METABOLITES AND HORMONES AS INDICATORS OF POSTPARTUM REPRODUCTIVE EFFICIENCY OF SUPPLEMENTED PASTURE- BASED FRIESIAN DAIRY COWS AT KALRO, LANET, NAKURU COUNTY, KENYA

ANNAH HOKA INDETIE (MSC)

184/21977/2012

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DECLARATION

I hereby declare that this thesis is my original work and has not been submitted to any other University for degree or other awards

Signature………………………… Date……………………………

ANNAH HOKA INDETIE
184/21977/2012
DEPARTMENT OF ZOOLOGICAL SCIENCES

SUPERVISORS

We confirm that the work reported in this thesis was carried out by the candidate under our supervision

Signature…………..…………… Date …………………………
Prof. MICHAEL GICHERU
DEPARTMENT OF ZOOLOGICAL SCIENCES
KENYATTA UNIVERSITY

Signature…………………………… Date…………….………….

Dr. SYPRINE OTIENO
DEPARTMENT OF ZOOLOGICAL SCIENCES
KENYATTA UNIVERSITY

DEDICATION

Dedication of this work is to God for enabling me to complete it; to my husband for his encouragement and support.

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ABBREVIATIONS AND ACRONYMS

ADF	Acid Detergent Fibre
AI	Artificial Insemination
ANOVA	Analysis of Variance
ATP	Adenosine Tri Phosphate
BCS	Body Condition Scores
BF	Butter Fat
BHB	β – Hydroxybutyrate
BSA	Bovine Serum Albumin
BUN	Blood Urea Nitrogen
CL	Corpus Luteum
CLA	Commencement of Luteal Activity
CP	Crude Protein
CRBD	Complete Randomized Block Design
DCCO	Degummed Canola Crude Oil
ECF	East Coast Fever
EDTA	Ethylene Diamine Tetra – Acetate
ENPD	Early Non -Pregnancy Diagnosis
FAO	Food and Agriculture Organisation
FSH	Follicle Stimulating Hormone
G - 6 - PDH	Glucose - 6- Phosphate Dehydrogenase
IGF-1	Insulin-like Growth factor-1
IVDMD	In Vitro Dry Matter Digestibility
LH	Luiteinizing Hormone
LYS	Lysine
M1	Mutant1
ME	Metabolisable Energy
MUN	Milk Urea Nitrogen
NDF	Neutral Detergent Fibre
NEB	Negative energy balance

NEFA	Non-esterified fatty acids
NFE	Nitrogen-free Extract
OM	Organic Matter
P	Protein
P4	Progesterone
PGF2α	Prostaglandin F2 α
PSP-B	Pregnancy Specific Protein-B
QC	Quality Control
RDP	Rumen Degradable Protein
RIA	Radioimmunoassay
RPM	Revolution Per Minute
SM-C	Somatomedin-C
SNF	Solid not Fat
SSA	Sub-Saharan Africa
TAG	Triacyglycerol
TC	Total Count
TCA	Tricarboxylic acid
TMR	Total Mixed Ratio
VLDL	Very Low Density Lipoprotein

ABSTRACT

High-yielding dairy cows experience a phase of substantial tissue catabolism in the early postpartum period due to a negative nutritional balance that is partially brought on by inadequate fodder quality and quantity. A significant obstacle to increasing cow production is the requirement for feed ingredients during late pregnancy to sustain the growth of the fetus and lactation following parturition. Metabolic signals and regulatory hormones are linked to the mechanism for the restart of ovulation. The ability of metabolites and hormone levels to predict nutritional condition and postpartum reproductive efficiency in dairy cows in Kenya is unknown. Programs for breeding and feed formulation may benefit from this data. The goal of the study was to determine the role of hormones and feed metabolites as markers of postpartum reproductive efficiency. Twenty cows were supplemented with total mixed ration feed blocks (TMR) twice daily while grazing on *Elba rhodes* grass for eight hours/day. Ten in-calf cows served as the control; they grazed on *Elba rhodes* grass for eight hours without supplementation. Body condition scoring was done every fifteen days. Gestation period, calf sex, milk production, and days to postpartum heat were recorded. Analysis of feeds' chemical composition and digestibility were conducted. Skimmed milk was prepared and kept at 4°C until time for laboratory analysis. Jugular venipuncture was used to obtain blood samples into ethylene diamine tetraacetate polystyrene tubes. The blood was spun at 1500 rpm for 15 minutes to release plasma which was stored at -20°C until time for laboratory analysis. Radioimmunoassay was used to track postpartum ovarian activity using skimmed milk. Data was analyzed using statistical analysis system (2010). The results showed that neutral detergent fibre of feeds used in this study ranged from 39.5±0.04 % to 41.7±6.7%. Nutrient components that highly positively correlated with metabolisable energy were protein, methionine, and lysine. Supplementation significantly influenced ($p<0.05$) metabolite and hormonal levels. Body condition score at calving was higher among test cows (2.9±0.1) than the control (2.5±0.1). Test cows produced significantly higher percentage (80%) of female calves than the control (20%). The test cows took significantly shorter gestation period (278.4±1.0 days) than the control (284.1±1.1 days). The test cows registered higher mean daily milk yield (9.1 L) than the control (5.3 L). Test cows cycled earlier (50.4±0.90 days) than the control (62.7 ±2.0 days). The test cows had significantly less number of insemination to conception (1.35) than the control cows (2.7). Insulin like growth factor-1(IGF) and insulin reduced with advanced pregnancy, with the test cows having significantly higher values than the control. Glucose decreased with advanced pregnancy reaching threshold day 15 postpartum with the test cows having higher values. Commencement of luteal activity concided with low levels of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB). Luteal activity progesterone was positively highly correlated to IGF-1($p < 0.001$, $r^2=0.672$), and glucose ($p < 0.001$, $r^2=0.634$) but highly negatively correlated to NEFA ($P=0.001$, $r^2 = -0.689$) and BHB ($P=0.001$, $r^2=0.679$). Glucose, NEFA, IGF-1, and BHB are good indicators of postpartum reproductive efficiency. Data generated from this study is useful in reducing calving interval. Supplementation of in calf cows with quality feed is recommended to reduce calving intervals.

CHAPTER ONE: INTRODUCTION

1.1 Background Information

In sub-Saharan Africa, agricultural production continues to be the primary source of income for rural people. It employs more than 60% of the population and accounts for around 30% of gross domestic product (GDP). One of sub-Saharan Africa's greatest dairy industry is found in Kenya. The 3.8 million milking cattle in Kenya's dairy business make it a vibrant sector with a significant economic and nutritional impact on the lives of many people, including farmers, milk vendors, processors, and consumers (Indetie *et al*., 2007)). Kenya and South Africa are the only nations in Africa that produce enough milk for both local use and export (Techno-serve, 2008). It is commonly known that dairy cow fertility is declining globally (Beam and Butler, 1999; Royal *et al*., 2000; Lucy, 2001). Energy needed for milk production and for heat expression compete for the same nutrients. Postpartum cows have low body condition score; this happens because energy intake is less than energy expenditure for reproduction and production.

To mitigate the negative energy balance, cow's metabolic and hormonal changes enable enhanced lipolysis (Reist *et al*., 2002; Bekuma and Galmessa, 2019). Metabolic adaptation hormones and metabolites transform the nutritional status to reproductive functioning. Reduced concentrations of estradiol and insulin-like growth factor 1 (IGF-1) are linked to ovulation failure (Denicol *et al*., 2012), Blood urea nitrogen (BUN) dynamics can be monitored quickly and non-invasively using milk urea nitrogen (MUN) (Gustafsson and Palmquist, 1993; Munyaneza *et al*., 2017). The six to eight-week transition or peri-parturient period is crucial in determining the welfare and profitability of individual cows over the next lactation (Drackley *et al*., 2005; Wankhade *et al*., 2017). Poor transition management programs increase the risk of production diseases in dairy cows (Mulligan and Doherty, 2008). Difficult transitions have negative effects on subsequent reproduction, and disturbances in metabolism during the transition and may have direct or indirect repercussions on fertility (Chapinal *et al*., 2012). Several nutritional regimens have been proposed to improve metabolic and physiological adaptations from gestation to suckling, with different results (Friggens *et al*., 2004; Roche *et al*., 2013). Insufficient and inconsistent diets are common characteristics of feeding regimens in low input systems resulting in large variation in milk yield (Walshe *et al*., 1991; Makau *et al*., 2020) and long calving intervals (Odima *et al*., 1994; Bell *et al*., 2011). Nutritional deficiency of up to 15 MJ/day of metabolisable energy and an effective rumen degradable protein of 235 g per day has been established (Topps, 1994).

Dairy cows fed on poor quality feeds are quite responsive to better nutrition (Makau *et al*., 2020). Most common dairy cattle breeds in Kenya are Friesian, Ayrshire, Guernsey and Jersey (Lukuyu *et al.*, 2019). Ovarian activity of postpartum lactating cows are thought to be re-initiated by feeds (Makau *et al*., 2020). However, the association is not clear (Keady *et al*., 2001; Pushpakumara *et al*., 2003; Chagas *et al*., 2006). Different dietary components are associated with successful postpartum reproduction, through their enhancement of follicular dynamics (Bisinotto *et al*., 2012). Brachiaria cultivars can support optimum ruminant livestock production, and, their high nutritive value supersedes minimum animal requirements (Sokupa *et al.*, 2023). The body's energy balance is indicated by blood hormone (IGF-1) and metabolites (NEFA, BHBA) (Xu *et al*., 2020).

1.2 Statement of the Problem

Improvement in milk yield has led to negative energy balance (NEB), leading to long calving intervals. To limit the negative effects of NEB, various nutritional methods have been employed (Gordon *et al*., 2013) with varying influences; positive influences (Chagas *et al*., 2007), negative influences (Keady *et al*., 2001), and no responses (Pushpakumara *et al*., 2003). Metabolite and hormone profiling are valuable for monitoring postpartum reproductive efficiency in dairy cows (Kabir *et al*., 2022). Detection of an association between metabolite and hormone concentrations with conception has not been widely researched (Schiller *et al*., 2015). Ovarian activity of post-partum lactating cows is thought to be re-initiated by feeds (Makau *et al*., 2020), however, the association is not clear (Keady *et al*., 2001; Pushpakumara *et al*., 2003; Chagas *et al*., 2006).

Brachiaria cultivars can support optimum ruminant livestock production, and, their high nutritive value supersedes minimum animal requirements (Sokupa *et al.*, 2023). There was need to establish the association between conception with metabolite and hormones when postpartum dairy cows were supplemented with total mixed ration feed blocks comprising Brachiaria *ruziziensis* as a fodder in the blocks. High associations would be used as indicators of postpartum reproductive efficiency and would be used to monitor performance of postpartum dairy cows for timely intervention leading to prompt exhibition of postpartum heat.

1.3 Justification of the Study

A transition period is crucial in the life of dairy cattle because it can affect the metabolic and health condition of dairy cows as well as their reproductive and yield potential in subsequent lactations (Roche *et al*., 2015). During the transition period, there are fluctuations in the dry matter intake (DMI). A pronounced decline in DMI is observed in the last 10 days of parturition hence presumably not sufficient to fulfill the increased nutrient and energetic demand of postpartum dairy cows (Pérez-Báez *et al*., 2019). This could lead to a negative energy balance in the early lactation phase. During negative energy balance, excessive lipolysis and rising levels of non-esterified fatty acids (NEFA) in the blood of dairy cows are frequently linked to the accumulation of triglycerides (TG) in hepatocytes and impairment of liver function. This results in elevated ketone production. Increasing blood NEFA levels may impair oocyte development and reproductive efficiency (Walsh *et al*., 2006).

Postpartum hormones and metabolites profiling can serve as early, objective indicators of postpartum reproductive efficiency in dairy cows. Early detection of reproductive deficiency using these markers can help prevent increased calving interval leading to increased production and reproduction sustaining dairy farming. The current study was done using Friesian dairy cattle because the breed is used for research at the KALRO-Lanet Centre and it is one of the most popular dairy breeds in Kenya. The study was done at Kenya Agricultural and Livestock Research Organization (KALRO), Lanet for ease of monitoring, sample collection and storing at (-20^{o}C) using the existing sample refrigeration systems.

1.4 Research Questions

(i) What is the effect of total mixed ration feed blocks (TMR) on cow and calf characteristics within 60 days postpartum among pasture-based Friesian dairy cows at KALRO-Lanet?

(ii) What is the influence of TMR feed blocks on selected hormones and metabolites profiles within the voluntary waiting period (60 days postpartum) among Friesian cows?

(iii) What is the relationship between selected hormones and metabolites with milk yield and postpartum reproductive efficiency, within 60 days among pasture-based Friesian cows in KALRO-Lanet?

(iv) What hormones and metabolites are indicative of postpartum ovarian cyclicity among pasture-based Friesian cows at KALRO-Lanet?

1.5 Null Hypotheses

(i) Total mixed ration feed blocks have no effect on cow and calf characteristics of pasture based Friesian dairy cows at KALRO-Lanet.

(ii) Total mixed ration (TMR) feed blocks have no effect on selected milk and plasma metabolites and hormones among pasture based Friesian dairy cows at KALRO-Lanet.

(iii)There is no relationship between selected metabolites and hormones on postpartum reproductive efficiency among pasture based Friesian cows at KALRO-Lanet.

(iv) Metabolites and hormones are not predictive of postpartum ovarian cyclicity among pasture based Friesian dairy cows at KALRO-Lanet.

1. 6 Objectives

1.6.1 General Objective

To determine the role of feed metabolites and hormones as indicators of postpartum reproductive efficiency among pasture based Friesian dairy cows in KALRO-Lanet.

1.6.2 Specific Objectives

(i) To determine the effect of total mixed ration (TMR) feed block on cow and calf characteristics among pasture-based Friesian dairy cows in KALRO-Lanet?

(ii) To determine the influence of total mixed ration (TMR) feed blocks on selected milk hormone plasma hormones and metabolites profiles within the voluntary waiting period (60 days postpartum) among Friesian cows

(iii) To assess relationship between selected hormones and metabolites with milk yield and postpartum reproductive efficiency, within 60 days among pasture-based Friesian cows in KALRO-Lanet?

(iv) To determine metabolic and hormonal indicators of postpartum reproductive efficiency and conception within 60 days.

1.7 Significance of the Study

Determining metabolite and hormone indicators of postpartum cyclicity in dairy cows is significant because it helps in early detection of metabolic and reproductive issues. This allows for timely intervention to improve fertility, prevent diseases, and optimize farm profitability. These indicators reveal a cow's energy status and overall health, which directly impact ovarian function, and can allow for dietary adjustments and management strategies to enhance reproductive success and milk/meat production. This will empower the farmer through sale of the surplus products.

CHAPTER TWO: LITERATURE REVIEW

2.1 Dairy Cows

The dairy cows are bovines bred and fed to produce large volumes of milk. They suffer metabolic stress of high performance and the physical effects of being milked and handled two or three times daily (Erickson and Kalscheur, 2020). Holstein breed is the most common and it is known for its high milk production. Other common dairy breeds include; Brown Swiss, Ayrshire, Jersey, Guernsey, Norwegian Red, and milking Shorthorn (Buchanan. 2016). Most common dairy breeds in Kenya include Friesian, Ayrshire, Guernsey, and Jersey (Lukuyu *at al*., 2019).

2.1.1 Reproductive Physiology of Dairy Cows

Optimizing reproductive performance is of crucial to dairy and beef producers. The understanding of the physiologic and endocrinological systems regulating the reproductive process is essential for the creation of novel and enhanced techniques to improve reproductive performance (Hafez. and Hafez, 2000; Magnusson and Persson, 2015; **Evans** *et al*., 2022).

2.1.2 Estrus Cycle of Dairy Cows

Estrus refers to the short period in which a cow/heifer is sexually receptive and will stand to be bred. Ovarian steroid hormones have a special impact on behavioural regions of the brain, which results in estrus. A Graafian follicle develops in response to pituitary gonadotrophic hormones, synthesizing and secreting increasing amounts of estradiol. Once estradiol reaches threshold concentration, it first initiates behavioural response and a surge in the release of pituitary gonadotrophic hormones, particularly lutinizing hormone (LH).

In "western" cow breeds, this results in a spike in gonadotrophins, which may be seen in the peripheral circulation, and prompts ovulation 20–30 hours later. The mature follicle controls the amount of estradiol produced, the timing of ovulation, and the concurrent development of the oocyte (Hunter, 2003). Characteristic like, mucus outpouring at estrus is caused by estradiol's effects on the vaginal canal. In the female vaginal canal, mucus is the first physiological medium that sperm cells encounter and must overcome before reaching the site of fertilization (Rutllant *et al*., 2005). A three-dimensional filamentous network of mucins (macromolecular glycoproteins) controls the physical characteristics of mucus throughout the secretory bulk. In the follicular phase of the ovarian cycle, cervical mucus becomes more copious, fluid, translucent, less thick, and easier for spermatozoa to pass through. The mucus becomes thin and viscous during the luteal phase, making it difficult for sperm to penetrate through.

Eight to twelve hours after onset of estrus, the mucus significantly reduce its mechanical barrier effect, making it less hostile for sperm movement (Rutllant *et al*., 2005). Although studies of temperature variations in the ovarian structures during estrus have been conducted (Greve *et al*., 1996; Hunter *et al*., 2006), research utilising contemporary technologies is urgently needed to examine temperature changes in the deeper parts of the genital tract. They could be triggering a range of molecular changes in genital epithelial tissues and growing embryos, and may not represent temperatures recorded in the vagina or rectum (Hunter *et al*., 2018). Bulling animals deploy polymorphonuclear leucocytes into the uterine lumen, where they vigorously phagocytose dead or decapacitated sperms. Bovine caudal isthmus operates as sperm storage site after mating for upto 30 hours (Hunter and Wilmut, 1983; Hunter and Wilmut, 1984; Wilmut and Hunter, 1984)

2.1.3 Theory on Regulation of Estrus

A recent theory has underlined the significance of gonadotropin-releasing hormone (GnRH) in the control of estrus behaviour (Van Eerdenburg, 2008). It is feasible since there are other hormones besides estradiol that govern estrus symptoms. Hypothalamic alpha type estradiol receptors completely disappear during estrus (Lyimo *et al*., 2000; Van Eerdenburg, 2000). The regulation of estrus behaviour, which includes licking, stroking, and aggressive behaviour like head butting, has thus been considered as being significantly influenced by GnRH (Kerbrat and Disenhaus, 2004). An increase in activity is another behavioural change seen during estrus, as evidenced by a cow taking more steps while in estrus. Without expert assistance, it is challenging to spot this change, yet it is present in many estrus cows (Roelofs *et al*., 2005). Low milk production is another occurrence that occasionally takes place during estrus (Schofield *et al.*, 1991). However, Rajamahendran *et al*. (1989) found no evidence of a significant decrease in milk output during estrus.

2.1.4 Hormonal Control of the Estrus Cycle

The hypothalamic and pituitary control of reproductive and metabolic hormones is necessary for the integration of the reproductive system and its function during important physiological periods such as the estrus cycle, pregnancy, and the transition (postpartum) phases, which results in the restoration of reproductive competence (Silva *et al*., 2018). Ability to quantify hormones using specialized, delicate, and precise methods has greatly advanced our understanding of the endocrine system using the broad principle that Trenkle (1972) introduced which permit radioimmunoassay (RIAs) to precisely and reliably detect hormones in plasma. Biological measurements based on RIA, ELISA, mRNA expression via polymorised chain reactions (PCR), and protein abundance all use such a principle.

The GnRH released by hypothalamic neurosecretory neurons terminating in the median eminence travels through the blood vessels of the hypophyseal portal system to the anterior pituitary. The primary role of GnRH is to stimulate the release of FSH and LH from gonadotrophins, which are essential for follicular development, maturation, and ovulation as well as the development of the corpus luteum (CL). Estrus responses in cows with adequate plasma estradiol can be easily recognized (Lopez *et al*., 2005). Modern dairy farming depends on dairy cows having good fertility. Although the fertilization rate is still higher than 70% in high and medium-producing dairy cows, fertility has unfortunately decreased over the past few decades (Bridges *et al*., 2013), indicating that there may be additional factors contributing to the observed diminishing fertility.

Losses of embryo occur before day 16 after insemination (Diskin *et al*., 2011) and appear to be associated with low progesterone (P4) levels. Low systemic P4 concentration has been linked to both a smaller corpus luteum (CL) (Moore *et al*., 2014) and a robust hepatic steroid hormone clearance (Witbank *et al*., 2012). Endometrial functions needed for embryonic survival, implantation, and growth are stimulated and sustained by progesterone (Spencer *et al.,* 2016). Regardless of the presence of the embryo, P4 increases the expression of essential genes in the endometrial epithelia, which is then further enhanced by molecules from the conceptus such as interferon tau and prostaglandins (Spencer, *et al*., 2016). .

2.1.5 Behavioral Patterns Associated with Estrus

A mature and competent egg is produced during estrus through ovarian follicular development (Forde *et al*., 2011). Insulin-like growth factor (IGF-1), growth hormone (GH), and leptin have been implicated in the regulation of reproduction in cattle during the ovarian follicle development (Ibtisham *et al*., 2018).

It is possible to modify the relationships between metabolic hormones and follicular growth in cattle to optimize milk output and reproductive success. This period, also known as the receptive phase of estrus or real estrus, is defined as the time when a cow expresses standing estrus (Hurnik, 1987; Sveberg *et al*., 2011). Majority of cows show estrus that last for few minutes, while few cows show estrus that last up to seven hours (Roelofs *et al*., 2005; Sveberg *et al*., 2011;). The main estrus signs are the desire to mount and the lowering of the back. Secondary symptoms such as anogenital sniffing, chin resting, restlessness, licking, and rubbing against objects are common, but they are viewed as less accurate because they can occur at any time during the estrus cycle, not only during the receptive phase (Diskin and Sreenan, 2000; ; Kerbrat and Disenhaus, 2004; Roelofs *et al*., 2010).

