LACTATION PERFORMANCE, PROFITABILITY AND MASTITIS SUSCEPTIBILITY OF HOLSTEIN FRIESIAN COWS FED RUMEN PROTECTED METHIONINE IN KENYA

By

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SEPTEMBER, 2023

DECLARATION

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

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DEDICATION

I dedicate my thesis work to my family and friends. A special feeling of gratitude to my loving wife Mercyline Owaga whose words of encouragement and push for tenacity kept me going throughout the period and to my son Shiloh Mitch Owaga whose quality time with daddy had to be cut short a few times for me to catch up with this work.

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ABSTRACT

The purpose of the research was to determine how rumen-protected methionine (Mepron®) affects milk output, cost efficiency, and mastitis resistance in dairy cows in Kenya. This research also aimed to address the issue of high cost of dairy concentrate feeds that emerges from formulating diets based on crude protein. Majority of diets intended for high-yielding dairy cows, are designed with crude protein of 17 to 18%. To achieve this high crude protein level, additional protein sources are required, resulting in more costly dairy feed. This study was carried out in a commercial dairy farm (Risa Farm) located in Ngecha ward, Limuru sub-county, Kiambu County, Kenya. Twelve Holstein Friesian dairy cows in their transition period (<100 in milk) usually when methionine need is highest, were recruited for this study. The cows chosen ranged in parity from the first to the fourth. Four replicates of each of the three treatments (T1, T2, and T3) were used in a completely randomized design (CRD). The treatment diets comprised dairy meal adjusted for amino acids using Mepron® (T1), commercial dairy meal prepared using crude protein (T2) and farm dairy meal developed using crude protein (T3). Near Infra-Red Reflectance (NIR) technique was used to determine the proximate and amino acid content of the ingredients used to create the treatment diets. AMINOCow software was used to design the rations and feed optimizer (Brill) was used to optimize the treatment rations. Milk production for each cow was recorded every day at 10 am, 5 pm, and 2 am. California mastitis test (CMT) was done on each cow every two days throughout the experimental period. Data was collected for 7 weeks, after one week of acclimatization of the cows to the treatment rations and procedures. The gathered data was submitted to analysis of variance, where means with $p < 0.05$ were considered significant and Student Newman Keuls (SNK) test used to distinguish the significant means. R software for Windows (Version 4.0.2) was used to run all statistical analyses. In the cow group given treatment T1 (dairy meal balanced for methionine using Mepron®), the findings revealed a numerical increase of milk output of 1.2 liters per day during the course of the trial. Throughout the course of the trial, the milk output of the cows given treatments T2 and T3 decreased by 2.62 and 1.60 liters per day, respectively. In T1, the total performance exhibited an upward trend, which was statistically significant ($p = 0.004$). Methionine levels in the treatment meals and cow milk output were shown to be positively correlated. Mastitis incidence was significant across the groups. Mepron® group (T1), followed by T3 (Farm dairy meal), and T2 had considerably greater score 0 (-ve Mastitis) and 1 (+ve mastitis in one quarter, respectively) (Commercial dairy meal). A score of 3 and 4 were not found in the Mepron® group (T1), which emphasizes the association between methionine adequacy and the prevalence of mastitis. Dairy ration balancing using Mepron (T1) permitted feed cost decrease of 19.44% compared to T3 and 0.04% compared to T2 rations. This research shows that using rumen protected such as Mepron®) to balance dairy rations lead to increased milk output, decreased mastitis occurrences, and is cost-effective.

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LIST OF ABBREVIATIONS

AA	Amino Acid
ADF	Acid Detergent Fiber
BHBA	β-hydroxybutyrate
CM	Clinical Mastitis
CMT	California Mastitis Test
CP	Crude Protein
DM	Dry Matter
DMI	Dry Matter Intake
EAA	Essential Amino Acids
GDP	Gross Domestic Product
Hcy	Homocysteine
Ile	Isoleucine
ILRI	International Livestock Research Institute
Leu	Leucine
MER	Metabolizable Energy for Ruminants
MUN	Milk Urea Nitrogen
NIR	Near Infra-Red Reflectance method
NRC	National Research Council
Phe	Phenylalanine
PRM	Partial Mixed Ration
RPAA	Rumen Protected Amino Acid
RP-Met	Rumen Protected Methionine
SCC	Somatic Cell Count
SCM	Subclinical Mastitis
SPSS	Statistical Package for Social Sciences
Tau	Taurin
Thr	Threonine
TMR	Total Mixed Ration

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CHAPTER ONE: INTRODUCTION

1.1 Background

Feed is a crucial component of the dairy production value chain since it influences the productivity and, therefore, the profitability of dairy operations (Britt, 2017). Animal output and health have been enhanced by the application of contemporary innovations and advanced technology in animal feeding. In dairy animal nutrition, amino acids may be one of the limiting dietary components, especially for lactating dairy cows. This is particularly true for cows in early lactation, when dry matter (DM) intake is low but amino acid needs are high (Ji & Dann, 2013).

In East Africa, cottonseed cake, sunflower meal, sunflower cake, soya bean meal, full-fat soya, alfalfa, and copra are employed as protein (amino acid) sources in the composition of dairy rations. However, these resources are expensive, seasonal, and scarce (ASARECA, 2013).

Traditionally, dairy ration formulation has been based on common parameters including calories, carbohydrates, fiber, protein, minerals, and vitamins (Appuhamy, 2014). The issue with this method is that the majority of these "nutrients" are not what the animal body needs, particularly for development and production (Batistel, 2017). This approach lacks the precision necessary for making reliable nutritional balance assessments. In addition, there is a continuous problem with component availability and rising protein source prices (Lukuyu & Gachuiri, 2019). The majority of dairy diets, especially those intended for high-yielding dairy cattle, are designed to include 17 to 18 percent crude protein (CP). To achieve this CP level, additional protein sources are required, resulting in more costly dairy feed (National Research Council (Cabrita, 2011).

The process by which "protein" is absorbed by the rumen bacteria is very difficult to predict and may be inefficient, therefore feeding a high-protein diet to a high-producing dairy cow is not a realistic approach of meeting its nutritional needs (Schwab, 2017). Protein is not required for animal survival. Instead, they need certain amino acids, the building blocks of proteins (Cabrita, 2011). The primary source of these amino acids in ruminants is ruminal bacterial biomass, which is controlled by a number of nutritional and management characteristics (Batistel, 2017). High levels of

CP in the diet are associated with increased levels of urea nitrogen in the blood, which is associated with decreased fertility in lactating dairy cows (Guo et al., 2014).

The amino acid composition of rumen microbial protein is analogous to that required for growth and milk production, making it a well-balanced protein source. However, rumen microbial protein synthesis cannot provide sufficient amino acids to satisfy the demands of cows generating tremendous quantities of milk (Pszczolkowski, 2020). It is crucial to optimize the amino acid supply in dairy feeds in order to increase protein intake, milk production, and milk solids.

Supplementing dairy rations with rumen-protected amino acids that limit milk production and milk protein synthesis may compensate for a deficiency of metabolizable protein in the majority of dairy cow diets, particularly those designed according to crude protein (CP) content (Sinclair, 2013). Methionine and lysine are typically the first amino acids to reduce milk production, according to Appuhamy (2014). The benefits of harmonizing amino acids in dairy cattle include enhanced milk production, liver function, fertility, reduced environmental impact, and increased profitability (Nocek, 2015).

There is mounting evidence that supplementing animal diets with specific amino acids increases their resistance to viral diseases (Yu-long Yin, 2017). The three sulfur amino acid factors glutathione (GSH), homocysteine (Hcy), and taurin (Tau) regulate the inflammatory component of the immune response in vitro and in vivo (Yu-long Yin, 2017). Methionine consumption temporarily increases plasma GSH, Hcy, and Tau levels (Robert, 2006). Glutathione conjugates with a variety of electrophiles and xenobiotics to eliminate reactive oxygen species and free radicals (Feng et al., 2017). There is evidence that intracellular GSH concentrations modulate cellular signaling pathways (particularly the nuclear transcription factor pathway) in response to immunological stresses (Fratelli et al., 2015). This study investigated the aforementioned benefit of methionine by comparing the incidence of mastitis in animals supplemented with rumen-protected methionine to that of animals not supplemented with rumen-protected methionine and total methionine levels.

In the absence of adequate quantities of an essential amino acid in the diet, the rate of protein synthesis is limited by the availability of that amino acid. The first limiting amino acid is the amino acid that becomes deficient in the diet first (Lapierre, 2017). The primary amino acid that inhibits milk production is methionine (Appuhamy et al., 2011). To meet methionine requirements, the ration must contain a high concentration of CP, which may result in expensive animal feed due to the expensive nature of CP sources. This requirement, however, can be met by supplementing the dairy diet with rumen-protected methionine, which has been shown to be more cost-effective (Batistel, 2017).

Schwartz and Broderick (2017) state that commercially produced amino acids fed to cattle must be protected from microbial degradation in the rumen. Rumen protected methionine is produced by coating a small pellet of methionine with a chemical that prevents rumen microbes from destroying the methionine while still providing sufficient methionine to the lower stomach. Amino acids have been commercially coated with ethyl-cellulose, copolymer, lipids, and fatty acids, among other polymers. It is crucial to evaluate the coating since it determines whether Methionine will undergo slow-release post-rumen, fast-release post-rumen, or no release (Cow Protected) in certain circumstances. Using rumen-protected methionine permits more cost-effective amino acid balancing close to dairy cow needs (Lapierre, 2017). This research aimed to determine how feeding rumen protected methionine affects lactation performance and mastitis resistance in lactating Holstein Friesian cows.

1.2 Statement of the Problem

In the milk production value chain, feed accounts for more than 70 percent of overall production costs. Rations prepared with unprocessed protein are expensive because protein supplies are scarce and compete with fodder for other species, such as chickens and pigs. It has been discovered that supplementing dairy cows with rumen-protected methionine improves lactation performance by decreasing the quantity of dietary protein required for milk production. This study sought to address the issue of the high cost of concentrate feeds that results from using unrefined protein as the primary constituent in diet formulation.

Worldwide, mastitis is a prevalent condition. It has significant economic implications for dairy operations. It diminishes milk quality and bovine welfare, agitates producers, and poses a public health risk. Methionine has been implicated as playing a significant role in enhancing the immune system of dairy cattle and providing rumen-protected amino acids. It has been demonstrated that a relatively high level of methionine supplementation increases the proliferative response of peripheral T-lymphocytes in dairy heifers.

1.3 Justification of the Study

Kenya mainly depends on Uganda, Tanzania, and sometimes India for the supply of oilseed cakes such as Sunflower Seed Cake, Cotton Seed Cake, and Canola cake, which are the principal protein source needed to create animal feed (Kenya Market Trust., 2017). Over time, these components have gotten more costly because to strong demand induced by the expanding feed businesses in Tanzania and Uganda, resulting in international rivalry for the commodities (Kenya Market Trust., 2017).

In Kenya, dairy feed formulation is still reliant on crude protein, with high-yielding dairy meal having a CP content of 17.5%. (EAS, 2017). To reach this high CP level, dairy rations must include high levels of the aforesaid pricey components, resulting in expensive concentrate feed (Kenya Market Trust, 2017). This has a detrimental effect on farm-level milk production costs.

Even at high protein levels, their diet is often low in Methionine and Lysine, the limiting amino acids for milk production that are essential for boosting milk protein and solids (Schab & Glen, 2017). Mastitis is the most common production illness in the world's dairy herds (Gera & Guha, 2011). Infectious agents, host resistance, and environmental variables interact to determine the incidence of illness (Gera & Guha, 2011). It is a worldwide issue that negatively impacts animal health, milk quality, and the economics of milk production, resulting in enormous financial losses (Sharma et al. 2017).

In the present study pure breed Holstein dairy were considered because it’s the main exotic breed used for milk production on large, medium and small farms in Kenya (Lukuyu, 2019). Holstein Friesian produce more milk their hence their nutrient requirement and hence amino acid requirement is higher than other breeds (ristensen, 2015) making it ideal for the present study. Selective pressure for increased milk production in Holstein Friesian has led to a higher propensity to mastitis (Berry, 2011) one of the parameters we were investigating in this study

Molito dairy farm was ideal for this study because it had pure breed Holstein Friesian cows bred locally over the years with genetics from reputable breeders like American Breeders' Service (ABS) and World-Wide Sires. The farm also had farm management software that enabled easy access to important data on cows' traits suitable for this experiment

1.4 Significance of the Study

The use of rumen-protected amino acids allows for the balancing of cow amino acid requirements while minimizing crude protein consumption (William & Charles, 2016). When rumen protected methionine is used in a well-balanced feed, the crude protein content is lowered to receive a cost advantage from the rumen protected methionine while also reaping the benefits of enhanced milk production and reduced nitrogen output (Arriola Apelo, 2014). Prudent concentrate feed composition to include rumen protected methionine plays an important part in guaranteeing feed cost efficiency, since low protein diets that are less expensive may be fed to achieve the same or better performance of the cow in terms of milk output.

