SERUM HORMONE LEVELS AND MILK YIELD AMONG FRIESIAN CATTLE AT DIFFERENT PARITIES – THIRTY DAYS POST - PARTURIENT

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DECLARATION

Declaration by the Candidate

This thesis is my original work and has not been presented for any academic work in any institution and shall not be reproduced in part or full, or in any format without prior written permission from the author and/or the University of Eldoret.

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DEDICATION

This thesis is dedicated to my family whose love and support has been my greatest source of inspiration. To my beloved children, Kevin Murgor, Allan Murgor and Ian Murgor for bringing joy and purpose to life and enlightening me on the meaning of perseverance.

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ABSTRACT

The study examined the interactions between feeds, parity, milk-yield and hormonal profile of Friesian cows during the 30- day into lactation. The study objective included: to investigate the impact of parity on weight change and milk yield of post - parturient Friesian Cows; to examine the effect of serum hormone levels on feed intake of post – parturient Friesian Cows; and to determine the effect of parity on serum hormone levels of post – parturient Friesian Cows in Uasin Gishu County, Kenya. Three farms namely Elfam, Elso and Betan in Uasin Gishu County were purposively selected with nine pedigree Friesian cows in three parities (2, 3 & 4) whose milk production averaged 20 litres/day. The study utilized a randomized complete block design and adopted a natural on–field experiment while adopting their nutritional diets. Each cow was fed daily on 40 kg of forage, mineral licks and water *ad libitum*. The daily milk yield was recorded. Feed intake was determined daily and blood samples collected at parturition and on 7-day interval and tested for cortisol, prolactin, oestrogen, and IGF-α1 levels. Resulting data were entered into Microsoft Excel application and analysed using Genstat 14 to generate descriptive statistics and ANOVA, and results presented in tabular and graphical formats. All the lactating cows irrespective of parity progressively lost weight daily with cows in parity 2 having the highest average daily weight loss. Milk yield significantly differed with cows in parity 2 having the lowest daily milk yield at 21.66 Kgs/day while cows in parity 4 had the highest daily milk yield at 24.99 Kgs/day. All the cows had on average low feed intake at parturition and progressively increased the feed intake to 35 Kgs/day. Serum cortisol levels gradually declined while serum IGF-α1, prolactin and oestrogen levels progressively rose. Parity had a significant effect on weight change ($p < 0.05$) and milk yield ($F = 8.27$, $p < 0.05$). Serum hormone levels; prolactin ($r = 0.760$, $p < 0.05$), Oestrogen ($r = 0.785$, $p < 0.05$) and IGF-α1 ($r = 0.692$, $p < 0.05$) significantly and positively correlated with feed intake which negatively correlated with serum cortisol ($r = -0.613$, $p < 0.05$). Only serum cortisol levels differed significantly with parity levels. The study concluded that a correlational and causal linkages exist between parity, serum hormonal profile, feed intake and milk yield of post-parturient Friesian cows in Uasin Gishu County. The study recommends that farmers improve herd management techniques for managing and sustaining the cow's physiological state (weight loss and milk yield) during the immediate post – parturient period. Further, there is need for the farmers to intensify the feed intake through improve feed palatability to support and maintain the growth profiles of the dairy cows and that farmers improve the nutritional diets by proper feed formulation to accommodate the changing physiological needs of the post – parturient lactating cow.

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ABBREVIATIONS, ACRONYMS AND SYMBOLS

ADF:	Acid Detergent Fibre
AOAC:	Association of Official Analytical Collaboration
BCS:	Body Condition Score
βHBA:	βeta – Hydroxybutyrate
CDVS:	County Director of Veterinary Services
CP:	Crude Protein
DMI:	Dry matter intake
EAA:	Essential Amino Acid
ELISA:	Enzyme-linked immunosorbent assay
ELFA:	Enzyme-linked fluorescence assay
FCM:	Fat – Corrected Milk yield
GHs:	Growth Hormone
HCC:	Hair Cortisol Concentration
HPA:	Hypothalamic Pituitary - Adrenal
IGF-1:	Insulin-like growth factor
IGFBP:	IGF-binding proteins
KALRO:	Kenya Agricultural Livestock Research Organization
LCD:	Liquid Crystal Display
LoD:	The lower limit of detection
N:	Nitrogen
ME:	Metabolizable Energy
MP:	Metabolizable Protein
MS Excel:	Microsoft Excel software
NDF:	Natural Detergent Fibre

NEB:	Negative energy balance
NIR:	Near InfraRed
NEFA:	Non – Esterified Fatty Acid
RIA:	Radio-immunoassay
PUFA:	Polyunsaturated fatty acid
RUP:	Rumen Undegradable Protein
SPR:	Solid phase receptacle
SARA:	Subacute ruminal acidosis
TAG:	Triacylglycerol

OPERATIONAL DEFINITION OF TERMS

Hormonal Fluctuations: Changes in the levels of specific serum hormones, including cortisol, insulin-like growth factor (IGF-1), prolactin and oestrogen as measured in blood samples collected from lactating Friesian cattle during the first thirty days post-parturient.

Immediate Post-parturient Period: Specifically refers to the thirty-day time frame following calving, during which the hormonal fluctuations and physiological changes in dairy cattle are observed and analysed.

Lactating Friesian Cattle: Female Friesian cattle that have recently calved down and are actively producing milk during the study period. This includes cows within the first thirty days postpartum.

Milk Production: Quantitative measurement of the amount of milk produced by lactating Friesian cattle, recorded at regular intervals during the first thirty days post-parturient. Measurements will be taken using established techniques, such as milking machines.

Feed Intake: Quantity of feed consumed by lactating Friesian cattle within a specified period, typically measured in kilograms. Feed intake will be assessed through observation and recording of the quantity of feed provided and residual feed in designated feeding areas.

Selected Farms in Uasin Gishu County: Refers to a purposively chosen subset of dairy farms located within the geographical boundaries of Uasin Gishu County, Kenya. The selection criteria was herd size, management practices, and owner’s consent to participate in the study.

Data Collection: Involves the systematic gathering of information through blood sample, milk production measurements, and observations on feed intake from the selected lactating Friesian cattle during the study period.

Data Analysis: The systematic examination of data using statistical techniques to identify patterns, associations, and trends related to hormonal fluctuations, milk production, and feed intake in lactating Friesian cattle during the first thirty days post-parturient.

Cortisol Check-1 test: This is a rapid quantitative assay for the detection of cortisol in the blood.

Prolactin-Check-1 test: This is also a rapid quantitative assay for the detection of prolactin in the blood.

SPR: The solid phase receptacle is the solid phase as well as the pipetting device for the assay.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The dairy industry serves as the foundation of global agriculture and provides essential nutrients as well as sustaining farmers economically. The Friesian cattle breed is predominantly known for its high milk production potential; however, optimal milk production requires a deeper understanding of hormonal dynamics during the post-parturient period (Djokovic et al., 2015). The dairy industry heavily relies on the efficient and consistent high milk production of lactating cows. Any suboptimal growth performance during the post-parturient period results in decreased milk yield, and prolonged calving intervals, all of which impact the economic viability of the dairy operation. Therefore, optimizing the nutritional strategy during the immediate post-parturient is imperative for milk productivity and feed intake (Muro et al., 2023).

Dietary formulation for cows in the immediate post-parturient period is a critical aspect of herd management, directly influencing transition success and subsequent reproduction (Cardoso et al., 2020). The immediate thirty - day period after parturition is characterized by elevated nutritional demands for the lactation as diets in the lactation stages is contingent on the dietary starch levels where the lower glucose availability significantly modifies the postprandial circulatory patterns of hormones (Piccioli-Cappelli et al., 2014). Therefore, identifying and comprehending the hormonal changes that influence dairy cow milk production and feed intake during early lactation is paramount (Djokovic et al., 2017).

The studies on hormone dynamics in post-parturient dairy cattle have gained prominence emphasizing the significance of hormonal balance for reproductive efficiency and milk production. Prolactin impacts the development of mammary gland and initiation of lactation, cortisol impact on stress response and metabolism, while oestrogen is involved in reproductive functions, and Insulin-like Growth (IGF-1) contribute to growth and milk synthesis (Gaiani et al., 2014). Stressful conditions during the peri-parturient period, such as changes in diet, environment interactions, can lead to elevated cortisol levels (Wu et al., 2019)..

Cortisol hormone regulates metabolic processes and mobilizes energy reserves to support lactation while prolactin and somatotropin regulate milk synthesis, mammary gland development, and other associated metabolic processes (Herosimczyk et al., 2013). For instance, a study on dairy farms in Italy, identified the linkages between cortisol levels and decreased milk production and thus demonstrated the need for herd management strategies (Sgorlon, 2015). On their part, Boer et al., (2013) researched on Friesian dairy cattle and underscored the influence of oestrogen levels in regulating the oestrous cycle and contributes to efficient reproductive performance in dairy cattle.

The wide-ranging metabolic changes during peri-parturition to early lactation period presents an immediate challenge to high-productive dairy cows because elevated nutrients and energy supply are required for lactation (Sammad et al., 2022). The transition to lactation comes with an elevated surge in the energy, mineral and protein requirements. A successful transition into lactation involves metabolic changes to multiple organs in order to expedite the availability of these nutrients to the mammary glands, often at the expense of other important tissues and organs (Roche et al., 2013).

Dairy cows in the peri-parturient period tend to decrease the dry matter intake (DMI) by between 10% to 30% (Drackley & Cardoso, 2014) a fact that is attributable to the foetal growth which reduces the abdominal cavity thus restricting the ruminal space (Chapinal et al., 2012). The quantity of DMI influences the digestibility of forage fibre and in turn this affects distension of the reticulo-rumen more than the specific forage to concentrate ratios (Piccioli-Cappelli et al., 2014). The dramatic increase in nutrient requirements following parturition is related to the demands of the mammary gland for the production of milk, milk fat, lactose and protein (Roche et al., 2013).

The transition to early lactation is coupled with metabolic challenge and the haemato-chemical profiles of the high-yielding dairy cow presents an opportunity for examining the nutritional health status during this transition period (Piccioli-Cappelli et al., 2014). The low dry matter intake (DMI) is concomitantly with low energy levels during the immediate post-parturient (Gross et al., 2011) resulting in an energy deficit during the transition to lactation (Djokovic et al., 2014). Further, the higher energy diets tend to elevate the blood sugar concentrations in both the basal and mean post-prandial states (Piccioli-Cappelli et al., 2014).

The key element in the peri-parturient homeorhetic mechanism is the regulation of energy intake and the post-absorptive nutrient partitioning (Roche et al., 2013). However, the high sugar diets for a lactating cow does not resolve the state of negative energy balance during the immediate post-parturient period because of the lactation requirements as the lactating cow in the initial post-parturient may redirect the nutrient to lactation at the expense of a reproductive function (Toledo-Alvarado et al., 2017).

Despite the consumption of the high energy diets, the concentration of non-esterified fatty acids (NEFA) is always higher (Piccioli-Cappelli et al., 2014).

Negative Energy Balance (NEB) may impair reproductive function (Kitilit et al., 2016; Drackley & Cardoso, 2014) as insufficient DMI in the immediate post-parturient period leads to a nutrient and energy deficiency resulting in NEB. This Negative energy balance (NEB) is indicated by the metabolic differences between energy requirements and energy intake for a substantial physiological requirements of a lactating cow in the immediate post-parturient period (Jorritsma et al., 2013). Furthermore, excessive energy feed resources may result in higher NEFA and β – Hydroxybutyrate (βHBA) concentrations in blood and more Triacylglycerol (TAG) in the liver in the post–parturient period (Drackley & Cardoso, 2014).

The insufficient nutrition coupled with stress-related factors in the post–parturient period result in decreased voluntary DMI and the concomitant elevated Triacylglycerols (TAG) mobilization and increased NEFA concentrations after parturition (Drackley & Cardoso, 2014). NEB is linked to the mobilization of body reserves, from the adipose tissue and skeletal muscle supplements the nutrient supply to the mammary gland for the synthesis of milk (Gruber et al., 2014) occurs because homeorhetic prioritization require the physiological changes in the nutrient allocation to the mammary gland (Gross et al., 2011).

The characteristic changes in lipid metabolism leads to concurrent lipogenesis and lipolysis which subsequently supports synthesis of the lipid reserves which are utilized at parturition and the initiation of lactation. Furthermore, the hydrolysis of lipoprotein to generate triglycerides is influenced by the parturiency and post–parturiency period (Ayoub & Allam, 2015). Thus, when synthesis exceeds utilization, triacylglycerol (TAG) accumulates in the liver (Litherland et al., 2011) is indicated by the β-Hydroxybutyrate (βHBA) and the non-esterified Fatty Acids (NEFA) in the blood serum (Cincović et al., 2012).

Low-energy diets result in significantly higher basal concentrations of non-esterified fatty acids (NEFAs) in early lactation periods with high NEFA concentrations being indicating of a NEB (Piccioli-Cappelli et al., 2014). NEB coupled with the metabolic changes concomitantly affects milk production and health status of a lactating dairy cow as the energy requirements exceed the available energy from feed intake (Jorritsma et al., 2013). Thus, a metabolically - stressed cow in early lactation period simultaneously experiences NEB with elevated likelihood of health disorders (Gross et al., 2011). Variations in feed management is a primal factor in NEB state more than the difference in milk production. Thus, highly productive cows have a pronounced NEB nadir (Jorritsma et al., 2013). Furthermore, the individual cow differences in nutrient and energy allocation mechanisms may translate to different NEB state (Jorritsma et al., 2013).

The body weight loss as a result of the NEB is linked to lipid mobilization from the adipose tissue to supplement energy deficiency (Gross et al., 2011) and varies with the specific hormone concentration. For instance, the release of insulin–growth factors (IGF) and thyroid hormones is an adaptative mechanism for a lactating cow as these hormones facilitate milk production as well as sustaining metabolic activity in lactating cow(Jorritsma et al., 2013) as an endocrine-metabolic system (Piccioli-Cappelli et al., 2014). Low-energy diets tend to increases the natural bovine somatotropin to insulin ratio which rapidly mobilize lipid reserves resulting in a higher concentration of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (βHBA) contributing to the higher butter fat content in milk (Piccioli-Cappelli et al., 2014).

This metabolic activity results in higher body weight loss during the early lactation period with commensurate slowed body weight recovery in lactating cows fed on low-starch diets. Body weight loss and lower body condition score (BCS) in lactating cows is attributable to both nutritional and environmental factors (Drackley & Cardoso, 2014). The post-parturient NEB is associated negatively with reproductive performance such that the degree of NEB nadir and the rate of change in NEB strongly indicates an impaired reproductive cycle (Ingvartsen & Moyes, 2013).

The competition for nutrients between early and late lactation periods tends to delay the animal reproductive function (Drackley & Cardoso, 2014) as the lactating dairy cows are vulnerable to milk production disorders and diseases in both the pre–parturient and post–parturient periods (Ingvartsen & Moyes, 2013) as cows may develop serious metabolic and physiological changes which subsequently affect their health and nutritional status (Tanaka et al., 2011). During the post–parturient period, series of hormonal changes coordinate the essential physiological adjustments (Khani & Alijani, 2013) of calving, feed intake and milk production (Djokovic et al., 2015).

It is this intricate hormonal dynamics that is essential to optimizing feed intake and lactational performance of a lactating cow during the transition period which last approximately 30 days after parturition. While the dry period or close-up period's dietary formulation is well-established to support ruminal adaptation to higher energy diets after parturition, the immediate post-parturient period poses unique challenges(Cardoso et al., 2020). Failure to address these challenges adequately may compromise early lactation productivity, affecting feed intake and milk yield (Sammad et al., 2022), thus adequate nutrition, proper management practices, and monitoring of hormone levels are essential to ensuring a smooth transition into the post-parturiency and mitigate the likelihood of milk fever (Wu et al., 2019).

Past studies have explored the hormonal dynamics during the post-parturient period in dairy cows, with a focus on specific hormones like insulin, IGF-1, cortisol, and thyroxine (Wankhade et al., 2017; Anderson et al., 2019). This energy imbalance leads to metabolic disorders such as ketosis, which adversely affect feed intake and milk production. The resumption of fertility and ovarian cyclicity is linked to the hormone profile during this post-parturient period and therefore and understanding the hormone influencing reproductive health is essential for achieving timely conception and calving intervals.

However, there is a need for more comprehensive investigations that elucidate the interplay of multiple hormones and their impact on various aspects of the cow including milk yield and reproductive success, (Kurpinska et al., 2016). While some research has contributed to our understanding of individual hormonal changes during the post-parturient period, there remains a knowledge gap regarding the comprehensive hormonal profile (Gaiani et al., 2014). This study sought to address this gap by examining a broader spectrum of hormonal changes and their implications for dairy cow milk production and feed intake 30 days post-parturient.

1.2 Statement of the Problem

The abrupt physiological changes occurring during pre- and post-parturient period pose a substantial risk to the overall well-being, milk yield, and reproductive functions of cows (Gaiani et al., 2014). This immediate post-parturient period in dairy cows is characterized by significant hormonal and metabolic changes. Despite the awareness, there is a gap in understanding the comprehensive hormonal dynamics during this critical period and their specific implications for the cow's feed intake and milk production during the early days of post-parturiency. The dearth of comprehensive insights into the nuanced hormonal fluctuations during the early post-parturient period represents a significant challenge for effective dairy cow management (Webster, 2020). Addressing the substantial gap in understanding of the comprehensive hormonal changes in the first 30-day post-parturiency is imperative for advancing the efficiency, profitability, and well-being of dairy operations. Thus, the study examined the physiological changes that include, the serum hormonal changes, and productive and reproductive performance of Friesian cows at different parities during the first thirty – day post – parturient period in Uasin Gishu county, Kenya.

1.3 Justification of the Study

This study helps in understanding the hormonal changes influencing cow feed intake and milk production immediately post-parturient which is crucial for optimizing dairy cow management. The study findings will enable farmers to develop informed and targeted nutritional strategies for sustaining the requirements of the animals and maximizing milk production for increasing profitability. By understanding how hormonal dynamics are pivotal in determining feed intake and milk synthesis, the study addresses this knowledge gap in order to optimize the efficiency and profitability of dairying operations.

For breeders and veterinary surgeons, the study findings are useful in appreciating the hormonal dynamics during the post-parturient period in fostering the development targeted herd management strategies. Such strategies can encompass nutritional interventions, herd management strategies and stress mitigation protocols tailored to support the specific states of dairy cows in different stages. Implementing evidence-based herd management practices can improve feed intake, the reproductive efficiency and milk production. With a deeper understanding of the hormonal changes during this phase, farmers can implement strategies for enhancing milk production and mitigate metabolic disorders of dairy cattle.

This study adds to the existing literature in the field of dairy science and animal husbandry. It contributes valuable insights into the hormonal fluctuations of post-parturient cattle, shedding light on physiological processes that have practical applications in the dairy industry. By addressing the identified knowledge gap, the results of this research will directly benefit the dairy industry, contribute to scientific advancement, and ultimately lead to more economically viable and sustainable dairy operations. The existing knowledge regarding these changes remains limited, thus underscore the significance of bridging this gap not only for research but breeders and veterinary surgeons too.

1.4 Broad Objective

To determine the examine factors affecting cyclicity and physiological changes of a lactating Friesian cows in the immediate post-parturient period in Uasin Gishu County, Kenya.

1.5 Specific Objectives

1. To investigate the impact of parity on milk yield and weight change of post - parturient Friesian cows in Uasin Gishu County, Kenya.
2. To examine the effect of serum hormones (cortisol, prolactin, oestrogen, and IGF-α1) levels on feed intake of post – parturient Friesian cows in Uasin Gishu County, Kenya.
3. To determine the effect of parity on serum hormone (cortisol, prolactin, oestrogen, and IGF-α1) levels of post – parturient Friesian cows in Uasin Gishu County, Kenya.

1.6 Null Hypotheses

H_{O1} : Parity has no significant effect on milk yield of post - parturient Friesian cows in Uasin Gishu County, Kenya.

H_{O2} :Serum hormones (cortisol, prolactin, oestrogen, and IGF-α1) changes have no significant effect on feed intake of Post – parturient Friesian cows in Uasin Gishu County, Kenya.

H_{O3} : Parity has no significant effect on serum hormone levels (cortisol, prolactin, oestrogen, and IGF-α1) of post – parturient Friesian cows in Uasin Gishu County, Kenya

CHAPTER TWO

LITERATURE REVIEW

2.1 Dietary requirements for post–parturient lactating cows

The physiological changes during the post–parturient period largely affects the total serum protein levels more than the late gestation. The differences reflect the maternal requirements of proteins requirements for milk production and synthesis of immunoglobulins (Cincović et al., 2012). As a nutritional dietary requirement, lactating cows require higher DMI during early lactation to maintain higher milk yield (Piccioli-Cappelli et al., 2014). Whereas the demands for energy and calcium for lactation increase, the dry matter intake tends to decrease around the parturition period. This results in negative energy balance (NEB) with subsequent increased lipid mobilization and production of ketone bodies (Chapinal et al., 2012).