A number of local genital signs, including vulvar swelling and redness, as well as vulvar discharge, are strongly linked to estrus (Stevenson *et al*., 1983; Roelofs *et al*., 2010). When the cows are unrestrained, their heat expressions are more visible, but this makes it difficult to observe local signs. Tethered cows are unable to stand or mount, farmers must rely on the detection of secondary and local estrus indications (Ranasinghe *et al*., 2009). When a confined cow comes into contact with a neighbouring animal or the herdsman, they lower their backs and raise their tails, indicating sexual receptivity (Gustafsson, 1984; Hurnik, 1987; Nyman *et al*., 2016). For the success of AI, the timing of ovulation is linked to the timing of insemination (Nalbandov and Casida, 1942).

2.1.6 Importance of the Corpus Luteum

In females, the corpus luteum (CL) is necessary for establishing and maintaining pregnancy. It secretes progesterone, a steroid hormone that is crucial for the endometrium's decidualization and maintenance. If the egg is not fertilized, the CL stops secreting progesterone and decays. It then degenerates into a corpus albicans. The uterine lining sloughs off without progesterone and is expelled through the vagina (Gordon, 1996; Thiyagarajan *et al*., 2022). In an estrus cycle, the lining degenerates back to normal size. If the egg is fertilized and implantation occurs, the introduction of prostaglandins at this point causes the degeneration of the CL and the abortion of the fetus. However, in placental animals such as bovines, the placenta eventually takes over progesterone production and the CL degrades into a corpus albicans without embryo/fetus loss (Thiyagarajan *et al*., 2022).

2.1.7 Biological Functions of Progesterone

Progesterone (P4) is produced by luteal tissue and the placenta. The hormone is present in bodily fluids like blood and milk and is essential for the control of female reproduction. Its primary function is to maintain pregnancy by influencing the endometrium, which creates a favourable environment for the developing foetus (Kumar *et al*., 2014). In skimmed milk, progesterone concentrations below 1 nmol/L demonstrate the lack of CL, while concentrations above 3 nmol/L indicate a functional CL (Hafez and Hafez, 2000).

Low levels of progesterone are a prerequisite for occurrence of estrus (Gordon, 1996; Abreu, 2015). Progesterone can be measured using available sensitive assays to check on estrus. In Germany, milk P4 assays are used to detect whether dairy cows undergoing artificial insemination (AI) are in estrus. These assays also help to detect reproductive deficiencies among milking multiparous cows. The assays are also used to detect 'silent heats, or ovulation without estrus, which are common in dairy cattle and contribute to the reproductive problem. Acceptable conception rates can be attained with AI even in the absence of estrus after a measurable decline in milk progesterone (McLeod *et al.*, 1991; Hafez and Hafez, 2000; Rao *et al.*, 2013).

Milk samples from a cow (two or three taken weekly) can be analyzed for progesterone to determine the ideal ovulation date and whether the cow is cycling or not. Cows whose milk is sampled daily for progesterone analysis can have fixed-time artificial insemination from 17 days after a previous heat phase. The daily checks will indicate when the P4 levels decline, and thus the cow will be artificially inseminated (AI) on the third day (Rao *et al.*, 2013).

2.2 Artificial Insemination

Quick expansion of artificial insemination services in the world, has been the most significant commercial advancement in cattle breeding. Farmers have a choice of bulls with a variety of desirable qualities due to artificial insemination (AI) (Gordon, 1996; Gaurang *et al.*, 2017). Cattle estrus lasts from 12 to 24 hours with an an average of 18 hours, (Gordon, 1996; Rao *et al.*, 2013). Cattle ovulate 10–12 hours after estrus. Cattle should be inseminated once daily, in the morning, under ideal semen quality conditions in order to achieve satisfactory conception rates. When based on standing heat, AI can be given once daily and should be administered 8 to 11 hours after the heat (Nebel *et al.,* 1992; Riaz *et al.*, 2018). When using frozen-thawed semen, it's crucial to inseminate at the optimum time (late estrus). This could be due to sperm survival within the reproductive tract when only a few sperms are deposited in the uterus during insemination.

Most inseminators place sperm at the junction of the uterine body and the internal cervical aperture. Location of semen deposition has no effect on bull sperm retention and on fertility rates (Senger, 1993). The cervix is a formidable anatomical barrier, use of frozen-thawed sperm intracervical implanted (AI) can lower conception rates. It is therefore, advisable to perform a second insemination if heat symptoms persist for more than 24 hours following previous insemination (Kumar *et al*., 2014). Whenever, a cow is in heat, its uterus feels firmer when palpated than it does at other times during the estrus cycle. This could be due to the endometrial edoema and the muscle spasms that take place during estrus. The uterus feels flaccid and relaxed to the touch during estrus and the rest of the cycle (Bonafos *et al*., 1995; Forde *et al*., 2011). Reproductive tract of a bulling cow should be examined as a useful complement to visual estrus detection. This could identify changes in uterine and contractility leading to increased conception rates in dairy herds. Palpation may reveal an increased tone from one to two days before estrus and peaking during estrus. An inseminator may need to reconsider the farmer's heat detection tool if the uterus is flaccid on examination (Forde *et al*., 2011).

2.3 Early Non-Pregnancy Detection

Some of the methods used for early detection of non-pregnancy in cows include; observation of cows returning to heat 21 days after insemination, rectal palpation performed 3 months after insemination. This technique gives 80% accuracy of incalf cows (Karen *et al*., 2011). An in-calf cow secretes P4 hormone from the CL, which maintains pregnancy and increases in amounts as the pregnancy progresses. This hormone is found in milk and plasma of pregnant lactating cows (Hafez and Hafez, 2000; Balhara *et al*., 2013). Early non-pregnancy diagnosis (ENPD), using P4 as an indicator of pregnancy, uses milk or blood 21 to 23 days after insemination (Noakes, 1985; Scully *et al*., 2014**).** Estrogen is antagonistic to P4; therefore, during the time of estrus there should be little or no P4 but after unsuccessful AI the P4 build up will be within the normal range of non-inseminated cows up to day 21 when it will be almost zero and the cows show signs of heat. Open (non-pregnant) cow's P4 is less than 1 nmol/L of skimmed milk ((Hafez and Hafez, 2000; Nyman *et al*., 2016). The estimation of P4 led to detection of luteal function and paved the way for large-scale studies of incidences and timing of pregnancy losses in individual cows (Ricci *et al*., 2017).

2.4 Dairy Cow Conception and Pregnancy

The process of conception and beginning of pregnancy include; development of follicles that lead to ovulation, fertilization of the oocyte, transportation and growth of the embryo, maternal recognition of pregnancy, and implantation. This is a coordinated series of actions involving multiple reproductive tract tissues (Butler, 1998). Estrus cycle has a number of significant time-dependent components that must be closely managed in order to increase the chances of successful pregnancy. Calving interval, which comprises: gestation period, active AI breeding phase, and elective waiting time, must be well managed for maximum reproductive efficiency (Borakhatariya *et a*l., 2018). Voluntary waiting period which is about 40-70 days postpartum is physiologically necessary for the healing of the birth canal. Gaps between calvings rely on services required for conception and the ability of the cow to sustain the pregnancy (Borakhatariya *et al*., 2018**).**

2.5. Influence of Metabolites and Hormones on Reproduction

A transition period is crucial in the life of dairy cattle because it can affect the metabolic and health condition of dairy cows as well as their reproductive and yield potential in subsequent lactations (Roche *et al*., 2015). One of the most significant challenges in maintaining metabolic homeostasis in dairy cows is probably caused by a reduction in dry matter intake around the time of calving. This could lead to a negative energy balance in the early lactation phase. During negative energy balance, excessive lipolysis and rising levels of non-esterified fatty acids (NEFA) in the blood of dairy cows are frequently linked to the accumulation of triglycerides (TG) in hepatocytes and impairment of liver function. This results in elevated ketone production. Increasing blood NEFA levels may impair oocyte development and reproductive effectiveness (Walsh *et al*., 2006).

2.5.1. Hormonal Influence

Progression of pregnancy, normal fetal development, and the timing of parturition all depend on the presence of steroid hormones in the optimal concentration and ratios to one another (Conley and Assis Neto, 2008). Progesterone slows the luteinizing hormone (LH) ovulatory surges, prepares the endometrium for implantation, and maintains myometrial, during pregnancy. It also stimulates the synthesis of a range of endometrial secretions that help the embryo to develop successfully (Goff, 2004). Progesterone is primarily produced in the ovaries and placenta, while the adrenal cortex also contributes a negligible amount to total production. Follicular phase of the estrus cycle has low circulating progesterone levels. The levels rapidly increase during the luteal phase and reach their highest level on day10 after mid-cycle phase (Goff, 2004). Estrogen controls uterine blood flow, perfusion, myometrium growth, and the production of actomyosin, which strengthens uterine contractions during labour and fetal delivery (Boos *et al*., 2006).

Estrogens and relaxin work together to soften the cervix, relax the birth canal, and stimulate the release of prostaglandin F2 (PGF2) from the endometrium, all of which are regarded to be essential for normal delivery of the offspring (Kindahl, 2007). Insulin-like growth factor 1 (IGF-1), IGF binding proteins, growth hormone, and growth hormone receptors form somatotropin axis which play a role in reproduction. Levels of IGF-1 in blood decrease shortly after calving; this promotes nutritional partitioning to milk production; however, the drop has a detrimental effect on reproductive success. Postpartum energy balance is indicated by plasma IGF-1 concentrations. Synthesis and secretion of liver IGF-1 relies on good nutrition. Liver and other tissues create insulin-like growth factor-1, which has endocrine, paracrine, and autocrine functions (Yakar and Adamo, 2012)

2.5.2 Metabolic Influences

To increase milk production, many dairy cows are fed high-protein diets (Cabrita *et al*., 2011). The peripheral urea nitrogen content in dairy cattle indicates protein metabolism since urea is a result of protein breakdown (Butler, 1998). Urea diffuses into body fluids including blood and milk to equilibrate in other parts of the body, particularly reproductive tissues (Melendez *et al*., 2003). Excessive intake of total nitrogen, including rumen degradable protein, is the main cause of elevated circulating urea (Laven *et al*., 2007; Santos *et al*., 2008). Energy deficiency may promote the breakdown of amino acids from tissue proteins, increasing urea synthesis (Bell, 1995; Cheng *et al*., 2015).

During NEB, decreased liver function lowers urea metabolic clearance (O'Callahan *et al*., 2001). Both high and low circulatory urea concentrations have been associated with reduced fertility in dairy cows, notably with an increased calving to conception delay (Cabrita *et al*., 2011). There is a lack of consistency in these results across studies, and no conclusive evidence has yet been found to relate protein metabolism to fertility. Although urea is toxic, Laven *et al*. (2007) concluded that much of the negative impact of higher degradable protein intake was likely mediated by postprandial increases in ammonia, which can affect both oocytes and early embryos (Rhodes *et al*., 2006). Another hypothesis is that too much dietary protein during the early luteal phase reduces the pH of the uterus or alters other aspects of the uterine environment, making the uterus less favourable for embryo survival (Gunaretnam *et al*., 2013). Little is known about what hormonal and metabolic signals transmit body condition score state of dairy cow to the hypothalamic pituitary gonadal axis (Chelikani *et al*., 2009). Postpartum reproductive indices are correlated with NEB which is based on the message conveyed by hormones and metabolites to the cow's central nervous system. High quality feed increases circulating IGF-1 hormone. Insulin, IGF-1, and glucose levels are higher in cows that ovulate within 35 days of calving. High NEFA concentrations are positively correlated to reproductive deficiencies following initial artificial insemination (Sammad *et al*., 2022).

High-energy feed promotes activities of rumen microbes leading to synthesis and absorption of volatile fatty acids. This leads to insulin levels rising while fatty acid mobilisation from adipose tissues decrease (Doepel *et al*., 2002; Mohammed and Mutassim, 2016). The pre-pubertal increase in growth hormone and IGF-1 levels is one of the hormonal signals that help to determine when puberty begins in beef heifers (Kumar and Laxmi, 2015). Leptin hormone appears crucial in controlling ruminant feed intake and reproductive function by communicating the metabolic condition to the central nervous system (Zieba *et al*., 2005). Early lactation glucose concentration and postpartum reproduction have a positive genetic association. High glucose levels are linked to shorter calving intervals and high conception rates. Plasma glucose levels and the voluntary waiting period are inversely correlated (Kumar and Laxmi, 2015; Sulieman *et al*., 2017).

2.5.3. Failure of Fertilization

Fertilization fails when a viable ovum and a sperm cannot unite. The failure of normal embryonic growth, maternal pregnancy recognition, or regular pregnancy maintenance results in embryonic death after fertilization (Moraes *et al*., 2018). Estrus detection technique and cow fertility status determine time taken from parturition to 1st AI postpartum. Fertility status of a cow is determined by various aspects, including the service sire's fertility, proper semen management, AI technology, and insemination timing. The main challenge to efficient fertilization is estrus determination, it is more challenging in higher-yielding Friesian dairy cows (Palmer *et al*., 2010). Cow’s ability to exhibit estrus signs is influenced by many factors among them bull availability, food, and temperature. Confined cows exhibit less estrus (Sumiyoshi *et al*., 2014).

Bovine egg has a viable life of less than 12 hours after ovulation if it is not fertilized. The estimated viable life duration of thawed semen (at 60°C to 80°C for 6 to 7 s) in the female reproductive duct is less than 48 hours. Sperm's maximum viable life is between 12 and 16 hours, after which it begins to degenerate (Lyashenko, 2015).

Embryo death or abortion can occur when pregnant Friesian cows are inseminated. This is due to palpation of the amniotic vesicle (Wisnicky and Cassida, 1948; Dale *et al*., 2005; Romano *et al*., 2016) and slipping of the chorioallantoic membranes between the thumb and forefinger (Zemjanis, 1970; Romano *et al*., 2016). About 10% of pregnant dairy cows express estrus (Erb and Morrison, 1957; Dijkhuizen and van

Eerdenburg, 1997). Semen should be deposited in the mid cervical region of these cows to prevent abortions of held pregnancies (Macmillan *et al*., 1977).

2.5.4 Losses of Cattle Embryos

Bovine fertilization rates can reach 95%, and embryonic death is the leading cause of reproductive waste (De Vries, 2006). Major causes of embryonic losses include; genetic abnormalities of the embryo, uterine factors, diet, rectal palpation, insemination, ionic imbalance, age of the cow, heat stress, and infection of Bos taurus breeds (Ball, 1997; Dale *et al*., 2005). Embryo death is promoted by decreased secretion of interferon tau from day 15 of the embryos, and an increased secretion of uterine PGF2α (Abecia *et al*., 1999; Dorniak *et al*., 2013). Animals can abort at various stages of their pregnancy. A bovine conceptus is an embryo that develops into a foetus following conception (Moraes *et al*., 2018). The rise in early and late embryonic losses has been attributed to a variety of factors, however, it has been determined that undernutrition is the most significant (Ball, 1997; Bindari, *et al*., 2013).

Following fertilization, early embryonic death can occur as early as day 3 or, if the blastocyst develops then it will occur as early as day 7 or day 8 (Mclaren (1974; Moraes *et al*., 2018). Then the embryo enters the uterus by day nine, this is another critical stage that could lead to embryonic loss. The embryo is sufficiently mature by day 15 or 16, to prevent prostaglandin F2-alpha (PGF2ά) secretion from the uterus on its own. Large amounts of interferon produced by fetal trophoblast maintain the established pregnancy. Failure of developing placenta to adhere to uterine wall can lead to late embryonic loss which occurs between days 25 and 40 after artificial insemination (AI). The embryonic loss occurs because the uterine caruncles of the endometrium, which carry gases, nutrients, and waste products between the uterus and developing calf, are not linked to the placental cotyledons (Perry, 1981; Adeyinka *et al*., 2014). Dairy cows have early embryonic losses at a rate of 38% between days 15 and 18 after fertilization and late fetal deaths of 5 to 8% during pregnancy (Sreenan and Diskin, 1986; Wijma *et al*., 2016). Conception failure and early embryonic deaths are not as serious as late embryonic losses (Diskins *et al*., 2011). Supplemental progesterone, particularly in sub-fertile animals with low plasma progesterone levels, can boost the survival rates of developing embryos. Due to inefficient production of progesterone by their corpora lutea, older cows are more vulnerable to this issue (Cerri *et al*., 2009).

2.5.5 Lactating Cows' Nutrient Requirement

The science of balancing feed consumption and digestibility with an animal's nutritional requirements is known as animal nutrition. In most production systems, forages are crucial to the diet of dairy cows and affect their intake of energy and carbohydrates (Eastridge, 2006). Ranching dairy cow’s herbage consumption is influenced by; grazing duration, biting rate, and intake per biting (Holmes, 1989; Perez-Ramirez *et al*., 2008). The key nutrient element influencing cow production is dry matter intake. The most accurate indicator in dairy cattle is neutral detergent fibre (NDF) (Kendall *et al*., 2008).

High-yielding dairy cows should have at least 20% acid detergent fibre (ADF) and 29% neutral detergent fibre (NDF). This helps to maintain body tissue balance and optimal rumen function. A high NDF content in the fibre encourages chewing. Chewing activities leads to rumen buffering by saliva which leads to easy fermentation of the NDF (Wang, 2017). Body condition, lactation stage, and metabolic rate are animal elements that affect consumption whereas herbage factors affecting consumption include digestibility, size, and structure (Hayirli *et al.*, 2002). Ordinary milking cows need more than just herbage, so adding concentrates to offset the shortage of protein and energy is crucial. A high yielding dairy cow needs more than 7 kg of glucose to produce 89 kg of milk per day. Propionate, lactate, amino acids, and glycerol are among the precursors required to support enhanced gluconeogenesis.

Metabolic adjustment is necessary to make these substrates available (Hammon *et al.,* 2009). Most ruminant diets have a strong correlation between their energy level and the nutrients' ability to be digested. Digestibility accounts for loss of nutrients in the faeces after ingestion of feed. Though there are other losses of nutrients incurred during metabolism, loss through faeces is by far the most important. This loss varies from near zero to all the energy contained in a feed (McDonald *et al.,* 1995). Digestibility gives a biologically significant measure that can be utilized in routine feed evaluation as a nutritional value index. It is also closely related to metabolisable energy, which accounts for further loss of nutrients via urine and methane. An example of such a relationship allows the metabolisable energy (ME) content of forages to be calculated (AFRC, 1993).

The in-vitro dry matter digestibility (IVDMD) method has been widely used to evaluate the nutritional content of ruminant diets. Since its introduction, the Tilley and Terry (1963) IVDMD method has been frequently used to analyze feedstuffs and has been shown to be the most reliable and practical laboratory approach for predicting ruminant digestibility data. Forages have been tested for crude protein (CP) and fiber concentrations because of their direct impact on diet formulation (Stern *et al*., 1997). Low nitrogen utilization is a limiting factor in cow protein nutrition (N). High milk yielding dairy cows have about 25% nitrogen utilization efficiency (Huhtanen and Hristov, 2009). Insufficient intake of specific amino acids limits the overall milk yield and milk protein production in high-yielding dairy cows. The most restricting amino acids for the synthesis of milk proteins are lysine (Lys) and methionine (Met) (Rulquin *et al*., 2001).

Lactation and reproduction result in increased nutrient needs. On the other hand, nutrient supply may have an impact on the cow's ability to reproduce and produce milk. Reproduction is an 'all or nothing' phenomenon, and failure has serious repercussions that should be avoided (Butler *et al*., 2003; Friggens *et al*., 2013). A good diet stimulates insulin production, which promotes peripheral tissue glucose uptake and ovarian steroid synthesis (De Koster and Opsomer, 2013). Lactation, estrus cycle onset, and establishment are postpartum processes that compete for energy (Butler and Smith, 1989). Milk protein composition is likely to be influenced by hormonal changes that occur during estrus. Comparing milk protein profiles at different estrus cycle stages, could lead to identifying proteins that are specific to estrus (Du *et al*., 2021). Understanding how lactation and reproduction are interrelated is crucial for maximizing genetic progress and the profitability of the livestock sector (Indetie, 2015).

In a single lactation, a high yielding dairy cow can produce five times more dry matter in milk than she has (Reist *et al*., 2002). This causes high yielding cows to have net energy shortage (Bauman and Elliot, 1983; De Vries and Veerkamp, 2000). Milk constituents and energy for their synthesis are provided for through feed intake. This means, volume and type of milk produced is determined by the feed (Kayo, 2019). Milk fat is a triacylglycerol mixture containing saturated and unsaturated fatty acids.

The balance of fat synthesis and mobilization influences milk fat yield. This is regulated by glucogenic chemicals found in digestion products. High concentrations of propionate, glucose, and amino acids promote fat accumulation (Blum *et al*., 2000; Erickson and Kalscheur, 2020). Milk fat fluctuation has an effect on total solid variation. However, this does not affect milk yield (Mech *et al*., 2008). The time when milk supply is less than 30% of the peak is called average lactation length (Chang *et al*., 2001). Density is the ratio of a given volume of any substance to the mass of an equivalent volume of water at a given temperature. Milk has a specific gravity of 1.032 kg per litre at 4^0C but in the presence of solutes then milk freezing point will be below 0^0C (Henno *et al*., 2008). Milk freezing point is determined by the concentration of water-soluble components. The freezing point of milk will drop closer to zero as it gets diluted.