Balancing rations with rumen protected methionine offers the added advantage of lowering mastitis occurrences. This could be related to the fact that methionine plays a key role in immunity because it is involved in the glutathione mechanism, which is the major antioxidant in cells that influences inflammatory aspects of immune response and improves liver function, allowing the animal to fight infections of economic importance like Mastitis more effectively.

1.5 Study Objectives

1.5.1 General Objective

To contribute to enhanced lactation and economic performance of dairy cattle production in Kenya by using rumen protected methionine (Mepron®) in dairy rations.

1.5.2 Specific Objectives

i. To determine the effect of inclusion of rumen protected methionine (Mepron®) in lactating dairy cow diets on milk production in Kenya.

ii. To determine the effect of inclusion of rumen protected methionine (Mepron®) in lactating dairy cow diets on their resistance to mastitis.

iii. To carry out cost benefit analysis of using rumen protected methionine (Mepron®) to balance ration of dairy cows in Kenya.

1.6 Hypotheses

i. Ho: Milk production is not significantly different for cows fed ration balanced for amino acid as compared to cows fed high CP ration.

ii. Ho: Resistance to mastitis is not significantly different for cows fed ration balanced for amino acid as compared to cows fed high CP ration.

iii. Ho: Dairy ration balanced for amino acid using rumen protected methionine is not significantly cheaper than rations formulated based on CP.

1.7 Conceptual Framework

In this research, the dependent variables include milk output, mastitis incidence, and the cost-effectiveness of rations. The independent variable is the diet balanced for amino acids using Mepron® and diets constructed on crude protein without rumen protected amino acid from a commercial animal feed manufacturer and a farm-formulated ration. Broderick (2013) predicted that cows fed diets balanced for amino acid with rumen protected methionine would have increased milk output and fewer instances of mastitis compared to cows fed diets prepared on the basis of crude protein with no RM-Met added (Curtis et al., 2015).

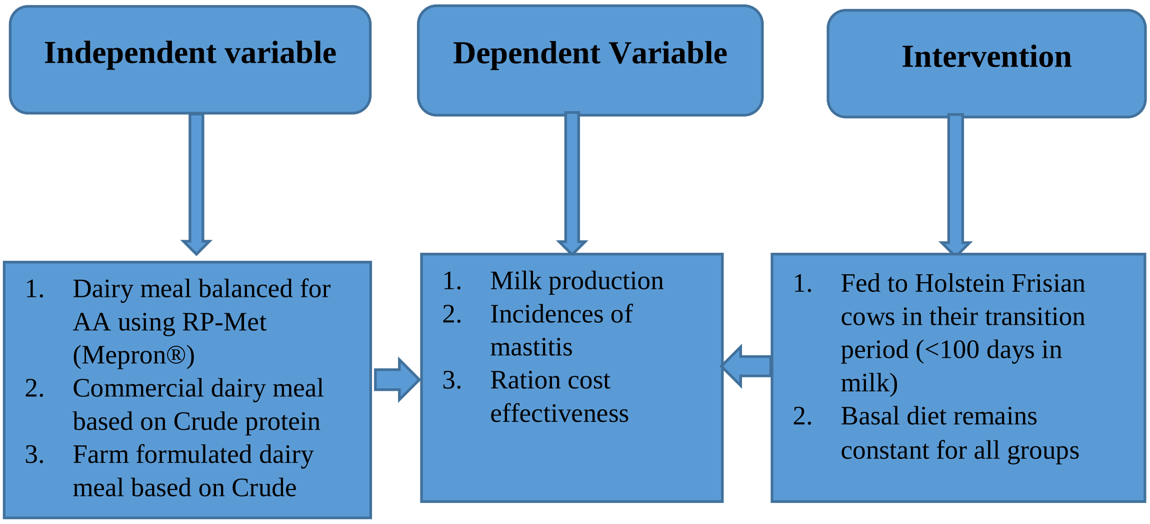

Figure 1.1 Conceptual Framework

Source: (Casanave, 2015)

1.8 Limitations to the Study

It was also assumed that the mixer wagon gave a homogenous mix of the Total Mixed Ration (TMR) throughout the experiment. However, this was not realized 100% because on two occasion the mixer wagon broke down and the farm did not have a spare one hence the TMR mixing was done manually on these two occasions.

The analyses of the amount of supplemented/added methionine in each treatment sample was carried out by Evonik Industries Ltd wet chemistry laboratory in Germany since the satellite lab in Kenya did not have calibration for analyzing added/supplement Methionine.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of the Dairy Industry

Kenya's dairy sector accounts for 6-8% of the country's gross domestic product (Muriuki, 2011). It is a large livestock sector activity and a significant source of income for approximately one million small-scale farmers (Muriuki, 2011). It accounts for more than 85 percent of the dairy cow population in East Africa and is the fastest-growing dairy subsector in Sub-Saharan Africa (Muriuki, 2011). Kenya is anticipated to have 4.3 million dairy cattle, producing 3.43 billion liters of milk, accounting for 18% of Sub-Saharan Africa's 3% global contribution (KNBS, 2019).

The sector is dynamic, with high growth rates for sold milk and investments by dairy societies and processors, primarily in cold chain, extended shelf-life (ESL) milk, and milk powder production. In 2011, approximately 80% of Kenya's milk production (5 billion liters) came from small-scale farmers (2021KDB). However, an increasing number of medium-sized producers and investors are investing in contemporary and commercial dairy production (KDB, 2012). The top four milk processors in Kenya are Brookside, NKCC, Githunguri, and Sameer, accounting for 85 percent of the daily milk processing volume of 1.5 million kilograms (KDB, 2012). The market is dominated by Brookside Dairy Ltd. (KDB, 2012). Even though the market for refined milk and milk products has grown substantially over the past decade, between 70 and 80 percent of milk is still supplied to consumers through the raw milk market.

.Friesian, Guernsey, Ayrshire, Jersey, and brown Swiss are the most popular dairy breeds in Kenya (Kibiego et al., 2015). To increase the dairy livestock, these exotic strains are crossed with native East African Zebu (Omore et al., 2013). Even though small-scale milk production in Kenya is a viable economic venture, it is hindered by a lack of nutritional and management knowledge, an inadequate quantity and quality of feeds, limited access to breeding, diseases, limited access to credit facilities, and limited access to output markets. The average annual cattle yield in Kenya is 4,000 kg (ILRI, 2017). This is lower than the worldwide average of 9000 kg per cow per year (Technology serve, 2013). Seasonality in milk production, milk quality, a substantial dearth of knowledge and skills, substandard service provision and input supply, excessive supply chain fragmentation, and the absence of inclusive business models all inhibit the industry's growth and competitiveness (KDB, 2012).

2.2 Overview of the Kenyan Animal Feed Industry

The magnitude of Kenya's animal feed industry has expanded steadily from 2013 to date, mostly due to the development of the livestock sector. In 2013, there were around 100 registered animal feed manufacturers; by 2014, there were over 150. (USDA, 2014). Twenty grain millers and eight oil seed farmers are included. Approximately fifty raw material importers and six feed premix and additive firms are also registered (USDA, 2014). In addition, there are hundreds of home/community-based formulators whose development is driven by farmers' desire to cut production costs.

Animal feed is composed of bulk basic materials, concentrates, minerals, and vitamins. The key fundamental components are cereals (corn, wheat, barley, oats, and millets), legumes and oil seed cakes (Soybean, Cotton Seed, and Sunflower Seed), and animal byproducts. This feed will supplement ruminants' basic diet of forages (grass, hay, silage). The bulk of commodities produced (41%) are poultry feed, followed by dairy feed (39%). As a consequence of competition, a number of businesses have developed unique products for certain markets (Kenya Market Trust report, 2017).

2.2.1 Amino acid requirements of dairy cattle

To satisfy the amino acid requirements of high-producing dairy cows, a substantial portion of feed protein must be metabolized post-ruminally into its component amino acids for assimilation (Kim, 2021). The tissue-level requirement for amino acids becomes a significant issue. Absorption of amino acids in a balanced manner from the gastrointestinal tract becomes a crucial factor in both high-producing dairy cows and non-ruminants. The amino acid requirements for milk production are understood, but the challenge is in predicting the formation of the primary source of these amino acids (ruminal biomass) and harmonizing this supply with other feed sources (Giovanni et al., 2017).

The amino acid profile of milk protein is presumably equivalent to the amino acid requirements of high-producing cows, such as those producing 45 kg of milk per day (Thomas et al., 2014). This conclusion is predicated on the premise that at these levels of milk production, 90 percent of the essential amino acid is utilized. Mammary organs absorb 11 amino acids, including the 10 amino acids commonly considered essential for non-ruminants and tyrosine in sufficient amounts for milk protein synthesis (Mepham, 2017). During lactation, a number of these amino acids, including methionine, phenylalanine, tyrosine, and tryptophan, are eliminated most efficiently (Zheng et al., 2017). A second group of amino acids, arginine, isoleucine, leucine, lysine, threonine, and valine, are typically excreted in greater quantities than they are produced due to increased tissue metabolic requirements for these amino acids (Zheng et al., 2017). Numerous studies indicate that providing high-producing dairy heifers with diets abundant in ruminal degradable proteins or ruminal protected amino acids increases milk production (Schingoethe, 2016).

Certain amino acids, but not crude protein or rumen bypass protein, are essential for lactating dairy heifers (Cabrit, 2011). A ration containing 16% CP and added RP-Met produced the same quantity of milk as a diet containing 17.3% CP without RP-Met, and both diets produced more milk than a meal containing 18.3% CP, according to Broderick (2013). As milk pricing systems place a greater emphasis on milk protein, milk protein% optimization is acquiring prominence (ILRI, 2017). Rumen protected amino acids (RPAA) are frequently introduced to diets in order to boost the protein content of milk. At peak lactation, cows reach a metabolic set point for milk, milk lactose, and milk protein synthesis (Bezman et al., 2015). Young cows may respond favorably to improved amino acid (AA) balance, but at this stage of lactation, cows reach a metabolic set point for milk, milk lactose, and milk protein synthesis.

2.2.2 Concept of Limiting Amino acid

When adequate amounts of an essential amino acid are lacking in the diet, the rate of protein synthesis is limited to the rate at which that amino acid is available. Subsequently, the essential amino acid is converted into a limiting amino acid. The first limiting amino acid is the essential amino acid that is in limited supply relative to its requirement for protein synthesis. Methionine and lysine are two amino acids that may inhibit the synthesis of milk and milk components in dairy cows (Lee, 2012).

2.3 The Role of Methionine in Dairy Nutrition

Prior research has focused on the function of methionine as a co-limiting amino acid for milk protein synthesis, as well as its role in modulating milk lipid synthesis and metabolic equilibrium (Fagundes, 2021; Seymour, 2016). Recent research has uncovered additional metabolic functions of methionine, including its role in liver function, oxidative balance, and immunology (Osorio et al., 2013). Sulphur amino acids are essential for the production of glutathione (GSH), homocysteine (Hcy), and taurin (Tau), which all influence inflammatory elements of the immune response in vitro and in vivo. Methionine consumption temporarily raises plasma levels of GSH, Hcy, and Tau (Robert, 2006). There is evidence that intracellular GSH concentrations modulate cellular signaling pathways (particularly the nuclear transcription factor pathway) in response to immunological stresses (Fratelli et al., 2015).

The increased demand for amino acids for foetal development, colostrum production, the mammary gland, the liver, and the gastrointestinal tract, combined with the natural decrease in dry matter consumption prior to calving, may result in a negative protein balance (Jaurana et al., 2017). During the transition period, methionine status influences the performance of dairy cows. Methionine enhances the performance of livestock. A supplementation of 5 to 10 grams of metabolizable methionine during pregnancy appears to promote optimal postpartum function. Methionine that is ruminally protected may enable producers to achieve a higher 0.05 to 0.1% unit of fat test (Batistel, 2017).

2.4 Methionine Requirement for Dairy Cattle

Methionine and lysine concentrations in feeds like soybean meal and maize are low in comparison to milk and ruminally produced bacterial protein. According to the (S.I. Arriola Apelo *, 2014) In general, these concentrations may be obtained in corn-based diets by combining high lysine protein supplements (e.g., blood, fish, and soya bean meal) with rumen protected methionine products, as well as restricting rumen un-degraded input protein to required levels (Gargallo, 2020). Methionine concentrations are greater in fish meal and grain milling byproducts. Several feed additive manufacturers have created rumen-protected forms of methionine and lysine (Abbasi et al., 2019). Such technologies enable the addition of methionine and lysine to diets in order to achieve the correct methionine and lysine balance in the protein that enters the small intestine and is available for absorption.

