^{bc}	140.8	169.3	141.6
SEM			30.63	10.31	14.03	31.06	20.44	22.49
Significance								
	Fertilisation (F)		ns	ns	ns	ns	ns	*
	Additives (A)		**	**	**	ns	ns	ns
	F x A		ns	**	*	ns	ns	ns

**: P <0.01; *: P <0.05; (ns) non-significant: P >0.05. ^{abcd} Column means with different superscripts differ significantly at P <0.05. 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.

Table 5.2: Crude protein disappearance (g kg^{-1}) after 12, 24 or 48 hour incubation in the rumen and subsequent *in vitro* digestibility of Napier grass silage after 90 days of ensiling

Fertilisation	Additives	N	Rumen incubation time (h)			*In vitro* crude protein digestibility		
			12	24	48	$IVCPD_{12}$	$IVCPD_{24}$	$IVCPD_{48}$
No slurry	No additive	6	96.3	140.5	230.1	241.7	216.9	222.2
	Molasses	6	197.7	270.2	334.3	123.9	94.1	162.3
	Maize meal	6	190.0	249.0	318.0	136.7	159.0	150.0
	Brown sugar	6	165.6	224.2	297.4	165.7	172.6	213.5
Slurry	No additive	6	164.3	226.1	264.7	273.7	173.7	225.1
	Molasses	6	212.2	288.9	370.0	118.9	141.4	119.4
	Maize meal	6	206.1	241.1	309.9	120.8	145.4	91.6
	Brown sugar	6	177.2	216.4	290.9	107.4	142.3	122.7
SEM			22.95	29.18	17.41	37.95	53.95	52.74
Fertilisation Means								
No slurry		24	162.4	221.0	294.9	167.0	160.6	187.0
Slurry		24	190.0	243.1	308.9	155.2	150.7	139.7
SEM			11.47	14.59	8.71	18.98	26.98	26.37
Additive Means								
	No additive	12	130.3^{b}	183.3^{b}	247.4^{c}	257.7^{a}	195.3	223.6
	Molasses	12	204.9^{a}	279.6^{a}	352.2^{a}	121.4^{b}	117.7	140.8
	Maize meal	12	198.1^{a}	245.0^{ab}	313.9^{ab}	128.8^{b}	152.2	120.8
	Brown sugar	12	171.4^{ab}	220.3^{ab}	294.2^{b}	136.6^{b}	157.4	168.1
SEM			16.23	20.64	12.31	26.84	38.15	37.29
Significance								
	Fertilisation (F)		ns	ns	ns	ns	ns	ns
	Additives (A)		**	**	**	**	ns	ns
	F x A		ns	ns	ns	ns	ns	ns

**: P <0.01; (ns) non-significant: P >0.05. ^{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, $IVCPD_{24}$: *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.

5.4 Discussion

5.4.1 *In vitro* dry matter digestibility

There is dearth of information on the effect of fertilising forages with bio-digester slurry or any other type of fertilisers on post ruminal silage IVDMD. The improvements in the digestibility of the silage associated with no bio-digester slurry fertilisation on residues after 48 hours of incubation might suggest increased feed intake as the IVDMD and feed intake are positively correlated (Van Soest, 1994). However, the higher potential degradability of bio-digester slurry silage (Table 4.2) might have caused the reduction on *in vitro* DM digestibility.

The present study results were in agreement with Kaldmäe *et al.* (2009) who reported that inoculant into silage had no effect on the IVDMD of red clover silages, and contradicted those of Zereu *et al.* (2015), who reported that using molasses in silage improved DM digestibility of ensiled vines of four sweet potato varieties. Even though IVDMD values were low with ranges of 124.8 - 239.4, 150.6 - 185.3 and 141.6 - 223.8 g kg^{-1} for residues after 12, 24 and 48 h of rumen incubation respectively, the trend increased in IVDMD for control silage compared to additives included. Silages agreed with Denek and Can (2007) results when wheat straw, molasses and urea were included in wet orange pulp.