2.5.6 Feed Conservation for Lactating Dairy Cows

Animal output is significantly affected by level of feeding. This is a particular issue during drought in the Arid and Semi-Arid Lands (ASAL) as feed is limited and also of low grade. There is recurrent loss of livestock during this period due to starvation. There are three major feed conservation methods used currently; Conserved hay bales are important feed resource during the dry period but they are scarce and expensive as they are brought from far distance farms in medium and high potentials areas. Hay bales are voluminous and thus fewer numbers are packaged in transportation containers and more often their quality may deteriorate due to risks during transportation such as rains causing dumpiness and hence moulding of the grass materials.

Feeds conserved as silage remain popular for dairy farms because they minimize loss of nutrients from harvest through storage, they allow for easier feeding, and often allow greater efficiency and timeliness of feed, mixing and handling on the farm, than dry forages (Mahanna and Chase, 2003). Measuring the chemical composition and physical properties of silages is important for proper ration formulation and troubleshooting silage quality problems (Heinrichs and Kononoff, 2013). Dairy cattle feeding behaviour and DMI are influenced by the amount and fermentability of silage's fibre, starch, and protein as well as fermentation byproducts (Oliveira *et al*., 2017). The conservation technique is expensive for an ordinary Kenyan farmer because of the farm machinery used. It is also not based on TMR approach hence concentrates have to be fed separately which could easily lead to wastage or contamination of the concentrates during handling. Developing alternative feed conservation technique would reduce this problem.

Feed block technique involves mixing various feed ingredients such as grass/legume pasture. The technique produces higher dry matter yields of better nutritive value than sole grass swards (Onifade and Akinola, 1986; Salman *et al*., 2017). The quality of conserved grasses and legumes may decline on exposure unlike feed materials conserved in feed block technique. Feed blocks are relatively small in size and easy to transport with minimal transportation risks. Although the nutritive qualities differ from those of fresh materials, adequate levels of nutrients are retained in feed blocks to merit their use in dry periods. The ingredients are economical for smallholder farms. In Kenya, complete meal feed blocks have not been used to improve dairy cow reproduction.

2.6 Effect of Nutrition on Reproduction

Nutritional effects on fertility may be particularly significant during the transition period (±4 weeks after calving) (Overton and Waldron, 2004). However, the impact of diet on reproduction during such a period seems complicated and multifactorial. Nutritional experiments are prone to confounding since adding one component affects other components in the diet. Nutritional interactions could also have an impact on the presence, absorption, and generation of other components as well as energy distribution and future milk production. Single nutritional intervention experiments are essential for comprehending the impact of micronutrients on fertility. However, because of the possibility of extraneous factors responses could be challenging to gauge (Lean *et al*., 2014).

2.6.1 Nutrient Partitioning to Aid Reproduction

Physiological balance is maintained through homeostasis, which is a multimodal compensatory mechanism, whereas; coordinated management of metabolism in support of physiological processes is referred to as homeorhesis. Homorhesis involves coordinated control in metabolism of body tissues necessary to support a physiological state. Postpartum milk production has a higher priority for dietary resources than postpartum estrus cycles. This leads to protracted postpartum anestrus through homoerotic regulation, due to insufficient feed intake which hardly supports milk production and initiates estrus cycles (Roche *et al.*, 2011).

Fat mobilization is aided by insulin's diminished capacity to promote lipogenesis and prevent lipolysis in late pregnancy. At the start of lactation, lipogenesis suppression is associated with low plasma insulin levels (McNamara, 1988; Contreras *et al*., 2017).

Massive tissue mobilization occurs prepartum and also at onset of lactation. This causes an increase in non-esterified fatty acids (NEFA). The acids decrease as lactation progresses (Danshea *et al*.; 1989; Wankhade *et al*., 2017). During early lactation, lipid metabolism changes from storing energy to significant mobilization of energy reserves (Wankhade *et al*., 2017). Homeorhetic control of the changes are effected through prolactin and somatotropin hormones (Remppis *et al*., 2011).

2.6.2 Effects of Malnutrition on Reproduction

Dairy cows face significant physiological strain when transiting from late pregnancy to early lactation. Dietary consumption cannot fulfil body requirements (Butler, 2000; Ribeiro *et al*., 2011). It is therefore, critical for the dairy industry to develop strategies to ease this peri-parturient transition. The strategies should be based on quality and quantity of nutrients required to sustain late conceptus growth and milk synthesis (Leiffers *et al*.; 2003; Ribeiro *et al*., 2011). Despite the fact that the consequences of feeding on blood metabolites have been defined, little is known about mechanisms that activate the various stages of reproduction (Robinson, 1990; Pelegrin-Valls *et al*., 2020). During times of feed scarcity, ruminants should receive at least one-third of their maintenance energy requirements. This is to prevent their lean tissues from being used to make precursors to glucose (Orskov, 1998; Reynolds *et al*., 2003).

In severe cases, undernutrition reduces colostrum production and slows lactogenesis. Maternal nutrition influences fetal metabolism, development, and colostrum production, thus plays an important influence in neonatal viability (Robinson, 1990; Puppel *et al*., 2019).

Feed nutrients are usually partitioned for cow growth, body reserves, maintenance, pregnancy, fetal growth, and lactation (Walker, 1993; Useni *et al.;* 2019). Glucose synthesis is reduced due to enhanced lipid mobilization and ketogenesis in animals that are severely underfed (Danfaer, 1994; Weber *et al.*, 2016). The best timing for insemination in nations with distinct dry and wet seasons is dictated by the corresponding changes in nutritional status (Lotthammer, 1991). Puberty is delayed by underfeeding throughout the development stage, as well as the onset of estrus after parturition in late pregnancy and a reduction in pregnancy rates in the postpartum period (McClure, 1994). The major cause of under nutrition is insufficient energy. In the tropics, smallholder dairy cattle rarely have access to sufficient quantity and quality feeds. The feeds have a low digestion rate and are deficient in some components, this causes little consumption.

The length of the postpartum anestrus period is influenced by body condition score (BCS) at calving and subsequent energy use. During the first four months of lactation, better condition score cows lose more weight than cows with poor condition score. However, compared to their leaner counterparts, they have bigger faster-growing calves, shorter anestrus durations and shorter calving intervals (Mukasa-Mugerwa *et al.*; 1997). High-producing dairy cows may experience nutritional deficiencies when the physical capacity of their stomachs restricts the amount of feed intake. As a result, there are less nutrients that can be extracted from the feed to meet their nutritional needs. Malnourished animals have a variety of physiological methods to deal with the circumstance (Grimaud *et al.*; 1998). The strategies include growth restriction, loss of fat, muscle, and visceral mass, reduced basal metabolic rate, and reduced total energy expenditure.

2.6.3 Pasture Improvement

Approximately one billion people live in Sub-Saharan Africa. This is one of the fastest expanding populations (World Bank, 2018), however Sub-Saharan Africa (SSA) has the lowest per capita consumption of livestock products in the world due to low animal productivity (Cardoso, 2012). Demand for dairy products is on the increase, due to improving per capita income, modernization, and westernization (Knips, 2006). To meet the demand for animal products animal productivity must be boosted using improved techniques for fodder productivity. There is an established strategy of using induced or natural genetic diversity to improve important food crops, and mutagenesis is particularly useful for creating novel variations in plants. An efficient strategy for increasing plant diversity and performance is mutation induction (Sutapa and Kasmawan, 2016). Chemical or physical mutagens can be used to cause mutations. Physical mutagenesis involves timed exposure to beta and alpha rays, non-ionizing gamma rays, and rapid and slow neutrons (Oladosu *et al*., 2015; Tadele, 2016).

Development of mutant varieties and expansion of genetic diversity of crops can be done through irradiation of seeds using gamma rays (Jan *et al*., 2012; Ali *et al*., 2015). The rays are more efficient because they have more penetrating strength and affordable because they are more accessible than other ionizing radiations (Moussa, 2006). They cause changes in physiological, morphological, and biochemical properties of plants thereby affecting growth of plant (Jan *et al.*, 2012; Haris *et al*., 2013).

The best method for enhancing plant performance is to irradiate seeds prior to planting. Increase in productivity of plants is related to gamma ray dose (Respati et al., 2018). The grass genus *Brachiaria* includes forage crops that are important for beef and dairy industries in tropical and sub-tropical Africa, South America and Oceania/Australia (Lizo *et al*. 2024). The grass has the potential of meeting the challenges of feed scarcity both in quantity and quality since it provides more forage per unit area and ensures regular forage supply due to its multi-cut nature (Schiek *et al.*, 2018).

Among the most important merits of genus *Brachiaria* grass include adaptation to drought and low fertility soils, ability to sequester carbon, increase nitrogen use efficiency through biological nitrification inhibition (BNI), arrest greenhouse gas emissions, thrives well on different soil types (clay to red soils) and distributed across tropical and sub-tropical regions. *Brachiaria* grass has recently been identified as an ideal fodder that can improve livestock production in eastern Africa (Cheruiyot et *al.*, 2020). There are several initiatives in the region aimed at promoting cultivation of this grass to support the emerging livestock industry (Maass *et al*., 2015). This is due to its high adaptability to low fertility soils. Irradiation of Brachiaria seeds will create diversity among the seedlings. Line selection of best performing Brachiaria will result in high productive cultivars. Gamma ray dose necessary to increase productivity in Kenyan native Brachiaria is not known.

2.7 Body condition as a Metabolic Status Indicator

A subjective tool to estimate body reserves is body condition scoring (Edmonson *et al*., 1989; Alapati *et al.*, 2010). Body condition is scored on a scale of 1 to 5, with 1 representing the least amount of fat and 5 denoting the most amount of fat. The scoring is based on a tactile and visual assessment of body fat reserves in the back and pelvic areas. The relationship between the change in body weight and the change in BCS is positive correlation (Broster and Broster, 1998; Berry *et al.,* 2011). High-yielding cows' postpartum calorie intake is less than half of what is needed for production (Van Arendonk *et al.,* 1991; Janovick and Drackley, 2010). As a result, the cows must mobilize body tissue, which causes them to lose a lot of weight. Long calving intervals are connected to loss in body condition between calving and 45 days in milk (DIM).

Cows with BCS of less than 3 at calving are less likely to be inseminated (Domecq *et al*., 1997; Montiel-Olgun *et al.,* 2019). Fat-to-protein ratio in milk is a more accurate predictor of sickness, fertility, and milk supply compared to BCS (Heuer, *et al.,* 1999; Toni *et al.,* 2011). A correlation exists between BCS and cows cycling at the end of the elective waiting time. The levels and variations of BCS during the lactation phase can have an impact on the success of reproduction and the restart of the estrus cycle. It also exposes the metabolic condition required for the cyclicity to resume (Pryce *et al.,* 2001; De Feu *et al.,* 2009).

To determine the relative level of energy balance in cows, their physical state needs to be evaluated regularly (Heuer *et al.,* 1999; Thorup *et al.,* 2012). Body reserves mobilization is increased due to genetic advancements that boost milk production resulting in a higher negative energy balance during early lactation. The mobilization of body reserves increases as genetic merit for production rises. This results in a bigger negative energy balance in the early lactation phase among thinner cows, lowering their fertility. Body condition score has negative genetic correlation of 0.40 with calving intervals therefore it can be used for fertility selection. There appears to be great change in BCS between calving and week 12 post-calving (Bastin and

Gengler, 2013). It is documented that there is a linear relationship between BCS and reproductive measurements whereas there is an inverse association between BCS and milk supply. Cows with high genetic merit lose more body condition and have lower BCS than cows with poor genetic merit (Bastin and Gengler, 2013).

2.8 Challenges to the Transition Cows

Dairy cows endure considerable metabolic and immune problems postpartum. At this period vital nutrients are crucial to the cows. Methionine levels in transition cows may be correlated with three critical metabolic indicators, including energy balance (lipid metabolism), protein balance, and antioxidant balance (Pedernera *et al.,* 2010). Subclinical or clinical illnesses or disorders, such as fatty liver, ketosis, insufficient passive immunity transfer to calf, impaired immunological function, and disease resistance in cows, can result from metabolic dysfunction (Bell, 1995; Overton and Waldron, 2004; Drackley *et al.,* 2011). Intake of dry matter is a crucial component of postpartum cow nutrition. The intake is influenced by management and environmental factors such as stocking density, heat stress, rumen adaptation, calcium metabolism, and overall disease challenge.

These factors need to be taken care of in order to maximize the benefits of adding critical nutrients like methionine as supplements throughout the transition phase (Overton and 'Waldron, 2004; Lean *et al*. 2013). During the first three to six weeks following calving, a normal Holstein cow mobilizes body fat and protein (; Bell, 1995; Komaragiri and Erdman, 1997; Thorup, *et al*., 2012). The liver participates in and controls the majority of this intense metabolic activity, mobilizing and directing food flow to the mammary gland and supporting organs including the gastrointestinal tract. Therefore, any decrease in liver function may affect lactation and conception. In many species, methionine is necessary for healthy liver function (Katoh, 2002; Schugar and Crawford, 2012).

2.9 Early Lactation Energy Balance

After calving, the mammary gland replaces the feto-placental unit as the main consumer of nutrients. As a result, there is an energy prioritizing that favors the start of the estrus cycle above the ingestion of nutrients for growth and milk production (Short and Adams, 1989; Baumgard *et al.,* 2017). Due to the negative energy balance, cows that consume less dry matter (DM) experience later first ovulation and first estrus, give less milk, and are less reproductive (Staples *et al.,* 1990). Intake of dry matter is mostly connected to body condition at calving, which in turn is associated to the severity and length of negative energy balance (NEB). There is high correlation between body condition score and days to 1st postpartum ovulation during the first 3 to 4 weeks (Butler, 2000; Gunmen *et al*., 2011). The interval between calving and the first ovulation is an important aspect in affecting energy level and reproductive efficiency.

After calving, the body's nutritional requirements shift dramatically from building up fetal mass and body reserves to rapidly mobilizing protein and lipid reserves to support increased postpartum lactation (Bauman and Currie, 1980; Rizos *et al.,* 2008). Appetite stimulation is required to ensure that normal, healthy cows receive enough DM for optimal milk production, follicular enlargement, ovulation, uterine involution, and cyclicity. Postpartum dairy cows have negative energy balance due to high energy requirements; the negative energy balance reaches nadir (lowest point) within two weeks postpartum (Roche, *et al.,* 2000; Gumen, *et al.,* 2011). The first ovulation takes place about 30 days after delivery and can occur anywhere between 17 and 42 days later. This has been connected to the energy nadir (Butler, 2000; Sakaguchi, 2009). Indicators of negative energy balance of postpartum cows include; delayed ovarian cyclicity, development of ketosis, and low conception rates.

Quick correction of the energy balance is crucial for health and productivity of the cow (Rutherford *et al.,* 2016). Ovulation occurs from 10 to 15 days after energy nadir. A cow has extremely high metabolic demands during early lactation, when milk production is at its peak. The mammary gland needs a lot of nutrients to produce lactose, protein, and triglycerides, which cannot be done from dietary consumption (Bell, 1995; Erickson and Kalscheur, 2020). Despite having a negative energy balance, dairy cows with high DM intake are higher milk yielders, thin and ovulate faster after calving than those with low DM ingestion (Bertoni *et al*., 2009).

Milking cows are in a state of negative energy balance 6-10 weeks after calving, despite adequate feed availability (Malt *et al*., 2013). This results in prolonged periods between initial ovulations. Feed capacity may not be the limiting factor for dry matter intake. It has been documented that, in the first six weeks postpartum, energy balance is negatively correlated to non-esterified fatty acids and positively correlated with insulin (Rutherford *et al.,* 2016). Obese cows mobilize more adipose tissue and produces increased glycerides in the liver postpartum leading to delayed postpartum ovulation (Rukkwamsuk *et al*., 1999). Negative energy balance is a physiological state of starvation that prevents ovulation by lowering ovarian reactivity to LH stimulation and affecting LH secretion. Follicles expand, become bigger and produce more estradiol after nadir. This increases the chance to ovulate. Cows with NEB have low amounts of insulin and glucose in the blood. Ovulating cows have circulation levels of insulin-like growth factor 1 (IGF-1) that are 40 to 50 percent higher than their non-ovulating contemporaries. Ovarian follicular development requires IGF-1 hormone (Beam and Butler, 1998; Walsh *et al*., 2011).

Follicular capability to generate adequate estradiol for ovulation during early NEB phase depends on the availability of serum insulin and IGF-1 concentrations (Garcia-Garcia, 2012). Feeds are important in reproduction, milk components (Heuer *et al*., 1999; Friggens *et al.,* 2007) as well as metabolites and hormones. Changes in the central and peripheral systems in postpartum cows reduce levels of reproductive hormones (Pethes *et al.,* 1985; Capuco *et al.,* 2001; Meikle *et al.,* 2004; Omari, *et al.,* 2020). The importance of hormones in controlling reproduction has been established, but information on how they affect ovarian function in -vivo is scanty, and debatable (Spicer, 2001; McNamara and Shields 2013).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Site

This research was conducted at KALRO-Lanet, Nakuru County (Appendix 1). There are two agro ecological zones: 3 and 4 at KALRO-Lanet. *Elba Rhodes* are among the well-established pastures located in agro-ecological zone 3 while *Pennisetum catabasis* (Manyatta grass) and *Pennisetum clandesteim* are the dominant species in agro-ecological zone 4. The Centre is at 1600 m above sea level. Average temperatures are 26 degrees Celsius and 10 degrees Celsius, for the highest and lowest temperatures respectively (Pratt and Gwyne, 1977). Small-scale dairy production is practiced by the Kenya Agricultural and Livestock Research Organization (KALRO)-Lanet. The centre has a herd of 70 Friesian cows grazed primarily in ecological zone 3, which has Elba Rhodes. The cows had unlimited access to salt, minerals, and water. To make experiment monitoring and control easy, the investigation was conducted at KALRO Lanet.

3.2 Sample Size Determination

Sample size of the dairy cows for this study was determined using Yamane (1967) method. The sample size was calculated as shown below.

$$n_o = \frac{N}{1 + N(\varepsilon^2)}$$

Where n is the sample size, N is the population size and ε is the level of precision (95%).

n=70/(1+(70*0.05^2) = 59.57. Approximate 60.

The above formular resulted into n = 60. Since the population was low, the sample size was reduced significantly, and because a given sample size provides proportionally more information for a small population than for a big population, the Finite Population Correction Formula was used (Cochran, 1963).

$$n = \frac{n_o}{1 + \frac{n_o - 1}{N}}$$

Where n_o is the sample size recommended, N is the population size and n is the new adjusted sample size.

n = 60/1+ (60-1)/70 =32.55; hence the new adjusted sample size used was thirty (30). This was due to available chemicals and reagents.

3.3 Maintenance of Experimental Cows

The experimental cows were pastured during the day on established *Elba rhodes*, Tickborne diseases were controlled weekly through plunge dip filled with diluted Almatix (Almandine Corporation, Switzerland). The test cows had seven days to become acclimated to the supplementary feeds before the 60-day data collection period. The average temperature and relative humidity for the duration of the experiment were 20^{o}C and 83%, respectively. Average annual rainfall was 800mm.

3.4 Study Design

Thirty in-calf Friesian dairy cows were purposively chosen based on the stage of pregnancy, only cows that were 7 months in-calf were enrolled. Using random numbers, the cows were divided into two treatments. Twenty (20) cows were randomly assigned to test group; this sample size is appropriate for providing the desired level of precision (Cochran, 1963). Ten experimental cows were put into control group. Test group cows received a supplement of 2 kilograms of total mixed ration feed blocks (one feed block) prepartum (3rd trimester) and a supplement of 4 kilograms of total mixed ration feed blocks (two feed blocks) at milking postpartum. None of the controls received supplements. The modest number of cows used was due to the negative control cows (10 cows), lack of supplementation results in reproductive wastage hence the need to minimize the number. In order to determine whether BCS has an effect on the mobilization of bodily energy reserves during the early phases of lactation (Schroder and Staufenbiel, 2006) the study used cows of BCS 1-4. In order to determine if parity has an effect on reproductive indices; cows of parity 1-4 were used Metabolite profiles, hormonal profiles, milk supply and quality were among the variables that were recorded for each group.

3.5 Feed Analysis

Grazing fields were sampled for pastures by randomly placing a 1 m^2 quadrant across the field and a sickle was used to cut the swards inside the quadrant. The procedure was repeated three times. The grasses were then well mixed and a representative sample along with samples of total mixed ration feed blocks were analyzed for nutrient contents. Association of official analytical collaboration (AOAC) (1990) technique was used to calculate the amount of dry matter (DM), measured in grams per kilogram, whereas the AOAC (2006a) method was used to calculate the amount of ash and organic matter (OM). Crude protein content (g/kg DM) was calculated using 6.25 x N (Kjeldahl nitrogen) concentration in the feed. The nitrogen concentration was calculated using the AOAC (2006b) method. Tilly and Terry (1963) technique was used to calculate in-vitro dry matter digestibility, while Van Soest *et al.* (1991) method was used to quantify neutral detergent fibre (NDF) and acid detergent fibre (ADF).

Fatty acids and amino. Acids were analyzed using the method described by Tie-xin and Wu Hong (2008).

