EFFECTS OF FEEDING BRACHIARIA-BASED DIETS SUPPLEMENTED WITH DESMODIUM AND DAIRY CUBES ON CATTLE MANURE GREENHOUSE GAS EMISSIONS

NAOMI JERUTO CHEPSUGE

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented in this or any other University for the award of a degree.

Signature: ______________________ Date: 01/06/2024

Naomi Jeruto Chepsuge

KM113/12033/17

Recommendation

This thesis has been submitted with our approval as university supervisors.

Signature: Date: 01/07/2024

Prof. James O. Ondiek, PhD

Department of Animal Sciences

Egerton University, Njoro, Kenya

Signature: ________________________ Date: 07/06/2024

Dr. Olivier B. Kashongwe,Ph.D.

Department of Animal Sciences

Egerton University, Njoro, Kenya

Signature: Sonja Leitner ________________________ Date: 15/06/2024

Dr. Leitner Sonja

Mazingira Centre

International Livestock Research Institute, Nairobi, Kenya

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DEDICATION

This work is dedicated to my beloved parents, brothers, and sisters for their support and prayers.

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ABSTRACT

The study evaluated the effect of protein concentrate (dairy cubes) and leguminous forage containing condensed tannins (*Desmodium intortum*) on diet digestibility and their impacts on manure greenhouse gas emissions. Leguminous forages containing condensed tannins (CT) have the potential to reduce manure nitrous oxide (N_2O) and methane (CH_4) emissions and improve diet digestibility and utilization. The objectives of the study were to determine the effects of two protein supplements on (1) the nutritional value and digestibility of diets, (2) manure characteristics, and (3) manure greenhouse gas (GHG) emissions. Twelve lactating crossbred cows were fed three dietary treatments: diet 1 (D_1) control (Brachiaria only), diet 2 (D_2) Brachiaria + dairy cubes, and diet 3 (D_3) Brachiaria + Desmodium in a completely randomized design with four cows per diet. Total feces and urine from individual cows per diet (n=4) were collected. The manure was incubated in glass jars at 20 °C for 84 days, and CH_4, N_2O, and CO_2 emissions were measured. Objective one involved proximate analysis, i.e. dry matter (DM), crude protein (CP), fibre analysis, and *in-vitro* degradability of dietary treatments. Objectives two and three involved analysis of manure samples taken at days 0, 7, 14, 28, 56, and 84 of incubation for water content, total carbon (TC), total nitrogen (TN), fibre, and CT and gas samples for GHG emissions. From the findings, apparent N digestibility differed significantly between dairy cube and desmodium supplemented diets (67.9 ± 2.59 % vs 55.3 ± 2.63%). Excreted N was higher in excreta from cows receiving N supplementation (dairy cubes and Desmodium) than control diet, both for fecal N (61.2 ± 2.62 and 62.5 ± 2.55 vs 42.7 ± 1.90g N day^{-1}), and urine N (66.9 ± 4.38 and 34.1 ± 0.93 vs 35.9 ± 0.87g N day^{-1}). Condensed tannins were higher in feces excreted by cows fed Desmodium supplemented diet than dairy cube supplemented diet (0.82± 0.07 vs 0.09±0.06% DM). Manure cumulative GHG emissions were similar across the diets. However, due to variations between experimental animals, diet effects on manure GHG emissions were not conclusive. From this study, diet composition influenced manure composition. Desmodium is recommended as an alternative protein supplement to expensive concentrates, but harvesting time should be considered for higher nutritional value and animal productivity.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ADL	Acid detergent lignin
ANOVA	Analysis of variance
C	Carbon
CH_4	Methane
CP	Crude protein
CT	Condensed tannins
DM	Dry matter
ECD	Electron capture detector
FID	Flame ionization detector
GHG	Greenhouse gas
N	Nitrogen
N_2	Dinitrogen gas
N_2O	Nitrous oxide
NDF	Neutral detergent fibre
NH_3	Ammonia
NH_4^+	Ammonium
NO	Nitric oxide
NO_3^-	Nitrate
OM	Organic matter
TT	Total tannins

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Livestock farming is one of the fastest-growing agricultural subsectors in developing countries. This is mainly due to the increased demand for animal-based products caused by population growth, urbanization, and higher incomes (Bruinsma & FAO, 2003; Thornton, 2010). In Kenya, the dairy industry is a growing subsector, contributing approximately 3.5% of the total GDP, with an annual growth rate of 4 to 5% (Government of Kenya, 2008; Makini et al., 2019). In addition to economic significance, the dairy sector serves as a source of income, milk – a significant contributor to food and nutrition security, and manure which can be used as fertilizer in crop and forage production (Makini et al., 2019). However, despite the benefits and Kenya's standing as a major milk producer, the sector is characterized by low productivity, which is attributed to inadequate feed supply and low nutritional value of diets, among other factors (FAO & New Zealand Agricultural Greenhouse Gas Research Cent, 2017). This results in economic inefficiencies and increases enteric methane emission intensities, contributing to climate change (Sakadevan & Nguyen, 2017).

To increase milk production per animal and reduce greenhouse gas (GHG) emissions per unit of product (i.e. GHG emission intensities), smallholder farmers are intensifying their production systems. This shift involves transitioning from free grazing on natural grasslands to stall feeding, incorporating planted improved pastures, crop residues, and protein supplements (Bebe et al., 2002; Brandt et al., 2018; Wilkes et al., 2020). However, dairy intensification could lead to an increase in manure production (Grossi et al., 2019) and nutrient excretion, which negatively impacts the environment via nutrient pollution (Lee et al., 2019; Petersen et al., 2013) and the production of CH_4 and nitrous oxide (N_2O) emissions (Grossi et al., 2019; Niu et al., 2016). Considering the increasing trend of dairy intensification (Brandt et al., 2018), an increase in amounts of waste (manure) excretions into the environment (FAO, 2009) is expected. Therefore, feeding practice is an important leverage to influence nutrient concentration in excreted manure in dairy systems.

The use of improved forages such as Brachiaria grass has been recommended to farmers as ideal fodder because of its desirable agronomic, nutritional, and environmental characteristics (Cheruiyot et al., 2020; Ghimire et al., 2015; Mutimura et al., 2018). However, Brachiaria grasses have relatively lower CP, and as a result, protein supplementation is required to improve feed quality and maximize milk production (Paul et al., 2020). Concentrates are the most widely used protein supplements in Kenyan dairy production, but they come at a cost

(Lukuyu et al., 2007). Due to this, there has been a growing interest in the use of affordable and locally grown protein rich leguminous forages and shrubs as alternative protein source to livestock feeds (Cheruiyot et al., 2020; Lukuyu et al., 2012; Mutimura et al., 2018). Their potential to increase milk production has also been shown by Makau et al. (2020) and Paul et al. (2020). In addition, most of the leguminous forages contain condensed tannins (CTs), which have been shown to improve N use in ruminants by decreasing protein degradability in the rumen and increasing its digestion and absorption in the small intestine (Aboagye et al., 2018).

The incorporation of these protein supplements into dairy diets or rations can have considerable follow-on effects on manure nutrient composition and subsequently on GHG emissions (Hindrichsen et al.,2006). They increase diet N and depending on their degradability in the rumen, they can interfere with degradation and flow of N and C sources in the digestive tract. As a result concentrations of these vary in excreta and can consequently affect GHG emissions (Gerber et al., 2013). Concentrates interfere with fibre digestibility leading to more excreted organic matter which is then available for microbial degradation during manure storage (Hindrichsen et al., 2005). The reduced enteric CH_4 emissions as a result of feeding concentrates can, therefore, be deprived by an increase in CH_4 emission during manure storage (Külling et al., 2002). Increasing the concentrate proportion in a diet also increases N intake, which results in higher N excretion, which increases N_2O fluxes from stored manure (Nampoothiri et al., 2018).

In contrast to concentrate additions, leguminous forages containing CTs shift urine N excretion from urine to feces, this lowers the volatile N concentration in manure resulting in reduced ammonia emissions (Misselbrook et al., 2005), and is also expected to reduce N_2O emissions from manure (Gerber et al., 2013). In a study by Min et al. (2020), direct inclusion of CTs in dairy manure reduced cumulative CH_4 and N_2O emissions. Hypothetically, the presence of CTs in the excreted manure from cattle fed CTs rich diets is expected to inhibit microbial activity, resulting in decreased N_2O and CH_4 emissions from the stored manure. However, some studies that included CTs in cattle diets showed no effect on manure CH_4 and N_2O emissions during composting and biodigestion (Fagundes et al., 2021; Hao et al., 2011). The authors attributed this finding to low CTs levels and the probability of microbes in the compost altering the biological activity of tannins.

The present study aims to evaluate whether protein supplementation affects manure characteristics and emissions, given the increasing use of protein supplements in Kenya. Specifically, the study will investigate the effect of two protein sources (concentrate - dairy cubes and tanniferous leguminous forage - *Desmodium intortum*) on diet digestibility, manure characteristics, and manure GHG emissions. The study's detailed objective is to determine the digestibility of diets based on Brachiaria hay supplemented with dairy cubes and *Desmodium intortum* and to evaluate the effect of feeding dairy cattle on a Brachiaria hay basal diet supplemented with dairy cubes and *Desmodium intortum* on excreted manure composition and manure CH_4 and N_2O emissions.

1.2 Statement of the Problem

The dairy sector in Kenya has become more commercialized and intensified, leading to an increase in the use of improved feeding practices to boost milk production. These practices include feeding animals with highly digestible forages and protein supplements, which not only enhance productivity but also reduces nitrogen excretion and potentially greenhouse gas (GHG) emissions. Manure is among the big sources of agricultural GHG emissions in the livestock sector, but the effect of diets on manure GHGs is still unclear, particularly in developing countries. In this context, leguminous forages have been identified as a promising intervention to enhance cattle productivity in Kenya and other developing countries, with the potential to reduce manure GHG emissions. Nevertheless, a knowledge gap remains, on whether and how improved diets affect the quantity and chemical composition of manure and subsequently manure GHG emissions in Kenyan dairy systems.

1.3 Objectives

1.3.1 Overall Objective

To contribute to the mitigation of climate change by providing scientific evidence that will improve understanding on the potential of diets to reduce manure GHG emissions

1.3.2 Specific Objectives

i. To determine the nutritional value and digestibility of Brachiaria hay-based diets supplemented with either dairy cubes or *Desmodium intortum*

ii. To determine the effects of feeding Brachiaria hay-based diet with either dairy cubes or *Desmodium intortum* supplementation on physico-chemical properties, fibre contents, and tannin contents of manure

iii. To estimate CH_4, N_2O, and CO_2 emissions from the manure of dairy cows fed Brachiaria hay with either dairy cubes or *Desmodium intortum* supplementation

1.4 Hypotheses

i. There is no significant difference in the digestibility of *Brachiaria brizantha* cv. *xaraes* hay-based diets supplemented with either dairy cubes or *Desmodium intortum*

ii. There is no significant effect of supplementing Brachiaria hay with dairy cubes or *Desmodium intortum* on the physico-chemical, fibre contents, and tannin contents of manure

iii. There is no significant effect of supplementing Brachiaria hay with dairy cubes or *Desmodium intortum* on CH_4, N_2O, and CO_2 emissions from the resultant manure

1.5 Justification

Currently, the rise in GHG emissions from livestock contributing to global warming is a matter of high concern in developing countries. In Kenya, the agricultural sector is the leading source of anthropogenic GHG emissions, with the livestock sub-sector contributing about half of the agricultural GHG emissions (Government of Kenya, 2018). Several initiatives, including the Kenya Dairy Sector Competitiveness Program, are actively engaged in the development of the sector to increase productivity and achieve climate change mitigation benefits by reducing GHG emission intensity (i.e. GHG emissions per unit of product). While the primary focus lies on enhancing productivity and mitigating enteric methane emissions, it is crucial to shift attention also towards reducing emissions from manure. The intensification of the dairy sector, as observed by the current increasing trends, may result in an increase in the production of manure into the environment. Additionally, there is a concern that a reduction achieved in enteric methane emissions due to diet manipulation might be counteracted by increased greenhouse gas emissions from manure. Diet or ration of the animal is a major deciding factor for manure GHG emissions (Nampoothiri et al., 2015), but there is need for more research to be conducted to assess the effect of diets used in Kenyan dairy production systems on manure GHG emissions *in situ*, to provide localized information on which diets can be used to mitigate manure GHG emissions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Current status of dairy farming in Kenya

Dairy farming is a crucial subsector of Kenya’s agricultural sector that plays a vital role in the country’s economy, food security, employment, and the livelihoods of millions of Kenyan farmers (Herrero et al., 2013; KDB, 2016). As a result of population growth, rising incomes, and urbanization, there is an increase in demand for dairy products, making livestock more important (Adesogan & Dahl, 2020). To meet the growing demand for milk, the dairy sector has been transitioning over time, moving towards commercialization and intensified systems (Bosire et al., 2019; Brandt et al., 2020). This involves increasing stocking densities, improving the genetic merit of the breeds, and feeding more concentrated diets (Chagunda et al., 2016). This is a promising intervention to increase milk production and reduce GHG emission intensity (Brandt et al., 2018), contributing to both the adaptation and mitigation of climate change (Campbell et al., 2014). However, this transition is likely to increase the volumes of manure (Grossi et al., 2019) and influence manure composition (Sorensen et al., 2003), which can negatively impact the environment through nutrient pollution and greenhouse gas emissions (Niu et al., 2016) if not well managed.

2.2 Dietary protein in dairy diets and its effects on animal performance

Protein supplementation is a common practice in dairy cow nutrition, aimed at enhancing growth, reproduction, and productivity of the animal. Protein is an important nutrient for cattle fed low-quality forages. It is essential for meeting the nitrogen requirements of both rumen microorganisms and the animals themselves.

Dietary protein is typically classified into two fractions: rumen-degradable proteins and rumen-undegradable proteins (Ali et al., 2009). Rumen-degradable protein serves as a substrate for microorganisms in the rumen for the synthesis of microbial protein, a valuable source of metabolizable protein for animals. However, the degradation of valuable protein in the rumen also results in losses of nitrogen as urea in the urine.

On the other hand, rumen-undegradable/bypass protein is not degraded in the rumen but is digested and absorbed in the small intestine and is available to the animal. Lactating animals require both microbial and bypass protein to meet their metabolizable protein requirements (Kamalak et al., 2005). Supplementation of dietary rumen-undegradable protein can increase milk yield (Gulati et al., 2005). However, some studies have reported little or no response, which could be due to poor digestibility postruminally (Kamalak et al., 2005), or deficiencies in the limiting amino acids for milk production (Schingoethe, 1996).

A study by Marghazani et al. (2012) indicated that protein supplements with varying rumen degradability characteristics influenced nutrient intake, digestibility, and yield performance during early lactation in Sahiwal cows. Diets rich in rumen-undegradable proteins during early lactation in tropical conditions have been shown to significantly improve milk yield and its composition (Kanjanapruthipong & Buatong, 2002).

2.3 Improved livestock feeding as manure GHG mitigation strategy

One promising technique for reducing greenhouse gas (GHG) emission intensities in dairy systems is to improve the nutritional quality of the rations fed to dairy cows. To reduce nitrous oxide (N_2O) and methane (CH_4) emissions from manure, the focus should be on improving nitrogen (N) use efficiency, balancing N input with production levels, and maintaining fiber digestibility while reducing enteric CH_4 fermentation (Montes et al., 2013). In East Africa, dairy production suffers from low productivity due to poor animal nutrition. Although available breeds have high milk potential, the generally low quality forages often fail to meet their nutritional requirements. Moreover, there is seasonality in the quantity and quality of available forage, with many regions experiencing shortages of supply during the dry season, and the forage available during this period is of very poor quality (Njarui et al., 2011).

Improved feeding practices can address this challenge by reducing the fluctuation of feed during the dry season. Ericksen and Crane (2018) proposed the use of improved forages and supplements among others as low GHG emissions strategies. For example, Brachiaria is an improved forage with desirable traits such as adaptation to acidic and low fertility soils, tolerance to drought, shade, and flooding, high biomass production potential, palatability, nutritional value, soil carbon sequestration ability, efficient use of soil N (through biological nitrification inhibition), and potential reduction of soil N_2Oemissions (Ghimire et al., 2015). In a study by Mutimura et al. (2018), *Brachiaria brizantha cv. Piata* grass had higher dry matter (DM), organic matter (OM), crude protein (CP), and less neutral detergent fibre (NDF) and acid detergent fibre (ADF) compared to Napier grass (Pennisetum purpureum), resulting in increased DM intake, CP intake, metabolizable energy intake, and higher milk yield. It is important to note that for all forage grasses, proper management practices such as manure fertilization to replenish soil nutrients and timely cutting are crucial for achieving good forage quality.

As part of feeding management, supplementation plays a critical role in dairy farming. Although optimized dairy production cannot rely on forage grasses alone, even if managed correctly, supplementation is essential to provide deficient nutrients in forages, mainly proteins, minerals, and trace elements that are required for body maintenance and milk production (Kellaway & Harrington, 2004). Supplements can be in the form of concentrates or forage supplements. Forage supplements, mainly legumes, are fed in addition to forages/grasses to compensate for the poor quality or as substitutes for concentrates (Lukuyu et al., 2007). Concentrates, such as dairy cubes, are nutrient-rich, providing more nutrients (energy and protein) than an equivalent amount of bulk forage. The downside is that they can interfere with the digestibility of forages in the rumen and are expensive. Supplementary forages, such as *Desmodium intortum,* which is an N- fixing tropical leguminous fodder plant that can be intercropped with other feed plants to increase the total protein content, are affordable because farmers can grow them themselves (Lukuyu et al., 2012). In a study by Mutimura et al. (2018) supplementation of Desmodium to both Brachiaria and Napier grass increased the diet CP, NDF and reduced ADF content. Desmodium also contains condensed tannins (CT), which are phenolic plant secondary compounds that can form complexes with proteins, polysaccharides, alkaloids, nucleic acids, and minerals due to the presence of a large number of phenolic hydroxyl groups. Due to their binding capacity, CTs improve N use in ruminants by decreasing the degradability of dietary protein in the rumen and increasing the amount of protein to be digested in the small intestine (Aboagye et al., 2018).