2.5 Rumen Protected methionine (RP-Met)

The initial effort to physically protect methionine and lysine from ruminal degradation relied on the preservation of methionine with lipids, which were commonly combined with organic components such as carbohydrates as stabilizers, softeners, and additives (Sandoval, 2015). The most challenging aspect of using lipids as the primary encapsulating material (Broderick et al., 2014) is identifying a combination of materials and techniques that had both high ruminal escape and intestinal Methionine release.

Lancaster & Jonson (2016) attempted to encapsulate Methionine with enzyme-resistant pH-sensitive synthetic polymers that are intractable in abomasal acid. The intestinal release coefficient of Met protected by polymer is greater than that of other formulations (Gialongo et al., 2015). Other rumen-protected methionine, such as Metasmart® (Noftsger et al., 2015), is composed of Methoxy Hydroxy Analogue (MHA), which is less degradable by rumen microorganisms (Osorio, 2013).

In this research, DL-methionine coated with Mepron® will be utilized. (Muhammad et al., 2020) Mepron® consists of a nucleus of DL-Met and starch surrounded by numerous thin layers of Ethyl-cellulase and stearic acid. Due to Ethyl-cellulase's low enzymatic degradation, the product is primarily destroyed by physical action and attrition. Therefore, the product degrades slowly in the rumen and gradually releases methionine in the intestine (Roger & Peter, 2013).

2.6 Studies on rumen protected methionine effect on Milk Production

Low methionine concentrations in milk protein constrained responses to rising lysine concentrations, whereas low lysine concentrations in milk protein constrained responses to rising methionine concentrations in MP (NRC, 2011). Plasma amino acid concentrations collected at different time intervals after administering a rumen-protected product can be used to evaluate and compare the amino acid availability of various products (Bach & Marshall, 2015).

Due to the high rumen protection and intestinal release coefficient of amino acids in pH-sensitive products, it appears to be the most effective method for increasing blood amino acid levels. Watanabe et al. (2016) discovered, however, that a fat-coated amino acid has the same capacity to improve production performance as pH-sensitive polymer products.

Lara et al. (2016) found that ruminal protected methionine supplementation had no effect on DM intake (20.38 kg per day), BW (599.78 kg), or BCS score (2.51 units). In contrast, RPM enhanced milk production (35.8 kg daily) and protein output (3.16 kg daily). The quantity of methionine influences milk production in a quadratic fashion. Guinard and Rulquin (2015) also reported a quadratic response in milk protein production, which was accompanied by a significant increase in casein synthesis and a decrease in urea nitrogen (Wu et al., 2017; Dinn et al., 2013). The addition of ruminally protected methionine (16 g per day) is required to increase milk production in Holstein cows with a mean daily milk production of 35 kg (Lara et al., 2016).

Davidson et al. (2013) found that multiparous cows supplemented with rumen-protected methionine had greater milk yield, milk protein and lipid yield, and dry matter utilization efficiency for milk production than primiparous cows. Broderick et al. (2014) discovered that rumen supplementation-maintained methionine in the diet while decreasing CP levels by substituting dietary soybean meal with high moisture maize had no effect on DMI, but increased milk production, milk lipid, and protein yield.

However, relatively few studies on RPL administration without concurrent rumen-protected methionine therapy have been published. Rogers et al. (2019) administered three doses of rumen-protected Lysine (5.9, 13.5, and 21.1 g/d) to three groups of cows at different phases of lactation and found that cows fed maize-based diets produced more milk and milk protein than those fed soybean meal. In addition, plasma concentrations of methionine and lysine were increased.

When rumen protected lysine and rumen protected methionine were added to a diet deficient in metabolizable Lysine and Methionine, Xu et al. (2013) observed an improvement in early lactation milk production, milk protein, and milk lipid content.

Socha et al. (2014) examined the effect of rumen protected methionine (10.2 g) and rumen protected lysine (16.0 g) supplementation at two levels of dietary CP, namely 18.5% and 16.0%, and discovered that there was an increase in milk yield, milk fat, and milk protein yield in both treatment groups, while the group receiving 16% CP and supplemented with RPM and RPL performed similarly to the group receiving 18.5% CP. Other studies demonstrated that supplementation with RPM and RPL increased milk yield, milk fat yield, and milk protein yield without affecting dry matter consumption (Armentano et al., 2017; Robinson et al., 2017; Wu et al., 2017). In contrast, numerous researchers (Berthiaume et al., 2016; Misciattelli et al., 2013; Girard et al., 2014; Noftsger et al., 2015; Broderick & Muck, 2013; Davidson et al., 2013; Benefield et al., 2014) discovered that RP-Met Mepron® supplementation had no effect on milk production.

The substantial increase in milk production following supplementation with rumen-protected methionine and lysine is consistent with the findings of a number of earlier studies. Noftsger and St-Pierre (2013) reported a considerable increase in milk production with RP-Met Mepron® supplementation (23,6 kg d-1) versus the control group (21,7 kg d-1). Similarly, Bach et al. (2015) demonstrated that lactating Holstein heifers produced more milk when rumen-protected methionine was administered (45.9 vs. 47.7 kg d-1). Similarly, Broderick et al., (2014) and Yang et al., (2015) reported that RP-Met Mepron® and lysine supplementation increased milk yield in crossbred cows (41.5 kg d-1) compared to the control (39.4 kg d-1) respectively. Socha et al. (2014) discovered that abomasal infusion of methionine and lysine increased milk production during the apex and early phases of lactation, but had no effect during the mid-lactation period.

2.7 Studies done on impact of rumen protected methionine on dairy cows Immunity

Worldwide, mastitis is a prevalent condition. It has significant economic repercussions for dairy farms (Halasa et al., 2012). It reduces milk quality (Bezman et al., 2015), bovine welfare (Kemp et al., 2013), irritates producers (Jansen, 2015), and poses a threat to public health. Mastitis is frequently treated with antibiotics (Royster & Wagner, 2015), which increases the likelihood of drug residues and the emergence of resistant bacteria in dairy cattle (Knappstein et al., 2015; Wendtland et al., 2013). These factors emphasize the significance of mastitis control on dairy farms.

Subclinical mastitis (SCM) and clinical mastitis (CM) are the two forms of mastitis (Krishnamoorthy et al., 2021). The somatic cell mass (SCM) of bovine milk is affected by the somatic cell count (SCC) (MMA et al., 2013). CM is distinguished by udder changes (redness, edema, pain, and heat), milk changes (color, fibrin, and clotting), and systemic involvement (fever, anorexia, and shock) (Smith, 2015). Typically, the incidence rate of CM is used to represent the frequency of CM on a farm, whereas SCC is used as a proxy for SCM (Huijps et al., 2015).

Leucocytes rely significantly on glutamine for nucleotide synthesis (Shah, 2020). Lactation decreases neutrophil and lymphocyte activity. Curtis et al. (2015) found that increasing dietary protein or amino acid supplementation decreased the prevalence of retained placenta and metabolic disorders; however, there is a paucity of research that has explicitly evaluated the influence of protein on cow health. Therefore, individuals with are limited in the number of repetitions, which diminishes the probability of discovering a therapeutic effect. However, these studies conclude that neither prenatal nor postnatal protein intake had a significant effect on milk somatic cell count (SCC), mastitis incidence, or other diseases (Wu & Satter, 2015; Garnsworthy & Jones, 2017). A rumen-protected Methionine supplement at a relatively high dosage (30g/d) was found to increase the proliferative response of peripheral T-lymphocytes in mid-lactation dairy cows, but had no effect on milk SCC levels (Shah, 2020).

Berthiaume et al. (2016) investigated the effect of RP-Met on splanchnic metabolism and discovered that it had no effect on milk and milk protein production, but increased actual protein content linearly. After supplementation with RP-Met, the Met concentration in the arterial blood increased. This result is consistent with the available scholarly evidence (Overton et al., 2016; Blum et al., 2009; Berthiaume et al., 2016). The linear increase in total splanchnic output of isoleucine, leucine, phenylalanine, and threonine indicated that RP-Met may induce a homeostatic response, resulting in decreased consumption of particular amino acids by the GIT and liver. Met extraction decreased linearly as arterial input increased in the mammary organ. Other studies have found that post-ruminal RP-Met administration increases Met blood levels (Pisulewski et al., 2016; Rulquin and Kovalezyk, 2013).

A successful lactation transition lays the groundwork for a profitable lactation with optimum production, reproduction, and health, thereby averting untimely slaughter. During this time period, metabolic abnormalities and health issues are prevalent, and they can quickly cancel out the economic potential of dairy bovine farms (Roche, 2013). Before giving birth, heifers undergo a period of pervasive immunosuppression (Kehrli et al., 2019; Waller, 2015). Periparturient immunological and metabolic failure in a cow, resulting in hyposensitivity and hyporesponse to antigens and increased susceptibility to infectious diseases such as mastitis.

Dry matter intake (DMI) of dairy heifers is quite low during late gestation and early lactation, despite the exceedingly high nutritional demand, notably postpartum (Grunner et al., 2014). As a result, cows have a negative protein and energy balance. Cows mobilize body fat and protein to provide the energy and amino acids required for fundamental maintenance and milk production. Protein deficit is temporary because: protein intake by cows can be quickly restored by increasing the protein content of their diet; and labile body protein reserves are rapidly depleted, resulting in a decrease in milk production to match protein supply (Broderick et al., 2014).

Immunological responses require amino acids for antibody production and cellular proliferation (Reza et al., 2016). However, compared to the kilogram of milk protein produced daily by cows in early lactation, the immune system's amino acid requirements are modest (Putnam et al., 2009). There is no conclusive evidence that rectifying the minor protein deficiency that develops during early lactation enhances immune function or mastitis resistance. Doepel et al. (2016) found that injecting peripartum heifers with 300 g of glutamine per day had very minor beneficial effects on immunological function, but this likely has little practical relevance. If protein intake is sufficient for milk production in the early stages of lactation, it is likely sufficient for normal immune function.

2.8 Economic benefit of using Rumen Protected Methionine (RP-Met)

The total protein concentration is the most influential dietary factor on milk nitrogen efficiency. Reducing dietary crude protein is the most significant means by which to increase efficiency of dietary protein utilization (Arriola Apelo, 2014). Although it is possible to use alternative, less expensive vegetable sources of protein or non-protein nitrogen sources such as feed grade or slow-release urea formulations (Sinclair et al., 2012), it is necessary to reduce protein levels in dairy cow diets in order to achieve significant cost reductions. 2010 (Yan et al.) The return on investment or cost-benefit ratio of deploying RP-Met products will vary based on a variety of dairy farm characteristics. RP-Met is a highly cost-effective feed additive when the milk producer is compensated more for milk protein content. To optimize the EAA profile in MP while using RP-Met, the whole component complements, particularly those contributing to the percentage of rumen undegradable protein (RUP), must be carefully chosen. When implementing RP-Met in a well-balanced diet, the crude protein level of the ration can and should be decreased to gain a cost advantage from the RP-Met while reaping the benefits of enhanced animal performance and reduced nitrogen production (Putnam et al., 2009).

The majority of RP-Met sources are imported and must compete against a number of locally produced proteinaceous substances. Since fishmeal is the only animal protein source approved for inclusion in dairy diets that are also well-balanced for Lys and Met, its use is restricted due to its higher price in comparison to alternative plant protein sources (Donaldson et al., 2011).

Mastitis has an effect on the reproductive function of a dairy bovine. Mastitis in cattle alters the endocrine and immune systems, resulting in aberrant estrous cycles, ovarian problems, metritis, and early embryo loss (Grohn et al., 2013; Siatka et al., 2018). It results in a prolonged gestational period, lower conception rates, a higher number of services per conception rate, and an increased risk of embryo loss (van Soest et al., 2017). Insufficient reproductive performance causes agricultural economic losses (Inchaisri et al., 2015; Rutten et al., 2014). Inchaisri et al. (2015) estimated annual net economic losses of €34 for a farm with average reproductive performance and €120 for a farm with low reproductive performance.