3.6 Irradiation of Landrace *Brachiaria* Seeds

Stalons of landrace *Brachiaria* (*Urochloa ruziziensis*) were picked at 1,919 meters above sea level in Kisii County (Oliveira, 2018). The stalons were established using approved agronomic techniques at KALRO-Lanet in Nakuru County. At maturity, seeds were harvested, manually cleaned placed in pre-labeled plastic petri-dishes that were marked with required irradiations doses, and the dishes were shipped to Seibersdorf laboratory in Austria where they were irradiated with doses of 0, 10, 20, 30, and 40 grey(gy), with the corresponding codes of K0gy, K10gy, K20gy, K30gy and K40gy. These formed five treatments (K0,K10,K20,K30 and K40). The irradiated seeds (M1) were brought back to Kenya, and planted at KALRO-Lanet using approved agronomic methods and each treatment was replicated three times. The plants were subjected to agronomic observation and selection.

3.6.1 Field Experimental Design for Irradiated *Brachiaria* Seeds Establishment

Tractors were used to plough and harrrow the ground before M1 *Brachiaria* seeds were planted in complete randomized block design with three replications for each treatment. Planting of the seeds was done using 250 kg/ha of single superphosphate fertilizer. The holes were spaced 60 cm apart and 30 cm within rows while the seeds were planted at 3cm deep. Calcium ammonium nitrate (CAN) was applied to the crop at a rate of 250kg/ha when the crop reached knee high. At booting, records of grass performances were taken (Rana and Kumar, 2014). At this stage 1.0^2 square quadrant was thrown upon each treatment three times and swards within the squares were cut, homogenized and a representative sample taken to a laboratory for nutritional profiles.

3.6.2 Mutant Selection

Seeds from M1 plants were evaluated for seed germination and planted. This continued up to 4th generation. Seeds from 4th generation were now planted as single plant progenies for effective determination of morphological and agronomical characteristics. Seeds from these plants were bulked (Kanimozhi *et al.,* 2021). Herbage, nutritional value, and seed yield were all taken into account when testing the M6 populations for suitable lines (Figure 3.1). At this moment, the plant phenotypic characteristics of the mutant lines were stable. After five months, the stable mutants were cut with a side mower and baled.

Figure 3.1: Schematic Presentation of *Brachiaria* Selection Methods Used

3.6.3 Preparation of Total Mixed Ration Feed Blocks

The components used to make the total mixed ration (TMR) feed blocks were: *Dolichos lablab* seed powder (15%), ground *Brachiaria* grass (55%), mineral premix (1%) lemon rinds (8%), cement (5%) common salt (1%), bentonite (5%) and molasses (8%). Water was poured into a plastic bowl with a capacity to hold 10 litres. Before adding the pulverised feed, the water, salt, lemon rind, and mineral premix were thoroughly blended. Cement was then gradually added to the above mixture. Molasses was finally poured in little amounts to the plastic basin as the entire mixture was stirred with a wooden rod.

The entire mixture was thoroughly kneaded then inserted into molds then compressed using hands. The TMR feed blocks were carefully taken out of the molds and laid out to cure on polythene paper (2 x 3 meter). The cured TMR feed blocks were taken to a greenhouse and placed on table grills measuring 1 x 4 meters each to dry. To allow for thorough drying and storage, the feed blocks were maintained in a high dry area with good ventilation. Each block was manually placed inside one self-sealing polythene paper, and all dried TMR feed blocks were subsequently hygienically packaged. Using enzyme-linked immunosorbent assay (ELISA) method, the blocks' purity was assessed after a month (Pirestani *et al.,* 2011).

3.7 Measurement of Variables

. Variables measured included body condition score, gestation period, calf sex, postpartum reproductive performance, milk composition, milk progesterone, plasma metabolites and hormones.

3.7.1 Determination of Body Condition Scores of Cows, and Calve Weights

Body condition score was calculated on a scale from 1 (very underweight) to 5 (extremely overweight) (Edmonson *et al.,* 1989). This was done from day 30 prepartum to day 60 postpartum. To the nearest quarter-point, the cows were accurately scored. The BCS at parturition was determined using the BCS that was closest to the event. According to the BCS, cows were categorized as lean (3) or obese (>3) at parturition. Birth weights of calves were measured using Smart Reader, BR Series G03113 (Gallagher group ltd, New Zealand).

3.7.2 Determination of Gestation Period, Calf Sex, Postpartum Reproductive Performance

Gestation period was calculated using the date of calving in relation to the date of artificial insemination (both dates inclusive). Calf sex was determined by visually inspecting the calf after birth. Milk progesterone was used to assess postpartum reproductive function. Luteal activity (OLA) was thought to begin on the first day milk progesterone concentration was above 1.572Nmol/L. (Martin *et al.*, 2010)

3.7. 3 Milk Composition

Seven days after calving, daily milk records were taken. Every week 20 mls of morning and evening milk samples were aseptically collected into pre-labelled sample tubes. The samples were promptly analyzed for milk components and properties, such as percent butter fat (percent BF), protein (percent P), solids not fat (percent SNF), density, and freezing point (percent SNF) using milkoscan minor (Ekomilk Horizon Ltd).

3.7.4 Blood Sampling

Sterile vacutainer tubes containing 1.8 mg/ml ethylene diamine tetraacetate (EDTA), were used to aseptically collect 10 ml of blood from the jugular vein of cows twice weekly. The blood was centrifuged for 15 minutes at 1500 rpm and blood plasma stored at -20°C for assaying of hormones and metabolites. Blood sampling was done from day 30 prepartum to day 60 postpartum in jumps of 15 days.

3.7.5 Measurement of Progesterone in Milk

Ten milliliters (ml) of evening milk was collected into collection vials each containing one sodium azide pill as a preservative. To separate fat from the milk samples, the samples were centrifuged for 30 minutes at 3,000 revolutions per minute using Heraeus® labofuge 200 (Thermo Fisher Scientific Inc.). Free fat milk was then aliquoted into 5mls labelled vials and stored at temperature of 4^0C. The milk was thawed and used to measure progesterone levels. Progesterone (P4) was measured using radioimmunoassay (RIA) protocol developed by FAO/IAEA (1999) (Appendix XIV).

3.7.6 Measurements of other Hormones and Metabolites

Every 15 days, free fatty acids (NEFA) were measured using a NEFA kit (Randox Lab. Ltd. Crumlin.co.Antrim, UK) from 30 days prior to calving to 60 days postpartum. Commercial Weiner laboratories (Rosario, Urgentina) assays were used to quantify urea, NEFA, proteins, and glucose on a spectrophotometer. The amount of plasma leptin was quantified in accordance with the instructions supplied by the makers of the 125IRIA kit used by Delavaud *et al*. (2000). Using paired samples, the inter and intra assay coefficients of variance for NEFA and leptin were determined. The 125I-RIA IGF-1 kit validated for bovine samples (Nikolic *et al.*, 2001) was used to determine IGF-1. Intra- and inter-assays were established for duplicate samples.

3.8 Statistical Analysis

Data on milk production, BCS, metabolites, and hormone concentrations were examined using one-way analysis of variance (ANOVA) (SAS, 2010). Pre and postpartum period, BCS, effect of parity and their interactions were all included in statistical model. During the study time, prenatal/postnatal days were divided into 15-day intervals (day 0 = parturition day). In order to evaluate reproductive factors, a generic linear model with parity and BCS at parturition as fixed variables was used. Parity, BCS at parturition, milk output, total protein, urea, NEFA, IGF-1, and leptin were the dependent variables, and the re-initiation of ovarian cyclicity was the independent factor. The Turkey Kramer test was used to indicate differences both within and between treatments. The correlation coefficient was calculated to investigate correlations between variables (SAS, 2010). Regression analysis was carried out to estimate effect of time on milk production. Hormones and milk production. Two statistical models were developed for analysis of data collected on pregnancy and calf and hormones and metabolites (Appendix II).

3.9 Logistical and Ethical Considerations

Approval to conduct research was granted by graduate school, Kenyatta University. The National Council for Technology and Innovations (NACOSTI) issued a research permit.

CHAPTER FOUR: RESULTS

4.1 Feed Analyses

Dry matter (DM) of the forages and total mixed ration (TMR) feed blocks varied from 45% (*Elba Rhodes) to 52.2% (Brachiaria grass),* and from 79.4% (feed blocks) to 89% (ground *Dolichos lablab* beans) respectively. Crude protein (CP) ranged from 6.84% (*Elba Rhodes* grass); 13.5% (*Brachiaria* grass); 16.8% ((feed blocks) to 24.9% (ground *Dolichos lablab* beans). Fat varied in the forages and feed block from 0.3% (*Elba Rhodes*; 0.57% (*Brachiaria* grass) 0.96% (Fedd block) to 1.22% (ground *Dolichos lablab*). Neutral detergent fibre (NDF) varied from 24.88% (ground *Dolichos lablab* beans); 39.5% (Feed block); 41.7% (*Elba Rhodes* grass); to 57.8% (*Brachiaria* grass) (Table 4.1). *Brachiaria* grass had the highest acid detergent fibre (ADF) (35.02%) whereas ground *Dolichos* beans had the least ADF (10.05%).

There was variation in lignin content of the feeds with *Elba rhodes* and complete meal feed blocks having the highest (above 11%) whereas *Brachiaria grass* and ground *Dolichos* beans had the least (less than 10%). Feed blocks had the highest amount of ash content (9.42%) whereas ground *Dolichos lablab* beans had the least ash content (3.8%). Ground *Dolichos lablab* beans had the highest organic matter digestibility (68.88%) but *Elba Rhodes* had the least (33.53%). *Dolichos lablab* beans had the highest in-vitro dry matter digestibility (IVDMD) (64.5%) whereas *Elba rhodes* had the least IVDMD) (55.3%). The forages and feed blocks had aflatoxin ranging from 1.1 (Elba Rhodes) to 7.4 (feed blocks) parts per billion (PPB). Feed energy ranged from 2515.7 metabolisable energy (ME) Kcal/Kg (*Elba rhodes*) to 3921.5 ME Kcal/Kg (feed blocks) (Table 4:1).

Table 4.1: Nutrient Content of Forages and TMR Feed Blocks (Mean±SE)

Nutritional Component	*Elmba rhodes*	*Brachiaria* Grass	Ground *Dolichos* Seeds	Complete Meal Feed Block(g/100g)
Dry Matter	45±0.06	52.2±0.52	89±2.1	79.4±3.7
C P (%)	6.84±0.04	13.5±0.51	24.9±1.25	16.8±1.1
Fat (%)	0.3±0.02	0.57±0.02	1.22±0.08	0.96±0.02
NDF (%)	41.7±6.7	57.8±2.01	24.88±0.11	39.5±0.04
ADF (%)	28.6±2.5	35.02±1.12	10.05±0.04	20.07±0.02
ADL (%)	11.7±1.6	8.43±0.02	6.04.00±.02	11.1±0.6
Ash (%)	9.1±0.8	8.99±0.09	3.8±0.07	9.42±2.1
OMD (%)	33.53	60.6±2.5	68.88	64.47
IVDMD (%)	55.3±0.05	61.07±1.2	64.5±0.04	59.79± 0.03
Methionine	1.8±0.01	2.29±0.01	3.93±0.01	3.35±0.07
Lysine	3.6±0.01	5.89±0.04	5.94±0.09	6.9±0.14
Total aflatoxin (ppb)	1.2±0.002	1.1±0.01	3.93±0.04	7.40±0.01
ME Kcal/Kg	2515.7±4.5	2539.7±3.94	3207.9±0.93	3921.5±9.5

4.1.1 Correlations of Feed Components

Nutritive components that affected in-vitro dry matter digestibility (IVDMD) were: Dry matter (DM) (P=0.0001, $r^2_{=-}$ -0.992) and ash (P=0.0001, r^2= -0.992). Neutral detergent fibre (NDF) was highly positively correlated to crude fibre (CF) ((P=0.000, r^2= 0.921), and acid detergent fibre (ADF) (P= 0.000, r^2=0.992), whereas NDF was negatively correlated to crude protein (CP)(P= 0.000, r^2= -0.921), methionine (P= 0.000, r^2= -0.978), lysine (P= 0.002, r^2= -0841) and metabolisable energy (P= 0.00, r^2 = -0.956). Crude protein was negatively correlated to CF (P=0.015, r^2 = -0.735), NDF

(P= 0.0001, r2= -0.902) and ADF (P= 0.002, r2= -0.848) but highly positively correlated to methionine (P= 0.001, r^2=0.865) and Lysine (P=0.0001, r^2=0.976). Dry matter (DM) was highly correlated to ash (P<0.001, r2= 0.943). Crude fibre was negatively correlated to; CP (P=0.015, r^2=-0.736), nitrogen free extract (NFE) (P=0.004, r^2=-0.812), methionine (P= 0.002, r^2= -847), and metabolisable energy (P=0.0001, r^2= -0.879) but positively correlated to NDF (P=0.000, r^2= 0.921) and ADF (P=0.000, r^2= 0.928) (Appendix III). Nutritive components that highly correlated positively with metabolisable energy were nitrogen-free extract (NFE), methionine, and lysine. Nutritive components that highly negatively correlated with metabolisable energy were CF, NDF, and ADF (Appendix III).

4.2 Effect of Supplementation on Dam and Calf Characteristics

4.2.1 Pregnancy Period

There was significant difference, (n=30, p <0.001, F= 30.09) in gestation period between treatments (Appendix IV). The test cows took a shorter gestation period (calved 6 days earlier). The average gestation periods for test cows and control cows, were 278.4±1.0, and 284.1±1.1 days respectively (Tables 4.2).

4.2.2 Body Condition Score at Calving

Cows in both treatments lost their body condition score with maximum loss occurring at day 30 postpartum (Figure 4.1). There was significant difference (P < 0.006, F= 13.26,) in body condition score (BCS) at calving between treatments (Appendix IV). The test cows had higher BCS at calving averaging 2.9±0.1 while the control cows BCS averaged 2.5±0.1 (Tables 4.2).

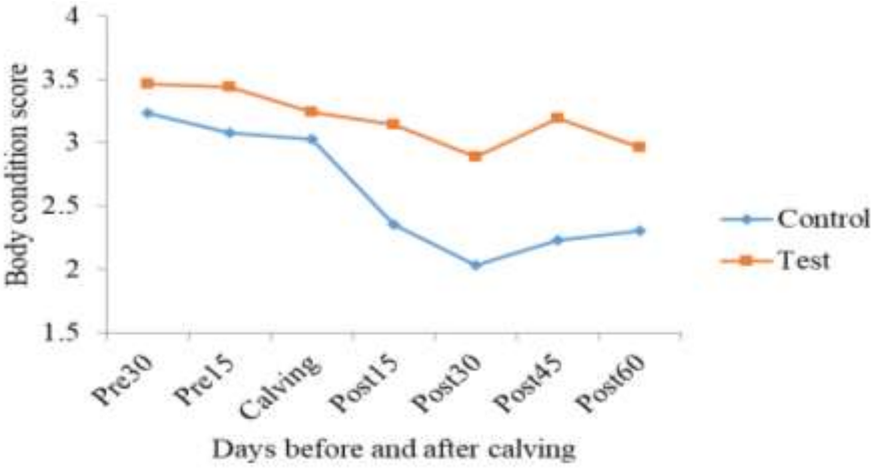

Figure 4.1: Pre and Postpartum Change in Body Condition Scores of Friesian Cows

4.2.3 Effect of Supplementation on Cow and Calf Characteristics

There were significant differences in gestation period between test and control cows with test cows having shorter gestation period **(**278.4±1.0 days) than control cows (284.1±1.1 days). At calving, the effect of supplementation was seen in the test cows' body condition score, which was substantially higher (2.9±0.1), than body condition score of the control cows (2.5±0.1).

The test cows' average calf weight was 27.1±0.1kg, while the control cows' average calf weight was 28.3±0.5 kg. The weights of the calves under the two treatments did not differ significantly (Table 4.2). Test cows cycled at an average of 50.4 days and the control cows cycled at an average of 62.7 days postpartum (Table 4.2). The average progesterone levels at estrus for the test and control cows were 0.53±0.39 and 0.57±0.06 Nmol/L, respectively. There was no significant difference in progesterone levels at estrus between test and control cows (Table 4.2).

Table 4.2: Effect of Supplementation on Cow and Calf Characteristics (Mean±SE)

Supplementation	Pregnancy period (days)	BCS at calving	Calf Weight (kg)	Days to visible heat	P4 at estrus (Nmol/L)
Test	278.4 ± 1.0^{b}	2.9 ± 0.1^{a}	27.1±0.1	50.4 ± 0.9^{b}	0.53±0.39
Control	284.1 ± 1.1^{a}	2.5 ± 0.1^{b}	28.3±0.5	62.7 ± 2.0^{a}	0.57±0.06

^{ab} Different subscripts in the same column are significant ($P<0.05$)

4.2.4 Effect of Supplementation on Calf Sex, Milk Production and Postpartum Reproductive Efficiency

The test cows had a higher proportion of female calves than male calves (Figure 4.2). Test cows conceived at a much higher rate (55 %) whereas the control cows had a higher percentage of non-fertilization (50%) than the test cows (20 %). The test cows recorded significantly higher milk production than the control cows. The mean milk production from the test animals was 9.1 Lday^{-1} compared to 5.3 Lday^{-1} for the control cows (F= 159.52, $p<0.0001$) (Figure 4.3). There was significant difference between test and control in reproductive indices. Cows in the test group had higher conception, and silent heat cases as compared to the control cows. Frequency of embryo loss and non-cyclicity were only seen among test cows. Non-fertilization was significantly higher among cows in control group (Figure. 4.4).

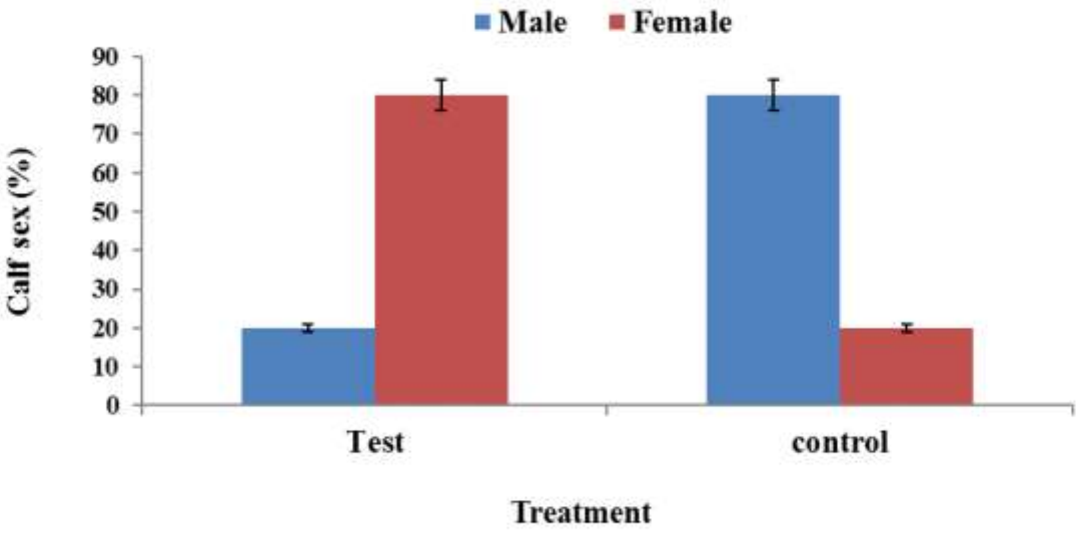

Figure 4.2: Effect of Supplementation on Calf Sex (%). Error Bars Represent Significance

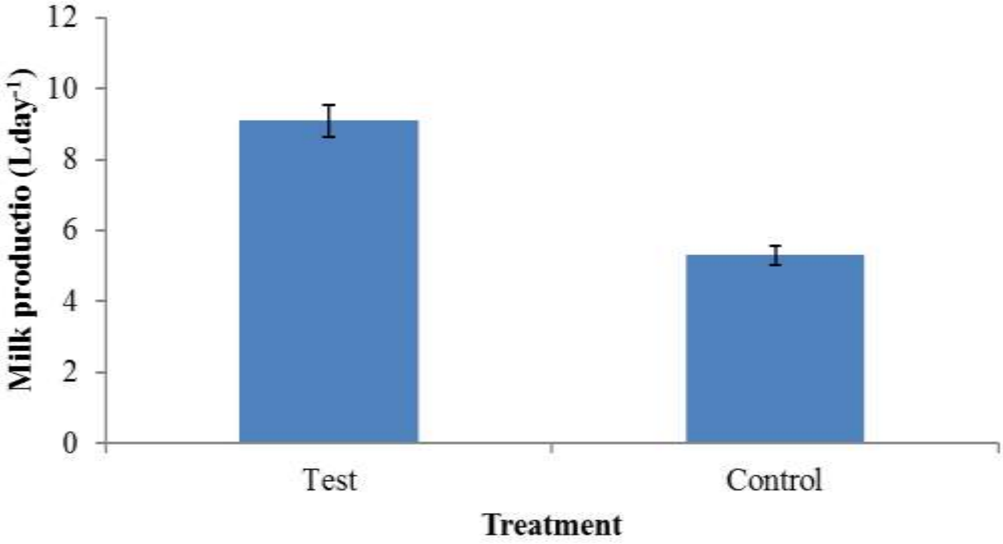

Figure 4.3: Effect of Supplementation on Milk Production (Lday^{-1}). Error Bars Represent Significance

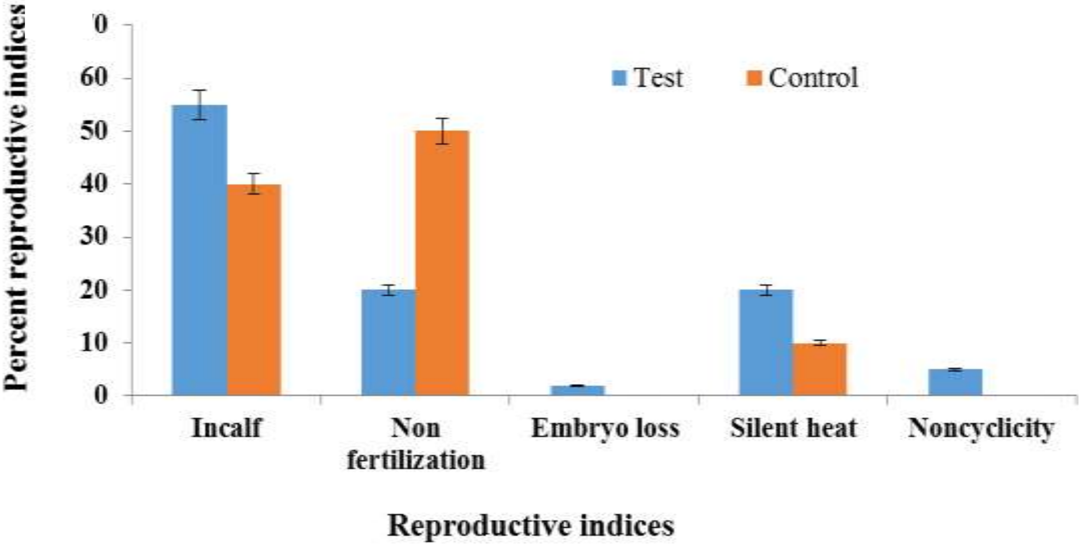

Figure 4.4: Effect of Supplementation on Reproductive Indices (%).Error Bars Represent Significance

4.2.5 Correlation Between Cow and Calf Characteristics

A significant correlation was observed between the pregnancy period and BCS at calving. The BCS at calving was negatively highly correlated ($P < 0.006$, $r^2 = -0.645$) with the days to visible heat (Appendix V).

