EFFECT OF SUPPLEMENTATION OF LACTATING DAIRY COWS WITH *Moringa oleifera* Lam. ON BASAL FEED UTILIZATION, MILK YIELD, AND QUALITY

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A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Master of Science Degree in Animal Nutrition of Egerton University

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DECLARATION AND RECOMMENDATION

Declaration

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

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Recommendation

This thesis has been submitted with our approval as University supervisor.

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DEDICATION

This work is dedicated to Almighty God for giving me the knowledge, understanding, and sound mind required to complete this project. To my parents, siblings, family, and friends for the love, support, and words of encouragement. Also, to the smallholder dairy farmers in Sub-Saharan Africa for their tireless effort in contributing to food and nutritional security amidst all challenges and for giving a reason to carry out this study.

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ABSTRACT

The primary factor limiting ruminant production in the tropics is the lack of a year-round supply of quality forage and protein supplements. Therefore, the use of underutilized shrubs and trees such as *Moringa oleifera* as a feed resource for ruminants is noteworthy due to its rich nutritional composition. This study investigated the use of Moringa as a feed supplement for dairy cows to improve feed utilization, milk yield, and quality. Proximate analysis and fibre assessments were used to assess the nutritional value of the feed ingredients. Nine lactating Holstein-Friesian cows (444±39.7 kg) in their early and mid-lactation were randomly assigned to three dietary treatments supplemented with 0, 5 and 10% of Moringa leaves on a dry matter basis in a randomised complete block design for eight weeks. The data was analysed using SAS, and the means were separated using Tukey's Test at $P<0.05$. Moringa had the highest crude protein (295.05 g/kg DM), ether extract (82.51 g/kg DM), dry matter digestibility (782.42 g/kg DM), digestible energy (3.62 Mcal/kg), and metabolizable energy (2.97 Mcal/kg) compared with Lucerne. However, it had the least crude fibre (109.28 g/kg DM) and fibre fractions. Cows supplemented with Moringa leaves showed increased ($P<0.05$) feed intake (15.33 kg DM/day), milk yield (4.14 L/day), milk energy output (12.03 MJ/day), energy corrected milk (3.87 kg/day), yield of milk components and milk production efficiency (0.26) compared to the control diet. There was no significant difference ($P>0.05$) in the vitamin C content, milk energy content, and milk composition in terms of the total solids, fat, solids non-fat, protein, and lactose across all the treatments. The volume of gas produced consistently increased and was higher in Moringa-supplemented treatment (T3) at 24 hr (8.93 ml/200mg DM). T3 had the highest short-chain fatty acids (0.19 ml/200mg DM), and there was no significant difference ($P=0.1087$) across the treatments, but the least organic matter digestibility (20.68%) was recorded in T3. There was no significant difference in the pH, volatile fatty acids, methane gas production, microbial protein, and microbial mass, across the treatments. Compared with the control, Moringa leaves decreased ($P<0.05$) the rumen protozoa and increased ($P<0.05$) rumen Ammonia-N concentration. Moringa reflects a desirable nutritional balance, and its supplementation in the diet of dairy cows increased the feed intake, milk production, digestibility, and rumen fermentation with no effect on the milk composition. The findings imply that Moringa leaves can serve as a protein supplement (at a 10% inclusion level) for ruminants, including dairy cows, and a substitute for Lucerne, the major protein supplement used by ruminant producers in the tropics.

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LIST OF ABBREVIATIONS AND ACRONYMS

ADC	Acid Detergent Cellulose
ADF	Acid Detergent Fibre
ADL	Acid Detergent Lignin
ANF	Anti-nutritional Factors
BC	Berseem clover
BCS	Body Condition Scores
CSC	Cottonseed Cake
DMI	Dry Matter Intake
DMM	Completely Defatted Moringa Seed Meal
EAZ	East African Zebu
GHG	Greenhouse Gas
KALRO	Kenya Agricultural and Livestock Research Organization
MCP	Microbial Crude Protein
ME	Metabolizable Energy
MOLM	*Moringa oleifera* leaf meal
MOS	Moringa whole Seeds
MP	Metabolizable Protein
NDF	Neutral Detergent Fibre
NH_3-N	Ammonia Nitrogen
NPN	Non-protein Nitrogen
NSC	Non-Structural Carbohydrates
OMD	Organic Matter Digestibility
PDMM	Partially Defatted Moringa Seed Meal
RDP	Rumen-Degradable Protein
RUP	Rumen-Undegradable Protein
VFA	Volatile fatty acids

CHAPTER ONE

INTRODUCTION

1.1 Background Information

About 10% of Kenya's Gross domestic product (GDP) and at least 50% of the agricultural GDP emanate from the country's livestock production (KALRO, 2022). Livestock consists of dairy cattle, goats, camels, and beef cattle, as well as non-ruminants, and emerging livestock such as quail. Milk production in Kenya accounts for a significant portion of the economy, estimated at about 5% of GDP, with 4.6 billion litres coming from cattle, 6.4 million litres from goats, and 340 million litres from camels (KALRO, 2022). Kenya accounts for 3% of Sub-Saharan Africa's 18% share of the global dairy market and the dairy cattle population is estimated to be 4.3 million, with production systems ranging from extensive to semi-intensive to intensive (Odero-Waitituh, 2017). Smallholder dairy farmers account for up to 70-80% of Kenya's dairy producers, implying that they can be used for economic development, food security, and poverty reduction (Wambugu *et al.,* 2011). However, smallholder dairy production is challenged by many factors, which include inadequate feed and feeding (Mutavi *et al.,* 2016). Most smallholder farmers are found in the highlands, where demand for food crops restricts access to grazing areas and the availability of feed and fodder (Bebe *et al.,* 2003).

About 40% of households in the smallholder regions offer dairy cattle improved or preserved fodder with supplementation (Muia *et al.,* 2011). Cattle are fed on forages such as Napier grass, Rhodes grass, Lucerne, and crop residues such as maize stover supplemented with concentrate or compound dairy feeds. Most fodders are collected from public or communal land or purchased, resulting in limited feed resources. Around 95% of smallholder dairy farmers use crop residues for their livestock, but the storage methods are insufficient to preserve the quality, resulting in seasonal variations in feed availability and a reduction in milk production (Njarui *et al.,* 2016). Studies have shown that feeds are vital to dairy productivity as fodder quality considerably affects milk productivity.

Despite its immense economic potential, the dairy industry in developing countries is constrained by numerous factors, including a shortage of year-round supply of high-quality feeds, poor milk-producing breeds, poor management, and the impacts of climate change. In most developing countries, the insufficiency and poor quality of forage are major constraints to improvement in livestock productivity (Hao *et al.,* 2017). In addition to an inadequate year-round supply of high-quality forage, the availability and cost of high-quality concentrates and supplementary feeds are significant global issues in the livestock sector, particularly for smallholder dairy farmers (Hao *et al.,* 2017). Consequently, one possible option to this challenge of inadequate supply of high-quality forage is the exploration and exploitation of non-conventional feed resources. Therefore, there is a need to explore alternative feed resources such as non-conventional protein-rich forage trees with high nutrient content, a balanced profile of amino acids, a reasonable cost, and a large proportion of digestible fibre, which can supplement protein and energy in the animals during periods of feed scarcity. Forage from some trees can be used as an alternative feed resource for ruminant livestock in the tropics and subtropics (Silanikove, 2000).

During the dry season, livestock farmers with limited access to feed resources may find it advantageous to use fodder trees and shrubs to improve the quality and availability of feed for their animals, as the trees are a good and inexpensive source of protein and micronutrients (Moyo *et al.,* 2012). In this regard, the drought-tolerant Moringa (*Moringa oleifera*) tree can be considered one of the numerous top feed resources in the tropics. Moringa is a tree native to India but has spread to many tropical and sub-tropical areas worldwide. It's a perennial evergreen tree (Palada *et al.,* 2007) whose forage contains approximately 5.9% moisture, 38.6% carbohydrates, 27.2% protein, and 17.1% fat (Yameogo *et al.,* 2011).

Benefits of using Moringa as a source of forage for ruminants include the plant's versatility as a forage tree, which allows for multiple harvests throughout the growing season and the storage of its dried leaves for extended periods without loss of nutritional content (Mendieta-Araica *et al.,* 2011). Numerous studies have demonstrated that Moringa can enhance the health and performance of ruminants, such as oxidative status and milk production (Babiker *et al.,* 2016), improve their nutrition via microbial protein synthesis (Soliva *et al.,* 2005), and regulate rumen microflora as it contains large quantities of active substances, such as flavonoids, and other phenolic compounds (Kholif *et al.,* 2016). The various nutrients in Moringa, including protein, fatty acids, minerals, and vitamins, make it a good feed resource for livestock (Moyo *et al.,* 2012).

1.2 Statement of the Problem

Kenya has the highest per capita milk consumption in Sub-Saharan Africa due to its relatively high milk production compared to other countries. However, the dairy industry has not achieved its full potential due to many constraints, key among them being the inadequate year-round supply of high-quality basal feed and those available being generally of low quality and with the cost of supplements being relatively high. This has led to limited feed resources, seasonal fluctuations in feed availability to dairy cows, and, therefore poor feeding regime that inevitably leads to low milk yield and quality. Therefore, there is a need to explore and exploit non-conventional feed resources for use in dairy cows' diets to alleviate this feed shortage. One such option is using Moringa as a feed supplement for dairy cows to improve milk yield and quality.

1.3 Objectives of the Study

1.3.1 Broad Objective

To contribute to food security by improving livestock productivity in the tropics through the use of Moringa leaves as a supplement for lactating dairy cows.

1.3.2 Specific Objectives

The specific objectives of the study are as follows: -

i. To determine the effect of supplementing Moringa leaves on the nutritional composition of the diets using proximate analysis, fibre fraction and anti-nutritional content.
ii. To determine the effect of supplementing Moringa leaves in dairy cows fed on basal Rhodes grass hay on feed intake, milk yield, and milk composition.
iii. To determine the effect of supplementing Moringa leaves in dairy cows fed on basal Rhodes grass hay on *in vitro* digestibility, rumen fermentation parameters (pH, NH_3-N, VFA, protozoa number), microbial protein production, and ruminal methane gas production.

1.4 Hypotheses

The following Null Hypotheses (H_0) have been postulated for the study: -

i. Supplementing with Moringa leaves does not significantly affect the nutritional composition of the diets based on proximate analysis, fibre fraction and anti-nutritional content.
ii. Supplementing Moringa leaves in dairy cows fed on basal Rhodes grass hay does not significantly affect feed intake, milk yield, and milk composition.
iii. Supplementing Moringa leaves in dairy cows fed on basal Rhodes grass hay does not significantly affect *in vitro* digestibility, rumen fermentation, microbial protein production, and ruminal methane gas production.

1.5 Justification of the Study

In developing countries, ruminant livestock production, particularly dairy and beef cattle is constrained by many factors, the major one being the lack of a year-round supply of quality forage, especially during the dry season. Most forages, especially crop residues, are generally of lower quality and need supplementation with protein and mineral sources to improve their utilisation. However, the cost of some supplements, such as oil seed meals and cakes from soybean, sunflower, and cotton seed that are used in the formulation of supplementary feeds, are considered to be high and outrageous to most dairy farmers, especially the smallholder dairy farmers who form the highest proportion of dairy farmers in Kenya.

To alleviate this problem, livestock scientists have been looking into several strategies, such as exploring and exploiting high-quality feeds that can increase milk and meat production while still being cost-effective and environmentally friendly. However, there are constraints, such as unavailability during certain times of the year, since green fodder is least available following harvest, resulting in reduced livestock production and negatively impacting milk and meat products. This is so even though there is a significant increase in food needs occasioned by the ever-growing human population and per capita disposable income in many developing countries, Kenya included. This also leads to unsustainable livestock production, which creates environmental challenges as the gap between available resources and the need to meet rising human needs widens.

Researchers have recently focused on underutilized shrubs and trees as a feasible option for diversifying feed resources available for feeding animals such as dairy and beef cattle. Moringa is one feed resource that has been overlooked for many years but has enormous potential as a feed resource for ruminants. However, in recent years, its potential as livestock fodder has been appreciated and investigated due to its outstanding and unique characteristics, such as its rapid growth, higher nutritional status, and higher biomass production. Moreover, it can be grown in marginal lands with high ambient temperatures and low water availability, where cultivating other crops is challenging. In addition to being safe for use as a ruminant, Moringa fodder has been demonstrated to improve ruminal fermentation patterns, and it has the potential to reduce greenhouse gas emissions in the form of methane and contribute to food security through sustainable livestock production.

CHAPTER TWO
LITERATURE REVIEW

2.1 Overview of Dairy Cattle Production in Kenya

Kenya's dairy industry accounts for the most significant share (50%) of the country's agricultural GDP, growing at a rate of 4.1% per year (KALRO, 2022). Kenya's dairy sector contributes 3% of the 18% of milk produced globally by Sub-Saharan Africa, making Kenya one of Africa's top milk producers, with over 85% of the East Africa dairy cattle population (Jabbar *et al.*, 2000). With an estimated 5.1 million dairy cattle and milk production of 3.8 billion litres, Kenya's dairy cattle represent around 25% of the country's overall cattle population (Table 2.1). Kenyan dairy cattle are a mixture of exotic and local breeds and crossbreeds. Dairy cattle are mostly kept on smallholder farms, and the most common breeds are the Friesian, Guernsey, Ayrshire, and Jersey, as well as crosses between these breeds and the native East African Zebu (EAZ) (Kibiego *et al.*, 2015).

Table 2.1: Trends in Kenya dairy cattle population and milk production from 2012-2018

Year	Dairy cattle (head)	Milk production (litres)
2012	4,340,278	3,906,250,200
2013	4,505,582	4,055,023,800
2014	4,316,153	3,884,537,700
2015	4,242,132	3,817,918,800
2016	4,505,733	4,055,159,700
2017	4,573,871	3,560,701,586
2018	5,135,875	3,778,206,805

Source: Mbae *et al.* (2020)

In Kenya's dairy industry, smallholder farmers account for up to 70-80% of the country's total milk producers (Mutavi *et al.*, 2016). The milk production cost reflects the essential inputs and degree of intensification, with feed and fodder accounting for the bulk of that cost (between 55 and 70%) (Kibiego *et al.*, 2015). Most dairy cows kept by smallholder farms are either pure exotic breeds or crosses between indigenous and exotic breeds. They are therefore considered to have relatively high genetic merit for milk production. However, they cannot realise their full production potential because of many factors, including nutritional and environmental constraints, significantly insufficient forages (Njarui *et al.*, 2016), concentrates, and mineral supplements (Mugambi *et al.*, 2015). Besides, there is also the seasonal fluctuation in feed quantity and quality following annual weather patterns. As a result, milk production is often high during the wet season because the animals can access adequate quantities of high-quality feed, followed by a rapid decline during the dry season when available forage is generally inadequate and of deficient quality. This results in poor production performance in smallholder dairy herds (Njarui *et al.,* 2016).

The dairy industry has a significant impact on farmer incomes, food, and nutrition security (Chand *et al.,* 2015), employment (Muriuki, 2011), and women's participation in household decision-making about expenditures (FAO, 2011). Most of the milk is consumed on farms or sold in the informal market, with only about 16% going to dairy processors in the formal sector (KNBS, 2020). Rural women cannot control their income from formal market sales, so informal market sales are crucial for their economic well-being (Tavenner & Crane, 2018). Many farmers are embracing modern dairy farming methods and technology. The nation is now geared towards dairy farming as a business due to the rising demand for milk and dairy products arising from changing demographic factors such as; the rapidly growing human population, expanding urbanisation, and a growing middle class (Odero-Waitituh, 2017). However, feed scarcity is typically experienced during the dry season, despite the government's efforts through the Kenya Agricultural and Livestock Research Organization (KALRO) to promote dairy production using improved forages (Odero-Waitituh, 2017). As a way out of this complex feed scarcity situation, exploring non-conventional feed resources that might act as an alternative during the dry season is essential. Moringa forage is a non-conventional low-cost feed resource that can be explored as a feasible feed resource.

2.2 Dairy Production System in Kenya

Kenya has two primary dairy production systems, the large-scale (or commercial) and the smallholder dairy production system, distinguished by the number of animals kept, the complexity of management, and the volume of milk produced. Human population density and agroecological zones determine the production system used in any area (Staal *et al.,* 2003). Dairy cattle are primarily raised on smallholder farms, while commercial farms and larger herds are prominent in some regions. These animals are raised in a variety of systems, including intensive (where animals are housed throughout the entire process), open grazing (where animals graze fields), and semi-intensive (where animals are partially zero-grazed and taken to the field) (Wahinya *et al.*, 2020).

According to Njarui *et al.* (2016), 44% of the smallholder dairy production systems in the Kenyan highlands with high population densities implement zero grazing, which comprises feeding livestock fodder crops and crop residues supplemented with concentrates in the stalls. Meanwhile, the remaining 33% and 23% of the systems practise semi-zero grazing and free-ranging systems, respectively (Bebe *et al.*, 2003). Over 80% of dairy cattle are owned by small-scale dairy farmers, who produce over 56% of all milk; the remaining 20% are owned by large-scale commercial dairy farms, which produce 30% of the milk (Njarui *et al.*, 2011). Before gaining independence, colonial settlers were Kenya's primary practitioners of large-scale dairy production. However, after the country gained independence in 1963, the Kenyan government adopted policies that greatly favoured the sub-division and selling of large-scale farms in the highlands at subsidised prices to smallholder farms (Odero-Waitituh, 2017). Most animals under the commercial production system are kept in a highly intensive system in the highland areas, while few are kept in a semi-intensive system (Lukuyu *et al.*, 2019).

2.3 Nutrient Requirements of Dairy Cattle

Carbohydrates, protein (amino acids), fatty acids, minerals, vitamins, and water are essential nutrients for dairy cattle to meet the requirement of the mammary gland to synthesize milk and milk components (Table 2.2). The use of feed additives may occasionally improve feed utilisation and, therefore, the performance of the animals. Knowledge of the physical features and combination interactions of these nutrients is required to formulate and feed dairy cattle (Erickson & Kalscheur, 2020).

Table 2.2: Nutrient requirements of lactating dairy cows (Live weight = 454-680 kg; Milk production = 25-54.4 kg)

Parameters	**Requirements**	**Parameters**	**Requirements**	**Parameters**	**Requirements**
DM intake (kg)	18.00-30.00	**Minerals**		**Vitamins**	
Energy		Absorbable Ca (g/day)	50.70-88.00	Vitamin A (IU/day)	49500-75000
NE_L (Mcal/kg)	1.37-1.80	Dietary Ca (%)	0.53-0.67	Vitamin A (IU/kg)	2123-3685
NE_L (Mcal/day)	27.70-48.30	Absorbable P (g/day)	41.4-80.30	Vitamin D (IU/day)	13500-21000
Protein		Dietary P (%)	0.32-0.44	Vitamin D (IU/kg)	579-1004
Metabolizable protein (g/d)	1862-3476	Mg (%)	0.18-0.21	Vitamin E (IU/day)	360-545
Diet %MP	9.20-13.00	Cl (%)	0.24-0.29	Vitamin E (IU/kg)	16.00-27.00
Rumen degradable protein (g/d)	1747-2947	K (%)	1.00-1.07		
Diet %RDP	9.50-10.00	Na (%)	0.19-0.23		
Rumen undegradable protein (g/d)	933-2089	S (%)	0.20		
Diet %RUP	4.60-8.40	Co (mg/kg)	0.11		
%CP	14.10-18.40	Cu (mg/kg)	10.00-11.00		
Fibre and carbohydrate		I (mg/kg)	0.34-0.60		
NDF, min %	25.00– 33.00	Fe (mg/kg)	12.30-18.00		
ADF, min %	17.00– 21.00	Mn (mg/kg)	12.00-14.00		
NFC, max %	36.00– 44.00	Se (mg/kg)	0.30		
		Zn (mg/kg)	43.00-55.00		

Source: NRC (2001)

2.3.1 Carbohydrates

Dietary carbohydrates make up the bulk of the diet of dairy cows and serve as the primary source of energy for these animals' basic needs as well as their development, production, and reproduction. It makes up as much as 70% of the diet for dairy cows during lactation and even more for growing heifers and non-lactating cows (Erickson & Kalscheur, 2020). Carbohydrates can be obtained from feedstuffs such as basal forages, roughages, and supplements in the form of grains and sugars. Cattle obtain their energy needs from volatile fatty acids (VFA) produced during the digestion of forages like pastures, hay, and silage by rumen microbes, including cellulolytic bacteria. Feeding forages with an appropriate particle size encourages rumination, producing copious quantities of saliva that buffers the rumen pH to a level (6-7) that aids in the maintenance of the rumen microorganisms and the optimization of the rumen's health (NRC, 2001). Some roughages, such as by-products and even some forages, have high neutral detergent fibre (NDF) content yet do not stimulate much saliva production because of their small particle size (Erickson & Kalscheur, 2020).