Mastitis has both direct and indirect positive effects on the social and economic factors of dairy farms. The primary factors that motivate Dutch farmers to enhance mastitis management are job satisfaction, farm status, and economic losses (Valeeva et al., 2012). Job contentment refers to a farmer's pleasure as a consequence of effective mastitis management. The overall condition of the farm reflects the benefits of improved mastitis management, such as fewer problems with other bovine maladies, a more efficient milking process, and a reduced daily dosage of antibiotics per animal (Valeeva et al., 2012). If providing desiccated cows garlic boluses decreases the incidence of CM on a farm, the farmer's job satisfaction may increase. This increased job satisfaction would motivate the farmer to enhance his administration, thereby enhancing the health of his livestock in terms of mastitis and other diseases such as acidosis and endometritis. When a farmer decides to use garlic boluses to enhance udder health on his farm, he assumes a different perspective regarding udder health. Changes in farmers' attitudes and knowledge, as well as changes in farmers' behavior, accounted for 24% and 5%, respectively, of the variance in the diminished incidence rate of clinical mastitis (CM). However, the management factors associated with mastitis control remained unchanged. This indicates that the incidence rate of CM decreases as a consequence of an increase in the quality of mastitis management as opposed to a change in mastitis management. In an effort to reduce the incidence of mastitis, the incidence of mastitis may decrease not only as a result of garlic boluses, but also as a result of a change in the producers' attitude toward mammary health.

2.9 Studies done on Rumen Protected Methionine in Africa

On the African continent, researchers have used numerous methodologies to evaluate the efficacy of rumen-protected products, with animal production trials being the most common (Rogers et al., 2019). Trials examining the effect on milk production, milk composition, and milk protein composition have been conducted with remarkable success (Wu and Satter, 2015). Several studies have also examined the impact of post-ruminal rumen-protected methionine infusion on blood plasma concentrations (Zhao et al., 2018). In situ methods, such as the movable bag / in sacco procedures, have also been routinely utilized to evaluate RPAA products (Berthiaume et al., 2016; Rossi et al., 2013).

In South Africa, the emphasis is on mass production of animal products in order to provide enough sustenance, and the environmental impact of animal husbandry is not a major concern (Wang et al., 2017). To maximize economic milk production, however, it is necessary to optimize the utilization of crude protein (CP) from the dairy feed in order to increase total animal efficiency and output while concurrently reducing operational costs (Siatka et al., 2018). This can be achieved by enhancing rumen function (maximizing the quantity of microbial protein synthesis) and increasing the amino acid (AA) composition of the metabolizable protein (MP) that is absorbable in the lower gastrointestinal tract (GIT). Diets limited in protein are an effective method for reducing N excretion from dairy cows (Wang et al., 2015); however, for every 1% decrease in dietary protein, milk production decreases by 0.7 to 1.2 kg/d (Broderick, 2013; Wang et al., 2017). Lapierre et al. (2015) suggest that emphasis should be placed on increasing the conversion of dietary nitrogen to high-quality milk protein. This can be achieved by supplying a dietary AA profile that closely resembles the AA profile required for milk synthesis (Noftsger et al., 2015), decreasing the CP content of the ration and increasing N efficacy without affecting milk production. Dietary composition, feed intake level, and consequently rumen outflow and dilution rate of microbial crude protein (MCP) have a substantial impact on the input of microbial protein to the MP. Therefore, protein should be administered post-ruminally with an AA profile that corresponds to the heifers' requirements for metabolically absorbable protein (Robinson et al., 2014). (2014) Robinson et al.

A South African company has developed a prototype for rumen-protected liquid methionine and has requested sufficient research to demonstrate its relative bioavailability in dairy cows (Rulquin and Kovalezy, 2013). It was decided to evaluate the product using a variety of available methodologies in order to ascertain the relative level of protection against rumen degradation and the absorption rate in the small intestine. On both the product and the mode of protection, only limited technical information was made available. The challenge was to identify cost-effective evaluation strategies that could effectively investigate this liquid rumen-protected methionine source. It was determined to compare the liquid rumen protected methionine prototype with the well-studied Methionine sources Smartamine (Adisseo, Inc., Antony, France) and DL-Methionine (DLMet) (Evonik Degussa, Theodore, Alabama, United States). Smartamine is completely protected from rumen degradation and available for assimilation in the abomasum, unlike DL-methionine.

In Kenya, the most limiting amino acids (Lys and Met) have been successfully supplemented with rumen-protected amino acids. Several technologies have been studied since the early 1990s to protect Methionine from rumen degradation, with the first attempts combining Methionine with lipids, inorganic materials, carbohydrates, softening agents, and additives (Schwab and Ordway, 2016). In order to increase the AA content of the metabolizable protein, technologies have been developed to chemically and/or physically protect these AA from degradation in the rumen without impairing digestion in the small intestine. Increasing the quantity of methionine delivered to the small intestines significantly increases protein production in the mammary gland.

2.10 Summary of Gaps in Literature

According to a study of the literature, most studies have been conducted using a total mixed ration (TMR) feeding approach, in which the complete ration is balanced, and both concentrate and forage are balanced together at the farm level. This research aims to address amino acid balancing ration in a partial mixed ration (PMR) system where concentrate feed/dairy meal is combined separately from forage, often by a commercial animal feed manufacturer in the form of dairy meal or dairy cubes. When amino acid supplementation is performed at the concentrate level in a feed production firm, the dairy meal is incorporated into the whole diet. In most farms in Kenya and East Africa, dairy meal is acquired separately from forage, generally from an animal feed producer.

In Kenya, dairy goat production is an alternative livestock industry that is suited for many small-scale or part-time livestock enterprises (Ogola, 2010). With rising urbanization resulting in limited arable land, dairy goat farming may be able to replace some dairy cow farming since goats are easy to maintain, inexpensive to purchase, and need less acreage for production. Dairy goat farming for profit focuses on milk production as its primary source of revenue, but little research has been done on the amino acid needs of small ruminants, particularly dairy goats.

CHAPTER THREE: METHODOLOGY

3.1 Study Site

The study was carried out at Molito Dairy Farm (Risa Farm) which is located in Ngecha ward, Limuru sub-county, Kiambu County, Kenya. Molito farm coordinates are as follows: -1.1860236683874361, 36.70393096944112. Limuru sub-county (Figure 2.1) has a Marine west coast, warm summer climate (Classification: Cfb). The sub-county's yearly temperature is 22.05ºC and it is -0.45% lower than Kenya's averages. Limuru typically receives about 137.44 millimeters (5.41 inches) of precipitation and has 226.84 rainy days (62.15% of the time) annually according to Koppen Geiger Climate Classification (Andrew, 2021).

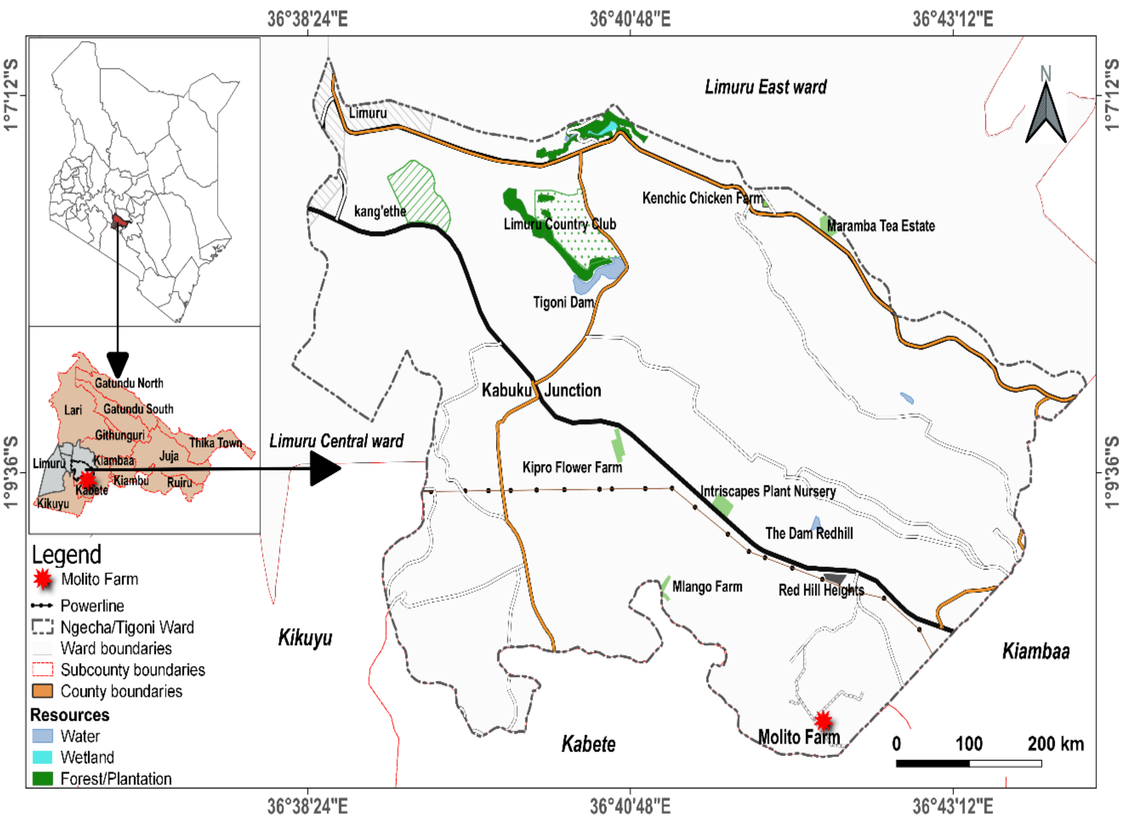

Figure 3.2 Study location map

Source: Koppen Geiger Climate Classification (2021)

3.3 Experimental diets

The ingredients for the trial diets were purchased from local sources. Wheat bran, maize, sunflower, cotton seed cake, soybean meal, canola cake, wheat pollard, maize germ, vitamin/mineral premix, and Kupakula® were among the components (Dairy protein concentrate). The byproducts of milling maize and wheat were utilized as energy sources, whereas the byproducts of milling oil seeds were used as protein sources. The rumen protected methionine (Mepron ®) was imported from Europe (Evonik Industries Ltd) and made accessible in East Africa via authorized local wholesalers (Essential Drugs Limited and Tamfeeds Limited). The nutrient content of the batches of feed materials used to make the experimental diets was evaluated and then included into the formulation. AMINOcow® ration formulator was utilized to construct the cows' whole feed according to breed, degree of production, lactation stage, and parity.

The ration formulator software includes a way for determining the amino acid need. The concentrate portion of the TMR was extracted from AMINOcow® formulator and entered into Brill formulation software (Van Amburgh et al., 2010) for least cost optimization of the ration, where for the test feed amino acid was balanced using Mepron® as a source of RP-Met and for the other ration the optimization was performed based on crude protein according to Kenya Bureau of Standards (KEBS) standard (FDEAS 75: 2018). The formulation and estimated nutritional content of the treatment meals are shown in Table 3.1. The concentrate component of the treatment diets was then integrated into a Total Mixed Ration (TMR) to generate the actual treatment diets provided to the lactating dairy cows. Actual inclusion levels of the TMR components used to formulate the diets are shown in Table 3.2 and are designated as follows: Treatment 1 (T1) – Dairy meal balanced for amino acid using Mepron® without increasing crude protein level; Treatment 2 (T2) – Commercial dairy meal formulated based on crude protein; and Treatment 3 (T3) – Farm formulated dairy meal formulated based on crude protein.

Table 3.1 Dairy meal Formulation and Calculated Nutrient Composition (As is Basis)

Feed	T1	T2	T3
Wheat bran	350.00	350.00	119.00
Maize	258.00	250.00	265.00
Sunflower cake	100.00	100.00	172.00
Cotton seed cake	0.00	0.00	66.00
Soybean meal	74.30	99.90	32.00
Canola cake	0.00	0.00	79.00
Wheat pollard	66.60	60.80	106.00
Maize germ	60.00	50.60	106.00
Rice Polish	60.00	50.00	0.00
Limestone	15.60	16.80	19.00
Macklik® super	0.00	0.00	21.00
Diamond V XPC	0.00	0.00	1.00
Toxin binder	0.00	0.00	1.00
Dairy premix	10.00	10.00	0.00
Kupakula®	0.00	0.00	13.00
Urea	0.00	8.40	0.00
DCP	3.50	3.50	0.00
Mepron	2.00	0.00	0.00
Total	**1,000.00**	**1,000.00**	**1,000.00**
Cost/Kg (KES)	**26.68**	**26.90**	**33.13**
Calculated nutrient composition of the experimental diets			
Crude protein (%)	15.50	16.16	17.43
Crude fiber (%)	9.09	11.44	8.66
Either extract (%)	5.10	4.83	5.22
MER (Kcal/Kg)	2,450.00	2,405.00	2,289.00
Calcium (%)	0.80	0.80	0.91
Total Phosphorus (%)	0.82	0.81	0.69
Lysine (%)	0.69	0.74	0.77
Methionine (%)	0.44	0.28	0.34

(T1) is dairy meal balanced for amino acid using Mepron® without increasing crude protein level. (T2) is commercial dairy meal formulated based on crude protein and (T3) is farm formulated dairy meal based on crude protein, DCP- Dicalcium Phosphate, MER-Metabolizable Energy for Ruminants. Exchange rate 1USD equals 101.99KES

Table 3.2 Total Mixed Ration (TMR) Formulation ("as fed" basis) of the Treatment Diets

	Treatment diets		
Ingredients	**T1 (%)**	**T2 (%)**	**T3 (%)**
Lucerne	14.25	14.25	14.25
Maize silage	56.98	56.98	56.98
Mepron® dairy meal	28.49	0.00	0.00
Commercial dairy meal	0.00	28.49	0.00
Farm dairy meal	0.00	0.00	28.49
Mineral lick	0.28	0.28	0.28
Total	100.00	100.00	100.00

(T1) is group fed dairy meal balanced for amino acid using Mepron® without increasing crude protein level. **(T2)** is group fed commercial dairy meal formulated based on crude protein and **(T3)** is group fed farm formulated dairy meal based on crude protein.