There was a significant correlation ($P<0.001$, $r^2= 0.501$) in heat exhibition between postpartum dams, with dams of heavier calves taking longer time to exhibit heat signs compared to dams of light calves (Appendix V). Days to visible heat and the BCS at calving had a strong negative correlation ($P<0.001$, -0.645) (Appendix V).

4.3 Influence of Parity on Calf and Cow Characteristics

Pregnancy period increased with parity. Parity 1 had an average gestation period of 273.4±0.8 days, while parity 4 had an average gestation period of 287.6±1.2 days (Table 4.3). The body condition score at calving improved with increased parities, for parity 1 and 4, the average body condition score at calving was 2.6±0.1 and 4.0±0.2

respectively (Table 4.3). Body condition score at calving was higher for the cows at parity 3 and 4 than for the lower parities (Table 4.3). Parity 1 and 2 took longer (57.1±1.5) days than parity 3 and 4, which took an average of 47.2±2.4 days; to exhibit apparent heat signs (Table 4.3). There were significant differences in progesterone levels between primiparous and multiparous cows during postpartum estrus.

Table 4.3: Parity Effect on Calf and Cow Characteristics (Mean±SE)

Parity	Gestation Period (days)	BCS at calving	Calf Weight (Kg)	Days to postpartum heat	P4 at estrus (Nmol/L)
Parity1	273.4±0.8^{c}	2.6±0.1^{c}	28.0±0.5	57.1±1.5^{a}	0.59±0.20
Parity2	282.2±07^{b}	2.3±0.2^{c}	27.7±0.8	58.4±1.8^{a}	0.57±0.7
Parity3	282.3±0.3^{b}	3.0±0.0^{b}	27.4±1.2	48.6±0.3^{b}	0.57±0.1
Parity4	287.6±1.2^{a}	4.0±0.2^{a}	26.0±1.9	47.2±2.4^{b}	0.37±0.04

^{abc} Different superscripts within columns are significantly different at $P<0.05$

4.3.1 Effect of Postpartum Days and Parity on Milk Production

The test cows had highest milk production recorded at 45 days after calving with the least milk production occurring on day 15 after calving (Figure 4.5). Parity significantly affected milk production with parity 3 of the test cows producing the highest amount of milk ($p<0.0001$, $F= 10.52$) (Fig. 4.6). Significant interactions existed between supplements, parity, and days to postpartum heat (Appendix VI).

There was a difference in amount of milk produced with parity 3 and 4 cows within the test cows producing much more milk than parity 2 cows. Parity 3 cows in the control group produced much more milk than cows of parities 1, 2 and 4 (Figure 4.6).

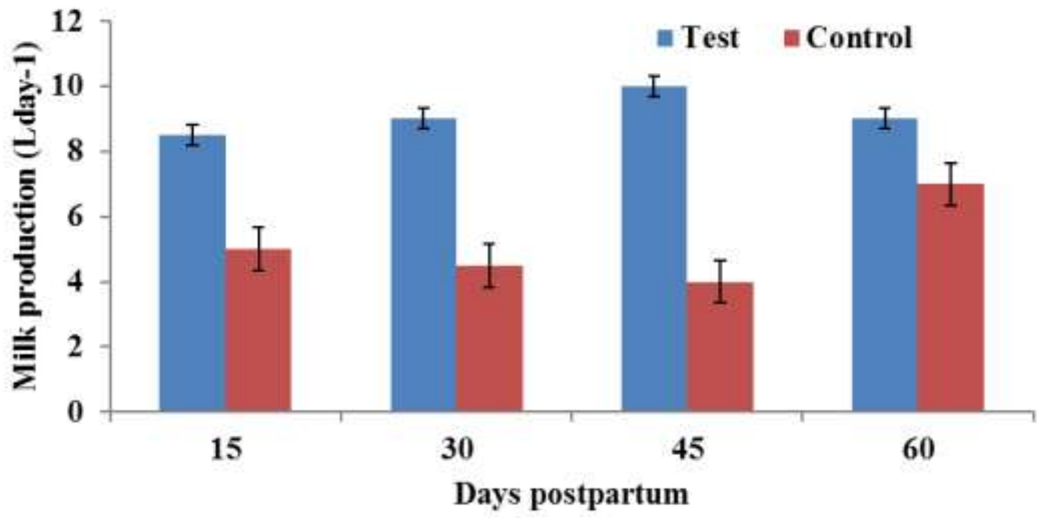

Figure 4.5: Effect of Postpartum Days on Milk Production. Error Bars Represent Significance

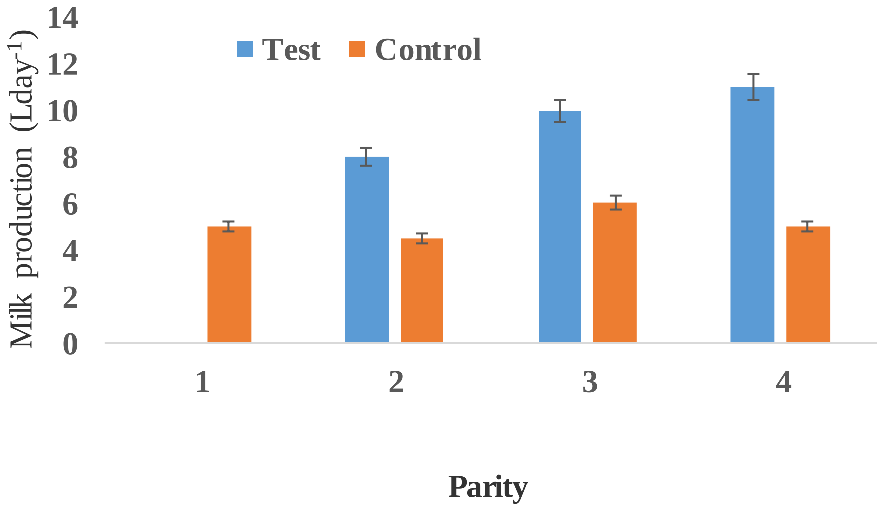

Figure 4.6: Effect of Parity on Milk Production. Error Bars Represent Significance

Feed supplementation had significant effect on milk production over the study period. Regression analysis showed that there was a linear increase in the mean periodic milk yield from 15 days postpartum to 60 days postpartum (Figure 4.7).

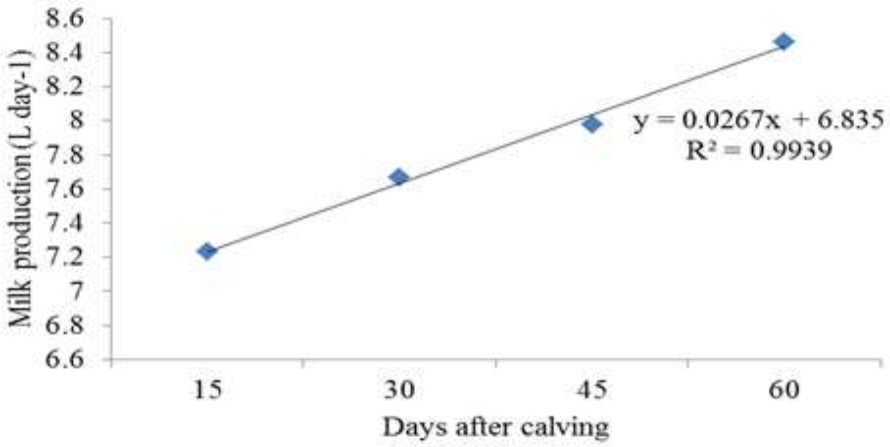

Figure 4.7: Line of Good Fit Showing Effect of Postpartum Days on Milk Production.

4.3.2 Effect of Supplementation on Milk Components

The test cows had greater protein percentages ((3.16±0.07)) than the control cows (3.07±0.02). Their milk had higher solid not fat (SNF) percentages ($P<0.05$) than the control. There was no significant variation in density, fat-to-protein ratio, or fat %. (Table 4.4). Milk protein had a strong association with freezing point (Appendix VII).

Table 4.4: Effect of Supplementation on Milk Components (Mean±S.E)

Treatment	% Protein	% Fat	%SNF	Freezing Point (^{o}C)	Density	Ratio: Fat/Protein
Test	3.16±.1	8.88±.1^{a}	3.16±.07^{a}	1.027±.1	-0.57±.1	1.11±.01
Control	3.07±.1	8.15±.1^{b}	3.07±.02^{b}	1.026±.02	-0.54±.1	1.13±.06

^{ab} *Different subscrips within colum are significant at P<0.05*

4.4 Milk Progesterone Profile Pre- and Post-Partum

Milk progesterone hormone started to decrease 30 days before calving and reached a threshold (nadir) at calving for both test and control cows. Day 45 revealed a significant difference ($P<0.05$), with the test cows mean progesterone level being higher at 6 Nmol/L compared to the control cows which had progesterone value of 3.8 Nmol/L. (Figure 4.8) at day 45 postpartum.

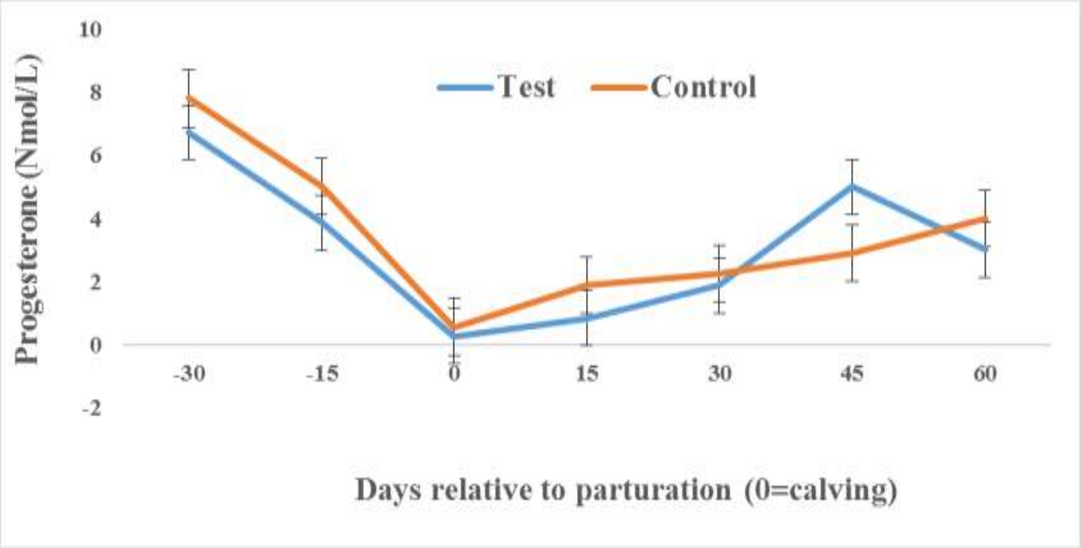

Figure 4.8: Effect of Supplementation on Progesterone Profile Within 30 Days Pre and 60 Days Postpartum. Error Bars Represent Significance

4.5 Effect of Parity on Days to Visible Heat

There was significant difference (P<0.05) between parity 1 and parity 2 in the number of days taken to show visible heat with cows in the test group taking shorter time to show heat signs. Cows of parity 3 and 4 in the control group took shorter time to exhibit visible heat signs as compared to parity 1 and 2 in the same treatment (Figure 4.9).

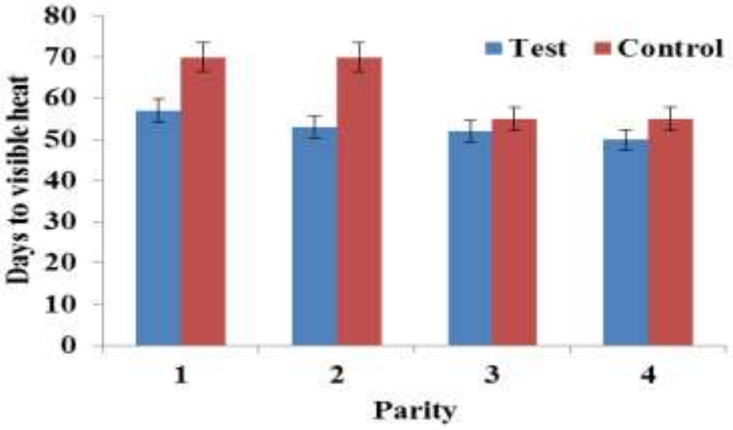

Figure 4.9: Effect of Parity on the Number of Days to Visible Heat. Error Bars Represent Significance

4.6 Effect of Supplementation and Parity on Inseminations and Conception

There was a significant difference in inseminations/conceptions between test and control cows, with test cows having fewer inseminations/conceptions (1.35) as compared to cows in the control group (2.7) (Figure 4.10). There was also significant difference in number of inseminations/conception between parities with parity 3 having the least number of inseminations/conception (1.2) whereas parity 2 had the highest (2.1) (Figure 4.11).

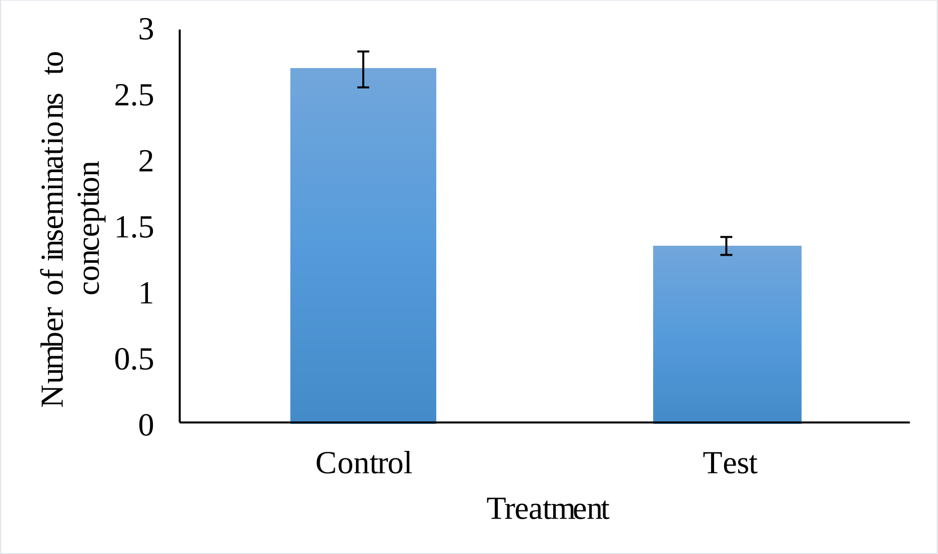

Figure 4.10: Effect of Treatment on Number of Inseminations/Conception. Error Bars Represent Significance

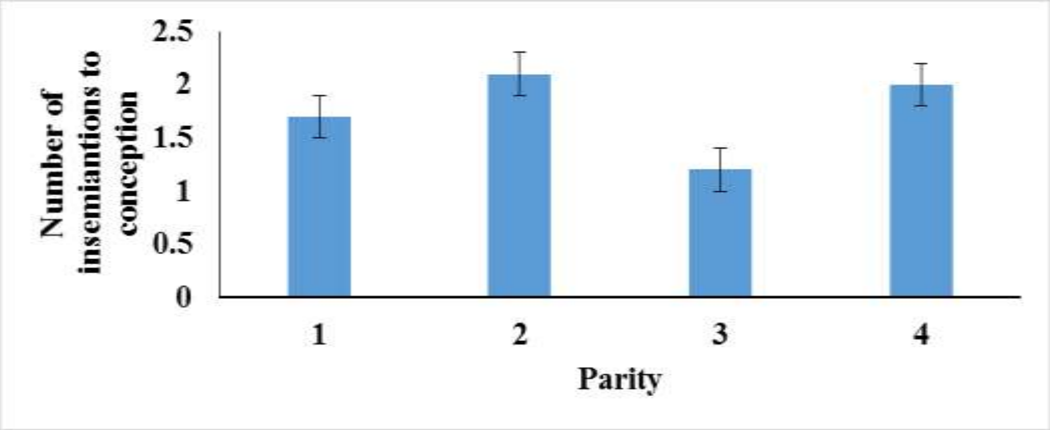

Figure 4.11: Effect Of Parity on Number of Inseminations/Conception.

4.7 Effects of Supplementation on Body Condition Score Metabolites and Hormones

Supplementation had a significant effect on glucose, urea, non-esterified fat acids, proteins except for Beta-hydroxybutyrate (BHB) (P=0.467, F= 0.53) (Appendix VIII). Parity had no significant effect on NEFA, leptin, glucose, or P4 levels. Insulin-like growth factor -1 and BHB hormones were significantly affected by their interactions with parity. Leptin and P4 levels were the only variables where the days by parity interaction was not significant (Appendix VIII). The test cows showed higher concentrations of the hormones and metabolites, while the control cows showed higher amounts of urea and progesterone (Table 4.5). Insulin-like growth factor -1 levels in the test group cows were higher (4.9 nmol/L) than in the control group cows (4.1 nmol/L). The test group cows had glucose and protein levels of 45.5 mg/dl and 79.2 g/L, compared to the control cows' values of 35.5 mg/dl and 73.6 g/L, respectively (Table 4.5). Progesterone levels were greater in the control cows (3.48 nmol/L) than in the test cows (2.94 nmol/L) (Table 4.5) (p.>0.0001, F= 356.94,).

There was significant difference in variables evaluated: body condition score, insulin-like growth factor -1, insulin, non-esterified fat acids, beta-hydroxybutyrate, leptin, glucose protein urea and protein evaluated for parity 1 and 2 with the exception of insulin and urea. (Table 4.6). There was no significant variation between parity 3 and parity 4 profiles of IGF-1, insulin, NEFA, BHB, leptin, glucose, and P4 in postpartum cows, despite parity significantly varying among them (Appendix VIII). The protein and urea profiles did, however, significantly differ, with parity 4 having greater values (Table 4.6).

Table 4.5: Effect of Supplementation on Body Condition Score, Hormones and Metabolites (Mean±SE) of Friesian Dairy Cows

Treatment	BCS	IGF-1	Insulin	NEFA	BHB	Leptin	Glucose	Protein	Urea	P4
Test	3.2±.02^{a}	4.9±.1^{a}	10±.04^{a}	.35±.0^{a}	.36±.2^{a}	.44±.01^{a}	45.5±.5^{a}	79.2±.4^{a}	4.42±.1^{b}	2.94±.1^{b}
Control	2.6±.04^{b}	4.1±.1^{b}	9.4±.09^{b}	.33±.01^{b}	.35±.0^{a}	.38±.01^{b}	35.5±.9^{b}	73.6±.6^{b}	4.52±.1^{a}	3.48±.2^{a}

Means ± standard error of the means. Means followed by different superscript letters within a column are significantly different at $\alpha<0.05$.

Table 4.6: Effect of Parity on Hormones and Metabolites (Mean±SE) of Friesians Dairy Cows

Parity	IGF-1	Insulin	NEFA	BHB	Leptin	Glucose	Protein	Urea	P4
1	3.23±.23^{c}	10.±.37^{a}	0.33±.02^{b}	0.29±.05^{b}	0.38±.02^{c}	36.6±2.7^{c}	64.4±1.4^{d}	4.0±.4^{c}	3.8±.7^{a}
2	4.21±.9^{b}	9.8±.7^{a}	0.34±.1^{a}	0.36±.1^{a}	0.41±.01^{b}	41.1±.8^{b}	71.9±0.4^{c}	4.0±.1^{c}	3.1±.2^{b}
3	5.23±.13^{a}	9.9±.08^{a}	0.35±.01^{a}	0.36±.02^{a}	0.43±.01^{a}	44.0±.9^{a}	80.5±.4^{b}	4.6±.1^{b}	3.0±.2^{c}
4	5.11±.13^{a}	9.9±.07^{a}	0.35±.01^{a}	0.36±.46^{a}	0.43±.01^{a}	43.1±.9^{a}	85.7±.3^{a}	5.2±.1^{a}	3.1±.2^{c}

Means ± standard error of the means. Means followed by different superscript letters within a column are significantly different at $\alpha<0.05$.