Carbohydrates can be categorised as structural and non-structural carbohydrates, although cellulose and starches are the most common carbohydrates used in dairy cattle feeding. The NDF measures structural carbohydrates that include cellulose, hemicellulose, lignin, and any Nitrogen bonded to the fibre (NRC, 2001). Lignin is not a carbohydrate but a component of the NDF closely associated with carbohydrates; it is also indigestible, and its proportion increases as the plant matures (Erickson & Kalscheur, 2020). Since lignin and acid detergent fibre (ADF) have inverse correlations with digestibility, dairy farmers should harvest fodder in the middle of their growth cycle to take advantage of its more excellent digestibility (Halachmi *et al.*, 2016) and simultaneously maximise dry matter yield.

Non-structural carbohydrates (NSCs) are made up of starches (amylose and amylopectin) and sugars (simple sugars and disaccharides). When feeding a lactating cow, they comprise approximately 35% of the diet's dry matter (DM), but in a far-off dry cow or post-weaned, growing heifer, they can make up a smaller percentage (NRC, 2001). More than 50% of a lactating cow's diet should consist of forage, with 17-21% ADF, 23-33% NDF, and 36-44% NSC (NRC, 2001) noting that roughages, hay, silage, and pasture are the primary sources of NDF (Erickson & Kalscheur, 2020).

2.3.2 Protein

Based on the assumption that feed protein contains around 16% N, feedstuff protein is measured as crude protein (CP) or Kjeldahl-N, which is the %N in a feed multiplied by 6.25. (Erickson & Kalscheur, 2020). Crude protein consists of true protein and non-protein nitrogen (NPN) compounds such as dipeptides, nucleic acids, NH_3-N, and others (Schwab & Broderick, 2017). Regarding digestibility, crude protein is typically classified into two categories: RDP (rumen-degradable protein) and RUP (rumen-undegradable protein). Rumen microorganisms break down RDP into dipeptides, amino acids, and finally to Ammonia-N and branched chain VFA, while RUP escapes microbial degradation in the rumen and flows to the small intestine to be enzymatically digested (NRC, 2001).

In the rumen, microorganisms utilize Ammonia-N, fermented carbohydrates (Fermentable OM), and minerals to synthesise microbial crude protein (MCP) (NRC, 2001). The rumen epithelium absorbs the excess ammonia, which then goes to the liver via the hepatic portal vein to be converted into urea, with some of the urea being excreted in urine and, therefore, a waste (Landau *et al.*, 2005). However, a significant amount is also recycled back to the gastrointestinal tract through secretion in saliva and gut epithelium, thus providing a valuable source of endogenous nitrogen (Kholif *et al.*, 2016).

These endogenous N sources are helpful, especially in ruminants subsisting on low N basal diets such as crop residues. The urea recycled through saliva is a significant source of Ammonia-N in the rumen, readily utilized by the microbes for microbial protein synthesis (Detmann *et al.,* 2014). Milk and other biological fluids are other avenues through which some urea leaves the body. Ruminants, including dairy cows, require Protein-N for the rumen microbial system (which essentially requires ammonia and some peptides) and the body tissues (which require amino acid nitrogen for various metabolic functions such as tissue synthesis and milk protein synthesis) (Kholif *et al.*, 2016). Balancing the dietary Protein-N to satisfy the two metabolic systems is critical for the optimal productivity of the ruminant animal. Ideally, including a substantial amount of rumen-degradable nitrogen source such as NPN can satisfy the NH_3-N requirements for the rumen microbes (Landau *et al.,* 2005). At the same time, the microbial crude protein and a significant proportion of dietary UDP can meet the animal’s body requirement for the critical amino acids needed for optimal tissue metabolism and milk synthesis (NRC, 2001).

Dairy cattle mainly utilize amino acids for tissue accretion (muscle growth), metabolic functions (e.g., production of antibodies, enzymes, hormones, etc.), and milk protein synthesis. Enterocytes in the small intestine absorb amino acids, which are then transferred to the liver for use or to various tissues for maintenance, growth, production (lactation), and reproduction. In other words, this proves that animals do not need proteins *per se* but require the amino acids that make up what we call "true proteins" (Paz & Kononoff, 2014). To accurately examine the protein utilised by dairy cows, it is typically advised to use metabolizable protein (MP). MP reflects amino acids absorbed in the small intestine and comprises RUP, MCP, and endogenous protein (such as sloughed cells) (Erickson & Kalscheur, 2020).

To meet the nitrogen needs of rumen microorganisms, the diets of dairy cows should include a variety of protein sources rather than just one. Additionally, the CP content of the diet should not be higher than 14.1-18.4%, with RUP making up 4.6-8.4% of the dietary DM and RDP accounting for 9.5-10%. (NRC, 2001). Suppose the microorganisms in the rumen have access to a sufficient amount of N. They are estimated to produce 200 g of MCP from the degradation of 1 kg of fermentable organic matter (OM). However, if N is limited, the MCP production is assumed to be less than 145 g/kg degraded OM (Landau *et al.,* 2005). It is well known that many dairy cows in the tropics struggle to produce enough milk due to their inability to properly absorb adequate quantities of essential amino acids (Jamarun *et al.,* 2020). Therefore, supplementation with some RUP can go a long way in supplying the limiting amino acids in dairy cows, thereby increasing milk production (NRC, 2001).

2.3.3 Fat

Fat has the highest energy density (2.25 times that of carbohydrates) and can be triacylglycerides or non-glycerides (Ni *et al.,* 2020). When cattle are under heat stress, fat can help maintain caloric intake as it is not significantly fermented in the rumen, producing little heat of fermentation (Erickson & Kalscheur, 2020). Rumen microbes can absorb fatty acids into their cell membranes and synthesise others, changing the number and structure of the main carbon chains in the lipid. However, hydrogenation of fats in the rumen can have detrimental effects on rumen microorganisms and lead to milk fat depression, decreased dry matter intake, and lower milk yield (Bauman *et al.,* 2011). These unfavourable outcomes directly result from the dietary fat's rapid availability to microorganisms in the rumen; an abundance of dietary fat reduces microbial populations and leads to poor fibre digestibility (Ni *et al.,* 2020). High levels of dietary lipids are also known to slow down microbes' attachment to fibre particles, thus undermining fibrolytic activity in the rumen and, therefore, depressing roughage digestion. Hence, lipids supplementation should be done cautiously because of this depression of fibre digestion in the rumen (Ni *et al.,* 2020).

Fat is commonly used as a supplement in developed countries to boost the calorie density of the diets of dairy cows without increasing the bulk of the diet (Erickson & Kalscheur, 2020). However, a suitable form of dietary fat, such as calcium salts of long-chain fatty acids that are insoluble in the rumen, must be used to avoid depressing fibrolytic activity. In early lactation, fats are transported to the mammary gland and incorporated into milk fat, increasing milk production (Ni *et al.,* 2020). However, after milk production reaches its peak, fat is retained in adipose tissue for subsequent lactation (Bauman *et al.,* 2011). To mitigate the unfavourable effects of dietary fat on rumen fermentation during late lactation, it is advised that fat be supplied at no more than 1% of the diet's dry matter, and the calcium level should be increased (Ni *et al.,* 2020). The optimum level of fat inclusion in the diet of lactating dairy cows is 4-5%, as levels greater than this can depress fibre digestion in the rumen and decrease feed intake (Rabiee *et al.,* 2012).

2.3.4 Vitamins

Vitamins are organic substances required for optimal metabolism and are categorised as water-soluble or fat-soluble vitamins (Erickson & Kalscheur, 2020). The B-complex and C vitamins are water-soluble vitamins that can be sufficiently synthesised in the rumen. However, some are required to be supplemented to have a significant effect. The fat-soluble vitamins are vitamins A, D, E, and K. Rumen microbes can synthesize Vitamin K and a wide range of B-complex vitamins, avoiding the necessity of their inclusion in the ration of ruminants, including dairy cows. In contrast, vitamins A, D, and E are supplemented at varying levels depending on the diet (Spears & Weiss, 2014).

2.3.5 Minerals

Mineral nutrition is vital for developing dairy cows since large quantities of milk produced result in high demand for minerals, leading to metabolic diseases such as milk fever and grass tetany (Goff, 2018). Minerals are inorganic compounds essential for structural support, enzyme activity, osmotic regulation, and the conduction of nerve impulses. Furthermore, just like protein-N, a dairy cow requires an adequate supply of minerals to sustain two systems: the rumen microbial ecosystem for optimal performance and the tissue metabolism level that includes lactation (Moyo *et al.,* 2012).

Minerals are categorised as either macro minerals or microminerals. Microminerals (or trace minerals) are needed in relatively small amounts, typically milligrams or micrograms per tonne (ppm). They include; cobalt, manganese, zinc, iron, copper, selenium, fluorine, molybdenum, and iodine, while macro minerals are needed in large quantities (typically in grams per tonne), and they include; calcium, phosphorus, magnesium, potassium, chlorine, sodium, and sulphur. (Erickson & Kalscheur, 2020). In particular, sulphur is very critical in herbivores subsisting on low-quality roughages such as crop residues as it is required for the proliferation of anaerobic fungi that are crucial in the degradation of refractory material in the forestomach (Chung *et al.,* 2012).

An adult lactating cow weighing 454-680 kg and producing an estimated 25-54.4 kg of milk per day requires 50.7-88 g/d of calcium and 41.4-80.3 g/d of phosphorus, with corresponding quantities of sodium (0.19–0.23%), potassium (1.07%), and sulphur (0.2%) in its diet (NRC, 2001). An insufficient supply of minerals, notably; sodium chloride (salt) and sulphur, might reduce feed intake by causing poor rumen microbiota growth and inefficient nutrition metabolism in the tissues (Goff, 2018). Microminerals should be added at the following concentrations: 12.3-18 mg/kg DM for Iron; 10-11 mg/kg DM for copper; 12-14 mg/kg DM for manganese; 0.11 mg/kg DM for cobalt; and 43-55 mg/kg DM for zinc as they are essential for rumen and tissue metabolism (NRC, 2001).

2.3.6 Water

Water is the second most crucial element for life after oxygen (Kononoff *et al.*, 2017). Water is crucial in maintaining temperature, providing insulation, transporting nutrients, and excreting waste metabolites from the body (Chapman *et al.*, 2017). Dairy cows obtain water via drinking, metabolism, and feed (Khelil-Arfa *et al.*, 2012). Dairy cows should always have access to adequate quantities of clean and cool water, as failure to supply sufficient water can limit feed intake and milk production. As milk production increases, so does water intake since 86-88% of milk is made up of water (Ammer *et al.*, 2018). Daily water consumption in lactating dairy cows can be estimated with the use of the equations developed by Appuhamy *et al.* (2016):

(i) $L/d = -91.1 + (2.93 \times DMI) + (0.61 \times DM\%) + (0.62 \times NaK(mEq/kg)) + (2.49 \times CP\%) + (0.76 \times \text{mean ambient temperature } (°C))$

(ii) $L/d = -60.2 + (1.43 \times \text{milk yield}) + (0.64 \times NaK(mEq/kg)) + (0.83 \times DM\%) + (0.54 \times \text{mean ambient temperature } (°C)) + (0.08 \times \text{days in milk})$

The first equation employs dry matter intake, while the second one utilizes milk yield, and it has been established that the two equations are sufficient for estimating daily water consumption (Kononoff *et al.*, 2017).

2.4 Feeding of Dairy Cows

Grass (including Napier grass, Brachiaria, Star grass, Maize, Sorghum, and Rhodes grass), crop residues, and by-products are the primary source of nutrients for dairy cows in the tropics. However, these sources are not always adequate for crude protein and digestible nutrients (Bezabih *et al.*, 2014). To boost the nutritional value and milk production response of grass-based diets for these animals, perennial legumes (such as Lucerne and Clovers) are typically supplemented with these grasses (McLennan *et al.*, 2016). Supplements in the form of commercial concentrates are also used, but they are not always accessible or affordable to smallholder dairy farmers (Muia *et al.*, 2011). The nutritional deficiencies and some of the feed shortages experienced by dairy farmers during the dry season have led to the utilization of fodder trees or shrubs legumes (such as Leucaena, Calliandra, Gliricidia, Sesbania, Mulberry, and Moringa) as feed resources due to their low cost and high availability (Suliman *et al.,* 2016). Most of these forages have mainly been used as protein-rich supplements to improve the utilisation of basal roughages, especially Boma Rhodes grass hay, Napier grass, and crop residues.

2.4.1 Rhodes Grass as a Basal Diet to Dairy Cows

Forages like Boma Rhodes grass (*Chloris gayana*) are often utilised as a basal diet for dairy cows in the tropics. According to Heuzé *et al.* (2016a), it is a perennial green grass that is drought-resistant and highly productive in terms of DM yield. However, the high moisture content and lack of water-soluble carbohydrates make it unsuitable for silage but excellent for grazing or hay production (Parvin *et al.*, 2010).

The nutritional values for Rhodes grass vary with maturity, variety, and season but on average, it contains about 8-10% crude protein, 32% crude fibre, 8-9 MJ/kg ME, 0.2-0.4% Ca, 0.1-0.2% P, and 55% total digestible nutrient (Heuzé *et al.,* 2016a). The low protein content reduces microbial activity in the rumen, resulting in depressed digestibility, decreased feed intake, and suboptimal performance, particularly in milk yield and composition (Osuga *et al.,* 2012). As a result, Rhodes grass hay is unlikely to provide the nutritional needs of productive dairy cows and must be supplemented with legumes such as Lucerne, trefoils, and clover.

2.4.2 Lucerne (Alfalfa) as a Supplement for Dairy Cows

For supplemental feeding to benefit the animal, the animal must have access to an adequate amount of its basal diet, browse, or hay. The function of the supplement is to provide the rumen microbes with the nutrients they require to digest the basal diet more effectively. Lucerne (*Medicago sativa*), also known as Alfalfa in the USA, is one of the essential legume forages in the world. It is a significant source of protein for livestock because it has a high protein content (16-18%) and a well-balanced profile of amino acids (Chaabena *et al.*, 2011).

The diets of dairy cows are based primarily on this herbaceous legume due to its high nutritional content, yield, and adaptability (Annicchiarico & Pecetti, 2010). Compared to other fodders, it has more calcium, magnesium, potassium, sulphur, iron, cobalt, manganese, zinc, and vitamins (Marković *et al.*, 2009). Since Lucerne's high protein content is easily digested and its crude fibre digests quickly, dairy cows fed on it have higher nutritional intake and digestibility than those fed on other forages (Brink *et al.,* 2010). Lucerne hay has a DM content of 76.1-94.8%, crude protein content of 11.3-25.3%, crude fibre content of 19.4-38.2%, NDF content of 31.3-59.4%, ADF content of 22.5-44.5%, ADL content of 4.8-11.1 %, and OM digestibility of 55-77%. This nutritional content varies based on the plant's age, the leaf: stem ratio, the frequency of harvests, the conditions of harvest, and the processing techniques utilized (Heuzé *et al.,* 2016b).

About 25-35% of the CP content in Lucerne hay is rumen undegradable and readily available for milk production, so feeding dairy cows a diet based on Lucerne hay will help minimise the expense of purchasing rumen-undegradable proteins (Veronesi *et al.,* 2010). When fed alone, Lucerne hay can produce up to 27 kg of milk per cow, but in high-yielding dairy cows (more than 45 kg/d), Lucerne hay can only give 50-60% of the total requirements. Therefore, additional protein and energy must be provided to sustain milk production (Heuzé *et al.,* 2016b). Based on several trials conducted in the United States, dairy cows fed 20 kg/d DM from Lucerne pasture produced about 25 kg/d milk without supplementation (Mauriès, 2003). It was also found that when cows grazing on tropical pasture were supplemented with Lucerne alone, their milk yield increased from 10-12 L/d to 14-16 L/d. An overall increase in feed intake was also observed when Lucerne was pen-fed in combination with maize silage at a 50% inclusion level in the dairy cow's diet (Cook *et al.,* 2005).

Forage crops like Lucerne are ideal for dairy cows because they give all the necessary nutrients, but Lucerne is in short supply for the global herbivorous animal sector (Hao *et al.,* 2017). There is a growing disparity between the availability of high-quality forage and its high demand, particularly in Sub-Saharan Africa, where the quantity of Lucerne produced falls far short of the dairy industry's needs (Falowo *et al.,* 2018). As a result, there is a need to assess the use of fodders from leguminous trees or shrubs such as Moringa as feed resources for ruminants since they have the potential to supplement the feeding of natural pastures and crop residues due to their high protein, mineral, and vitamin content and availability throughout the dry season.

2.5 Use of Non-Conventional Feed Resources in Livestock Production

In most developing countries, the livestock sector is an integral part of the economy and essential for the population's food security. Dairy farming accounts for 50% of Kenya's agricultural GDP and has an annual growth rate of 4.1%, making it the largest subsector of livestock production (KALRO, 2022). However, the growth of this subsector is constrained by the absence of year-round supply, inadequacy, and low quality of the existing protein and energy feed resources (Njarui *et al.,* 2016). It has been found that feed costs typically account for the largest share (up to 70%) in the cost of milk production (Alqasi *et al.,* 2011), and feed quality and quantity are the most critical factors contributing to efficient and profitable dairy farming, especially on smallholder farms (Wanapat *et al.,* 2013).

However, the lack of adequate and low-quality feed, especially during the dry season, is the greatest challenge constraining the production in dairy farms in most developing countries (Wanapat *et al.,* 2013). The low livestock productivity is mainly due to the low quality and limited quantity of feed (FAO, 2010). It is necessary to supplement the available poor-quality basal feed resources with a certain amount of concentrates to increase farm animal productivity through increased intake and digestion of basal roughages (Nurfeta, 2010). On the other hand, smallholder farmers rarely use conventional feeds as supplements because of the feed ingredients' high price and restricted availability.

Conventional feed resources (cereal grains, legumes) are considered to be expensive and in short supply in many regions worldwide. Increasing competition between humans and livestock has recently led to consistent price increases for these commodities (Hao *et al.,* 2017). In recent years, another critical competition has emerged in the form of the diversion of cereal grains, especially maize, to biofuel/ethanol production, exerting more pressure on the available grain for livestock production. The current situation makes it impossible to meet consumers' long-term demand for livestock products. As a result, there is a pressing need to explore other novel, low-cost feed sources that can provide essential nutrients to livestock while readily accessible to farmers all year round (Ondiek *et al.,* 2010). Leguminous tree fodder is one viable option for overcoming these constraints (Muia *et al.,* 2011).