3.3 Experimental Design, Animals and their Care

A completely randomized design (CRD) was used in this experiment. Each treatment had four lactating cows of different parity, body condition and body size. There were therefore three treatments (T1, T2 and T3) with four replicates per treatment. The feeding trial was conducted over a period of 7 weeks after one week of acclimatization to the diets and experimental procedures.

Twelve Holstein Friesian dairy cows were recruited for this study and the characteristics of the experimental animals were as outlined in Table 3.3. The average body size was 558.6 Kgs, body score of 2.8 units, in the second parity and <100 days post calving. The animals are housed under zero grazing system where feed and water were supplied in stalls where the animals were, and they only needed to move to the adjacent milking pallor during milking time. The animals were milked three times per day at 10.00 am, 5.00pm and 2.00am using a milking machine. Milking hygiene was observed, and teats were dipped into a teat dip using Mastrite® after every milking to control mastitis.

Table 3.3 Characteristics of the Study Animals Allocated to the Treatment Diets

Treatment	Cow ID	Body size (Kg)	Body condition	Parity
T1	Karendi	504	2.50	1
	Peggy	560	2.60	2
	Mara	587	2.75	3
	Lona	587	2.75	2
T2	Natella	480	2.50	1
	Malia	618	3.00	2
	Ramona	630	3.15	3
	Maua	578	2.75	4
T3	Malaika	487	2.50	1
	Selma	512	2.50	2
	Rosy	640	3.25	3
	Bianca	520	3.00	2

T1 is Treatment 1 balanced for amino acid using Rumen protected Methionine. T2 and T3 are Treatment 2 and Treatment 3 formulated based on crude protein as commercial dairy meal and farm formulated dairy meal respectively. (BCS Scale 1 being lean and 5 being over conditioned)

Feeding was based on semi total mixed ration where all forage feed were mixed using a mixer wagon and the concentrate portion of the ration was mixed separately and added to the other portion of the ration at a rate of 10 kg per cow per day. This was then divided into four equal portions and served four times a day.

The animals were sprayed with acaricide (Amitraz®) on Friday every week for tick control. Deworming was done before the start of the experiment using Nilverm (3.0% w/v Oxyclozanide, 1.5% w/v Levamisole Hydrochloride, 0,382% Cobalt Sulphate).

3.4 Data and Sample Collection

3.4.1 Body weight

The live weight of each cow was taken at the beginning of the feeding trial and weekly on Fridays by measuring from the base of the withers, down under the belly, just behind the elbow and foreleg, and all the way back around using a weighing band. This approach was developed from measurements of Holstein heifers (Heinrichs and Hargrove, 2017).

3.4.2 Body condition score

Body condition scoring was carried out on all the study animals at the beginning of the feeding trial and then weekly on Fridays to the end of the experiment. The scoring method used was as described by Marija et al. (2011) which involves observing and handling of the backbone, loins, ramp area, pin bone, hip bone, top of the backbone and end of the short ribs. The grind used had values ranging from 1 to 5, where 1 being a very thin cow with no fat reserves and 5 being a severely over conditioned cow (Marija *et al*., 2011). The body scores were carried out by two independent persons, the researcher and either by the farm manager or a team member from Unga Farm Care Limited, then the average score was calculated and recorded.

3.4.3 Milk sampling and samples

The total milk produced per milking for each individual animal was weighed using hook type Hanson weighing scale that has an accuracy of 10grams and can weigh up to a maximum of 100kgs.

California Mastitis Test (CMT) was done for all experimental animals in each group for the four quarters after every two days to monitor incidences of mastitis. Individual milk samples (250mls) for each cow were collected in plastic sample bottle then packed in ice box ready for transportation to the laboratory for analysis. This was done every Friday and submitted to the laboratory for milk quality tests.

3.4.4 Feed samples

Feed ingredient samples were collected per batch as they are delivered in the factory. This involved collection of samples from several bags in a batch from the front, middle and end of the truck. The portions were then mixed, and a 200 grams sample was drawn and used for analysis.

Feed intake was calculated by weighing the feed provided and weighing the feed left after feeding in the evening for each treatment group every day.

3.5 Laboratory Analysis

Feed components used to build the treatments were evaluated at the Evonik East Africa Satellite Laboratory in Nairobi for Crude Protein, Crude fiber, Starch, Sugar, ADF, NDF, Ash, Energy, and Total Amino Acids using Near Infrared Reflectance (NIR) Method. The NIR technique of analysis determines the nutritional value of feed using light reflection as opposed to chemistry to detect and quantify the levels of chemicals in a sample. The reflectance data are then incorporated into calibration formulae that determine nutrient concentrations. The equations are based on comparative examinations of split samples for wet chemistry and NIR reflectance. This method of examination is quick and precise, requiring minimum sample preparation, often merely grinding through a 0.5 mm sieve. The procedure is highly repeatable and has a fast turnaround time, making it a practical and inexpensive alternative to chemical feed analysis. It employs infrared light with an electromagnetic spectrum ranging from 700 to 2500 nm. This information is then utilized to determine the sample's qualities, in this instance its nutritional makeup. (Sun et al., 2016)

Wet chemistry was used to assess the three treatment diets for Crude Protein, Crude Fiber, Starch, Sugar, ADF, NDF, Ash, Energy, and Total Amino Acids in accordance with the protocol of the Association of official analytical chemists (AOAC, 2013).

3.6 Data Management and Data Analysis

All the data collected from the feeding trails was recorded into a hard copy data sheet (Appendix IV) and later transferred to an excel spread sheet.

Analysis of variances was done for data on milk production, resistance to mastitis and milk composition. The significance of means was separated using, the Student Newman Keuls (SNK) at $p < 0.05$. All statistical analyses were performed using R software (Version 4.0.2) for windows (R Core Team, 2020).

3.7 Ethical Considerations

The management and general care of the lactating cows followed accepted guidelines as outlined by the Federation of Animal Science Societies (FASS, 2015). The research proposal was reviewed by Kenyatta University Graduate School and the approval granted to carry out the study (Appendix I). Authority to conduct the experiment and collect data was also granted by National Commission for Science, Technology, and Innovation (NACOSTI) (Appendix II). The management of Molito Farm granted agreed to the procedures of the experiment and granted the permission to use their lactating Friesian cows and carry out the trial on the farm (Appendix III).

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Chemical Composition of the Feed Ingredients and Diets

Table 4.1 displays the results of the approximate analysis of the components utilized to make the concentrate part of the treatment diets. The DM content of the components ranged from 88.31% for maize to 92.366% for cotton seed cake and Kupakula. This was within the suggested range of more than 85 percent (Moniruzzaman1, 2022). The high DM assures a low moisture level that does not allow mold development, hence decreasing the likelihood of mycotoxin contamination. The DM level of a feed may have a significant effect on animal intake, particularly in grazing circumstances after heavy rainfall, when the feed water content will not only dilute the calorie value but also limit intake (Morrison, 2007).

Soybean meal and other oil seed cakes from canola, cotton, and sunflower were the primary sources of protein for the concentrate part of these test diets. On a DM basis, the CP content of these components varied from 26.15 percent (sunflower cake) to 50.73 percent (soybean meal). The protein source constituents assessed in this research were suitable for use in dairy cow diets (Salfer et al., 2019). The predominant energy source for the concentrate component of the trial diet was whole maize and by-products of grain milling, with energy content varying from 2,105 to 3,803 Kcal/kg on a DM basis. The majority of components utilized to manufacture the concentrate part were fibrous, with neutral detergent fiber (NDF) which influences DM intake, ranging from 16.04% in Kupakula® to 46.05% in sunflower cake for the protein ingredients and from 14.45% in maize to 44.75% in wheat bran. The amount of acid detergent fiber (ADF) that effects digestibility varies from 4.07% in maize to 34.12% in sunflower cake. The low fiber content of these components allows them to be used with other roughages in dairy cow diets as dietary supplements. Mepron® had 91.00% DM, 54.95% DM CP, and 3.30% DM CF, whereas Kupakula® contained 92.36% DM, 52.98% DM CP, and 6.53% DM CF.

Metabolisable energy for ruminants (MER) is the energy left after accounting for utinary energy losses; hence, it is the energy maintained in the body for productivity and metabolic system support, such as breathing. Wheat bran has a MER of 2105 Kcal/kg, whereas Mepron® has a MER of 4735 Kcal/kg.

Table 4.1 Ingredients Proximate Analysis (%DM basis).

Ingredient	DM (%)	CP (%)	EE (%)	CF (%)	Ash (%)	Starch (%)	NDF (%)	ADF (%)	Sugar (%)	MER (Kcal/Kg)
Mepron®	91.00	54.95	1.10	3.30	1.64					4735.00
Kupakula®	92.36	52.98	5.41	6.53	7.16	0.65	16.04	9.42	8.79	2608.00
Sunflower cake	92.00	26.15	10.50	30.38	6.28	0.11	46.05	34.30	4.67	2623.00
Cotton cake	92.42	32.94	7.71	23.89	6.14	0.11	44.16	31.99	3.46	2675.00
SBM	89.96	50.73	4.51	6.70	7.35	0.67	15.76	9.67	9.06	2678.00
Canola cake	92.09	39.33	8.74	11.91	7.67	0.11	28.37	20.36	8.25	2423.00
Maize	88.31	9.18	5.21	2.54	1.41	71.63	14.45	4.07	1.17	3803.00
Maize germ	88.55	11.23	10.53	5.85	3.39	46.23	27.67	7.54	3.16	3217.00
Wheat bran	88.89	17.10	4.58	10.15	5.76	23.56	44.70	13.31	5.37	2105.00
Wheat pollard	89.59	17.43	4.51	7.82	4.54	29.98	36.28	10.89	5.78	2533.00
Rice polish	90.38	13.50	14.91	10.46	10.73	29.40	26.06	12.78	3.37	3010.00

SBM- Soybean Meal, DM- Dry Matter, CP- Crude Protein, EE- Either Extract or Crude fat, CF-Crude Fiber, ADF- Acid detergent fiber (residue containing cellulose, lignin and insoluble minerals), NDF- Neutral Detergent Fiber (insoluble fibers, hemicellulose, cellulose, lignocellulose, and lignin**)**, and MER- Metabolizable Energy for Ruminants.

4.1.1 Amino Acid Composition of the Feed Ingredients

Table 4.2 lists the amino acid composition of the ingredients used in the diets. The methionine content of the feed ingredients ranged from 0.19% in maize to 0.76% in canola cake among the common ingredients used to formulate dairy rations while Mepron® had methionine content of 85%. The quantities of methionine in the common ingredients used to formulate dairy rations are not sufficient to meet the requirement for milk production based on amino acid requirement of lactating dairy cows (Zanton, 2014) hence the need to supplement from synthetic sources such as Mepron®.

The lysine content in the ingredient ranged from 0.29% in maize to 3.06% in soybean meal. The histidine content of the concentrate portion of the ingredients was ranging from 0.27% in maize and 1.30% in soybean meal. Methionine and lysine concentration in feed ingredients such as soybean meal and maize are low compared to their concentration in milk and ruminally synthesized bacterial protein. The concentrations of lysine and methionine used to produce metabolizable proteins are 7.2 and 2.4%, respectively (Zanton, 2014).

Table 4.2 Ingredients Total Amino Acid Analysis (%DM basis).