5.4.2 *In vitro* crude protein digestibility

There is also dearth of information on the effect of fertilising forages with bio-digester slurry on post ruminal silage IVCPD. Surprisingly, the untreated silages which had higher NDF and ADL had superior IVCPD after 12 h incubation. This can be attributed to higher ruminal undegradable protein of treated silages compared to untreated silages. Present study results were agreement with Tokita *et al.* (2015) who reported that the digestibility of crude protein in no additive silage material outperformed glucose, formic acid and tannic acid treatments silages. On the other hand, present study results contradicted with those of Nowak *et al.* (2004) who earlier reported a significant increase of intestinal digestibility of undegraded protein in formic acid treated silage.

5.5 Conclusion

It can be concluded that higher ruminal degradability results in lower post rumen digestibility. As a result, carbohydrate additives inclusion increased ruminal DM and CP degradation of treated silages but subsequently causes drop in post rumen digestion of CP after 12 h incubation.

CHAPTER 6: GENERAL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

6.1 General discussions

The study's specific objectives were to determine the effect of fertilisation with bio-digester slurry and molasses, brown sugar and maize meal additives inclusion on chemical composition and fermentation characteristics, DM and CP ruminal degradability and *in vitro* digestibility of Napier grass (*P. purpureum*) silage. Bio-digester slurry fertilisers were applied with the aim to improve the quality of forage for ensilage since the quality of silage depends on original quality of preserved crop. However, nutrient source additives were added when ensiling in order to stimulate the LA fermentation, hence, resulting in a decrease in pH content and thus improving silage quality (McDonald *et al.*, 1991). Farmers and researchers have been using additives for decades with the aim of driving fermentation processes towards rapid growth of LAB, which inhibit the activities of undesirable bacteria (Dean *et al.,* 2005*).* It has been generally reported that additives to silage had a positive effect on the silage fermentation by increasing LA, the preferred acid in reducing the pH of silage (Seglar, 2003 Lubisi, 2014; Mutavhatsindi, 2015). Therefore, in the present study, it was hypothesized that fertilisation with bio-digester slurry and carbohydrate additives inclusion would not improve chemical composition and fermentation characteristics, DM and CP ruminal degradability and *in vitro* digestibility of Napier grass silage.

Fertilising Napier grass material with bio-digester slurry for period of 12 weeks increased the CP concentrations to 118.6 g CP kg^{-1} DM and reduced DM and WSC concentrations of the material to 270.6 g kg^{-1} and 53.6 g WSC kg^{-1} DM, respectively (Table 3.4). Even though the DM and WSC were reduced, they still fell within the recommended range of 250 - 400 g kg^{-1} DM (Wilkinson, 2005) and minimum required concentration of 37 g WSC kg^{-1} DM (Haigh, 1990) for effective fermentation. However, it was difficult to compare our results others because of the lack of information on application of bio-digester slurry.

After 90 days of ensiling, the results indicated that the bio-digester slurry fertilisation with molasses inclusion improved DM content of Napier grass silage. This was due to the availability of WSC attributed to high levels (28.3 g kg^{-1}) of residual WSC (Table 3.5), which was consumed by LAB responsible for directing the fermentation process towards production of LA as the main fermentation product (Seglar, 2003; McDonald *et al.*, 2011) and subsequently caused the decline in pH to 4.2 (Table 3.5). Additionally, these desirable characteristics means decreased the fermentation losses and hence, more digestible substances were preserved.

Regarding the mineral composition of the Napier grass silages, no bio-digester slurry fertilisation with no additive inclusion increased Mn content of Napier grass silage (Table 3.7). Contrastingly, Lubisi (2014) reported that fertilisation with bio-digester slurry and carbohydrate additives interaction had no effect on Mn content of Napier grass silage. Regardless of treatment, all silages provided inadequate amounts of Ca, Na and P to maintain ruminants, suggesting supplementation of silage for such minerals.