Lipid metabolism during peri-parturient and post-parturient is characterized by the concurrent lipolysis and lipogenesis where lipid reserves are subsequently utilized at parturition and the commencement of milk synthesis (Litherland et al., 2011). The increased lipolysis with concomitant decreased lipogenesis rapidly mobilizes body fat and re-esterifies fatty acids in adipose tissue, leads to a net release of glycerol and non-esterified fatty acids (NEFA) into the bloodstream (Roche et al., 2013).

Studies have also observed the influence of genetic factors on levels of body adiposity, where regulation of stored energy reserves and any variations from the optimal levels of adiposity, triggers the satiety and hunger regulators and reduces energy expenditure until the desired levels of body fat is restored. This metabolic activity points to a regulating effect of DMI to adipose tissue for maintaining basal adipose tissue (Melendez et al., 2019).

During the early lactation period, the energy demands for maintaining body tissues and milk production surpass the energy obtainable from dietary sources in cows (Reinhardt et al., 2011). As examined by Piccione et al., (2012), the metabolic changes on the selected biochemical blood parameters of Holstein Friesian dairy cows during the transition to post–parturient period indicate a statistically significant lower level of urea, triglycerides, NEFA and Calcium minerals in the immediate post – parturient period. However, following late lactation, the study observed a substantial elevated levels of protein, creatinine, cholesterol and phosphorus minerals (Nozad et al., 2014).

Thus, TAG production is influenced by the parturiency and post–parturiency period (Ayoub & Allam, 2015) as indicated by the βHBA and NEFA in the blood serum (Cincović et al., 2012). Increased blood NEFA concentration coupled with a decreased body fat mass in the immediate post-parturient period is consistent with the decline in several lipogenic enzymes in adipose tissue and correspond with lower enzymatic rates and milk synthesis (Roche et al., 2013).

In a study by Gross et al., (2011) the effect of NEB through feed restriction on the metabolic activity and lactational performance of lactating cows in the post – parturition period and observed that feed restriction resulted in a noticeable decline in the milk yield of -3.1 ± 1.1 Kg and that the cows under restricted feed encountered a greater NEB and reduction of dietary energy density. Usually, Non-Essential Fatty Acid (NEFA) concentrations tend to increase within a week of feed restriction and gradually decrease thereafter (Gross et al., 2011). The NEB in the post–parturient period arises from the commencement of milk synthesis; however, the duration and the depth of the NEB nadir highly correlated with DMI (Drackley & Cardoso, 2014).

Higher concentrate-to-forage ratio is associated with higher levels of energy and lower levels of fibre (Piccione et al., 2012) and any low-energy diets result in higher average daily concentrations of urea in plasma during lactation periods (Piccioli-Cappelli et al., 2014) as lactating dairy cow only converts 24% to 32% dietary nitrogen (N^2) to milk protein, while excreting the rest of the dietary N^2 in urine and faeces (Sánchez et al., 2023). Importantly, higher dietary crude protein (CP) intake tends to increases the CP digestibility due to metabolic faecal protein as increasing the dietary CP from 11% to 14% increases organic matter digestibility with concomitant change in the rumen microbial composition. (Balance & Gas, 2023).

An average of 25% of dietary N is captured in milk in the current dairy production systems, while the remaining N is excreted in faeces and urine with about 60% Nitrogen (N) losses occurring in post-absorptive mechanism (Apelo et al., 2014a). The CP chain of the ruminants require individual amino acids (AA) in specific amounts and therefore the N excretion in faeces and urine can be substantially reduced through dietary formulation practices that improve N efficiency. Consequently, adequate AA supply helps to ensure unwanted N is not consumed, absorbed and then excreted (Prestegaard-Wilson et al., 2021).

In terms of CP content, different studies give varied average CP content for a lactating cow with a lower figure of 13.5% (Apelo et al., 2014a), 15.1% (Broderick, 2003) and

15.6% (Tedeschi et al., 2015; Chen et al., 2011), 16.1% (Leonardi, Stevenson and Armentano, 2013) with a peak at about 16 to 16.5% (Chen et al., 2011)). However, a maximum of 17.0% CP is recommended in the early lactation period as this is the peak of the lactation curve (Chen et al., 2011), as a US study observed that dietary CP content >13.5 % in dry matter (DM) does not affect milk production in lactating cows(, Apelo et al., 2014a).

There is no further increase in milk production in terms of yield and/or milk components beyond 16.7% CP as the N is lost through undigested Amino Acids (AA), microbial nucleic acids, catabolism of absorbed AA and the endogenous losses in urine and faeces (Chen et al., 2011). Thus, the gross N efficiency can be increased to 30% or greater if the dietary CP of 15% or lower is supported by individual Essential AA(EAA) supplementation (Apelo et al., 2014a). Dietary protein content > 17.0% CP always ends up being excreted in a nitrogenous form in faeces, unabsorbed by the animal (Chen et al., 2011). Further, the remaining fraction of N is lost through urine (Prestegaard-Wilson et al., 2021) and it is estimated that 1.36 million tonnes of reactive N are released by US dairy industry (Apelo et al., 2014b).

It has empirically validated that lactating cow require higher EAA levels for the development of the mammary gland (Zhang et al., 2023), as the AA profile of the protein linearly translates into milk N (Nichols et al., 2022; Raggio et al., 2004). Therefore, higher dietary CP intake translates into higher AA absorption rates up to a certain levels where insufficient dietary energy intake cannot support the rumen microbial proteins synthesis (Raggio et al., 2004). Due to the reduced DMI, lactating cows in the immediate post-parturient period faced are nutritionally deficient in AA however, the ruminal microbial activity complements the synthesizing the EAA (Weekes et al., 2006).

This microbial transformation of nutrients in the gut largely defines the minimum basic AA requirements for a lactating cow (Prestegaard-Wilson et al., 2021) as deficiencies in the digestible AA limits the efficient protein synthesis in milk (Nichols et al., 2024). However, excess EAA supply to the mammary glands revert to general blood flow circulation through splanchnic catabolism which may equal or surpass the EAA requirements for milk protein synthesis (Apelo et al., 2014a). Thus, the relative ratios of rumen ammonia and post-absorptive AA catabolism to the quantities of excreted urea depends on N supply matching ruminal and post-absorptive requirements As a result, optimum CP levels, translate into minimal N wastages (Apelo et al., 2014a)..

Any dietary N that is not incorporated into milk synthesis is excreted in faeces or urine as the microbial protein digestibility is presumed to average 80%. Any rumen microbes in cattle only utilizes the required basal N and either recycles or excretes excess N (Fuller, 2012). These faecal which makes up 18% to 31% of faecal N includes endogenous N, undigested dietary N, and microbial N either as undigested proteins in the gut, shed off gut mucosa cells, and recycled urea in the hindgut (Røjen et al., 2012). Further, faecal N is derived from excess nutrient supply considering that there is constant intestinal digestibility of bacterial protein and Rumen Undegradable Protein (RUP) (Apelo et al., 2014a).

Consequently, dairy cows utilize adipose fat tissue as an energy source but, there is an upper limit to which the fatty acid oxidation occurs (Reinhardt et al., 2011). This intensified lipid mobilization for milk synthesis tend to increase the blood lipid concentration thereby predisposing the dairy cow to inflammatory-based diseases such as fatty liver, and ketosis (Ayoub & Allam, 2015). Furthermore, lactating cows requires mineral (phosphorus, calcium and magnesium) for pregnancy and milk synthesis (Cincović et al., 2012). In particular, calcium and phosphorus mineral requirement depends in the physiological status and the lactational potential of the cow (Cincović et al., 2012) as the levels of calcium and phosphorous minerals are expected to reduce at parturition due to the high demand for colostrum and milk synthesis (Ayoub & Allam, 2015).

Metabolic disturbances such as hyperketonemia and hypocalcemia are common in post-parturient dairy cows, leading to detrimental effects on health, milk yield, and fertility (Hubner et al., 2022). The presence of ketones in the milk, blood and urine indicates ketosis, which typically become clinically evident 10 days to 3 weeks after calving. The severity of hyperketonemia due to the increased βHBA concentration explains the increased incidence of clinical ketosis may which concurrently occur with hypoglycemia (Hubner et al., 2022). Thus, gluconeogenesis is hindered, leading to hypoglycaemia. The cow becomes more lethargic, further decreasing feed intake and milk production (Churakov et al., 2021).

Any non-nutritional stressors reduce DMI and predispose the dairy cattle to post-parturient health complications such as metritis or ketosis (Drackley & Cardoso, 2014). However, these post-parturient health complications may not be caused by low peri-parturient DMI as Drackley and Cardoso (2014) observed that cows restricted to 80% daily energy requirements have a lowered risk of ketosis or metritis. Other significant metabolic complication during the transition period includes milk fever, fatty liver, retained placenta, lameness left-displaced abomasum, and clinical mastitis (Ingvartsen & Moyes, 2013) which may threaten both animal health and welfare if these metabolic challenges exceed the coping mechanisms of the animal (Colditz & Hine, 2016).

2.2 Milk Production of post–parturient lactating cows

Milk yield and composition in dairy cattle fluctuates with changes in either protein or energy supplies. In practice, dietary protein and energy supplies vary depending on the feed but any increase in energy supply increases milk production linearly or curvilinearly depending on the lactational stage (Kitilit et al., 2016). Increasing dietary CP supply results in increased milk production (Brun-Lafleur et al., 2010), however, highly productive cows tend to have higher NEB which in turn leads to reproductive failure (Poncheki et al., 2015).

The lactating dairy cows rely to a greater extent on the mobilization of body reserves during the transition period, thus increasing energy supply in the peri-parturient led to increase in milk yield in the post-parturient period (Gruber et al., 2014). Diet supplementation through the use of concentrate increases the energy density of post-calving diets thereby improving milk production but this relationship is curvilinear. Beyond certain yield curve, the milk production reduces considerably to any additional quantities of concentrate consumed, (Gruber et al., 2014).

In a study of New Zealand dairy farms, Handcock et al., (2019) observed a positive relationship between body weight and milk production, as lactating cows with higher body weight tending to produce higher milk quantities up to three lactational cycles.

Moreover, in Brazil study on the influence of body weight on growth performance of lactating cows, Poncheki et al., (2015) observed statistical differences in body weight as cows with higher parities tending to have higher body weights than those with lower parities. However, the study noted that there was not statistically difference in body weight loss between parities (Poncheki et al., 2015).

In an experimental study, examining protein–energy balance versus milk yield, Brun-Lafleur et al., (2010) observed that milk yield responded to the dietary changes in high productive cow than in low productive cows. The study also noted that primiparous cows produce milk with higher protein content than multiparous cows. Moreover, a study linking dietary starch content and milk levels showed higher starch contents translate to higher milk yield in late lactation, while high protein diets tend to improve milk production but is negatively associated with reproductive performance (Piccioli-Cappelli et al., 2014). These studies show the adequacy of the dietary during the transition phase, however, quantifying the "adequacy" of the dietary format remains a challenge (Grove-White, 2015).

When corn starch diets are compared, Dann and Nelson (2011) observed that milk production is optimized at 21.0% starch content in DMI. In a similar study on energy diets, Silva-del-Rio et al., (2010) compared higher-energy diet versus normal diets during the transition period and observed that higher-energy diet produces significantly less milk when compared against normal energy diet throughout the entire dry period. The study highlights the delicate nature of balancing nutritional diets while emphasizing the importance of starch content alongside adequate forage fibre in feeds during this critical transition phase and underscored the need for a more refined understanding of dietary requirements for lactating cow during the post-parturient period.

The supplementary use of polyunsaturated fatty acid (PUFA) from sunflower oil before calving to calcium minerals from fish oil after calving is a novel strategy as cows subjected to diet exhibited higher conception rates and increased milk production (Thatcher, 2017). The higher conception rates is attributable to higher amounts of linoleic acid (omega-6 PUFA) which is linked to improved uterine health and lowered early embryonic loss, which further improve reproductive success. The negative impact of poor transitioning on reproductive performance a well-understood, particularly during the first two weeks post-parturiency (Grove-White, 2015). Therefore, implementing targeted peri-parturient and post-parturient strategies is crucial to easing the NEB state, minimizing health problems and improving subsequent fertility. Ideally, by 8 weeks post-parturient, over 95% of the lactating cows should have a positive energy balance, emphasizing the importance of proactive nutritional strategies.

2.3 Hormonal profile in post–parturient lactating cows

At the onset of lactation, the nutritional and energetic requirements of dairy cow increase four-fold for the highly productive dairy cows immediately after parturition. The underlying NEB state is largely attributable to low blood insulin levels which in turn stimulates the release of growth hormones (GHs) which in turn mobilize lipids into NEFAs (Roche et al., 2013). Lipid mobilization is an adaptative mechanism to NEB state that is regulated by Growth Hormone (GH) and via IGF-I to prioritize milk synthesis (Sharma et al., 2014). However, low-energy diets cause an increased hepatic uptake of catabolised AA but with no observable changes in milk yield and composition (Piccioli-Cappelli et al., 2014).

The Growth hormone through IGF-I and IGF-binding proteins (IGFBP) accelerates gluconeogenesis in the liver while concurrently slowing down lipogenesis, and indirectly acts on mammary gland and muscle to increase NEFA utilization and intensification blood flow to the mammary glands (Gruber et al., 2014). The resultant plasma NEFA is efficiently utilized in the synthesis of mammary triacylglycerol (TAG) and is oxidised in non-mammary tissues (Roche et al., 2013) in a lipolysis process that results in NEB of a lactating cow (Song et al., 2021).

Insulin-like growth factor 1 (IGF-1) stimulate cell division or differentiation (Taylor et al., 2004) thereby regulating metabolism, growth and lactation in dairy cattle as well as stimulating the anabolic activity of growth hormones (Mullen et al., 2011). As the serum IGF-I increases, cows in early lactation stage have the highest milk yield more than those in the mid or late lactation stages (Sharma et al., 2014). Thus, cows are in a NEB state, the serum IGF-I synthesis declines during the early lactation period (Sharma et al., 2014). The IGF-I system is also linked to the oestrus as low IGF-1 and insulin levels during NEB state is linked to eventual increases in days to first oestrus (Drackley & Cardoso, 2014).

However, after parturition, the increased secretion of growth hormone (GH) depresses the IGF-I concentration, thereby preserving the homeostatic balance in an energy-deficient dairy cows as the elevated plasma GH mobilizes lipid from adipose tissue (Rhoads et al., 2004). Furthermore, insulin hormone is critical for the increased liver synthesis of IGF-1 and serves as a response to elevated concentrations of Somatotropin (growth hormone; GH). Therefore, the serum insulin concentrations serve as a primary link between the metabolic and reproductive systems and indicates the adequacy to the dietary requirement of energy and protein (Drackley & Cardoso, 2014).

IGF-1 is a critical player in metabolism and growth during the post-parturient phase. According to Leury *et al.* (2013) reported IGF-1 concentrations in cows one month to parturition at 3.5 ng.ml–1, with fluctuations observed in the peri-calving period. Understanding the changes in IGF-1 levels thirty days post-parturient is crucial for elucidating its role in metabolic adaptations and nutrient utilization during early lactation. IGF-I may be possibly be associated with fertility disturbance due to non-adaption (Jorritsma et al., 2013). The low of insulin and IGF-1 concentrations during the NEB state results in follicular changes in ovaries thus reducing the secretion of oestradiol (E2) and luteinizing hormone (LH). Any decline in LH and E2 levels in turn delays the resumption of the oestrus cycle (Rhoads et al., 2004). However, its concentration varies with age, breed and stage of lactation (Taylor et al., 2004).

The IGF-I concentrations during the post – parturient period occurs due to lactation and highly productive cows are higher risk of non – adaptation due to low insulin levels during early lactation periods (Jorritsma et al., 2013). This scenario has been observed in studies examining the linkages between IGF-I, reproductive and lactational performance of Holstein-Friesian dairy cows. Taylor et al., (2004) observed that plasma IGF-1 concentrations significantly vary between different lactational cycles and that primiparous lactating cows have higher IGF-I plasma concentrations than multiparous animals. In addition, milk production is non-linearly linked to plasma IGF concentrations as such IGF-1 largely influences the oestrus cycle since multiparous cows take longer for ovarian cyclicity.

IGF-1 is involved in the uncoupling of the somatotropic axis where Insulin-like growth factor-I inhibits the release of GH as there is approximately 70% reduction in plasma IGF-I concentration during the transition to lactation, with the commensurate increase in plasma GH concentrations (Roche et al., 2013). It is this growth hormone (GH) that plays a critical role in the homeorhetic coordination of energy utilization through lipid metabolism to facilitate the energy release from adipose stores immediately after parturition(Drackley & Cardoso, 2014).

During the immediate post-parturient period, the reduced dry matter intake (DMI), parturition labour, uterine involution, lactation demands, and initiation of the reproductive cycle all led to NEB. The NEB state alters the metabolic and endocrine status as observed by high NEFA levels, high glucose consumption, low insulin levels, reduce IGF-1 levels coupled with high levels of growth hormone (GH)(Toledo-Alvarado et al., 2017).

Additionally, IGF-1 is known to influence energy metabolism through the potential feed intake (Reinhardt et al., 2011; Gunnink, 2005). Fluctuations in IGF-1 levels may impact mammary tissues' responsiveness to lactogenic stimuli, influencing milk production (Ceriani, 2004). In general, the hormonal interactions interconnected, and intricate thereby influencing multiple dimension of the physiology of a lactating cow. Therefore, studies show that the intricate balance of prolactin, cortisol, oestrogen, and

IGF-1 hormones is pivotal for feed intake and milk production during the critical thirty days post-parturient (Jaetzold, 2010).

These endocrine-metabolic interactions influence the type and quantity of absorbed by a lactating dairy cows such that any differences in the available nutrient affects milk yield and its composition (Piccioli-Cappelli et al., 2014). As observed, Poncheki et al., study (2015) the body weight changes during the post-parturient period influences reproductive performance of a lactating as cows with lesser weight loss were more likely to return to oestrus cycle than cows with higher body weight loss.

2.4 Hormone profile and milk production of post–parturient lactating cows

Numerous studies have demonstrated the association between higher milk productivity and elevated prolactin levels and during the early lactation period as prolactin hormone plays a critical role in mammary gland development and the initiation and maintenance of lactation (Desrivieres et al., 2003). Thus, any disturbances in prolactin levels, such as inadequate release or excessive suppression is associated with decreased milk production (Sgorlon, 2015) while increased milk yield leads to a higher insulin resistance that is linked to elevated mobilization of lipid and a lower BCS base (Mezzetti et al., 2021).

Prolactin hormone was indirectly linked to the regulation of the reproductive cycle in cattle (Nowak et al., 2018) but recent findings have linked prolactin to oestrus cycle. Prolactin hormone is known to directly influences mammary glands, but the responsiveness mammary gland to prolactin is moderated by systemic and local factors (Lacasse et al., 2016). The local factors include stimulation, light, olfaction, and stress while the systemic factors include the central nervous system, the immune system, the uterus, and the mammary glands and hormones; thyrotropin-releasing hormone (TRH), oestrogen and dopamine (Al-Chalabi et al., 2018).

In linking serum prolactin levels and subsequent milk output, Lanctôt et al., (2024) observed that the length of the dry period is associated with prolactin hormone as low serum prolactin levels during a short dry period restores milk production to normal levels. This influence of prolactin has been identified in several studies where the sustained increase in prolactin concentration which peaks at 234 $ng \cdot ml^{-1}$ at parturition is followed by a gradual decline to 69 ng.ml–1 at the 6th week into lactation (Edgerton & Hafs, 2003). On his part, Convey (2003) observed that prolactin concentration stabilizes at 9 $ng \cdot ml^{-1}$ 90 days into lactation as this observed prolactin levels underscore its pivotal role in initiating and sustaining lactation in dairy cows (Convey, 2003). This studies highlight the importance of, prolactin in milk synthesis besides sustaining the proper functioning of the corpus luteum (Kurpińska & Skrzypczak, 2020).

IGF-1 is also associated with growth, fertility, and lactation in cows (Mullen et al., 2011).The systemic IGF-1 is linked to reproductive performance in cattle from age at first calving, conception rate to first service, ovarian cyclicity, and embryo development (Mullen et al., 2011). As the IGF–1 plasma concentrations are linked to longer calving-to-conception intervals in primiparous cows, while low IGF-I levels after parturition in multiparous cows is linked to longer time to return to the oestrus cycle as it affects the oocytes and compromise the embryonic growth (Taylor et al., 2004). The low IGF-1 levels also influence calcium absorption and negatively affects milk yield (Li et al., 2016). Therefore, lower IGF-1 levels translate into milk fever (Horst et al., 2012) as the

IGF-I works in concert with oestrogen to influence calcium homeostasis (Correa et al., 2015).