4.7.1 Effect of Supplementation on Insulin-Like Growth Factor 1 (IGF- 1) profile

There was significant difference in insulin growth factor 1 (IGF-1) values between test and control cows from 30 days pre-partum to 60 days postpartum (n=30, $p<0.0001$, F= 57.13). The IGF-1 decreased gradually in both treatments reaching threshold on day 15 postpartum. Highest IGF-1 values were taken on day 60 postpartum for both treatments (Figure 4.12)

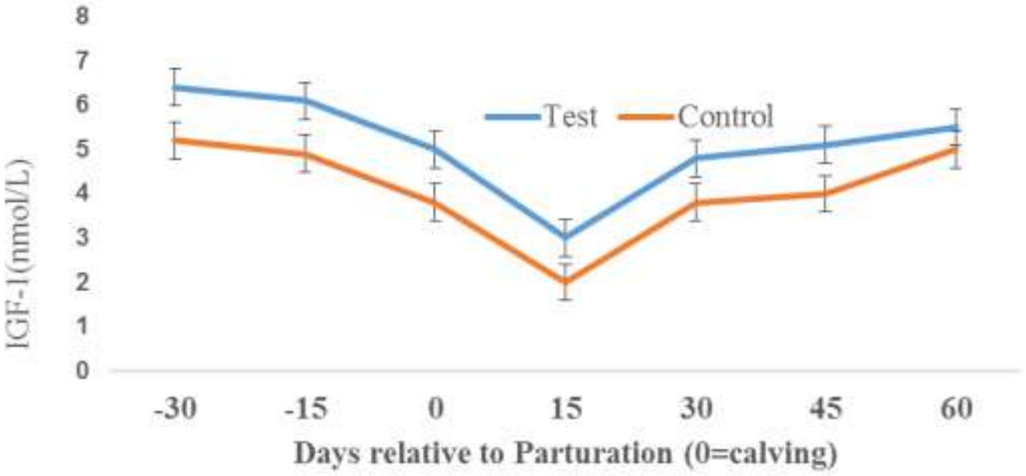

Fig. Effect of supplimentation on IGF levels

Figure 4.12: Effects of Supplementation on IGF-1 Profiles. .

4.7.2 Effect of Supplementation on Glucose Profiles

There was no significant difference in glucose levels between treatments before parturition, but after parturition there was significant difference in the levels with the test cows having higher values of glucose up to day 60 postpartum.

Both treatments had prepartum reductions in their glucose levels, which reached a threshold on day 15 postpartum. Glucose values gradually increased in both treatments until they reached their highest levels on day 60 postpartum (Figure 4.13).

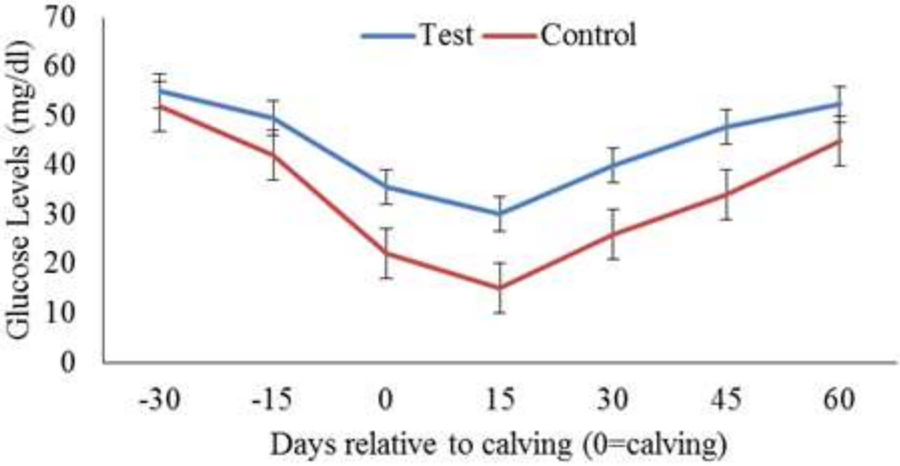

Figure 4.13: Effect of Postpartum Days on Glucose Profile. .

4.7.3 Effect of Supplementation on Non-Esterified Fatty Acids (NEFA)

Non-esterified fatty acids (NEFA) peaked on day 15 after calving and then steadily decreased until they reached a threshold on day 45 after calving (Figure 4.11). Control cows had significantly higher values of NEFA than the test cows on day 15 and 30 postpartum ($p<0.001$, F=57.74) (Figure 4.14).

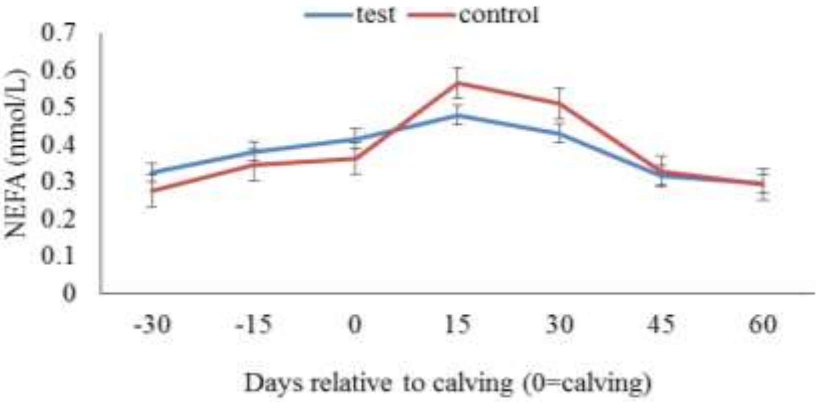

Figure 4.14: Effect of Supplementation on NEFA Profiles.

4.7.4 Effect of Supplementation on β-hydroxybutyrate Profile

B-hydroxybutyrate levels began to rise at calving, and they continued to rise gradually until parturition in both treatments. After calving, the values grew sharply until day 15 postpartum, then tabled off from day 30 to day 60, then it declined sharply until day 45 and subsequently decreased on day 60 postpartum (Figure 4.15).

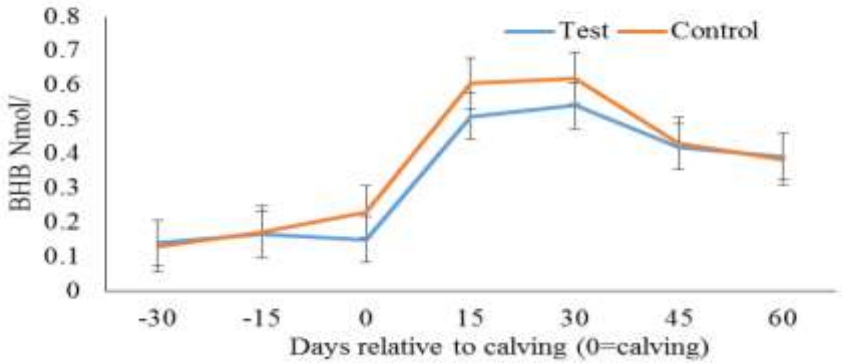

Figure 4.15: Effect of Supplementation on BHB Profiles. .

4.7.5 Effect of Supplementation on Insulin Profile

The plasma insulin concentrations were low during prepartum period. The levels in test cows started to increase significantly from day 15 prepartum to day 30 postpartum whereas the hormone levels in the control cows reduced from day 15 prepartum reaching minimal levels at calving then the hormone rapidly increased from day 15 postpartum reaching nadir on day 30 and day 60 postpartum (Figure 4.16) for test and control cows respectively. The insulin levels were significantly different (F=112.06, $p<0.0001$), with the test cows having significantly higher levels on day 30, whereas the control cows had the highest levels on day 60 (Figure 4.16).

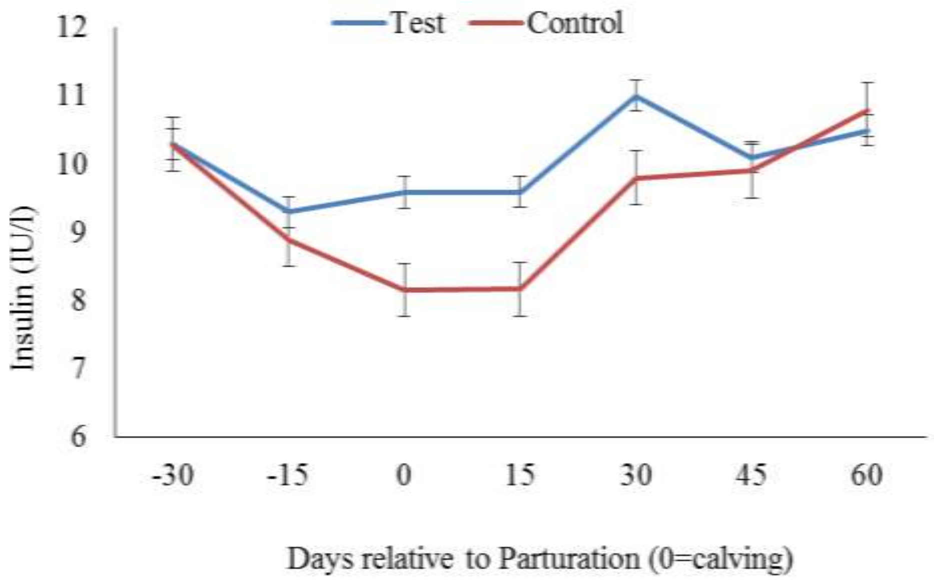

Figure 4.16: Effect of Postpartum Days on Insulin Profile.

4.7.6 Effect of Supplementation on Leptin Profile

Both the test and control cows experienced a decline in plasma leptin from day 30 prepartum to basal levels on day 15 postpartum, there was a significant variation in the hormone profiles between the two groups from day 30-15 prepartum (Figure 4.18). There was no significant difference in leptin levels from calving to day 60 postpartum in both groups (Figure 4.18).

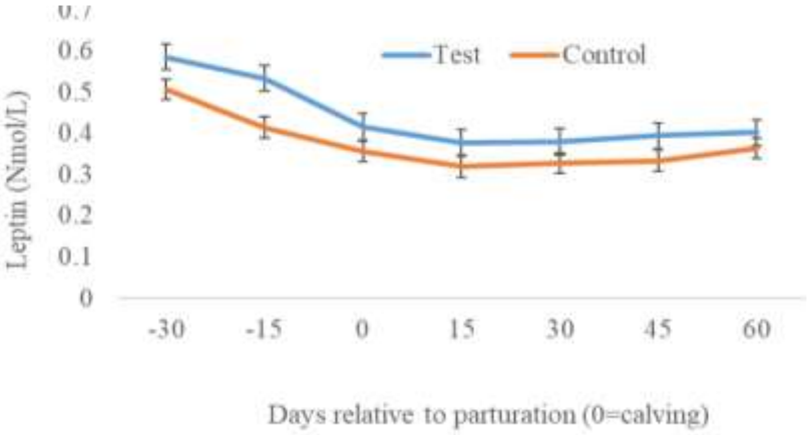

Figure 4.17: Effect of Postpartum Days on Leptin Profile.

4.7.7 Effect of Supplementation on Urea Profile

Plasma urea steadily decreased from day 30 prepartum reaching basal levels day 30 postpartum in both groups, with significant difference in concentration of the hormones on day 15 postpartum. The control cows showed higher values (4.81Nmol/L) of urea than the test cows (3.08 Nmol/L) (Figure 4.18).

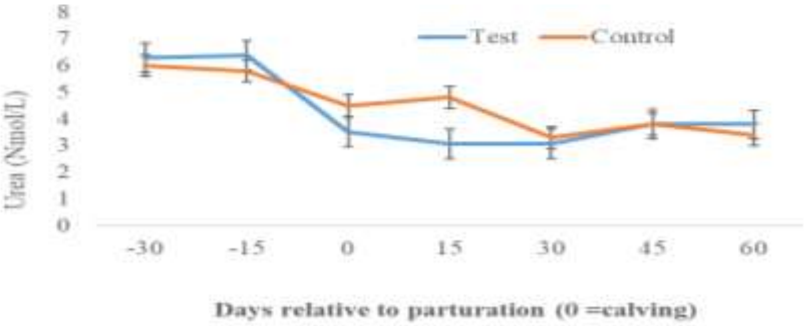

Figure 4.18 Effect of Postpartum Days on Urea Profile. .

4.7.8 Correlation of Milk Progesterone, Selected Plasma Hormones and Metabolites Prepartum, Term, and Postpartum

Before calving, the BCS was significantly correlated with the hormones and metabolites except for insulin, BHB, and P4 levels. Insulin-like growth factor-1 was positively correlated with NEFA ($P<0.0001$, $r^2 = 0.531$), leptin ($P<0.0001$, $r^2 =$

0.557), glucose (P<0.0001, $r^2 = 0.443$,), urea (P<0.0001, $r^2 = 0.505$,) and proteins (P<0.0001, $r^2 = 0.719$,). The insulin level was positively correlated with the leptin, glucose and P4 levels. There was a significant negative correlation between NEFA and P4 levels (P=0.001, $r^2 = -0.628$,) (Appendix IX).

At calving, body condition score was negatively correlated with urea but strongly correlated with insulin, NEFA, leptin, and glucose. The IGF-1 highly correlated positively with protein (P<0.0001, $r^2 = 0.637$), whereas insulin correlated negatively with BHB, urea, and P4. Non-esterified fatty acids correlated significantly with leptin and glucose but non-significantly with BHB, urea, and P4. Leptin, glucose, and protein were all significantly inversely correlated with BHB. Glucose and leptin had a positive correlation, although urea and P4 had a negative correlation. Urea and P4 had a negative correlation with glucose. Urea and protein had a positive correlation, while P4 and urea had a positive correlation (Appendix X).

Day 45 after calving, the BCS was negatively correlated with NEFA (P = 0.0007, $r^2 = -0.217$) and P4 levels (P= 0.0407, $r^2 = -0.132$). Insulin-like growth factor 1 was negatively correlated with the NEFA and BHB levels. Milk production was positively correlated with BCS, IGF-1, insulin, leptin, glucose, protein and urea. There was a negative correlation (P< 0.001, $r^2 = -0.306$) between the milk production and the NEFA levels. Luteal activity (P4) was positively highly correlated to IGF-1(p < 0.0001, $r^2=0.672$,), and glucose (P< 0.001, $r^2=0.634$) but highly negatively correlated to NEFA (P=0.001, $r^2 = -0.689$) and BHB (P<0.00, $r^2=-679$) (Appendix XI).

CHAPTER FIVE: DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Effect of TMR Feed Blocks on Cow and Calf Characteristics

Elba rhodes and *Brachiaria ruziziensis* had low values as compared to values reported by Kozloski *et al*. (2005). They reported values of CP and ADF to be 15.2% and 46%, respectively. The current study utilized Brachiaria ruziziensis and Elba rhodes grsses cut at 5 months while the earlier study by Kozloski *et al.* (2005) used dwarf elephant grass hay cut at 3 months, which could account for the difference in ADF values. Differences in the nutritional content were anticipated since the grasses' chemical composition and nutritive values varied.

The amount of neutral detergent fiber (NDF) in the feeds utilized in this study indicated that the pastures were of high-quality and were good at improving rumen function (Russel *et al.*, 1992, Waghorn and Clark, 2004). The value of NDF was reported to be 71.5 percent by Kozloski *et al.* (2005). This was higher than values of the feeds used in the current study. The difference could result from utilization of different feeds. In the current investigation, TMR feed blocks made from *Brachiaria* grass and ground *Dolichos lablab* seeds were used. In the earlier investigation, dwarf elephant grass was used. The digestibility of the feeds decreased as ADF values increased. This was anticipated due to the fact that ADF indicates the presence of cellulose and lignins in a feed. These elements inhibit digestion. This finding agreed with the finding of Osho *et al*. (2013).

The protein level of the feeds employed in the study had a significant impact on their in-vitro dry matter digestibility (IVDMD). The high levels of lignin and fiber in the grasses may have reduced in-vitro dry matter digestion (De la Mora *et al.*, 2016). Ash levels increased with dry matter content. There was high correlation of metabolisable energy (ME) with methionine, lysine, and nitrogen free extract (NFE). This indicated that an increase in these nutrients contributed to an increase in ME. There was negative relationship between metabolisable energy with NDF, ADF, and CF. This indicated that a rise in these fibers was associated with a decrease in metabolisable energy. This was anticipated since the fast rate of transit from the rumen is correlated with decreased fiber digestibility. The resident duration of the fiber in the rumen is shortened by a high rumen passage rate. The rumen also fills up more quickly when intake is increased.

Volatile fatty acid (VFA) concentrations increase as a result, and the pH of the rumen decreases. Cellulolytic bacteria are severely harmed by low rumen pH leading to Low metabolisable energy (Faniyi *et al.*, 2019). Metabolisable energy (ME) was adversely linked with NDF and ADF and the finding agreed with earlier authors (Kamalac *et al*., 2005). Neutral detergent fibre (NDF) was highly positively correlated to CF and ADF. This implied that there was positive correlation of NDF to CF and ADF. This agreed with the results of Li *et al.* (2013) who reported the same association in a dult Cecectomised rooster. Neutral detergent fibre (NDF) was negatively correlated to CP, methionine, lysine and metabolizable energy ($P < 0.00$, $r^2 = -0.956$). This indicated increase in NDF led to decrease in the above components.

The fiber component, had a negative correlation with *in-vitro* dry matter digestibility (IVDMD). This is due to the fact that fibers are less easily digested than non-fibrous components (protein, methionine, lysine and NFE). The results were in agreement with findings of Mahyuddin (2008) who reported that crude fiber (CF), neutral detergent fiber (NDF), and acid detergent fiber had a strong negative correlation with metabolisable energy. This is due to the fact that their high fiber content prevented *in-vitro* digestibility. The NFE, Methionine, and Lysine had strong positive correlations with metabolisable energy. This is as a result of their non-fibrous nature and quick in-vitro digestion. These findings agreed with the finding of Mahyuddin (2008) and Barton *et al*. (1976), who found that protein was the best indicator of digestibility for tropical grasses.

The average gestation period was shorter (278.4± 1.0 days) within the test cows than the control cows (282.1±1.1 days). The difference could be due to supplementation which may have stimulated the fetal hypothalamic-pituitary-adrenal axis, leading to early parturition (Matthews and Challis, 1996). This finding was consistent with the finding of Norman *et al.* (2009), which stated that a Friesian dairy cow's average gestation time was 279.4 ± 5.7days.

Physical body condition of the cows decreased in both treatments postpartum, with the highest decrease occurring at day 30 postpartum. (Figure. 4.1). The finding contrasted with that of (Chagas *et al*., 2007) who reviewed nadir to occur on day 50 after calving. Body condition score improved after nadir. The test cows had a higher body condition score at calving (2.9±0.1)) than the control cows (2.5±0.1). Supplementation may have boosted their feed intake, which would explain the difference in the Body Condition Score (Haffaf, 2016). The results demonstrated that nutritional supplementation was an efficient means of maintaining milk supply while shielding test cows from the detrimental impacts of decreasing body condition brought on by lactation. This finding contrasted with that of Ibrahim *et al*. (2010), who reported supplementing lactating cattle with live yeast had no effect on dry matter intake (DMI), milk production, metabolic state, or mobilization of body reserves. The difference in the findings could be because the author used live yeast to supplement postpartum cows; the current investigation began supplementation prepartum using TMR feed blocks.

There was no significant difference in calf birth weights between the test cows and control cows this finding implies that a higher level of nourishment merely improves the physical state of the dam and has no impact on the progeny's development during the last 30 days of pregnancy. The very high conversion rates and the minimal responses of the fetus to additional concentrates can only be explained by the fact that cows were well-fed on established *Elba rhodes*, which is the only explanation for the current findings. The aforementioned reactions could be taken into consideration as the very minimum predicted from concentrate supplementation regulating calf birth weights.

Lack of significant difference in calf birth weights between test and control groups of dairy cows was also reported by Prima *et al.* (2018) but contrasted with that of Bayemi *et al.* (2014), who came to the conclusion that calves' birth weights were higher when cows were given more concentrate feed before calving. The test cows had a higher percentage (80%) of female calves (16) than the control cows (20%), indicating a significant difference in calf sex. This was unexpected because test cows were supplemented during the latter trimester of pregnancy, when the gender of the calf was thought to have been determined. It was unclear what led to this finding. The results differed from those of Salehi *et al*. (2016). He observed that Friesian dairy cattle treated with sun flower seed prepartum gave birth to more male than female calves (26 males and 19 females).

The test group of cows produced 71.2 percent more milk than the control cows. This could be because the test cows received greater protein TMR feed blocks as a supplement so the cows had enough protein for maintenance and milk production. Milk peaked 45 days postpartum and was lowest on day 15 postpartum. Supplementing postpartum cows with TMR feed blocks during the pre- and post-partum periods boosted milk production. It appeared like the supplement TMR feed blocks provided enhanced availability of ammonia levels in the rumen which the rumen microorganisms accessed and broke down in the gut to increase milk production. This result was consistent with that of Otto *et al.* (2016), who reported that supplementing dairy cows with degummed crude canola oil (DCCO) increased milk output.

Supplementation considerably changed the milk's constituents, with the test cows showing increased levels of protein, solid not fat (SNF), and freezing points. This suggests that providing TMR feed blocks to test cows improved microbial protein, increasing propionic acid synthesis and absorbing the acids via the mammary glands, increasing milk protein (Beever *et al.,* 2001).

Milk protein and freezing point had strong correlations (Appendix VI). Protein influences freezing point due to the low-molecular weight components that accumulate in the concentration polarization layer (Jan *et al.*, 2021). This finding was consistent with that of Martono *et al.* (2016), who found that the combined effect of feed additives increased milk's protein, SNF, total solids, density, and lactose content.