No bio-digester slurry with no additive included and bio-digester slurry with no additive included recorded lower DM degradability at 24 incubation h (Figure 4.1), indicating that microbial activity was favoured by carbohydrate additives inclusion, acting on the substrate and in the assimilation of soluble carbohydrates and easily digestible compounds. Carbohydrate inclusion might have caused the reduction of plant cell wall fractions in the silages. In literature, Granzin and Dryden (2005) reported that adding additives had no effect on DM degradability, while other studies (Sahoo and Walli, 2008; Shellito *et al.*, 2006) had reported that diets with molasses inclusion had higher (P <0.05) ruminal DM degradability. Furthermore, no bio-digester slurry with molasses improved the DM potential degradability of silage (Table 4.2) but did not differ from no bio-digester slurry with maize meal silage, bio-digester slurry fertilisation with molasses, maize meal and brown sugar silage. This could be because carbohydrate inclusion to above treatments combination during ensiling improved the solubility and degradability of silage in the rumen.

Bio-digester slurry fertilisation with molasses inclusion improved the DM degradability at 24 and 48 h incubation due to high solubility and digestibility of silages. Subsequent pepsin-pancreatin digestion of DM on residues after 12, 24 and 48 h of ruminal incubation were higher (P >0.05) in no bio-digester slurry fertilisation with no additive included and bio-digester slurry fertilisation with no additive included (Table 5.1), probably due to their lower potential ruminal degradability (Table 4.3). Contrary to Xu *et al.* (2011), Han *et al.* (2013) and Moselhy *et al.* (2015) the application of molasses or molasses with urea as well as inoculation, enzymes, significantly influenced improved IVDMD, while Keskin *et al.* (2005) did not observe effects from additives on IVDMD.

6.2 General conclusion and recommendations

6.2.1 General conclusion

Fertilising Napier grass with bio-digester slurry improved the DM content of fresh cut grass at pre-ensiling. It was apparent that fertilising Napier grass with bio-digester slurry and molasses inclusion improved DM content of silage, thus causing subsequent improvement in fermentation of the Napier grass silage by increasing LA content and causing a drop in pH and NH_3-N. however, the best results regarding the DM ruminal degradability of Napier grass silages was obtained with each fertilisation treatment (bio-digester slurry and no bio-digester slurry) with carbohydrate additives (molasses, maize meal and brown sugar). Furthermore, it seemed that no bio-digester slurry with no additive inclusion and bio-digester slurry with no additive inclusion were effective in improving the IVDMD and IVCPD due to their low ruminal degradability. Therefore, ensiling of Napier grass could be a good alternative for ruminant feeding during winter dry season.

6.2.2 Recommendations

It is recommended that ensiling of forage can be practiced as a substitute for natural pasture during winter. Therefore,

i. There is need to encourage farmers to adopt the practice of ensiling cultivated or surplus fodder for use during winter season.

Further research is necessary to study the effect of:

ii. Different application rates of bio-digester slurry fertilisers and carbohydrate additives on chemical composition and fermentation characteristics of Napier silage.
iii. Longer application period of bio-digester slurry fertilisers to improve chemical composition and fermentation characteristics of Napier silage
iv. Fertilisation with bio-digester slurry and carbohydrate additives on nutrient digestion and performance in ruminants.

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APPENDICES

Appendix 1: Analysis of variance for chemical composition of fresh cut, pre-ensiled Napier grass irrigated with and without bio-digester slurry

Source	df	DM	pH	WSC	CP	Ash	Fat	NDF	ADF	ADL
Fertilisation	1	87.00	0.041667*	0.8067	240.94*	42.83	58.020*	8.916	503.6	0.000382
Error	4	45.80	0.004867	28.3167	26.38	20.34	3.760	174.418	104.4	0.054293

*: *P* <0.05. df: Degree of Freedom, DM: Dry Matter, WSC: Water Soluble Carbohydrates, CP: Crude Protein, NDF: Neutral Detergent Fibre, ADF: Acid Detergent Fibre, ADL: Acid Detergent Lignin.

Appendix 2: Analysis of variance for fermentation characteristics of Napier grass silage after 90 days of ensiling

Source	df	pH	WSC	LA	NH_3-N
Fertilisation (F)	1	0.000204	6.409	34.60	44.1
Additives (A)	3	0.290349	227.297*	168.11	1415.2*
F x A	3	0.025982	12.506	10.81	51.2
Error	16	0.219900	45.894	133.10	434.2

*: *P* <0.05. df: Degree of Freedom, WSC: Water Soluble Carbohydrates, LA: Lactic Acid and NH_3-N: Ammonia Nitrogen.