Besides, the IGF-1, oestrogen plays a crucial role in the resumption of ovarian activity and oestrous cyclicity and fluctuation in oestrogen concentration disrupt reproductive function, potentially affecting milk production and overall well-being (Kalita et al., 2025). Notably, Stevenson and Britt (2017) suggest a complex interplay between oestrogen and IGF-1 in dairy cows during the post-parturient period as a rise in oestrogens at parturition can adversely affect energy balance by enhancing TAG in the liver, especially when plasma NEFA are low (Churakov et al., 2021).

Fluctuations in oestrogen levels during the peri-parturient period affect calcium absorption and utilization (Kalita et al., 2025), as persistently higher oestrogen concentrations is marked by a pronounced surge in the final two weeks and peaks on parturition day (Convey, 2003) before a subsequently rapid decrease in the immediate post-parturient period (Kindahl et al., 2012). Oestrogen also contributes to the development of the myometrium, the synthesis of actomyosin which aids in uterine contractions during parturition. An increase in oestrogen levels in the amniotic fluid contributes to the commencement of the uterine contraction and prepares the reproductive tissues for calving (Kurpińska & Skrzypczak, 2020). As observed by, Henricks et al., (2012), progressive rise in oestrogen concentrations is witnessed 14 days to parturition from 0.5 to 2.66 $ng \cdot ml^{-1}$, and this is followed a gradual decrease at a rate of 0.248 ng per day occurred during five days into calving.

As peri-parturient homeorhetic mechanism regulates the energy intake and the post-absorptive nutrient partitioning (Roche et al., 2013), the calving and/or environmental factors induces stressors to the dairy thereby elevated the cortisol levels which is linked to the changes in metabolic processes leading to decreased milk production (Boer et al., 2013). This increased Hypothalamic pituitary–adrenal (HPA) axis activity raises the metabolic stress that is associated with the physiological load of lactation and/or inflammation (Otten et al., 2023).

The normal functioning of the HPA axis is impacted by the chronic stress levels and the prolonged elevation of cortisol levels consequently affect the feed intake, hormonal balance, and milk production (Boer et al., 2013).The dynamic changes in the functioning of the hypothalamic-pituitary-adrenal (HPA) axis often indicates animal's response to a potential stressor through the increased plasma cortisol concentrations as an adaptive mechanism to stressful and environmental factors like calving (Huzzey et al., 2011). Studies have linked NEB state in lactating cows to higher serum cortisol concentrations with peripheral cortisol concentrations exhibiting weak circadian rhythms (Forslund et al., 2010).

During the transition period, cows often face metabolic stressors as a such the cortisol concentration also fluctuates during the post-parturient period (Convey, 2003). Serum cortisol levels influence several physiological processes as indicated by several studies For instance, Ezzat et al., (2013) observed that serum cortisol levels decline after parturition, while Otten et al., (2023) observed that cortisol concentration levels positively correlate with milk production after parturition till 300 days into lactation. Studies have also

Other studies have linked cortisol concentration to cow breeds, breeding concentration and on milk productions. In this instance, Nedić et al., (2017) observed that Friesian cow breeds tend to have higher cortisol concentrations than Busha breeds, an attribute that can be explained by intensive herd management practises and the physiological high milk production capabilities. Endo et al., (2017) share similar findings with Friesian cows having higher cortisol concentration levels than Brown–Swiss cows, 60 to 90 days into lactation.

Moreover, Endo et al., (2019) study on cortisol levels and cow's physiology state in Friesian dairy cows in the transition period observed that the cortisol levels negatively correlated with body condition scores (BCS) and that cows on first parity had the highest cortisol concentrations. When milk cortisol concentration was compared to the lactation stage and parity, Fukasawa et al., (2008) observed that milk cortisol concentrations in Holstein cows in early lactation were significantly higher than in other stages. While, milk cortisol levels correlated lowly daily milk yield, it did not vary with the age and parity of the cow.

Concerning cow's physiology of Holstein–Friesian dairy cows during transition period, Ayoub and Allam (2015) observed that triglycerides, phospholipids, lipoprotein and plasma total cholesterol significantly increased at peri-parturient and in the immediate post – parturient period. Huzzey et al., (2011) focused on the linkages between cortisol levels and inflammation and health complications in Friesian cows during post–parturient period and observed that there is an elevated risk inflammation associated with higher cortisol levels during post–parturient period. Further, elevated serum cortisol levels can indicate with endotoxin mastitis and metritis in cows with hypocalcemia (Forslund et al., 2010) as high milk production may translate to high stress levels (Ingvartsen & Moyes, 2013). It is also reported that some haemato-chemical liver parameters are indicative of physiological stress during parturition and the post-parturient periods (Piccione et al., 2012).

In conclusion, the post-parturient period necessitates careful dietary formulation to ensure a smooth transition and optimal reproductive performance. The challenges associated with sudden shifts in dietary energy density, the potential for SARA, and the delicate balance of nutrient content underscore the complexity of post-parturient nutrition (Cardoso et al., 2020). Advances in understanding the interplay between diet, nutrient content, and milk yield during this period are crucial for the ongoing improvement of dairy herd management practices. Robust research efforts are needed to refine dietary recommendations, address gaps in knowledge, and contribute to the favourable productive and reproductive of dairy cows during this critical phase. The return to oestrus cyclicality is linked to metabolic and hormones profile which primarily includes insulin and IGF-1, which links dietary nutritional status with gonadotropin secretion, recouples the GH-IGF system, and ensures follicle maturation and ovulation (Bisinotto et al., 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The current study was carried out at Uasin Gishu County, a region marked by diverse agricultural landscape, defined by three distinctive agroecological zones (AEZs) that significantly influence the region's farming practices and productivity. These zones which are categorized based on altitude, temperature, and precipitation levels, play a pivotal role in shaping the county's rich agricultural heritage. The Lower Highlands (LH), spanning from LH2 to LH4, experience an annual average precipitation of 1150-1220 mm, with temperatures ranging between 15.7˚C and 25.10˚C at an altitude range of between 2350 and 2450m. The Upper Midlands (UM3), falls at a moderate elevation, fostering diverse agricultural activities. The upper midlands experience annual precipitation between 1000-1400mm, with temperatures ranging from 18.0˚C to 20.5˚C and an altitude range of between 1550 and 1950 m above sea level. The Upper Highlands (UH), divided into UH1 and UH2, represent higher elevations. Upper highlands have an annual precipitation of between 1100 and 1400 mm, with temperatures of 15.0 ˚C to 23.0 ˚C and an altitude range of between 2350 and 2750 m above sea level(Akeng'a et al., 2017).

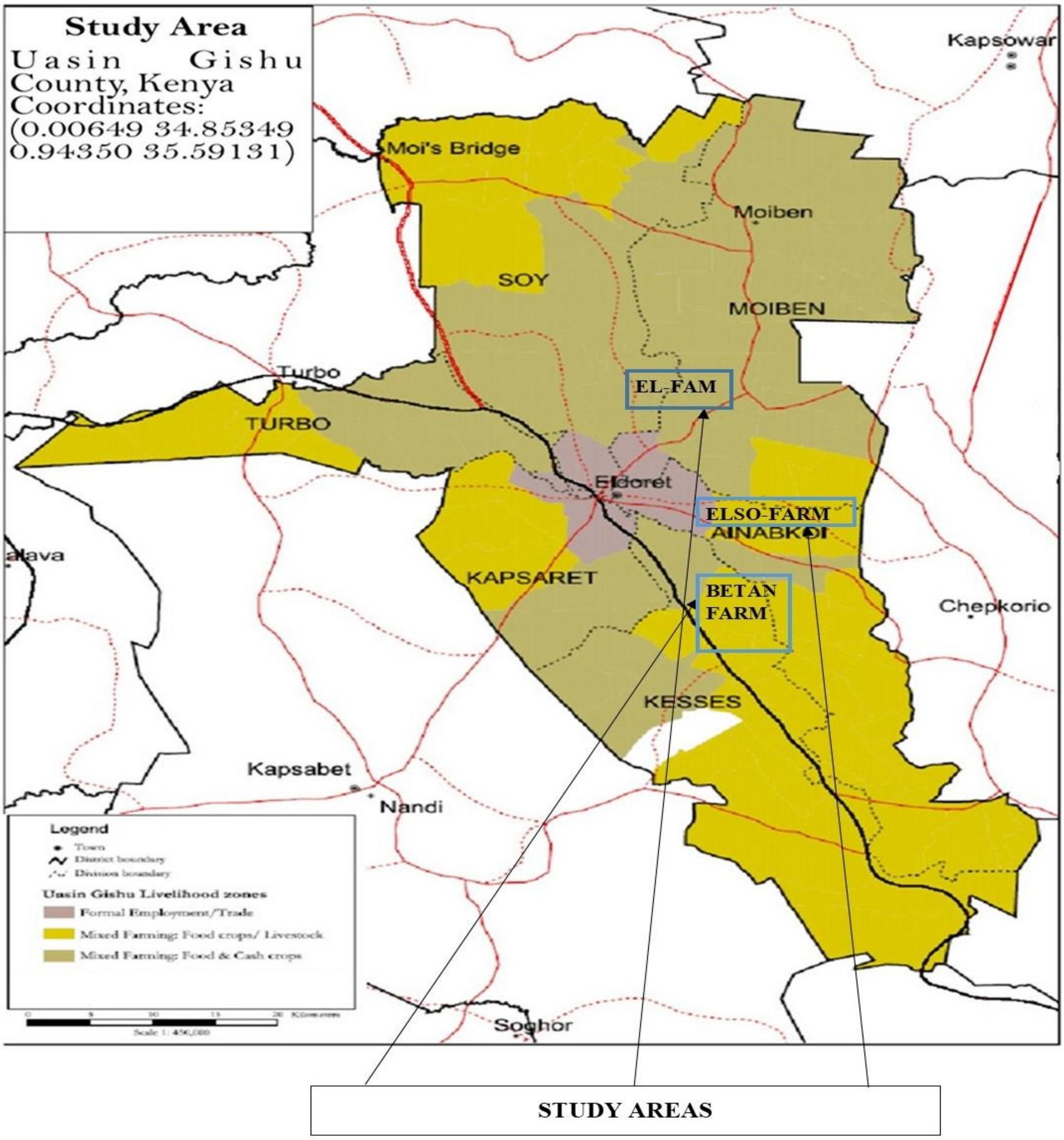


Figure 3.1: Map of the Study Area

3.2 Study Design

A research design is a procedural arrangement used by the study to collect and analysis data in a manner that aligns data relevance to the research objective (Kothari, 2014). The study utilized a randomized complete block design where experimental farm effects were blocked. The study blocked farm management and diets to eliminate the variability introduced by these factors leading to more precise estimates of the treatment effects (Montgomery, 2021). The study adopted a natural on–field experiment where nutritional diets in each farm were adopted without standardization during the experiment period.

The randomized complete block design (RCBD) is indicated by the linear model shown in Equation 1.

$$Y_{ijk} = \mu + \tau_i + \beta_j + \varepsilon_{ijk} \quad \text{(Equation 1)}$$

Where:

Y_{ijk}= Observation of the i^{th} Treatment in the j^{th} block;

μ = Overall population mean;

τ_i = Effect due to the i^{th} treatment;

β_j = Effect due to the j^{th} block;

ε_{ijk} = Random error associated with Y_{ijk}

3.3 Experimental Diet

The experimental diets took the following notations; Elfam (Diet 1), Elso Farm (Diet 2) and Betan Farm (Diet 3) as indicated in the table 3.1

Table 3.1: Diets in the Experimental Farms

Name	Main feed	Feed supplements	Mineral supplements	Feeding set up
Elfam Ltd	Silage, hay and Napier	Lucerne and Desmodium	Mineral block	Forage, feed supplementation given twice a day Mineral and water given ad-lib
Elso Farm	Silage, hay and Napier	Concentrates (Dairy meal)	Mineral Adlib and block	Animal given 6 Kgs of concentrates at (8:00 AM) and main feed immediately and at 3:00 PM. Minerals and Water given ad-lib
Betan Farm	Hay, Silage and Napier	Concentrates (Dairy meal)	Mineral supplementation	Main feed and concentrates mixed and given together Minerals and Water given ad-lib

3.4 Description of Experiment

The current study was carried out at Elfam, Elso and Betan farms, which were purposively selected based on specific criteria that included size, management practices, cattle breeds and owners' willingness to participate in the study. In each of the selected farms, three lactating Friesian cows with the averagely similar calving moment and post–parturient period but with different parities (2, 3 and 4) and with an average milk production of 20 litres per day were selected. For purposes of this study, parity in each farm therefore served as a factor while diets were blocked since each farm employed a different feed formulation. The three selected cows kept in an isolated pen where they were fed, and given water and minerals ad libitum. The cows were milked twice between 4 am and 3 pm daily during the thirty days of the study. The milk yield was recorded for each cow per day including the colostrum.

3.5 Measurement of Bodyweight

To determine the requirements, the selected cows were initially weighed at the onset of the experiment using the weight band and then weighed daily for the next thirty days. All the weights were recorded in plain paper datasheet. Each cow was given 3% allowance of its body weight to cater for the measurement errors.

3.6 Blood Sample Collection and Preparation

Before blood sample collection, the vacutainer tube was labelled using a permanent marker according to the date, parity and farm. The first blood sample was collected on the day the selected cow calved down and thereafter, after a 7^{th} -day interval between 7:00 AM and 8:00 AM for the subsequent thirty days. A blood sample of 4ml per cow was drawn aseptically from the jugular vein into a 7cc vacutainer tube using a 23-gauge needle. The blood was transferred before being stored in a cooler box at 2 - 8 ºC and delivered to the analytical laboratory within 2 hours. The blood samples were then refrigerated at temperatures ranging between 0 – 4 ºC awaiting for the determination of the respective serum hormonal levels.

3.7 Blood Sample Analyses

Immediately the blood was delivered to the laboratory, the vacutainer tube was kept in a 45^0 tilt to allow the serum to settle at the top and easily obtain the serum. The following hormonal tests were carried out on the blood serum at the Nairobi Annex Laboratory, Eldoret.

3.7.1 The Serum Cortisol Check-1 Test

The Cortisol check-1 test was used to determine the serum cortisol levels. The serum 25ng (1 drop) was put into a cartridge and 100ng/L cortisol diluent(reagent) was added. The cartridge was put into a VEDA-LAB *Easy Reader+* semi-automated machine and allowed to run for 9 minutes and the results were displayed on the display Liquid crystal display (LCD) screen of the RIA reader. The limitation of this machine is that it can only analyse one serum test at a time making it time-consuming. The hormone levels were compared for the 30-day post-parturient period using descriptive statistics (means, graphs, bar charts) and F - statistics was used to compare hormone levels between different groups (parities).

3.7.2 The Serum Prolactin-Check-1 Test

The Prolactin-Check-1 test was used for testing the serum prolactin levels. The collected serum of 100µg/L was transferred into a cartridge and the acridinium ester (reagent) was added and allowed to incubate for 35min in a fully automated Chemiluminescence Immunoassay Analyzer (ICMA 2000). The readings were displayed on the Liquid crystal display screen of the analyzer. This machine analyses eight serum samples at once. The hormone levels were compared for the 30-day post-parturient period using descriptive statistics (means, graphs, bar charts) and F - statistics was used to compare hormone levels between different groups (parities).

3.7.3 The Enzyme-Linked Fluorescent Assay(ELFA) Test

The ELFA test was used for testing the quantity of serum oestrogen of the lactating cows. From the centrifuged content, 100µg/L of serum was put into a cartridge and the horse radish peroxidase (HRP) (reagent) was allowed to incubate the serum for 35min in a fully automated Chemiluminescence Immunoassay Analyzer (ICMA 2000). The readings of oestrogen were displayed on the Liquid crystal display screen of the analyzer. This machine analyses eight serum samples at once. The hormone levels were compared for the 30-day post-parturient period using descriptive statistics (means, graphs, bar charts) and F - statistics was used to compare hormone levels between different groups (parities).

3.7.4 The Radioimmunoassay(RIA) Test

Insulin-like growth factor (IGF-α1) was tested using radioimmunoassay (RIA). The researcher transferred 100 µg/L of serum into a cartridge, added alkaline phosphatase (AP) reagent and was allowed to incubate for 35min in a fully automated Chemiluminescence Immunoassay Analyzer (ICMA 2000). The readings were displayed on the Liquid crystal display screen in order. This machine analyses eight serum samples at once (Siemens, 2018).

3.8 Feed Analysis

The study did not interfere with the farm's feeding regime but described it. Feed samples were collected and subjected to feed analysis for protein, carbohydrate, and dry matter content at Kenya Agricultural Livestock Research Organization (KALRO, Naivasha). The feed samples were collected from Elfam, Elso and Betan farms and air dried under a shade for two days to effect uniform drying before being packaged in cotton paper bags and labelled accordingly.

A 200 grammes sample was put in a hot air oven at 65°C for 24 hrs for further drying before being ground into a fine powder and sieved through a 1mm sieve. The ground samples were then packaged in airtight polythene bags and labelled. These ground feeds were subjected to proximate analysis according to the procedure of the Association of Official Analytical Collaboration Formerly Association of Official Analytical Chemists (AOAC) (2019). The samples were subjected to the analysis for the determination of the amounts of ash, crude fibre, crude protein, dry matter and lipids (ether extracts) (AOAC, 2019).

3.8.1 Crude Fibre

The crude fibre content was analysed based on the Van Soest fibre analysis technique (Van Soest et al., 1991). Once, proximate analysis procedures were completed, the data was analysed through the ANOVA, F- test and with Tukey test at $p \leq 0.05$ significance level to separate the means.

3.8.2 Crude Protein

Feed analysis for crude proteins was conducted using the Near-infrared (NIR) spectrometry at Kenya Agricultural and Livestock Research Organization (KARLO), Naivasha. A sample of 2 grams of feed was selected and ground to pass a 2 mm sieve. The ground samples were subjected to NIR spectroscopy, and the validated calibration model was applied to predict protein concentrations in the samples (AOAC, 2019).

3.8.3 Crude Fiber

Feed analysis for carbohydrates/crude fibre was conducted using the Near-infrared (NIR) spectrophotometry method used at KALRO. For carbohydrates analysis using NIR spectroscopy, 2 grams of the sample were ground to pass through a 1 mm sieve, and then measured using an Ft97900 F7 NIR Analyzer (Albrecht et al., 1987). A calibration set with known carbohydrate concentrations was used during this measurement. The resultant spectra were reference to standard values to establish a validated calibration model. This model was then applied to predict carbohydrate concentrations in new feed samples, with the final results being reviewed and interpreted.

3.8.4 Dry Matter

The dry matter analysis was done by oven-drying method at KALRO, Naivasha. A representative sample of 2 grams was placed in an oven with temperature ranging between 105-110°C to eliminate moisture. As recommended by AOAC (2019) the drying process is continued until the sample achieves a constant weight.

After drying, the samples were cooled in a desiccator to prevent moisture reabsorption before being weighed again to determine their dry weight. The dry matter was estimated using the following formula:

$$\text{Dry Matter (\%)} = (\text{Dry weight} / \text{Original weight}) \times 100$$

The dry matter content was presented as a percentage of the total weight.

3.9 Data Analysis and Presentation

Upon completion of the laboratory analysis, the collected data underwent a comprehensive quantitative analysis using GenStat (2014). The initial step involved cleaning up of the raw data in Microsoft Excel. Here, any inconsistencies, outliers, or missing values were addressed through meticulous data-cleaning procedures that include data consistency and completeness checks to ensure the data set's integrity. Both descriptive and inferential statistical techniques were used to analyse weight change, hormone levels feed intake, and milk yield data

3.9.1 Feed Intake

The study gave a 40 Kgs mixture of silage, hay, Napier grass and concentrates (or substitute plant protein) once a day to the animal and after a day, the feed leftovers were weighed and discarded and new feed was introduced. The study then determined the feed consumed using the following formula and the data was recorded for the next 30 days.

$$\text{Feed consumed} = \text{Feed given} - \text{feed leftover.}$$

The feed intake data from the different animals were recorded manually in a squared grid rule book before being collated and entered into a Microsoft Excel worksheet. The data in the worksheet was analysed descriptively (mean and standard deviation) to determine the trends which were presented in graphical format and then analysed inferentially using ANOVA and correlation statistics. The ANOVA statistics determined the differences between feed intake in different parities while correlation statistics determined the association between serum hormonal change and feed intake, and milk yield as indicated in objective 2.

3.9.2 Milk Yield

The study weighed and recorded daily milk yield in Kgs from the cows under the experiment during the study period. The milk yield data were collated and entered into a Microsoft Excel worksheet and analysed descriptively (mean and standard deviation) to determine the trends which were presented in graphical format and then analysed using ANOVA and Pearsons' correlation statistics. The ANOVA statistics determined the differences between milk yield and different parities while Pearsons's correlation statistics determined the relationship between serum hormonal changes, feed intake, and milk production as indicated in objective 3.