The average percentage of fat, protein and ratio of fat to protein suggested feed ration was stable and that there was greater room to raise milk output (Guinee *et al.*, 2007). These values were lower than those provided by Raovi *et al.* (2013), the could be due to the earlier author using pasteurized milk, the current study used fresh non-pasteurized milk.

Progesterone profiles detected from skimmed milk were used to assess ovarian activity. Significant differences in postpartum ovarian cyclicity were caused by supplementation, with the test cows cycling earlier (50.4±0.90 days) than the control group (62.7±2.0 days). This finding was in agreement with Prima *et al*. (2018) who observed that the test and control cows took 55.33±7.78 and 66.83±5.34 days respectively to show postpartum heat. The finding of the current study are consistent with earlier findings that prepartum nutritional supplementation affected postpartum reproduction positively (Tyagi *et al*., 2010; Khalil *et al.*, 2012; and Aungier *et al.*, 2014). The findings are consistent with Gillah *et al.* (2014) research findings that supplementing concentrates before calving decreased the calving interval of lactating crossbred cows. The results also concurred with the findings of Elmetwally *et al.* (2016), who reported that the number of days to heat for the supplemented cows dropped after the first parturition.

The average progesterone levels at estrus for the test cows and the control cows were not significantly different. This indicated that the amount of progesterone produced by the corpus luteum during estrus was not considerably affected by supplementation. These findings implied that postpartum CL function is one of the most important elements affecting reproductive efficiency. The findings agreed with those of Corah *et al.* (1974), who found that progesterone levels dropped from 6.48 and 8.1 nmol/l during late gestation to 2.27 nmol/l at parturition in the test and 2.92 nmol/l in the control cows respectively, and remained low until just before the first postpartum estrus.

Prior to the first postpartum estrus, progesterone levels were high and reached 1.94 nmol/l. Supplementation had a substantial impact on the frequency of conceptions and inseminations, with test cows having fewer number of inseminations /conceptions than the control cows. Supplementation shortened the time between calves and lowered breeding expenses brought about by the high price of additional AI dosages. The results agreed with those of Haile and Yoseph (2018), who found that the average number of inseminations per conceptions among Friesian cows was 1.9.

Multiparous cows had better body condition scores than younger cows. Body condition score was highly correlated to calf birth weight. The results were different from those of Mulliniks *et al.* (2012), who observed that cow calving BCS had no effect on calf birth weight, branding (55-d BW; $P = 0.76$), or weaning (205-d BW; $P < 0.001$). The days to visible heat and the BCS at calving had a strong negative correlation ($P = 0.60$, $r^2 = -0.645$,). This is because skinny cows must first replenish their BCS before they can use that energy to express their heat. This result varied from that of Mulliniks *et al.* (2012), who found that total pregnancy rates were unaffected by body condition score.

There was a substantial positive association between calf birth weight and postpartum heat, with heavier-calf dams taking longer to go into heat than the lighter-calf dams. This could be due the fact that dams with bigger calves required more time for uterine involution. This result was different from that of Guedon *et al*. (1999) who reported that calf weight at parturition was not related to timing of first ovulation postpartum.

The variation could result from the utilization of several breeds. The current study used Friesian cows that are milked both for human and animal consumption, the previous study used beef cows that are not milked. Days to visible heat was highly negatively correlated P<0.001, $r^2 = -0.645$) with BCS at calving, with low BCS cows requiring longer time to manifest visual heat. This could be because lean cows had to regain their BCS before using energy to show their heat. This indicates that BCS affected pregnancy rates. The finding differed with the results of Mulliniks *et al*. (2012) who reported pregnancy rates to be unaffected by body condition score.

The results of this study suggest that gestational periods in both the test and control cows were affected by parity, with gestation periods increasing with parity. This may be because lower parity cows have less energy balance for growth since they cannot eat enough energy from their feed and because their uteruses have a limited ability to allow further fetus growth. This result agreed with the finding of Foote's (1981) who indicated that changes in gestation length were connected to the cow's parity. Body condition score at calving increased as parity increased. The finding further concurred with the results of Sharma *et al*. (2018) who found postpartum BCS of experimental cows to be considerably higher in higher parity than in low parity cows. Calf birth weights were unaffected by parity. This result was unexpected because it was anticipated that higher parity cows would have bigger calves than lower parity cows due to lower competition for nutrients necessary for growth. The finding could only indicate that the established *Elba Rhodes* was able to give lower parity cows enough nutrition for milk and for growth. The results concurred with an earlier study which reported that parity has no discernible impact on the calf birth weight of the Fogera cow breed (Melaku *et al.*, 2011).

Cows of parity 1 and 2 and parity 3 and 4 took about the same amount of time to show clear signs of heat. These results may be explained by the fact that young cows' metabolic profiles differ from those of older cows since they are still growing and produce less milk (Wathes *et al.,* 2007). Another possible alternative theory is that primiparous and multiparous cattle have different progesterone metabolisms. In this study, there was no discernible variation in postpartum estrus progesterone levels between primiparous and multiparous cows.

In spite of parity, postpartum follicular waves developed and maintained progesterone levels because all the cows' physiological characteristics were the same. The results concurred with those of Carr *et al*. (1994) who found progesterone (P4) levels at estrus in multiparous suckled beef cows were not affected by isocaloric and isonitrogenous diets, with increasing levels of fat. The results differed from earlier researchers (Spicer *et al*., 1993; Son *et al.,* 1996, Petit *et al.,* 2006; Arellano-Rodriguez *et al.,* 2009; Kawashima *et al.,* 2010;) who found out that food supplements raised progesterone levels during estrus. The mean progesterone levels in the test and control cows did not differ significantly by day 30. However, by day 45 there was clearly a difference, with the test cows having a higher mean progesterone level. This indicated that test cows had started their luteal activity (CLA) early. This could have been because the test cows received supplements, and the feed could have promoted the early postpartum cyclicity.

5.1.2 Influence of TMR Feed Blocks on Selected Milk Hormone, Plasma Hormones and Metabolites.

There was significant difference in milk progesterone levels on day 45 postpartum, with the test cows having higher mean values. This indicated early commencement of luteal activity (CLA) among test cows. This was expected since the test cows were supplemented and the feed could have mitigated against the effect of negative energy balance among the test cows leading to early resumption of cyclicity among them within day 45 postpartum. The results concurred with the results of Kalasariya *et al*. (2017). This finding differed with the finding of Horan *et al*. (2005) who reported CLA mean interval as 32.9 days. The difference could be due to different diets used; the current study used total mixed ration feed blocks as supplements on pasture based system whereas the former study used a high concentrate pasture based system.

Insulin-like growth factor-1 (IGF-1) steadily reduced in both treatments and reached threshold on day 15 postpartum. During this time, the IGF-1 production in both treatments was quite low, but it gradually grew and peaked on day 45 postpartum. The increase in IGF-1 production led to increase in progesterone levels, culminating in ovarian cyclicity. The test cows had higher IGF-1 levels throughout the entire experiment. This might be as a result of supplementation given to the test cows, which mitigated against the severity of NEB's effects by reducing the rate of tissue mobilization during parturition and early postpartum period, as an alternative source of energy needed in response to high milk production (Lu *et al*., 2013). This finding was consistent with the results of Obese *et al*. (2011) who showed that postpartum supplementation with cereal grain concentrate altered plasma levels of IGF-I.

Plasma insulin levels were significantly different with the test cows having noticeably higher levels. This hormone plays a major role in controlling energy metabolism during postpartum period (De Koster *et al*., 2013). It also, has antilipolytic effect inhibiting the release of NEFA during this time (Hayirli, 2006). This finding would mean control cows, having low levels of the hormone, were not able to sufficiently control energy metabolism and levels of NEFA in the blood (Qiao *et al*., 2024**).** Leptin hormone profiles in both treatments did not significantly differ. The hormone leptin, which is mostly produced by adipose cells, regulates how much feed an animal eats and how much energy it utilizes (Houseknecht *et al.*, 1998). This outcome demonstrated that cows in both treatments experienced a 45-day reduction of the desire to feed. This result differed with that found by Csillik *et al.* (2017), who reported that plasma leptin levels in test cows remained significantly higher than those in control groups.

In the current experiment, TMR feed blocks (Brachiaria and Dolichos lablab) were used to feed only the test cows, while Elba Rhodes was used to feed both treatments whereas supplementation with lipid-encapsulated conjugated linoleic acid were used in a previous study (Csillik *et al*., 2017). The differing results could be due to different ingredients used. There was no observable variation in leptin concentration when cyclicity was found in both treatments at day 45 postpartum. The lack of a significant difference in leptin concentration between the treatments may be due to leptin concentrations that are not low enough to adversely affect ovulatory cycles. These results were consistent with the finding of Holtenius *et al.*, (2002) who showed no correlation between postpartum leptin concentrations and the time it took for cyclicity to resume.

There was significant difference in pre and postpartum plasma glucose concentration between the treatments. Glucose is a substantial byproduct of carbohydrate digestion. Therefore, the liver of postpartum cows need gluconeogenesis to produce glucose. Numerous homeorhetic processes are used by cows to increase blood sugar levels. Glucose may be able to coordinate overall animal metabolism due to its capacity to orchestrate changes in circulating insulin and IGF1 concentrations (Lucy, 2008). The difference in glucose concentrations between the two treatments may have been influenced by supplementation of the test cows and which provided more feed for gluconeogenesis and led to higher concentrations of the metabolite. This finding agreed with that of Sun *et al*. (2016), who found that post-ruminal choline and methionine administration increased glucose levels during calving (day 0) and the postpartum period in cows.

There was high positive correlation between IGF-1 and glucose than there was between IGF-1 and insulin. Weak association of IGF-1 and insulin could be because direct effects of IGF-I on insulin actions are tissue specific, occurring principally in skeletal muscle and kidney (Constantin *et al*., 2023). Non-esterified fatty acids (NEFA) peaked on day 15 after delivery and then gradually declined until they reached a threshold on day 45 postpartum. This could suggest that supplementation had an impact on NEFA levels between days 15 and 30 postpartum. This indicated that all postpartum cows mobilized their adipose tissue through lipolysis during the first 30 days postpartum for survival and milk production.

Following a significant increase in adipose tissue mobilization over the first 7 to 30 days postpartum, NEFA levels for both treatments decreased as lactation progressed. The results agreed with the finding of Marette *et al.* (2017) who reported that basal plasma NEFA levels dropped as lactation progressed after a low dosage of epinephrine was infused (up to 70 percent). These results may be related to physiological, metabolic, and endocrine changes that support milk production, including food partitioning for fetal needs, the start of lactogenesis, the stress of giving birth, and different physiological, metabolic, and endocrine changes. All of the body's tissues receive energy through the diffusion of NEFA into the circulation. However, too much NEFA might be poisonous (Overton, 2001). The bovine liver has a limited capacity to convert NEFA to triacylglycerol (TAG). They may be exported as very low density lipoprotein (VLDL) or may be oxidized (Spain and Scheer, 2001). When the limit is reached, accumulated TAG in the liver and unutilized acetyl CoA from fatty acid oxidation is converted into ketone bodies such as acetone, acetoacetate, and beta-hydroxybutyrate (BHB), which can be seen in the blood, milk, and urine (Goff and Horst, 1997; Nelson, 2005).

A high concentration of TAG impairs the liver's normal operation. The amount of NEFA in the blood affects TAG synthesis and accumulation in the liver. As a result, fatty liver syndrome in cows with lipolysis is highly likely to occur (Rukkwamsuk *et al.,* 2000). Beta-hydroxybutyrate (BHB) levels in both treatments started to slowly rise from day 15 prepartum up to calving, then the levels rose sharply up to day 15 postpartum and tabled off from day 15 to day 30 postpartum reaching threshold day 45 postpartum. This metabolite is a sign of lipomobilization (Gonzalez *et al*., 2011). Therefore, lipomobilization occurred in both treatments. This occurs because dairy cows frequently do not consume enough feed to meet their energy requirements at the start of lactation. They consequently mobilize their body fats and produce ketone bodies, particularly BHB (Cheput and Sirard, 2020). The levels achieved here are low, this may suggest a natural adjustment to the new energy requirements for both treatment's reproduction and milk production.

Plasma urea significantly reduced in both treatments, reaching nadir 15 days after calving. Plasma urea levels significantly varied between treatments, being higher in the control than the test cows. This could indicate that the control cows had a lot of energy deficit which could have stimulated catabolism of amino acids from tissue proteins yielding increased amount of urea in blood (Bell, 1993) as opposed to the test cows.

5.1.3 Relationship between Selected Hormones and Metabolites with Milk Yield and Postpartum Reproductive Efficiency,

Milk production was positively correlated with BCS, IGF-1, insulin, leptin, glucose, protein, and urea.

Insulin-like growth factor 1 was negatively correlated with the NEFA and BHB levels.

There was a non-significant negative correlation between milk production and the NEFA levels.

Luteal activity (P4) was positively highly correlated to IGF-1($p < 0.0001$, $r^2=0.672$,), and glucose ($P< 0.001$, $r^2=0.634$) but highly negatively correlated to NEFA ($P=0.001$, $r2 = -0.689$) and BHB ($P<0.00$, $r^2=-679$).

5.1.4 Indicators of Postpartum Ovarian Cyclicity

There was high correlation between IGF-1 ($P<0.001$; $r^2=0.672$), glucose ($P<0.001$; $r^2=0.634$), NEFA ($P<0.001$; $r^2 =-689$) and BHB ($P<0.001$; $r^2-0.679$) at day 45 postpartum. The relationship of P4 with other hormones and metabolites were negligible at day 45 postpartum. This is the time the test cows showed heat. The above hormones might indicate postpartum ovarian cyclicity at day 45 postpartum.

5. 2 Conclusions

1. Supplementation increased milk production whereas it reduced gestation period and days to postpartum cyclicity
2. Feed supplementation had positive influence on milk progesterone levels on day 45 effecting early postpartum heat exhibition.
3. Supplementation increased concentrations of plasma IGF-1, insulin, glucose, and progesterone.
4. Luteal activity (indicated by progesterone levels) highly correlated with IGF-1, glucose, NEFA, and BHB.
5. Glucose, IGF-1, NEFA and BHB are good indicators of postpartum reproductive efficiency among pasture based Friesian dairy cows.

5.3 Recommendations

1. Ministry of livestock and fisheries should develop animal production protocols that will incorporate supplementation of dairy cows with TMR feed blocks postpartum to enhance animal productive and reproductive efficiency.
2. The breeder's society of Kenya should develop breeding protocols using postpartum day 45 levels of P4 as a good indicator of postpartum fertility.

iii. Kenya agricultural livestock and research organization should develop animal nutrition protocols incorporating IGF-1 and glucose in performance trials among postpartum dairy cows**.**

5.4 Areas for Further Research

More research is required to confirm the importance of glucose, calf sex, calf birth weight, P4, IGF-1, BHB, and NEFA in dairy cattle breeding programs.

This is due to the following:

i. **Glucose is** a primary nutrient for fetal growth, maternal blood glucose levels influence glucose transport to the fetus. High milk production requires significant glucose, and maintaining adequate levels of glucose after calving is crucial for the cow to cycle and conceive

ii. **Calf sex** influences fetal growth, which can affect birth weight and calving ease.

iii. **Calf birth weight is** a key indicator of calving ease in selection and culling decisions to minimize the risk of dystocia. Poor neonatal growth increases costs and can impact performance.

iv. **Progesterone (P4) is a h**ormone that correlates with pregnancy probability and is a strong predictor of successful conception.

v. **Insulin-like Growth Factor 1 (IGF-1)** plays a role in fetal development and overall calf growth. Higher IGF-1 levels in the cow are linked to improved reproductive success and a higher probability of getting pregnant

vi. **Beta-hydroxybutyrate (BHB) is** a measure of energy status in the cow, which affects the health and growth of the calf during the critical transition period.

vii. **Non-esterified Fatty Acids (NEFA)** are indicative of energy balance and fat mobilization in the cow, which are critical for the health and metabolic state of the calf.

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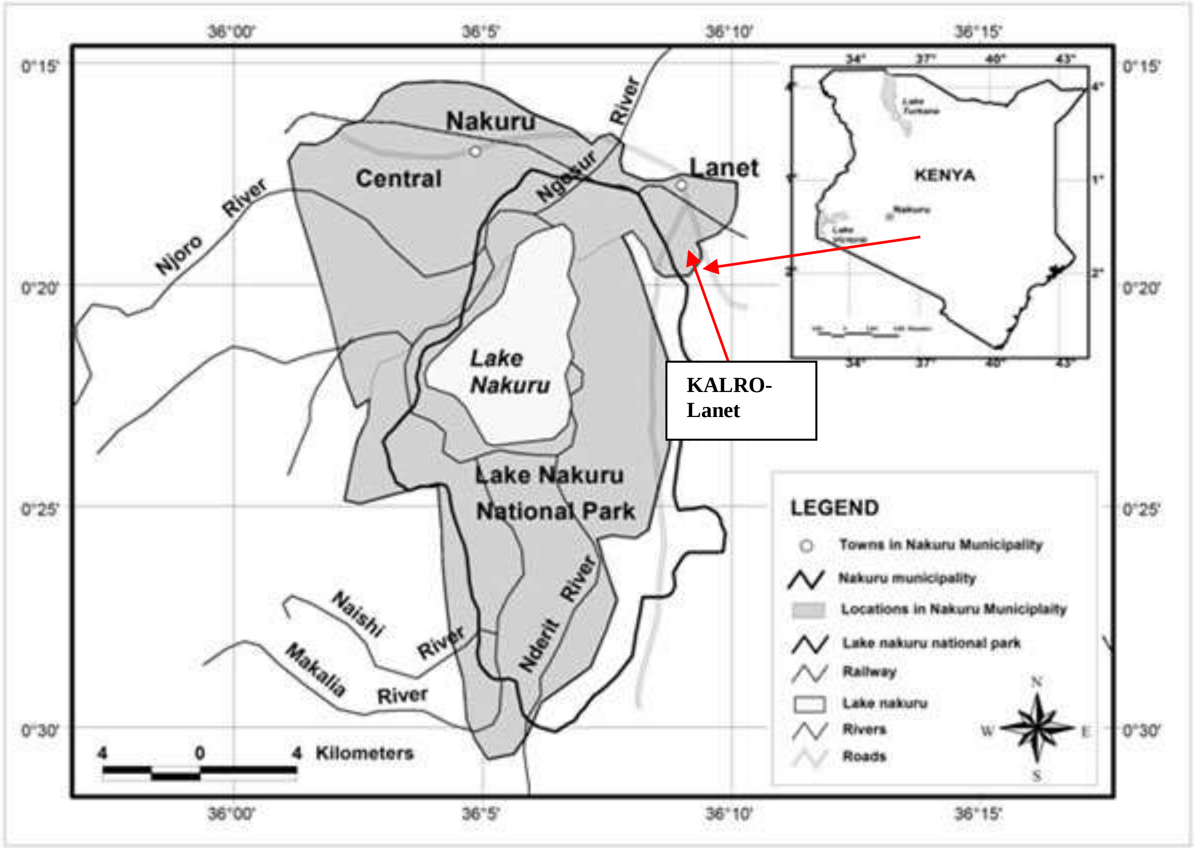
APPENDICES

APPENDIX I: Map of Nakuru County, Kenya

Map showing the location of KALRO-Lanet farm

(https://assembly.nakuru.go.ke/web/about-assembly/nakuru-county-on-the-map/)



APPENDIX II: Statistical Model

(i) Hormones and milk production

$$Y_{ijklm} = \mu + R_i + \alpha_j + \beta_k + \alpha\beta_{jk} + \tau_l + \alpha\tau_{il} + \alpha\beta\tau_{ikl} + \Sigma_{ijkl}$$

Where;

Y_{ijklm} = Response variable

μ = overall mean

R_i = Effect due to the ith replicate

α_j = Effect due to the jth supplementation

β_k = effect due to the kth days

$\alpha\beta_{jk}$ = Interactive effect due to the jth supplementation and the kth days

τ_l = Effect due to the lth parity

$\alpha\tau_{il}$ = Interactive effect due to the kth supplementation in the lth parity

$\alpha\beta\tau_{ikl}$ = Interactive effect due to the jth supplementation with the kth days in the lth parity

Σ_{ijkl} = Random experimental error

(ii) Pregnancy characteristics

$$Y_{ijkl} = \mu + R_i + \alpha_j + \beta_k + \alpha\beta_{jk} + \tau_l + \alpha\tau_{il} + \alpha\beta\tau_{ikl} + \Sigma_{ijkl}$$

Where;

Y_{ijkl} = Response variable

μ = overall mean

R_i = Effect due to the ith replicate

α_j = Effect due to the jth supplementation

β_k = effect due to the kth parity

$\alpha\beta_{jk}$ = Interactive effect due to the jth supplementation and the kth parity

Σ_{ijkl} = Random experimental error

APPENDIX III: Correlations of Feed Components

CORRELATIONS	DM	ASH	Fat	CP	CF	NFE	NDF	ADF	Methionine	Lysine	IVDMD	ME_Kcal_Kg
DM	1.000	0.943	-0.681	0.319	-0.036	-0.413	0.003	0.111	-0.167	0.261	-0.941	-0.263
ASH	0.943	1.000	-0.455	0.582	-0.230	-0.325	-0.272	-0.161	0.126	0.543	-0.992	0.006
Fat	-0.681	-0.455	1.000	0.297	-0.074	0.093	-0.373	-0.415	0.556	0.431	0.493	0.524
CP	0.319	0.582	0.297	1.000	-0.736	0.242	-0.902	-0.848	0.865	0.976	-0.520	0.774
CF	-0.034	-0.230	-0.074	-0.736	1.000	-0.812	0.921	0.928	-0.847	-0.603	0.187	-0.879
NFE	-0.413	-0.325	0.093	0.242	-0.812	1.000	-0.617	-0.688	0.590	0.099	0.349	0.713
NDF	0.003	-0.272	-0.373	-0.902	0.921	-0.617	1.000	0.992	-0.978	-0.841	0.217	-0.956
ADF	0.111	-0.161	-0.415	-0.848	0.928	-0.688	0.992	1.000	-0.983	-0.785	0.108	-0.979
Methionine	-0.167	0.126	0.557	0.865	-0.847	0.590	-0.978	-0.983	1.000	0.839	-0.068	0.979
Lysine	0.261	0.543	0.431	0.976	-0.603	0.099	-0.841	-0.785	0.839	1.000	-0.484	0.731
IVDMD	-0.941	-0.992	0.493	-0.520	0.187	0.349	0.217	0.108	-0.068	-0.484	1.000	0.047
ME_Kcal_Kg	-0.263	0.006	0.524	0.774	-0.879	0.713	-0.656	-0.979	0.979	0.731	0.047	1.000

DM, dry matter, CP, crude protein   CF, crude fibre   NFE,   nitogen -free extract, NDF, neutral detergent fibre,
ADA, acid detergent fibre IVDMD, invitro dry matter digestibility ME, Metabolizable energy

APPENDIX IV Pregnancy Period, BCS at Calving, Calf Birth Weight and Days to Visible Heat.