Appendix 3: Analysis of variance for chemical composition (g kg^{-1} DM) of Napier grass silage after 90 days of ensiling

Source	df	DM	CP	NPN	Fat	Ash	NDF	ADF	ADL
Fertilisation (F)	1	789.3	115.45	3.989	12.67	178.47	324.5	647.1	883
Additives (A)	3	8048.0**	447.77	4.972	42.11*	2402.64**	33817.9**	25212.8**	238
F x A	3	2265.0*	27.49	3.271	41.49*	36.30	1437.0	866.1	43
Error	16	665.5	186.93	3.303	11.14	77.28	22385.8	3037.2	3056

**: *P* <0.01; *: *P* <0.05. df: Degree of Freedom, DM: Dry Matter, CP: Crude Protein, NPN: Non Protein Nitrogen,NDF: Neutral Detergent Fibre, ADF: Acid Detergent Fibre, ADL: Acid Detergent Lignin.

Appendix 4: Analysis of variance for macro- (g kg^{-1}) and micro- (mg kg^{-1}) minerals of Napier grass silage after 90 days of ensiling

Source	Df	Ca	Mg	K	Na	P	Zn	Cu	Mn	Fe
Fertilisation (F)	1	0.2017	0.8563*	7.956	0.006170	0.15028*	36.98	0.3759	542.85**	57706*
Additives (A)	3	12.6272**	0.47561**	362.857**	0.488236**	0.08253	30.59*	0.9494	385.59**	25087
F x A	3	0.0810	0.2265	6.030	0.047611	0.04733	15.84	0.7978	261.98*	14578
Error	16	0.0775	0.1606	10.157	0.021214	0.02622	18.89	1.3834	53.03	8381

**: *P* <0.01; *: *P* <0.05. df: Degree of Freedom, Ca: Calcium, Mg: Magnesium, K: Potassium, Na: Sodium, P: Phosphorus, Zn: Zinc, Cu: Copper, Mn: Manganese and Fe: Iron.

Appendix 5: Analysis of variance for degradability constants and calculated effective degradability at three passage rates for dry matter disappearance

Source	df	Degradability constants				ED (%) at three outflow rates		
		a	*b*	*c*	*a* + *b*	*K* = 0.02	*K* = 0.05	*K* = 0.05
Animal		31.651	26.21	0.0003976	0.70	36.86	34.57	33.38
Fertilisation (F)	1	8.250	213.79	0.0000422	306.03**	181.82*	107.55*	74.60*
Additives (A)	3	48.687**	423.22	0.0001111	707.37**	394.75**	249.15**	186.64**
F x A	3	45.239**	299.97	0.0000589	196.80**	52.69	38.80	36.13
Error	16	7.248	31.10	0.0001311	31.62	29.24	19.58	15.48

**: *P* <0.01; *: *P* <0.05. df: Degree 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, and *k*: rumen outflow rate (h^{-1}).

Appendix 6: Analysis of variance for degradability constants and calculated effective degradability at three passage rates for crude protein disappearance

Source	df	Degradability constants				ED (%) at three outflow rates		
		a	*b*	*c*	*a* + *b*	*K* = 0.02	*K* = 0.05	*K* = 0.05
Animal		60.12	180.7	0.0000138	33.3	25.69	28.92	31.91
Fertilisation (F)	1	0.37	45.7	0.0000166	76.7	78.48	52.57	41.03
Additives (A)	3	40.49	196.2	0.0000104	399.1*	250.66	165.24	126.40
F x A	3	10.31	64.5	0.0000126	36.8	21.88	14.08	11.00
Error	16	68.77	261.0	0.0000163	110.2	77.22	67.11	62.46

*: *P* <0.05. df: Degree 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, and *k*: rumen outflow rate (h^{-1}).