3.10 Ethical Considerations

Prior to the experiment, the researcher sought permission from the owners of the three farms; Elso, Elfam and Betan farms to be allowed to conduct the experiment in their farms. Once accepted, farm managers of the selected farms were fully informed on the nature, purpose, and potential risks of the study. Subsequently, to safeguard the welfare of the cows during the study, the study took appropriate measures to ensure they were not exposed to any physical, psychological, or emotional harm.

Permission was obtained from the National Commission for Science, Technology and Innovation (NACOSTI), the County Director of Veterinary Services (CDVS), and the university introduction letter before conducting the study. Throughout the research process, strict adherence to guidelines and recommendations was observed, ensuring that the study aligns with established ethical standards. Compliance with data protection regulations and guidelines to safeguard the confidentiality and security of the collected data was observed.

CHAPTER FOUR

RESULTS

4.1 Objective One: Impact of parity on body weight and milk yield of Post - parturient Friesian Cows in Uasin Gishu County, Kenya

4.1.1 Body weight trends of the post–parturient Friesian Cows

The average weight of the Friesian cows at each parity is presented in graphical illustration in Figure 4.1. The dairy cows in parity 4 had the highest average weight with a minimum of 640 Kgs and were significantly ($p < 0.05$) heavier than the dairy cows in parity 3 and parity 2. The cows in parity 3 had a comparatively average minimum weight of 570 Kgs but were not significantly different ($p > 0.05$) from the cows in parity 2 which weighed 538 Kg.

Based on the graphical trends, the cows irrespective of their parities progressively lost weight daily during the immediate post–parturient period.. The cows with parity 4 losing weight began with an initial of 660 Kgs after parturition dropping to 640 Kgs in day 19 and slowly recovering to record 654 Kgs at day 30. Cows with parity 3 began with an average of 588 Kgs before gradually losing weight dropping to 570 Kgs by day 19 and easing to 580 Kgs by day 30. Cows with parity 2 began with an average initial weight of 552 Kgs after parturition then slowly losing body weight to record 538 Kgs by day 17 and slowly recovering to record 541 Kgs by day 30.

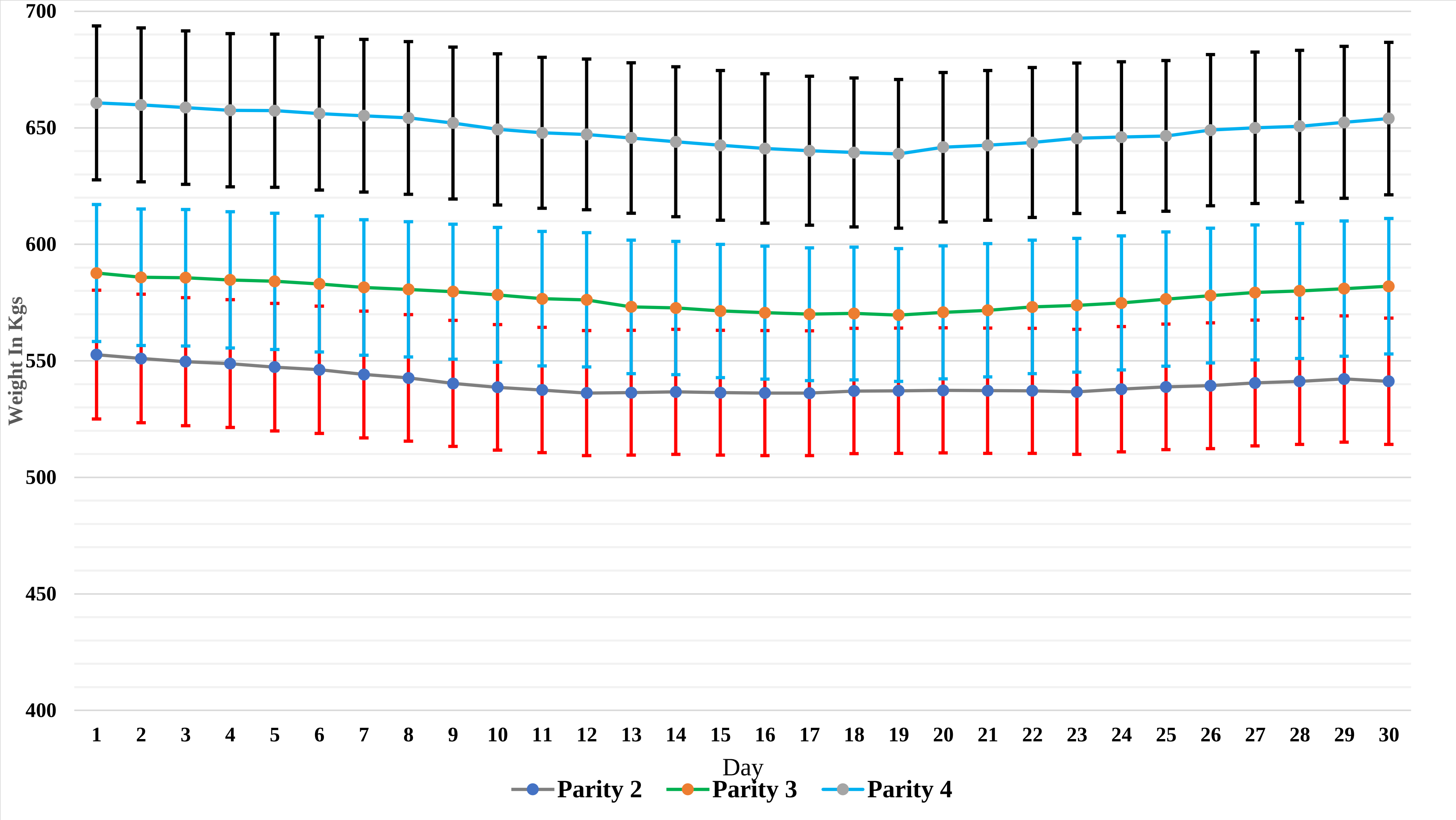


Figure 4.1: Body weights of the dairy cows of different parities for thirty days

4.1.2 Milk yield in parities during post – parturient period

The milk production of the lactating cows among parities is graphically presented in Figure 4.2. The figure showed that cows in parity 4 had an initial average milk production of 16 Kgs/day which drastically increased to 27 Kgs/day on day 13 and stabilised at 27 – 28 Kgs/day by day 30.

The cows in parity 3 had an initial milk production of 14 Kgs/day before gradually increasing to 26 Kgs/day by day 20 and then easing and stabilising at 28 Kgs/day by day 30. The cows in parity 2 had an initial milk production of 11Kgs/day and gradually increased to 28Kgs/day by day 25 and then easing and stabilizing at 27 Kgs/day by day 30.

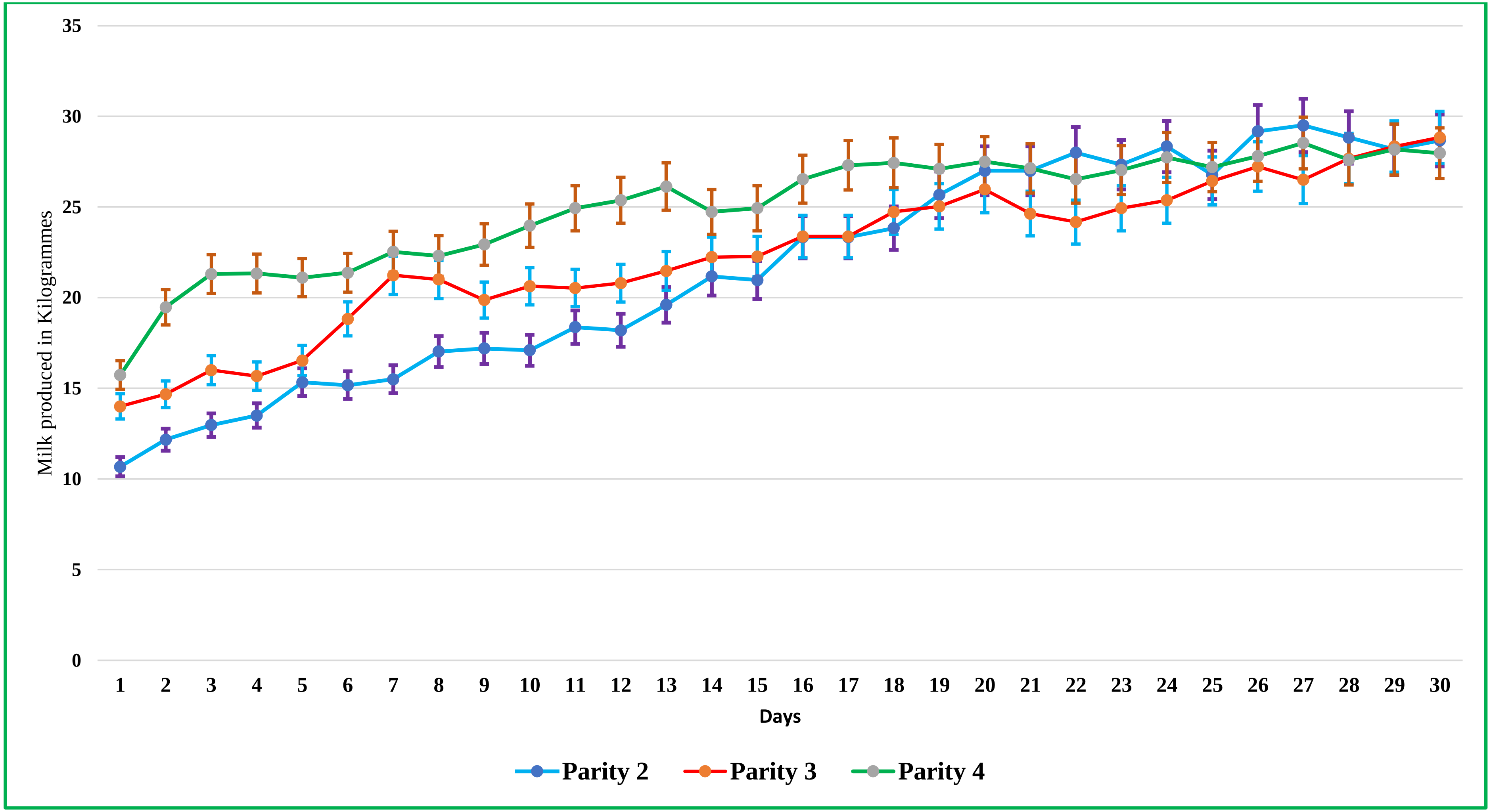


Figure 4.2: Milk yield trend of cows in different parities during the 30 days post parturient period

Table 4.1: The average milk yield of cows in different parities

Parity	Mean
2	21.66^{a}
3	22.41^{a}
4	24.99^{b}

Table 4.1 shows the results for the effect of parity on milk yield. The results indicated that parity levels were statistically significant in explaining the differences in milk yield. Cows in parity 2 had the low($p \geq 0,05$) daily milk yield at 21.66 $Kgs.day^{-1}$ which was not statistically different from cows in parity three at 22.41 $Kgs.day^{-1}$ with cows in parity 4 having the highest($p \leq 0.05$) daily milk yield at 24.99 $Kgs.day^{-1}$. These statistics showed that cows in parity 4 are more likely to produce more milk than the cows in lower parities.

4.2 Objective Two: Influence of serum hormonal levels on feed intake of Post – parturient Friesian Cows

The study first examined the nutritional composition of the diet and presented the information in section 4.2.1, then graphically illustrating the trends in the levels of four serum hormonal levels; cortisol, insulin like – growth factor, prolactin and oestrogen.

4.2.1 Nutritional Composition of the Experimental Farms

The results shown in Table 4.2 show Betan farm diets had better values in Ether extracts (4.52%), Acid Detergent Lignin (ADL)(4.66%), crude protein content (9.62%), Ash content (9.59%) and ME (3.001 Mj/KgDM) while Elso Farm had the highest values in Acid Detergent Fibre(ADF) (35.52%) and Neutral Detergent Fibre (NDF) (62.41%)

with crude protein (CP) content (7.91%) and crude fibre content (30.81%). Elfam had the lowest values in Ash content (6.50%) and crude protein content (7.62%). The nutritional diets significantly differed ($p< 0.05$) with Betan Farm seemingly having higher nutritional requirements in terms of Ash, CP, EE contents and metabolizable energy.

Table 4.2: Nutrient Composition of the Experimental Farms

	Nutrient Composition in %								**ME** in
Farm	**DM**	**Ash**	**CP**	**CF**	**EE**	**NDF**	**ADF**	**ADL**	MJ/KgDM
Elfam	91.90^{a}	6.50^{c}	7.62^{c}	25.53^{b}	1.84^{b}	58.60^{a}	34.49^{a}	4.10^{a}	2.370^{b}
Elso	91.75^{a}	7.91^{b}	7.91^{b}	30.81^{a}	1.89^{b}	62.41^{a}	35.22^{a}	3.27^{b}	2.230^{b}
Betan	92.03^{a}	9.59^{a}	9.62^{a}	23.16^{c}	4.52^{a}	53.26^{b}	30.52^{b}	4.66^{a}	3.001^{a}
F	0.18	154.24	1390.45	118.53	383.01	15.95	29.21	14.25	244.80
p-value	0.840	0.000	0.000	0.000	0.000	0.004	0.001	0.005	0.000

[a, b, c,] Means with different superscripts are significantly different (p<0.05)

The correlation statistics in Table 4.3 indicate that milk yield statistically and positively correlates with feed intake ($r = 0.615$), ($p \leq 0.01$) with the coefficient of determination ($R^2 = 0.378$) indicating that feed intake determines 37.8 % of variations in milk yield. This indicates that one Kg unit of feed intake consumed by the lactating cow explains a 0.378 Kg increase in milk yield.

Table 4.3: Correlation between Feed intake and Milk yield

Feed Intake	Feed Intake	Milk yield
Feed Intake	1	
Milk yield	0.65**	1

**Significance at 0.01 level (2-tailed).

4.2.2 Feed Intake by dairy cows in different parities

The daily feed intake of the cows was determined and the findings were graphically illustrated as shown in Figure 4.3. The average feed intake showed that cows in all parities had their initial intake of between 15 and 17 Kgs/day and did not significantly ($p \geq 0.05$) differ among parities. The trends showed a gradual increase in feed intake from 15 Kgs/day to an average of 35 Kgs/day for all the cows. The feed included both dry (hay, concentrate) and wet (silage, Lucerne and Napier grass).

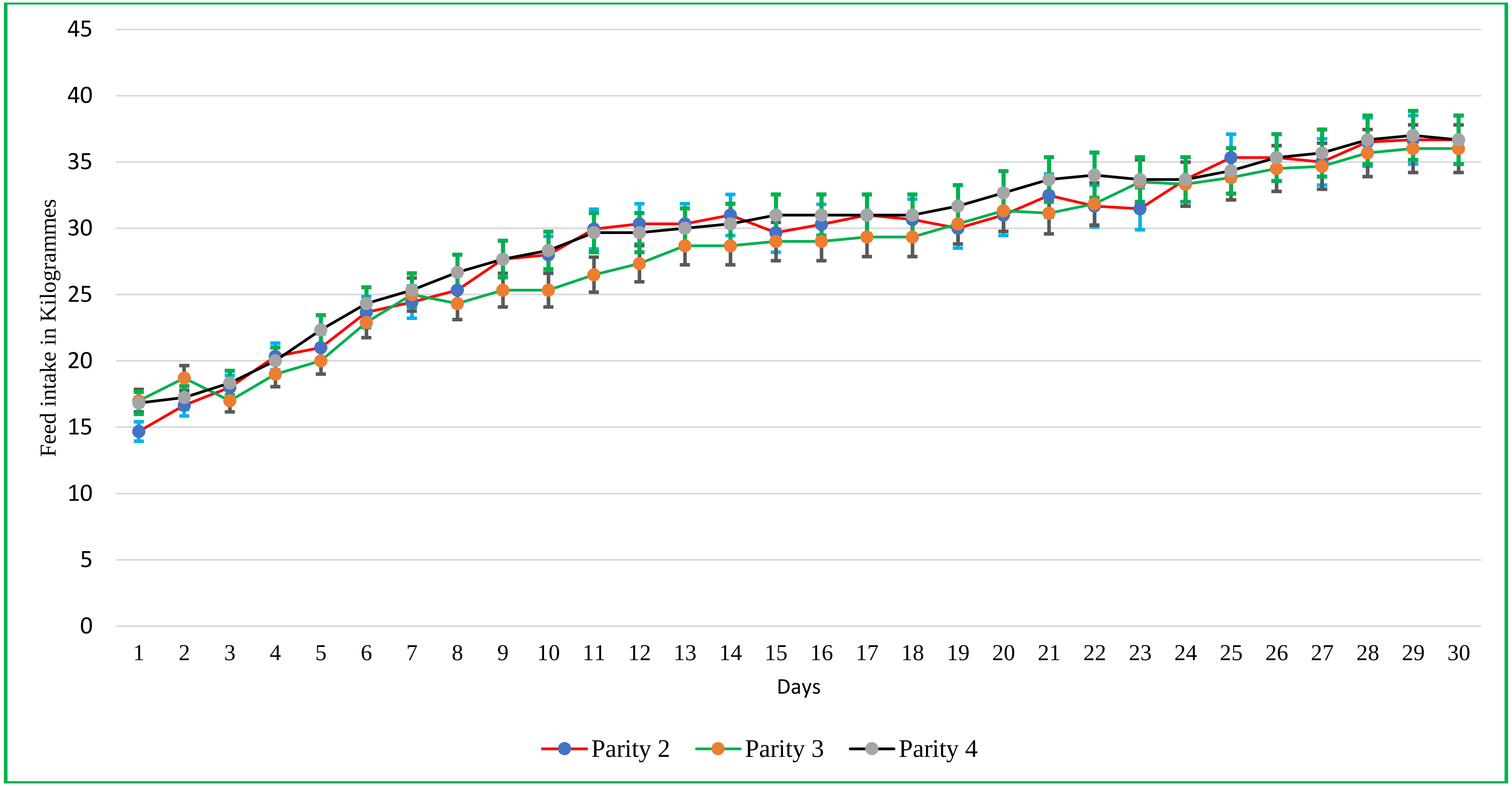


Figure 4.3:Feed intake for Friesian Cattle in different parities through the study period

4.2.3 Serum Hormone Levels and Feed intake of Post – parturient Friesian Cows

The study examined the association between serum hormone levels and feed intake using correlation statistics and the results are presented below.

Table 4.4: Correlations between serum hormonal levels and feed intake of the Post - parturient Friesian Cows

	Feed Intake	Serum Cortisol	Serum Prolactin	Serum Oestrogen	Serum IGF-α1
Feed Intake	1				
Serum Cortisol	-0.613**	1			
Serum Prolactin	0.760**	-0.692**	1		
Serum Oestrogen	0.785**	-0.464**	0.851**	1	
Serum IGF-α1	0.692**	-0.645**	0.851**	0.735**	1

**. Significance at the 0.01 level (2-tailed).

Table 4.4 indicated that feed intake significantly and positively correlated to prolactin hormone ($r = 0.760$, $p < 0.01$), oestrogen hormone ($r = 0.785$, $p < 0.01$), and IGF-α1 ($r = 0.692$, $p < 0.01$) but negatively correlated ($r = -0.613$. $p < 0.01$) to cortisol hormone. This indicated that serum hormone levels (Prolactin, IGF-α1 and Oestrogen) largely and positively influence feed intake such that, where there is an increase in the increase in serum hormone levels by the lactating cows in the immediate post–parturient period, there is a commensurate amounts of feed consumed. Only the serum cortisol hormone levels had a highly significant ($p\leq0.01$) negative effect on feed intake implying that the higher levels of serum cortisol hormone have an inverse effect with lactating cows tending to have lowered feed intake and on the converse lower levels of serum cortisol hormone tend to lead to higher feed intake.

4.3 Objective Three: Effect of parity on milk yield of post - parturient Friesian Cows

4.3.1 Hormone Levels of Post – parturient Friesian Cows

As indicated in Figure 4.4, the levels of serum cortisol hormone of the Friesian cows in different parities gradually declined from week 1 to week 4. At parturition, cows in parity 2 had elevated serum cortisol levels of 201 $ngmL^{-1}$ and were significantly different ($p < 0.05$) from cows in parity 3 (142 $ngmL^{-1}$) and parity 4 (120 $ngmL^{-1}$). All the cows in different lactation cycles(parities) significantly reduced the levels of serum cortisol hormone to reach low levels of 112 $ngmL^{-1}$ (parity 2), 84 $ngmL^{-1}$ (parity 3) and 72 $ngmL^{-1}$(parity 4) by week 4.

The trends in the levels of serum cortisol given in Figure 4.4 showed that lactating cows in parity 2 had significantly ($p \leq 0.05$) higher levels of serum cortisol than cows in parity 3 and 4 before significantly dropping with time in all the parities.