In the tropics and subtropics, using multipurpose trees and other fodder legumes as an alternative feed resource in ruminant nutrition has gained popularity (Aye, 2016). This is due to their low cost and potential for increasing milk production and growth rates, especially during the dry season (Adebayo *et al.,* 2019). These trees' protein, vitamins, and minerals (Bakhashwain, 2010) make them a potential year-round supplement to ruminants feeding on crop residues and natural pastures (Philip *et al.,* 2019). One such tree is the Moringa (*Moringa oleifera*) tree, a multipurpose tree with multiple industrial and feeding purposes.

One of the biggest challenges with using leguminous trees and shrubs as feedstuff for ruminants is that they contain high tannin, which could affect the animals' performance (Simbaya *et al.,* 2020). Methods such as wilting, sun-drying, chemical treatment, ammoniation, stall feeding, and blending tree/shrub fodder with basal diets have been developed to ameliorate these adverse effects (Franzel *et al.,* 2014). Consequently, to justify their usage as an alternative fodder for dairy cows, the potential of these multipurpose tree species as a non-conventional feed resource to alleviate the animal feed supply problem during the dry season must be objectively evaluated.

2.6 Use of *Moringa oleifera* as Livestock Feedstuff

Moringa (*Moringa oleifera*) is a plant of the family *Moringaceae* and is a fast-growing, drought-resistant, multipurpose tropical tree (Figure 2.1). It is native to northern India and Pakistan but has spread throughout the tropics and subtropics and is now a widespread plant in many African countries (Oyeyinka & Oyeyinka, 2018). It was rediscovered in the 1990s and has become one of Asia and Africa's most economically valued crops (FAO, 2014).

Figure 2.1: *Moringa oleifera* tree showing different parts

Source: Zainab *et al.* (2020)

Moringa has become very important around the globe because it can be utilized for different purposes. Furthermore, it develops well in different soils and climates and is easy to propagate. It is therefore expected to rise in importance as a versatile crop in Africa (William *et al.,* 2014). In Africa, Moringa is primarily grown for its leaves, whereas in Asia, the pods are valued for their numerous benefits (Bosch, 2004). Its leaves are rich in nutrients, including proteins, total phenols, vitamins A, B, C, and E, and minerals, including calcium, potassium, copper, iron, manganese, and magnesium (Hekmat *et al.*, 2015). The leaves are rich in phytonutrients, including carotenoids and ascorbic acid, all of which have been shown to scavenge free radicals and have immunosuppressive effects (Saini *et al.,* 2014).

Moringa leaves have been reported to have a moderate level of palatability, despite their high nutritional value for ruminants (Bancessi *et al.,* 2020). They are widely used in various African countries for small-scale rabbit husbandry. However, it is not recommended to incorporate Moringa leaves into the diets of chickens, pigs, and fish in large quantities due to their high fibre content and anti-nutritional factors (Heuzé *et al.,* 2019). Moringa has a rapid growth rate and can be harvested for its leaves in less than two and a half months. Depending on the environment, ideal cutting intervals range from 15 to 75 days (Nasrin *et al.,* 2014). Fodder yields are highly variable, ranging from 27 to 120 t/ha of fresh matter at the initial cutting (Nouman *et al.,* 2014), with up to 9 cuttings/year is feasible (Bancessi *et al.,* 2020). Forage yield and growth rate are maximised in warm, dry conditions, especially when supplemented with fertilizers and irrigation (Radovich, 2013).

Moringa leaves are a rich source of digestible protein, digestible OM, and energy; as a result, they are an essential source of easily digested protein in the rumen and gut of ruminants (Gutiérrez *et al.,* 2012). They are also beneficial mineral supplements when minerals are deficient or unavailable (Heuzé *et al.,* 2019). Supplementing low-quality basal forage diets with Moringa leaves may benefit animal health and productivity. Incorporating Moringa in dairy cow diets at the same protein and energy levels as concentrates (commercial, sunflower meal, soybean meal) has increased animal performance (Mendieta-Araica *et al.,* 2011). Compared to other tree and shrub species in Venezuela, the Moringa leaf was found to have a moderate level of palatability, as cattle, sheep, and goats were observed to consume it at a moderate level (García *et al.,* 2008; Ojeda *et al.,* 2012). The DM intake was equivalent to or higher than that of Leucaena (*Leucaena leucocephala*) or Gliricidia (*Gliricidia sepium*) when Moringa was given as a sole supplement in the diet of growing goats (Asaolu *et al.,* 2012).

2.6.1 Nutritional Composition of Moringa

Reports of Moringa's high nutrient and low anti-nutritional factor content have sparked a growing interest in the plant's potential as an animal feed (Su & Chen, 2020). A literature review on the nutritional content of the leaves revealed that it is extremely rich in proteins, minerals, vitamins, and other secondary metabolites (Table 2.3).

Table 2.3: Nutritional composition of Moringa leaves

Nutritive value	**Composition (%)**
Moisture	9.53
Crude protein	30.29
Fat	6.50
Ash	7.64
Crude fibre	5.90
Neutral Detergent Fibre	11.40
Acid Detergent Fibre	8.49
Acid Detergent Lignin	1.80
Acid Detergent Cellulose	4.01
Condensed tannins	0.31
Total polyphenols	2.02

Source: Moyo *et al.* (2012)

According to Wu *et al.* (2013), the CP content of the leaves ranges from 23.0-30.3% on a DM basis, which is higher than the levels in Lucerne and Mulberry (Tang *et al.*, 2021). It is widely known that low crude fibre content implies excellent palatability of the feed ingredient (Su & Chen, 2020). The crude fibre content of the leaf is as low as 5.9% (Moyo *et al.*, 2012), which is practically similar to soybean meal that is regarded as a standard feedstuff (Wu *et al.*, 2013). The leaves are highly digestible, evidenced by their low NDF, ADF, ADL, and Acid Detergent Cellulose (ADC) content of 11.4%, 8.49%, 1.8%, and 4.01%, respectively (Moyo *et al.*, 2012) (Table 2.3). The ash content of the leaf (about 12%) is said to be higher than that of corn meal or soybean meal, indicating a high mineral content (Teixeira *et al.*, 2014). The leaf has a fat content of about 6.50% (Moyo *et al.*, 2012), which is higher than the fat content of other woody plant forages, and about 57% of these fats are unsaturated (Teixeira *et al.*, 2014). It was discovered that the dried Moringa leaves contain 17 fatty acids (Table 2.4), with α-linolenic acid constituting 44.57% while heneicosanoic, g-linolenic, palmitic, and capric acids constitute 14.41%, 0.20%, 0.17%, and 0.07% respectively (Moyo *et al.*, 2012).

Table 2.4: Fatty acid composition of Moringa leaves

Fatty acid	**Composition (%)**	**Fatty acid**	**Composition (%)**
Ether extract	6.50	Arachidic (C20:0)	1.61
Capric (C10:0)	0.07	Heneicosanoic (C21:0)	14.41
Lauric (C12:0)	0.58	Behenic (C22:0)	1.24
Myristic (C14:0)	3.66	Tricosanoic (C23:0)	0.66
Palmitic (C16:0)	11.79	Lignoceric (24:0)	2.91
Palmitoleic (C16:1c9)	0.17	Total saturated fatty acids (SFA)	43.31
Margaric (C17:0)	3.19	Total mono-unsaturated fatty acids (MUFA)	4.48
Stearic acid (C18:0)	2.13	Total poly-unsaturated fatty acids (PUFA)	52.21
Oleic (C18:1c9)	3.96	Total Omega-6 fatty acids (n-6)	7.64
Vaccenic (C18:1c7)	0.36	Total Omega-3 fatty acids (n-3)	44.57
Linoleic (C18;2c9,12(n-6)	7.44	PUFA: SFA	1.21
α-Linolenic (C18:3c9,12,15(n-3)	44.57	PUFA: MUFA	14.80
g-Linolenic (C18:3c6,9,12 (n-6)	0.20	n-6:n-3	0.17

Source: Moyo *et al.* (2012)

Due to the high concentration of amino acids in Moringa, it can be utilised as the primary source of amino acid supplementation when combined with other conventional forage sources (Su & Chen, 2020). Moringa has a higher lysine, glutamine, histidine, leucine, isoleucine, arginine, phenylalanine, alanine, and valine than other woody plants (Shi *et al.*, 2018). It contains 16-19 amino acids, including the 9 essential amino acids (Table 2.5) that animals cannot synthesise (Moyo *et al.*, 2012). The methionine level of the leaf is reportedly higher than Lucerne, very close to corn meal, but lower than soybean meal (Farkhoy *et al.*, 2012).

Table 2.5: Amino acid composition of Moringa leaves

Essential	**Composition (%)**	**Non-essential**	**Composition (%)**
Threonine	1.357	Arginine	1.780
Methionine	0.297	Serine	1.087
Valine	1.413	Aspartic acid	1.430
Phenylalanine	1.640	Glutamic acid	2.530
Leucine	1.960	Glycine	1.533
Lysine	1.637	Alanine	3.033
Tryptophan	0.486	Proline	1.203
Isoleucine	1.177	Hydro-Proline	0.093
Histidine	0.716	Cysteine	0.010
		Tyrosine	2.650

Source: Moyo *et al.* (2012)

Minerals, including phosphorus, calcium, potassium, zinc, and iron, are abundant in Moringa leaves (Table 2.6), making them an excellent source of these minerals compared to other tree leaves. These minerals are essential for growth and development, milk production, and control of metabolic and enzymatic processes (Shi *et al.*, 2018). Dried Moringa leaves contain 3.65% calcium, 0.30% phosphorus, 0.50% magnesium, 1.50% potassium, 8.25% copper, 0.63% sulphur, 0.164% sodium, 490 mg/kg iron, 363 mg/kg selenium, 86.8 mg/kg manganese, and 13.03 mg/kg zinc (Moyo *et al.*, 2012).

Table 2.6: Mineral composition of Moringa leaves

Macro-elements	**Composition (%)**	**Micro-elements**	**Composition (mg/kg)**
Calcium	3.650	Zinc	31.03
Phosphorus	0.300	Copper	8.25
Magnesium	0.500	Manganese	86.80
Potassium	1.500	Iron	490.00
Sodium	0.164	Selenium	363.00
Sulphur	0.630	Boron	49.93

Source: Moyo *et al.* (2012)

Natural antioxidants consist primarily of polysaccharides, polyphenols, vitamins, and alkaloids (Moyo *et al.*, 2012). The use of plant-derived antioxidants as feed additives in livestock production has been encouraged by reports that animals fed plant-based supplements with antioxidant qualities have a strong anti-oxidation ability (Lahucky *et al.,* 2010). Nkukwana *et al.* (2014) found that the high concentration of secondary metabolites in Moringa leaves made them sound like feed additives, increasing the meat's resistance to oxidation and extending its shelf life. This can also be attributed to the abundance of vitamins, flavonoids, phenols, and carotenoids in Moringa leaves (Moyo *et al.,* 2012).

The total amount of carotenoids in Moringa leaf is 40,139 μg/100 g of fresh weight, and about 47.8% of this amount is β-carotene (Seshadri & Nambiar, 2003). β-carotene is one of the most important precursors for vitamin A, an active substance required to maintain physiological functions and promote growth, development, and reproduction (Su & Chen, 2020). Additionally, Moringa leaves contain high levels of vitamin E (α-tocopherol). This antioxidant helps protect cells from harmful free radicals (reactive oxygen molecules) and boosts cellular immunity (Moyo *et al.,* 2012).

The high fibre content and presence of anti-nutritional factors (ANFs), which have a detrimental influence on palatability and digestibility, place significant limitations on using trees and shrubs as a feed source for livestock (Simbaya *et al.*, 2020). ANFs through various methods, interfere with the metabolic processes that occur within the body and influence how nutrients are utilised. The most important ones include phytate, lectins, polyphenols, and protease inhibitors (Shi *et al.,* 2018). However, Moringa leaves are suitable for animal feed due to their low ANF content (Table 2.7), which varies with cultivar and growing climate (Nasrin *et al.,* 2014).

Table 2.7: Anti-nutritional content of Moringa leaves

Anti-nutritional factors	**Composition (%)**
Alkaloids	1.56
Flavonoids	5.42
Oxalates	1.42
Phytate	2.23
Saponin	2.06
Tannin	1.63

Source: Stevens *et al.* (2015)

Moyo *et al.* (2012) reported a value of 3.12% for condensed tannins and 2.02% for total phenols. Compared to fresh foliage, drying the leaves can reduce the condensed tannins by 15% to 30% (Chebli *et al.,* 2021), and animal consumption of these total phenols has no adverse effects. However, the phenols have been found to have favourable biological effects on animals, such as antimicrobial, anti-inflammatory, antioxidant, and anti-tumour properties, as well as the suppression of platelet aggregation (Thurber & Fahey, 2009).

Moreover, saponin (2.06%) contributes to the bitter flavour of the leaves (Stevens *et al.,* 2015), yet this quantity is not enough to negatively affect livestock (Moyo *et al.*, 2012). Moringa leaves contain 2.23% phytate and 1.42% oxalate, respectively. Furthermore, because oxalate is insoluble, it is not linked to the development of kidney stones (Stevens *et al.,* 2015). It is important to emphasize that the nutritional composition of Moringa leaves makes them an excellent alternative feed resource for livestock production.

2.7 Dietary Application of *Moringa oleifera* in Ruminant Feeding

Feed shortages are one of the most severe challenges, affecting livestock production in many developing countries in the tropics. Leguminous browses, which can be cultivated by small-scale farmers and have more protein than grasses, seem promising in this regard to overcome the feed sources' limitations (Soltan *et al.*, 2012).

Moringa is a fascinating tree species studied extensively due to its high nutritional content and high biomass output, making it suitable for livestock fodder (Sun *et al.*, 2017). When the leaves are incorporated into the diet, the animals eat them voluntarily. Moringa leaves are the ideal tree portion to be used as a source of protein for ruminants due to their high levels of digestible protein and well-balanced amino acid profiles (Babiker *et al.*, 2017). When administered as a protein supplement, the leaves enhanced the growth performance of growing lambs, milk yield, and composition of cows, sheep, and goats (Babiker *et al.*, 2017). However, the high ruminal degradability of the leaves' protein prevents them from being recommended as a source of rumen-undegradable protein (Soliva *et al.*, 2005).

In addition to having a high concentration of crude protein, the Moringa plant includes some bioactive substances that have been identified to have distinct nutritive value. The plant has enhanced several livestock species' health, growth performance, milk production, and meat quality (Soltan *et al.*, 2017). In a study by Sarwatt *et al.* (2002), goats in East Africa were supplemented with Moringa leaves in addition to a diet of *Chloris gayana* hay. In the trial, when sunflower seed cake was replaced with Moringa leaves at 0, 25, 75, and 100%, it was noted that there was a significant increase in basal diet DM intake as Moringa inclusion increased.

The use of Moringa (MO) fodder in combination with Leucaena (LEU) and *Gliricidia sepium* (GLI) fodders on West African Dwarf (WAD) goats was evaluated (Asaolu *et al.,* 2011). In the study, it was concluded that the experimental groups (50% MO+50% GLI, 50% MO+50% LEU, and 100% MO) did not differ significantly in DM intake, indicating that

Moringa leaves can completely replace LEU and GLI in a goat's diet without having any adverse effects on DM intake. In another study by Mahmoud (2013), it was found that replacing some of the clover hay in a concentrate feed mixture with Moringa stems could be done without affecting the performance of Rahmani lambs. Adegun & Aye (2013) experimented on West African Dwarf sheep in Nigeria. They concluded that cotton seed cake could be replaced with Moringa leaf meal as a protein source in a concentrate mix when provided as a supplement to Guinea grass (*Panicum maximum)*. In South Africa, Moyo *et al.* (2014) investigated the impact of feeding Moringa leaves rather than sunflower cake on the growth performance, physicochemical traits, and sensory qualities of chevon from crossbred Xhosa lop-eared goats. They observed that goats fed sunflower cake or Moringa leaves experienced significantly higher average daily weight gains. In contrast, goats fed Moringa leaves produced chevon with the best physicochemical characteristics and sensory scores.

A study conducted by Babeker & Abdalbagi (2015) to determine the impact of Moringa on the performance of Sudan Nubian goats found that Moringa leaf meal (MOLM) can be used as a feed resource at a 20% inclusion level for small ruminants without affecting productivity. In another study conducted by Allam *et al.* (2015) to assess how effectively Moringa leaves might replace Soybean meal for fattening lambs, the findings indicated that DM intake increased as the proportion of Moringa leaf in the diet increased. Jiwuba *et al.* (2016) found that integrating 15% MOLM in the diets of WAD goats resulted in improved haematological characteristics and performance. Fadiyimu *et al.* (2016) conducted a study to evaluate how adding Moringa, *Gliricidia sepium*, or cassava fodder affected the feed intake, growth efficiency, and carcass characteristics of WAD sheep consuming *Panicum maximum* as their basal diet. Based on the result, it was found that Moringa can appropriately replace *Gliricidia sepium* as a supplement in the diets of small ruminants.

Kholif *et al.* (2017) experimented in Egypt to ascertain the impact of replacing Berseem clover (BC) with Moringa (MO) leaf on feed utilisation and lactation performance in Nubian goats. According to the findings, MO diets enhanced feed intake, nutritional digestibility, total serum protein, albumin, and glucose. Elaidy *et al.* (2017) discovered that replacing up to 15% of the starter diet of suckling buffalo calves with dried Moringa increased the calves' growth performance. The blood biochemical profile of grazing Mehsana goat kids fed various amounts of Moringa leaves was also investigated by Damor *et al.* (2017), and they found out that the group fed Moringa had higher serum total protein and albumin levels than the other groups. However, the glucose levels were similar across all groups. An experiment was carried out by Korsor *et al.* (2017) to assess the indirect effects of adding Moringa to the does' diet on the growth rates of pre-weaning kids. The study found that the body condition scores (BCS) and the growth rate factors differed significantly. According to a study conducted in Saudi Arabia by Babiker *et al.* (2017), ewes and goats' milk production and composition improved when Lucerne hay was partially replaced with Moringa leaves. According to research conducted by Choudhary *et al.* (2018) in Bihar, replacing 50% of a doe's concentrate feed with Moringa leaf significantly impacted milk production and overall performance. Sultana *et al.* (2018) concluded in their study in Bangladesh that Moringa leaves can be incorporated into the diet of goats up to 67.2% or 3.4% of live weight to produce lean meat without influencing dietary intake or daily gain. Based on their research in Jharkhand, Kumar *et al.* (2018) found that feeding goats a diet with Moringa leaf powder increased their productivity, with the highest performance at a 15% inclusion level.

Although numerous studies have been conducted to examine the impact of Moringa on the productive performance of dairy cows, sheep, and goats in terms of milk production, milk quality, growth performance, carcass quality, rumen fermentation, and methane emission (Falowo *et al.,* 2018; Soltan *et al.,* 2017), there have not been enough investigations on the utilization of Moringa in the diet of lactating dairy cows found in Kenya. Therefore, more research on feeding Moringa to these animals is required to assess its potential as a substitute feedstuff in periods of feed shortage.