Appendix 7: Analysis of variance for dry matter disappearance (g kg^{-1}) after 12, 24 or 48 hour incubation in the rumen and then *in vitro* digestibility of Napier grass silage

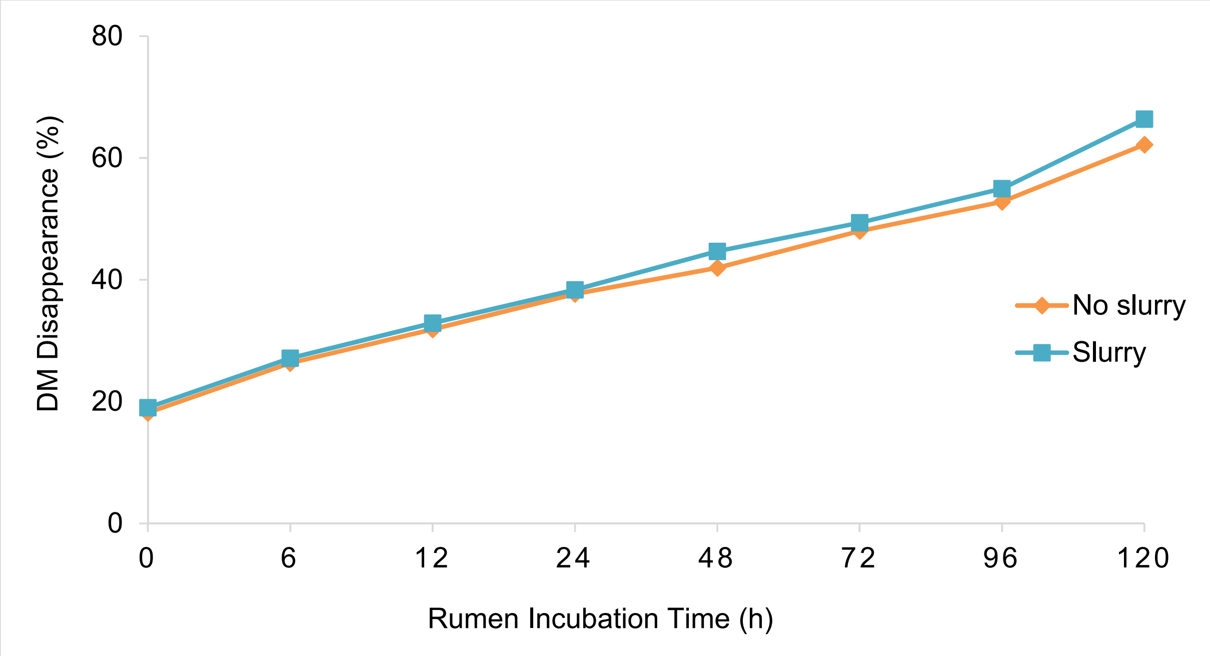
Source	df	Rumen incubation time (h)			*In vitro* dry matter digestibility		
		16	24	48	$IVDMD_{12}$	$IVDMD_{24}$	$IVDMD_{48}$
Fertilisation (F)	1	6706	191	7983	13780	163	37825**
Additives (A)	3	121954**	41038**	48444**	47782	4395	21782
F x A	3	8590	13500**	13052*	526	1041	10812
Error	16	16882	1913	3545	17366	3761	9107