2.4 Manure production and characteristics

Livestock production naturally generates manure, which is influenced by the amount of feed intake (Oenema et al., 2005). Manure is composed of animal feces and urine, often mixed with bedding materials, spilled feeds, and drinking water. Fecal material contains a variety of organic compounds, including undigested feed components and endogenous losses. Urine contains waste products and excess nutrients not utilized by the animal, primarily urea and other water-soluble nitrogenous compounds (Teenstra et al., 2015). Livestock manure is a valuable source of nutrients essential for crop and pasture production, such as nitrogen (N), phosphorus (P), and potassium (K) as well as micronutrients such as copper (Cu), manganese (Mn) and zinc (Zn). Moreover, manure can be utilized as an energy source through anaerobic digestion for biogas production or thermochemical processes such as pyrolysis, gasification, or combustion for heat or electricity generation (Borhan et al., 2011; Eckhardt et al., 2018). However, manure is also a significant contributor to agricultural GHG emissions, ranking second to enteric methane (CH_4) (O’Mara, 2011). Major GHGs (CO_2, CH_4, and N_2O) can be produced from manure due to the presence of organic matter and microbes (Li et al., 2012). Manure is responsible for 7% of both agricultural CH_4 and nitrous oxide (N_2O) emissions (Waldrip et al., 2015). CO_2 from manure is usually not accounted for in GHG emission inventories because the CO_2 from the manure originates from partly digested plant material. Since plants fix CO_2 during their growth through photosynthesis, the emitted CO_2 from manure and the CO_2 fixed through photosynthesis cancel each other out, resulting in net zero CO_2 emissions.

The quality of manure is influenced by several factors including, the type of animal (ruminant or non-ruminant), diet (forage-based or grain-based), animal age (which can influence the amount of feed consumed), animal environment, and animal productivity (Mathot et al., 2012; Oenema et al., 2005; Tongwane & Moeletsi, 2018).

According to a study by Borhan et al. (2011), the chemical properties of manure are primarily influenced by the chemical composition of the feed given to the animals. In smallholder dairy systems, the quantity and quality of manure differ significantly due to the variability in fodder resources in terms of amount and distribution across different seasons (Rukiko et al., 2018).

Manure is commonly classified based on the total solids content, as follows;

i. Liquid contains less than 4% solids

ii. Slurry manure contains 4% to 10% solids

iii. Semi-solid manure contains between 10 and 20% solids

iv. Solid manure contains over 20% solids content (Meer, 2008).

2.5 Effects of livestock feeds on manure characteristics and GHG emissions

Diets differ in composition and nutritional quality depending on the formulations, influencing the composition of excreted manure (Boadi et al., 2004). Globally, the total mixed ration is the most predominant feeding method for dairy cows indoors (Gulati et al., 2018). However, in Kenyan smallholder dairy farming, diverse feed resources are utilized, including on-farm grown forages such as Napier grass, Kikuyu grass, Brachiaria grass (currently adopted), and occasionally Rhodes grass, crop residues, externally sourced forages, forage legumes (though not widely adopted) and commercial dairy meal (Gachuiri et al., 2017; Kashongwe et al., 2017). The seasonal and rain-fed production of forages often results in shortages during dry seasons. Moreover, the feeding value of most of these tropical forages is comparable to that of older grasses, having high fiber, low energy, and crude protein, and often failing to meet animal nutrient requirements (Creemers & Aranguiz, 2019). As a result, purchased concentrates are used to supplement the low energy and protein from forages, but are fed in limited amounts, as a result, forage to concentrate ratio is high (Kashongwe et al., 2017; Wilkes et al., 2020).

Cattle fed high forage diets, as is common in smallholder systems, excrete manure with a higher content of plant cell wall material but reduced N concentrations. This manure is rich in C and has a high C: N ratio, influencing both CH_4 emissions (Boadi et al., 2004) and N_2O emissions (Leitner et al., 2021) from manure. Additionally, its lignin-rich nature makes it more resistant to microbial degradation.

The amount of nitrogen excreted in urine is influenced by the protein concentration and degradability of the diet. The use of rumen-undegradable protein sources reduces excessive protein breakdown in the rumen and consequently manure N (Boadi et al., 2004). Conversely, high urinary N excretion rates result from the use of highly degradable protein supplements and high CP diets (Wattiaux & Karg, 2004).

In temperate regions, the feeds for dairy animals are usually richer in N than in the tropics. As a result, when high protein forage-based rations are given to these animals, they tend to excrete more N due to low energy contents and high protein content (Külling et al., 2001), and a higher proportion is usually portioned to urine than feces (Rufino et al., 2006). In smallholder farming systems in the tropics, where animals are fed low quality basal diet with supplements, N excretion is lower, suggesting that less urinary-N is lost through ammonia volatilization and leaching (Delve, 2001). In tropical regions, the concentration of fecal N is often less than 1% due to low protein diets, whereas in temperate regions, it is often above 3% due to higher quality diets (Rufino et al., 2006).

In a study conducted in Kenya by Delve (2001), where Friesian-Ayrshire steers were fed on barley straw supplemented with either *Macrotyloma axillare* (Leguminous forage: high N and low polyphenol content), *Calliandra calothyrsus* (leguminous forage: high in both N and polyphenol content) or poultry manure at 15 or 30% of the DM, found that N intake increased with supplementation. However, urinary N excretion was lower for legume supplementation compared to poultry manure. Nonetheless, only a small percentage of ingested N was excreted in urine, and the animals were under-nourished at the beginning of the experiment, resulting in high N retention during the feeding trial. Faecal N was higher for all the supplemented diets.

Regarding plant secondary compounds, the level of protein-binding polyphenols affects the chemistry of excreta, including concentrations of N in feces and urine, N excretion rates, ratios of fecal-N to urinary-N, concentrations of fiber fractions in feces, and the relative partitioning of N into fecal fiber fractions (Powell et al., 2009). From the aforementioned study, cows fed alfalfa containing high levels of tannins had the lowest fecal N and urinary N. On the other hand, the fiber fraction relative to fecal N was significantly higher in feces from cows fed high tannin birdsfoot trefoil.

Understanding the impact of dairy diet on manure N composition and transformation can contribute to various short and long-term management decisions. Over the short term, better predictions of manure N mineralization and plant availability can be made. In the long-term, impacts such as soil N accumulation and associated hazards of nitrate leaching and denitrification, sequestration of C in soil, and impacts (e.g., fertilizer use and soil erosion) of the cropping systems designed to provide diet components (e.g., alfalfa vs. corn grain) should be considered (Magdoff & Weil, 2004; Wattiaux & Karg, 2004), but they are beyond the scope of this study.

2.6 Greenhouse gas emissions from manure

Greenhouse gas emissions from manure are biogenic and can be controlled through appropriate handling, treatment, and storage conditions. After excretion manure undergoes a series of reactions, specifically decomposition, hydrolysis, fermentation, nitrification, and denitrification, which transform C- and N-compounds leading to N_2O, CH_4, and CO_2 emissions (Chadwick et al., 2011; Külling et al., 2001). The global warming potential (GWP) of CH_4 and N_2O is 28 and 265 times greater than that of CO_2, respectively, when calculated over 100 years (Pachauri et al., 2015). The IPCC excludes CO_2 emissions from animal and manure sources from greenhouse gas accounting because the carbon dioxide produced is part of natural carbon cycling and is offset by CO_2 fixed through plant photosynthesis (Rotz et al., 2010). However, CO_2 emissions from manure are important indicators of decomposition processes as they provide information on microbial activity.

2.6.1 Carbon dioxide emissions- sources, processes, controls and substrates

Carbon dioxide (CO_2) is released from manure during aerobic decomposition via microbial respiration, as well as from anaerobic decomposition of organic matter in the manure (Li et al., 2012; Moller et al., 2004). Emissions can occur from solid manure piles, anaerobic lagoons with a crust, slurry tanks, and barns (Eckhardt et al., 2018). When fresh manure is exposed to oxygen, organic matter starts to decompose, and organic polymers, such as carbohydrates, proteins, and fats, break down into their smaller constituents like sugars, amino acids, or fatty acids. Microbial decomposers in organic matter utilize the available C as an energy source and produce CO_2 as a byproduct (Li et al., 2012). Under anaerobic conditions, anaerobic microbes like acidogenic bacteria break down organic compounds to produce CO_2 using other electron receptors than O_2 (e.g. SO_4^{2-} or NO_3^-) (Li et al., 2012).

Temperature and moisture influence CO_2 emissions from manure, and the decomposition rate slows down if the environmental temperature or moisture content deviates from the optimum (Li et al., 2012). Environmental conditions and the chemical composition of the organic matter (i.e., the substrate) explain around 70% of the observed variation in the decomposition of organic matter in terrestrial ecosystems (Ayres et al., 2009). Similarly, in the case of manure, cellulose, lignin, total N, and C: N influence decomposition rates (Zhu et al., 2020).

2.6.2 Methane emissions - sources/sinks, processes, controls, and substrates

Methane (CH_4) emissions from manure result from anaerobic decomposition of organic matter (Moller et al., 2004). It is emitted from fresh or compacted solid manure, slurry, and liquid manure due to low oxygen levels (Chadwick et al., 2011). Methane can also be oxidized through methanotrophic bacteria in the presence of oxygen, for example, in well-aerated compost heaps or in the outer crust of manure heaps, or on slurry tanks.

The formation of CH_4 involves methanogenic archaea, through a series of biochemical processes which involve four steps. In the hydrolytic phase (step one), organic compounds are decomposed into simpler forms (monomers) by hydrolase (exoenzyme) present in facultative and obligatory hydrolytic and fermentative anaerobic bacteria. In the acidic phase (step two), these monomers are converted into short-chain volatile fatty acids (mainly lactic, butyric, propionic, and valeric acid) by hydrogen-producing acetogenic bacteria. The acid phase continues in step three, where homoacetogenic microorganisms consume the volatile fatty acids to produce acetic acid, CO_2, and hydrogen. Finally, in step four, the methanogenesis phase occurs, during which methanogens (strictly anaerobic) reduce acetate, CO_2, and H_2 into CH_4 via one of three processes:

i) Acetotrophic pathway → $4CH_3COOH \rightarrow 4CO_2 + 4CH_4$

ii) Hydrogenotrophic pathway → $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$

iii) Methylotrophic pathway → $4CH_3OH + 6H_2 \rightarrow 3CH_4 + 2H_2O$ (Tauseef et al., 2013).

The production of CH_4 from manure is influenced by both physiochemical and biological factors, with temperature, moisture, manure chemical composition, and microbial activity being the most important (Guo et al., 2020; Li et al., 2012). Additionally, the concentrations of the organic matter (substrates) and CO_2 also regulate methane production (Kebreab et al., 2006).

2.6.3 Nitrous oxide emissions – sources/sinks, processes, controls, and substrates

Nitrous oxide emissions from manure result directly from nitrification and denitrification processes, occurring in stacked manure, the crust on the surface of stored manure slurry, bedded-pack manure on barn floors, and on manure-laden dry lot surfaces (Rotz et al., 2010). In addition, manure contributes to indirect N_2O emissions through volatilization, leaching, and runoff of manure-N which is deposited and converted to N_2O elsewhere (Moeletsi & Tongwane, 2015).

The respiratory N_2O reductase found in denitrifying bacteria, serves as a dominant sink for N_2O, as it reduces N_2O to N_2 (Thomson et al., 2012). However, the relative contribution of N_2O to N_2 emissions from manure in SSA is unknown.

During decomposition, organic N in the manure undergoes mineralization to ammonium (NH_4^+) (Li et al., 2012). Subsequently, in the presence of oxygen, NH_4^+ is oxidized to nitrite (NO_2^-) and further to nitrate (NO_3^-) as shown in **Equation 1** a process referred to as nitrification. Nitrification is a microbe-mediated process, and the bacteria involved require CO_2, H_2O, O_2, and either NH_4^+ or NO_2^- for growth, as well as a pH above 5 (Kebreab et al., 2006).

Under anaerobic conditions, denitrification occurs where NO_3^- is reduced stepwise to NO_2^-, nitric oxide (NO), N_2O, and finally dinitrogen (N_2) as shown in **Equation 2**. N_2O is produced when the reduction is incomplete (Chadwick et al., 2011). Conditions necessary for denitrification include the presence of heterotrophic bacteria, reductants such as organic C, lack of oxygen, and the presence of N substrates (Kebreab et al., 2006).

Equation 1 : Nitrification

$NH_4^+ + O_2 \rightarrow H^+ + H_2O + NO_2^- \rightarrow NO_2^- + H_2O \rightarrow NO_3^- + H_2$

Equation 2: Denitrification

$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$

Microbial nitrification and denitrification are the main processes that utilize inorganic N compounds leading to N_2O formation, but there are several other microbial processes such as autotrophic and heterotrophic nitrification, co-denitrification, chemodenitrification, and dissimilatory nitrate reduction to ammonia (DNRA), which can also lead to N_2O formation, albeit usually have a lower contribution (Braker & Conrad, 2011; Butterbach-Bahl et al., 2013).

2.7 Factors controlling manure decomposition and GHG emissions

2.7.1 Manure chemistry

The composition of animal manure can differ depending on factors such as the type of animal, feed composition, bedding materials used, and the method of manure management (Rotz, 2004; Sorensen et al., 2003). Cattle that consume diets high in forage produce manure with a higher content of partially digested cell wall material, which is more resistant to microbial degradation compared to manure from cattle that consume high grain diets (Amon et al., 2001). The composition of manure plays a role in influencing the processes of C and N mineralization/immobilization during decomposition (Forge et al., 2005). The key properties of manure affecting mineralization include N content, C content, polyphenol content, and the main structural components such as cellulose and lignin (Morvan et al., 2006). The initial ratios of these components (C: N, lignin: N, cellulose: N, and lignin: cellulose) determine the decomposers involved, the pathways, and the rates of the decomposition process (Talbot & Treseder, 2012). According to the study, interactions among lignin, cellulose, and N were identified as the primary controls over litter decomposition. In this study, it was found that litter N alleviates N limitation of C use by decomposers, cellulose serves as a co-substrate for lignin degradation, and lignin protects cell wall polysaccharides from decay. Lower initial N and high C: N in cattle manure results in slower decomposition rates, and lignin: N and ADF: ADL regulate the decomposition rate (Zhu et al., 2020).

2.7.2 Temperature and moisture

Temperature and moisture are important factors in regulating decomposition rates largely due to their effects on microbial metabolic rates and population structure. The decomposition rate declines if the environmental temperature or moisture content deviates from its optimum range. They collectively affect biogeochemical reactions, such as enzyme activity, respiration, methanogenesis, nitrification, and denitrification (Li et al., 2012). Temperature controls enzyme activity and metabolic activity of microbes, where increased temperatures can result in an increase in enzyme activity leading to increased decomposition rates, and subsequently an increase in GHG emissions due to enhanced microbial activity (Conant et al., 2011). Moisture affects O_2 availability, stimulates microbial activity and enzyme diffusion, and thereby regulates decomposition rates. In a study by Zhu et al. (2020), an increase in moisture accelerated decomposition rates in semi-arid drylands. This study identified moisture as a main environmental driving factor for the manure decomposition process. The research showed a positive correlation between decomposition rates and cumulative precipitation and a negative correlation with mean temperature, this was attributed to the region of the study (a tropical region), where temperature variation is minimal throughout the year and is generally not a limiting factor for decomposition.

2.7.3 Tannins

Feeds containing natural phytocompounds, such as condensed and hydrolyzable tannins, offer a sustainable approach to mitigate the environmental impacts of ruminants. Tannin-containing feeds have demonstrated the ability to reduce both enteric methane emissions and urinary nitrogen excretion in ruminants (Maamouri et al., 2011; Tan et al., 2011). Tannins are water-soluble phenolic plant secondary compounds of relatively high molecular weight that can form complexes, mainly with proteins, polysaccharides, alkaloids, nucleic acids, and minerals due to the presence of a large number of phenolic hydroxyl groups (Kamra et al., 2006). They are widespread in nutritionally important shrubs, legumes, forage trees, cereals and grains commonly consumed by ruminants. Due to their capacity to form complexes with proteins, they often limit the utilization of these feedstuffs (Frutos et al., 2004; Patra, 2014). Tannins exist in two forms; condensed tannins (CT) and hydrolyzable tannins (HT). Hydrolyzable tannins occur mainly in fruit pods and plant galls, and upon degradation, their products are absorbed in the small intestine of animals and are potentially toxic to ruminants. In contrast, CT, commonly known as proanthocyanidins (PA), are commonly found in forage legumes, trees, and shrubs. Conventionally, the two forms are differentiated based on their molecular weight, with CT having a higher molecular weight than HT (Frutos et al., 2004).

Condensed tannins improve N use in ruminants, they increase the amount of protein digested in the small intestine by decreasing its degradability in the rumen as a result of their binding capacity (Aboagye et al., 2018; Waghorn et al., 1987). This mechanism results in reduced levels of NH_3 produced in the rumen, consequently lowering urinary N and increasing fecal N. At the environmental level, this change contributes to the reduction of N_2O emissions from manure (Hristov et al., 2013).

The combined effects of protein binding and inhibition of nitrification indicate that tannins have the potential to reduce reactive N, thus reducing total N_2O emissions from manure. Feeding tannins to animals can lead to better nitrogen utilization in forage cropping systems and improve the quality of milk products (Beauchemin et al., 2009). Though previous research has explored tannin feeding trials and their implications on enteric methane emissions and soil nitrogen, there is still a significant gap in understanding the impact on greenhouse gas emissions from manure during storage, especially in developing countries.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study site

The study was conducted in two laboratories - Egerton University Animal Science Laboratory, located in Njoro at coordinates 00° 19' 00’ S 36° 06' 00" E, and the Mazingira Centre for Environmental Research and Education laboratory hosted by the International Livestock Research Institute (ILRI), located in Nairobi at coordinates 1° 16' 11.73’ S, 36° 43' 26.0472". The climate is marked by a mean annual temperature of 17°C and a mean annual rainfall of 875 mm that shows a bimodal pattern (long rains from March to June and short rains from October to December).

3.2 Experiment 1: To determine the nutritional value and digestibility of *Brachiaria brizantha cv. xaraes* hay based diets supplemented with either dairy cubes or *Desmodium intortum*

This experiment involved proximate analysis, fibre analysis, and *in-vitro* degradability of the feed ingredients (Brachiaria hay, dairy cubes, and *Desmodium intortum* hay) and formulated experimental diets (formulated from the ingredients). The apparent digestibility of DM, N, and NDF were also determined. The experimental diet samples included:

- D_1 (Diet 1): Control, Brachiaria + molasses + urea,
- D_2 (Diet 2): D_1 + Dairy cubes
- D_3 (Diet 3): D_1 + Desmodium.

The samples were collected during an animal feeding trial (companion study), where cross-bred lactating dairy cows were fed on the three stated experimental diets.

3.2.1 Feed ingredients

The individual feed ingredients were obtained from different locations; Brachiaria was harvested from ILRI farm at the late bloom stage and sun-dried for 9 days, while *Desmodium intortum* was obtained from smallholder farmers in western Kenya, and dairy cubes were purchased. Brachiaria and Desmodium hay were chaffed into about 10 cm lengths.

3.2.2 Diet formulation

The experimental diets were formulated using chopped *Brachiaria brizantha* cv. *xaraes* hay, urea **(**as a protein supplement), molasses (as an energy supplement), and experimental protein supplements (dairy cubes and *Desmodium intortum*). Three diets were formulated to meet the required crude protein and energy requirement for crossbred cows weighing on average 350 kg, supporting the production of 3 litres for the cows on the control diet and 4 litres for the cows on diets 2 and 3 per day as described by John Moran (2005). Diet 1 (D_1) control consisting of 87% brachiaria hay (*Brachiaria brizantha* cv. *xaraes*) with 12.2% molasses and 0.8% urea supplement, diet 2 (D_2) consisting of 65.7% brachiaria hay, 8.5% molasses, 0.8% urea and 25% dairy cubes and diet 3 (D_3) consisting of 35% brachiaria, 6.4% molasses, 0.8% urea and 57.7% Desmodium hay as shown in **Table 1** below.