2.7.1 Effect of *Moringa oleifera* on Feed Intake and Digestibility of Dairy Cows

Ruminants can digest fibrous material due to their digestive system structure and the microbes' activity in their rumen. Due to their unique digestive mechanism, they can digest tree forages like Moringa (Su & Chen, 2020). According to Sánchez *et al.* (2006), adding Moringa as a protein supplement to poor-quality diets increased DM intake and digestibility in Creole dairy cows. Comparing diets containing soybean or rapeseed meal to those containing diets made from Moringa leaves, the apparent *in vitro* digestibility of organic matter (OM) and fibre in the Moringa diets were equal or even greater (Soliva *et al.,* 2005).

Mendieta-Araica *et al.* (2011) also reported their findings on comparisons between fresh or ensiled Moringa and elephant grass in the diet of dairy cows and found that Moringa silage had higher protein and fibre digestibility. However, the metabolizable energy intake was the same for all diets. However, there was a difference in the protein and fibre intake between the diets, with the fresh Moringa diet having the highest DM intake and the elephant grass diet having the lowest. *In vivo* nutrient apparent digestibility has also been unaffected by the partial substitution of Moringa silage for Lucerne hay (50%) and maize silage (Zeng *et* *al.,* 2018). Moringa can improve the intake and apparent digestibility of nutrients in dairy cows, as evidenced by the findings of Li *et al.* (2019). They found a significant increase in the DM intake and apparent digestibility of dry matter, crude protein, acid detergent fibre, and neutral detergent fibre in the Moringa diet.

Therefore, Moringa can be utilized as a feed resource for dairy cows because of its high DM intake and digestibility. The dairy cow's voluntary feed intake or DM intake is crucial in dairy management because it makes it easier to formulate economically and nutritionally sound rations. When combined with milk yield, it can determine the economic worth of a single cow at any given stage of lactation, helping the operation as a whole to make more cost-effective decisions (Halchimi *et al.,* 2004). Hence, there is a need to look into the effect of Moringa leaves on dairy cows' DM intake and digestibility if it will be recommended as a feed resource available all year round.

2.7.2 Effect of *Moringa oleifera* on Rumen Fermentation and Methane Gas Production of Dairy Cows

Animal feeding is one of the variables with the strongest influences on greenhouse gas (GHG) emissions and production efficiency (Hristov *et al.,* 2013). High-quality forages are required to maximise production performance in dairy production, even though enteric methane emission from forage digestion means a waste of feed energy for ruminants. Taking into account measures linked to animal nutrition and feed can reduce increases in methane emissions from ruminant livestock (Dong *et al.,* 2019). Natural methanogen inhibitors found in Moringa leaves are being studied as potential substitutes for antibiotics in feed for ruminant animals (Elghandour *et al.,* 2017).

Soliva *et al.* (2005) reported that when comparing un-extracted Moringa leaves (ML) and extracted Moringa leaves (EML) with rapeseed and soybean meal, ammonia content was very low in ML diets, and there was high microbial protein synthesis in ML and EML diets, which is equivalent to soybean or rapeseed meal diets. Their study also revealed that daily methane emission was 17% lower in the EML diet than in diets containing soybean or rapeseed meal, suggesting that EML and ML have a high potential as alternatives to soybean and rapeseed meal as protein sources in ruminant diets. However, due to their substantial contents of readily fermentable N and energy, they are not recommended as a source of rumen-protected protein. Dong *et al.* (2019) used the methyl-coenzyme M reductase α-subunit gene to investigate the impact of dietary supplementation with Moringa on the faecal methanogenic community in dairy cows. The secondary metabolites of Moringa in the diet were responsible for the increased abundance of the *Methanosphaera* genus, *Methanosphaera* spp, *and ISO3-F5* spp that were detected. The findings suggest that supplementing with Moringa may help to lower methane emissions.

Compared to the control diet (Lucerne hay), the Moringa diet increased total ruminal VFA, acetate, propionate, and butyrate, and a lower concentration of NH_3-N in the cows' rumen. Microbial analysis of the rumen revealed that the *Fibrobacter succinogenes, Butyrivibrio fibrisolvens, Ruminobacter amylophilus, Streptococcus bovis,* and *Prevotella ruminicola* relative expression levels were higher in the cows fed Moringa diet. These results suggest that Moringa may stimulate more efficient rumen fermentation in dairy cattle (Li *et al.,* 2019). Lactating dairy cows were used in a study to compare the *in sacco* fermentation kinetics of Moringa leaf meal (MOLM), whole seeds (MOS), and partially (PDMM) or completely defatted seed meal (DMM). The findings revealed that DMM and PDMM contained more crude protein and fibre. In contrast, MOLM and DMM contained less condensed tannins, MOS and PDMM contained more true soluble protein, MOLM contained more non-protein nitrogen, and oleic acid was the most prevalent fatty acid. The results indicated that MOLM had the highest rate of CP disappearance, effective degradability, and intestinal digestibility of CP. The study concluded that MOLM had a higher protein nutritive value than MOS, DMM, or PDMM and proposed its usage in practical applications (Ebeid *et al.,* 2020).

Ruminal microbial fermentation is essential for maintaining milk production because it may be altered by increasing the number and activity of the rumen bacteria, which improves nutrient digestion (Li *et al.,* 2018). Consideration of the ecological impact of feeding ruminants fodder and shrub species (like Moringa) is essential. Therefore, it is crucial to investigate alternate feed sources that would offer essential nutrients and reduce greenhouse gas emissions in the production of ruminant animals. Moringa can regulate rumen microbiota and fermentation because of its high concentration of active chemicals, including flavonoids and other phenolic components (Kholif *et al.,* 2016).

2.7.3 Effect of *Moringa oleifera* on Milk Yield and Milk Composition of Dairy Cows

Worldwide demand for livestock products is rising steadily because of the ever-increasing human population. High-quality protein (milk and meat) is crucial to the human diet, and ruminants play a critical role in producing these protein sources. Thus, it is essential to investigate animal nutrition and feed methods to sustainably raise livestock productivity, particularly in the dairy industry (Soltan *et al.,* 2017).

Sarwatt *et al.* (2004) investigated the effect of Moringa and cottonseed cake (CSC) supplementation on smallholder dairy cows fed Napier grass. Regarding milk production, the results suggested that the Moringa meal prepared from sun-dried leaves was more valuable than cottonseed cake. At the same time, a blend of 40% Moringa and 60% CSC was more effective than either ingredient fed separately. They determined that Moringa's strong performance is attributable to its rumen-modulating function. According to Sánchez *et al.* (2006), adding Moringa as a protein supplement to low-quality basal diets boosted milk production but did not affect milk composition. Mendieta-Aracia *et al.* (2011) also determined that Moringa silage can be fed to dairy cows in significant amounts to yield the same quantity and quality of milk as traditional diets when comparing fresh or ensiled Moringa with elephant grass. According to Cohen-Zinder *et al.* (2017), in assessing the impact of feeding Moringa silage in place of maize silage on dairy cow milk production, cows fed Moringa silage had the highest milk yield implying that ensiling Moringa is the ideal procedure to improve dairy cow production. Research has also revealed that replacing up to 50% of the Lucerne hay and maize silage with Moringa silage had no adverse effects on milk production (Zeng *et al.*, 2018).

Including Moringa supplements (rachis and twigs) into the diet of lactating multiparous cows resulted in a modified milk fatty acid profile and increased milk production. These findings suggested that using Moringa supplements as a diet supplement could produce higher-quality and healthier milk (Zhang *et al.*, 2018). Transition Holstein cows fed Moringa leaf meal (MOLM) have improved milk composition and antioxidant capacity but not milk yield. The maximum increment was recorded at a 60% inclusion level (Kekana *et al.*, 2020).

2.8 Research Gaps

The use of Moringa leaves to improve feed utilization and productivity in ruminant animals has gained attention in recent years. However, there are still some research gaps that need to be addressed. These include:

i. Investigation of the interactions of Moringa leaves with other commonly used feed resources in the tropics. Ruminant diets are composed of more than one feed resource, and there is a need to understand the interaction between Moringa leaves and these feed resources to optimize ruminant nutrition and performance in terms of productivity.

ii. Several studies have investigated the effect of Moringa leaves on milk yield and composition, but there is still a need to evaluate its impact on milk quality.

iii. Most studies focused on feed intake and milk production, but there is still a need to investigate the impact of Moringa supplementation on the rumen fermentation kinetics, digestibility parameters such as nutrient utilization and fibre digestibility, and overall feed efficiency.

Addressing these gaps will provide a more comprehensive understanding of the potential benefits and limitations of utilising Moringa leaves to improve ruminant nutrition and productivity.

CHAPTER THREE

EFFECT OF MORINGA LEAVES ON NUTRITIONAL COMPOSITION OF THE DIETS

Abstract

The primary factor limiting ruminant production in the tropics is the lack of a year-round supply of quality forage and protein supplements. Therefore, the use of underutilized shrubs and trees such as *Moringa oleifera* is noteworthy due to its rich nutritional composition and potential as a ruminant feed resource. This study investigated the nutritional composition of *Moringa oleifera* leaves, Lucerne hay, and Rhodes grass hay. The ingredients were grouped into seven in ratios 90:10:0, 90:5:5, 90:0:10, 0:100:0, 0:50:50, 0:0:100, and 100:0:0 of Rhodes grass hay, Lucerne hay and Moringa leaves respectively. Proximate and fibre assessments, including total phenolic and condensed tannin contents, were used to determine the nutritional value of these feed ingredients. The crude protein content ranged from 106.74 g/kg DM in Boma Rhodes to 295.05 g/kg DM in Moringa, and adding Moringa to other ingredients increases the crude protein. The ash content ranges from 127.85 g/kg DM in Lucerne to 102.67 g/kg DM in Boma Rhodes, and there was no significant difference ($P<0.05$) between Lucerne, Moringa, and the combination of the two. The ether extract and non-structural carbohydrates were significantly ($P<0.05$) higher in Moringa (82.51 g/kg DM. The crude fibre ranged from 356.46 g/kg DM in Boma Rhodes to 109.28 g/kg DM in Moringa, and adding Lucerne or Moringa to Boma Rhodes reduces the crude fibre. The fibre fractions were significantly ($P<0.05$) lower in Moringa than in others. The organic matter content and hemicellulose were significantly ($P<0.05$) lower in Moringa (788.89 g/kg DM and 1.19 g/kg DM) and higher in Boma Rhodes (883.05 g/kg DM and 330.78 g/kg DM). The dry matter digestibility, digestible energy, and metabolizable energy were significantly ($P<0.0001$) higher in Moringa (782.42 g/kg DM, 3.62 Mcal/kg DM, and 2.97 Mcal/kg DM) and lower in Boma Rhodes (567.56 g/kg DM, 2.70 Mcal/kg DM, and 2.22 Mcal/kg DM). Moringa has the highest total phenolic (6.39%) and the least condensed tannins (0.13%) compared to its combination with Lucerne. The proximate values and the fibre contents of Moringa reflect a desirable nutritional balance which shows that it can serve as a protein supplement for ruminants, including dairy cows, and also a substitute for Lucerne which is the major protein supplement utilized by ruminant producers in the tropics.

3.1 Introduction

Milk production in Kenya accounts for a significant portion of the economy, estimated at about 5% of GDP, and most producers are smallholder dairy farmers who account for 70-80% of Kenya's dairy producers (KALRO, 2022). This implies that they can be used as a tool for economic development, food security, and poverty reduction (Wambugu *et al.,* 2011). In Kenya, dairy cows are mainly fed on forages such as Napier grass, Rhodes grass, Lucerne, and crop residues supplemented with concentrate or compound dairy meal. However, most smallholder farmers are found in the highlands, where demand for food crops restricts access to grazing areas and the availability of feed and fodder (Bebe *et al.,* 2003). Most of these farmers can also not afford high-quality concentrates and supplementary feeds (Mutavi *et al.,* 2016), leading to inadequate feed and feeding in dairy production.

One possible option to this challenge of inadequate supply of high-quality forage is the exploration and exploitation of non-conventional feed resources such as protein-rich forage trees with high nutrient content, a balanced profile of amino acids, a reasonable cost and a large proportion of digestible fibre, which can serve as a supplement for dairy cows during periods of feed scarcity. Fodder trees and shrubs have been reported to improve the quality and availability of animal feed as they are a good and inexpensive source of protein and micronutrients (Moyo *et al*., 2012). In this regard, the drought-tolerant Moringa tree can be considered one of the numerous top feed resources in the tropics.

Moringa is a tree native to India but has spread to many areas worldwide. It's a perennial evergreen tree (Palada *et al.,* 2007) whose forage contains 5.9% moisture, 38.6% carbohydrates, 27.2% protein, and 17.1% fat (Yameogo *et al*., 2011). Benefits of using Moringa as a source of forage for ruminants include the plant's versatility that allows multiple harvests throughout the growing season and the storage of its dried leaves for extended periods without loss of nutritional content (Mendieta-Araica *et al.,* 2011).

Numerous studies have demonstrated that Moringa can enhance the health and performance of ruminants, such as oxidative status and milk production (Babiker *et al.,* 2016), improve their nutrition via microbial protein synthesis (Soliva *et al.,* 2005), and regulate rumen microflora as it contains large quantities of active substances, such as flavonoids, and other phenolic compounds (Kholif *et al*., 2016). The various nutrients in Moringa, including protein, fatty acids, minerals, and vitamins, make it a good feed resource for livestock (Moyo *et al.,* 2012). Therefore, it is essential to assess the nutritive value of *Moringa oleifera* as a possible option for feeding dairy cows and compare it with the commonly fed basal diet (Rhodes grass hay) and a protein supplement (Lucerne).

3.2 Materials and Methods

3.2.1 Preparation of Samples

Rhodes grass hay was acquired from neighbouring stores in the university, Lucerne hay was acquired from Kenya Agriculture and Livestock Research Organisation (KALRO), Naivasha while Moringa leaves were sourced from Kiorimba/Machegene, Meru County, Kenya. Before chemical analysis, dried samples of the three feed ingredients (Rhodes grass hay, Lucerne hay, and Moringa leaves) were ground using a hammer mill manufactured by JXSC Mine Machinery Factory, China and passed through a screen with 3 mm holes (Plate 3.1).

Plate 3.1: Grinding a sample of feed ingredients using a hammer mill

These ground ingredients were used in the formulation of the samples for the diets and supplements as stated in Table 3.1, where BL is 90% Rhodes grass hay and 10% Lucerne hay; BLM is 90% Rhodes grass hay, 5% Lucerne hay, and 5% Moringa leaves; BM is 90% Rhodes grass hay and 10% Moringa leaves; L is 100% Lucerne hay; LM is 50% Lucerne hay and 50% Moringa leaves; M is 100% Moringa leaves; and B is 100% Rhodes grass hay.

Table 3.1: Composition of the samples used in the determination of the nutritional value of diets and supplements

Ingredients	**Diets**			**Supplements**			**Basal diet**
	BL	BLM	BM	L	LM	M	B
Rhodes grass hay (%)	90	90	90	0	0	0	100
Lucerne hay (%)	10	5	0	100	50	0	0
Dried Moringa leaves (%)	0	5	10	0	50	100	0
Total (%)	**100**	**100**	**100**	**100**	**100**	**100**	**100**

BL=90% Rhodes grass+10% Lucerne; BLM=90% Rhodes grass+5% Lucerne+5% Moringa; BM=90% Rhodes grass+10% Moringa; L=100% Lucerne; LM=50% Lucerne+50% Moringa; M=100% Moringa; B=100% Rhodes grass

3.2.2 Determination of the Nutritional Composition of the Feed Ingredients

Before starting the feeding trial, the nutritional composition of the feed ingredients was carried out at the Department of Animal Sciences laboratory at Egerton University. The dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF), and ash of the diets and supplements were determined using method 934.01, method 984.13, method 920.39, method 978.10, and method 942.05 of the Association of Analytical Chemists' official procedures respectively (AOAC, 2005) and the Organic Matter (OM) was calculated as DM-Ash. The nitrogen-free extract (NFE) was calculated as 100 - (Ash+CP+EE+CF), while the non-structural carbohydrate content (NSC) was calculated as 100 - (Ash+CP+EE+NDF) (NRC, 2001).

Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), and Acid Detergent Lignin (ADL) were determined in accordance with Van Soest *et al.* (1991). Hemicellulose (HC) was calculated as NDF-ADF, while the Dry matter digestibility (DMD) was calculated as 88.9-(0.779xADF) (Rohweder *et al.*, 1978). The DMD values were used to estimate digestible energy (DE) using the regression equation reported by Fonnesbeck *et al.* (1984): DE (Mcal/kg) = 0.27+(0.0428xDMD). Then DE values were converted to metabolizable Energy (ME) using the formula reported by NASEM (2016): ME (Mcal/kg) = 0.821×DE.

The total phenolic and condensed tannins of M and LM were also analysed following the procedure described by Makkar (2003) where butanol-HCl-iron was used to ascertain the presence of condensed tannins in the feed ingredients and the interflavan bonds in the mineral acids were broken into pink colored anthocyanidins at a temperature around 95°C.

3.3 Statistical Analysis

Each nutrient analysis was done in triplicates, and the data were subjected to an analysis of variance (ANOVA) following a Completely Randomised Design (CRD) using the Generalized Linear Model (GLM) procedure of SAS (SAS, 2009). The different means were separated using Tukey's Studentized Range (HSD) Test at $P<0.05$.

The statistical model of Completely Randomised Design (CRD) was used:

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

where: Y_{ij} - the jth observation of the ith treatment

μ - the population mean

τ_i - the effect of the ith treatment, i = {BL, BLM, BM, L, LM, M, B}

ε_{ij} - the random error

3.4 Results

The nutritional composition of the feed ingredients is presented in Table 3.2**.** The result shows that the DM ranged from 913.01-985.72 g/kg DM, with the highest being found in B and M having the least DM. The Ash content ranged from 102.67-127.85 g/kg DM, but there was no significant difference between L, LM, and M and between BL, BLM, and BM. The CP content ranged from 106.74-295.05 g/kg DM, where M has the highest CP and B has the least CP. The CF ranged from 109.28-356.46 g/kg DM, and BM and BL had no significant difference. The EE ranged from 12.41-82.51 g/kg DM, with M having the highest EE content and BL having the least.

The NDF ranged from 138.01-743.41 g/kg DM, and there was no significant difference between BL and BM. The ADF ranged from 136.82-412.63 g/kg DM, but no significant difference between BM and BL exists. The ADL ranged from 12.13-52.68 g/kg DM; there was no significant difference between BL, BLM, LM, BM, and B. The NFE ranged from 352.36-405.01 g/kg DM, with L having the highest and BLM having the least. The NSC ranged from 1.53-360.32 g/kg DM; there was no significant difference between B, BLM, BM, and BL. The OM ranged from 788.89-883.05 g/kg DM, with B having the highest OM and M having the least. The HC ranged from 1.19-330.78 g/kg DM, with B having the highest and M having the least.

The DMD ranged from 567.56-782.42 g/kg DM, with no significant difference between BL and BM. The DE ranged from 2.70-3.62 g/kg DM, with M having the highest and B having the least. The ME ranged from 2.22-2.97 g/kg DM, and there was no significant difference between BL and BM.

The result shows that LM had more (0.18%) condensed tannins compared to M (0.13%), and the highest total phenolic was observed in M (6.39%), while LM (5.55%) had the least.