**: *P* <0.01. 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.

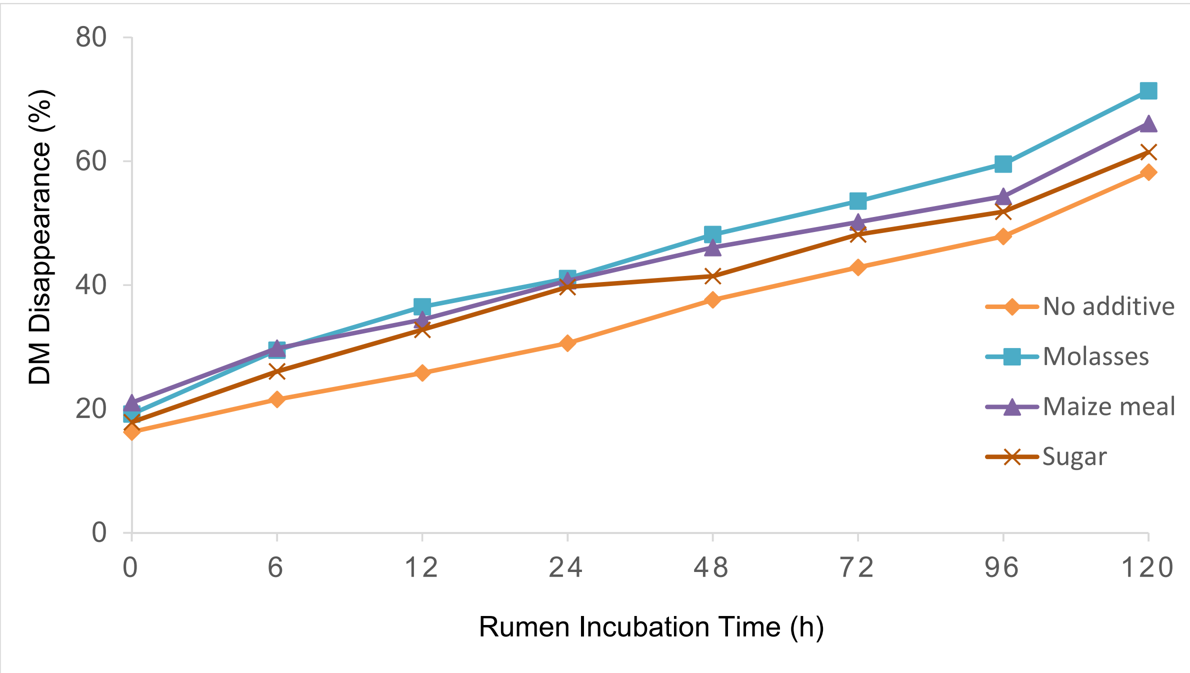
Appendix 8: Analysis of variance for crude protein disappearance (g kg^{-1}) after 12, 24 or 48 hour incubation in the rumen and then *in vitro* digestibility of Napier grass silage

Source	df	Rumen incubation time (h)			*In vitro* crude protein digestibility		
		16	24	48	$IVCPD_{16}$	$IVCPD_{24}$	$IVCPD_{48}$
Fertilisation (F)	1	9116	5890	2326	1654	1188	26846
Additives (A)	3	13733**	19754**	22803**	50214**	12087	22136
F x A	3	2193	5839	1805	4145	4810	6336
Error	16	3159	5110	1819	8643	17466	16690

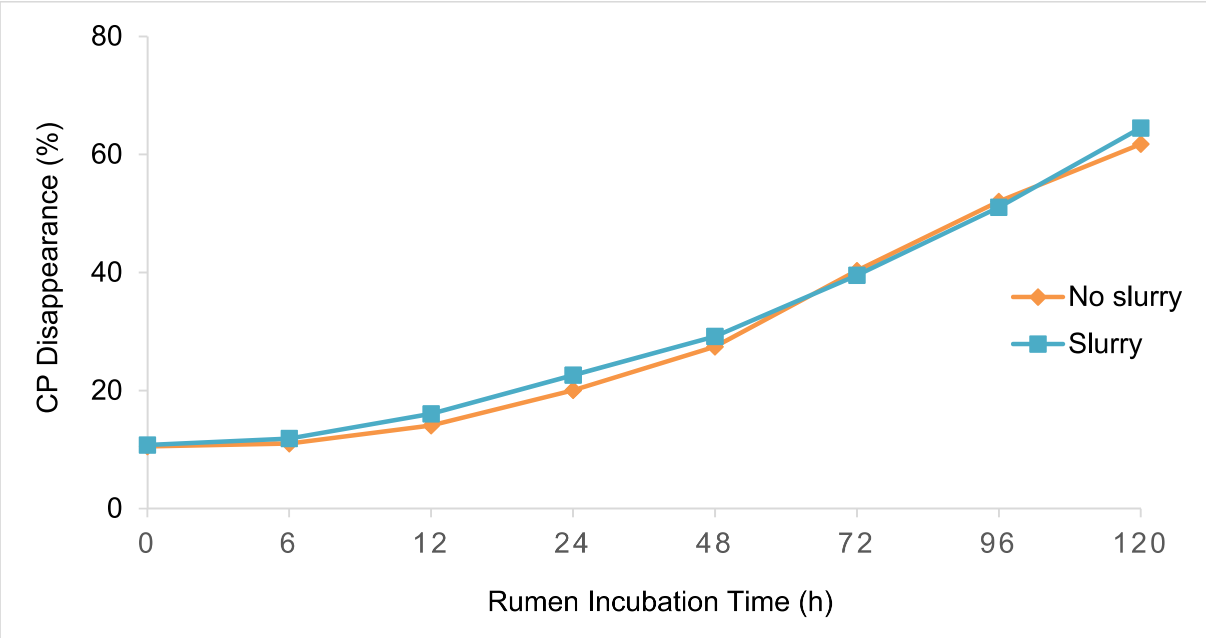
**: *P* <0.01. df: Degree of Freedom, $IVCPD_{12}$: *In vitro* crude protein digestibility after 12 hours of rumen incubation, $IVCPD_{24}$: *In vitro* crude protein digestibility after 24 hours of rumen incubation and $IVCPD_{48}$: *In vitro* crude protein digestibility after 48 hours of rumen incubation.