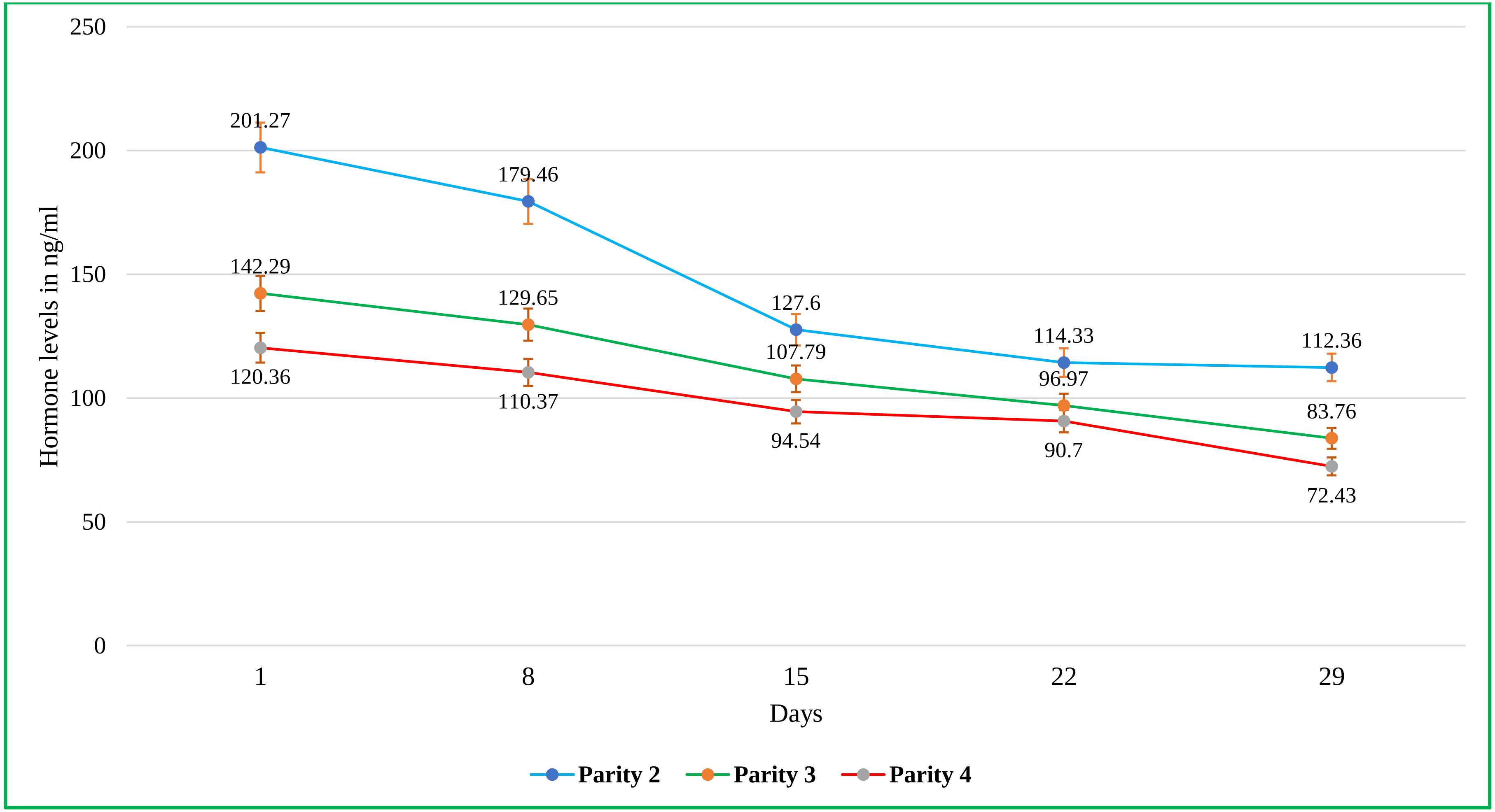


Figure 4.4: Serum Cortisol Levels in parities among Friesian cattle at Uasin Gishu County

Figure 4.5 shows that the levels of serum prolactin quadrupled from an average of 84.3 $ngmL^{-1}$ in week 1 to 350.8 $ngmL^{-1}$ in week 4 for different parities accordingly. At parturition, the cows in parity 2 had the lowest serum prolactin levels at an average of 61 $ngmL^{-1}$ and were significantly different ($p < 0.05$) from cows in in parity 3 (76 $ngmL^{-1}$) and in parity 4 (116 $ngmL^{-1}$). The serum prolactin levels gradually rose with the increase in feed intake (Figure 4.3) to an optimal amount of 307 $ngmL^{-1}$ (parity 2), 344 $ngmL^{-1}$ (parity 3) and 400 $ngmL^{-1}$(parity 4) by week 4. The levels of serum prolactin in all the parities gradually increased with the cows in parity 4 having the highest levels while cows in parity 2 had the lowest serum prolactin levels. Generally, serum prolactin showed an increasing trend among all the cows. As the feed intake increased, there was a significant rise in serum prolactin levels to optimise milk production as the lactating cows stabilized the nutritional requirement in the post–parturient period.

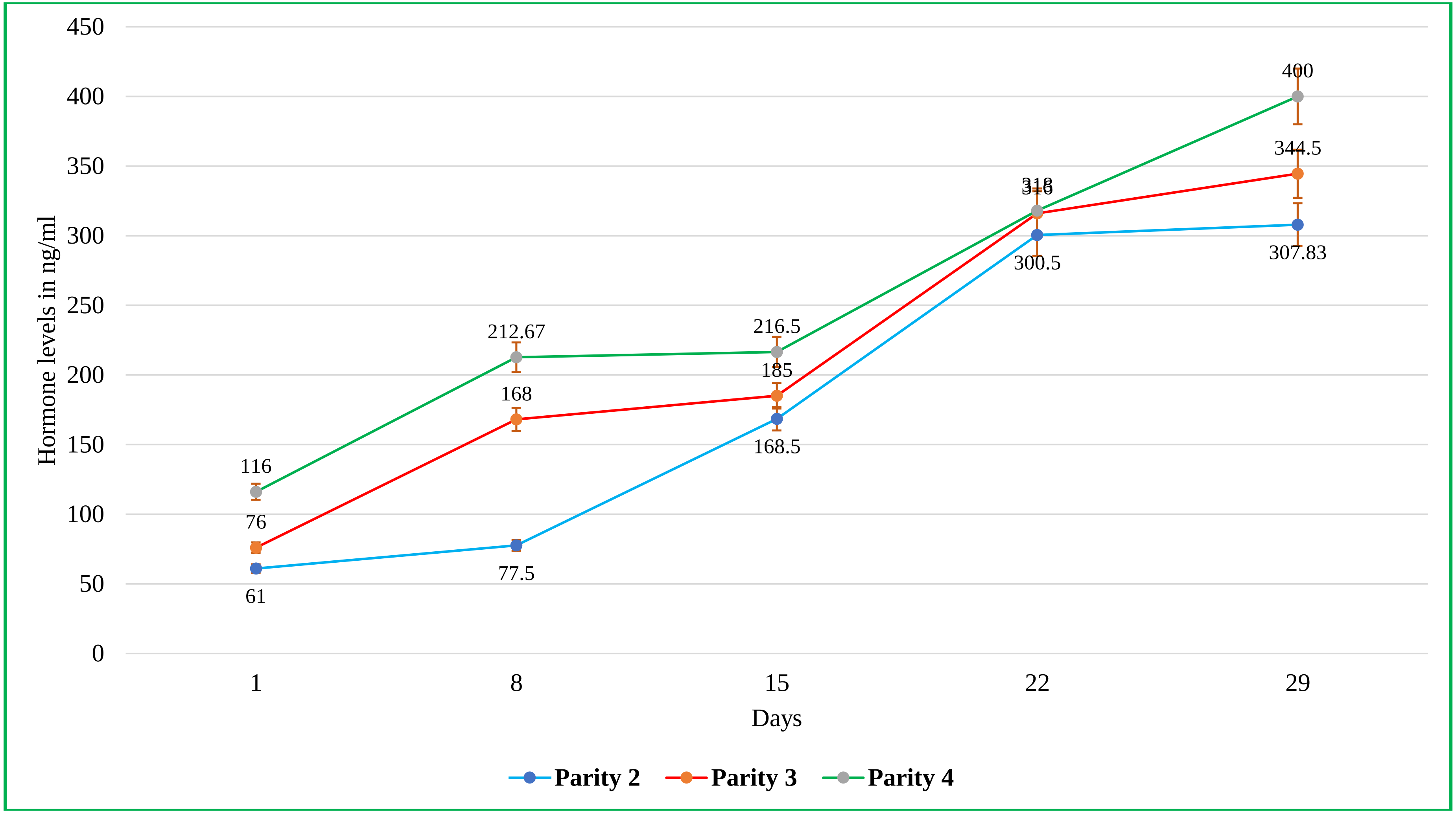


Figure 4.5: Serum Prolactin Levels among parities of Friesian cattle at Uasin Gishu County

The levels and trends of serum oestrogen of the lactating cattle in parities are illustrated graphically in Figure 4.6. Figure 4.6 shows that the levels of serum oestrogen tripled from an average of 1.2 $ngmL^{-1}$ in week 1 to 3.6 $ngmL^{-1}$ in week 4 for different parities accordingly. At parturition, the cows in parity 2 had the highest levels of serum oestrogen at an average of 1.41 $ngmL^{-1}$ and were significantly different ($p < 0.05$) from cows in parity 3 (1.14 $ngmL^{-1}$) and parity 4 (1.05 $ngmL^{-1}$). The serum oestrogen levels gradually rose with the increase in feed intake (Figure 4.3) to an optimal amount of 4.07 $ngmL^{-1}$ (parity 2), 3.48 $ngmL^{-1}$ (parity 3) and 3.15 $ngmL^{-1}$(parity 4) by week 4. Initially, the cows of parity 3 had the lowest levels of the serum oestrogen up to day 15, when, the serum oestrogen levels gradually increased and surpassed the levels of serum oestrogen in parity 4. As the feed intake rose, there was a gradual increase in the serum oestrogen levels as the Friesian cows sought to stabilise the oestrus cyclicity for the next lactation cycle.

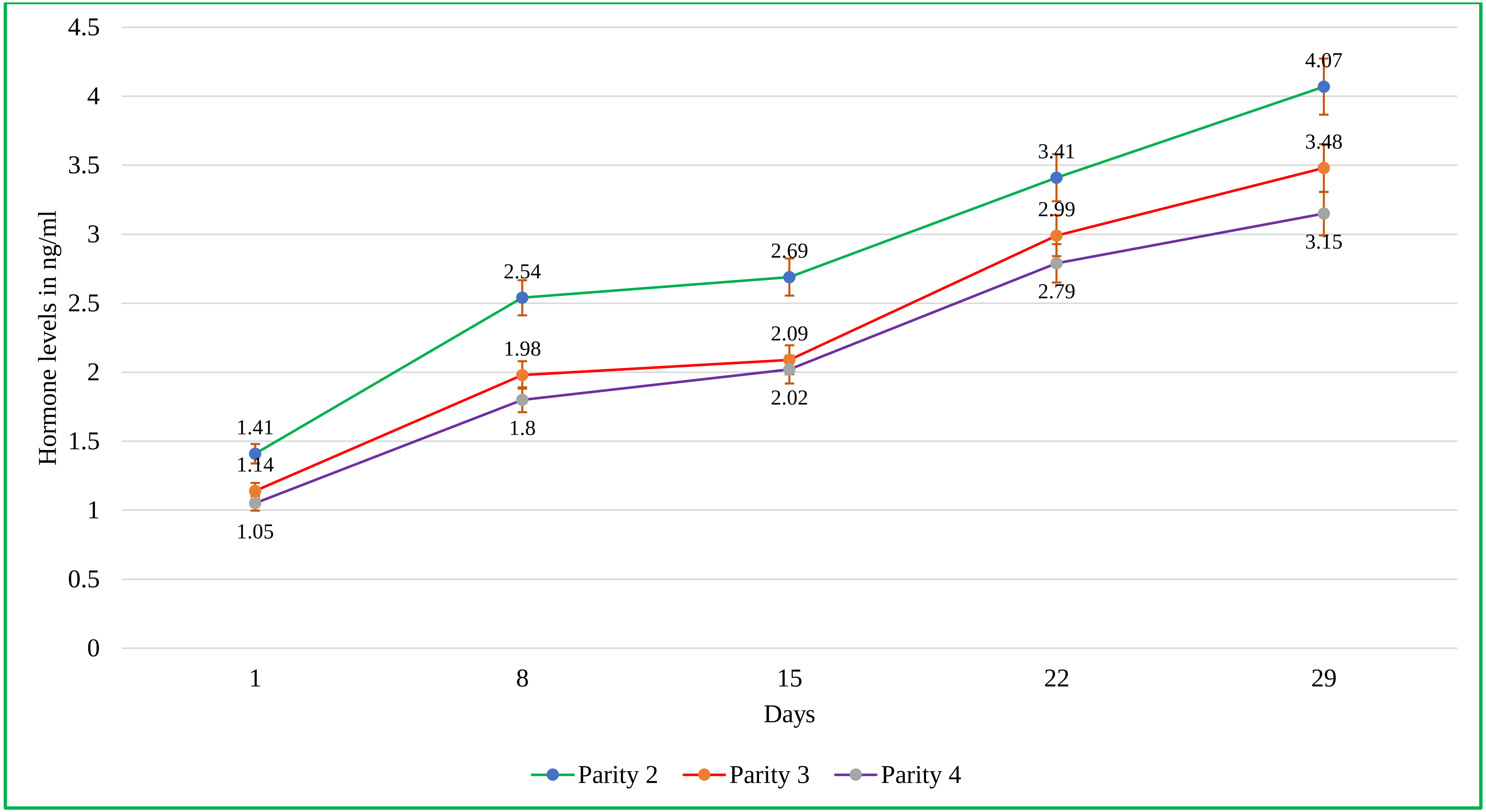


Figure 4.6: Serum Oestrogen Levels among Friesian cattle in different parities at Uasin Gishu County.

The levels of serum Insulin Growth Factor hormones in plasma are illustrated graphically in Figure 4.7. Figure 4.7 shows that the average serum IGF-α1 levels of the lactating cows tripled from 109.8 $ngmL^{-1}$ in week 1 to 324.6 $ngmL^{-1}$ in week 4. The figure shows that the levels of serum IGF-α1 tripled from an average of 110 $ngmL^{-1}$ for the different parities in week 1 to 325 $ngmL^{-1}$ in week 4 for different parities. At parturition, the cows in parity 4 had the lowest serum IGF-α1 levels at an average of 81 $ngmL^{-1}$ and were significantly different ($p < 0.05$) from cows in parity 3 (115 $ngmL^{-1}$) and parity 2 (133 $ngmL^{-1}$). The serum IGF-α1 levels gradually rose with the increase in feed intake (Figure 4.3) to an optimal amount of 280 $ngmL^{-1}$ (parity 4), 327 $ngmL^{-1}$ (parity 3) and 367 $ngmL^{-1}$ (parity 2) by week 4. As the feed intake rose, there was a discernible increase in the serum IGF-α1 levels as the Friesian cows sought to support their normal bodily growth functions and needs. As the serum IGF-α1 levels rose there was a significant increase in the feed intake to compensate for the NEB, stabilized nutritional requirements and optimum milk production.

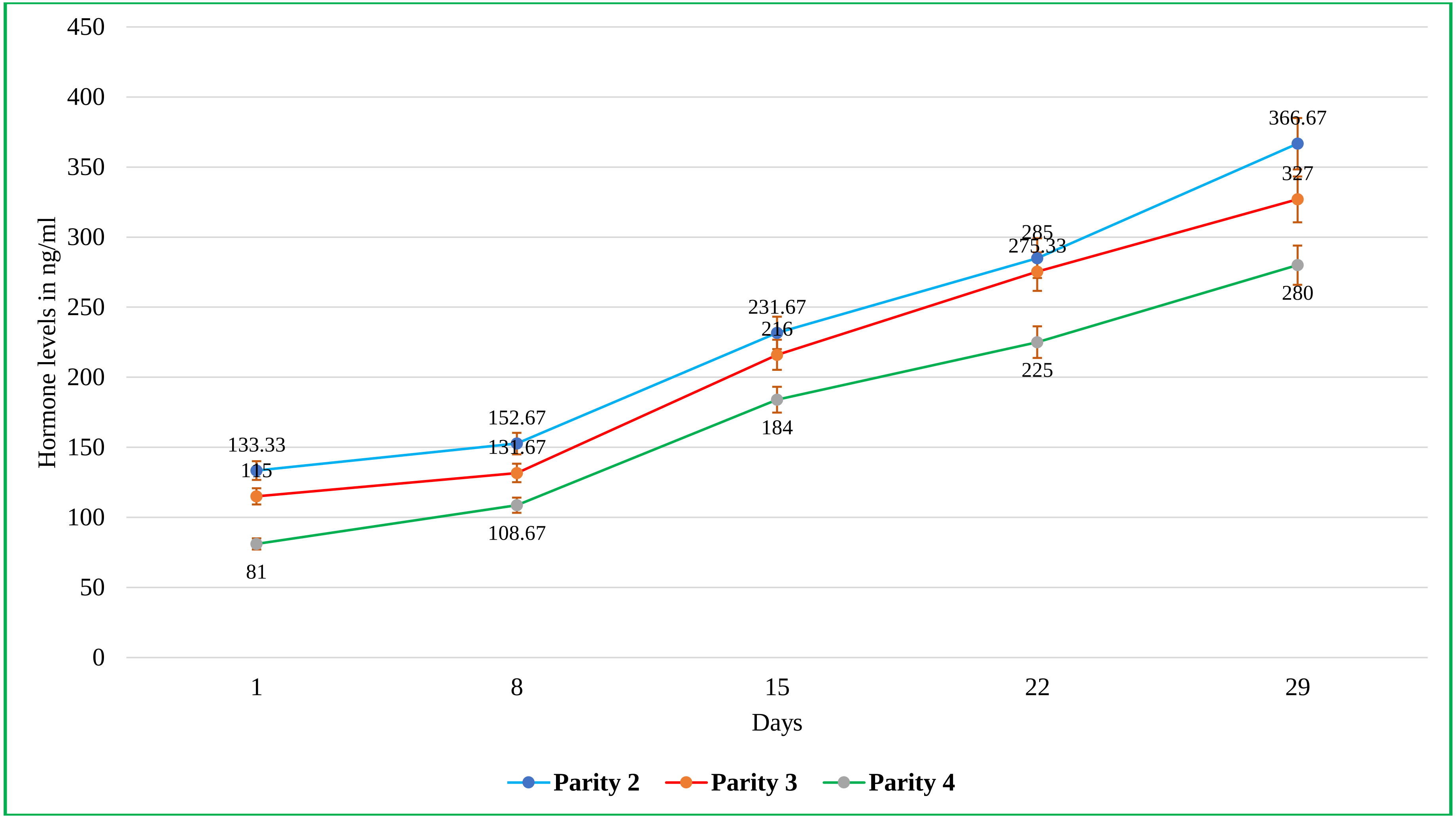


Figure 4.7: Serum Insulin Growth Factor Levels in Friesian cattle of different parities at Uasin Gishu County

4.3.2 Correlation between serum Hormone Levels and milk yield in Post – parturient Friesian Cows

Table 4.5: Association between serum hormonal levels and milk yield of the Post - parturient Friesian Cows

Correlation coefficient

	Milk yield	Cortisol	Prolactin	Oestrogen	IGF-α1
Milk yield	1				
Cortisol	-0.737**	1			
Prolactin	0.744**	-0.692**	1		
Oestrogen	0.723**	-0.464**	0.851**	1	
IGF-α1	0.740**	-0.645**	0.851**	0.735**	1

**.Significance at the 0.01 level (2-tailed).

The correlation statistics given in Table 4.5 show that milk yield significantly ($p < 0.01$), positively correlated ($r = 0.744$), to prolactin hormone, oestrogen hormone, ($r = 0.723$) and IGF-α1 ($r = 0.740$), but significantly ($p < 0.01$) and negatively ($r = -0.737$) correlated to cortisol hormone. This indicated that milk yield is largely and positively influenced by the serum hormone levels (Prolactin, IGF-α1 and Oestrogen) such that as these hormone levels increase, there is bound to be an increase in the amounts of milk produced by the lactating cows in the immediate post – parturient period. Only the serum cortisol has a significantly ($p \leq 0.01$) negative effect on milk yield implying that higher levels of serum cortisol have an inverse effect on lactating cows tending to have lowered feed intake and on the converse lower levels of serum cortisol tend to lead to higher milk yield.

4.3.3 Parity and Serum Hormone Levels of Post – parturient Friesian Cows

Table 4.6: Serum Cortisol levels at different parities

Parity	$ngmL^{-1}$
4	108.3[a]
3	112.3[a]
2	159.2[b]

Columns with different superscripts are significantly different ($P \leq 0.05$)

Table 4.6 illustrates the influence of parity levels on serum cortisol concentration and the statistics showed that parity levels were significant ($p \leq 0.05$) in explaining the differences in levels of serum cortisol. Cows in parity 4 had the lowest levels of serum cortisol at 108.3 $ngmL^{-1}$ but not statistically different from cows in parity 3 at 112.3 $ngmL^{-1}$, however, cows in parity 2 had the highest levels of serum cortisol at 159.2 $ngmL^{-1}$. These showed that cows with lower parity levels are more likely to have higher serum cortisol levels than cows in higher parities.