Table 3.2: Nutrient composition of the diets and supplements (g/kg DM)

Source of Variation	**BL**	**BLM**	**BM**	**L**	**LM**	**M**	**B**	**SEM**	***P***
DM	977.44^{b}	969.26^{d}	972.15^{c}	978.46^{b}	949.73^{e}	913.01^{f}	985.72^{a}	5.18	<.0001
Ash	112.32^{b}	108.36^{b}	109.12^{b}	127.85^{a}	126.28^{a}	124.12^{a}	102.67^{c}	2.10	<.0001
CP	152.40^{d}	171.73^{d}	155.58^{d}	207.16^{c}	252.93^{b}	295.05^{a}	106.74^{e}	13.49	<.0001
CF	335.95^{c}	345.43^{b}	336.89^{c}	239.60^{d}	185.68^{e}	109.28^{f}	356.46^{a}	19.97	<.0001
EE	12.41^{f}	22.11^{d}	15.71^{e}	20.37^{d}	38.81^{b}	82.51^{a}	34.77^{c}	4.99	<.0001
NDF	721.34^{b}	693.50^{c}	716.12^{b}	428.37^{d}	318.45^{e}	138.01^{f}	743.41^{a}	50.15	<.0001
ADF	403.08^{ab}	397.80^{b}	405.16^{ab}	335.74^{c}	247.36^{d}	136.82^{e}	412.63^{a}	21.81	<.0001
ADL	39.86^{b}	36.04^{b}	34.63^{b}	52.68^{a}	35.10^{b}	12.13^{c}	32.53^{b}	2.53	<.0001
NFE	386.92^{ab}	352.36^{c}	382.70^{b}	405.01^{a}	396.30^{ab}	389.04^{ab}	399.37^{ab}	3.83	<.0001
NSC	1.53^{d}	4.29^{d}	3.48^{d}	216.24^{c}	263.53^{b}	360.32^{a}	12.41^{d}	31.66	<.0001
OM	865.12^{b}	860.90^{b}	863.03^{b}	850.61^{c}	823.45^{d}	788.89^{e}	883.05^{a}	6.57	<.0001
HC	318.26^{ab}	295.70^{c}	310.96^{bc}	92.64^{d}	71.09^{e}	1.19^{f}	330.78^{a}	29.33	<.0001
DMD	575.00^{de}	579.11^{d}	573.38^{de}	627.46^{c}	696.30^{b}	782.42^{a}	567.56^{e}	16.99	<.0001
DE	2.73^{de}	2.75^{d}	2.72^{de}	2.96^{c}	3.25^{b}	3.62^{a}	2.70^{e}	0.07	<.0001
ME	2.24^{de}	2.26^{d}	2.24^{de}	2.43^{c}	2.67^{b}	2.97^{a}	2.22^{e}	0.06	<.0001
CT	-	-	-	-	0.18^{a}	0.13^{b}	-	0.01	<.0001
TEPH	-	-	-	-	5.55^{b}	6.39^{a}	-	0.19	0.0002

^{abcde}Means in the same row with the same letter are not significantly different at P<0.05; SEM= Standard error of the mean; BL=90% Rhodes grass+10% Lucerne; BLM=90% Rhodes grass+5% Lucerne+5% Moringa; BM=90% Rhodes grass+10% Moringa; L=100% Lucerne; LM=50% Lucerne+50% Moringa; M=100% Moringa; B=100% Rhodes grass; DM=dry matter; CP=crude protein; CF=crude fibre; EE=ether extracts; NDF=neutral detergent fibre; ADF=acid detergent fibre; ADL=acid detergent lignin; NFE=nitrogen free extracts; NSC=non-structural carbohydrates; OM=organic matter; HC=hemicellulose; DMD=dry matter digestibility; DE=digestible energy; ME=metabolizable energy; CT=condensed tannins; TEPH=total extractable phenolic.

3.5 Discussion

Moringa oleifera is one of the alternative forage protein sources, and the nutritional composition can be compared to *Leucaena leucocephala* and *Gliricidia sepium* mostly in terms of crude protein (Oduro *et al.*, 2008). The result shows that Moringa leaves have the highest CP (295.05 g/kg DM) compared to Lucerne, which is within the range reported by Wu *et al.* (2013). It can also be observed that its combination with Lucerne and Boma Rhodes at different ratios improves the CP of these ingredients, showing its suitability as a protein source for dairy cows and a replacement for Lucerne hay.

The result shows that Moringa has an ash content of 124.12 g/kg DM, which is within the range reported by Teixeira *et al.* (2014), and there was no significant difference in the ash content of Lucerne, Moringa, and their combination which implies that Moringa also has a high mineral content and can serve as an option for Lucerne as a protein supplement in the diet of dairy cows.

Crude fibre enhances chewing, rumination, and saliva production in ruminant animals, which helps maintain the rumen pH and normal rumen function. This result shows that Moringa has the least crude fibre (109.28 g/kg DM), which is higher than the value reported by Moyo *et al.* (2011), while Boma Rhodes hay has the highest (356.46 g/kg DM). This result shows the suitability of Moringa leaves as a supplement rather than a basal diet in ruminant nutrition. It is also good to note that low CF will improve the diet's palatability, making a Moringa-supplemented diet highly acceptable by dairy cows.

Fat is essential in ruminant nutrition as it increases the palatability of a diet and the rate of passage, increasing the digestibility of feed. This result shows that Moringa has the highest crude fat (82.51 g/kg DM) compared to Lucerne, Boma Rhodes, and their different combinations. This result is slightly higher than what Moyo *et al.* (2011) reported, which might result from different stages of harvest, soil type, the season of harvesting, post-harvesting treatment, and agro-climatic conditions. This high crude fat content indicates the presence of fatty acids, which have been reported to be mostly unsaturated fatty acids (Teixeira *et al.*, 2014) that can improve feed efficiency and energy available from the diet despite it being a protein supplement.

Fibre fraction determines the extent and rate at which feed is digestible (Rubanza *et al.*, 2005). The NDF is the structural component of the plant, specifically the cell wall, and serves as a predictor of voluntary intake because it provides rumen fill. Adequate NDF in dairy cow diets enables proper rumen function, but a high NDF diet may lead to excessive gut fill and limited feed intake. The study showed that Moringa leaves have the least NDF

(138.01 g/kg DM) when compared to Lucerne, Rhodes grass, and their different combinations, and this result is lower than the findings of Foidl *et al.* (2001) and Moyo *et al.* (2011). It was also observed that adding Moringa leaves to Lucerne and Boma Rhodes hay at different ratios reduces the NDF.

The ADF is a measure of the least digestible component of forage, and as the ADF increases, the digestibility of the forage decreases. The ADF and ADL of the Moringa used in this study differ from the findings of Foidl *et al.* (2001) and Moyo *et al.* (2011), but it was lower (136.82 g/kg DM and 12.13 g/kg DM) than Lucerne, Boma Rhodes, and their different combination. This shows that Moringa leaves have higher digestibility than Lucerne hay, and its combination with Boma Rhodes hay improves its digestibility.

This result also showed no significant difference between the NFE value of Lucerne, Moringa, and their combination, which implies that they have the same amount of soluble carbohydrates and that Moringa can replace Lucerne as a protein supplement in dairy cow diets. NSC is an important factor in the diet of dairy cows as they are required for propionate and microbial protein synthesis, which are essential for milk and milk protein synthesis. The observed content of NSC of the Moringa used in this study differs from the findings of Kholif *et al.* (2018) but is within the acceptable range (30-40% on a DM basis) for lactating dairy cows, and its combination with Lucerne increases the value.

It can also be noted from this study that Moringa has a high content of mineral elements, evident from its low OM (788.89 g/kg DM) when compared with Lucerne, Boma Rhodes, and their different combinations and its addition to Lucerne, and Boma Rhodes at different ratios increases their mineral contents. This value differs from the study of Kholif *et al.* (2018), and the mineral composition explains Moringa's nutritional and therapeutic role in animal diets (Al-Kharusi *et al.,* 2009).

Hemicellulose (HC) is a polysaccharide whose hydrolysis serves as a source of energy in ruminal fermentation (Miranda-Romero *et al.,* 2020). The findings from this study show that Moringa has the least (1.19 g/kg DM) HC, and its combination with Lucerne and Boma Rhodes reduces the HC contents of these ingredients, which shows that it’s a good source of protein rather than energy. This also differs from the findings of Kholif *et al.* (2018).

This result shows that Moringa has the highest DMD, DE, and ME (782.42 g/kg DM, 3.62 Mcal/kg, and 2.97 Mcal/kg, respectively), evident from its fibre fractions and CF content when compared to Lucerne, Boma Rhodes and their combinations at different ratios. This result is consistent with the values reported by Kholif *et al.* (2018).

This result also indicates that Moringa has negligible anti-nutritional factors, evident from the low percentage of condensed tannins (0.13%) and total phenolic (6.39%). This study differs from Moyo *et al.* (2011), that reported the value of condensed tannins and total phenolic as 3.12 mg/g and 2.02%, respectively, and Kholif *et al.* (2018), that reported it has 26 g/kg DM and 45 g/kg DM respectively. The CT content can reduce protein degradation in the rumen and increase protein flow to the intestine (Min *et al.,* 2003). The concentration of phenols in this study has been reported to have no adverse effect when included in animal diets (Foidl *et al.,* 2001) but rather have beneficial biological effects such as antimicrobial and antioxidant activities (Islam *et al.,* 2021).

3.6 Conclusion

Supplementation with Moringa leaves significantly affects the nutritional composition of the diets as it can be observed that its combination with Lucerne and Boma Rhodes at different ratios improves the crude protein content and this shows its suitability as a protein source for dairy cows to improve their productivity when fed with fibrous basal fodders or crop residues in arid and semiarid regions.

CHAPTER FOUR

EFFECT OF SUPPLEMENTING MORINGA LEAVES ON FEED INTAKE, MILK YIELD, AND MILK COMPOSITION IN DAIRY COWS

Abstract

The major factor affecting dairy production in the tropics is the cost and availability of quality feed resources. Thus utilization of non-conventional feed resources like trees and shrubs may improve dairy performance and productivity in terms of milk production. This study investigated the effect of supplementing Moringa leaves on feed utilization and lactation performance of Holstein-Friesian cows. A total of 9 lactating cows weighing 444±39.7 kg were randomly assigned to three dietary treatments supplemented with 0, 5, and 10% of Moringa leaves in place of 10, 5, and 0% of Lucerne hay on a DM basis in a randomised complete block design. The data on feed intake, milk yield and quality were subjected to an ANOVA procedure following an RCBD using the GLM procedure of SAS, and the different means were separated using Tukey's Studentized Range Test at $P<0.05$. Cows supplemented with Moringa leaves showed increased ($P<0.05$) feed intake (15.33 kg DM/day), milk yield (4.14 L/day), milk energy output (12.03 MJ/day), energy corrected milk (3.87 kg/day), yield of milk components and milk production efficiency (0.26) compared the control diet. There was no significant difference ($P>0.05$) in the vitamin C content, milk energy content, and composition of the milk in terms of the total solids, solid non-fats, fat, protein, and lactose across all the treatments. The supplementation of dairy cow diets with Moringa leaves increased the feed intake and milk production without negative effects on the milk composition. The findings imply that Moringa leaves can be used as a protein supplement (at 10% inclusion level) for dairy cows and a substitute for Lucerne hay to improve feed utilisation and production efficiency.

4.1 Introduction

Despite the immense economic potential of the livestock industry in developing countries, it is constrained by numerous factors, including the inadequate year-round supply of high-quality feeds. In addition, the availability and cost of high-quality concentrates and supplementary feeds are significant global issues in the livestock sector, particularly for smallholder dairy farmers (Hao *et al.,* 2017). Consequently, one possible option to this challenge of inadequate supply of high-quality forage is the exploration and exploitation of non-conventional feed resources with high nutrient content, a balanced profile of amino acids, a reasonable cost, and a large proportion of digestible fibre which can supplement protein and energy in the animals during periods of feed scarcity. Forage from some trees can be used as an alternative feed resource for ruminant livestock in the tropics and subtropics (Silanikove, 2000).

During the dry season, livestock farmers with limited access to feed resources may find it advantageous to use fodder trees and shrubs to improve the quantity and quality of the available feed for their animals, as the trees are a good and inexpensive source of protein and micronutrients (Moyo *et al.,* 2012). Moreover, fodder trees and shrubs, including multipurpose trees, are deep-rooted and can access subterranean water resources, providing high-quality forage during dry and drought periods (Silanikove, 2000). In this regard, the drought-tolerant Moringa (*Moringa oleifera*) tree can be considered one of the numerous top feed resources in the tropics.

Moringa is a tree native to India but has spread to many areas worldwide. It's a perennial evergreen tree (Palada *et al.,* 2007) whose forage contains 5.9% moisture, 38.6% carbohydrates, 27.2% protein, and 17.1% fat on a DM basis (Yameogo *et al.,* 2011). The benefits of using Moringa as a source of forage for ruminants include its versatility which allows for multiple harvests throughout the growing season, and the storage of its dried leaves for extended periods without loss of nutritional content (Mendieta-Araica *et al.,* 2011).

The various nutrients in Moringa, including protein, fatty acids, minerals, and vitamins, make it a good feed resource for livestock (Moyo *et al.,* 2012), and numerous studies have demonstrated that Moringa can enhance the performance of ruminants, such as milk production (Babiker *et al.,* 2016) as well as improving their nutrition and feed utilization (Soliva *et al.,* 2005). Therefore, this study aimed to investigate the effect of supplementing Moringa leaves at different levels as a substitute for Lucerne hay on feed intake, milk yield, and composition of lactating dairy cows.

4.2 Materials and Methods

4.2.1 Study Site

This research was conducted at Tatton Agriculture Park (TAP), Egerton University, Njoro, Kenya. It is located between longitude 35º 57’E and latitude 0º 23’S at an elevation of 2,200 and 2,280 m above sea level, with a daily mean temperature of 21ºC. The precipitation pattern is bimodal, with mean annual precipitation between 900 and 1,020 mm. The long rain occurs between March and May, occasionally extending into June, and the short rain occurs between September and November.

4.2.2 Experimental Animals and Design

A total of 9 lactating Holstein-Friesian cows in their early and mid-lactation, weighing 444±39.7 kg and producing 3.01±0.06 L/day were housed in sheltered cubicles within the vicinity of the University Biogas unit. They were randomly assigned to three experimental groups of three animals each, using a randomised complete block design (RCBD). During the first week of the feeding trial, the animals were drenched with an anthelminthic to control internal parasites and sprayed weekly to control external parasites (Plate 4.1).

Plate 4.1: Spraying of experimental animals to control ectoparasites

4.2.3 Experimental Diets and Treatment

Rhodes grass (*Chloris gayana*) hay served as the basal diet, while Lucerne (*Medicago sativa*) hay and dried Moringa (*Moringa oleifera*) leaves were used as supplements. Rhodes grass hay was acquired from neighbouring stores in the university, and Lucerne hay was acquired from Kenya Agriculture and Livestock Research Organisation (KALRO), Naivasha. The hay was shredded using a fodder chopper and stored in gunny bags (Plate 4.2).

Plate 4.2: Shredding and bagging of hay

Moringa leaves were sourced from Kiorimba/Machegene, Meru County, Kenya. The leaves were removed from the branches, air-dried on plastic sheets under partial shade, and then stored in gunny bags (Plate 4.3).

Plate 4.3: Drying and mixing of Moringa and Lucerne

The cows were fed three different experimental diets, as stated in Table 4.1, and were randomly assigned to one of three treatments as follows: -

i. T1: The basal diet with 0% Moringa (10% Lucerne); control
ii. T2: The basal diet with 5% supplementation of Moringa (5% Moringa + 5% Lucerne);
iii. T3: The basal diet with 10% supplementation of Moringa (10% Moringa).

Table 4.1: Composition of dietary treatments

	Dietary treatments		
Ingredients	**T1 (Control)**	**T2**	**T3**
Dried Moringa leaves (%)	0	5	10
Lucerne hay (%)	10	5	0
Rhodes grass hay (%)	90	90	90
Total (%)	**100**	**100**	**100**

Dry matter intake (DMI) was estimated as 4% of the live weight of each cow, the supplements (Moringa leaves and Lucerne hay) were fed at 10% of the total estimated daily DMI for each cow, and the basal diet (Rhodes grass hay) was offered *ad libitum*.

4.2.4 Estimation of Feed Intake

The basal diets were offered to each cow *ad libitum* at 08:00 hr, 13:00 hr, and 16:00 hr, and to ensure that the supplement was wholly consumed, it was provided prior to *ad libitum* feeding of the basal diet. The basal diet was also adjusted based on the previous day's intake to ensure that the cow receives sufficient basal feed without leaving too many leftovers and to avoid selective feeding (Plate 4.4). There was also unlimited access to water and a complete mineral supplement. The feeding trial lasted for eight (8) weeks, with two prior weeks assigned to adaptation to the treatments.

Plate 4.4: Weighing and offering of supplements and basal diet to experimental animals

Daily records of the quantity of feed supplied and the amount of leftovers were kept. The feed intake on a dry matter basis and the total dry matter intake (TDMI) were estimated based on the daily differences between the amount of feed offered and the refusals:

a) DMI of basal diet (kg DM/day) = Basal diet offered (kg) - Basal diet refused (kg)
b) TDMI (kg DM/day) = DMI of the basal diet (kg) + DMI of the supplement (kg)

4.2.5 Estimation of Milk Yield and Determination of Milk Composition

The cows were hand milked twice daily, early in the morning at 04.00 and in the afternoon at 16.00 hr (Plate 4.5). During the eight weeks of the feeding trial, milk yield was recorded daily, and a mixed sample of morning and evening milk was obtained from each cow for laboratory analysis of the components.

Plate 4.5: Measuring of milk yield after milking

Milk samples were analysed for total solids, fat, protein, lactose, and solids-non-fat using Ultra Scan Kurien Milk Analyzer, manufactured by Hindustan Thermostatics, India. The vitamin C content of the milk was determined using the method described by Roe & Kuether (1943) with some modifications by Tomovska *et al.* (2018). The whey fraction of the milk was prepared by adding 15 ml of 6% trichloroacetic acid (TCA) into a tube, followed by slow addition of 5 ml of milk with continuous stirring to make a fine suspension. This mixture was kept at room temperature for 5 minutes and then centrifuged at 6000 rpm to obtain the supernatant. Ascorbic acid (vitamin C) was used as the standard, and it was also used in preparing the calibration curve at concentrations of 0.10, 0.40, 0.80, 1.20, 2.0, 3.0, and 4.0 mg/L. The standard solutions and 1.2 ml of the clear supernatant of the samples were placed in the test tubes then 1.2 ml of 6% TCA and 0.4 ml of 2,4-Dinitrophenylhydrazine were added. The tubes were closed and incubated in a water bath at 37°C for 3 hr, then cooled in an ice bath for 10 minutes. These test tubes were constantly stirred after cooling, and 2 ml of cold sulphuric acid was added gradually before it was mixed with a vortex. The absorbance of these mixtures was measured using a 117 Controller based UV-Visible Spectrophotometer at 520 nm (Gjorgievski *et al.,* 2014).

The average yield (g/d) of each milk component was calculated for individual cows by multiplying milk yield by the component content (g/kg) of milk (Kholif *et al.,* 2016). The gross energy content in the milk was calculated as Milk energy content (MJ/kg) =4.184×

[(41.63×fat (g/kg) +24.13×protein (g/kg) +21.60×lactose (g/kg)–117.2)/10000] ×2.204 (Tyrell & Reid 1965). The milk energy output was calculated as milk energy output (MJ/d) =milk energy content (MJ/kg) ×milk yield (kg/d) (Kholif *et al.*, 2016). Energy-corrected milk (ECM) was calculated as ECM (kg/d) =milk (kg/d) × [38.3×fat (g/kg) +24.2×protein (g/kg) + 16.54×lactose (g/kg) +20.7]/3140 (Sjaunja *et al.,* 1991).