Source of variation	df	Gestation period	BCS at calving	Calf birth weight	Days to visible heat
Supplementation	1	440.83^{***}	2.49^{***}	208.3^{ns}	2033.63^{***}
Parity	3	468.11^{***}	6.95^{***}	11.55^{ns}	423.75^{***}
Supplementation*parity	3	7.56^{ns}	0.37^{*}	1.94^{ns}	137.81^{***}
Error	52	7.15	0.10	5.61	9.32
Total	59				
CV (%)		0.95	11.5	8.6	5.6
R^2		0.83	0.82	0.17	0.88

*, **, *** - significant at p = 0.05, 0.01 and 0.001 respectively

APPENDIX V: Correlation between Cow and Calf Characteristics

	Pregnancy period	BCS at calving	Calf Weight	Days to visible heat	P4 at estrus
Pregnancy period	1	0.382^{*}	-0.043^{ns}	-0.082^{ns}	-0.09^{ns}
BCS at calving		1	-0.197^{ns}	-0.645^{**}	-0.197^{ns}
Calf Weight			1	0.501^{**}	0.086^{ns}
Days to visible heat				1	0.137^{ns}
P4 at estrus					1

*, ** - significant at p =0.01 and 0.001 respectively; ns- not significant

APPENDIX VI: ANOVA Mean Squares for Milk Production

Source of variation	**df**	**Milk production**
Supplementation	1	763.65***
Days to postpartum heat	3	16.05***
Parity	3	28.69***
Supplementation*days	3	49.38***
Supplementation*parity	2	9.16*
Days *parity	9	7.49**
Supplementation*days*parity	6	2.43^{ns}
Error	212	2.80
Total	239	
CV (%)		19.4
R^2		0.66

*, **, *** - significant at p = 0.05, 0.01 and 0.001 respectively; ns- not significant

APPENDIX VII: Correlation of Milk Components

	fat	SNF	Density	AWA	Freez	Protein
Fat	1					
Pearson correlation		0.445	0.237	0.3.5	0.269	0.462
Sig (2tailed)		0.014*	0.208	0.101	0.167	0.010*
SNF						
Pearson correlation	0.445	1	0.764	0.263	0.963	0.978
Sig (2tailed)	0.014		0.000**	0.16	0.000**	0.000**
Density			1			
Pearson correlation	0.237	0.764		0.644	0.861	0.73
Sig (2tailed)	0.208	0.000**		0.737	0.000**	0.000**
AWA				1		
Pearson correlation	0.305	0.263	0.064		0.295	0.07
Sig (2tailed)	0.101	0.13	0.737		0.114	0.714
Freez						
Pearson correlation	0.259	0.963	0.851	0.285	1	0.92
Sig (2tailed)	0.167	0.000**	0.000**	0.114		0.000**
Protein						1
Pearson correlation	0.462	0.978	0.73	0.07	0.92	
Sig (2tailed)	0.010*	0.000**	0	0.714	0	

*, ** - significant at p = 0.05, and 0.01 respectively.

APPENDIX VIII: ANOVA Means Square Values for the BCS, Hormones and Metabolites

Source of variation	df	BCS	IGF-1	Insulin	NEFA	BHB	Leptin	Glucose	Protein	Urea	P4
Supplementation	1	31.79***	60.82***	38.86***	0.04***	0.009^{ns}	0.37***	9224.7***	2924.0***	0.86**	26.0***
Days	6	5.36***	78.19***	18.82***	0.16***	2.25***	0.37***	5073.1***	694.5***	86.14***	325.1***
Parity	3	0.19**	27.78***	1.65**	0.0007^{ns}	0.12*	0.0004^{ns}	1.02^{ns}	4718.9***	36.76***	0.1^{ns}
Supplementation*parity	2	0.33***	1.23*	0.47^{ns}	0.0004^{ns}	0.02**	0.0002^{ns}	2.0^{ns}	9.7^{ns}	0.43*	0.03^{ns}
Supplementation*days	6	1.30***	1.64***	5.66***	0.09***	0.03***	0.010***	380.4***	75.6***	10.86***	2.5***
Days*parity	18	0.09**	1.42***	1.10***	0.0008*	0.01**	0.0003^{ns}	8.54***	75.7***	0.62***	0.05^{ns}
Error	382	0.04	0.36	0.35	0.0004	0.003	0.0004	2.76	6.8	0.11	0.05
Total	419										
CV (%)		6.8	12.9	6.0	5.8	16.3	4.7	3.9	3.4	7.6	6.9
R^2		0.82	0.83	0.61	0.91	0.91	0.95	0.98	0.90	0.94	0.99

*, **, *** - significant at p = 0.05, 0.01 and 0.001 respectively; ns- not significant

APPENDIX IX: Correlation of the Cow Body Condition, Hormones and Metabolites Before Calving

	BCS	IGF-1	Insulin	NEFA	BHB	Leptin	Glucose	Protein	Urea	P4
BCS	1	0.38***	0.004^{ns}	0.502***	-0.055^{ns}	0.568***	0.468***	0.456***	0.327***	-0.072^{ns}
IGF-1		1	0.022^{ns}	0.531***	-0.096^{ns}	0.557***	0.443***	0.719***	0.505***	-0.068^{ns}
Insulin			1	-0.107^{ns}	-0.136^{ns}	0.195*	0.320***	0.117^{ns}	0.073^{ns}	0.359***
NEFA				1	0.046^{ns}	0.524***	0.227*	0.561***	0.318***	-0.628***
BHB					1	-0.103^{ns}	-0.123^{ns}	-0.033^{ns}	-0.065^{ns}	-0.116^{ns}
Leptin						1	0.862***	0.534***	0.289**	0.247**
Glucose							1	0.372***	0.169^{ns}	0.532***
Protein								1	0.833***	-0.140^{ns}
Urea									1	-0.090^{ns}
P4										1

*, **, *** - significant at $p = 0.05$, 0.01 and 0.001 respectively; ns- not significant

APPENDIX X: Correlation of the Cow Body Condition, Hormone Levels and Metabolite Concentration at term

	BCS	**IGF-1**	**Insulin**	**NEFA**	**BHB**	**Leptin**	**Glucose**	**Protein**	**Urea**	**P4**
BCS	1	0.055^{ns}	0.511***	0.348**	-0.089^{ns}	0.358**	0.431***	-0.152^{ns}	-0.533***	-0.339**
IGF-1		1	0.199^{ns}	0.256*	-0.345**	0.165^{ns}	0.246^{ns}	0.637***	0.245ns	-0.078^{ns}
Insulin			1	0.665***	-0.537***	0.715***	0.861***	0.172^{ns}	-0.541***	-0.697***
NEFA				1	-0.569***	0.624***	0.733***	0.204^{ns}	-0.424***	-0.638***
BHB					1	-0.437***	-0.621***	-0.445***	0.162^{ns}	0.501***
Leptin						1	0.762***	0.102^{ns}	-0.452***	-0.659***
Glucose							1	0.251^{ns}	-0.537***	-0.759***
Protein								1	0.594***	-0.185^{ns}
Urea									1	0.406**
P4										1

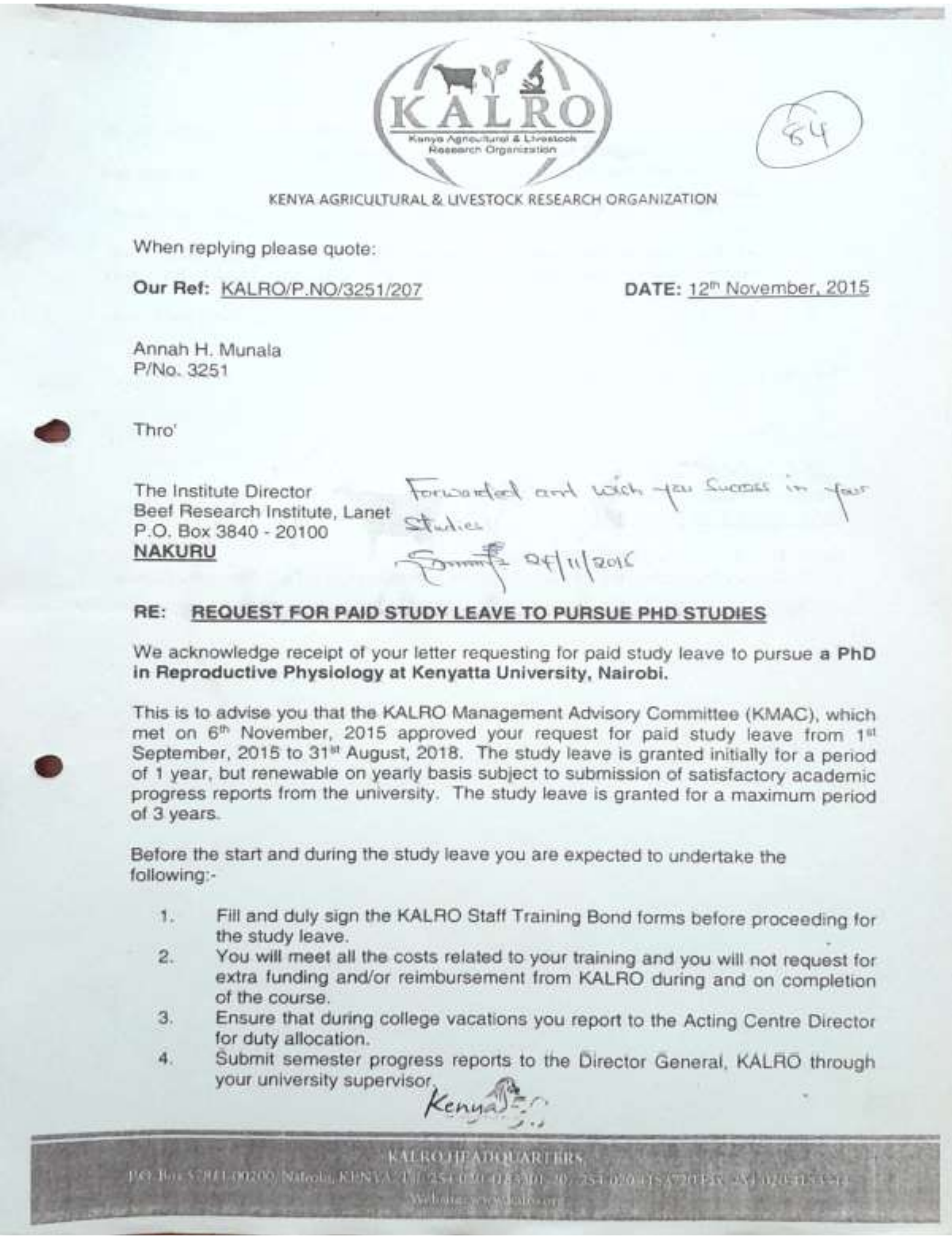
*, **, *** - significant at $p = 0.05$, 0.01 and 0.001 respectively; ns- not significant

APPENDIX. XI: Correlation of the Cow body Condition, Hormones and Metabolites and Milk Production Postpartum

	BCS	IGF-1	Insulin	NEFA	BHB	Leptin	Glucose	Protein	Urea	P4	Milk
BCS	1	0.273***	0.131*	-0.217***	0.221***	0.554***	0.448***	0.324***	0.016^{ns}	-0.132*	0.566***
IGF-1		1	0.289***	-0.722***	-0.490***	0.169**	0.738***	0.484***	0.272***	0.672***	0.449***
Insulin			1	-0.732***	-0.251***	0.159*	0.435***	0.053^{ns}	-0.119^{ns}	0.116^{ns}	0.268***
NEFA				1	0.612***	-0.143*	-0.786***	-0.209**	0.118^{ns}	-0.689***	-0.306***
BHB					1	0.158*	-0.456***	-0.011^{ns}	0.066^{ns}	-0.679***	0.789^{ns}
Leptin						1	0.365***	0.204**	-0.078^{ns}	0.072^{ns}	0.400***
Glucose							1	0.323***	0.094^{ns}	0.634***	0.541***
Protein								1	0.649***	0.153*	0.486***
Urea									1	0.134*	0.181**
P4										1	0.096^{ns}
Milk											1

*, **, *** - significant at p = 0.05, 0.01 and 0.001 respectively; ns- not significant

APPENDIX XII: Study Leave Approval



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KENYA AGRICULTURAL & LIVESTOCK RESEARCH ORGANIZATION

When replying please quote:

Our Ref: KALRO/P.NO/3251/207 **DATE:** 12th November, 2015

Annah H. Munala
P/No. 3251

Thro'

The Institute Director
Beef Research Institute, Lanet
P.O. Box 3840 - 20100
NAKURU

Forwarded and wish you success in your studies
24/11/2015

RE: REQUEST FOR PAID STUDY LEAVE TO PURSUE PHD STUDIES

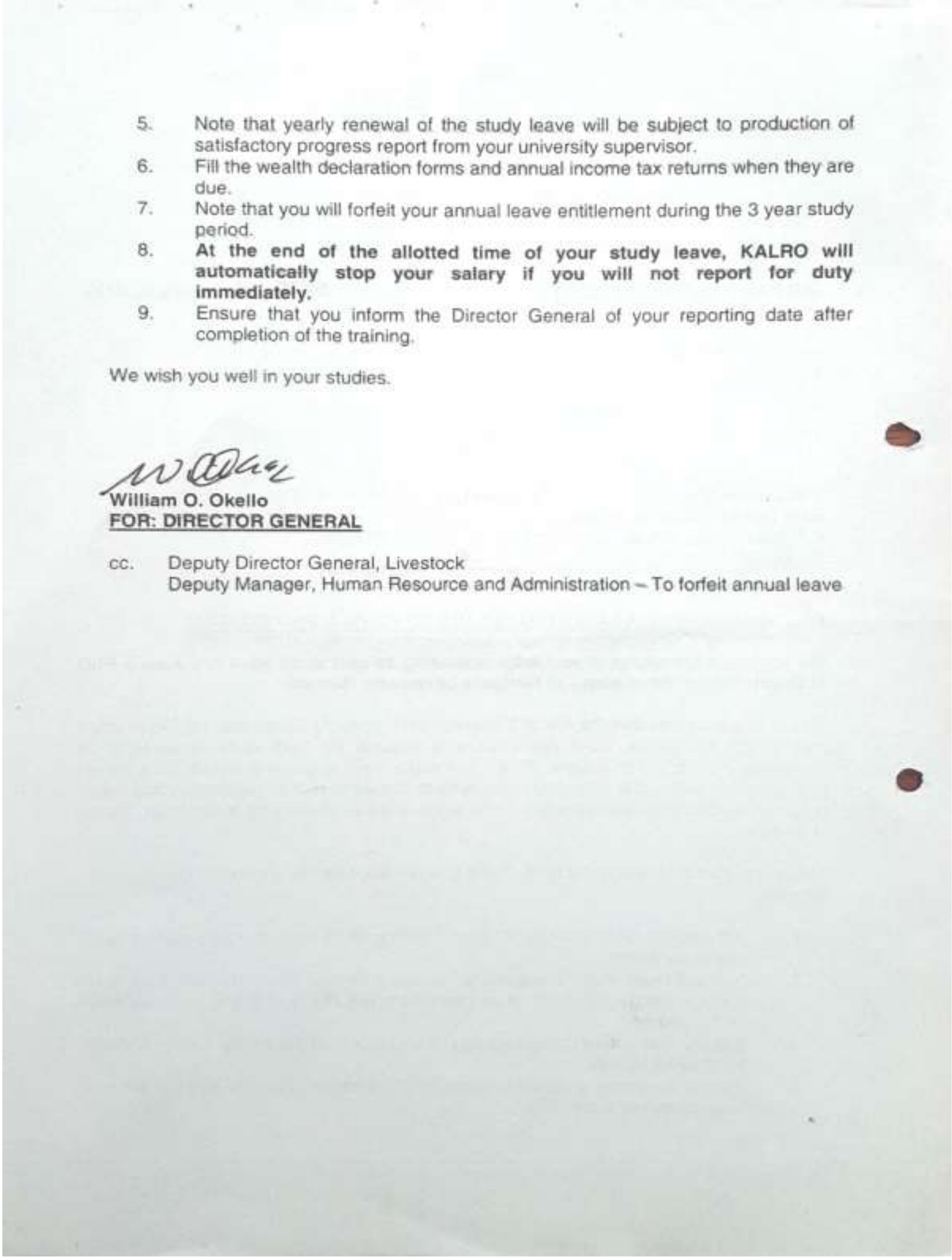
We acknowledge receipt of your letter requesting for paid study leave to pursue **a PhD in Reproductive Physiology at Kenyatta University, Nairobi.**

This is to advise you that the KALRO Management Advisory Committee (KMAC), which met on 6th November, 2015 approved your request for paid study leave from 1st September, 2015 to 31st August, 2018. The study leave is granted initially for a period of 1 year, but renewable on yearly basis subject to submission of satisfactory academic progress reports from the university. The study leave is granted for a maximum period of 3 years.

Before the start and during the study leave you are expected to undertake the following:-

1. Fill and duly sign the KALRO Staff Training Bond forms before proceeding for the study leave.
2. You will meet all the costs related to your training and you will not request for extra funding and/or reimbursement from KALRO during and on completion of the course.
3. Ensure that during college vacations you report to the Acting Centre Director for duty allocation.
4. Submit semester progress reports to the Director General, KALRO through your university supervisor.

Kenya



5. Note that yearly renewal of the study leave will be subject to production of satisfactory progress report from your university supervisor.
6. Fill the wealth declaration forms and annual income tax returns when they are due.
7. Note that you will forfeit your annual leave entitlement during the 3 year study period.
8. **At the end of the allotted time of your study leave, KALRO will automatically stop your salary if you will not report for duty immediately.**
9. Ensure that you inform the Director General of your reporting date after completion of the training.

We wish you well in your studies.

William O. Okello
FOR: DIRECTOR GENERAL

cc. Deputy Director General, Livestock
Deputy Manager, Human Resource and Administration – To forfeit annual leave

APPENDIX XIII: Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR
SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 997390

Date of Issue: 30/July/2020

RESEARCH LICENSE

This is to Certify that Mr.. ANNAH INDETIE BOKA of Kenyatta University, has been licensed to conduct research in Nakuru on the topic: METABOLITES AND HORMONES AS INDICATORS OF POSTPARTUM REPRODUCTIVE EFFEICIENCY OF PASTURE BASED FRIESIAN DAIRY COWS IN KARI-LANET, KENYA for the period ending : 30/July/2021.

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APPENDIX XIV : Summary of Publications and Conference Papers

i. **Hoka, AI, Gicheru, M., Otieno, S (2023)**. Effect of total mixed ration supplementation of transition cows on reproductive indices of Friesian dairy cows. Animal Production society of Kenya. Annual scientific symposium, 24th-27th October, Best western hotel Kisumu.

ii. **Hoka, AI, Gicheru, M., Otieno, S (2023).** Indicators of postpartum ovarian cyclicity among Friesian cows. 1st KALRO scientific conference and exhibition. 27th-31st March, KALRO-headquarters, Nairobi.

iii. **Hoka, AI, Gicheru M,, Otieno S (2022).** Postpartum reproductive performance of dairy cows fed on mutant *Brachiaria* and *Dolichos lablab* feed blocks (TMR). Animal Production society of Kenya. Annual scientific symposium, 27th-28th October, Bamburi beach hotel Mombasa.

iv. **Hoka, AI, Gicheru, M, Otieno, S (2019).** Effect of cow parity and calf characteristics on milk production and reproduction of Friesian dairy cows. *J. Nat. Sci. Res*. Vol.9, No.10. DOI: 10.7176/JNSR

v. **Hoka, AI, Gicheru M,, Otieno, S. Korir, H. (2019).** Effect of gamma irradiation of local *Brachiaria* ruziziensis (germain & evrard) seeds on agronomic performance and yield. J. *Plant Breed. Genet*. 07 (01), 09-17. DOI: 10.33687/pbg.007.01.2836

APPENDIX.XV: Experimental Illustrations

Milking Friesian Dairy Cow

Gamma Counter, Gloves, Milk, and Blood Samples.