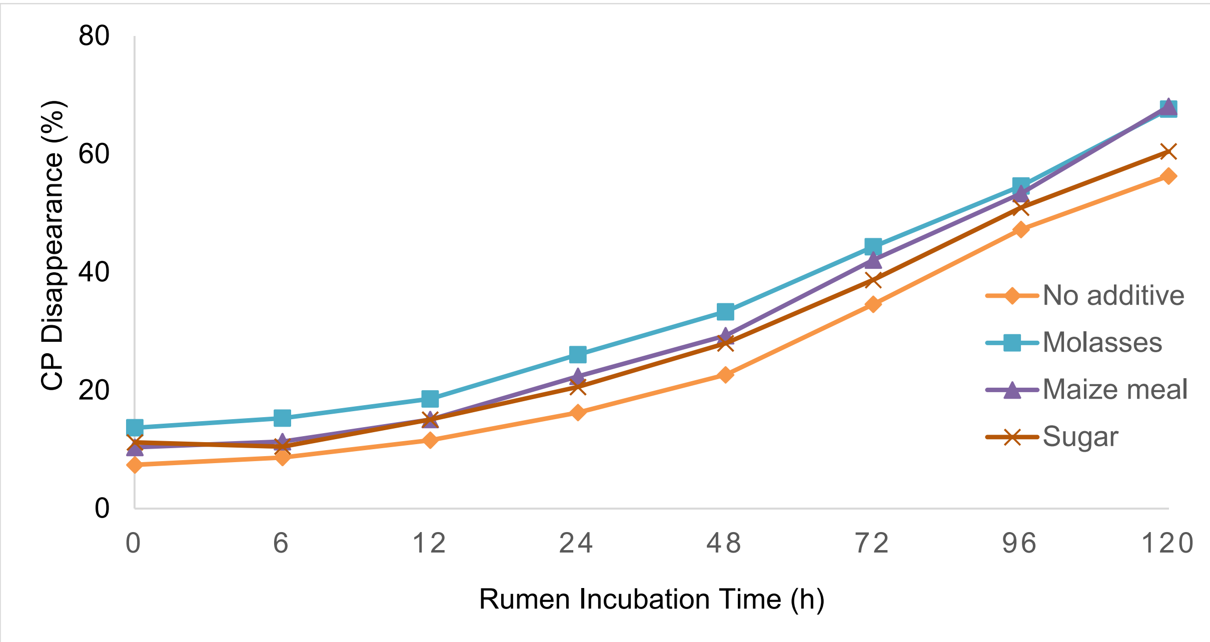
Appendix 9: Effects of fertilisation with bio-digester slurry on *in sacco* dry matter disappearance of Napier grass silage after 90 days of ensiling



Appendix 10: Effects of carbohydrate additives on *in sacco* dry matter disappearance of Napier grass silage after 90 days of ensiling



Appendix 11: Effects of fertilisation with bio-digester slurry on *in sacco* crude protein disappearance of Napier grass silage after 90 days of ensiling



Appendix 12: Effects of carbohydrate additives on *in sacco* crude protein disappearance of Napier grass silage after 90 days of ensiling