4.3 Statistical Analysis

Data were subjected to an analysis of variance (ANOVA) procedure following a Randomised Complete Block Design (RCBD) using the Generalized Linear Model (GLM) procedure of SAS (SAS, 2009). The different means were separated using Tukey's Studentized Range (HSD) Test at $P<0.05$.

The statistical model of Randomised Complete Block Design (RCBD) was used:

$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$

where: Y_{ij} - the j^{th} observation of the i^{th} treatment

μ - the population mean

α_i - the effect of the i^{th} treatment, $i = \{1,2,3\}$

β_j - the effect of the j^{th} block

ε_{ij} - the random error of the i^{th} treatment on the j^{th} block

4.4 Results

4.4.1 Feed Intake

The feed intake was assessed based on the DMI of the basal diet and the TDMI for each cow. The result (Table 4.2) shows that cows supplemented with Moringa leaves (T3) had the highest (13.69 kg DM/day) DMI followed by those supplemented with Lucerne hay (T1). The highest (15.33 kg DM/day) TDMI was also recorded in the cows supplemented with Moringa leaves (T3), followed by those supplemented with Moringa leaves and Lucerne hay (T2). The DMI of the basal diet and TDMI was significantly different ($P<0.05$) among all the treatments, and the highest feed intake was recorded in the treatment supplemented with Moringa leaves (T3). This study shows that supplementation with Moringa leaves significantly enhanced feed intake ($P<0.05$).

4.4.2 Milk Yield and Composition

The milk yield and composition of lactating Holstein-Friesian cows supplemented with different levels of Moringa leaves and Lucerne hay are presented in Table 4.2. Compared with the control diet, supplementation with Moringa leaves increased ($P<0.05$) milk yield, milk energy output, energy-corrected milk, milk component yield, and milk production efficiency.

Table 4.2: Feed intake, milk yield, and composition of lactating Holstein-Friesian cows supplemented with different levels of Moringa leaves and Lucerne hay

Parameters	**Dietary treatments**			**SEM**	***P***
	T1 (control)	**T2**	**T3**		
Feed intake (kg DM/day)					
DMI of the basal diet	13.32^{b}	12.45^{c}	13.69^{a}	0.09	<.0001
TDMI	15.12^{a}	14.34^{b}	15.33^{a}	0.09	<.0001
Milk					
Milk yield (L/d)	3.35^{c}	3.63^{b}	4.14^{a}	0.05	<.0001
Milk energy content (MJ/kg)	3.06^{a}	3.09^{a}	3.08^{a}	0.11	0.3021
Milk energy output (MJ/d)	9.93^{c}	10.84^{b}	12.03^{a}	0.13	<.0001
Energy-corrected milk (kg/d)	3.19^{c}	3.48^{b}	3.87^{a}	0.04	<.0001
Milk composition (g/kg)					
Total solids	117.93^{a}	125.74^{a}	123.70^{a}	2.94	0.4378
Solids non-fat	77.89^{a}	82.52^{a}	85.15^{a}	1.86	0.3383
Fat	40.04^{a}	43.22^{a}	38.56^{a}	1.46	0.0623
Protein	29.22^{a}	31.22^{a}	31.89^{a}	0.70	0.3380
Lactose	44.15^{a}	46.89^{a}	48.26^{a}	1.04	0.3520
Vitamin C (mg/L)	4.50^{a}	4.34^{a}	4.26^{a}	0.10	0.7310
The average yield of milk components (g/d)					
Total solids	395.61^{c}	430.85^{b}	487.33^{a}	5.07	<.0001
Solids non-fat	261.60^{c}	285.23^{b}	332.81^{a}	3.48	<.0001
Fat	134.00^{c}	145.62^{b}	154.52^{a}	1.74	<.0001
Protein	98.15^{c}	107.91^{b}	124.85^{a}	1.31	<.0001
Lactose	148.26^{c}	161.91^{b}	188.64^{a}	1.97	<.0001
Milk production efficiency					
Milk yield/DMI	0.22^{c}	0.25^{b}	0.27^{a}	0.00	<.0001
ECM/DMI	0.21^{c}	0.25^{b}	0.26^{a}	0.00	<.0001

^{abc}Means in the same row with the same letter are not significantly different at P<0.05; T1= Rhodes grass hay supplemented with Lucerne hay; T2= Rhodes grass hay supplemented with Lucerne hay and Moringa leaves; T3= Rhodes grass hay supplemented with Moringa leaves; SEM= Standard error of the mean; DMI= Dry matter intake; TDMI= Total dry matter intake; ECM= Energy corrected milk.

However, there was no significant difference (P>0.05) in the total solids, solid non-fat, fat, protein, lactose, vitamin C, and milk energy content across all the treatments. This study shows that supplementation with Moringa leaves enhanced milk yield significantly (P<0.05), but there was no significant difference (P>0.05) in the milk composition across all the treatments.

4.5 Discussion

4.5.1 Feed Intake

Feed intake is essential in ruminant production because it’s the major factor influencing production efficiency and health of the animals, but it depends on some factors such as palatability, digesta flow rate, and fibre content (Kholif *et al.,* 2016). The result of this study shows that there was a significant increase in the feed intake in dairy cows supplemented with Moringa leaves (13.69 kg DM/day) compared to those supplemented with Lucerne hay (13.32 kg DM/day) or the combination of Moringa leaves and Lucerne hay (12.45 kg DM/day).

The higher feed intake in Moringa-supplemented cows indicates that it is more palatable and acceptable to dairy cows. The increased feed intake can also result from the low rumen degradability of Moringa leaves, as it has been reported that a decrease in ruminal protein degradation increases feed intake (Kholif *et al.,* 2014). The increased intake observed may also result from increased microbial activity in relation to the increased essential nutrients available to the rumen microbes (Su & Chen, 2020).

This result agrees with a study that reported that supplementation of Moringa at different levels in the dairy cow diet increases feed intake (Dong *et al.,* 2019). Kholif *et al.* (2018) also reported a higher feed intake with Moringa than Berseem clover when fed to dairy goats. However, Mendieta-Araica *et al.* (2011) reported no significant differences in the feed intake among the treatments when Moringa leaf meal was fed to cows.

Protein supplementation has been shown to increase TDMI in diets with low-quality roughage (Patra, 2009), and this study shows that Moringa leaves supplementation has an additive effect on the intake of Rhodes grass hay, increasing its efficiency and utilization. The beneficial effect on feed intake and feed utilisation efficiency could have been mediated through enhanced rumen digestion and tissue metabolism.

4.5.2 Milk Yield and Composition

The result of this study shows that supplementation with Moringa leaves increases milk yield, milk energy output, energy-corrected milk, and the average yield of each milk component when compared with the control. The increase observed is a result of increased feed intake, ruminal undegraded protein, and improved rumen fermentation (Sarwatt *et al.,* 2004). This increase may also be attributed to the high ruminal propionate level in the Moringa diet, as propionate serves as a precursor of gluconeogenesis and lactogenesis (Kholif *et al.,* 2018), and propionate also provides more energy for milk production than acetate (NRC, 2001). These findings agree with the study of Zhang *et al.* (2018) that reported that supplementation of the basal diet of lactating multiparous cows with Moringa improved milk production and the health status of the cows. Khalel *et al.* (2014) also reported an increase in milk yield and milk composition in cows fed Moringa as a protein supplement, which was attributed to the enhanced synthesis of Microbial-N and SCFA. However, Mendieta-Araica *et al.* (2011) reported a decrease in milk yield and no significant effect on the milk composition when soybean meal was replaced with Moringa leaf in the diet of dairy cows.

Nutrition impacts milk composition and has been reported to account for about 50% of the variation in milk protein and fat content (Freeden, 1996). The end product of microbial fermentation in the rumen (VFAs) determines the composition of the milk. Acetic and butyric acid production determines the milk fat content, while propionate is the precursor for milk lactose content. On the other hand, milk protein synthesis is determined by rumen-fermentable carbohydrates and the optimal supply of usable crude protein (Mikołajczyk *et al.,* 2019).

It can be observed from this study that there were no significant differences among all the treatments, which shows that supplementation with Moringa leaves has no significant effect on the milk composition and milk energy content. This is consistent with the study of Mendieta-Aracia *et al.* (2011), which shows that there was no significant difference between the treatments in milk composition when soybean meal was replaced with Moringa in the diet of dairy cows. Although, it was observed from this study that the solids non-fat, protein, and lactose concentration increases while the total solids and fat slightly decrease as the level of Moringa in the diet increases. This agrees with the study of Sánchez *et al.* (2006) that concluded that including Moringa as a protein supplement in low-quality diets did not affect milk composition.

This study also observed that Moringa had no significant effect on the vitamin C content of the milk as the concentration of vitamin C decreases with increasing levels of Moringa in the diet. This shows that there was no transfer of bioactive compounds (vitamin C) from fodder to milk, and this opposes the findings of Al-Juhaimi *et al.* (2020), where Moringa leaves were utilized to improve the quality of milk from Aardi goats, they observed a higher vitamin C content in goats fed diets containing Moringa leaves than those fed Lucerne hay.

Milk production efficiency is a measure that helps determine the dairy cow's ability to turn feed nutrients into milk or milk components and is correlated to a farm's profitability and sustainability (Lapierre *et al.,* 2020). In this study, milk production efficiency was measured in terms of Milk yield/DMI and ECM/DMI, and the highest efficiency was recorded in Moringa-supplemented cows. The result shows that the cows supplemented with Moringa leaves had higher milk production efficiency than the control, and supplementation with Moringa leaves can improve the profitability and sustainability of a dairy farm.

4.6 Conclusion

Supplementation of poor-quality basal forage such as Rhodes grass with Moringa leaves (at 10% inclusion level) has a positive effect on the feed intake, milk yield, and milk production efficiency in dairy cows as opposed to Lucerne hay, but it does not affect the composition and vitamin C content of the milk. Therefore, Moringa leaves can be used as a protein supplement for dairy cows in place of Lucerne hay during period of feed scarcity to improve feed utilization and reduce production costs.

CHAPTER FIVE

EFFECT OF SUPPLEMENTING MORINGA LEAVES ON *IN VITRO* DIGESTIBILITY AND RUMEN FERMENTATION KINETICS IN DAIRY COWS

Abstract

Alteration of the microbial ecosystem and ruminal function can be used to improve feed utilization and production performance of ruminant animals. This study aimed to assess the effect of supplementing Moringa leaves in dairy cows fed on basal Rhodes grass hay on *in vitro* digestibility and rumen fermentation kinetics. Nine lactating Holstein-Friesian cows (444±39.7 kg) were randomly assigned to three dietary treatments supplemented with 0, 5 and 10% of Moringa leaves in place of 10, 5 and 0% of Lucerne hay on a DM basis in a randomised complete block design for 8 weeks. The rumen fluid was collected in the last week of the feeding trial to determine digestibility, rumen fermentation kinetics and methane gas production. The volume of gas produced consistently increased and was higher in Moringa-supplemented treatment (T3) at 24 hr. T3 produced the highest short-chain fatty acids, and there was no significant difference ($P>0.05$) across the treatments, but the least organic matter digestibility was recorded in T3. Moringa leaves have the highest metabolizable energy, while the Lucerne-supplemented treatment (T1) has the least, and no significant difference exists between other treatments. There was no significant difference in the pH, VFAs, estimated methane, microbial protein, and microbial mass, even though there was a variation in the Moringa-supplemented treatment and the control. Compared with the control, Moringa leaves decreased ($P<0.05$) the rumen protozoa and increased ($P<0.05$) rumen Ammonia-N concentration. It is concluded that Moringa leaves enhanced digestibility and rumen fermentation in lactating Holstein-Friesian cows and can be used as a protein supplement for dairy cows as well as a substitute for Lucerne hay.

5.1 Introduction

Despite the economic potential of the dairy industry in developing countries, they are constrained by numerous factors, including a shortage of year-round supply of high-quality feeds, low-quality milk-producing breeds, poor management, and their impacts on climate change, and this has led to poor livestock productivity (Hao *et al.,* 2017). Smallholder dairy farmers constitute the higher percentage of dairy producers in the tropics, and they use crop residues for their livestock with little or no supplementation and their storage methods are insufficient to preserve the quality of the feeds, which results in seasonal variations in feed availability and a reduction in milk production (Njarui *et al.,* 2016).

Animal nutritionists and microbiologists have altered the microbial ecosystem and rumen function to improve animal feed utilization and performance by including ionophores and antibiotics. This intervention's metabolism and residual effect have led to anti-microbial resistance, which has caused great public concern and strict regulations on ruminant production (Matloup *et al.,* 2017). Hence there is a need to explore alternative resources, such as fodder trees and shrubs, that can replace antibiotics in manipulating the rumen function to increase productivity on a sustainable basis. In this regard, the drought-tolerant Moringa (*Moringa oleifera*) tree can be considered one of the numerous top feed resources in the tropics.

Moringa's versatility allows multiple harvests throughout the growing season, and the dried leaves can be stored for extended periods without losing nutritional content (Mendieta-Araica *et al.,* 2011). Numerous studies have demonstrated that Moringa can enhance the health and performance of ruminants, such as oxidative status and milk production (Babiker *et al.,* 2016), improve their nutrition via microbial protein synthesis (Soliva *et al.,* 2005), and regulate rumen microflora as it contains large quantities of active substances, such as flavonoids, and other phenolic compounds (Kholif *et al.,* 2016). The various nutrients in Moringa, including protein, fatty acids, minerals, and vitamins, make it a good feed resource for livestock (Moyo *et al.*, 2012).

Several studies have reported that the inclusion of Moringa leaves in ruminant diets has improved animal performance quantitatively and qualitatively (Babiker *et al.,* 2016; Cohen-Zinder *et al.,* 2016; Kholif *et al.,* 2016; Kholif *et al.,* 2017; Mendieta-Araica *et al.,* 2011; Sultana *et al.,* 2015; Zeng *et al.,* 2018). Therefore, this study aimed to assess the effect of supplementing Moringa leaves in dairy cows fed basal Rhodes grass hay on *in vitro* digestibility and ruminal fermentation.

5.2 Materials and Methods

5.2.1 Collection of Rumen Liquor

During the last week of the feeding trial, rumen liquor was collected manually from the animals using a stomach tube connected to a vacuum pump 3 hr after the morning feeding (Lodge-Ivey *et al.,* 2009) (Plate 5.1). Feed particles were removed from the rumen liquor by straining it through four layers of cheesecloth. This was used to evaluate digestibility and determine the pH, NH_3-N, VFA, protozoa number, and microbial protein synthesis.

Plate 5.1: Collection of rumen liquor from experimental animals

5.2.2 Determination of *in vitro* Dry Matter Digestibility

To evaluate digestibility, the experimental diets, and supplements were subjected to *in vitro* gas production following the procedures of Tilley & Terry (1963) as modified by Menke & Steingas (1988). The samples were placed in calibrated glass syringes with Vaseline-lubricated pistons and then incubated with the rumen liquor and buffer mixture in a water bath maintained at 39°C. The gas production at 0, 3, 6, 9, 12, 24, 48, 72, and 96 hr were recorded and used to determine IVDMD (Plate 5.2).

Plate 5.2: Measurement of gas production

Cumulative gas production data were fitted into the exponential model of Ørskov & McDonald (1979) using the Neway Excel program, and the degradation curve was described as $Y= a+b\ (1-e^{-ct})$

where: Y – the gas produced

a – the soluble fraction

b – the degradable fraction that is degraded at time t

a+b – the potential gas produced

c – the gas production rate constant

t – the incubation time

A graph was plotted to illustrate the progression of the gas production. The gas generated was also utilized to estimate short-chain fatty acids (SCFA), metabolizable energy (ME), and organic matter digestibility (OMD) as

SCFA (mmol/200mg DM) =(0.0222*GP24)-0.00425, where GP24 is 24h net gas production (Getachew *et al.,* 2002);

OMD (%) =18.53+(0.9239*GP48) +(0.0540*CP), where GP48 is 48h net gas production, and CP is crude protein (Menke & Steingas, 1988);

ME (MJ/kg DM) =2.2+(0.1357*GP24) +(0.0057*CP) +(0.0002859*EE^2) where GP24 is 24h net gas production, CP is crude protein, and EE is ether extract (Menke & Steingas, 1988).

5.2.3 Determination of pH

The pH of the rumen liquor was measured immediately using a pH metre calibrated at pH 4.0 and pH 9.0 using respective pH buffers.

5.2.4 Determination of NH_3-N

A portion (8 ml) of the rumen liquor was preserved by adding 2 ml of freshly prepared 25% metaphosphoric acid to prevent further microbial activity and NH_3 loss through volatilization (Plate 5.3).

Plate 5.3: Fixation of NH_3 with metaphosphoric acid

The mixture was stored in tightly capped tubes at a temperature of -20°C until further analysis. The rumen Ammonia-N was determined using the procedure of Smith & Murphy (1993). It is based on the principle that ammonia reacts with alkaline hypochlorite and phenol in the presence of a catalyst (sodium nitroprusside) to form indophenol (blue), and the concentration of ammonia is directly proportional to the absorbance of indophenol, which is measured spectrophotometrically using 117 controller based UV-Visible Spectrophotometer (Plate 5.4).

Plate 5.4: Reading of sample absorbance with a spectrophotometer

5.2.5 Determination of VFA

An aliquot of the rumen liquor was frozen at -20°C for analysis of the volatile fatty acids (VFA). The concentration of the VFAs (acetate, propionate, and butyrate) was determined using the spectrophotometric method for determining high-range VFA concentration in mixed-acid fermentation samples developed by Aramrueang *et al.* (2022) using acetic, propionic, and butyric acid as standards. The calibration curve for each VFA was prepared at 0, 500, 1000, 1500, 2000 and 2500 mg/L by diluting individual VFA standards with distilled water. A portion (0.4 ml) of the rumen fluid was mixed with 0.4 ml of concentrated ethylene glycol and 0.1 ml of 90 g/L H_2SO_4 in a screwed tube. The mixture was heated at 100°C for 10 minutes. After cooling down, 0.5 ml of 18 g/L $NH_2OH{\cdot}HCl$, 0.5 ml of 75 g/L NaOH, 0.5 ml of 140 g/L HCl, and 2 ml of 3.5 g/L $FeCl_3$ were added to the mixture and mixed vigorously. Then the absorbance of this mixture was measured using a 117 controller based UV-Visible Spectrophotometer at 513 nm (Aramrueang *et al.,* 2022).

5.2.6 Determination of Protozoa Number

A subsample of the rumen liquor was fixed with 18.5% formalin immediately (Dehority, 1984), and a portion of each sample was stained and stored in methyl green formalin saline (MFS) solution (Gürelli, 2014) as shown in Plate 5.5. A well-mixed sample was used for the determination of the population of protozoa in the rumen fluid (Gürelli & Göçmen, 2012) with the aid of a light microscope.

Plate 5.5: Determination of protozoa number using a light microscope

5.2.7 Determination of Microbial Protein

Microbial protein was determined using Lowry's procedure, where centrifugation was used to separate bacterial cells from the rumen liquor. The supernatant was discarded and the solid part (obtained cells) was washed with a buffer mixture and then centrifuged at 25,000 x g for 20 minutes to remove the remaining buffer. Proteins from cells were released by suspending the washed cell in 0.25 N NaOH and heating them in a boiling water bath for 10 minutes. Microbial crude protein was then determined by the Folin phenol method by adding 0.7 ml of Lowry's solution into 0.5 ml of the sample and standards (BSA solution). This was mixed thoroughly and incubated for 20 minutes in the dark. After incubation, 0.1 ml of diluted Folin reagent was added and then incubated for 30 minutes at room temperature. After incubation, the absorbance of the solutions was read using a 117 controller based UV-Visible Spectrophotometer at 750 nm (Lowry *et al.,* 1951).