Table 1. Dietary treatments and their proportions in the rations

Diet	**D_1** **Control**	**D_2** **Control + Dairy cubes**	**D_3** **Control + *Desmodium intortum***
Diet ingredients and proportions			
Expected DM intake (kg)[1]	8.2	8.0	7.83
Basal diet - chopped *Brachiaria brizanta cv. xaraes* hay	87 % (7.14 kg)	65.7 % (5.25 kg)	35 % (2.75 kg)
N supplement - urea	0.8 % (0.07 kg)	0.8 % (0.07 kg)	0.8 % (0.07 kg)
Energy supplement - molasses	12.2 % (1.0 kg)	8.5 % (0.68 kg)	6.4 % (0.5 kg)
Experimental supplements			
Chopped *Desmodium intortum* hay	0 %	0 %	57.7 % (4.52 kg)
Dairy cubes	0 %	25% (2 kg)	0 %
Diet composition (%DM)			
CP	7.5	10.3	10.3
NDF	57.6	52.4	61.3
Total tannins	< 0.2	< 0.2	1.6
Condensed tannins	< 0.1	< 0.1	

3.2.3 Sample collection

The individual feed ingredient samples were collected before feed formulation and the experimental diets were sampled during the animal feeding trial. The samples were dried at 50 °C for 96 h, ground to pass through a 1 mm sieve, and stored in plastic bags for proximate, fibre, tannins, and digestibility analysis.

3.2.4 Laboratory analysis

Proximate analysis

Dried ground feed ingredients and diet samples were analyzed using AOAC analytical methods as follows dry matter (DM) (method 934.01), ash (method 942.05), where 2 g of the samples were dried in duplicates at 105 °C for 24 h**,** cooled in a desicator and weighed then transferred into a muffle furnace for ashing at 550^0 C for 4 h. DM was calculated as shown in **Equation 3** and ash in **Equation 4**.

Equation 3 $\% \boldsymbol{DM} = \frac{[(\boldsymbol{Dried\ sample\ \ + dish\ weight})\boldsymbol{g} - (\boldsymbol{empty\ dish\ weight})\boldsymbol{g}}{(\boldsymbol{initial\ sample\ weight})\boldsymbol{g}} \times \mathbf{100}$

Equation 4 $\% \boldsymbol{Ash} = \frac{[(\boldsymbol{Ashed\ sample{+}dish\ weight})\boldsymbol{g}-(\boldsymbol{empty\ dish\ weight})\boldsymbol{g}]}{(\boldsymbol{initial\ sample\ weight})\boldsymbol{g}} \times \mathbf{100}$

The crude protein (CP) was determined as described by AOAC (method 990.03), this procedure involved digestion, distillation, and titration; where 0.3 g of the dried ground feed sample was digested with sulphuric acid (H_2SO_4) in the presence of a catalyst to breakdown all N bonding converting them to ammonium ions, the digest was then alkalinized using sodium hydroxide (NaOH) and distillated, the distillate (ammonia trapped in boric acid) was finally titrated with 0.1 *N* hydrochloric acid (HCL). The amount of acid used in titration (corresponds to the amount of nitrogen in the sample) was multiplied by 6.25 to convert to CP as shown in **Equation 6.**

Equation 5: $\% \ \mathbf{N}\ (\mathbf{on\ DM\ basis}) = \frac{[\mathbf{1.4007}\times(\mathbf{Va}-\mathbf{Vb})\times\mathbf{N}]}{\mathbf{W}\times\mathbf{100}}$

Equation 6: $\% \ \boldsymbol{CP}\ (\boldsymbol{on\ DM\ basis}) = \ \% \ \boldsymbol{N}\ (\boldsymbol{on\ DM\ basis}) \times \mathbf{6.25}$

where:

1.4007 = conversion factor (1 ml of the acid is equivalent to 1.4007 mg of N)

Va = Volume of acid used for titration

Vb = Volume of acid used for titration for blank

N = Normality of acid

W = Weight of sample in g

6.25 = Conversion factor for crude protein for all forages

For ether extract (EE) (method 920.39) was used, where 2g of the sample was weighed into a thimble and placed on a soxhlet extractor, and petroleum ether was used as a solvent. Cell wall constituents were analyzed by the methods of Van Soest et al. (1991). This method allows for sequential extraction of the fibre fractions as neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL). NDF measures hemicellulose, cellulose, and lignin, while ADF measures cellulose and lignin, and ADL measures lignin content; from these measures, the 'hemicellulose' fraction is defined as NDF-ADF, the 'cellulose' fraction is defined as ADF-ADL and the 'lignin' or 'acid-unhydrolizable residue' fraction is defined as ADL. Gross energy (GE) was measured using a bomb calorimeter (Harris, 1970) and condensed tannins (CT) were determined using Near-infrared spectroscopy (NIRS).

In vitro degradability

The gas production technique was used to determine the fermentative activity of the mixed microbial population as described by Menke and Steingass (1988). Rumen liquor was collected in the morning (6 am) from fistulated animal before feeding and watering into a pre-warmed thermos-flask and taken to the laboratory. One litre of rumen fluid was filtered through two layers of cheesecloth to obtain strained rumen fluid. This was then flushed with carbon dioxide (CO_2) and mixed with buffers to simulate the action of saliva.

Feed samples weighing 200 mg (1mm screen) were prepared in duplicate and placed into 100 ml glass syringes. The rumen fluid and buffer medium were mixed in the ratio of 1: 2 (v/v). A buffer-rumen fluid mixture of 30 ml was then passed into syringes holding samples, shaken gently and any air bubbles were released. The syringes were then incubated in a thermostatically controlled water bath at 39^{o} C for 0 - 96 hours. Both the samples and blank (rumen fluid + buffer) were run in duplicates.

The volume of gas produced was determined at 0, 3, 6, 9, 12, 18, 36, 48, 72, and 96 hours by reading the calibration of the piston. Two blank syringes containing only 30 ml of buffered rumen fluid were incubated to estimate gas production due to endogenous substrates for the blank corrections. The total increase in volume minus the blank value was used to calculate the gas produced. The calculated values of gas produced were then used to calculate the cumulative gas produced. The mean cumulative gas produced was fitted in the exponential equation by Ørskov and McDonld (1989) as shown in **Equation 7**.

Equation 7 : $\mathbf{Y= a + b (1-e^{-ct})}$

The equation was used to determine gas production characteristics a, b, a+b, c, and RSD constants. These constants explain the degradability potential of the feed ingredients, where Y is the gas produced at time t, a is the gas produced from the immediately soluble fraction, b is the gas produced from the insoluble but degradable fraction, a+b is the potential extent of gas production, and c is the rate constant of gas produced per hour (h^{-1}).

The gas produced at 48 hour was used to calculate *in-vitro* organic matter digestibility (OMD %), metabolizable energy ME (MJ/kg DM) content, and short chain fatty acids (SCFA mmol/200mg DM) of feed ingredients and experimental diets using Equation 8Equation 9Equation 10Equation 11Equation 12 shown below of Menke and Steingass (1995) and Menke and Steingass (1988).

Equation 8: SCFA (mmol/200mg DM) = 0.0222 GP - 0.00425

For concentrates:

Equation 9: ME (MJ/kg DM) = 1.06 + 0.1570 × Gas produced (ml/200 mg DM) + 0.0084 × CP (g/kg DM) + 0.022 × EE (g/kg DM) – 0.0081 × Ash (g/kg DM)

Equation 10: OMD (%) 48 HR = 9 + 0.9991 × Gas produced (ml/200 mg DM) at 48hrs) +0.0595 × CP (g/kg DM) + 0.0181× Total ash (g/kg DM)

For roughages

Equation 11: ME (MJ/kg DM) = 2.20 + 0.1357 × Gas produced (ml/200 mg DM) + 0.0057 × CP (g/kg DM) + 0.0002859 × (EE)^2 (g/kg DM)

Equation 12: OMD (%) 48 HR = 16.49 + 0.9042 × Gas produced (ml/200 mg DM) at 48hrs) + 0.0492 × CP (g/kg DM) + 0.0387 × Total ash (g/kg DM)

Apparent digestibility

Apparent digestibility of DM, N, and NDF were determined for the experimental diets for the period in which the diet samples were taken. Apparent digestibility was determined by the difference between the amount ingested and the amount excreted in feces, as shown in Equation 13 and nitrogen utilization was calculated using Equation 14 (Barbosa et al., 2018).

$$\textbf{Equation 13 : Apparent digestibility } (\%) = \frac{\textbf{Nutrient ingested} - \textbf{Nutrient excreted}}{\textbf{Nutrient ingested}} \times \mathbf{100}$$

$$\textbf{Equation 14: Nitrogen utilization } (\%) = \frac{\textbf{N intake} - \textbf{Fecal excreted N –Urine excreted N}}{\textbf{N Intake}} \times \mathbf{100}$$

3.2.5 Data analysis

Data from proximate analysis; (DM, CP, EE, NDF, ADF & ADL) and digestibility analysis (OMD, ME, and SCFA) were presented as analyzed as there were no statistical replicates. The effect of dietary treatment on DM, N, and NDF intake, excretion, and apparent digestibility was analyzed using analysis of variance (ANOVA); PROC GLM of SAS, (2002), following the model:

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

where, Y_{ij} = DM, N and NDF intake, excretion, and apparent digestibility, μ = Overall mean, D_i = effect of i^{th} diet, and ε_{ij} = random error term. Significant means were separated using Tukey's test at 5% significance.

3.3 Experiment 2: To determine the effects of feeding *Brachiaria brizantha cv. xaraes* hay with either dairy cubes or *Desmodium intortum* supplementation on physico-chemical, fibre, and tannin contents of manure

This experiment involved an animal feeding trial (companion study) and a laboratory manure incubation experiment. At the end of the second period of the companion study, samples for the analysis and manure incubation experiment were collected.

3.3.1 Animal feeding trial (companion study)

The study consisted of three dietary treatments and three feeding periods. Each period lasted for four weeks, with a three-day washout period before the start of each period. The diets were changed in each subsequent period for all animal groups so that all groups had consumed all the dietary treatments by the end of the feeding period. The cows selected for the study were in parity 1 and 2 and were grouped based on milk yield and days in milk into three groups, each with four cows. These groups were then randomly assigned to the dietary treatments. Each animal was housed and fed individually in partitioned open pens measuring 1.90m x 2.87m, which were roofed and bedded with rubber mats. The animals had free access to clean water supplied by automatic waterers.

3.3.2 Feces and urine collection

The manure used in this study was collected during the second feeding period of the experiment. The study collected the feces and urine excreted by each animal within a dietary treatment over 24 hours as shown in **Table 2**. Feces were scooped from the pen floor six times per day and stored in plastic buckets with lids. Urine was collected using non-invasive urine collection devices designed for female animals. These devices were attached using adhesive glue around the shaved perineum areas (Korir et al., 2022). A silicon tube was attached to the base of the urinals to direct the flow of urine into prefilled 10 L plastic containers with 200 ml of 5 *M* HCl to lower urine pH from 7.0 – 8.7 to 2 and below, preventing N loss through volatilization.

Table 2. Treatment allocation of experimental animals from which excreta was collected for the laboratory incubation trial.

(D_1) **Control**	**(D_2)** **D_1 + Dairy cubes**	**(D_3)** **D_1 + Desmodium**
Animal B1	Animal C2	Animal A3
Animal B3	Animal C4	Animal A1
Animal B4	Animal C3	Animal A4
Animal B2	Animal C1	Animal A2

3.3.3 Feces and urine sampling

Approximately 500 g of feces were transferred into labeled aluminum foil trays and dried at 50 °C for DM, total organic C and total N (CN), fibre analysis (NDF, ADF, and ADL), and CT analysis. About 100 g of the fresh samples were stored at +4 °C in zipped plastic bags for nitrate (NO_3^-), ammonium (NH_4^+), and pH analysis, and approximately 2 kg was immediately used for the laboratory incubation (see **Section 3.3.4**). Of the urine aliquots, 20 ml was stored at +4 °C for analysis of NO_3^- and NH_4^+, 20 ml was stored frozen at –20 °C for analysis of Kjeldahl-N, and 1 L was transferred into 1.5 L plastic bottles and immediately used for the laboratory incubation.

3.3.4 Manure preparation for incubation

In this study, it was found that the mean ratio of feces to urine was 70:30 as calculated using **Equation 15** and **Equation** 16. This ratio was also equivalent to their percentage proportions in the manure to be stored, which was 350g. Therefore, the amount of feces and urine to be stored was 70% and 30% of 350g, respectively.

Equation 15: Mean ratio of dung (%) = $\mathbf{{}^{Av.\,dung\,excreted}/_{Av.\,total\,excreta} \times 100}$

Equation 16: Mean ratio of urine (%) = $\mathbf{{}^{Av.\,urine\,excreted}/_{Av.\,total\,excreta} \times 100}$

Where total excreta is the sum total of excreted feces and urine.

Manure preparation was conducted as described in the following steps:

Step 1: 1.96 kg of excreted fresh feces was weighed and transferred into a beaker

The total amount of feces required per jar was 70% of 350 g which is 245 g, for each cow there were 5 jars for manure sampling at different times, but for allowance, feces required for 8 jars was calculated as 245 g × 8 =1.96 kg.

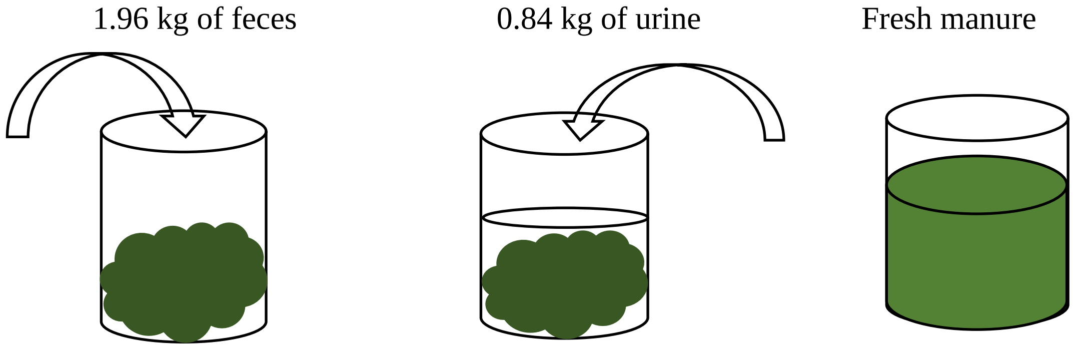
Step 2: Neutralization of urine to a pH of 7.6 using 5 *M* NaOH:

To prevent nitrogen loss, urine from animals was collected in acidified containers during total collection. Acidified urine slows down urea hydrolysis, which reduces the availability of nitrogen and could alter N_2O emissions during laboratory incubation. Therefore, the urine was neutralized to pH as excreted, as described below:

A sample weighing 0.84 kg of acidified urine was transferred into a beaker and placed onto a magnetic stirrer. The pH was then measured using the pH meter (Jenway model) pre-calibrated with pH buffers 4.0 and 7.0 and drops of 5 *M* NaOH were added until a pH of 7.6 was attained.

The total amount of urine required for a jar is 30% of 350 which is 105 g, as calculated for fresh dung, urine required for 8 jars was also calculated as 105 g × 8 = 0.84 kg

Step 3: The neutralized urine was transferred into the beakers containing faeces and gently mixed using a spatula.



Step 4: Aliquots of 350 g of the mixed manure from the 12 cows were then transferred into five replicates of labeled 1 L mason jars and transferred into a temperature-controlled chamber set at 20 °C, where they were incubated for 12 weeks. Four of the five replicate jars

were used for destructive manure sampling after 1, 2, 4, and 8 weeks of incubation, while the remaining jar was used for continuous manual gas sampling and the final manure sampling after 12 weeks as shown in the illustration below **Figure 1** (beaker represents a mason jar)

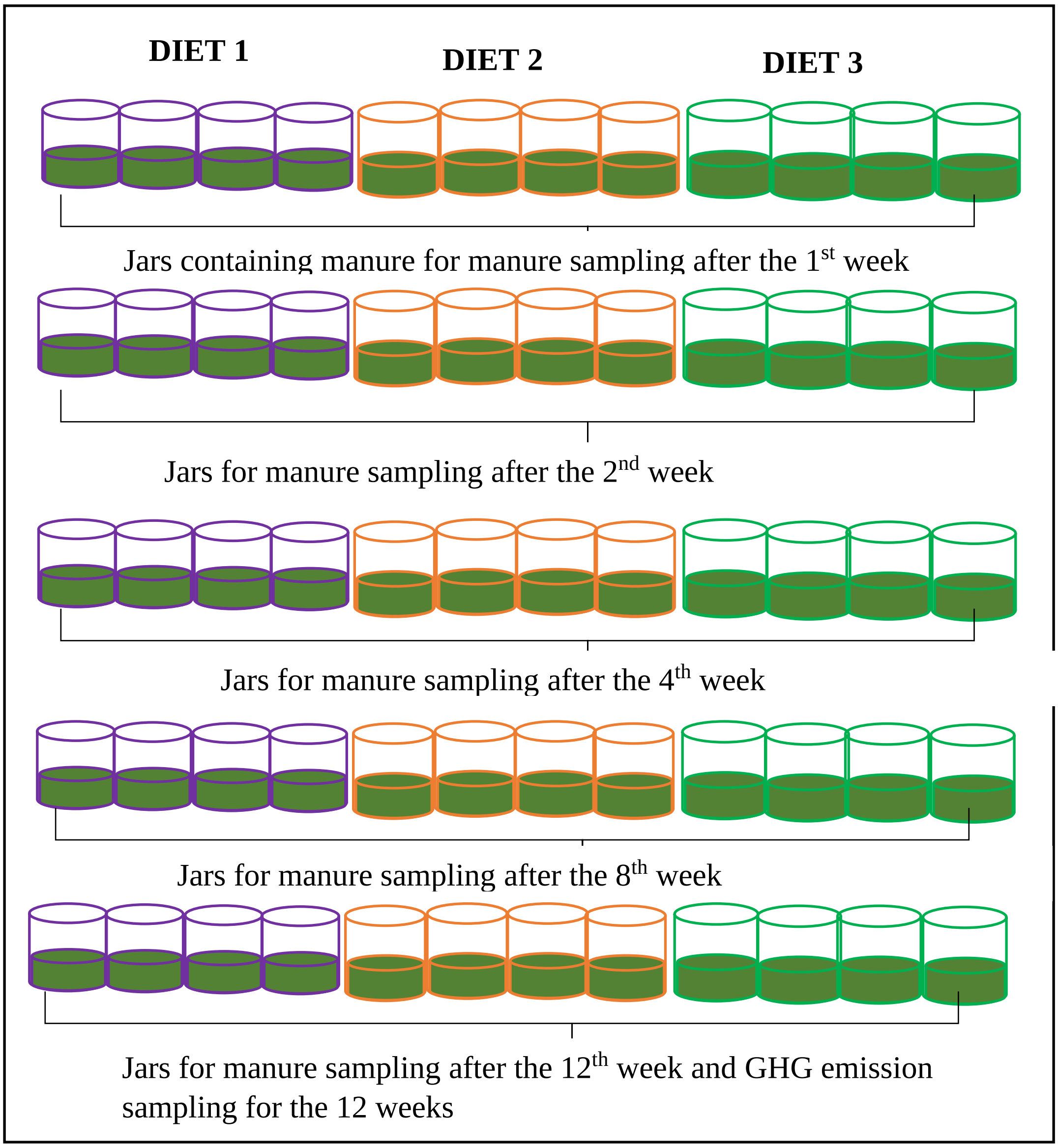


Figure 1. Illustration of the setup of mason jars containing manure for manure sampling and gas sampling for the 12 weeks of manure storage

3.3.5 Manure sampling

At each manure sampling time point, aliquots of the manure were sampled and stored at +4 °C for pH, NO_3^- and NH_4^+ analysis, and 300 g was transferred into aluminum dishes and dried in the oven at 50 °C for 72 h and ground to pass through a 1 mm sieve and stored in zipped plastic bags at air temperature until fibre and CT analysis.