Ingredient	DM (%)	Met (%)	Lys (%)	His (%)	M+C (%)	Thr (%)	Trp (%)	Arg (%)	Ile (%)	Lue (%)	Val (%)
Wheat bran	88.89	0.25	0.68	0.46	0.59	0.54	0.28	1.18	0.53	1.02	0.79
Maize	88.31	0.19	0.29	0.27	0.39	0.33	0.07	0.45	0.31	1.05	0.44
Sunflower	92.00	0.54	0.89	0.62	0.97	0.90	0.35	2.06	1.05	1.63	1.23
Cotton cake	92.42	0.46	1.35	0.85	1.01	1.03	0.41	3.46	1.01	1.82	1.38
SBM	89.96	0.66	3.06	1.30	1.38	1.93	0.67	3.69	2.30	3.81	2.38
Canola Cake	92.09	0.76	2.11	1.02	1.75	1.66	0.53	2.43	1.54	2.68	1.99
Wheat Pollard	89.59	0.27	0.73	0.45	0.61	0.57	0.26	1.18	0.55	1.06	0.80
Maize germ	88.55	0.20	0.51	0.33	0.44	0.42	0.11	0.72	0.36	1.01	0.59
Rice polish	90.38	0.25	0.62	0.35	0.53	0.50	0.17	1.04	0.46	0.92	0.71
Kupakula®	91.00	0.34	0.55	0.45	1.02	0.61		2.38	0.66	1.16	0.86
Mepron®	92.36	85.0									

SBM- Soybean meal, Met-Methionine, Lys-Lysine, His-Histidine, M+C- Methionine + Cysteine, Thr-Threonine, Trp-Tryptophan, Arg-Arginine, Ile-Isoleucine, Lue-Leucine and Val-Valine

4.1.2 Calcium and Phosphorus Content of the Feed Ingredients

The calcium and phosphorous content of the feed materials used in experimental dairy diets is presented in Table 4.3.

Table 4.3 Calcium and Phosphorus Analysis (%DM basis) of Ingredients used to formulate the Experimental Diets.

Ingredient	Calcium (%)	Phosphorous (%)
Wheat bran	0.16	1.12
Maize	0.05	0.29
Sunflower cake	0.38	1.00
Cotton seed cake	0.19	0.82
Soya bean meal	0.38	0.69
Canola cake	0.49	0.68
Wheat pollard	0.15	0.97
Maize germ/bran	0.05	0.91
Rice polish	0.09	1.74
Limestone	38.00	0.02
Macklik® super	20.36	11.00
DCP	24.50	18.20

DCP-Dicalcium phosphate

The plant-based feed ingredients had calcium levels ranging from 0.05% in maize and maize germ to 0.38% in sunflower cake and soybean meal. Limestone had a calcium level of 38% and low levels of phosphorus. The Kupakula® which is a protein supplement used in formulation of dairy cattle feeds had calcium to phosphorous ratio of 1.85: 1. The dicalcium phosphate (DCP) had a calcium to phosphorous ratio of 1.23:1. Calcium requirements of lactating dairy cows are high relative to other species or to non-lactating cows because of the high calcium concentration in milk. Thus, inorganic sources of calcium, such as calcium carbonate or dicalcium phosphate, must be added to the rations of lactating dairy cows. Dietary calcium from organic sources is generally absorbed with greater efficiency than that from inorganic sources. Furthermore, cows in negative calcium balance (usually 6 to 8 weeks post-calving) absorb calcium more efficiently than cows in positive calcium balance. Generally, diets with forages from primarily grass (including corn silage) sources as is the case in this trial, will usually have minimum calcium concentration requirements in the range of 0.42%–0.47%. This is below the recommended levels of 0.75%. (Duplessis, 2023).

The calcium and phosphorus composition of the ingredient used to formulate rations for this trial compared favorably with ingredient composition data compiled by (Kalscheur et al., 2012; Moreau et al., 2015) and was hence considered to be within the normal range.

4.2 Nutrient Composition of the Treatment Diets

The DM, CP and amino acids content of the three treatment diets are presented in Table 4.4. Treatment diet T1 had the lowest crude protein level at 16.45% followed by T2 (commercial dairy meal) at 19.83%, then T3 (farm dairy meal) at 20.06%. Treatment diet T1 had the highest total methionine content at 0.42% followed by T3 at 0.33% and T2 had the lowest methionine content at 0.29%. Treatment diet T1 was the only one with added synthetic methionine (Mepron®) at 0.14%. Both T2 and T3 had negligible amounts of synthetic methionine (Mepron®) <0.011%.

Increasing the CP content in treatment diets T2 and T3 (up to 19.83%, and 20.06% respectively) did not meet the recommended methionine requirement level of 49 gram/head/day (Pacheco *et al*., 2012). This was consistent with experiment done by Batistel et al., (2017), who found that lowering the crude protein content of a diet but at the same time supplementing with rumen protected methionine achieves the same or better milk production compared with high crude protein diet.

Table 4.4 Nutrient Content (%DM) of the Three Treatment Diets.

Material type	T1	T2	T3
Dry Matter	90.26	90.51	90.44
Crude Protein	16.45	19.83	20.06
Methionine	0.42	0.29	0.33
Lysine	0.72	0.80	0.77
Histidine	0.41	0.45	0.50
Cysteine	0.29	0.32	0.36
Methionine + Cysteine	0.71	0.61	0.70
Threonine	0.58	0.63	0.71
Arginine	1.10	1.22	1.42
Isoleucine	0.61	0.66	0.72
Leucine	1.22	1.29	1.42
Valine	0.78	0.84	0.93
Phenylalanine	0.73	0.79	0.90
Glycine	0.79	0.86	0.92
Proline	0.94	0.99	1.05
Alanine	0.82	0.87	0.94
Aspartic acid	1.38	1.25	1.61
Glutamine	2.96	3.20	3.56
NH3	0.38	0.56	0.50
SUM_WITH_NH3	14.81	16.21	17.58
SUM_WO_NH3	14.43	15.64	17.08
Supplemented Methionine	0.14	<0.011	<0.011
Supplemented Lysine	<0.022	<0.022	<0.022

(T1) is group fed dairy meal with Mepron®. (T2) is group fed commercial dairy meal and (T3) is group fed farm formulated dairy meal.

4.3 Dairy Cow Feed Intake

The results of feed intake by the lactating dairy cows are presented in table 4.5. The animals consumed the diets well throughout the experiment period. The feed intake was fairly uniform across the treatment diets. The diets were formulated into a TMR and therefore the various components of the diets were consumed according to the intake of the diets.

Table 4.5 Average Feed Intake (As fed basis) by the Lactating Cows

Feed	T1	T2	T3
Lucerne	5.00	5.00	5.00
Maize silage	20.10	21.50	19.50
Dairy meal	10.00	10.00	10.00
Mineral supplement	0.10	0.10	0.10
Total	**35.20**	**36.60**	**34.60**

(T1) is group fed dairy meal with Mepron®. (T2) is group fed commercial dairy meal and (T3) is group fed farm formulated dairy meal.

Feed intake in dairy cows is highly regulated by animal nutrient requirements and metabolic state, and by the type and temporal pattern of absorbed fuels (Harvatine & Allen, 2006). In the current study, the feed intake of the diets by the cows did not differ across the treatments. This implies that any differences in production by the dairy cows could be due to the utilization efficiency of the diets especially for milk production. However, the results show that the dairy cows consuming treatment diet T2 consumed numerically more feed followed by those consuming treatment diet T1 and those cows consuming treatment diet T3 consumed the least amount of feed. The diets were generally palatable to the cows as they were freely consumed by the animals.

4.4 Lactation Performance of the Cows

The average lactation and weekly milk production of the lactating Holstein Friesian cows during the experiment period are presented in Table 4.6 and 4.7. The trends in milk production is also presented in Figure 4.1. The results of this study showed an increase ($p>0.05$) in milk production of cows fed dairy meal balanced for amino acid RP-Met (Mepron®). This result is in line with Lee (2012) and Appuhamy (2014), who reported that methionine and lysine are most often the first limiting amino acids for milk production. However, the milk production of cows fed commercial dairy meal declined compared to cows fed farm dairy meal formulated based on crude protein (Table 4.6).

Table 4.6 Lactation performance of Friesian Cows Fed Experimental Diets

	T1	T2	T3	SEM	*p*-value
Initial milk production	19.7	18.2	19.8	0.64	0.53
Final milk production	20.9[a]	15.6[b]	18.2[c]	0.67	0.00
Change in milk production	+	-	-		
Overall milk production	144[a]	116[b]	140[ab]	4.49	0.08
Mean milk production	20.91[a]	15.59[b]	18.19[ab]	0.26	0.00

SEM is Standard Error Mean, T1, dairy meal balanced for amino acids using Mepron®; T2, commercial dairy meal formulated based on crude protein; T3, farm formulated dairy meal based on crude protein

During the first 2 weeks of the experiment, there was no statistical difference in milk production by the dairy cows fed on the different treatment diets. However, from week 3 until the end of the experiment, milk production differed significantly (p <0.05) among the treatment groups. The dairy cows consuming treatment diet T1 generally maintained higher milk production especially from the 4th week of the experiment followed by treatment T3. The dairy cows consuming treatment T2 consistently produced the least milk throughout the experiment period. The dairy cows consuming diet T1 produced the highest milk (20.91 l/day) which was statistically different from the dairy cows consuming diet T3 (18.19 l/day) and significantly different from milk production from dairy cows consuming diet T2 (15.59 l/day). Consequently, the dairy cows consuming diet T1 increased mean milk production per day during the experimental period while those consuming diet T2 and T3 showed a decline in milk production.

Table 4.7 Weekly Lactation Performance (l/day) of Friesian Cows Fed Experimental Diets.

Week	(T1)	(T2)	(T3)	*p- Value*
1	19.71	18.28	19.79	0.535
2	20.93	18.08	20.68	0.14
3	21.24^{b}	17.02^{a}	21.46^{b}	0.009
4	21.00^{b}	15.16^{a}	20.93^{b}	0.001
5	21.65^{b}	16.25^{a}	20.43^{ab}	0.009
6	21.24^{b}	15.85^{a}	18.52^{ab}	0.005
7	20.91^{b}	15.59^{a}	18.19^{ab}	0.004

(T1) is group fed dairy meal with Mepron®. (T2) is group fed commercial dairy meal and (T3) is group fed farm formulated dairy meal. Means with the same row followed by different lower- case are significantly different (p<0.05).

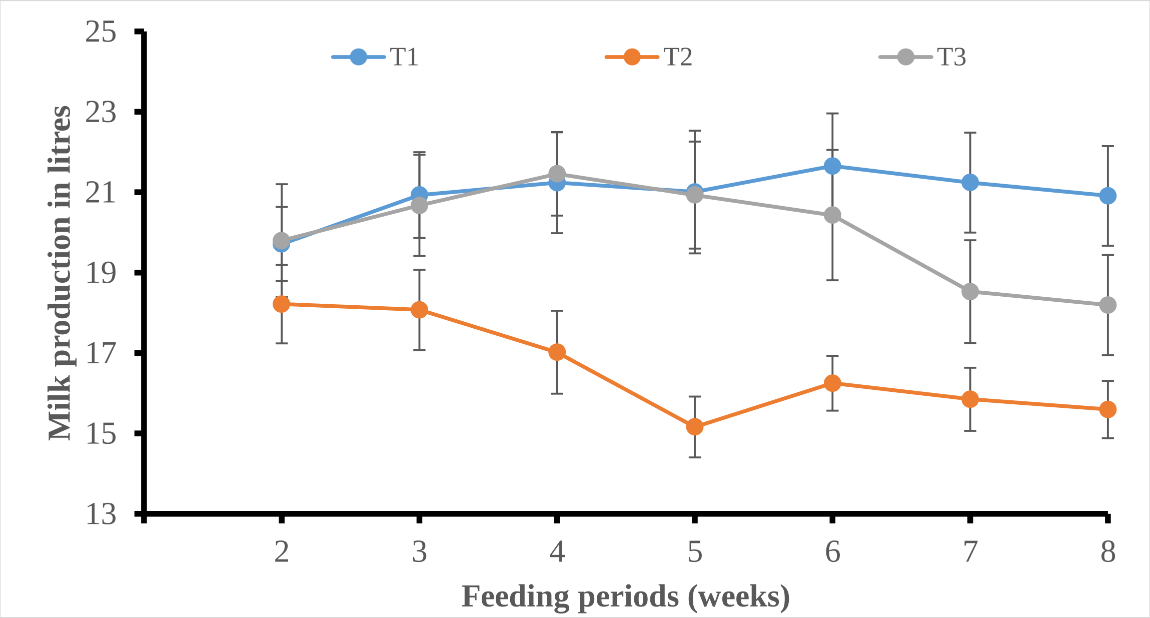

Figure 4.1 Milk production Trends during the Experiment Period

(T1) is group fed dairy meal with Mepron®. (T2) is group fed commercial dairy meal and (T3) is group fed farm formulated dairy meal.