5.2.8 Estimation of Microbial Mass

The concentration of the VFAs was used to estimate the values of ATP using the equation described by Widiawati & Thalib (2009):

$ATP_{pr} =[2.5x(A)] +[2.75x(P)] +[3.5x(B)]$

where: ATP_{pr} – is the amount of ATP produced (mole)

(A)– is the concentration of acetate (mole)

(P) – is the concentration of propionate (mole)

(B) – is the concentration of butyrate (mole)

As Widiawati & Thalib (2009) suggested, the values of ATP produced and the values of ATP required for synthesizing a unit of the microbial cell were employed to predict the microbial protein synthesized in the rumen during fermentation. It is based on the assumption that each mole of ATP produces 10 g of the microbial cells. As a result, the microbial mass was computed using the equation described by Widiawati & Thalib (2009):

Microbial mass = 10 x ATP_{pr}

where: Microbial mass – is the amount of microbial cells produced (g)

ATP_{pr} – is the amount of ATP produced (mole)

5.2.9 Estimation of Ruminal Methane Gas Production

Ruminal methane gas produced during the fermentation of the diets was estimated from the VFA proportions using the equation described by Widiawati & Thalib (2009):

$CH_4 = [0.5x(A)] + [0.5x(B)] - [0.25x(P)]$

where: CH_4 – is the amount of methane produced (mole)

(A) – is the concentration of acetate (mole)

(B) – is the concentration of butyrate (mole)

(P) – is the concentration of propionate (mole)

5.3 Statistical Analysis

Data were subjected to an analysis of variance (ANOVA) procedure following a Randomised Complete Block Design (RCBD) using the Generalized Linear Model (GLM) procedure of SAS (SAS, 2009). The different means were separated using Tukey's Studentized Range (HSD) Test at P<0.05.

The statistical model of Randomised Complete Block Design (RCBD) was used:

$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$

where: Y_{ij} - the j^{th} observation of the i^{th} treatment

μ - the population mean

α_i - the effect of the i^{th} treatment, i = {1, 2, 3}

β_j - the effect of the j^{th} block

ε_{ij} - the random error of the i^{th} treatment on the j^{th} block

5.4 Results

5.4.1 *In vitro* Gas Production and Fermentation Characteristics

The result of the *in vitro* gas production and fermentation characteristics of the experimental diets and supplements are presented in Table 5.1. It shows that T3 (8.93 ml/200mg DM) produced the highest gas at 24 hr of incubation, while T1 (2.38 ml/200mg DM) produced the least. At 48 hr of incubation, T3 (1.42 ml/200mg DM) produced the least gas, and there was no significant difference between T4, T5, and T6.

The initial gas produced (A) was highest in T5 (1.41 ml/200mg DM), and no significant difference was observed across the treatments. The highest actual gas production (B) was recorded in T2 (5.6 ml/200mg DM), and there was no significant difference between T1, T3, T4, T5, and T6. The rate of gas production (C) was highest in T6 (8.18 ml/200mg DM), but there was no significant difference across the treatments. The total gas production (A+B) does not follow the same pattern as A and B as the highest gas was produced by T2 (7.18 ml/200mg DM), and there was no significant difference between T1, T3, T4, T5, and T6. The residual standard deviation (RSD) also varies, with T3 (2.84) having the highest and other treatments showing no significant difference.

The T3 diet (0.19 ml/200mg DM) produced the highest short-chain fatty acids (SCFA), and there was no significant difference across the treatments. The organic matter digestibility (OMD) was highest in T2 (29.99%), and the least was recorded in T3 (20.68%). T6 (6.86 MJ/kg DM) has the highest metabolizable energy (ME) while T1 (3.44 MJ/kg DM) has the least, and there was no significant difference between T2, T3, T4, and T5.

Table 5.1: Effect of supplementing lactating Holstein-Friesian cows with different levels of Moringa leaves and Lucerne hay on *in vitro* gas production and fermentation characteristics

Dietary treatments	**GP24**	**GP48**	**A**	**B**	**C**	**A+B**	**RSD**	**SCFA**	**OMD**	**ME**
T1	2.38^{a}	9.02^{b}	0.57^{a}	4.09^{b}	0.04^{a}	4.66^{b}	2.82^{a}	0.05^{a}	27.69^{b}	3.44^{c}
T2	5.02^{a}	11.40^{a}	1.22^{a}	5.60^{a}	0.05^{a}	7.18^{a}	2.54^{a}	0.11^{a}	29.99^{a}	3.99^{bc}
T3	8.93^{a}	1.42^{d}	0.27^{a}	3.40^{b}	0.10^{a}	3.66^{b}	2.84^{a}	0.19^{a}	20.68^{d}	4.37^{b}
T4	4.08^{a}	5.61^{c}	0.21^{a}	3.04^{b}	0.07^{a}	3.25^{b}	1.64^{a}	0.09^{a}	24.83^{c}	4.05^{bc}
T5	5.62^{a}	6.64^{c}	1.41^{a}	3.17^{b}	7.47^{a}	4.58^{b}	2.23^{a}	0.12^{a}	26.03^{bc}	4.83^{b}
T6	7.59^{a}	4.88^{c}	1.08^{a}	3.05^{b}	8.18^{a}	4.13^{b}	2.56^{a}	0.16^{a}	24.63^{c}	6.86^{a}
SEM	0.68	0.78	0.17	0.27	1.08	0.32	0.19	0.02	0.71	0.27
P	0.1086	<.0001	0.3877	0.0018	0.0216	<.0001	0.5788	0.1087	<.0001	<.0001

^{abcd}Means in the same column with the same letter are not significantly different at P<0.05; T1=Rhodes grass hay supplemented with Lucerne hay; T2=Rhodes grass hay supplemented with Lucerne hay and Moringa leaves; T3=Rhodes grass hay supplemented with Moringa leaves; T4=Lucerne hay; T5=Lucerne hay and Moringa leaves; T6=Moringa leaves; SEM= Standard error of the mean; GP24=gas production at 24 hr; GP48=gas production at 48 hr; A=initial gas produced; B=actual gas produced during degradation; C=the rate of gas production per hr; A+B=the total gas produced during fermentation; RSD=Residual Standard Deviation; SCFA=short chain fatty acids; OMD=organic matter digestibility; ME=Metabolizable energy.

Figure 5.1 presents the *in vitro* gas production measured at 0, 3, 6, 9, 12, 24, 48, 72, and 96 hr and shows the trends in the fermentation of the diets and supplements. This shows that T2 has the highest degradability. It can also be observed that the highest level of degradation for all the samples except T3 was observed at 48 hr before they started declining.

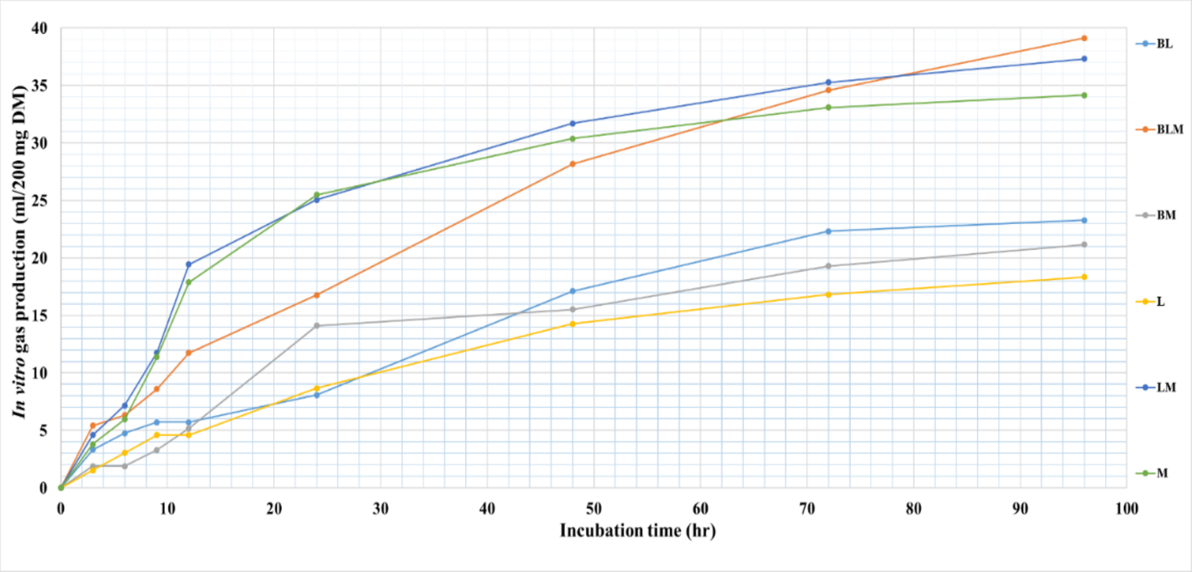

Figure 5.1: *In vitro* gas production of supplementing lactating Holstein-Friesian cows with different levels of Moringa leaves and Lucerne hay

5.4.2 Rumen Fermentation Kinetics

The effect of supplementing lactating Holstein-Friesian cows with different levels of Moringa leaves and Lucerne hay on their rumen fermentation kinetics is presented in Table 5.2. The highest pH was observed in T2 (6.5), and no significant difference was across the treatments. The Ammonia-N content significantly differed across the treatments, and the highest was recorded in T2 (41.03 mg/L). Supplementing with Moringa leaves did not affect the concentration of acetate, butyrate, or propionate ($P=0.0092$), and there was no difference ($P<0.0001$) in the acetate: propionate concentration across the treatments.

There was a decrease in protozoa number and an increase in microbial protein synthesis in the Moringa-supplemented treatment compared to the control. There was no significant difference ($P=0.0092$) across the treatments regarding the estimated methane gas and microbial biomass.

Table 5.2: Effect of supplementing lactating Holstein-Friesian cows with different levels of Moringa leaves and Lucerne hay on rumen fermentation parameters

Parameters	**Dietary treatments**			**SEM**	***P***
	T1 (control)	**T2**	**T3**		
pH	6.17^{a}	6.50^{a}	6.30^{a}	0.07	0.2563
Ammonia-N (mg/L)	10.31^{c}	41.03^{a}	24.25^{b}	4.67	0.0092
Acetate (mmol/L)	27.51^{a}	28.93^{a}	27.67^{a}	1.47	0.9912
Propionate (mmol/L)	41.27^{a}	43.40^{a}	41.50^{a}	2.21	0.9912
Butyrate (mmol/L)	55.02^{a}	57.86^{a}	55.33^{a}	2.95	0.9912
Acetate: Propionate	0.67^{a}	0.67^{a}	0.67^{a}	0.00	<.0001
Protozoa number (x10^3 cells/ml)	70.57^{a}	14.21^{b}	4.26^{b}	10.56	0.0017
Microbial protein (mg/L)	92.45^{a}	92.38^{a}	100.10^{a}	4.28	0.3697
Methane (mmol/L)	30.95^{a}	32.55^{a}	31.12^{a}	1.66	0.9912
Microbial mass (mmol/L)	3748.30^{a}	3941.70^{a}	3769.30^{a}	200.88	0.9912

^{abc}Means in the same row with the same letter are not significantly different at P<0.05; T1= Rhodes grass hay supplemented with Lucerne hay; T2= Rhodes grass hay supplemented with Lucerne hay and Moringa leaves; T3= Rhodes grass hay supplemented with Moringa leaves; SEM= Standard error of the mean.

5.5 Discussion

5.5.1 *In vitro* Gas Production and Fermentation Characteristics

The result from this experiment shows that the *in vitro* gas production and fermentation characteristics of the diets and supplements varied widely, and it showed that Moringa had the least digestibility, evident from its low gas production. This can result from anti-nutritional factors inhibiting microbial degradation but simultaneously increasing the flow of undegradable nutrients, such as RUP, to the small intestine for enzymatic degradation. These results show that supplementation with Moringa will improve the proper utilization of the nutrients by increasing the ratio of rumen undegradable protein to the rumen degradable protein, which leads to improved feed utilisation efficiency.

Microbial fermentation of carbohydrates results in SCFA, which is used as the main energy source by ruminants, and it is estimated that they produce around 75% of the metabolizable energy required by cattle (Bergman, 1990). This result showed that Moringa produced low SCFA and has low OMD, emphasizing its low susceptibility to microbial degradation in the rumen and increased flow of nutrients to the small intestine for enzymatic degradation.

5.5.2 Rumen Fermentation Kinetics

The rumen microbiome is critical to the host animal's effective feed breakdown and utilisation, ultimately affecting its nutritional status. It is believed that the rumen microbiota of cattle produces around 70% of the total energy substrates ingested and utilised by the animal through their fermentation characteristics (Hungate, 1966).

The rumen is an ideal habitat for the growth of anaerobic microbes, and the normal pH of grass-fed ruminants is between the range of 6-7 (Russell *et al.,* 2009). The pH observed in this experiment is within the range of 6.1-6.8, which shows that the animals had a healthy rumen, and the diets did not have a significant effect (P=0.2563) on the rumen pH. The result is consistent with that of Kholif *et al.* (2017), who studied the effect of Moringa leaf extract on rumen fermentation, and Soliva *et al.* (2005), who compared Moringa leaves with soybean and rapeseed meal.

The rumen Ammonia-N concentration ranged from 10.31-41.03 mg/L, and it falls within the range reported by Satter & Slyter (1974) to maximize microbial growth and activity in the rumen. The result showed an increase in rumen Ammonia-N concentration with supplementation with Moringa than the control diet. This shows that the level administered can enhance rumen fermentation by making N available for microbial growth and enhancing microbial protein synthesis (Soliva *et al.,* 2005). It explains the high microbial protein content observed in the Moringa-supplemented diet. However, Li *et al*. (2019) reported a decrease in ruminal Ammonia-N concentration in cows fed the Moringa diet compared to the control diet. Kholif *et al.* (2016) also observed a decrease in the concentration when they fed Moringa as fresh leaves, hay, or silage to lactating goats.

Ruminants can derive energy from complex carbohydrates through microbial fermentation to yield volatile fatty acids (VFAs), estimated to provide up to 75% of the total metabolizable energy (Bergman, 1990). The VFA production in the rumen depends on nutrient digestibility, the rate of absorption, the rate of digesta passage from the rumen, and the activity of the microbial population in the rumen (Li *et al.,* 2019).

This study showed no significant difference in the acetate, propionate, butyrate, and acetate: propionate concentration of the Moringa-supplemented treatments compared to the control. It can also be observed that there was an increase in the concentration of the VFAs with the addition of Moringa leaves, although the combination of Moringa and Lucerne showed the highest increase. This increase indicates an improvement in fermentation due to the increase in the microbial mass, mostly bacteria (Li *et al.*, 2019). This result agrees with Kholif *et al.* (2015) and Li *et al.* (2019), who reported that feeding Moringa improves the VFA concentration in lactating goats and cows.

The rumen protozoa have been demonstrated to enhance methanogenesis and impact intraruminal recycling of microbial protein even though they contribute to fibre degradation and stabilize ruminal pH changes (Puniya *et al.*, 2015). Some of the methanogenic bacteria have a symbiotic relationship with rumen ciliate protozoa and remain either inside the body of the protozoa or are attached to their surface, and this is the reason why defaunation is usually associated with reduced production of methane in the rumen as the methanogens lose their symbiotic association resulting in their reduced biological activity (Santra & Karim, 2003).

In this study, there was a drastic decrease in protozoa number in the Moringa-supplemented diet compared to the control diet, and this can be attributed to the active substances in Moringa leaves such as saponin, which has been documented as an antiprotozoal agent (Ebeid *et al.*, 2020). This decrease can also result from the unsaturated fatty acids present in Moringa leaves, as they are toxic to rumen ciliate protozoa (Moyo *et al.*, 2011) and cause defaunation. This result is consistent with the result of Abdel-Raheem & Hassan (2021), Ebeid *et al.* (2020), and Sultana *et al.* (2015), who reported that Moringa supplementation decreases the amount of rumen ciliate protozoa in buffalo calves, water buffaloes, and goats respectively.

As a consequence of the decrease in the protozoa numbers, an increase in the total bacterial population is expected as there is no predation of bacteria by the protozoa, and this will decrease the energy loss through methanogenesis by 5.5–7.9 % of gross energy intake (Mathieu *et al.*, 1996). The decrease in the estimated methane production in the Moringa-supplemented diet can also result from the secondary metabolites present in the leaves because they have an inhibitory effect on ruminal methanogenic bacteria (Bodas *et al.*, 2012). This result agrees with the report from Kholif *et al.* (2017) and Li *et al.* (2019), who used lactating goats and cows as their model animals to investigate the effect of Moringa on methane emission.

In this study, Moringa leaves generally had a positive effect on rumen fermentation, and studies have shown that low or medium levels of secondary metabolites in ruminant diets have a positive effect on rumen fermentation and productivity compared to high levels

(Salem *et al.,* 2014). The variation among the results of studies might be related to the nature and concentration of secondary metabolites in different leaves.

5.6 Conclusion

It is concluded that supplementation with Moringa leaves (at a 10% inclusion level) improves digestibility and rumen fermentation as they enhance fermentation kinetics and decreased methane production through effective modulation of the rumen microbiome. Moringa leaves can be used as supplements for dairy cows feeding on low-quality basal diets during periods of feed scarcity in agro-pastoral areas and therefore help to mitigate the feeding crisis as a non-conventional protein source for ruminants.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General Discussion

Milk production in Kenya accounts for a significant portion of the economy with the largest share coming from dairy cows (KALRO, 2022). Despite being a major driver of economic development, the growth of this key sector is constrained by seasonal fluctuations in feed availability which leads to a decrease in productivity (Njarui *et al.*, 2016). There is therefore a need to explore alternative feed resources such as non-conventional protein-rich forage trees with high nutrient content and available at an affordable cost, which can supplement protein and energy in dairy cattle during periods of feed scarcity (Silanikove, 2000). In this regard, the drought-tolerant Moringa (*Moringa oleifera*) tree can be considered one of the numerous top feed resources in the tropics as it is readily available and has been reported to enhance the health and performance of ruminants, such as oxidative status and milk production (Babiker *et al.*, 2016). It also improves their nutrition via microbial protein synthesis (Soliva *et al.*, 2005), and regulates rumen microflora as it contains large quantities of active substances, such as flavonoids, and other phenolic compounds (Kholif *et al.*, 2016). This study was conducted to investigate the use of Moringa as a feed supplement for dairy cows to improve basal feed utilization, milk yield, and quality.

Chapter three of this thesis dealt with the evaluation of the nutritional composition of each feed ingredient (Boma Rhodes hay, Lucerne hay and Moringa leaves) and when they were combined at different ratios to formulate diets. The assessment was carried out through proximate analysis, determination of fibre fractions, condensed tannins and total extractable phenolic. The nutritional evaluation showed that Moringa leaves have high crude protein, ether extract, ash content, dry matter digestibility, digestible energy and metabolizable energy but low crude fibre, organic matter neutral detergent fibre, acid detergent fibre and acid detergent lignin while the content of anti-nutritional factor was generally negligible when compared with Lucerne and Boma Rhodes. These results show that Moringa is a suitable protein supplement rather than a basal diet as it improves the crude protein content and reduces the fibre content when combined with other ingredients, it’s also a good source of mineral, unsaturated fatty acids and rumen undegradable protein evident from the low amount of tannins.