3.3.6 Laboratory analysis

Feces, urine, and manure sample analysis

The feces and manure sub-samples were analyzed for chemical composition, specifically DM, water content (WC), ash, NH_4^+ and NO_3^- concentration, total N, organic C, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and CT content.

Dry matter and WC were determined as recommended by Peters et al. (2003), where 50 g of fecesand manure was weighed into pre-weighed aluminum trays using a Mettler Toledo weighing balance at 0.01 g precision and dried at 50 °C for 72 h using a GenlabThermocoupled drying oven. The DM and WC were then calculated as shown in **Equation 17** and **Equation 18**.

Equation 17
$$\% \mathbf{DM} = \frac{[(\mathbf{Dry\ sample\ weight+tray})-(\mathbf{empty\ tray\ weight})]}{[(\mathbf{wet\ sample\ weight + tray})-(\mathbf{\ empty\ tray\ weight})]} \times \mathbf{100}$$

Equation 18
$$\% \mathbf{WC} = \frac{[(\mathbf{wet\ sample\ weight+tray})-(\mathbf{dry\ sample\ weight + tray})]}{[(\mathbf{wet\ sample\ weight + tray})-(\mathbf{\ empty\ tray\ weight})]} \times \mathbf{100}$$

Crude ash was determined as described by AOAC (Association of Official Analytical Chemists) (1990), where crucibles with 0.5 g of the samples were dried first at 105 °C for 24 h then transferred into the muffle furnace for incineration at 550 °C for 4 h. NDF, ADF and ADL were done following Van Soest et al. (1991). Condensed tannins were determined using Near-infrared spectroscopy (NIRS). Ammonium (NH_4^+) and NO_3^- content were determined using colorimetric method as described by Hood-Nowotny et al. (2010), where 1 g of manure was extracted using 25 ml of 0.5*M* K_2SO_4, shaken for 30 mins, then centrifuged for 30 mins at 3000 rpm and filtered using ashless Whatman Filter Paper No. 42. The extract was then stored in the freezer for analysis.

For NH_4^+ determination, standards, colour reagent (0.3 *M* NaOH: Salicylate solution: deionized H_2O in the ratio 1:1:1), and oxidation solution (dichloroisocyanuric acid sodium solution) were prepared. 100 µl of blanks (extractant), standards, and samples were then pipetted in triplicates into a transparent 96-well microplate, and in each well 50 µl of the colour reagent was added first then 20 µl of the oxidation solution. The plates were then covered using microplate lids, and incubated at room temperature for 30 mins for colour development, after which the absorbance was measured photometrically without the lids at 660 nm.

NH_4^+ concentration calculations were done as follows:

Step 1: The standards were used for calibration; where a linear curve was plotted using the standards absorbance (x-axis) against the concentration (y-axis). The slope obtained was used as the calibration slope.

Step 2: The blank absorbance average was calculated

Step 3: Blank-corrected concentrations were calculated for all samples as shown in **Equation 19**:

Equation 19: Blank-corrected concentration (µg NH_4^-N ml^{-1})

$$= (\mathbf{sample\ absorbance - av.\,blank\ absorbance}) \times \mathbf{calibration\ slope}$$

Step 4: Concentration in µg NH_4^-N g^{-1} DW was calculated as shown in **Equation 20**:

Equation 20$= \frac{\text{Blank−corrected concentration} \times \text{Dilution factor} \times \text{Extraction volume (ml))}}{\text{Dry matter (g)}}$

For NO_3^- determination, standards, Griess reagent (N-Naphtylethylendiamin solution and sulfanilic acid in the ratio 1:1) and Vanadium(III)chloride were prepared. 100 µl of blanks, standards, and samples were pipetted in triplicates into a transparent 96-well microplate and into each well 100µl of the griess reagent was first added then 100 µl of vanadium(III)chloride solution. Microplates were then covered using the microplate lids and incubated at room temperature for 60 minutes, after which the absorbance was measured photometrically without the lids at 540 nm. The calculation of NO_3^- concentration was done following the steps used for NH_4^+ where the final calculation was done as shown in **Equation 21** below:

Nitrate (µg NO_3^-N g^{-1} DW)

Equation 21 $= \frac{\text{(Blank−corrected concentration} \times \text{Dilution factor} \times \text{Extraction volume (ml))}}{\text{Dry matter (g)}}$

Total nitrogen (N) and carbon (C) content were measured using the elemental combustion method described by Pella (1990) and Watson et al. (2003). Triplicate samples of 200 mg dried manure were analyzed on a Vario Max cube elemental analyzer.

The pH was measured according to Peters et al. (2003). Fresh feces and manure samples (5 g each) were weighed in triplicate and placed in 50 ml plastic screw cap bottles. Then, 25 ml of 0.01 *M* $CaCL_2$ (manure to CaCl2 ratio of 1:5) was added. The bottles were shaken for 10 minutes on a shaker and allowed to stand for 20 minutes. The pH meter was calibrated using pH buffers 4.0 and 7.0. The electrode of a Jenway model pH meter was then immersed in each bottle containing the supernatant solution to measure the pH.

3.3.7 Statistical analysis

Differences in excreta (feces and urine) and manure composition caused by different diets were analyzed by ANOVA, using PROC GLM of SAS (2002) using the model;

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

where, Y_{ij}= excreta and manure composition (WC, pH, total C, total N, C/N ratio, fibre, and CT) µ = Overall mean, D_i= effect of i^{th} diet, and ε_{ij} = random error term. Significant means were separated using Tukey's test at 5% significance.

Correlation analysis was also conducted using the Pearson correlation of SAS (2002) between nutrient intake and nutrient excreted to determine the relationship between diet composition and excreta composition. Additionally, for manure properties measured at different time points during storage, repeated measures analysis was done using PROC GLM of SAS to test for the effect of diet, time, and their interactions using the model:

$Y_{ijk} = \mu + D_i + T_j + (DT)_{ij} + \varepsilon_{ijk}$

where, Y_{ijk}= manure property at $_j$th time from cows fed $_i$th diet, D_i= effect of i^{th} diet, T_j= effect of $_j$th time, $(DT)_{ij}$= effect of interaction between diet and time and ε_{ijk}= random error term.

Experiment 3: To estimate CH_4, N_2O, and CO_2 manure emissions from dairy cattle fed *Brachiaria brizantha cv. xaraes* hay supplemented with either dairy cubes or *Desmodium intortum*

3.4.1 Data collection

Gas sampling

Gas sampling was done daily for the first 15 days of incubation, then four times a week for the next 15 days, and three times a week for the remaining 8 weeks. To this end, the mason jars were fitted with lids and rubber seals to allow for air-tight closure during gas sampling, and a sampling port containing a rubber septum through which gas samples were drawn with a syringe. At each sampling date, jars were taken out of the incubation chamber, placed onto the lab bench, and left open for ventilation for 10 minutes. Lids were then closed and five gas samples of 30 ml each were drawn from each mason jar headspace using 60 ml plastic syringes through the sampling port and transferred to pre-evacuated 10 ml gas chromatography (GC) vials. The gas sampling protocol involved sampling at intervals of 2 min (0, 2, 4, 6, and 8 min) for the first 4 weeks, and at intervals of 3 min for the last 8 weeks (0, 3, 6, 9, and 12 min). After each time a gas sample was drawn from the jar, the same volume of air of known GHG concentration was injected into the mason jar to refill the initial headspace volume and avoid under-pressure build up.

3.4.2 Laboratory analysis

Gas sample analysis and calculations

The CO_2, CH_4, and N_2O concentrations were determined using a gas chromatograph (SRI 8610C, SRI Instruments, Torrance, CA, USA) equipped with an electron capture detector (ECD) for N_2O detection, and a flame ionization detector (FID) with a methanizer for CO_2 and CH_4 detection. The peak areas measured by the GC were converted into concentrations using calibration curve equations generated from standard gas concentrations (2.03 to 49.8 ppm for CH_4, 4003 to 2420 ppm for CO_2, and 329 to 2530 ppb for N_2O) relative to measured peak areas of standards. Linear equations were used for CO_2, CH_4, and a power function for N_2O.

Calculation of manure-atmosphere GHG fluxes

Before gas flux calculation, the volumentric gas concentrations (ppm or ppb) for the second to last sampling time point were corrected to account for the air refilled during gas sampling for pressure equilibration (**Equations Equation** 22**, Equation** 23**,** and **Equation** 24);

$$\textbf{Equation 22} \quad c_{diluted_t} = \frac{\left(c_{measured_t} \times \frac{AW}{22.41}\right) \times (V_{HS} - V_{spl}) + \left(c_{refilled} \times \frac{AW}{22.41}\right) \times V_{spl}}{V_{HS}} \times \frac{22.41}{AW}$$

$$\textbf{Equation 23} \quad \Delta c_t = c_{measured_t} - c_{diluted_t}$$

$$\textbf{Equation 24} \quad c_{corrected_{t+1}} = c_{measured_{t+1}} + \Delta c_t$$

In the first step (Equation 22), the dilution of the air concentration at timepoint t ($c_{diluted_t}$) was determined by using the relative contributions of the measured gas sample concentration at timepoint t ($c_{measured_t}$) and the concentration of the air used for refilling the headspace volume ($c_{refilled}$) weighed for their respective volumes (V_{HS}, headspace volume, V_{spl}, sample volume). For this, concentrations were converted from mixing ratios (ppm for CO_2, and ppb for CH_4 and N_2O) to a mass-per-volume basis using the ideal gas law ($PV=nRT$). Because pressure (P), temperature (T), and the ideal gas constant (R, 8.314 $JK^{-1}mol^{-1}$) are the same for both concentrations, they can be disregarded, leaving only the volume (V) and the number of atoms or mixing ratios (*n*). Therefore, the mixing ratios were converted to a mass-per-volume basis by multiplying with the atomic weights (AW) of C (12 g mol^{-1}) for CH_4 and CO_2, or 2*N (28 g mol^{-1}) for N_2O, and dividing by the ideal gas volume (V, 22.41 L mol^{-1}).

In the second step (Equation 23), the change in concentration due to the dilution (Δc_t) was calculated by subtracting the diluted concentration at timepoint t ($c_{diluted_t}$) from the measured concentration at timepoint t ($c_{measured_t}$). Finally (Equation 24), the change in concentration was considered for the gas concentration at the next timepoint, $t+1$ to give the corrected concentration ($c_{corrected_{t+1}}$) by adding the change in concentration (Δc_t) to the measured concentration at the next timepoint ($c_{measured_{t+1}}$).

Emissions were then computed from the change in concentrations with time using the measured concentration at the first time point (t_0 = 0 minutes, before any dilution steps had been conducted) and the corrected concentrations at the subsequent time points. For this, a linear regression approach was used as described by Butterbach-Bahl et al. (2011) to estimate the slope of headspace (gas concentration change over time). The slope was then used to calculate the flux rate following **Equation 25** for CO_2 and CH_4, and

Equation 26 for N_2O:

Equation 25 $$\boldsymbol{Gas\ flux\ (CO_2\ \&\ CH_4) = \frac{dConc}{dt} \times \frac{P}{1013} \times \frac{273}{T+273} \times \frac{12}{22.41} \times \frac{V_{HS}}{DM} \times 60}$$

Equation 26 $$\boldsymbol{Gas\ flux\ (N_2O) = \frac{dConc}{dt} \times \frac{P}{1013} \times \frac{273}{T+273} \times \frac{28}{22.41} \times \frac{V_{HS}}{DM} \times 60}$$

where $dConc/dt$ is the change in gas concentration over time (slope, ppm min^{-1}), P is the air pressure (hPa), 1013 is the sea level air pressure (hPa), T is the incubation chamber temperature(°C), 22.41 is the ideal gas volume (L), 12 and 28 are the molecular weight of one atom of C and 2 atoms of N, respectively, V_{HS} is the headspace volume of the glass jar (ml), V_{HS} was obtained by subtracting the volume of the jar from the volume occupied by the manure. The volume occupied by manure was determined from the measured density and mass of the manure in the jar, where volume is equivalent to mass over density. DM is the manure dry matter (g) and 60 is the conversion factor from minutes to hours. Units of gas fluxes calculated were mg CH_4-C g^{-1} DM h^{-1}, mg CO_2-C g^{-1} DM h^{-1}, and µg N_2O-N g^{-1} DM h^{-1}.

For quality check, the R^2 of the calculated slopes were considered; where CO_2 slopes with $R^2 < 0.9$ and CH_4 and N_2O slopes with $R^2 < 0.7$ were considered not valid. The limit of detection (LoD) of the GC was also calculated based on Parkin et al. (2012), and fluxes below the LOD were set at 0.001. The gas fluxes were used to calculate cumulative GHG emission or uptake for the 84 days of storage using trapezoidal interpolation and finally expressed per 100 g of stored manure.

CHAPTER FOUR

RESULTS

4.1 Nutritive value of Brachiaria hay based diets supplemented with either dairy cubes or *Desmodium intortum*

4.1.1 Chemical Composition of the Feed Ingredients

As shown in **Table 3,** results indicate the content of dry matter (DM), crude protein (CP), crude fat (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin (ADL), and ash of the feed samples that were analyzed. From the data, it is evident that dairy cubes (17.7 ± 0.06 % DM) and Desmodium (14.9 ± 0.41 % DM) had a higher percentage of CP compared to Brachiaria (5.09 ± 0.07 % DM). On the other hand, Brachiaria had a higher cellulose content (34.0 ± 0.24 % DM) compared to Desmodium (32.7 ± 0.24 % DM) and dairy cubes (12.3 ± 0.10 % DM). Conversely, Desmodium had a higher lignin content (18.6 ± 0.02 % DM) compared to Brachiaria (4.18 ± 0.02 % DM) and dairy cubes (3.60 ± 0.02 % DM).

Table 3. Nutrient composition of feed ingredients

	Feed ingredients		
	Brachiaria	Dairy cubes	Desmodium
DM (%)	95.3 ± 0.02	93.5 ± 0.17	94.6 ± 0.28
CP (% DM)	5.09 ± 0.07	17.7 ± 0.06	14.9 ± 0.41
CF (% DM)	2.80 ± 0.03	21.3 ± 0.48	7.62 ± 0.10
NDF (% DM)	69.2 ± 0.01	42.4 ± 1.03	67.5 ± 1.27
ADF (% DM)	38.2 ± 0.25	15.9 ± 0.11	51.2 ± 0.37
[1]Hemicellulose (% DM)	31.0 ± 0.25	26.5 ± 1.14	16.3 ± 0.89
[2]Cellulose (% DM)	34.0 ± 0.24	12.3 ± 0.10	32.7 ± 0.24
ADL (% DM)	4.18 ± 0.02	3.60 ± 0.02	18.6 ± 0.02
ASH (%)	10.8 ± 0.09	7.65 ± 0.14	6.46 ± 0.14

[1] Calculated as NDF – ADF, [2] ADF – ADL. DM= dry matter, CP= crude protein, CF= crude fat, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin. Values are mean ± standard deviation for the analyzed duplicates.

The feed ingredients mentioned above were used to formulate three experimental diets for the study. Brachiaria was used as the control and basal diet, which was supplemented with either dairy cubes or Desmodium as protein sources. The basal diet (Brachiaria) used in this experiment had lower CP than expected, so urea was added to the diets as an N supplement, and molasses was included as an energy supplement. The proportions of the feed ingredients allocated to each experimental diet are presented in

Table 4 below.

Table 4. Proportions of feed ingredients allocated to the experimental diets

	D_1	**D_2**	**D_3**
Feed ingredients	**Control**	**D_1 + Dairy cubes**	**D_1 + *D. intortum***
Basal diet (*B. brizanta xaraes* hay)	87 %	65.6 %	35 %
N supplement - urea	0.8 %	0.8 %	0.8 %
Energy supplement - molasses	12.2 %	8.5 %	6.4 %
Experimental supplements			
Chopped *Desmodium intortum* hay	0 %	0 %	57.7%
Dairy cubes	0 %	25%	0 %

4.1.2 Chemical composition of experimental diets

The nutrient composition of the experimental diets is presented in Table **5** below. The dry matter (DM) was found to be similar for all diets and ranged between 94.2 ± 0.26 and 94.6 ± 0.02%. As expected, the diets that were supplemented with protein had a higher crude protein (CP) content. Diet 2 (D_2) had a CP content of 12.2 ± 0.30% DM, and Diet 3 (D_3) had a CP content of 11.3 ± 0.48% DM, while Diet 1 (D_1) had a CP content of 10.1 ± 0.36% DM. The cellulose content was found to be higher in D_3 (39.5 ± 0.25% DM) as compared to D_1 (36.8 ± 0.25% DM) and D_2 (36.0 ± 0.01% DM). Additionally, the lignin content was higher in D_3 (10.2 ± 0.03% DM) as compared to D_2 (3.98 ± 0.01% DM) and D_1 (3.17 ± 0.00% DM).