CHAPTER FIVE

DISCUSSION

5.1 Objective One: Parity, weight change and milk yield of post–parturient Friesian cows

Whereas dietary nutrients and energy availability are considered to be limiting lactational performance during immediate post-parturient period, a lactating cow is subjected to physiological changes. Higher milk production in a lactating cow is demanding in terms of nutritional requirements and herd management and is linked to greater metabolic load, with an elevated risk to different metabolic disorders in early lactation period (Macmillan et al., 2021; McArt & Neves, 2020).

5.1.1 Weight change of the post–parturient Friesian Cows

The dairy cows in parity 4 had the highest average weight with a minimum of 640 Kgs and were significantly ($p < 0.05$) heavier than the dairy cows in parity 3 and parity 2. The cows in parity 3 had a comparatively average minimum weight of 564 Kgs but were not significantly different ($p > 0.05$) from the cows in parity 2 which weighed 537 Kg. After day 19, parity 3 and 4 had a discernible average weight gain while parity 2 fluctuated around the mean. In summary, parity 2 had a statistically significant($p < 0.05$) higher weight loss while cows in parity 4 had the highest average daily weight gain. The cows in different parities tend to have statistically and significantly different weights, a fact that is explained by the typical growth performance of cows in different parities. Cows in higher parities 3 or 4 tend to have significantly higher body weight than cows with lower parities. The results are supported by empirical studies which show that feed intake among lactating cows tends to increase up to a body weight of 750 kg and then stagnate or even decline based on the sub-genotypes of dairy cow breeds (Ledinek et al., 2019). A greater negative energy balance (NEB) is experienced by post–parturient cows lead to a daily loss of about 0.5 kg of body weight (Montoya et al., 2017).

The differences between dietary intake and outlay determines the nutrient balance and a negative energy balance (NEB) only occurs when the endogenous energy reserves are depleted. Higher weight loss is positively associated with the highest lactation performance indicating that cows with highest milk production tend to have greater weight loss (Poncheki et al., 2015). The severity and length of a NEB is largely shaped by dry matter intake (DMI) immediately after parturition (Rodney et al., 2018) and any significant drop in DMI exacerbates the energy deficiency while enhances lipolysis with the consequent generation of NEFA and this overwhelms the local oxidative capacities. Further, non- esterified fatty acid (NEFA) and β-hydroxybutyrate(βHBA) has an inhibiting effect on immunity function (McArt et al., 2012).

The concomitant metabolic load exceeds the adaptive capacities of lactating cows resulting in an elevated risk of metabolic complications (Hernández-Castellano *et al*. 2017). The metabolic load arises because of the increasing lactational performance of dairy cows with commensurate or higher nutritional requirements (Gross & Bruckmaier, 2019). The metabolizable energy of a lactating cows support that rumen microbial activity which increases the efficiency of microbial protein production and protein utilization (Rodney et al., 2018).

Body weight in post-parturient cattle is affected by factors such as parity, stage of lactation, frame size, gestation, and breed (Roche et al., 2013), however, significant differences in the body weight of a lactating cow is attributable to the genetic makeup which contributes up to 60% of the variation in weight with cow-related factors being; age within parity, breed or genetic merit, calving season, calving weight heterosis, and calving year (Roche et al., 2013). It is also important to attribute differences in the weight gain/loss to parity levels as cows with higher parities tending to experience lower comparable weight losses(Ledinek et al., 2019). Higher milk production has been linked to weight loss but this is inconsequential because of the influence of nutritional diets (Boudelal & Niar, 2020).

The study findings showed that NEB occurred immediately after parturition and ended in week 3 as the feed intake more than doubled. This finds support in Gross et al., (2011) indicated that physiological NEB peak between the 4th and 8th week post-parturient, however, the study cautioned that NEB may last up to the 14th week. However, Lean et al., (2023) observed that the lactation-induced NEB may occur later during lactation due insufficient feed intake and/or low feed quality. Further, Boudelal and Niar, (2020) indicated that the weight loss occurs up to the 4th week post–parturient until the cow passes the natural period of insulin resistance.

The early post-parturient period is specifically known for adipose tissue metabolism. On-going lipolysis is characterized by the increase blood NEFA) and the losses in body weight and body condition score (BCS) (Kenéz et al., 2015). Studies have approximated around 120 kg of lipids in visceral and subcutaneous adipose tissue (Raschka et al., 2016) which may be mobilized to compensate for the NEB (Gross, 2022). Further, lipid mobilization may be derived from skeletal muscle proteins to form a key source of Amino Acids for milk production in early lactation period (Sadri et al., 2016; Yang et al., 2020). Simultaneously the reduced DMI coupled with transient NEB is accompanied by concomitant lipid mobilization to maintain the metabolic energy requirements of a lactating cow more than milk production (Gross, 2022).

The catabolic process associated with an intense lipid mobilization results in well-defined metabolic and physiological processes geared towards the maintenance of sufficient energy requirements for sustaining life processes and milk production. This cycle of lipid mobilization and storage is a typical metabolic phenomenon in a lactating cows that is necessary as it induces extensive modification of adipose tissue (Kenéz et al., 2015). When lipolysis occurs at an excessive levels, it aggravated metabolic load will likely result in the development of ketosis and steatosis (Gross, 2022).

Over-conditioning/steaming up of cows as a herd management of cows during the peri-parturient period does not equate to a larger reservoir of lipids in body tissue, as it adversely affects animal health after parturition as over-conditioned mobilizes substantially lipid tissues when compared to unconditioned leaner cows (Locher et al., 2015; Schuh et al., 2019). This scenario was observed by Moraes et al., (2015) who noted that high-producing lactating cows with genetically higher BW tend to consume higher DMI and have lower back fat thickness in comparison to cows with medium or low genetic traits. However, it is these high-producing lactating cows that tend to have higher NEB leading to higher BW loss during the post-parturient period.

5.1.2 Milk Yield of the post – parturient Friesian cows

The observation shows that cows in parity 4 had significantly ($p < 0.05$) the highest milk yield until day 20 when the milk production curve stabilized while cows with parity 2 and 3 were significantly different from one another ($p < 0.05$) until day 16 when different became insignificant. The study observed an average of between 27 and 29 Kgs of Milk produced at day 30 which is indicative of the progress of the lactating cow. Apelo et al., (2014a) observed that the average milk production per US Holstein Friesian lactating cows was 34.5 ± 0.3 kg/day. The differences between the study and literature could be attributable to the differences in the nutritional composition and as observed by Buza and Holden, (2016) a 60:40 forage-to-concentrate ratio given to US dairy cattle result in an average daily milk yield of between 31.9 ± 5.1 Kg and 33.1 ± 4.61 Kg. Considering the average milk production for the cows in the study fell within the range of the US dairy industry (Buza & Holden, 2016), then it can be inferred that the cows in the study had a comparable sufficient nutritional requirement for a lactating cow.

It has been observed from extant literature that a lactating cow can sustain milk production at a highest level despite the NEB. Thus, higher milk yield during the post-parturient period requires high amounts of energy and nutrients with the additional energetic requirements for milk production being assumed to range between 50 and 60 kg/d for a 650-kg cow which is about four- to five-fold increase in the recommended energy and nutrient supply for lactating post-parturient dairy cows (Gross, 2022). AS indicated by literature, there is a significant efficient mobilization of adipose tissue to support milk production (Moraes et al., 2015).

Blood glucose levels is a constraining factor for milk production as 85% of the glucose primarily supports the mammary gland in milk synthesis (Thompson-Crispi et al., 2014) and therefore, low glucose concentrations at the onset of lactation are complemented by hepatic gluconeogenesis and glycogenolysis (Hernández-Castellano et al., 2017) to support increase milk production (Hötger et al., 2013). As glucose concentrations surge lipolysis is reduced (Grossen-Rösti et al., 2018; Malacco et al., 2020) as the cow prioritise milk production. However, milk protein declines due to the energy and protein deficiency due to feed restriction during the transition period (Gross et al., 2011).

The concomitant prioritization of milk production after parturition tend to inhibits the reuptake of glucose and fatty acid into adipose tissue (Gross, 2022). Thus, fatty acids are eliminated from blood stream through the formation of ketone bodies such as acetone and βHBA. Simultaneously, the physiological limits of milk production during the post-parturient period due to ketosis as the cows tend to exhibit depressed DMI with consequent reduction in milk production (Raboisson et al., 2014) as ketosis in lactating cows is as a result of excessive βHBA arising from the inability of the liver to oxidise all the mobilised NEFA (Zarrin et al., 2014). Consequently, the delayed endocrine adaptation to calcium requirements during the post-parturient period may result in hypocalcemia that reduced DMI and milk yield, and elevated risk diseases such as ketosis, displaced abomasum, and mastitis (Venjakob et al., 2017).

5.1.3 Hypothesis One: Parity and weight change and milk yield

Since the F- test result was statistically significant, $F (2, 267) = 8.27$, $p = 0.001$, the study finding therefore rejects the null hypothesis that parity has no significant effect on milk yield of post-parturient Friesian Cows and concluded that parity levels of the lactating cows have a significant influence on milk production of a lactating Friesian cow in the immediate post – parturient period.

The hypothesis is explained by the correlations between milk production and BCS (Ledinek et al., 2019) and BW (Rodney et al., 2018). The differences in milk productivity among the cows with different parities are attributable to the parities and higher weight losses (Ledinek et al., 2019). This suggests a curvilinear association between body condition and milk production. An increasing BCS up to 3.5 on a scale of 5 leads to a substantial increase in the milk and fat yield of the cows with compensatory availability of energy for the cow. The body condition score and the body weight loss in the post-parturient period is associated with the productive and reproductive health of the cow (Boudelal & Niar, 2020). Further, correlational studies show that cows that have a higher weight loss tend to have highest milk production (Roche et al., 2013) with reduce reproductive performance (Rodney et al., 2018).

Instantly after parturition, milk production is accompanied by changes in blood metabolites (Ayoub & Allam, 2015). During NEB, key hormones promote increase lipolysis and reduces lipogenesis, thus optimizing NEFA production to maintain physiological equilibrium. The net result is the mobilization of lipid in the adipose tissue to cover for the energy deficit (Boudelal & Niar, 2020). The onset of lactation increases the demand for minerals such as calcium (Hernández Castellano et al. 2017) and usually, several kilograms of calcium is stored in the skeleton and during the post-parturient period, the abrupt demand for calcium mineral at the onset of lactation exceeds the circulating plasma calcium pool which consist of only a few grams (Goff, 2014). Increasing energy supply enhanced the quantity and composition of the milk and that the energy balance corresponds to the change in bodyweight and BCS (Gruber et al., 2014).

5.2 Objective Two: Serum hormonal levels and feed intake of post – parturient Friesian Cows

The nutritional influences during the transition period to parturition is multifactorial and complex, thus, nutrient availability and uptake influence energy partitioning and milk production (Rodney et al., 2018).

5.2.1 Dietary Requirements of post – parturient Friesian Cows

In the study, the nutritional diets significantly differed ($p< 0.05$) with Betan Farm seemingly having higher nutritional requirements in terms of Ash, CP, EE contents and metabolizable energy. Based on the nutritional information, the study estimated the daily metabolizable energy requirements based on earlier estimates of daily metabolizable energy requirements. The study observed a minimum of between 33.45 $MJ \cdot day^{-1}$ for Elso Farm, 35.55 $MJ \cdot day^{-1}$ for Elfam 45 $MJ \cdot day^{-1}$ for Betan Farm assuming a minimum average of 15 Kgs of DMI as consumed by cows with parity two immediately after parturition. This would signify a NEB for cows in Elso Farm and Elfam but a positive energy balance in Betan Farm, fact that may be attributable to NEB or through the stress related to parturition as observed by the cortisol levels.

Considering that a lactating cows weighing between 350 to 400 Kg require daily metabolizable energy requirements of between 39.96 and 44.99 $MJ \cdot day^{-1}$ for their daily maintenance energy requirements and that the metabolizable energy requirement for one Litre of milk is approximately 4.69 $MJKg^{-1}$ per unit of DM with a daily ME of 8.17 $MJKg^{-1}$ per unit of DM (Montoya et al., 2017).

However, the highest CP content was 9.65% which is comparatively lower for the nutritional requirements of the lactating cow which is about 14.3% CP to allow for an adequate ruminal bacterial growth and efficient fermentation of structural carbohydrates with a concurrent production of significant quantities of microbial protein (Montoya et al., 2017). Apelo et al., (2014b) observed that the average CP content in the diets for the dairy cows in US farms is about 17.8 ± 0.1% CP with a mean gross N efficiency of 24.7 ± 3.99%. The dietary CP content for immediate post-parturient lactating cows > 14.7% (Montoya et al., 2017). To begin with, the peri-parturient period requires an increase of 20–25% in nutrient and energy requirements for sustenance and pregnancy maintenance (Hernández Castellano et al. 2017).

Montoya et al., (2017) observed that lactating cows in the immediate post-parturient period require NDF at almost 0.87-1.0% of BW and this study, the figures would translate to a minimum of 4.67 Kgs for cows in parity two and a maximum of 6.4 Kgs for cows in parity four and as per the diets, the average lowest NDF offered by farms ranged between 7.989 Kgs to about 8.79 Kgs immediately after parturition. These figures satisfy the NDF nutritional requirement for the lactating cow. Usually, high quality forages have low NDF levels with commensurate high DMI while higher-energy forages have lower ADF levels. Empirical evidence shows that concentrates offer sufficient nutritional requirements for lactating cows, however, excess concentrates tend to increase feed costs (Buza & Holden, 2016).

Thus, higher dietary NDF% levels increase endogenous N excretion while higher DMI increases the passage rates while suppressing digestibility (Balance & Gas, 2023). The DMI with the plant fibres such as ADF and NDF are biodegraded by the microbes to produce metabolizable energy, while microbial growth is supported by dietary crude protein (CP) and minerals (Gross, 2022). Empirical evidence shows that lactating dairy cows rely on adipose tissue reserves for milk production during the post-parturient period (Friggens et al., 2017).

Trends in feed intake for a lactating cow is largely influenced by the BCS at parturiency (Boudelal & Niar, 2020) and later on explained by physiological changes during the post-parturient period (Jensen & Proudfoot, 2017). The low DMI, which gradually rises through two or three weeks into peak lactational performance is as a result of increased lactational nutritional requirements (Montoya et al., 2017). Thus, different nutritional strategies impact lactational performance as the energy requirements of a dairy cow tend to increase two- to three-fold from 21-day periparturient to 21-day post-parturient period (Gruber et al., 2014). Increasing the concentration in a diet to 55% has no negative effect on body weight and increases milk yield while a low forage-to-concentrate ratio of 35:60 tends to increases milk yield and protein content but decreases fat content (Buza & Holden, 2016).

Before parturition, cows reduce their DMI and at parturition, there is further reduction in DMI that is coupled with lactational demands accounting for the high nutritional requirements in terms energy, protein and minerals (Montoya et al., 2017). The nutrient availability before and after parturition is linked to the DMI but also by the endogenous lipid reserves in the body tissue as reflected by the alterable body weight and BCS (Rodney et al., 2018). The cow's adaptive mechanisms largely influence the metabolism of adipose tissue which serves as the main energy storage organ. However, during the dry period and late lactation anabolic processes allows storage of TAG in the adipose tissues (Kenéz et al., 2015).

5.2.2 Feed Intake by post – parturient Friesian Cows

The study observed a gradual increase in average feed intake from the day 1 from 15 kg of DMI for cows with parity two to 17 Kgs for cows with parity four immediately after parturition and a progressive and gradual increase in average feed intake to 35 Kg in the day 30. The gradual increase in the feed intake could be explained by the cow's physiological state and nutrient availability as the lactating cows continuously adapts its energy metabolism to suit varying energy requirements (Kenéz et al., 2015). Thus, dairy cows with higher BCS slowly increase their DMI and achieve a maximum DMI between 12 to 16 weeks into lactation (Jorritsma et al., 2013).

While effective rumen activity requires a balance of ADF and NDF content (Zebeli et al., 2012), only high-quality pastures and forages than can support milk production levels of around 30 kg/d as high milk production is accompanied by considerable lipid mobilization supported by concentrate supplementation (Zbinden et al., 2017). In contrast, high energy diets (starch-based concentrates) relative to the forage content (> 60%) may result in (subclinical) rumen acidosis (SARA) which consequently retards rumination activity i.e., DMI and rumen passage rate (Neubauer et al., 2020; Humer et al., 2018).

5.2.3 Hypothesis Two: Serum hormone levels and feed intake

Since the correlation statistic indicated that feed intake was statistically and positively correlated with prolactin hormone ($r = 0.760$, $p < 0.01$), Oestrogen hormone ($r = 0.785$, $p < 0.01$), and IGF-α1 ($r = 0.692$, $p < 0.01$) while negatively correlating with cortisol hormone($r = -0.613$, $p < 0.01$), the study finding therefore rejects the null hypothesis that serum hormone changes has no significant effect on feed intake of post – parturient

Friesian Cows and concluded that serum hormone changes have a significant influence on diet levels of post – parturient Friesian Cows.

The finding to the hypothesis is explained by both metabolic and physiological changes which imposes metabolic and lactational load on a dairy cow in the immediate post-parturient period. Milk production after parturition requires sudden increase in the nutritional and energy requirements for milk production and milk production surges further despite energy deficiency (Hernández-Castellano et al., 2017). Initial milk production after parturition is associated generally with the amounts and peak values of prolactin hormone (Zhang et al., 2024) which then interacts with IGF-1 to mobilize lipolytic activity in adipose tissue in the post-parturient period (Bisinotto et al., 2018).

The dietary composition alters the available nutrients for tissue regeneration and milk synthesis (Moraes et al., 2015) and thus cows in immediate post-parturient period derive their nutritional requirements from the DMI and require greater Metabolizable Protein, Energy, and minerals than cows in early lactation translating to NEB (Montoya et al., 2017). These changes in BCS and body weight serve as proxies for the state of energy balance in the lactating cow in addition to reflecting the nutrient balances between energy, minerals, protein, specific fatty acids and vitamins (Rodney et al., 2018).

5.3 Objective Three: Parity and serum hormonal levels of post – parturient Friesian Cows

5.3.1 Serum Hormonal profiles of post – parturient Friesian Cows

Based on the trends in the levels of serum IGF-α1, the cows with parity 2 will more likely return to regular growth rates than those in parity 3 and parity 4. Various hormone profiles including IGF-α1 are dramatically modified by the onset of lactation period in order to provide an adaptive feedback to modulate the metabolic changes (Singh et al., 2014). However, IGF-α1 like other hormones is also involved in the follicular development and the renewal of the cyclic oestrus activity, and any alterations during the adaptative metabolic mechanism negatively influence the ovarian activity (Kawashima et al., 2012).

At parturition, the cows had low serum prolactin levels at an average of 84.3 $ngmL^{-1}$ with concurrent NEB as indicated by figure 4.2, and low feed intake in figure 4.4 because of serum cortisol. Prolactin, a hormone crucial for milk secretion during lactation, plays a multifaceted role in the development, differentiation, and functioning of mammary tissues, as well as in supporting the corpus luteum's function (Henricks et al., 2012). Prolactin directly affects mammary gland functions but the responsiveness of the mammary gland to prolactin appears to be modulated by local and systemic factors (Zhang et al., 2024). The basal prolactin concentration is affected by the environment and changes throughout the year without similar changes in milk yield suggesting that the mammary gland adapts to the prolactin levels (Tong et al., 2018).

The trends in the levels of oestrogen hormone indicate that cows of parity 2 tend to return to the oestrus cycle earlier than those in parity 3 and parity 4. An increase in the oestrogen concentration during the periparturient period in dairy cattle is associated with the development and increased enzymatic activity of the mammary gland. A higher concentration of estrone sulphate and other oestrogens have been observed in mid-pregnancy in cattle until parturition. This also happens in concert with the rapid surge in serum prolactin levels in the last two weeks of pregnancy (Kurpińska & Skrzypczak, 2020). In the 14 days to parturition and at parturition the serum oestrogen concentration rapidly increases and the highest levels are recorded on parturition and declines thereafter (Kurpińska & Skrzypczak, 2020).

5.3.2 Hypothesis Three: Parity and serum hormone levels

Since the F- statistic was statistically significant, $F (2, 44) = 10.55$, $p = 0.001$, the study finding, therefore, rejects the null hypothesis that parity has no significant effect on hormone changes of Post – parturient Friesian Cows and concluded that parity levels have a significant influence on the serum hormonal profile of a lactating Friesian cow in the immediate post – parturient period

At parturition, the cows encountered higher stress levels as indicated by elevated serum cortisol levels at 154.6 $ngmL^{-1}$ with concurrent NEB as indicated by figure 4.2, and low feed intake in figure 4.4 that was plateauing by day 12 and then gradually increasing to an optimum amount by day 25 as the serum IGF-α1 levels rise to new levels of 261.8 $ngmL^{-1}$.