Chapter four described the effect of Moringa supplementation on feed intake, milk yield and milk composition in lactating dairy cows. The supplementation of Moringa leaves (up to 10%) increased the feed intake which might be associated with its low rumen degradability and increased microbial activity in relation to the increased level of essential nutrients that are available to the rumen microbes. The increase in milk yield is also a result of the increased feed intake, higher level of ruminal undegraded protein and generally the improved rumen fermentation. However, Moringa supplementation did not have a significant effect on the milk composition even though the solids non-fat (SNF), protein, and lactose concentration increased while the total solids and fat slightly decreased as the level of Moringa in the diet increased. The result shows that the cows supplemented with Moringa leaves had higher milk production efficiency than the control, and supplementation with Moringa leaves can improve the profitability and sustainability of dairy production.

Chapter five examined the effect of Moringa supplementation on *in vitro* digestibility and rumen fermentation kinetics in terms of pH, NH_3-N, volatile fatty acids (VFA), protozoa population, microbial protein synthesis, microbial mass and rumen methane gas emission. The results from this experiment showed that the Moringa-supplemented treatments generally had low gas production which may explain its low ruminal degradability and high enzymatic digestion in the small intestine. Moringa leaves generally had a positive effect on rumen fermentation kinetics as it increased the NH_3-N available to the rumen microbes. This led to the increased synthesis of microbial protein which is utilized by the ruminant animals. There was also a notable decrease in protozoa numbers in the rumen and this is likely to translate to an increase in the population of beneficial bacteria such as cellulolytic bacteria and a reduction of methanogenic bacteria as evident from the improvement of fibrolytic activity in the rumen and decrease in ruminal methane production. This study has shown that the presence of low to medium rather than high levels of secondary metabolites from forage plants such as Moringa may have a positive effect on rumen fermentation and productivity in ruminants.

6.2 Conclusions

i. The nutritional composition of the diets and supplements varied significantly, and the nutritive value of Moringa leaves showed its potential as a protein supplement for ruminant animals feeding on coarse fodders (such as Rhodes grass hay) or crop residues in arid and semiarid regions.
ii. Supplementation of Rhodes grass hay with Moringa leaves improved the feed intake, milk yield, and milk production efficiency with no effect on the milk composition

compared to the Lucerne-supplemented treatment. Moringa leaves can be used to improve feed utilization efficiency and reduce production costs.

iii. Supplementation of Rhodes grass hay with Moringa leaves improved digestibility and rumen fermentation as it enhanced fermentation kinetics and decreased methane production through effective modulation of the rumen microbiome. Moringa leaves can serve as a non-conventional protein source for ruminants to improve productivity.

6.3 Recommendations

i. Dairy farmers in the arid and semi-arid regions where high-quality forage is limited should consider adding Moringa leaves to the feeding regimen to optimize the nutritive value and overall herd health.

ii. An inclusion rate of 10% Moringa leaves (replacing 10% Lucerne) in the diets of dairy cows can improve performance in terms of feed intake and milk production of the lactating cows under the current experimental conditions.

iii. Moringa leaves can be used as a protein supplement for dairy cows fed on low-quality diets during feed scarcity as it can effectively modulate the rumen microbiome, enhance fermentation kinetics, and decrease methane production, which will help to mitigate the feeding crisis serve as a climate-smart feed resource.

6.4 Areas for further studies

i. A study should be conducted to determine the cost-benefit analysis of supplementing the recommended combinations in this study when compared to the use of conventional concentrates that are available in the market when feeding them to lactating dairy cows.

ii. A study should be conducted to explore further the effect of using Moringa leaves as a sole supplement at different inclusion levels on the performance of dairy cows.

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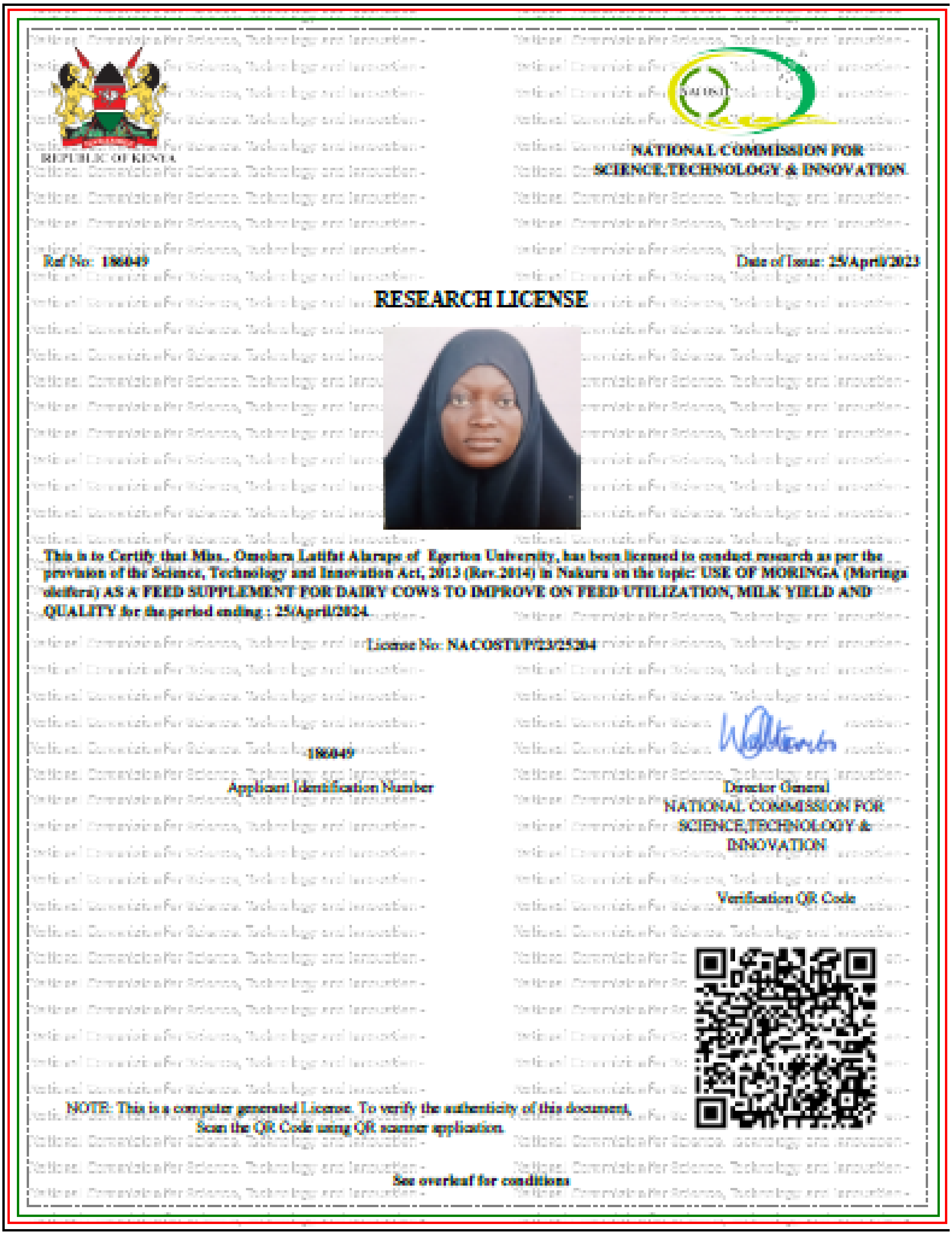
APPENDICES

Appendix A: NACOSTI Permit



REPUBLIC OF KENYA

NATIONAL COMMISSION FOR
SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 180049

Date of Issue: 25/April/2023

RESEARCH LICENSE

This is to Certify that Miss.. Omolara Latifat Alarape of Egerton University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Nakuru on the topic: USE OF MORINGA (Moringa oleifera) AS A FEED SUPPLEMENT FOR DAIRY COWS TO IMPROVE ON FEED UTILIZATION, MILK YIELD AND QUALITY for the period ending : 25/April/2024.

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Appendix B: Ethical Clearance

EGERTON UNIVERSITY

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EGERTON

EGERTON UNIVERSITY INSTITUTIONAL SCIENTIFIC AND ETHICS REVIEW COMMITTEE

EU/RE/DVC/009
Approval No. EUISERC/APP/213/2023 ***3rd February, 2023***

Alaope Omolara Latifat
Department of Animal Sciences
Egerton University
P. O. Box 536
Egerton
Telephone: [illegible]
E-mail: [illegible]

Dear Alaope,

RE: ETHICAL APPROVAL: USE OF MORINGA (MORINGA OLEIFERA) AS A FEED SUPPLEMENT TO IMPROVE ON FEED UTILIZATION, MILK YIELD AND QUALITY OF DAIRY COWS

This is to inform you that ***Egerton University Institutional Scientific and Ethics Review Committee*** has reviewed and approved your above research proposal. Your application approval number is ***EUISERC/APP/213/2023***. The approval period is ***3rd February, 2023 – 4th February, 2024***.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. You are required to adhere Institutional Experimental Animals use and Care policy.
iii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***Egerton University Institutional Scientific and Ethics Review Committee***
iv. Death and life-threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***Egerton University Institutional Scientific and Ethics Review Committee*** within 72 hours of notification
v. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***Egerton University Institutional Scientific and Ethics Review Committee*** within 72 hours.
vi. Clearance for Material Transfer of biological specimens must be obtained from relevant institutions.
vii. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
viii. Submission of an executive summary report within 90 days upon completion of the study to ***Egerton University Institutional Scientific and Ethics Review Committee***.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely

Date. [illegible]

Prof. Raphael M. Nguru
CHAIRMAN, EGERTON UNIVERSITY INSTITUTIONAL SCIENTIFIC AND ETHICS REVIEW CTTEE
RMN/BC

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Appendix C: ANOVA Statistical Output

The GLM Procedure

Dependent Variable: Crude protein (CP)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	75407.14463	12567.85744	180.37	<.0001
Error	14	975.49467	69.67819		
Corrected Total	20	76382.63930			

R-Square	Coeff Var	Root MSE	CP Mean
0.987229	4.355375	8.347346	191.6562

Tukey's Studentized Range (HSD) Test for CP

Note: This test controls the Type I experiment wise error rate, but it generally has a higher Type II error rate than REGWQ.

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Trt
A	295.047	3	T6
B	252.933	3	T5
C	207.163	3	T4
D	171.733	3	T2
D	155.577	3	T3
D	152.403	3	T1
E	106.737	3	T7

The GLM Procedure

Dependent Variable: Milk yield (MKYD)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	66	542.5193070	8.2199895	43.27	<.0001
Error	464	88.1366121	0.1899496		
Corrected Total	530	630.6559190			

R-Square	Coeff Var	Root MSE	MKYD Mean
0.860246	11.74565	0.435832	3.710584

Tukey's Studentized Range (HSD) Test for MKYD

Note: This test controls the Type I experiment wise error rate, but it generally has a higher Type II error rate than REGWQ.

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Trt
A	4.14266	177	T3
B	3.63429	177	T2
C	3.35480	177	T1

The GLM Procedure

Dependent Variable: Feed intake (FI)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	63	1489.744036	23.646731	15.78	<.0001
Error	440	659.456707	1.498765		
Corrected Total	503	2149.200743			

R-Square	Coeff Var	Root MSE	FI Mean
0.693162	9.305252	1.224241	13.15645

Tukey's Studentized Range (HSD) Test for FI

Note: This test controls the Type I experiment wise error rate, but it generally has a higher Type II error rate than REGWQ.

Means with the same letter are not significantly different.			
Tukey Grouping	**Mean**	**N**	**Trt**
A	13.6943	168	T3
B	13.3213	168	T1
C	12.4538	168	T2

The GLM Procedure

Dependent Variable: Protozoa number (PRZ)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	7833.132271	1958.283068	40.23	0.0017
Error	4	194.687741	48.671935		
Corrected Total	8	8027.820012			

R-Square	Coeff Var	Root MSE	PRZ Mean
0.975748	23.50705	6.976527	29.67844

Tukey's Studentized Range (HSD) Test for PRZ

Note: This test controls the Type I experiment wise error rate, but it generally has a higher Type II error rate than REGWQ.

Means with the same letter are not significantly different.			
Tukey Grouping	**Mean**	**N**	**TRT**
A	70.565	3	T1
B	14.208	3	T2
B	4.262	3	T3

Appendix D: Publications

International Journal of Veterinary Sciences and Animal Husbandry 2023; 8(4): 232-237

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Nutritional evaluation and *in vitro* dry matter digestibility (IVDMD) of *Moringa oleifera, Medicago sativa, Chloris gayana* and their combinations at different ratios

OL Alarape, PK Migwi and JO Ondiek

DOI: https://doi.org/10.22271/veterinary.2023.v8.i4d.602

Abstract
The major factor limiting ruminant production in the tropics is the lack of a year-round supply of quality forage and protein supplements. Therefore, the use of underutilized shrubs and trees such as *Moringa oleifera* is noteworthy due to its rich nutritional composition and potential as a feed resource for ruminants. This study investigated the nutritional composition of *Moringa* leaves, Lucerne hay, and Rhodes grass hay with their combinations at different ratios. The ingredients were grouped into seven in ratios 90:10:0, 90:5:5, 90:0:10, 0:100:0, 0:50:50, 0:0:100, and 100:0:0 of Rhodes grass hay, Lucerne hay and *Moringa* leaves respectively. Proximate, fibre, total phenolic and condensed tannin contents were used to determine the nutritional value of these feed ingredients. The *in vitro* dry matter digestibility was also determined and the result was used in estimating the metabolizable energy (ME), organic matter digestibility (OMD) and short-chain fatty acids (SCFA). *Moringa* has the highest CP, EE and NSC. There was no significant difference ($p<0.05$) in the ash content of Lucerne, *Moringa* and the combination of the two. The NDF, ADF, ADL and CF contents were significantly lower ($p<0.05$) in *Moringa* than in others which explains the increased ($p<0.05$) dry matter digestibility, DE and ME observed in *Moringa*. The organic matter content and hemicellulose were significantly lower ($p<0.05$) in *Moringa*. *Moringa* has the highest total phenolic (6.39%) and the least condensed tannins (0.13%) when compared with the combination of Lucerne and *Moringa*. The gas production was significantly lower ($p<0.05$) in *Moringa* leaves despite its high ME. The results show that *Moringa* reflects a desirable nutritional balance which shows that it can serve as a protein supplement for ruminants including dairy cows and also a substitute for Lucerne which is the major protein supplement utilized by ruminant producers in the tropics.

Keywords: Digestibility, fibre fractions, lucerne, *Moringa*, proximate, ruminants

1. Introduction

Milk production in Kenya accounts for a significant portion of the economy estimated at about 5% of GDP, and the majority of the producers are smallholder dairy farmers who account for 70-80% of Kenya's dairy producers [18]. This implies that they can be used as a tool for economic development, food security, and poverty reduction [33].

Dairy cows are fed on forages such as Napier grass, Rhodes grass, Lucerne, and crop residues which are supplemented with concentrate or compound dairy meal. However, most smallholder farmers are found in the highlands, where demand for food crops restricts access to grazing areas and the availability of feed and fodder [4]. Most of these farmers are also unable to afford high-quality concentrates and supplementary feeds [28] which has led to the challenge of inadequate feed and feeding in dairy production.

One possible option to this challenge of inadequate supply of high-quality forage is the exploration and exploitation of non-conventional feed resources such as protein-rich forage trees with high nutrient content, a balanced profile of amino acids, a reasonable cost and a large proportion of digestible fibre which can serve as a supplement for dairy cows during periods of feed scarcity.

Fodder trees and shrubs have been reported to improve the quality and availability of feed for animals as they are a good and inexpensive source of protein and micronutrients [19].

ID: 163

Effect of Replacing Alfalfa Hay with Dried Moringa Oleifera Leaves on Rumen Fermentation, Rumen Microbial Protein Synthesis and Methane Production in Lactating Dairy Cows

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Abstract

Alteration of the microbial ecosystem and ruminal function can be used to improve feed utilization and production performance of ruminant animals. This study aimed to assess the effect of supplementing Moringa leaves in dairy cows fed on basal Rhodes grass hay on rumen fermentation, microbial protein production, and ruminal methane gas production. Nine lactating Holstein-Friesian cows (444±39.7 kg) were randomly assigned to three dietary treatments supplemented with 0, 5 and 10% of Moringa leaves in place of 10, 5 and 0% of Lucerne hay on a DM basis in a randomised complete block design for 8 weeks. There was no significant difference in the pH, VFAs, estimated methane, microbial protein, and microbial mass, even though there was a variation in the Moringa-supplemented treatment and the control. Compared with the control, Moringa leaves decreased (P=0.0017) the rumen protozoa and increased (P=0.0092) rumen Ammonia-N concentration. It is concluded that Moringa leaves enhanced rumen fermentation kinetics in lactating Holstein-Friesian cows and can be used as a protein supplement for dairy cows and a substitute for Lucerne hay.

***Keywords**: Ammonia-N, Feed efficiency, Methane, Moringa oleifera, Ruminal fermentation*

Introduction

Despite the economic potential of the dairy industry in developing countries, they are constrained by numerous factors, including a shortage of year-round supply of high-quality feeds, low-quality milk-producing breeds, poor management, and their impacts on climate change, and this has led to poor livestock productivity (Hao et al., 2017).

Smallholder dairy farmers constitute the higher percentage of dairy producer in the tropics, and they use crop residues for their livestock with little or no supplementation and their storage methods are insufficient to preserve the quality of the feeds, which results in seasonal variations in feed availability and a reduction in milk production (Njarui et al., 2016).

Animal nutritionists and microbiologists have altered the microbial ecosystem and rumen function to improve feed utilization and performance by including Ionophores and antibiotics. This intervention's metabolism and residual effect have led to anti-microbial resistance, which has caused great public concern and strict regulations on ruminant production (Matloup et al., 2017).

Hence, there is a need to explore alternative resources, such as fodder trees and shrubs, that can replace antibiotics in manipulating the rumen function to increase productivity on a sustainable basis. In this regard, the drought-tolerant Moringa (*Moringa oleifera*) tree can be considered one of the numerous top feed resources in the tropics. Moringa is a tree native to India but has spread to many areas worldwide. It's a perennial evergreen tree (Palada et al., 2007) whose forage contains 5.9% moisture, 38.6% carbohydrates, 27.2% protein, and 17.1% fat (Yameogo et al., 2011). Moringa's versatility allows multiple harvests throughout the growing season, and the dried leaves can be stored for extended periods without losing nutritional content (Mendieta-Araica et al., 2011).

Numerous studies have demonstrated that Moringa can enhance the health and performance of ruminants, such as oxidative status and milk production (Babiker et al., 2016), improve their nutrition via microbial protein synthesis (Soliva et al., 2005), and regulate rumen microflora as it contains large quantities of active substances, such as flavonoids, and other phenolic compounds (Kholif et al., 2016). The various nutrients in Moringa, including protein, fatty acids, minerals, and vitamins, make it a good feed resource for livestock (Moyo et al., 2012).

Several studies have reported that the inclusion of Moringa leaves in ruminant diets have improved animal performance quantitatively and qualitatively (Mendieta-Araica et al., 2011; Sultana et al., 2015; Babiker et al., 2016; Cohen-Zinder et al., 2016; Kholif et al., 2016; Kholif et al., 2017; Zeng et al., 2018). Therefore, this study aimed to assess the effect of supplementing Moringa leaves in dairy cows fed basal Rhodes grass hay on ruminal fermentation, microbial protein production, and ruminal methane gas production.