Table 5. Nutrient composition of experimental diets

	Experimental diets		
Nutrient (%DM)	**D_1 (Control)**	**D_2 (D_1+ Dairy cubes)**	**D_3 (D_1+ Desmodium)**
DM (%)	94.2 ± 0.26	94.5 ± 0.21	94.6 ± 0.02
CP	10.1 ± 0.36	12.2 ± 0.30	11.3 ± 0.48
CF	3.68 ± 0.46	12.9 ± 0.12	8.11 ± 0.42
NDF	64.0 ± 0.03	64.7 ± 0.44	68.7 ± 0.15
ADF	39.9 ± 0.24	40.0 ± 0.02	49.7 ± 0.17
Hemicellulose	24.0 ± 0.21	24.8 ± 0.42	19.0 ± 0.31
Cellulose	36.8 ± 0.25	36.0 ± 0.01	39.5 ± 0.25
ADL	3.17 ± 0.00	3.98 ± 0.01	10.2 ± 0.03
ASH	9.76 ± 0.03	9.57 ± 0.06	8.18 ± 0.03

DM= dry matter, CP= crude protein, CF= crude fat, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin. Values are mean ± standard deviation for the analyzed duplicates.

4.1.3 Phenol content of experimental diets

According to **Table 6**, the total tannin content (TT) differed among the diets. D_3 had a higher content (2.16 ± 0.12%) than D_1 (1.77 ± 0.06%) and D_2 (1.71 ± 0.03%). Condensed tannins also varied and were higher in D_3 (0.42 ± 0.04%) compared to D_1 (0.27 ± 0.00%) and D_2 (0.23 ± 0.04%)

Table 6. Phenol content of experimental diets

	Experimental diets			ANOVA	
Phenol type (%)	**D_1 (Control)**	**D_2 (D_1+ Dairy cubes)**	**D_3 (D_1+ Desmodium)**	**P**	**R^2**
TT	1.77 ± 0.06^{b}	1.71 ± 0.03^{b}	2.16 ± 0.12^{a}	**0.0533**	0.86
CT	0.27 ± 0.00^{b}	0.23 ± 0.04^{b}	0.42 ± 0.04^{a}	**0.051**	0.86

TT= total tannins, CT= condensed tannins. Values are mean ± SE, (n=2). Different superscripts within a row indicate significant differences ($P < 0.05$).

4.1.4 *In vitro* dry matter degradability of the feed ingredients and experimental diets

According to **Table 7**, the gas production was higher in dairy cubes (51.9 ml) in comparison with Brachiaria (40.9 ml) and Desmodium (28.5 ml). In the case of experimental diets, the gas production was comparatively lower in D_3 (34.4 ml) as compared to D_2 (47.6 ml) and D_1 (47.8 ml).

Table 7. *In-vitro* gas production and characteristic parameters of feed ingredients and experimental diets

	Cumulative gas (ml) produced at				**Parameters of gas production**			
	12 h	**24 h**	**48 h**	**96 h**	**A**	**b**	**a+b**	**c**
Feed ingredients								
Brachiaria	14.2	23.6	31.0	40.9	1.3	39.4	40.8	0.03
Dairy cubes	18.7	32.1	41.7	51.9	0.5	50.7	51.2	0.04
Desmodium	9.51	16.4	22.7	28.5	1.0	28.6	29.7	0.03
Experimental diets								
D_1 (Control)	19.6	30.8	38.2	47.8	1.5	43.9	45.4	0.04
D_2 (D_1+Dairy cubes)	21.2	30.7	38.1	47.6	3.9	41.5	45.4	0.04
D_3 (D_1+ Desmodium)	12.7	20.6	27.0	34.4	0.9	32.9	33.8	0.04

a, b, c are constants in the equation (Ørskov and McDonld, 1989); a = gas production (ml) from readily soluble fraction, b = gas production (ml) from insoluble but degradable fraction, (a+b) = potential gas production, c = gas production rate. Values are mean.

4.1.5 Short chain fatty acids, metabolizable energy, and organic matter digestibility by *In vitro* gas production

As shown in **Table 8**, dairy cubes had higher levels of short-chain fatty acids (SCFA) (0.92 mmol/200mg DM) compared with Brachiaria (0.68 mmol/200mg DM) and Desmodium (0.50 mmol/200mg DM). Additionally, dairy cubes had a higher organic matter digestibility (OMD) (62.7 %) compared to Brachiaria (51.4 %) and Desmodium (47.0 %). The experimental diets also showed that SCFA was higher in both D_1 and D_2 (0.84 mmol/200mg DM) compared to D_3 (0.59 mmol/200mg DM). Similarly, OMD was higher in D_2 (60.8 %) and D_1 (60.0 %) compared with D_3 (49.8 %).

Table 8. Short chain fatty acids, metabolizable energy, and organic matter digestibility of feed ingredients by *In vitro* degradability

	SCFA (mmol/200mg DM)	**ME MJ/Kg DM**	**OMD% 48 h**
Feed Ingredients			
Brachiaria	0.68	6.92	51.4
Dairy cubes	0.92	13.1	62.7
Desmodium	0.50	7.99	47.0
Experimental diets			
D_1 (Control)	0.84	8.35	60.0
D_2 (D_1+ Dairy cubes)	0.84	12.80	60.8
D_3 (D_1+ Desmodium)	0.59	8.38	49.8

4.1.6 Total apparent digestibility of dry matter, nitrogen, and fibre of the experimental diets

The data presented in **Table 9** shows that there was no significant difference in DM digestibility among the experimental diets, which ranged from 55.2 ± 2.86 to 61.8 ± 1.83%. However, there was a significant difference in nitrogen digestibility, with D_2 (67.9 ± 2.59%) and D_1 (66.6 ± 1.33%) having higher digestibilities compared to D_3 (55.3 ± 2.63%). Nitrogen utilization was also significantly different, with cows fed D_1 (38.1 ± 2.23%) having a higher utilization rate compared to those fed D_3 (15.8 ± 4.71%), cows fed D_2 (24.9 ± 5.86%) had a utilization rate in between. NDF digestibility was higher in cows fed D_2 (66.3 ± 1.68%) compared to those fed D_3 (59.9 ± 1.82%).

Table 9. Apparent digestibility of DM, N, and NDF of three experimental diets fed to cows

Item		**Experimental diets**			**ANOVA**	
		D_1(Control)	**D_2(D_1 + Dairy cubes)**	**D_3(D_1 + Desmodium)**	***P***	***R^2***
DM	**Intake, kg/d**	8.52 ± 0.53 ^{a}	10.6 ± 0.74 ^{a}	8.58 ± 0.79 ^{a}	0.1017	0.40
	Feces excretion kg/d	3.41 ± 0.17 ^{a}	4.05 ± 0.29 ^{a}	3.41 ± 0.15 ^{a}	0.1684	0.33
	Digestibility %	59.7 ± 1.15 ^{a}	61.8 ± 1.83 ^{a}	55.2 ± 2.86 ^{a}	0.1220	0.37
N	**Intake,g/day**	133.6 ± 8.58 ^{b}	203.1 ± 14.2 ^{a}	148.7 ± 13.6 ^{b}	**0.0077**	0.66
	Feces excretion, g/day	44.4 ± 2.04 ^{b}	64.2 ± 2.73 ^{a}	65.5 ± 2.67 ^{a}	**0.0003**	0.83
	Digestibility, %	66.6 ± 1.33 ^{a}	67.9 ± 2.59^a	55.3 ± 2.63^b	**0.0063**	0.68
	Urine excretion g/day	37.8 ± 2.76 ^{c}	86.0 ± 2.91 ^{a}	57.8 ± 2.72^b	**<.0001**	0.94
	Net utilization %^{1}	38.1 ± 2.23 ^{a}	24.9 ± 5.86 ^{ab}	15.8 ± 4.71^b	**0.0213**	0.57
	Intake, kg/d	5.45 ± 0.34 ^{a}	6.88 ± 0.48 ^{a}	5.90 ± 0.55 ^{a}	0.1339	0.36
NDF						
	Feces excretion kg/d	1.93 ± 0.09 ^{a}	2.32 ± 0.21 ^{a}	2.35 ± 0.16 ^{a}	0.1829	0.31
	Digestibility %	64.5 ± 1.00 ^{ab}	66.3 ± 1.68 ^{a}	59.9 ± 1.82 ^{b}	**0.0399**	0.51

^{1}Calculated as N intake - Fecal excreted N – Urine excreted N/N Intake *100,DM= dry matter, N= nitrogen, NDF= neutral detergent fibre. Values are mean ± SE, (n=4). $^{a, b, c}$ Within a row, least square means without a common superscript differ (P<0.05).

Effects of feeding dietary treatments (Brachiaria hay with dairy cubes or *Desmodium intortum* supplementation) on physico-chemical, fibre, and tannin contents of manure

This objective determined excreta properties (feces, urine, and their mixture), specifically N, C, C: N ratio, fibre, and CT content, as a result of feeding three experimental diets. For manure, initial properties of freshly excreted manure as well as changes over time until the end of the incubation period of 12 weeks were determined.

4.2.1 Effect of feeding experimental diets on nitrogen (N) excretion and fecal chemical composition

Cows fed on the D_2 diet had higher N excretion rates than those fed D_1. Specifically, the urinary N increased by 46% and 49% compared with the D_1 and D_3, respectively. Consequently, the urinary N to fecal N ratio was higher ($P<0.001$) for cows fed the D_2 compared with the D_1 and D_3.

When expressed as a proportion of the total N excreted, the amount of N in cow's feces was 12% lower for those fed the D_2 diet and 16% higher for those fed the D_3 compared with those fed D_1. Conversely, urinary N was 12% higher for cows fed the D_2 and 23% lower for cows fed D_3 compared with D_1, (**Table 10)**.

Table 10. Nitrogen (N) excretions of dairy cows fed three experimental diets

Parameter	Experimental diet			
	D_1 (Control)	**D_2 (D_1+ Dairy cubes)**	**D_3 (D_1+Desmodium)**	***P*-value**
Excreta Output				
Urine (kg day $^{-1}$)	10.9 ± 0.26^{a}	12.2 ± 0.80^{a}	7.58 ± 0.21^{b}	<0.001
Feces (kg DM day $^{-1}$)	3.28 ± 0.14^{a}	3.87 ± 0.28^{a}	3.62 ± 0.17^{a}	0.191
Nitrogen excretion (g day $^{-1}$)				
Feces	42.7 ± 1.90^{b}	61.2 ± 2.62^{a}	62.5 ± 2.55^{a}	<0.001
Urine	35.9 ± 0.87^{b}	66.9 ± 4.38^{a}	34.1 ± 0.93^{b}	<0.001
Total N (Fecal + Urine)	78.6 ± 1.69^{c}	128 ± 6.93^{a}	96.6 ± 3.03	<0.001
Urinary N: Fecal N	0.85 ± 0.05^{b}	1.09 ± 0.03^{a}	0.55 ± 0.02^{c}	<0.001
N, % of total N excretions				
Feces	54.3 ± 1.45^{b}	47.9 ± 0.67^{c}	64.7 ± 0.88^{a}	<0.001
Urine	45.7 ± 1.45^{b}	52.0 ± 0.67^{a}	35.3 ± 0.88^{c}	<0.001

DM= dry matter, N= nitrogen. Values are mean ± SE, (n=4). $^{a, b, c}$ Within a row, least square means without a common superscript differ (P<0.05).

This study found that feces from cows fed with D_3 had 4% higher total carbon (C) concentrations compared with those on D_2 and D_1 (**Table 11**). Additionally, the lignin content in the feces of cows fed with D_3 was 71% higher (P<0.001) than those fed with D_2 and D_1. The feces of cows fed D_3 also contained 89% more condensed tannins (CTs) than those fed D_2.

Table 11. Water, carbon, C: N, condensed tannin, and fibre content of feces excreted by dairy cows fed three experimental diets

Parameter	Experimental diet D_1 (Control)	D_2 (D_1+ Dairy cubes)	D_3 (D_1+ Desmodium)	*P* -value
Water content (%)	83.2 ± 0.38^a	82.7±0.48^a	.6±0.61^a	0.126
Total Carbon (% DM)	42.1 ± 0.11^b	41.9± 0.21^b	.0 ± 0.24^a	<0.001
Carbon: Nitrogen	31.2 ± 0.51^a	25.3 ± 1.41^b	.3 ± 0.35^b	0.001
Hemicellulose (% DM)	24.3 ± 0.61^b	26.4 ± 0.51^a	.7 ± 0.31^c	<0.001
Cellulose (% DM)	28.6 ± 0.87^a	26.1 ± 0.77^a	.2 ± 0.74^a	0.055
Lignin (% DM)	3.53 ± 0.17^b	4.57 ± 0.06^b	.9 ± 0.62^a	<0.001
Condensed Tannins (% DM)	^{BLOD}	0.09 ± 0.06^b	32± 0.07^a	<0.001

$_{BLOD}$= below limit of detection. Values are mean ± SE, (n=4). $^{a, b, c}$ Within a row, least square means without a common superscript differ (*P*<0.05).

4.2.2 Correlation between diet composition and excreta composition

The data in Table 12 shows that there was a strong positive correlation between excreta (fecal) DM and diet DM (r = 0.8, P = 0.003), N (r = 0.8, P = 0.002), hemicellulose (r = 0.7, P = 0.018) and cellulose (r = 0.8, P = 0.002). In addition, there was a strong correlation between fecal N and diet lignin content (r = 0.7, P = 0.009), as well as diet N (r = 0.6, P = 0.039). Urine N was strongly related to diet N (r = 0.8, P = 0.001). Furthermore, for fibre, fecal hemicellulose was strongly correlated with diet hemicellulose (r = 0.9, P < 0.000), DM (r = 0.8, P = 0.003), N (r = 0.8, P = 0.003), and cellulose (r = 0.7, P = 0.007). Cellulose was strongly correlated with diet cellulose content (r = 0.7, P = 0.014) and DM (r = 0.6, P = 0.025), while fecal lignin was strongly related to diet lignin content (r = 0.98, P < 0.0001).

Table 13. Pearson correlation coefficients and *P values* between diet and excreta dry matter, nitrogen content, and fiber content

	Diet composition				
Excreta composition	**DM**	**N**	**Hemicellulose**	**Cellulose**	**Lignin**
DM	0.78440	0.79715	0.66673	0.79591	0.33504
	0.0025	**0.0019**	**0.0179**	**0.0020**	0.2871
Fecal Nitrogen	0.46024	0.60019	0.26608	0.48377	0.71024
	0.1322	**0.0391**	0.4032	0.1110	**0.0096**
Urinary Nitrogen	0.62627	0.81366	0.61597	0.59722	0.16209
	0.0293	**0.0013**	**0.0330**	0.0403	0.6147
Total excreted N	0.61445	0.79910	0.53855	0.60176	0.36737
	0.0335	**0.0018**	0.0708	**0.0384**	0.2401
Hemicellulose	0.78041	0.77674	0.90830	0.73387	-0.45466
	0.0027	**0.0030**	**<.0001**	**0.0066**	0.1375
Cellulose	0.63881	0.57205	0.44007	0.68125	0.53887
	0.0254	0.0520	0.1522	**0.0147**	0.0706
Lignin	-0.07388	-0.03987	-0.37323	-0.00268	0.98476
	0.8195	0.9021	0.2321	0.9934	**<.0001**

DM=dry matter, N= nitrogen content.

4.2.3 Manure properties: dry matter, nitrogen, carbon, and condensed tannin concentrations during incubation

The manure dry matter (DM) of cows fed D_3 was initially 9.6% higher than those fed D_1 (**Table 14**). The DM concentrations decreased slightly during incubation. At the end of the 12-week incubation period, manure from cows fed D_3 still had higher DM (45.9 ± 1.49) compared with D_2 (42.1 ± 1.07) and D_1 (40.0 ± 0.70).

Initial manure total nitrogen (TN) was 31% and 34% higher ($P<0.001$) in manure from cows D_2 and D_3, respectively, compared with D_1. After 12 weeks of incubation, manure TN had decreased slightly to 0.94 ± 0.03 for D_3 and increased to 0.63 ± 0.01 for D2, as well as for D_1 (0.63 ± 0.01).

Initial manure total carbon (TC) was 9.91 and 15.1% higher ($P=0.004$) in manure from cows fed D_3 compared with D_2 and D_1. After 12 weeks of incubation, manure TC had decreased but was still higher for D_3 (19.8 ± 0.85), compared with D_2 (17.1 ± 0.33) and D_1 (16.1 ± 0.33).

Initial lignin content was 67% and 73% higher ($P<0.001$) in manure from cows fed D_3 compared with those fed D_2 and D_1. After the 12-week incubation period, the lignin content had decreased but was still higher ($P=0.004$) in incubated manure from cows fed D_3 (5.21 ± 0.33) compared with D_2 (1.31 ± 0.08) and D_1 (1.03 ± 0.04).

The initial manure condensed tannins (CTs) content was 89% higher ($P=0.008$) in manure from cows fed D_3 compared with D_2 and D_1. During the incubation period, CTs decreased over time, and at the end of incubation, 54% of CTs in manure from cows fed D_3 had been degraded.

Table 14. Average Dry matter, total nitrogen, carbon, lignin, and condensed tannins content of fresh manure and after incubation

	DM	**TN**	**TC**	**Lignin**	**CT**
	g jar^{-1}	g jar^{-1} DM	g jar^{-1} DM	g jar^{-1} DM	g jar^{-1} DM
Start (day 0)					
D_1: Control	44.5 ± 0.75^{b}	0.63 ± 0.01^{c}	18.0 ± 0.47^{b}	1.47 ± 0.05^{b}	0.00 ± 0.00^{b}
D_2: D_1 + Dairy cubes	46.2 ± 1.29^{ab}	0.83 ± 0.07^{b}	19.1 ± 0.57^{b}	1.80 ± 0.12^{b}	0.03 ± 0.02^{b}
D_3: D_1+ Desmodium	49.2 ± 1.22^{a}	0.96 ± 0.04^{a}	21.2 ± 0.76^{a}	5.41 ± 0.25^{a}	0.26 ± 0.08^{a}
End (Day 84)					
D_1: Control	40.0 ± 0.70^{b}	0.63 ± 0.01^{c}	16.1 ± 0.33^{b}	1.03 ± 0.04^{b}	0.00 ± 0.00^{b}
D_2: D_1 + Dairy cubes	42.1 ± 1.07^{b}	0.87 ± 0.09^{b}	17.1 ± 0.33^{b}	1.31 ± 0.08^{b}	0.00 ± 0.00^{b}
D_3: D_1+ Desmodium	45.0 ± 1.49^{a}	0.94 ± 0.03^{a}	19.8 ± 0.85^{a}	5.21 ± 0.33^{a}	0.12 ± 0.01^{a}

Values are mean ± SE, (n=4). $^{a, b, c}$ Within a row, least square means without a common superscript differ ($P<0.05$).

During incubation, both diet had a significant effect on the concentration of DM, and time had a significant effect on total nitrogen and lignin as shown in **Table 15**.