The findings indicate that serum cortisol concentration levels declined immediately at parturition and remained at a low level until the first oestrus. While there is a surge in plasma prolactin concentration prior to parturition, the blood cortisol concentration peaks at parturition time (Gao et al., 2023). The cortisol concentrations tend to peak sharply at parturition and then declines to the basal level at peri-parturient concentrations after 3-5 days into lactation and then significantly decline around 10 days into post-parturition period (Forslund et al., 2010). The NEB in high-productive cows tend to lead to elevate cortisol release to induce protein catabolism and gluconeogenesis. Elevated cortisol concentration levels for a lactating cow may be associated with physiological burdens such as metabolic stress and/or inflammation complication such as subclinical mastitis (Otten et al., 2023).

Insulin Growth Factor (IGF) and binding proteins are important to mammary development and function as both Insulin Growth Factor – 1 (IGF-1) and Insulin Growth Factor – II (IGF-II) regulate cell function, growth and differentiation as well as and prevention of cell apoptosis (Akers, 2016). Insulin and IGF-1 concentrations decline shortly before parturition as the growth hormone (GH) are released with the concomitant mobilisation of lipid of adipose tissue. The decline in insulin and IGF-1 initiate lipolysis, while simultaneously inhibiting lipogenesis (Hernández Castellano et al., 2017) as the systemic IGF-1 has linked to milk yield and milk fat concentrations (Burgos & Cant, 2010).

Prolactin concentration peaks at parturition as the suppression of prolactin prevents lactogenesis however, basal prolactin concentration, is affected by the environmental changes without any drastic change in milk production (Ponchon et al., 2017).

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Based on the findings, the study makes the following conclusions;

i. The study found parity to affect milk production in cows. The cattle in parity four produced higher amount of milk on average than cows in parity three or two. Lactating cows in the immediate post – parturient period undergo weight loss across all parities, which is attributed to reduced dry matter intake and increasing milk production despite the current negative energy balance. Since Considering the finding the study concludes that parity has a significant effect on milk yield with cows having higher parities of four or five having higher milk production on average.

ii. The study observed a positive effect of serum hormone levels on feed intake of the lactating Friesian cows in the immediate post – parturient period. The levels of serum prolactin, oestrogen and IGF-α1 hormones gradually rose after parturition with commensurate increase of the amount of feed consumed by the cow. But the levels of serum cortisol levels reduced gradually.

iii. The study observed variations in serum hormone levels according to parity. Serum Cortisol levels were lower for Parity 4 and 3, but high for cows in parity 2. The study concludes that the parity levels have a significant influence on the serum hormonal profile of the lactating Friesian cows in the immediate post parturient period. The serum cortisol plays a significant role in determining the physiological state of a lactating cows.

6.2 Recommendation

6.2.1 Recommendation for the study

Since parity levels has a causal effect on milk yield of a lactating cows, there is need for the farmers to improve and refresh their herd management techniques in order to maximize the productive and reproductive potential of the heifers and lactating cows.

Since the hormonal profile of a lactating cows influence the cow's physiological state during lactation period, the farmers should improve or alter the nutritional diets to accommodate the changing physiological needs of the post – parturient lactating cow. Each physiological state of the cow should be accompanied by appropriate nutritional requirement such as increase in the protein content to an average of 15 %, altering the ratio of concentrate to forage to 60:35 to accommodate the peri-parturient to post – parturient period and reengineering the said ratios of protein to less than 10 % during the late lactation to improve on the nutritional efficiencies of the dairy cow.

Since the parity levels, feed intake and hormone levels interact and are mutually dependent on each other, there is need for the farmers to intensify the feed intake through improve feed palatability to support and maintain the growth profiles of the cows.

6.2.2 Recommendation for further studies

The study was limited in sample size of nine cows because of the limitations in the form and nature of the experiment(lactational cycles take a whole year) and therefore its findings cannot be generalized to large population buts serves as an important guide to large experimental sample.

Since the study was carried out over a 30 – days period, the inference is limited to the short period and may not be indicative of the long – term physiological state of the cow. The measurement of the hormones within the 30 – day period is dynamic and therefore other studies may examine the hormone profile of the cows immediately after parturition until the dry period to determine the hormone profiles.

Since the study only capture the serum hormone profiles of the only cortisol, prolactin, oestrogen and IGF-α1 hormones, other studies may measure the state of the energy balance in the lactating cow and the hormonal interplay of other hormones like the insulin, progesterone and other significant hormones in concert with dynamic metabolic changes during the early lactation till dry periods.

Since the study only used standard feeding arrangements, other studies may measure the feeding efficiency of the dairy cows in early lactation period.

REFERENCES

Akenga, T. S. (2018). A Survey on Use of Agrochemicals in Farms in Uasin Gishu and Homa Bay Counties, Kenya. *Africa Environmental Review Journal, 2*(2), 1-11.

Akers, R. M. (2016). Major advances associated with hormone and growth factor regulation of mammary growth and lactation in dairy cows. *Journal of dairy science*, *89*(4), 1222-1234.

Albrecht, K. A., Marten, G. C., Halgerson, J. L., & Wedin, W. F. (1987). Analysis of Cell-Wall Carbohydrates and Starch in Alfalfa by near Infrared Reflectance Spectroscopy 1. *Crop science*, *27*(3), 586-588.

Al-Chalabi, M., Bass, A. N., & Alsalman, I. (2018). Physiology, prolactin. StatPearls Publishing, Treasure Island (FL);. PMID: 29939606.

Anderson, L. L., Perezgrovas, R., O'Byren, E. M., & Steinetz, B. G. (2019). Biological actions of relaxin in pigs and beef cattle. *Annals of the New York Academy of Sciences*, *380*(1), 131-150.

AOAC International(2019). Official Methods of Analysis. 21st ed. Association of Official Analytical Chemists., Washington, D.C.

Apelo, S. A., Knapp, J. R., & Hanigan, M. D. (2014a). Invited review: Current representation and future trends of predicting amino acid utilization in the lactating dairy cow. *Journal of Dairy Science*, *97*(7), 4000-4017.

Apelo, S. A., Bell, A. L., Estes, K., Ropelewski, J., De Veth, M. J., & Hanigan, M. D. (2014b). Effects of reduced dietary protein and supplemental rumen-protected essential amino acids on the nitrogen efficiency of dairy cows. *Journal of Dairy Science*, *97*(9), 5688-5699.

Ayoub, A. G., & Allam, A. T. (2015). Alterations of Lipid and Mineral Metabolism during Late Pregnancy and Early Lactation in Holstein–Friesian cows. *Egyptian Journal of Chemistry and Environmental Health*, *1*(1), 883-898.

Balance, N. D., & Gas, G. (2023). Level and pattern of crude protein feeding: effects on rumen fermentation, nitrogen balance, nutrient digestibility, and greenhouse gas emissions. *Research in protein nutrition of lactating dairy cows and animal and dairy sciences education*, 65.

Bisinotto, R. S., Greco, L. F., Ribeiro, E. S., Martinez, N., Lima, F. S., Staples, C. R., ... & Santos, J. E. P. (2018). Influences of nutrition and metabolism on fertility of dairy cows. *Animal Reproduction (AR)*, *9*(3), 260-272.

Boer, H. M. T., Veerkamp, R. F., Beerda, B., & Woelders, H. (2013). Estrous behavior in dairy cows: identification of underlying mechanisms and gene functions. *Animal*, *4*(3), 446-453.

Boudelal, S., & Niar, A. (2020). Risk factors associated with reproductive disorders in dairy cows in Algeria. *Journal of the Hellenic Veterinary Medical Society*, *71*(2), 2213-2218.

Bradford, A. P., Jones, K., Kechris, K., Chosich, J., Montague, M., Warren, W. C., ... & Polotsky, A. J. (2015). Joint MiRNA/mRNA expression profiling reveals changes consistent with development of dysfunctional corpus luteum after weight gain. *PLoS One*, *10*(8), e0135163.

Broderick, G. A. (2003). Effects of varying dietary protein and energy levels on the production of lactating dairy cows. *Journal of dairy science*, *86*(4), 1370-1381.

Bruckmaier, R. M., & Gross, J. J. (2017). Lactational challenges in transition dairy cows. *Animal Production Science*, *57*(7), 1471-1481.

Brun-Lafleur, L., Delaby, L., Husson, F., & Faverdin, P. (2010). Predicting energy× protein interaction on milk yield and milk composition in dairy cows. *Journal of Dairy Science*, *93*(9), 4128-4143.

Burgos, S. A., & Cant, J. P. (2010). IGF-1 stimulates protein synthesis by enhanced signaling through mTORC1 in bovine mammary epithelial cells. Domestic animal endocrinology, 38(4), 211-221.

Buza, M. H., & Holden, L. A. (2016). A survey of feeding management practices and by-product feed usage on Pennsylvania dairy farms. *The Professional Animal Scientist*, *32*(2), 248-252.

Cardoso, F. C., Kalscheur, K. F., & Drackley, J. K. (2020). Symposium review: Nutrition strategies for improved health, production, and fertility during the transition period. *Journal of Dairy Science*, *103*(6), 5684-5693.

Ceriani, R. L. (2004). Hormones and other factors controlling growth in the mammary gland: A review. *Journal of Investigative Dermatology*, *63*(1), 93-108.

Chapinal, N., LeBlanc, S. J., Carson, M. E., Leslie, K. E., Godden, S., Capel, M., ... & Duffield, T. F. (2012). Herd-level association of serum metabolites in the transition period with disease, milk production, and early lactation reproductive performance. Journal of dairy science, 95(10), 5676-5682.

Chen, Z. H., Broderick, G. A., Luchini, N. D., Sloan, B. K., & Devillard, E. (2011). Effect of feeding different sources of rumen-protected methionine on milk production and N-utilization in lactating dairy cows. *Journal of dairy science*, *94*(4), 1978-1988.

Churakov, M., Karlssona, J., Rasmussena, A.E and Holteniusa, K. (2021). Milk fatty acids as indicators of negative energy balance of dairy cows in early lactation. Animal. 15(7):100253.

Cincović, R. M., Belić, B., Radojičić, B., Hristov, S., & Đoković, R. (2012). Influence of lipolysis and ketogenesis to metabolic and hematological parameters in dairy cows during periparturient period. Acta veterinaria, 62(4), 429-444.

Cohick, W. S. (1998). Role of the insulin-like growth factors and their binding proteins in lactation. *Journal of Dairy Science*, *81*(6), 1769-1777.

Convey, E.M. (2003). Blood hormone concentrations in ruminants during mammary growth, lactogenesis, and lactation: a review. Journal of Dairy Science, 57, 905–917.

Correa, S. M., Newstrom, D. W., Warne, J. P., Flandin, P., Cheung, C. C., Lin-Moore, A. T., ... & Ingraham, H. A. (2015). An oestrogen-responsive module in the ventromedial hypothalamus selectively drives sex-specific activity in females. *Cell reports*, *10*(1), 62-74.

Dann, H. M., & Nelson, B. H. (2011). Early lactation diets for dairy cattle–focus on starch. *Department of Animal Science at the New York State College of Agriculture and Life Sciences (A Statutory College of the State University of New York) Cornell University*, *46*.

Dann, H. M., Litherland, N. B., Underwood, J. P., Bionaz, M., D'angelo, A., McFadden, J. W., & Drackley, J. K. (2006). Diets during far-off and close-up dry periods affect periparturient metabolism and lactation in multiparous cows. *Journal of dairy science*, *89*(9), 3563-3577.

Desrivieres, S., Prinz, T., Laria, N. C. P., Meyer, M., Boehm, G., Bauer, U., chäfer, J., Desrivières, S., Prinz, T., Laria, N. C. P., Meyer, M., Boehm, G., Bauer, U., ... & Groner, B. (2003). Comparative proteomic analysis of proliferating and functionally differentiated mammary epithelial cells. *Molecular & Cellular Proteomics*, *2*(10), 1039-1054.

Djoković, R., Cincović, M., Kurćubić, V., Petrović, M., Lalović, M., Jašović, B., & Stanimirovic, Z. (2014). Endocrine and metabolic status of dairy cows during transition period. *The Thai Journal of Veterinary Medicine*, *44*(1), 59-66.

Djoković, R., Cincović, M., Belić, B., Toholj, B., Davidov, I., & Hristovska, T. (2015). Relationship between blood metabolic hormones, metabolites and energy balance in Simmental dairy cows during peripartum period and lactation. Pakistan Veterinary Journal, 35(2): 163-167

Djokovic, R., Kurcubic, V., Ilic, Z., Cincovic, M., Lalovic, M., Jasovic, B. and Bojkovski, J. (2017). Correlation between blood biochemical metabolites milk yield, dry matter intake and energy balance in dairy cows during early and mid-lactation. Advanced Diabetes Metabolism. 5(2): 26-30.

Drackley, J. K., & Cardoso, F. C. (2014). Prepartum and postpartum nutritional management to optimize fertility in high-yielding dairy cows in confined TMR systems. *Animal*, *8*(s1), 5-14.

Endo, N., Kitamura, T., Okubo, M., & Tanaka, T. (2019). Hair cortisol concentration in pre-and postpartum dairy cows, and its association with body condition, hock health, and reproductive status. *Animal Science Journal*, *90*(8), 924-931.

Edgerton, L.A., Hafs, H.D. (2003). Blood luteinizing hormone, prolactin, glucocorticoid, and progestin in dairy cows from calving to gestation. Journal of Dairy Science, 56, 451–458.

Forslund, K. B., Ljungvall, Ö. A., & Jones, B. V. (2010). Low cortisol levels in blood from dairy cows with ketosis: a field study. *Acta Veterinaria Scandinavica*, *52*(1), 31.

Friggens, N. C., Blanc, F., Berry, D. P., & Puillet, L. (2017). Deciphering animal robustness. A synthesis to facilitate its use in livestock breeding and management. *Animal*, *11*(12), 2237-2251.

Fukasawa, M., Tsukada, H., Kosako, T., & Yamada, A. (2008). Effect of lactation stage, season and parity on milk cortisol concentration in Holstein cows. *Livestock Science*, *113*(2-3), 280-284.

Fuller, M. (2012). Determination of protein and amino acid digestibility in foods including implications of gut microbial amino acid synthesis. *British Journal of Nutrition*, *108*(S2), S238-S246.

Gaiani, R., Chiesa, F., Mattioli, M., Nannetti, G., Galeati, G. (2014). Androstenedione and testosterone concentrations in plasma and milk of the cow throughout pregnancy. Journal of Reproduction and Fertility, 70, 55–59. .

Gao, J., Marins, T. N., Calix, J. O. S., Qi, Z., Bernard, J. K., & Tao, S. (2023). Hormonal and immunological responses of Holstein dairy cows from late lactation to the dry period and from the dry period to early lactation. *Domestic Animal Endocrinology*, *83*, 106790.

Goff, J. P. (2014). Calcium and magnesium disorders. *Veterinary Clinics: Food Animal Practice*, *30*(2), 359-381.

Götze, A., Honnens, A., Flachowsky, G., & Bollwein, H. (2010). Variability of mammary blood flow in lactating Holstein-Friesian cows during the first twelve weeks of lactation. *Journal of dairy science*, *93*(1), 38-44.

Gross, J. J. (2022). Limiting factors for milk production in dairy cows: perspectives from physiology and nutrition. *Journal of animal science*, *100*(3), skac044.

Gross, J. J., & Bruckmaier, R. M. (2019). Metabolic challenges in lactating dairy cows and their assessment via established and novel indicators in milk. *Animal*, *13*(S1), s75-s81.

Gross, J., van Dorland, H. A., Bruckmaier, R. M., & Schwarz, F. J. (2011). Performance and metabolic profile of dairy cows during a lactational and deliberately induced negative energy balance with subsequent realimentation. *Journal of dairy science*, *94*(4), 1820-1830.

Grossen-Rösti, L., Kessler, E. C., Tröscher, A., Bruckmaier, R. M., & Gross, J. J. (2018). Hyperglycaemia in transition dairy cows: Effects of lactational stage and conjugated linoleic acid supplementation on glucose metabolism and turnover. *Journal of animal physiology and animal nutrition*, *102*(2), 483-494.

Grove-White, D. (2015). Rumen Health in the Dairy Cow. *Bovine Medicine*, 297-304.

Gunnink, J. W. (2005). Pre-partum leucocytic activity and retained placenta. *Veterinary Quarterly*, *6*(2), 52-54.

Gruber, L., Urdl, M., Obritzhauser, W., Schauer, A., Häusler, J., & Steiner, B. (2014). Influence of energy and nutrient supply pre and post-partum on performance of multiparous Simmental, Brown Swiss and Holstein cows in early lactation. *Animal*, *8*(1), 58-71.

Grummer, R.R. (2005). Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. Journal of Animal Science, 73, 2820–2833.

Hafez, E.S.E. 2004. Page 196 in Reproduction in Farm Animals. 3rd ed. Lea & Febiger, Philadelphia, PA.

Handcock, R. C., Lopez-Villalobos, N., McNaughton, L. R., Back, P. J., Edwards, G. R., & Hickson, R. E. (2019). Positive relationships between body weight of dairy heifers and their first-lactation and accumulated three-parity lactation production. *Journal of dairy science*, *102*(5), 4577-4589.

Haugejorden, G., Waage, S., Dahl, E., Karlberg, K., Beckers, J. F., & Ropstad, E. (2006). Pregnancy associated glycoproteins (PAG) in postpartum cows, ewes, goats and their offspring. *Theriogenology*, *66*(8), 1976-1984.

Hendricks, D.M., Dickey, J.F., Hill, J.R., Johnston, W.E. (2002). Plasma oestrogen and progesterone levels after mating, and during late pregnancy and postpartum in cows. Endocrinology., 90, 1336–1342. Henrick, B. M., Nag, K., Yao, X. D., Drannik, A. G., Aldrovandi, G. M., & Rosenthal, K. L. (2012). Milk matters: soluble Toll-like receptor 2 (sTLR2) in breast milk significantly inhibits HIV-1 infection and inflammation. *PloS one*, *7*(7), e40138.

Hernández-Castellano, L. E., Hernandez, L. L., Sauerwein, H., & Bruckmaier, R. M. (2017). Endocrine and metabolic changes in transition dairy cows are affected by prepartum infusions of a serotonin precursor. *Journal of dairy science*, *100*(6), 5050-5057.

Herosimczyk., G., Waage, S., Dahl, E., Karlberg, K., Beckers, J.F., Ropstad, E. (2013) Pregnancy-associated glycoproteins (PAG) in postpartum cows, ewes, goats and their offspring. Theriogenology, 66, 1976–1984..

Horst, J. P., de Kloet, E. R., Schächinger, H., & Oitzl, M. (2012). Relevance of stress and female sex hormones for emotion and cognition. *Cellular and molecular neurobiology*, *32*(5), 725-735.

Hötger, K., Hammon, H. M., Weber, C., Görs, S., Tröscher, A., Bruckmaier, R. M., & Metges, C. C. (2013). Supplementation of conjugated linoleic acid in dairy cows reduces endogenous glucose production during early lactation. *Journal of Dairy Science*, *96*(4), 2258-227

Hubner, A., Canisso, I. F., Peixoto, P. M., Coelho Jr, W. M., Ribeiro, L., Aldridge, B. M., ... & Lima, F. S. (2022). Characterization of metabolic profile, health, milk production, and reproductive outcomes of dairy cows diagnosed with concurrent hyperketonemia and hypoglycemia. *Journal of Dairy Science*, *105*(11), 9054-9069.

Humer, E., Aschenbach, J. R., Neubauer, V., Kröger, I., Khiaosa-Ard, R., Baumgartner, W., & Zebeli, Q. (2018). Signals for identifying cows at risk of subacute ruminal acidosis in dairy veterinary practice. *Journal of animal physiology and animal nutrition*, *102*(2), 380-392.

Huzzey, J. M., Nydam, D. V., Grant, R. J., & Overton, T. R. (2011). Associations of prepartum plasma cortisol, haptoglobin, fecal cortisol metabolites, and nonesterified fatty acids with postpartum health status in Holstein dairy cows. *Journal of dairy science*, *94*(12), 5878-5889.

Ingvartsen, K. L., & Moyes, K. (2013). Nutrition, immune function and health of dairy cattle. Animal, 7(s1), 112-122

Ingvartsen, K. L., Dewhurst, R. J., & Friggens, N. C. (2003). On the relationship between lactational performance and health: is it yield or metabolic imbalance that cause production diseases in dairy cattle? A position paper. Livestock production science, 83(2-3), 277-308.

Jadav, P. V., Dhami, A. J., Patel, D. M., & Kavani, F. S. (2010). GnRH and its Applications in Bovine Reproduction. Journal of Advanced Research 1, 74–80.