Balancing dairy ration for amino acids (AA) is increasingly accepted in dairy nutrition. This is due to the desire to feed lower protein diets, high prices for protein supplements, an overall trend of higher milk protein prices, continued refinement and improvement of nutrition models, and increased availability of rumen-protected amino acids (Cabrita, 2011). From the result of this study, it was observed that balancing ration for amino acid using RP-Met (Mepron®) sustained and enhanced high milk production as compared to production from cows fed commercial dairy meal and farm dairy meals formulated based on crude protein.

One of the most critical determinants in milk production is the dietary quantity of crude protein (CP). The quantity of nitrogen (N) expelled in manure from dietary CP is about two to three times that excreted in milk (Broderick, 2003). Overfeeding of CP costs the animal in terms of energy and water. This cost is connected with the conversion of excess protein to urea and its subsequent removal from the body as urine (Dinn et al., 2013). The waste of energy in CP excretion might explain why cows given T2 and T3 showed a decrease in milk output while having a greater CP content in their diets.

Metabolizable protein is defined as genuine protein that is digested post-ruminally and the intestine absorbs the component amino acids (AA) (Cabrita, 2011). The essential nutrients for the dairy cow are AA rather than protein (Lee, 2012). Acceptance of some fundamental characteristics of AA nutrition and 'letting go' of balancing for crude protein are required for successful balancing for AA (Gilbreath et al., 2021). This is consistent with a research that found cows fed T1 balanced for amino acids with rumen protected Methionine (Mepron®) produced more milk than cows fed T2 and T3 formulated on CP. Another research showed that Met is more limiting than Lys, and cows reacted strongly to rumen-protected Met (RP-Met) supplementation (Socha et al., 2015).

Precision feeding may help dairy farmers increase milk output and composition by optimizing profit margins over feed costs and giving amino acid-balanced diets. The key focus in dairy nutrition is no longer crude protein (CP), but rather rumen degradable proteins (RDP) and amino acid concentrations in RUP (Arriola Apelo, 2014). Rumen undegradable protein (RDP) offers appropriate amino acid concentrations, while RDP is used by rumen microorganisms to produce their own amino acid, which is then given to the cow as microbial amino acid (Schwab, 2017). The key aims of this study are to reduce feed costs, increase milk output, and reduce environmental pollution (Tamura et al., 2019).

According to Toledo et al. (2021), the two most limiting amino acids for milk protein synthesis are lysine and methionine. When compared to milk protein and mixed rumen microorganisms, the two amino acids are typically low in most feed proteins. The importance of Lysine and Methionine concentrations in milk production and milk quality has been demonstrated in vitro using mammary epithelial cells by studying amino acid transport and signal transduction pathways that influence the expression of genes related to milk protein synthesis (Tamura et al., 2019). Both amino acids have been shown to boost milk protein transcription and translation by raising gene expression, although the maximum casein levels and cell proliferation rates were only detected when Lysine and Methionine supplements were administered in a 3:1 ratio. Various nutritional models have established appropriate Lysine and Methionine amounts in metabolizable protein, which has been helpful in balancing diets for these two amino acids.

Feeding rumen-protected Methionine (RP-Met) has been shown to increase post-ruminal Methionine availability (Arriola Apelo, 2014). Lactation performance in dairy cows has been enhanced by supplementing with RP-Met, which has been shown to lower the quantity of dietary protein required for milk production. The addition of RP-Met to transition cattle diets has lately received attention. Tamura et al. (2019) found that a lysine:methionine ratio of roughly 2.9:1 as a percentage of MP increased milk supply, perhaps owing to an increase in DMI and maybe improved use of body lipid stores. Supplementation with essential amino acids has been found to boost immune function, and these results support the fine-tuning of EAA content in dairy cow diet design.

Cysteine, homocysteine, and taurine, all required in methylation activities, may be synthesized from methionine, the body's only other sulfur-containing amino acid. The first stage in methionine metabolism is S-adenosylmethionine (SAM), a key cofactor in Met intermediate metabolism. SAM functions as a methyl donor in addition to amino acid residues in proteins, DNA, RNA, and small molecules (Gana, 2013). Methionine, in addition to being a precursor for SAM, is a source of hydrogen sulfide, taurine, and glutathione, among other things. Various oxidants, including those included in these products, minimize oxidative stress and protect tissue (Toledo et al., 2021). When milk production is at its greatest, rumen-protected methyl donors such as RP-Met may be required to satisfy the nutritional demands of the cows.

Most studies on the effects of methionine supplementation on otherwise nutritionally balanced meals have been carried out under tightly controlled settings during the transition period or at the start of lactation. There is a scarcity of data on the effects of dietary methionine supplementation throughout the peak of lactation and the remainder of the lactation, particularly in tropical cows. The addition of RP-Met to a corn-based, soybean meal lactation diet was expected to assist nursing cows perform better in the early stage of lactation and impact the plasma AA profile to achieve a 3.0:1 lysine: methionine ratio in metabolizable protein. The addition of rumen protected methionine to a high-milk-producer diet was necessary to study the influence of RP-Met on mid-lactation dairy cows' milk supply and milk component responses, notably peak milk yields. (Toledo and colleagues, 2021).

4.5 Effect of Inclusion of Rumen Protected Methionine on the Cows Resistance to Mastitis

Results on mastitis incidences is shown on figure 4.2 below. Cross tabulation analysis of mastitis incidence was significant among groups. Score Zero and one (-ve and +ve mastitis in one quarter, respectively) were significantly more frequent in Mepron® group (T1) followed by T3 (Farm dairy meal) and T2 (Commercial dairy meal). Interestingly, score 3 and 4 were not detected in Mepron® group (T1) a situation that may highlight the positive correlation between methionine adequacy from using RP-Met and mastitis incidence. Meanwhile, score 3 was significantly greater in T2 than T3. High incidence of mastitis with score 4 was only found in T3.

Figure 4.6 showed the apparently free mastitis and the +ve mastitis. Mepron® (T1) was 100% apparently free mastitis followed by T2 (75%) and T3 (74%) ($X^2 < 0.0001$). Additionally, Mepron® group (T1) showed zero incidence for score 3 and 4 mastitis. T2 was significantly affected compared to T3. The latter finding can be explained according to (Shah, 2020) there is no definitive link between protein content in diets and mastitis incidence however, there is more evidence regarding the harmful effect of N that is not in a protein form such as urea and ammonia on the mastitis incidence (Shah, 2020).

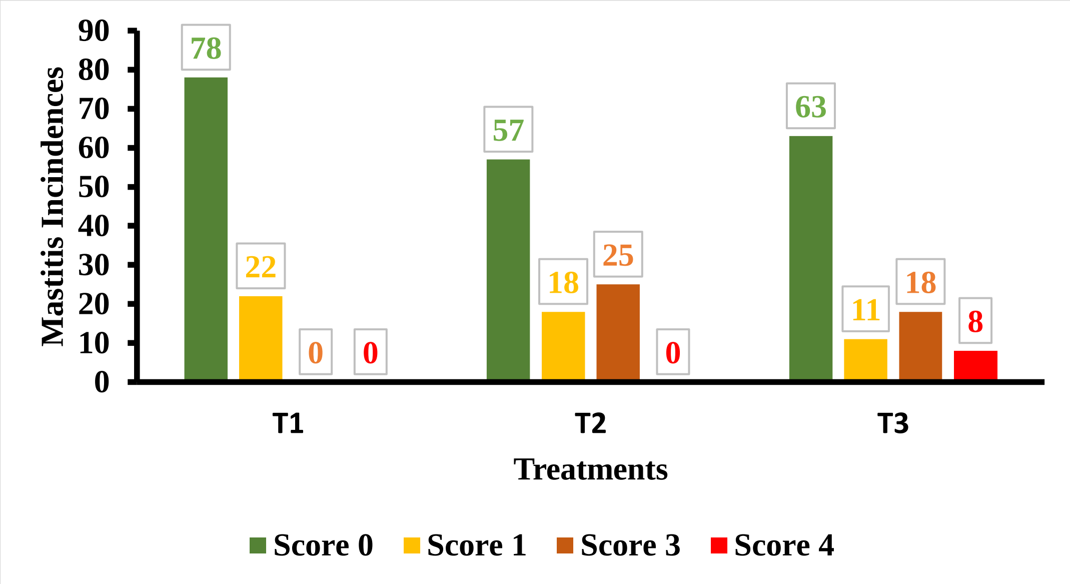

Figure 4.2 Mastitis incidences across treatments

(T1) is group fed dairy meal with Mepron®. (T2) is group fed commercial dairy meal and (T3) is group fed farm formulated dairy meal. Score 0 = -Ve Mastitis, Score 1= Mastitis in one quoter, score 2 = Mastitis in two quarters, Score 3 = Mastitis in three quoters and Score 4 = Mastitis in all the four quarters.

Cross tabulation analysis of mastitis incidence was significant among groups. Figure 4.3 showed that score Zero (negative mastitis in all the four quoters) were significantly more frequent in T1 (group fed ration balance for amino acid using rumen protected methionine Mepron®) followed by T3 (group fed farm ration formulated based on Crude protein) and T2 (group fed commercial ration formulated based on crude protein). Interestingly, score 3 and 4 were not detected in T1 a situation that may highlight the positive correlation between methionine adequacy from using rumen protected methionine and mastitis incidence. Meanwhile, score 3 was significantly greater in T2 than T3. High incidence of mastitis with score 4 was only found in T3.

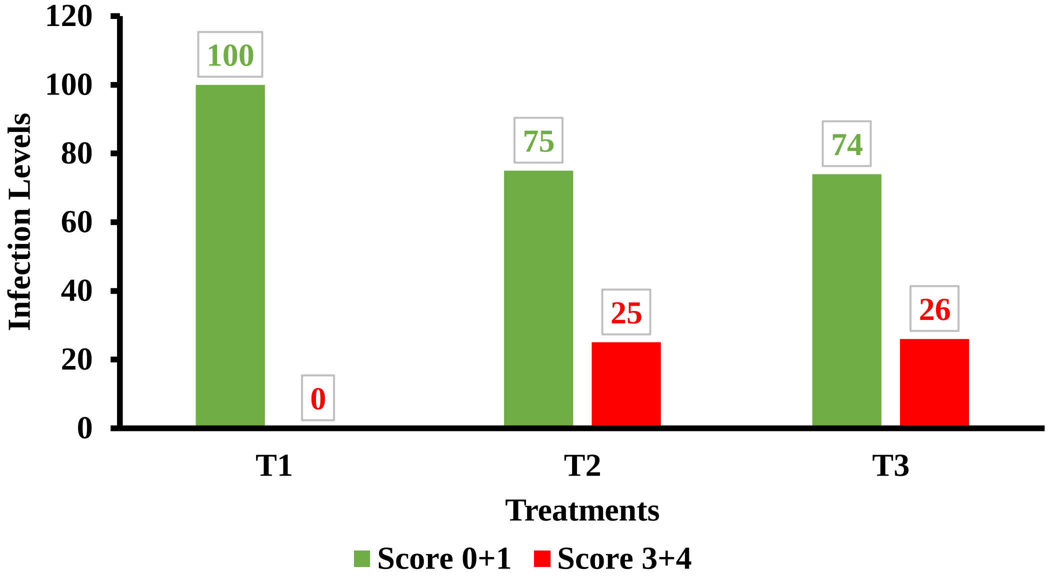

Figure 4.3 Mastitis infection levels across treatments.

(T1) is group fed dairy meal with Mepron®. (T2) is group fed commercial dairy meal and (T3) is group fed farm formulated dairy meal. Score 0 +1 = Negative Mastitis and positive mastitis in one quoter, Score 3+4 = Positive Mastitis in three quoters and positive mastitis in all the four quoters.

Figure 4.3 showed the apparently free mastitis and the +ve mastitis. Mepron® group was 100% apparently free mastitis followed by T2 (75%) and T3 (74%) ($X^2 < 0.0001$). T1 showed zero incidence for score 3 and 4 mastitis. T3 was significantly affected compared to T2. The latter finding can be explained according to (Shah, 2020) there is no definitive link between protein content in diets and mastitis incidence however, there is more evidence regarding the harmful effect of N that is not in a protein form such as urea and ammonia on the mastitis incidence.

Methionine has been linked to improved immune systems in dairy cattle, and supplying a rumen-protected Met supplement at a relatively high level (30g/d) was shown to increase the proliferative response of peripheral T-lymphocytes in mid-lactation dairy cows, despite no effect on milk SCC (Soder and Holden, 2009) might explain why dairy calves whose diets were supplemented with RP-Met had decreased occurrences of mastitis in our research.

4.6 Cost Benefit Analysis

The total cost of production includes feed, labor, medicine, water, power, and housing, among other things, but only the cost of feed was included during the production cost calculation since the rest were believed to remain constant throughout all treatments. Feed costs were determined using ingredient prices and the amounts of each included in the dietary feed regimens. All revenue advantages obtained were supposed to be recovered through the sale of milk produced. The cost-benefit analysis findings are shown in Table 4.8.