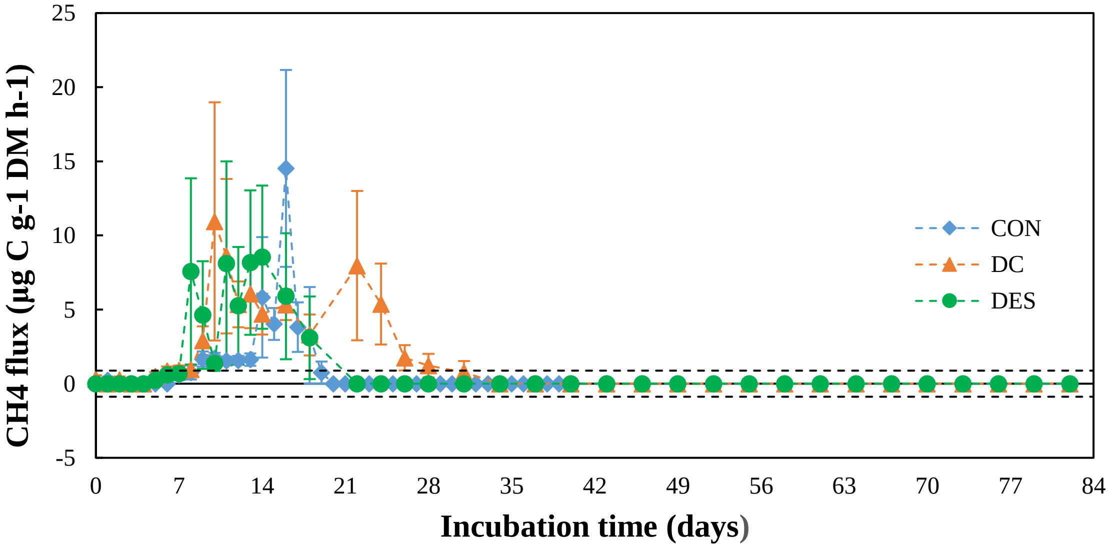
Table 15. Effect of time, diet, and their interactions on manure DM, N, C, and lignin during the 12 weeks of incubation (Repeated Measures)

Manure property	**Time (F, df, P-value)**	**Time*Diet (F, df, P-value)**	**Diet (F, df, P-value)**
DM	6.32, 2.27, 0.006	1.35, 4.54, 0.285	0.34, 2, 0.719
Total Nitrogen	0.77, 1.36, 0.435	0.44, 2.71, 0.711	6.39, 2, 0.019
Total Carbon	2.67, 1.35, 0.121	0.41, 2.70, 0.730	1.38, 2, 0.301
Lignin	1.24, 1.27, 0.304	0.42, 2.54, 0.714	158.3, 2, <.001

Manure CH_4 and N_2O emissions from dairy cattle fed Brachiaria hay supplemented with dairy cubes or *Desmodium intortum*

4.3.1 Methane emissions

Manure CH4 flux rates from manure for all experimental diets were initially below the limit of detection (LOD) of ± 0.88 in the first five days of incubation. Subsequently, CH_4 flux rates gradually increased and peaked between days eight and 16. For cows on the Desmodium diet, the peak was at 8.54 mg C g^{-1} DM h^{-1}, for cows on the dairy cube diet, it was at 10.9 mg C g^{-1} DM h^{-1}, and for cows on the CON diet, it was at 14.5 mg CH4-C g^{-1} DM h^{-1}. After this period, the emission rates declined and remained below the detection limit until the end of the incubation period as shown in (**Figure 2**).

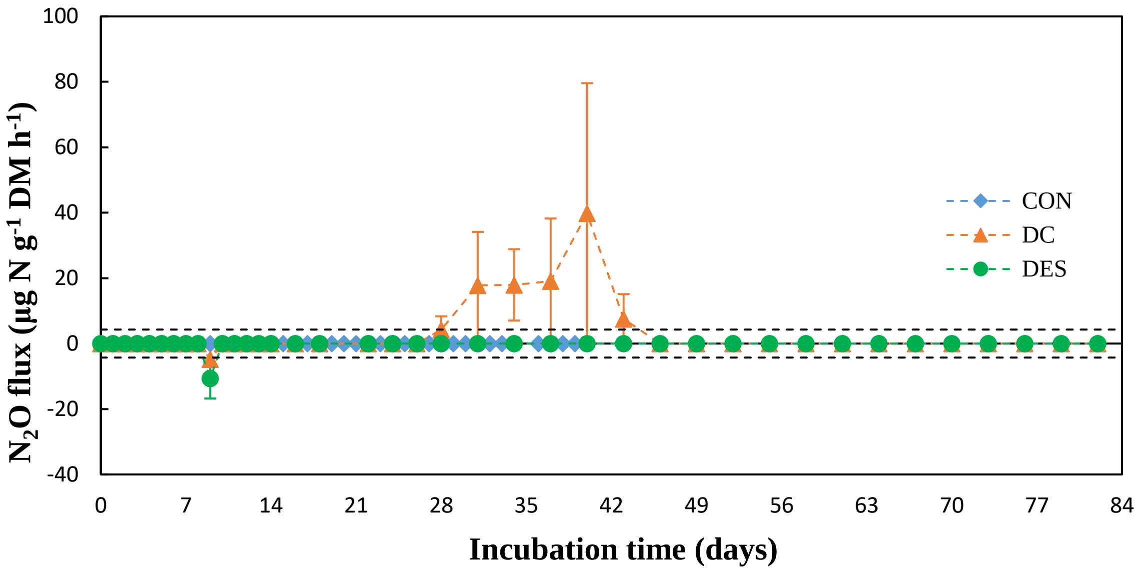


CON= Control, DC= Dairy cubes, DES=Desmodium, LOD= limit of detection. Flux values are mean ± SE, (n=4). Limit of Detection (= ±0.88).

Figure 2: Methane (CH_4) emissions during incubation of manure from cows fed three dietary treatments

4.3.2 Nitrous oxide emissions

The manure N_2O flux rates were found to be below the LOD of ± 4.28 in the incubated manure from cows fed with the control diet and Desmodium diet throughout the entire study period. In contrast, manure from cows fed with the dairy cubes diet showed N_2O emissions ranging between 17.8 – 39.8 µg N g^{-1} DM h^{-1} from day 24 until day 42 (week 6), with high variability between replicates as indicated by the large error bars.



CON= Control, DC= Dairy cubes, DES=Desmodium, LoD= limit of detection. Flux values are mean ± SE, (n=4). Limit of Detection (= ±4.28)

Figure 3: Nitrous oxide (N_2O) emissions during incubation of manure from cows fed three dietary treatments

There were no significant differences in manure CH_4 and N_2O cumulative emissions across the diets.

Table 16: Cumulative GHG emissions from incubated manure from cows fed three experimental diets

	Experimental diet			
	D_1 (Control)	**D_2 (D_1+ Dairy cubes)**	**D_3 (D_1+ Desmodium)**	***P*-value**
mg CH_4-C 100 g^{-1} stored manure for 12 weeks	35.1 ± 8.32^a	62.1 ± 33.0^a	49.1 ±13.7^a	0.6760
mg N_2O-N 100g^{-1} stored manure for 12 weeks	0.09 ± 0.00^a	-4.20 ± 2.51^a	195.2 ± 136.0^a	0.1780

CHAPTER FIVE
DISCUSSION

5.1 Digestibility of experimental diets

5.1.1 Chemical composition of feed ingredients and experimental diets

Chemical composition is an indication of the nutritive value of forages and feedstuffs. It involves measuring the amounts of DM, CP, and fibre content, with CP being a critical measure of the quality of forages and feeds (Speedy & FAO, 1991). In this study, Brachiaria grass had a lower CP content (4.85%) compared to another study by Nguku et al. (2016), where the CP of Brachiaria varied between 8.0 and 11.1% depending on the harvest period. This difference in CP content could attributed to late harvesting, which was done at a late bloom stage. The CP content of tropical forages tends to decrease rapidly as plant growth progresses due to the accumulation of cell wall carbohydrates (Van Soest, 1994). In contrast, dairy cubes had a CP of 16.6% because it is a commercial compounded protein supplement formulated to meet the nutritional requirements of dairy cows. Similarly, the legume *Desmodium intortum* had a lower CP (14.1%) compared to the 18.2% reported by another study (Ntakyo et al., 2020). The difference in CP content of the legume could also be attributed to late harvesting time, which leads to an increase in cell wall components and a reduction in CP contents (Gedamu et al., 2019).

Compared to the grass, the levels of CP in dairy cubes and *Desmodium intortum* indicate their potential as protein supplements for ruminants offered diets low in CP. To ensure a higher protein content, the right stage of harvesting should be considered for both Brachiaria and Desmodium. Regarding the diet mixtures, as expected the diets supplemented with dairy cubes (D_2) and Desmodium (D_3) had a higher CP (12.2 and 11.3 % DM) compared to the control diet (D_1) with no protein supplement (10.1 % DM).

Another important measure of forage and feed quality is the fibre content. Feed quality decreases as fibre content increases, but it is also an important dietary component for ruminants as some level of fibre content is required for normal rumen functioning (Van Soest et al., 1991). Fibre fractions measured typically include NDF, ADF, and ADL. In this study, for Brachiaria the NDF (65.9%), ADF (36.4%), and lignin (3.98%) were within the ranges reported in a study by Nguku et al. (2016). In Desmodium, NDF (63.9%) and ADF (48.5% were higher in this study compared to values reported by Ntakyo et al. (2020).

5.1.2 In-vitro digestibility of feed ingredients and experimental diets

The technique used in this study measures the volume of gas produced as a result of fermentation. When feedstuffs are mixed with buffered rumen fluid in vitro, the organic matter is fermented into SCFA and gases, mainly CO_2 and CH_4 (Getachew et al., 1998). The differences in gas production among feed ingredients and experimental diets are due to differences in the amount of degradable substrate and soluble fractions being fermented.

In this study, diet with no supplement (D_1) and diet supplemented with dairy cubes (D_2) had higher gas production compared to the diet supplemented with Desmodium (D_3). The low gas production in the Desmodium diet could be due to the higher fibre content and the presence of tannins. Fiber and tannin concentrations are strongly inversely related to gas production (Debela et al., 2011). Tannins can bind with dietary proteins and carbohydrates making them unavailable for degradation (Getachew et al., 2002).

Regarding apparent digestibility, it was found that the diet supplemented with Desmodium had lower DM, N, and NDF digestibility compared to the diet supplemented with dairy cubes and the control diet. This could be explained by the high lignin and tannin content of the Desmodium. High lignin contents lower the digestibility of a diet (Rasby & Anderson, 2008) and tannins can reduce fibre digestion by directly inhibiting cellulolytic microorganisms and preventing microbial digestion as a result of forming complexes with lignocellulose (McSweeney et al., 2001). Moreover, tannins form complexes with protein (Van Soest, 1994), thus reducing the availability of N (Rufino et al., 2006) and lowering N digestibility.

In summary, the results indicate that the diet with dairy cube supplement had higher nutritional value and digestibility compared to Desmodium supplemented diet. However, this is largely due to late harvesting and losses during transportation where a lot of leafy parts were lost. Therefore, since dairy cubes are expensive and not affordable to many smallholder dairy farmers, the use of Desmodium is highly recommended as it is cheaper to grow. To ensure its benefits as a supplement, harvesting time and postharvest management practices should be considered.

5.1.3 Effect of experimental diets on excreta and manure properties

In this study, there were strong positive correlations between excreta composition and diet composition. This is logical because during digestion, nutrients that are ingested by the animal but are not digested and absorbed, or are absorbed but not utilized, are excreted (Sutton & Lander, 2003; Teenstra et al., 2015). Therefore, the composite nutrients ingested by an animal will be reflected in the excreta. In this study, there was a strong correlation between fecal DM and diet DM, N, hemicellulose, and cellulose. Similar findings were observed in a different study by Wassie et al. (2019), where steers were fed diets at 100%, 80%, 60%, and 40% of their metabolisable energy requirement for maintenance, fecal DM was strongly correlated to DM intake ($r = 0.97$; $p < 0.001$). In another study by Nennich et al. (2005), where regression equations were developed to predict manure DM and nutrient excretion, DM intake was directly related to DM excreted.

The strong correlation between diet fibre (hemicellulose and cellulose component) and excreta DM in this study could be attributed to the strong relationship between diet NDF (hemicellulose + cellulose + lignin) and voluntary DM intake (DMI).

Nitrogen excretion in dairy cows is directly related to and predictable from N intake and DM intake (Nennich et al., 2005; Tomlinson et al., 1996; Vérité & Delaby, 2000). The concentration of N in cattle feces is correlated with the digestibility and concentration of N in the feed (FAO, 2010). In this study, fecal N was strongly correlated to diet N and lignin. The correlation of fecal N with diet ADL could be attributed to the effect of lignin on diet digestibility, a high lignin content reduces diet digestibility (Rasby & Anderson, 2008), and therefore more nutrients are excreted. For urinary N, both protein concentration and degradability of the diet influence the amount excreted (Wattiaux&Karg, 2004). In this study, urinary N was highly correlated to diet N, DM, and hemicellulose, indicating that an increase in N, DM, and hemicellulose intake increased urinary N excretion. In the model prediction studies by Nennich et al. (2005) and Tomlinson et al. (1996) on dietary effects on manure nutrient excretion, urinary N could be accurately predicted with good estimates of diet N and DM content in addition to animal body weight.

In this study, feces and manure from cows supplemented with Desmodium had a higher CT content compared to cows supplemented with dairy cubes and those fed the control diet. This was expected because the CT contained in Desmodium cannot be completely degraded by digestive enzymes (Frutos et al., 2004), resulting in excretion through both feces and urine. However, not all CT in the diet are usually recovered in the excreta (Patra & Saxena, 2011). The detergent action of bile salts and a pH >8 in the duodenum, dissociates bound CT into free CT, which undergoes conformational changes during lower intestinal passage, and the flavonoid ring structure is microbially degraded, this makes it undetectable by current analytical techniques (Perez-Maldonado & Norton, 1996).

In the present study, after excretion CTs were degraded during the manure incubation, likely due to anaerobic and/or aerobic microbial decomposition. These bacterial strains have been isolated and are capable of degrading condensed tannins both aerobically and anaerobically, as described by Bhat et al. (1998). In a study by Tahmourespour et al. (2017), tannin-degrading bacterial strains isolated from goat feces after grazing on tannin-rich diets were able to degrade fecal tannins during laboratory incubation. In the present study, we did not measure the microbial community in the manure, therefore we can only speculate about the mechanisms and agents of tannin decomposition during our lab incubation.

As expected, due to increased N intake, the excreted N was higher in excreta from cows receiving protein supplementation with dairy cubes and Desmodium. However, although both diets were supplemented at the same CP level, cows fed dairy cubes supplemented diet had higher urinary N excretion compared to cows receiving Desmodium supplemented diet. The lower urinary N from cows fed Desmodium can be attributed to the reduction of ruminal protein degradation due to the presence of CTs in the diet. In a study by Kariuki et al. (2001), cattle supplemented with *Desmodium intortum* had low levels of rumen ammonia-N, indicating low protein degradation in the rumen. It is known that CTs reduce the degradability of protein in the rumen, thereby increasing the fraction of protein available for digestion in the small intestine, and this shift in the site of protein digestion results in reduced urinary-N excretion and a shift towards fecal-N (Mueller-Harvey, 2006).

In this study, the manure N concentration decreased over time during the incubation. Some of this might be attributed to losses via ammonia volatilization, which is the largest pathway of manure N loss during the first week of manure storage (Lee et al., 2011) but was not measured in this study. Other pathways of gaseous N loss are via nitrification resulting in NO_x emissions, and via denitrification resulting in dinitrogen (N_2) and N_2O emissions (Montes et al., 2013). Only N_2O emissions were measured in this study as the focus was on GHG emissions, and the other gases would have required a different analytical setup that was unavailable. Because the laboratory incubation was conducted in glass jars, no leaching losses of manure N were expected.

Carbon content was higher in feces and manure from cows supplemented with Desmodium compared with cows supplemented with dairy cubes and with no supplement (control diet). This could be explained by the low apparent DM and NDF digestibility for Desmodium supplemented diet as a result of high lignin and CT content. In the present study, during manure incubation, C content decreased over time due to gaseous losses through CO_2 and CH_4 emissions (Shan et al., 2019). During incubation, the marked reduction in concentrations of manure properties and the significant effect of time could be attributed to biochemical processes that lead to continuous changes in manure quantity and quality (Li et al., 2012).

5.1.4 Effect of Manure Properties on CH_4 and N_2O Emissions during Incubation

On methane emissions, in contrast to our expectation, we found that CH_4 emissions were below LOD for the first five days of incubation. Fresh manure contains metabolically active methanogens from the hindgut and is high in degradable organic matter and moisture content, theoretically creating anaerobic conditions that favour the production of CH_4 (Hindrichsen et al., 2005). Consequently, others have found that CH_4 fluxes were highest immediately after manure excretion and decreased as the manure dried out (Leitner et al., 2021; Zhu et al., 2021). It is possible that in our study, methanogens may have been disturbed by the manure preparation (mixing of dung for homogenization, stirring to mix urine and dung) for the incubation setup, for example by mixing air into the manure during sample preparation, which suppressed methanogen activity.

At the end of the first week, CH_4 emissions gradually increased and remained elevated until the end of the third week, after which they returned to levels <LOD until the end of the incubation. This could be explained by the drying of the manure over time, as well as the formation of a crust, which has the potential to consume and significantly reduce CH_4 emissions during storage via bacterial CH_4 oxidation (Ambus & Petersen, 2005; Nielsen et al., 2013). In this study, crust formation was observed after 18 days, and after 49 days (7 weeks) the manure in the jars had dried out completely (i.e., no further moisture loss was observed).

During the manure incubation period, we found that most of the N_2O fluxes were below the LOD, except for a short period of N_2O emissions in the dairy cube supplemented diet. For N_2O emissions to occur, several conditions must be met: the presence of enough NO_3^- for denitrification, presence of labile C for heterotrophic denitrifiers, and the right moisture content (i.e., moderately moist but not too dry nor too wet) to give the right proportion of aerobic versus anaerobic microsites (Butterbach-Bahl et al., 2013). In fresh manure, most N is present as organic N, which first needs to be broken down into NH_4^+ and then nitrified to NO_3^-. Consequently, several studies have reported a time lag of N_2O emissions of several days (Petersen et al., 2004; Zhu et al., 2021) to weeks (Leitner et al., 2021) after the start of manure incubation, similar to what we have found in this study (emission window from day 28 to 42). Furthermore, the manure C:N ratio in the present study (24.3-31.2) was relatively high compared to other studies, which have simulated a more “Western” diet (e.g. C:N = 23.8 in

Chadwick (2005); C:N = 20.6 in Parkinson (2004). Therefore, it seems plausible that in the present study, manure N-content was limiting denitrification in the control diet (C: N = 31.2 ± 0.5) whereas sufficient N was present for N_2O production in the dairy cube supplemented treatment (C: N = 25.3 ± 1.4). In addition, there might have been an additional effect of CTs on N_2O emissions, which might explain the fact that we did not see N_2O emissions in the Desmodium supplemented treatment (C: N = 24.3 ± 0.4), even though the C: N ratio was similar compared to the dairy cube treatment. As mentioned earlier, the Desmodium supplemented diet led to a shift from urinary-N to faecal-N excretion. Previous studies in Kenya have found that urinary-N is more labile and promotes N_2O emissions from cattle urine patches, whereas dung patches led to lower or negligible N_2O emissions (Zhu et al., 2021, 2021a). In addition, the DES diet likely led to the formation of CT-protein complexes, which has been shown to inhibit microbial decomposition (Min et al., 2022) and potentially led to a slower release of mineral N in the Desmodium supplemented diets, further suppressing N_2O emissions. Finally, the manure incubation simulated aerobic decomposition, as no water was added during incubation to replace water lost through evaporation, and manure dried out over time. Consequently, the conditions in the manure might have been too dry for denitrification (Lee et al., 2011; Montes et al., 2013).

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i. The diet supplemented with dairy cubes and control had higher organic matter digestibility compared to the diet supplemented with Desmodium.

ii. Diets supplemented with dairy cubes and Demodium resulted in higher N excretion compared to the control diet with no supplement. Although both diets were formulated to have the same CP content, urinary N was higher for dairy cube than for Desmodium supplementation

iii. Manure CH_4 and N_2O emissions were lower for incubated manure from cows supplemented with Desmodium compared to dairy cubes, albeit not significantly due to large variability between experimental animals.

6.2 Recommendations

i. The use of improved forage such as Brachiaria grass can be adopted by dairy farmers due to the high organic matter digestibility, but care should be taken to ensure the grass is harvested before late bloom stage for higher CP contents. Desmodium can be used as a protein supplement but similarly, harvesting time should be considered for higher CP, lower fibre contents, and higher digestibility.

ii. Protein supplements influence N excretion, to lower N excreted and lost, the use of *Desmodium intortum* can be adopted by dairy farmers as it lowers urinary N, which is the N fraction that is most easily lost from the manure.

iii. *Desmodium intortum* (leguminous forage) can be used as a protein supplement in dairy diets to lower manure CH_4 and N_2O emissions.

6.3 Further Research

More studies on the effect of diets and animal breed on manure properties and manure GHG emissions in Kenya should be done for conclusive information and if possible quantification of all gases including ammonia. Care should be taken to either increase the number of experimental animals or to mix and homogenize the manure from all treatments to account for variability between individual animals. Given that this was a laboratory incubation, only limited conclusions on absolute GHG emissions can be drawn. Studies that investigate GHG emissions on a larger scale, e.g. from manure stockpiles or slurry tanks are needed to inform on absolute GHG emissions and derive emission factors that are representative of African smallholder dairy systems.

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APPENDICES

8.1 Appendix 1: Statistical analysis output

Analysis of variance
The GLM Procedure
Dependent Variable: Diet Total phenol

```
                                   Sum of
Source              DF         Squares     Mean Square    F Value    Pr> F
Model                2      0.12093333     0.06046667       4.03    0.1412
Error               3      0.04500000     0.01500000
Corrected Total     5      0.16593333

               R-Square     Coeff Var      Root MSE       tp Mean
               0.728807      5.609518      0.122474      2.183333

     Source                DF      Type I SS     Mean Square    F Value    Pr> F
        diet                 2      0.12093333      0.06046667       4.03    0.1412

Tukey Grouping         Mean       N    diet

                       A               2.3800      2    3
                       A               2.1200      2    1
                       A               2.0500      2    2
```

The GLM Procedure
Dependent Variable: Total tannins

```
                                   Sum of
Source              DF         Squares     Mean Square    F Value    Pr> F
Model               2      0.23663333     0.11831667       9.09    0.0533
Error               3      0.03905000     0.01301667
Corrected Total     5      0.27568333

               R-Square     Coeff Var      Root MSE       tt Mean
               0.858352      6.063274      0.114091      1.881667
     Source                DF      Type I SS     Mean Square    F Value    Pr> F
     diet                   2      0.23663333      0.11831667       9.09    0.0533

Tukey Grouping      Mean       N    diet
                       A       2.1600      2    3
                       A       1.7750      2    1
                       A       1.7100      2    2
```

The GLM Procedure
Dependent Variable: Condensed tannins

```
Sum of
Source              DF         Squares     Mean Square    F Value    Pr> F
Model               2      0.04013333     0.02006667       9.41    0.0510
Error               3      0.00640000     0.00213333
Corrected Total     5      0.04653333
```

```
                    R-Square    Coeff Var     Root MSE      ct Mean
                    0.862464     15.06131     0.046188     0.306667

   Source                DF      Type I SS     Mean Square   F Value   Pr> F
    diet                  2     0.04013333      0.02006667      9.41   0.0510

Tukey Grouping       Mean      N   diet
                      A      0.42000     2   3
                      A      0.27000     2   1
                      A      0.23000     2   2
```

The GLM Procedure
Dependent Variable: Dry Matter Digestibility

```
                                  Sum of
Source                 DF        Squares    Mean Square   F Value   Pr> F
Model                   2     91.9980667     45.9990333      2.68   0.1220
Error                   9    154.3380250     17.1486694
Corrected Total        11    246.3360917
                    R-Square    Coeff Var     Root MSE     DMD Mean
                    0.373466     7.030821     4.141095     58.89917

   Source                DF      Type I SS     Mean Square   F Value   Pr> F
   DIET                   2    91.99806667     45.99903333      2.68   0.1220

 Tukey Grouping      Mean      N   DIET
                      A       61.793     4   2
                      A       59.738     4   1
                      A       55.168     4   3
```

The GLM Procedure
Dependent Variable: Nitrogen Digestibility

```
                                  Sum of
Source                 DF        Squares    Mean Square   F Value   Pr> F
 Model                   2    384.7748167    192.3874083      9.37   0.0063

 Error                  9    184.8662750     20.5406972

 Corrected Total        11    569.6410917


                    R-Square    Coeff Var     Root MSE      ND Mean

                    0.675469     7.163150     4.532185     63.27083

Source                 DF      Type I SS     Mean Square    F Value   Pr> F

DIET            2    384.7748167    192.3874083      9.37   0.0063

              Tukey Grouping  Mean      N   DIET
```

```
A        67.945      4    2
                              A        66.565      4    1
                              B        55.303      4    3
```

The GLM Procedure
Dependent Variable: Nitrogen Utilization

```
                                     Sum of
Source                 DF         Squares     Mean Square    F Value    Pr> F
Model                   2     998.674817      499.337408       6.09    0.0213
Error                  9     738.362850      82.040317
Corrected Total       11     1737.037667

                  R-Square    Coeff Var      Root MSE       NU Mean
                  0.574930     34.48767      9.057611      26.26333

      Source                    DF       Type I SS     Mean Square    F Value    Pr> F
      DIET                       2     998.6748167     499.3374083       6.09    0.0213

          Tukey Grouping          Mean     N    DIET
                         A       38.063     4    1
B  A        24.883     4    2
                       B           15.845     4    3
```

The GLM Procedure
Dependent Variable: NDF Digestibility

```
                                     Sum of
Source                 DF         Squares     Mean Square    F Value    Pr> F
Model                   2     89.3558000      44.6779000       4.71    0.0399
Error                  9     85.4273000      9.4919222
Corrected Total       11     174.7831000

                  R-Square    Coeff Var      Root MSE     NDFD Mean
                  0.511238     4.846844      3.080896      63.56500

      Source                    DF       Type I SS     Mean Square    F Value    Pr> F
      DIET                       2     89.35580000     44.67790000       4.71    0.0399

     Tukey Grouping    Mean      N    DIET
                         A       66.340     4    2
B  A        64.500     4    1
                       B           59.855     4    3
```

Excreta properties ANOVA

The GLM Procedure
Dependent Variable: Dung Carbon

```
                                     Sum of
Source                 DF         Squares     Mean Square    F Value    Pr> F
Model                   2     804.9097167     402.4548583      21.22    0.0004
```

```
Error                  9    170.7044500     18.9671611
Corrected Total       11     975.6141667

                  R-Square    Coeff Var     Root MSE      C Mean
                  0.825029     1.066080     4.355130     408.5183

         Source               DF      Type I SS     Mean Square    F Value    Pr> F
         DIET                  2    804.9097167     402.4548583      21.22    0.0004

                 Tukey Grouping   Mean     N   DIET
                        A       419.758    4   3
                        B       405.323    4   1
                        B       400.475    4   2
```

The GLM Procedure
Dependent Variable: Dung Nitrogen

```
                                       Sum of
Source                 DF          Squares      Mean Square    F Value    Pr> F
Model                   2     38.73090417     19.36545208      16.18    0.0010
Error                  9    10.77151875      1.19683542
Corrected Total        11     49.50242292

                  R-Square    Coeff Var     Root MSE      DN Mean
                  0.782404     7.085682     1.094000     15.43958

         Source               DF      Type I SS     Mean Square    F Value    Pr> F
         DIET                  2    38.73090417     19.36545208      16.18    0.0010
                Tukey Grouping  Mean      N   DIET
                        A       17.3038    4   3
                        A       16.0025    4   2
                        B       13.0125    4   1
```

The GLM Procedure
Dependent Variable: Urine Nitrogen

```
                                 Sum of
Source                 DF          Squares      Mean Square    F Value    Pr> F
Model                   2     43.65851667     21.82925833      20.13    0.0005
Error                  9     9.75855000     1.08428333
Corrected Total       11     53.41706667

                  R-Square    Coeff Var     Root MSE      UN Mean
                  0.817314     16.88577     1.041289     6.166667

         Source               DF      Type I SS     Mean Square    F Value    Pr> F
         DIET                  2    43.65851667     21.82925833      20.13    0.0005

            Tukey Grouping       Mean      N   DIET
                        A        7.5725    4   3
                        A        7.4575    4   2
                        B        3.4700    4   1
```

The GLM Procedure
Dependent Variable: Dung Hemicellulose

```
                                 Sum of
Source              DF          Squares     Mean Square    F Value    Pr> F
Model                2      209.0767167     104.5383583     108.18    <.0001
Error                9        8.6969750       0.9663306
Corrected Total     11      217.7736917

              R-Square     Coeff Var      Root MSE      HEM Mean
              0.960064      4.376601      0.983021      22.46083
     Source              DF      Type I SS     Mean Square    F Value    Pr> F
     DIET                 2    209.0767167     104.5383583     108.18    <.0001

        Tukey Grouping      Mean      N    DIET
                     A      26.3675   4    2
                     B      24.3400   4    1
                     C      16.6750   4    3
```

The GLM Procedure
Dependent Variable: Dung Cellulose

```
                                 Sum of
Source              DF          Squares     Mean Square    F Value    Pr> F
Model                2      21.16295000     10.58147500       4.17   0.0523
Error                9      22.84025000      2.53780556
Corrected Total     11      44.00320000

              R-Square     Coeff Var      Root MSE     CELL Mean
              0.480941      5.695564      1.593049      27.97000

     Source              DF      Type I SS     Mean Square    F Value    Pr> F
     DIET                 2    21.16295000     10.58147500       4.17   0.0523

          Tukey Grouping   Mean      N    DIET
                     A      29.225   4    3
                     A      28.553   4    1
                     A      26.133   4    2
```

The GLM Procedure
Dependent Variable: Dung Lignin

```
                                 Sum of
Source              DF          Squares     Mean Square    F Value    Pr> F
Model                2      374.9556500     187.4778250     337.84    <.0001
Error                9        4.9943750       0.5549306
Corrected Total     11      379.9500250
              R-Square     Coeff Var      Root MSE     LIGN Mean
              0.986855      9.326280      0.744937      7.987500

     Source              DF      Type I SS     Mean Square    F Value    Pr> F
     DIET                 2    374.9556500     187.4778250     337.84    <.0001
```

```
              Tukey Grouping    Mean      N    DIET
                         A      15.8700      4    3
                         B       4.5650     4    2
                         B       3.5275     4    1
```

The GLM Procedure
Dependent Variable: Dung Total Phenols

```
                                    Sum of
Source              DF         Squares      Mean Square    F Value    Pr> F
Model              2      2.74155000      1.37077500      20.23    0.0005
Error               9      0.60995000      0.06777222
Corrected Total     11      3.35150000

              R-Square    Coeff Var      Root MSE      TP Mean
              0.818007      22.93665      0.260331      1.135000

     Source                DF       Type I SS     Mean Square    F Value    Pr> F
     DIET                   2      2.74155000      1.37077500      20.23    0.0005

  Tukey Grouping      Mean      N    DIET
                         A       1.5725      4    3
                         A       1.3625      4    2
                         B       0.4700      4    1
```

The GLM Procedure
Dependent Variable: Dung Total Tannins

```
                                    Sum of
Source              DF         Squares      Mean Square    F Value    Pr> F
Model               2      2.59935000      1.29967500      26.12    0.0002
Error               9      0.44775000      0.04975000
Corrected Total     11      3.04710000

              R-Square    Coeff Var      Root MSE      TT Mean
              0.853057      20.18526      0.223047      1.105000

     Source                DF       Type I SS     Mean Square    F Value    Pr> F
     DIET                   2      2.59935000      1.29967500      26.12    0.0002

          Tukey Grouping        Mean      N    DIET
                         A       1.5725      4    3
                         A       1.2725      4    2
                         B       0.4700      4    1
```

The GLM Procedure
Dependent Variable: Dung Condensed Tannins

```
                                    Sum of
Source              DF         Squares      Mean Square    F Value    Pr> F
Model               2      1.38165000      0.69082500      75.32    <.0001
Error               9      0.08255000      0.00917222
```

```
Corrected Total       11      1.46420000


                     R-Square     Coeff Var      Root MSE       CT Mean
                     0.943621      34.20419      0.095772      0.280000

        Source                DF       Type I SS     Mean Square    F Value    Pr> F
        DIET                   2     1.38165000      0.69082500      75.32    <.0001

          Tukey Grouping        Mean       N    DIET
                         A     0.75750     4    3
                         B     0.08250     4    2
                         B     0.00000     4    1
```

Correlation analysis between nutrient intake and nutrient excreted

```
Pearson Correlation Coefficients, N = 12
                              Prob > |r| under H0: Rho=0
Pearson Correlation Coefficients, N = 12
                             Prob > |r| under H0: Rho=0

                         DMI          DMO           NI           FN           UN           NO

DMI        1.00000      0.78440      0.95536      0.46024      0.62627      0.61445
         0.0025       <.0001       0.1322       0.0293       0.0335

    DMO        0.78440      1.00000      0.79715      0.73824      0.57470      0.67646
         0.0025                     0.0019       0.0061       0.0506       0.0157

    NI         0.95536      0.79715      1.00000      0.60019      0.81366      0.79910
<.0001       0.0019                     0.0391       0.0013       0.0018


                   HEMI         HEMO        CELLI        CELLO         LIGI         LIGO

    DMI         0.94978      0.78041      0.99634      0.63881      0.07111     -0.07388
<.0001       0.0027       <.0001       0.0254       0.8262       0.8195

    DMO         0.66673      0.65056      0.79591      0.87563      0.33504      0.25943
0.0179       0.0220       0.0020       0.0002       0.2871       0.4155

    NI          0.91860      0.77674      0.94031      0.57205      0.10667     -0.03987
<.0001       0.0030       <.0001       0.0520       0.7414       0.9021


                    DMI          DMO           NI           FN           UN           NO

    FN          0.46024      0.73824      0.60019      1.00000      0.69307      0.85334
0.1322       0.0061       0.0391                     0.0125       0.0004

    UN          0.62627      0.57470      0.81366      0.69307      1.00000      0.96722
0.0293       0.0506       0.0013       0.0125                     <.0001
```

```
    NO        0.61445     0.67646     0.79910     0.85334     0.96722     1.00000
0.0335      0.0157      0.0018      0.0004      <.0001

    HEMI      0.94978     0.66673     0.91860     0.26608     0.61597     0.53855
<.0001      0.0179      <.0001      0.4032      0.0330      0.0708

    HEMO      0.78041     0.65056     0.77674     0.15909     0.53843     0.44561
0.0027      0.0220      0.0030      0.6214      0.0709      0.1466

    CELLI     0.99634     0.79591     0.94031     0.48377     0.59722     0.60176
<.0001      0.0020      <.0001      0.1110      0.0403      0.0384

    CELLO     0.63881     0.87563     0.57205     0.62299     0.28036     0.42336
0.0254      0.0002      0.0520      0.0305      0.3774      0.1703

    LIGI      0.07111     0.33504     0.10667     0.71024     0.16209     0.36737
0.8262      0.2871      0.7414      0.0096      0.6147      0.2401

    LIGO     -0.07388     0.25943    -0.03987     0.64812     0.04267     0.25934
0.8195      0.4155      0.9021      0.0226      0.8953      0.4157

               HEMI        HEMO        CELLI       CELLO        LIGI        LIGO

    FN        0.26608     0.15909     0.48377     0.62299     0.71024     0.64812
0.4032      0.6214      0.1110      0.0305      0.0096      0.0226

    UN        0.61597     0.53843     0.59722     0.28036     0.16209     0.04267
0.0330      0.0709      0.0403      0.3774      0.6147      0.8953

    NO        0.53855     0.44561     0.60176     0.42336     0.36737     0.25934
0.0708      0.1466      0.0384      0.1703      0.2401      0.4157

    HEMI      1.00000     0.90830     0.92126     0.44007    -0.23444    -0.37323
              <.0001      <.0001      0.1522      0.4633      0.2321

    HEMO      0.90830     1.00000     0.73387     0.37576    -0.45466    -0.54894
      <.0001                  0.0066      0.2287      0.1375      0.0645


               HEMI        HEMO        CELLI       CELLO        LIGI        LIGO

    CELLI     0.92126     0.73387     1.00000     0.68125     0.14065    -0.00268
      <.0001      0.0066                          0.0147      0.6628      0.9934

    CELLO     0.44007     0.37576     0.68125     1.00000     0.53887     0.48187
      0.1522      0.2287      0.0147                  0.0706      0.1127

    LIGI     -0.23444    -0.45466     0.14065     0.53887     1.00000     0.98476
      0.4633      0.1375      0.6628      0.0706                          <.0001
```

```
LIGO     -0.37323     -0.54894     -0.00268      0.48187      0.98476      1.00000
           0.2321       0.0645       0.9934       0.1127       <.0001
```