Table 4.8 Cost benefit analysis

Parameters	T1	T2	T3	SEM	p-value
Average Milk Production (L)	20.91[a]	15,59[b]	18.19[ab]	0.64	0.012
Farm gate Milk prices (KES/L)	28.00	28.00	28.00	-	-
Revenue Per day (KES)	585.48[a]	436.52[b]	509.32[ab]	17.94	0.011
Concentrate feed rate (Kg)	10	10	10	-	-
Cost (KES/Kg)	26.68[c]	26.90[b]	33.13[a]	0.33	0.000
Concentrate feed expenses per day (KES)	**266.8[c]**	**269[b]**	**331.3[a]**	**3.28**	**0.000**
Total Income (KES)	**318.68[a]**	**167.52[b]**	**178.02[ab]**	**17.95**	**0.011**
Margin from Feed (%)	**54.43[a]**	**38.38[ab]**	**34.95[b]**	**2.10**	**0.009**

(T1) is group fed dairy meal with Mepron®. (T2) is group fed commercial dairy meal and (T3) is group fed farm formulated dairy meal Means within rows with different superscripts differ ($p<0.05$), SEM, standard error of means. Exchange rate KES to USD = 0.0098

Cows fed a treatment diet (T1) balanced for amino acids with RP-Met (Mepron®) produced an average of 20.91 liters of milk per day, while cows fed commercial dairy meal (T2) produced an average of 15.59 liters of milk and cows fed farm Dairy Meal (T3) produced an average of 18.19 liters of milk per day. At the time of the trial, the farm gate price of milk was KES 28 per liter. The income received each day from cows given Mepron® DM (T1), commercial dairy meal (T2), and farm dairy meal (T3) was projected to be Ksh585.48, Ksh436.52, and Ksh 509.32, respectively.

Table 4.8 also shows the cost per kilo of T1, T2 and T3 which is 26.68Ksh/kg, 26.90Ksh/kg and 33.13Ksh/kg respectively. Given that each day the cows were fed 10kg of the concentrate, then the cost of concentrate feeds given in T1 feeds, T2 feeds and T3 was KES 266.80, 269.00 and 331.30 respectively. The profit gained when giving T1, T2 and T3 is KES 318.68, 167.52 and 178.02 respectively. Balancing dairy ration using RP-Met (Mepron®) as the case with T1 ration allowed reduction of feed cost by 0.82% against T2 and by 19.47% against T3. This together with better performance in terms of milk production, resulted to margin of 54.43%, 38.38% and 34.95% for T1, T2 and T3 respectively.

$$\textbf{Margin from feed} = \frac{\text{Revenue} - \text{Cost}}{\text{Revenue}} \times 100$$

CHAPTER FIVE: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary of Significant Findings

Using rumen-protected Methionine (Mepron®) to balance dairy cow feed for amino acids increased milk output by 2.72L compared to crude protein-based ration formulation. Dietary amino acid balancing with rumen-protected methionine (Mepron®) allowed for a decrease in crude protein without affecting the lactation performance of the cow. The reduced protein diet was less expensive than crude protein-based diets, making it cost-effective. Balanced dairy rations including rumen-protected methionine (Mepron®) improved the resistance of dairy cattle to mastitis by boosting their immune. This demonstrated a connection between methionine sufficiency from RP-Met and decreased mastitis incidence.

5.2 Conclusions

1. Balancing dairy ration for amino acids using rumen protected amino acid (Mepron®) enhanced milk production by 2.72L compared to ration formulated based on crude protein
2. Balancing dairy rations for amino acid using rumen protected methionine (Mepron®) enhanced dairy cattle immunity seen through resistance to mastitis. There cos a positive correlation between methionine adequacy from using rumen protected methionine and reduction in mastitis incidences.
3. Balancing dairy rations for amino acid using rumen protected methionine (Mepron®) allowed for reduction of crude protein without compromising the cow's lactation performance. The low protein diets gave better performance at a lower cost.

5.3 Recommendations

Based on the study findings the following recommendations were made:

1. When formulating dairy ration ensure sufficient supply of methionine in form that bypasses microbial degradation in the rumen to support high milk production by balancing dairy rations for amino acids using rumen protected methionine (Mepron®).
2. Dairy nutritionist, vets and farm managers should apply amino acid nutrition to manage diseases such a mastitis by balancing rations for amino acid using rumen protected amino acid like Mepron®.

3. Dairy nutritionist to formulate low protein dairy meal at KEBs minimum 14.5%CP for both commercial and on-farm dairy meal to make it cost effective but ensure that the first and second limiting amino acid Methionine and Lysine are met through use of rumen protected methionine like Mepron® and or rumen protected lysine. To balance the ration first determine amino acid requirement of the dairy cattle using TMR formulator like AMINOCow® then extract the concentrate portion of the diet and optimize it using a least cost feed formulation software like Brill®

5.4 Areas for Further Studies

More study needs to be done with other commercial dairy breeds like Ayrshire and Jersey. In addition, studies need to be done using somatic cell count as a better way of detecting incidences of subclinical and clinical mastitis

Overall, this experiment shows that balancing AA in lactating dairy cows improves milk and milk component yields and production efficiency. Rumen protected methionine has made great strides in recent years. Rumen protected methionine is poorly understood. Molecular approaches to identify individual bacterial strains will help us understand rumen's biochemical network. We will soon have credible models to predict bacterial responses to dietary changes with the current interest in this subject across many scientific disciplines. Increasing public awareness of production agriculture, environmental concerns resulting in strict guidelines on nutrient disposal, and the changing nature of the dairy business will challenge ruminant nutritionists and microbiologists to understand rumen energetics better. Biological reactions are inefficient. With current knowledge and future research in the effects of rumen protected methionine, the potential to improve rumen function is huge.

Animal biology causes nutrients to accumulate where animals are. Growing public awareness raises environmental concerns. Consultants, educators, and producer advisors must help clients develop environmentally friendly business management systems. These systems should include agricultural goals. As consultants and educators, we must instill confidence in farmers, agribusinesses, and other stakeholders.

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APPENDICES

Appendix I: KU Graduate School Research Approval

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Our Ref: A150/OL/CTY/25996/2018

DATE: 31st August, 2021

Director General,
National Commission for Science, Technology
and Innovation
P.O. Box 30623-00100
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR MR. JOHN OWINO OWAGA – REG. NO. A150/OL/CTY/25996/18

I write to introduce Mr. John Owino Owaga who is a Postgraduate Student of this University. He is registered for M.Sc. degree programme in the **Department of Agricultural Science & Technology.**

Mr. Owaga intends to conduct research for a M.Sc. thesis Proposal entitled, **"Effect of Rumen Protected Methionine on Milk Production and Quality of Dairy Cattle in Kenya."**

Any assistance given will be highly appreciated.

Yours faithfully,

PROF. ELISHIBA KIMANI
DEAN, GRADUATE SCHOOL

JG/oww

Appendix II: Permit for Research from NACOSTI

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **805481**

Date of Issue: **13/September/2021**

RESEARCH LICENSE

This is to Certify that Mr.. John Owino Owaga of Kenyatta University, has been licensed to conduct research in Nairobi on the topic: Effect of rumen protected methionine on milk production and quality of dairy cattle in Kenya for the period ending : 13/September/2022.

License No: **NACOSTI/P/21/12888**

805481

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix III: Collaboration with Unga Farm Care

EVONIK
POWER TO CREATE

Protocol For Feeding Trial with Dairy Cows

Title of Study;	Impact of balancing diets and inclusion of Mepron® (Rumen Protected DL-Methionine) on dairy cow performance in Kenya
Trial Number;	002
Trial Sponsor;	Martin Smith Evonik Industries AG; Health & Nutrition GmbH Rodenbacher Chausee 4 63457 Hanau Wolfgang Germany Email martin1.smith@evonik.com
Trial Monitor;	John Owaga Evonik East Africa 14 Riverside Drive Business Park, Hanover Block, 5th Floor, Suite 5B P.O Box 2171-00606 Nairobi, Kenya Email john.owaga@evonik.com
Trial Investigator;	Harrison Juma Unga Farm Care Ltd Ngano House Commercial Street Industrial Area P.O Box 41788-00100 Nairobi, Kenya Cell Phone: +254721489686 Email hjuma@unga.com)

1/7

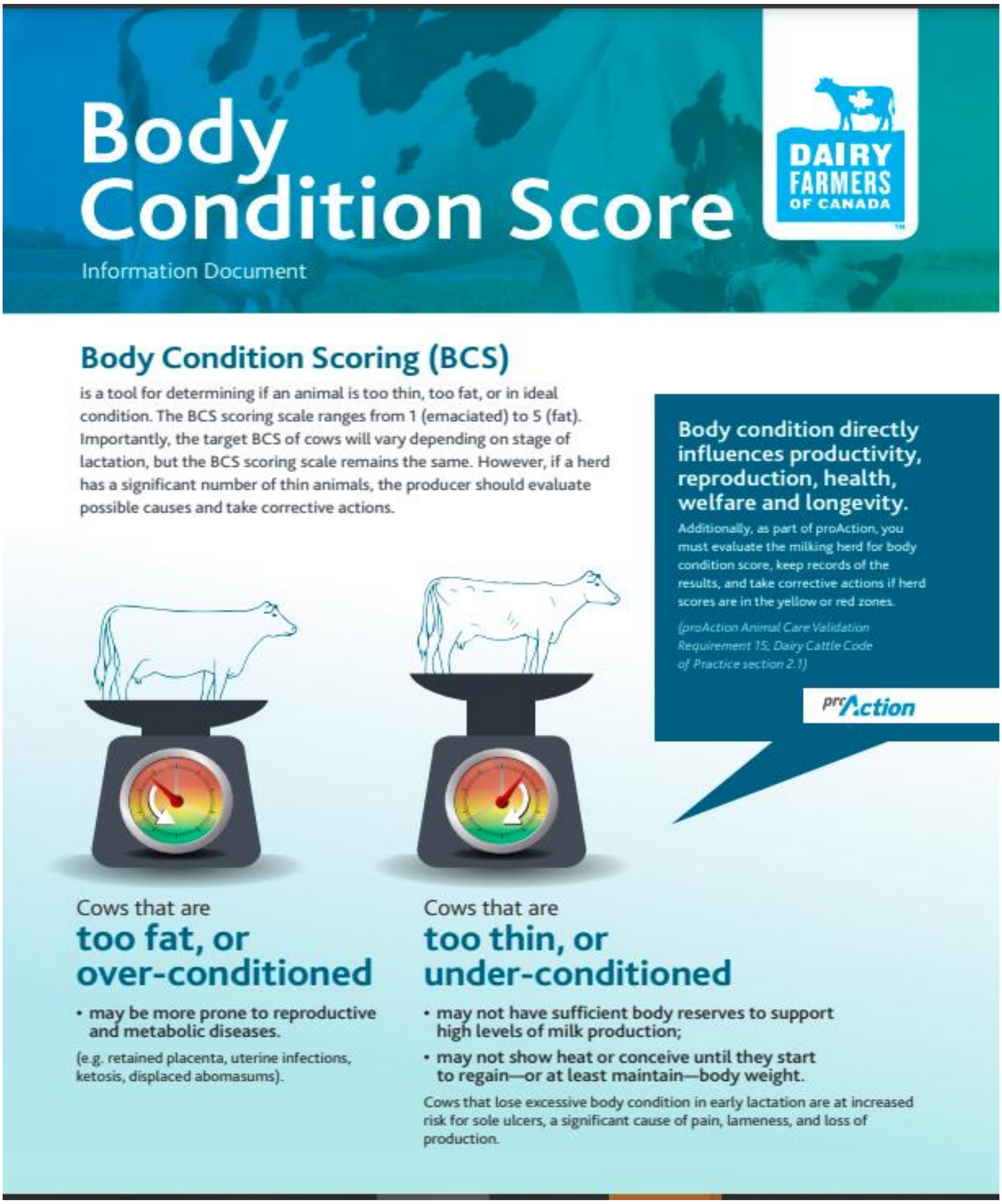
Trial Investigator;	Phiona Gichuru Unga Farm Care Ltd Ngano House Commercial Street Industrial Area P.O Box 41788-00100 Nairobi, Kenya Cell Phone:+254722483142 Email pgichuru@unga.com)

Appendix IV: Data collection tools

a) Milk production data sheet

Mepron trail data end of week Viii (2).xlsx

b) Grind for body score



c) Dairy meal specifications- KEBS

KENYA STANDARD

KS EAS 75:2019
ICS 65.120

APPROVED
2019-07-23

Compounded cattle feeds — Specification

Second Edition 2019

Appendix V: Experimental lay out and animals

a) Experimental animals