Repeated measures analysis

The GLM Procedure
Repeated Measures Analysis of Variance
Dependent Variable: Manure Water content (wc)

```
                   Repeated Measures Level Information
       Dependent Variable       wc1     wc2     wc3     wc4     wc5     wc6
           Level of time          1       2       3       4       5       6

                 Tests of Hypotheses for Between Subjects Effects
    Source               DF     Type III SS     Mean Square    F Value    Pr> F
    diet                  2     540.6081333     270.3040667      10.90    0.0039
    Error                 9     223.1207042      24.7911894

 Sphericity Tests                               Mauchly's
         Variables                DF    Criterion    Chi-Square    Pr>ChiSq
         Transformed Variates     14    0.0030923    41.029808       0.0002
         Orthogonal Components    14    0.0011658    47.955844       <.0001

MANOVA Test Criteria and Exact F Statistics for the Hypothesis of no time Effect
                      H = Type III SSCP Matrix for time
                          E = Error SSCP Matrix
                          S=1    M=1.5    N=1.5
       Statistic                Value     F Value    Num DF    Den DF    Pr> F
       Wilks' Lambda             0.0002520            3967.22         5         5    <.0001
       Pillai's Trace           0.9997480      3967.22        5         5    <.0001
Hotelling-Lawley Trace   3967.2206681   3967.22        5         5    <.0001
       Roy's Greatest Root            3967.2206681   3967.22        5         5    <.0001

     MANOVA Test Criteria and F Approximations for the Hypothesis of no time*diet
Effect
                     H = Type III SSCP Matrix for time*diet
                          E = Error SSCP Matrix
                           S=2    M=1    N=1.5
       Statistic                Value     F Value    Num DF    Den DF    Pr> F
       Wilks' Lambda            0.03652697            4.23       10        10    0.0162
       Pillai's Trace           1.28318305      2.15       10       12             0.1052
Hotelling-Lawley Trace  17.62432101      8.46       10    5.3333      0.0121
       Roy's Greatest Root       17.11285090      20.54        5         6           0.0010
```

The GLM Procedure
Repeated Measures Analysis of Variance
Dependent Variable: Manure Total Carbon (TC)

```
                      Repeated Measures Level Information
      Dependent Variable       TC1     TC2     TC3     TC4     TC5     TC6
      Level of TIME         1       2       3       4       5       6

      The GLM Procedure
      Repeated Measures Analysis of Variance
                    Tests of Hypotheses for Between Subjects Effects
          Source                 DF     Type III SS     Mean Square    F Value    Pr> F
          DIET                    2    129.9455083     64.9727542      1.59    0.2558
          Error                   9    367.2134292     40.8014921

      Sphericity Tests                                    Mauchly's
              Variables                 DF    Criterion    Chi-Square    Pr>ChiSq
              Transformed Variates        14    0.0068715     35.360688        0.0013
              Orthogonal Components         14    0.0090384      33.41451        0.0025

           MANOVA Test Criteria and Exact F Statistics for the Hypothesis of no TIME
Effect
                          H = Type III SSCP Matrix for TIME
                              E = Error SSCP Matrix
                              S=1    M=1.5    N=1.5
           Statistic                 Value            F Value  Num DF    Den DF    Pr> F
           Wilks' Lambda               0.0002874              3478.03               5                 5
      <.0001
           Pillai's Trace            0.9997126      3478.03       5        5            <.0001
      Hotelling-Lawley Trace  3478.0300651    3478.03       5        5      <.0001
           Roy's Greatest Root       3478.0300651    3478.03        5        5       <.0001

          MANOVA  Test  Criteria  and  F  Approximations  for  the  Hypothesis  of  no
TIME*DIET Effect
                        H = Type III SSCP Matrix for TIME*DIET
                              E = Error SSCP Matrix
                               S=2    M=1    N=1.5
           Statistic                 Value    F Value    Num DF    Den DF    Pr> F
           Wilks' Lambda             0.30545681      0.81       10        10       0.6277
           Pillai's Trace            0.80749264      0.81       10        12       0.6237
      Hotelling-Lawley Trace 1.90401304      0.91        10    5.3333    0.5767
           Roy's Greatest Root       1.68449827      2.02        5        6          0.2083
```

The GLM Procedure
Repeated Measures Analysis of Variance
Dependent Variable: Manure Total Nitrogen (TN)

```
                      Repeated Measures Level Information
             Dependent Variable       TN1      TN2      TN3      TN4      TN5      TN6
                Level of TIME          1        2        3        4        5        6
```

The GLM Procedure
Repeated Measures Analysis of Variance

```
                    Tests of Hypotheses for Between Subjects Effects
```

```
          Source               DF     Type III SS     Mean Square    F Value    Pr> F
          DIET                  2     11.90396944      5.95198472       8.65    0.0080
          Error                9      6.19101250      0.68789028

      Sphericity Tests                                Mauchly's
              Variables              DF    Criterion    Chi-Square    Pr>ChiSq
              Transformed Variates        14     0.002543     42.418254       0.0001
              Orthogonal Components       14    0.0163695     29.197593       0.0098

           MANOVA Test Criteria and Exact F Statistics for the Hypothesis of no TIME
Effect
                        H = Type III SSCP Matrix for TIME
                              E = Error SSCP Matrix
                              S=1    M=1.5    N=1.5
           Statistic                  Value    F Value    Num DF    Den DF    Pr> F
           Wilks' Lambda              0.0002103            4754.13        5        5    <.0001
           Pillai's Trace             0.9997897            4754.13        5        5    <.0001
      Hotelling-Lawley Trace  4754.1302549    4754.13        5        5    <.0001
           Roy's Greatest Root       4754.1302549          4754.13        5        5    <.0001



          MANOVA  Test  Criteria  and  F  Approximations  for  the  Hypothesis  of  no
TIME*DIET Effect
                      H = Type III SSCP Matrix for TIME*DIET
                              E = Error SSCP Matrix
                              S=2    M=1    N=1.5
           Statistic                  Value    F Value    Num DF    Den DF    Pr> F
           Wilks' Lambda             0.00671074      11.21        10        10    0.0004
           Pillai's Trace           1.49710055       3.57        10        12    0.0203
      Hotelling-Lawley Trace     72.93948683      35.01        10    5.3333    0.0004
           Roy's Greatest Root       71.89525430      86.27         5         6    <.0001
```

The GLM Procedure
Repeated Measures Analysis of Variance
Dependent Variable: Manure Hemicellulose (HEM)

```
                          Repeated Measures Level Information
            Dependent Variable      HEM1    HEM2    HEM3    HEM4    HEM5    HEM6
                Level of TIME          1       2       3       4       5       6

      The GLM Procedure
      Repeated Measures Analysis of Variance
                        Tests of Hypotheses for Between Subjects Effects
          Source               DF     Type III SS     Mean Square    F Value    Pr> F
          DIET                  2     4131.821769    2065.910885     124.77    <.0001
          Error                9      149.022825      16.558092

      Sphericity Tests                                Mauchly's
              Variables              DF    Criterion    Chi-Square    Pr>ChiSq
```

```
            Transformed Variates        14    0.0332396     24.168485       0.0437
            Orthogonal Components       14    0.0347177     23.859588       0.0476

          MANOVA Test Criteria and Exact F Statistics for the Hypothesis of no TIME
Effect
                         H = Type III SSCP Matrix for TIME
                              E = Error SSCP Matrix
                              S=1    M=1.5    N=1.5
          Statistic                   Value    F Value    Num DF    Den DF    Pr> F
          Wilks' Lambda                    0.00104834              952.89           5            5
<.0001
          Pillai's Trace              0.99895166               952.89        5        5    <.0001
      Hotelling-Lawley Trace    952.89266801  952.89        5        5    <.0001
          Roy's Greatest Root              952.89266801    952.89        5        5    <.0001

         MANOVA  Test  Criteria  and  F  Approximations  for  the  Hypothesis  of  no
TIME*DIET Effect
                        H = Type III SSCP Matrix for TIME*DIET
                              E = Error SSCP Matrix
                               S=2    M=1    N=1.5
          Statistic                   Value    F Value    Num DF    Den DF    Pr> F
          Wilks' Lambda             0.01439276       7.34        10        10    0.0021
          Pillai's Trace           1.38845129       2.72        10        12    0.0517
      Hotelling-Lawley Trace     40.49003136      19.44        10    5.3333    0.0016
          Roy's Greatest Root       39.78654329      47.74         5         6    <.0001
```

The GLM Procedure
Repeated Measures Analysis of Variance
Dependent Variable: Manure Cellulose (CELL)

```
                        Repeated Measures Level Information
           Dependent Variable     CELL1   CELL2   CELL3   CELL4   CELL5   CELL6
                Level of TIME         1       2       3       4       5       6

      The GLM Procedure
      Repeated Measures Analysis of Variance
                      Tests of Hypotheses for Between Subjects Effects
         Source                 DF     Type III SS     Mean Square    F Value    Pr> F
         DIET                    2     160.2384694      80.1192347       3.13    0.0932
         Error                   9     230.7088458      25.6343162

      Sphericity Tests                                Mauchly's
            Variables                 DF    Criterion    Chi-Square    Pr>ChiSq
            Transformed Variates        14    0.0163939     29.187022       0.0099
            Orthogonal Components       14    0.0182328     28.432197       0.0125

          MANOVA Test Criteria and Exact F Statistics for the Hypothesis of no TIME
Effect
                         H = Type III SSCP Matrix for TIME
                              E = Error SSCP Matrix
```

```
                         S=1    M=1.5    N=1.5
         Statistic                Value    F Value    Num DF    Den DF    Pr> F
         Wilks' Lambda              0.00146616           681.05        5         5    <.0001
         Pillai's Trace             0.99853384           681.05        5         5    <.0001
    Hotelling-Lawley Trace 681.05276069     681.05        5         5    <.0001
         Roy's Greatest Root       681.05276069     681.05        5         5    <.0001

        MANOVA  Test  Criteria  and  F  Approximations  for  the  Hypothesis  of  no
TIME*DIET Effect
                      H = Type III SSCP Matrix for TIME*DIET
                         E = Error SSCP Matrix
                          S=2    M=1    N=1.5
         Statistic                Value    F Value    Num DF    Den DF    Pr> F
         Wilks' Lambda                   0.08072313      2.52       10       10       0.0805
         Pillai's Trace             1.41210555      2.88       10       12      0.0430
    Hotelling-Lawley Trace   5.28285021      2.54       10    5.3333    0.1491
         Roy's Greatest Root        3.57520957      4.29        5        6         0.0524
```

The GLM Procedure
Repeated Measures Analysis of Variance
Dependent Variable: Manure Lignin (LIG)

```
                        Repeated Measures Level Information
           Dependent Variable       LIG1     LIG2     LIG3     LIG4     LIG5     LIG6
               Level of TIME          1        2        3        4        5        6

    The GLM Procedure
    Repeated Measures Analysis of Variance
                     Tests of Hypotheses for Between Subjects Effects
        Source                  DF     Type III SS     Mean Square    F Value    Pr> F
        DIET                     2     5347.037053     2673.518526    1152.02    <.0001
        Error                   9       20.886458       2.320718
    Sphericity Tests                                 Mauchly's
            Variables                 DF    Criterion    Chi-Square    Pr>ChiSq
            Transformed Variates        14    0.0231641     26.732588       0.0209
            Orthogonal Components       14    0.0167076     29.052427       0.0103

         MANOVA Test Criteria and Exact F Statistics for the Hypothesis of no TIME
Effect
                       H = Type III SSCP Matrix for TIME
                         E = Error SSCP Matrix
                         S=1    M=1.5    N=1.5
         Statistic                Value    F Value    Num DF    Den DF    Pr> F
         Wilks' Lambda              0.0006715            1488.15        5         5    <.0001
         Pillai's Trace             0.9993285      1488.15        5         5    <.0001
    Hotelling-Lawley Trace  1488.1528800    1488.15        5         5    <.0001
         Roy's Greatest Root       1488.1528800         1488.15        5         5    <.0001
```

```
          MANOVA Test Criteria and F Approximations for the Hypothesis of no
TIME*DIET Effect
                    H = Type III SSCP Matrix for TIME*DIET
                          E = Error SSCP Matrix
                          S=2    M=1    N=1.5
          Statistic              Value    F Value    Num DF    Den DF    Pr> F
          Wilks' Lambda            0.00152045         24.65             10              10
<.0001
          Pillai's Trace           1.27573744         2.11              10              12
0.1100
Hotelling-Lawley Trace   474.34764004   227.69      10      5.3333 <.0001
          Roy's Greatest Root          473.96289987     568.76           5              6
<.0001
```

ANOVA
The GLM Procedure
Dependent Variable: Manure condensed tannin at day 0

```
                                     Sum of
        Source                 DF        Squares     Mean Square    F Value    Pr> F
        Model                   2     0.56051667      0.28025833       8.79    0.0077
        Error                   9     0.28697500      0.03188611
        Corrected Total        11     0.84749167
                   R-Square     Coeff Var      Root MSE       ct Mean
                   0.661383      96.95936      0.178567      0.184167

        Source                 DF      Type I SS     Mean Square    F Value    Pr> F
        diet                   2     0.56051667      0.28025833       8.79    0.0077

  Tukey Grouping       Mean       N    diet
                        A       0.4875      4    3
                        B       0.0650      4    2
                        B       0.0000      4    1
```

The GLM Procedure
Dependent Variable: Manure condensed tannins at day 28

```
                                     Sum of
        Source                 DF        Squares     Mean Square    F Value    Pr> F
        Model                   2     0.12403333      0.06201667      45.94    0.0056
        Error                   3     0.00405000      0.00135000
        Corrected Total         5     0.12808333

                    R-Square     Coeff Var      Root MSE       ct Mean
                    0.968380      36.14001      0.036742      0.101667

        Source                 DF      Type I SS     Mean Square    F Value    Pr> F
        diet                   2     0.12403333      0.06201667      45.94    0.0056

                 Tukey Grouping          Mean       N    diet
                        A       0.30500      2    3
```

```
                  B       0.00000      2    2
                  B       0.00000      2    1
```

Cumulative emissions ANOVA

The GLM Procedure
Dependent Variable: Methane (CH_4)

```
        Sum of
Source                  DF        Squares     Mean Square   F Value    Pr> F

Model                    2      2819344.34      1409672.17      1.04    0.3931

Error                   9     12226724.83      1358524.98

Corrected Total         11     15046069.18


                 R-Square     Coeff Var      Root MSE       CH Mean

                 0.187381      60.01303      1165.558      1942.175


Source                  DF       Type I SS     Mean Square    F Value    Pr> F

DIET                     2     2819344.343     1409672.172      1.04    0.3931

              Tukey Grouping          Mean       N    diet
    A         2607.5      4    2
               A        1752.2      4    1
               A        1466.7      4    3
```

The GLM Procedure
Dependent Variable: Nitrous oxide (N_2O)

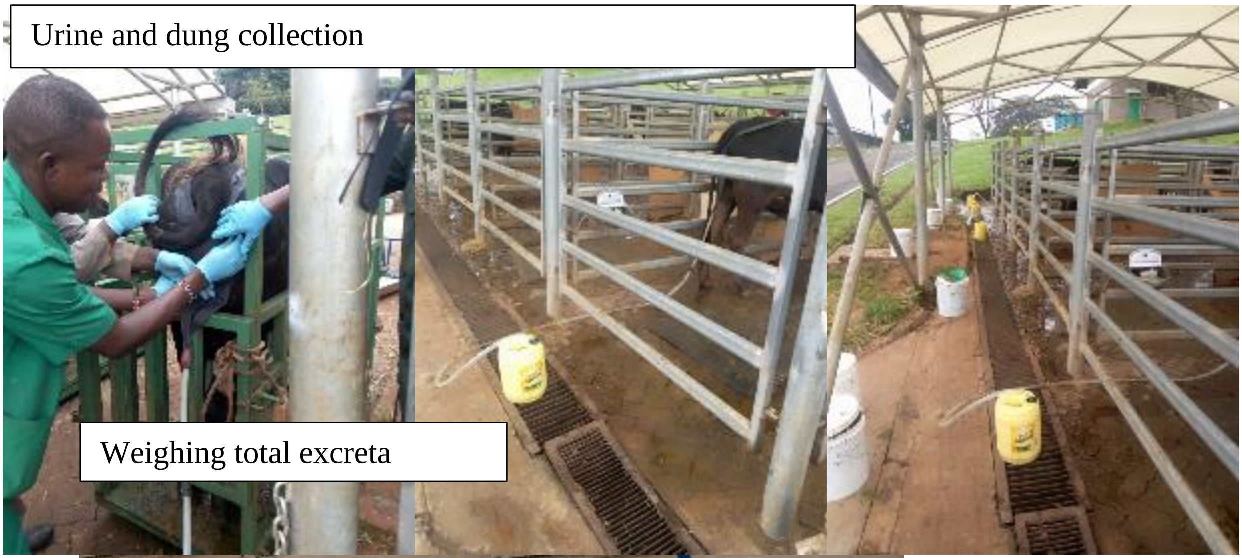
```
                                          Sum of
Source                  DF        Squares     Mean Square   F Value    Pr> F

Model                   2     154484341.0      77242170.5      2.03    0.1874

Error                   9     342674163.6      38074907.1

Corrected Total        11     497158504.6


                 R-Square     Coeff Var      Root MSE       NO Mean

                 0.310735      243.0154      6170.487      2539.134
```

```
Source              DF     Type I SS     Mean Square    F Value    Pr> F

DIET                 2   154484341.0     77242170.5       2.03    0.1874

     Tukey Grouping   Mean      N    diet
                A       7613            4    2
                A         2       4    1
                A         2       4    3
```

8.2 Appendix 2: Photos of invitro gas production and manure incubation

Invitro gas production

Urine and dung collection



Weighing total excreta

Manure incubation

Gas sampling

Air for refilling

Crust formation 3rd week of storage

8.3 Appendix 3: Research permit

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8.4 Appendix 4: Publication Abstract

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Naomi Chepsuge
[a] Egerton University, P.O. Box 536-20115 Egerton-Njoro, Kenya
[b] Mazingira Centre, International Livestock Research Institute (ILRI), Naivasha Rd, PO 30709, 00100 Nairobi, Kenya

Claudia Arndt
Mazingira Centre, International Livestock Research Institute (ILRI), Naivasha Rd, PO 30709, 00100 Nairobi, Kenya

Daniel Korir
Mazingira Centre, International Livestock Research Institute (ILRI), Naivasha Rd, PO 30709, 00100 Nairobi, Kenya

Effects of supplementing tropical dairy cows with different dietary protein sources on nitrogen excretion and manure greenhouse gas emissions

Naomi Chepsuge, Claudia Arndt, Daniel Korir, Daniel Girma Mulat, James O Ondiek, Olivier B Kashongwe and Sonja Maria Leitner

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Abstract

In Kenya, commercial concentrates and leguminous forages are widely used as protein sources in dairy production. However, little is known on their impact on manure greenhouse gas emissions. This study compared manure chemical composition, nitrogen excretion rates, and methane and nitrous oxide emissions in a controlled laboratory experiment. Fresh manure was collected from lactating dairy cows fed on a basal diet of Brachiaria *(Brachiaria brizantha* cv. *Xaraes*) hay (control, CON) and supplemented with either Desmodium (*Desmodium intortum*) hay (DES) or dairy cubes (CUBES) and incubated in 1 L mason jars at 20 ˚C for 84 days. Results showed that DES and CUBES supplementation increased nitrogen excreted both in faeces (1.81±0.02% DM, 1.68±0.10% DM) and urine (0.76±0.05%, 0.75±0.07%) compared to the CON (faeces: 1.35±0.02% DM, urine: 0.35±0.02%). These findings suggest that protein supplements affect manure composition, though in-situ studies are needed to understand their impact on manure emissions.