Jaetzold, R. S. (2010). Farm Management Handbook of Kenya. *Natural Conditions and Farm Management Information; Annex Atlas of Agro-Ecological Zones, Soils and Fertilising by Group of Districts; Subpart B2: Central Province Kirinyaga County., II.*

Jensen, M. B., & Proudfoot, K. L. (2017). Effect of group size and health status on behavior and feed intake of multiparous dairy cows in early lactation. *Journal of dairy science*, *100*(12), 9759-9768.

Jorritsma, R., Wensing, T., Kruip, T., Vos, P., & Noordhuizen, J. (2013). Metabolic changes in early lactation and impaired reproductive performance in dairy cows. *Veterinary research*, *34*(1), 11-26.

Jung, S. R. (2003). Comparison of Kjeldahl and Dumas methods for determining protein contents of soybean products. *Journal of the American Oil Chemists' Society*, 1169-1173.

Kanchev, L.N., Dobson, H. (2006). Plasma concentration of androstenedione during the bovine estrous cycle. Journal of Endocrinology, 71, 351–354.

Kawashima, C., Matsui, M., Shimizu, T., Kida, K., & Miyamoto, A. (2012). Nutritional factors that regulate ovulation of the dominant follicle during the first follicular wave postpartum in high-producing dairy cows. *Journal of Reproduction and Development*, *58*(1), 10-16.

Kalita, M. K., Soren, S., Das, J., & Patowary, P. (2025). Lactation. *Elements of Reproduction and Reproductive Diseases of Goats*, 133-148.

Kenéz, Á., Kulcsár, A., Kluge, F., Benbelkacem, I., Hansen, K., Locher, L., ... & Huber, K. (2015). Changes of adipose tissue morphology and composition during late pregnancy and early lactation in dairy cows. *PloS one*, *10*(5), e0127208.

Kesler, D. J., Garvericka, H.A., Caudlea, A.B., Bierschwala, C.J., Elmorea, R.G., Youngquista, R.S. (2009). Testosterone Concentrations in Plasma of Dairy Cows with Ovarian Cysts. Journal of Dairy Science, 62.

Khani, H., & Alijani, S. (2013). Reproductive performance and blood metabolites concentration in Iranian Afshari ewes fed calcium salts of fatty acids (CSFA) in flushing period. *International journal of Advanced Biological and Biomedical Research*, *1*(6), 669-676.

Kindahl, H., Knudsen, O., Madej, A., & Edqvist, L. E. (2012). Progesterone, prostaglandin F-2 alpha, PMSG and oestrone sulphate during early pregnancy in the mare. *Journal of reproduction and fertility. Supplement*, *32*, 353-359.

Kitagawa, D.; Gouda, M.; Kirii, Y (2014). Quick evaluation of kinase inhibitors by surface plasmon resonance using single-site specifically biotinylated kinases. Journal of Biomolecular. Screen, 19, 453–461.

Kitilit, J. K., Cheruiyot, A. C., & Kios, D. K. (2016). Effects of negative energy balance on health and milk yield of lactating dairy cattle. *Egerton Journal of Science & Technology*, *15*.

Kothari, C. R. (2014). Research Methodology: Methods and Techniques. (4th ed.). New Delhi: New Age International Publishers.

Kurpinska., D.J., Garvericka, H.A., Caudlea, A.B., Bierschwala, C.J., Elmorea, R.G., Youngquista, R.S. (2016). Testosterone Concentrations in Plasma of Dairy Cows with Ovarian Cysts. Journal of Dairy Science., 62.

Kurpińska, A., & Skrzypczak, W. (2020). Hormonal changes in dairy cows during periparturient period. *Acta Scientiarum Polonorum Zootechnica*, *18*(4), 13-22.

Lacasse, P., Ollier, S., Lollivier, V., & Boutinaud, M. (2016). New insights into the importance of prolactin in dairy ruminants. *Journal of dairy science*, *99*(1), 864-874.

Lanctôt, S., Blouin, R., Thibault, C., & Lacasse, P. (2024). Effect of milk stasis on mammary gland involution and the microRNA profile. *Journal of Dairy Science*, *107*(9), 7435-7445.

Ledinek, M., Gruber, L., Steininger, F., Fuerst-Waltl, B., Zottl, K., Royer, M., ... & Egger-Danner, C. (2019). Analysis of lactating cows on commercial Austrian dairy farms: the influence of genotype and body weight on efficiency parameters. *Archives Animal Breeding*, *62*(2), 491-500.

Lean, I. J., Golder, H. M., LeBlanc, S. J., Duffield, T., & Santos, J. E. P. (2023). Increased parity is negatively associated with survival and reproduction in different production systems. *Journal of Dairy Science*, *106*(1), 476-499.

Leonardi, C., Stevenson, M., & Armentano, L. E. (2013). Effect of two levels of crude protein and methionine supplementation on performance of dairy cows. *Journal of dairy science*, *86*(12), 4033-4042.

Li, Y., Ding, H.Y., Wang, X.C., Feng, S.B., Li, X.B., Wang, Z., Liu, G.W. and Li, X.W. (2016). An association between the level of oxidative stress and the concentrations of NEFA and BHBA in the plasma of ketotic dairy cows. Journal of Animal Physiology and Animal Nutrition. 100(5): 844-851.

Litherland, N. B., Dann, H. M., & Drackley, J. K. (2011). Prepartum nutrient intake alters palmitate metabolism by liver slices from peripartal dairy cows. Journal of dairy science, 94(4), 1928-1940.

Locher, L., Häussler, S., Laubenthal, L., Singh, S. P., Winkler, J., Kinoshita, A., ... & Dänicke, S. (2015). Effect of increasing body condition on key regulators of fat metabolism in subcutaneous adipose tissue depot and circulation of nonlactating dairy cows. *Journal of dairy science*, *98*(2), 1057-1068.

Macmillan, K., Gobikrushanth, M., Behrouzi, A., Hoff, B., & Colazo, M. G. (2021). Prevalence of early postpartum health disorders in Holstein cows and associations with production, reproduction, and survival outcomes on Alberta dairy farms. *The Canadian Veterinary Journal*, *62*(3), 273.

Malacco, V. M. R., Erickson, M., Cardoso, F. F., Biese, B. P., Laguna, J. G., & Donkin, S. S. (2020). Effect of glucose infusion dose and stage of lactation on glucose tolerance test kinetics in lactating dairy cows. *Journal of dairy science*, *103*(8), 7547-7554.

McArt, J. A. A., Nydam, D. V., & Oetzel, G. R. (2012). Epidemiology of subclinical ketosis in early lactation dairy cattle. *Journal of dairy science*, *95*(9), 5056-5066.

McArt, J. A. A., & Neves, R. C. (2020). Association of transient, persistent, or delayed subclinical hypocalcemia with early lactation disease, removal, and milk yield in Holstein cows. *Journal of dairy science*, *103*(1), 690-701.

Melendez, P., Poock, S. E., Pithua, P., Pinedo, P., Manriquez, D., Moore, S. G., ... & Taylor, J. F. (2019). Genome-wide study to detect single nucleotide polymorphisms associated with visceral and subcutaneous fat deposition in Holstein dairy cows. *Animal*, *13*(3), 487-494.

Mezzetti, M., Cattaneo, L., Passamonti, M. M., Lopreiato, V., Minuti, A., & Trevisi, E. (2021). The transition period updated: A review of the new insights into the adaptation of dairy cows to the new lactation. *Dairy*, *2*(4), 617-636.

Mishra, S., & Gupta, A. (2018). Chapter 7 - Experimental Design and Response Surface Analysis. *Applied Statistical Modeling and Data Analytics*, 169-193.

Montgomery, D. C. (2021). *Design and analysis of experiments* (10th ed. ed.). John Wiley & Sons.

Montoya, E. S., Chará, J. D., & Barahona-Rosales, R. (2017). The nutritional balance of early lactation dairy cows grazing in intensive silvopastoral systems. *Ciência Animal Brasileira*, *18*, e40419.

Moraes, L. E., Kebreab, E., Strathe, A. B., Dijkstra, J., France, J., Casper, D. P., & Fadel, J. G. (2015). Multivariate and univariate analysis of energy balance data from lactating dairy cows. *Journal of Dairy Science*, *98*(6), 4012-4029.

Mullen, M. P., Berry, D. P., Howard, D. J., Diskin, M. G., Lynch, C. O., Giblin, L., ... & Waters, S. M. (2011). Single nucleotide polymorphisms in the insulin-like growth factor 1 (IGF-1) gene are associated with performance in Holstein-Friesian dairy cattle. Frontiers in genetics, 2, 3.

Muro, B. B., Carnevale, R. F., Leal, D. F., Almond, G. W., Monteiro, M. S., Poor, A. P., ... & Garbossa, C. A. (2023). The importance of optimal body condition to maximise reproductive health and perinatal outcomes in pigs. *Nutrition research reviews*, *36*(2), 351-371.

Naylor, R. L. (2021). A 20-year retrospective review of global aquaculture. *Nature, 591* (7851), 551-563.

Nedić, S., Pantelić, M., Vranješ-Đurić, S., Nedić, D., Jovanović, L., Cebulj-Kadunc, N., ... & Kirovski, D. (2017). Cortisol concentrations in hair, blood and milk of Holstein and Busha cattle. *Slovenian Veterinary Research*, *54*(4), 163-172.

Neubauer, V., Petri, R. M., Humer, E., Kröger, I., Reisinger, N., Baumgartner, W., ... & Zebeli, Q. (2020). Starch-rich diet induced rumen acidosis and hindgut dysbiosis in dairy cows of different lactations. *Animals*, *10*(10), 1727.

Nichols, K., Wever, N., Rolland, M., & Dijkstra, J. (2024). Effect of source and frequency of rumen-protected protein supplementation on mammary gland amino acid metabolism and nitrogen balance of dairy cattle. *Journal of Dairy Science*. Journal of Dairy Science,107(9), 6797-6816,

Nichols, K., Dijkstra, J., Breuer, M. J. H., Lemosquet, S., Gerrits, W. J. J., & Bannink, A. (2022). Essential amino acid profile of supplemental metabolizable protein affects mammary gland metabolism and whole-body glucose kinetics in dairy cattle. *Journal of Dairy Science*, *105*(9), 7354-7372.

Nowak, M., Boos, A., & Kowalewski, M. P. (2018). Luteal and hypophyseal expression of the canine relaxin (RLN) system during pregnancy: implications for luteotropic function. PLoS One, 13(1), e0191374.

Nozad, S., Ramin, A. G., Moghaddam, G., Asri-Rezaei, S., & Kalantary, L. (2014). Monthly evaluation of blood hematological, biochemical, mineral, and enzyme parameters during the lactation period in Holstein dairy cows. *Comparative Clinical Pathology*, *23*, 275-281.

Otten, W., Heimbürge, S., Tuchscherer, A., & Kanitz, E. (2023). Hair cortisol concentration in postpartum dairy cows and its association with parameters of milk production. *Domestic Animal Endocrinology*, *84*, 106792.

Oxender, W. D., Askew, E. W., Benson, J. D., & Emery, R. S. (2012). Biopsy of liver, adipose tissue and mammary gland of lactating cows. *Journal of Dairy Science*, *54*(2), 286-288.

Piccione, G., Messina, V., Marafioti, S., Casella, S., Giannetto, C., & Fazio, F. (2012). Changes of some haemato-chemical parameters in dairy cows during late gestation, post-partum, lactation and dry periods. *Veterinary Medicine. Zoot*, *58*(80), 59-64.

Piccioli-Cappelli, F., Loor, J. J., Seal, C. J., Minuti, A., & Trevisi, E. (2014). Effect of dietary energy level and high rumen-undegradable protein on endocrine-metabolic status, milk yield, and milk composition in dairy cows during early and late lactation. *Journal of Dairy Science*, *97*(12), 7788-7803.

Poncheki, J. K., Canha, M. L. S., Viechnieski, S. L., & Almeida, R. D. (2015). Analysis of daily body weight of dairy cows in early lactation and associations with productive and reproductive performance. *Revista Brasileira de Zootecnia*, *44*(5), 187-192.

Ponchon, B., Zhao, X., Ollier, S., & Lacasse, P. (2017). Relationship between glucocorticoids and prolactin during mammary gland stimulation in dairy cows. *Journal of dairy science*, *100*(2), 1521-1534.

Prestegaard-Wilson, J. M., Daley, V. L., Drape, T. A., & Hanigan, M. D. (2021). A survey of United States dairy cattle nutritionists' practices and perceptions of reducing crude protein in lactating dairy cow diets. *Applied Animal Science*, *37*(6), 697-709.

Raboisson, D., Mounié, M., & Maigné, E. (2014). Diseases, reproductive performance, and changes in milk production associated with subclinical ketosis in dairy cows: A meta-analysis and review. *Journal of dairy science*, *97*(12), 7547-7563.

Raggio, G., Pacheco, D., Berthiaume, R., Lobley, G. E., Pellerin, D., Allard, G., ... & Lapierre, H. (2004). Effect of level of metabolizable protein on splanchnic flux of amino acids in lactating dairy cows. *Journal of dairy science*, *87*(10), 3461-3472.

Raschka, C., Ruda, L., Wenning, P., von Stemm, C. I., Pfarrer, C., Huber, K., Meyer, U., Dänicke, S. & Rehage, J. (2016). In vivo determination of subcutaneous and abdominal adipose tissue depots in German Holstein dairy cattle. *Journal of Animal Science*, *94*(7), 2821-2834.

Reinhardt, T. A., Lippolis, J. D., McCluskey, B. J., Goff, J. P., & Horst, R. L. (2011). Prevalence of subclinical hypocalcemia in dairy herds. *The Veterinary Journal*, *188*(1), 122-124.

Rhoads, R. P., Kim, J. W., Leury, B. J., Baumgard, L. H., Segoale, N., Frank, S. J., ... & Boisclair, Y. R. (2004). Insulin increases the abundance of the growth hormone receptor in liver and adipose tissue of periparturient dairy cows. The Journal of nutrition, 134(5), 1020-1027.

Roche, J. R., Friggens, N. C., Kay, J. K., Fisher, M. W., Stafford, K. J., & Berry, D. P. (2009). Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. *Journal of dairy science*, *92*(12), 5769-5801.

Roche, J. R., Bell, A. W., Overton, T. R., & Loor, J. J. (2013). Nutritional management of the transition cow in the 21st century–a paradigm shift in thinking. Animal Production Science, 53(9), 1000-1023.

Rodney, R. M., Celi, P., Scott, W., Breinhild, K., Santos, J. E. P., & Lean, I. J. (2018). Effects of nutrition on the fertility of lactating dairy cattle. *Journal of Dairy Science*, *101*(6), 5115-5133.

Røjen, B. A., Larsen, M., & Kristensen, N. B. (2012). Effect of abomasal infusion of oligofructose on portal-drained visceral ammonia and urea-nitrogen fluxes in lactating Holstein cows. *Journal of dairy science*, *95*(12), 7248-7260.

Sadri, H., Giallongo, F., Hristov, A. N., Werner, J., Lang, C. H., Parys, C., ... & Sauerwein, H. (2016). Effects of slow-release urea and rumen-protected methionine and histidine on mammalian target of rapamycin (mTOR) signaling and ubiquitin proteasome-related gene expression in skeletal muscle of dairy cows. *Journal of dairy science*, *99*(8), 6702-6713.

Sammad, A., Khan, M. Z., Abbas, Z., Hu, L., Ullah, Q., Wang, Y., ... & Wang, Y. (2022). Major nutritional metabolic alterations influencing the reproductive system of postpartum dairy cows. *Metabolites*, *12*(1), 60 - 74

Sánchez, A. Á., Martínez, A. G., & Portillo, B. A. (2023). Productive and economic performance of Brown Swiss cows at different stages of lactation fed two crude protein levels. *Tropical and Subtropical Agroecosystems*, *26*(3).

Schuh, K., Sadri, H., Häussler, S., Webb, L. A., Urh, C., Wagner, M., ... & Sauerwein, H. (2019). Comparison of performance and metabolism from late pregnancy to early lactation in dairy cows with elevated v. normal body condition at dry-off. *Animal*, *13*(7), 1478-1488.

Sgorlon, S, F. M. (2015). Factors affecting milk cortisol in mid-lactating dairy cows. *National Library of Medicine, 11*(259).

Sharma, B. K., Vandehaar, M. J., & Ames, N. K. (2014). Expression of insulin-like growth factor-I in cows at different stages of lactation and in late lactation cows treated with somatotropin. *Journal of dairy science*, *77*(8), 2232-2241.

Singh, P., Alex, J. M., & Bast, F. (2014). Insulin receptor (IR) and insulin-like growth factor receptor 1 (IGF-1R) signaling systems: novel treatment strategies for cancer. *Medical oncology*, *31*, 1-14.

Silva-del-Río, N., Fricke, P. M., & Grummer, R. R. (2010). Effects of twin pregnancy and dry period feeding strategy on milk production, energy balance, and metabolic profiles in dairy cows. *Journal of animal science*, *88*(3), 1048-1060.

Song, Y., Wang, Z., Zhao, C., Bai, Y., Xia, C., & Xu, C. (2021). Effect of negative energy balance on plasma metabolites, minerals, hormones, cytokines and ovarian follicular growth rate in Holstein dairy cows. Journal of veterinary research, 65(3), 361-375

Stevenson, J. S., & Britt, J. H. (2017). A 100-Year Review: Practical female reproductive management. *Journal of dairy science*, *100*(12), 10292-10313.

Tanaka, M., Kamiya, Y., Suzuki, T., & Nakai, Y. (2011). Changes in oxidative status in periparturient dairy cows in hot conditions. Animal Science Journal, 82(2), 320-324.

Taylor, V. J., Cheng, Z. P. G. A., Pushpakumara, P. G. A., Wathes, D. C., & Beever, D. E. (2004). Relationships between the plasma concentrations of insulin-like growth factor-I in dairy cows and their fertility and milk yield. *Veterinary Record*, *155*(19), 583-588.

Tedeschi, L. O., Fox, D. G., Fonseca, M. A., & Cavalcanti, L. F. L. (2015). Models of protein and amino acid requirements for cattle. *Revista Brasileira de Zootecnia*, *44*, 109-132.

Thatcher, W. W. (2017). A 100-Year Review: Historical development of female reproductive physiology in dairy cattle. *Journal of dairy science*, *100*(12), 10272-10291.

Thompson-Crispi, K. A., Sargolzaei, M., Ventura, R., Abo-Ismail, M., Miglior, F., Schenkel, F., & Mallard, B. A. (2014). A genome-wide association study of immune response traits in Canadian Holstein cattle. *BMC genomics*, *15*(1), 559.

Toledo-Alvarado, H., Cecchinato, A., & Bittante, G. (2017). Fertility traits of Holstein, Brown Swiss, Simmental, and Alpine Grey cows are differently affected by herd productivity and milk yield of individual cows. *Journal of dairy science*, *100*(10), 8220-8231.

Tong, J. J., Thompson, I. M., Zhao, X., & Lacasse, P. (2018). Effect of the concentration of circulating prolactin on dairy cows' responsiveness to domperidone injection. *Journal of Dairy Science*, *101*(3), 2579-2587.

Van Soest, P. V., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of dairy science*, *74*(10), 3583-3597.

Venjakob, P. L., Borchardt, S., & Heuwieser, W. (2017). Hypocalcemia—Cow-level prevalence and preventive strategies in German dairy herds. *Journal of dairy science*, *100*(11), 9258-9266.

Wankhade, Jadav, P.V., Patel, D.M., Kavani, F.S., Dhami, A.J. (2017). GnRH and its Applications in Bovine Reproduction. International Journal of Advanced Research and Development., 1, 74–80.

Webster, J. (2020). *Understanding the Dairy Cow* (3 ed.). John Wiley & Sons.

Weekes, T. L., Luimes, P. H., & Cant, J. P. (2006). Responses to amino acid imbalances and deficiencies in lactating dairy cows. *Journal of dairy science*, *89*(6), 2177-2187.

Wu, X., Sun, H. Z., Xue, M., Wang, D., Guan, L., & Liu, J. (2019). Days-in-milk and parity affected serum biochemical parameters and hormone profiles in mid-lactation holstein cows. *Animals*, *9*(5), 230.

Yang, Y., Sadri, H., Prehn, C., Adamski, J., Rehage, J., Dänicke, S., ... & Sauerwein, H. (2020). Proteasome activity and expression of mammalian target of rapamycin signaling factors in skeletal muscle of dairy cows supplemented with conjugated linoleic acids during early lactation. *Journal of dairy science*, *103*(3), 2829-2846.

Zarrin, M., Grossen-Rösti, L., Bruckmaier, R. M., & Gross, J. J. (2017). Elevation of blood β-hydroxybutyrate concentration affects glucose metabolism in dairy cows before and after parturition. *Journal of dairy science*, *100*(3), 2323-2333.

Zbinden, R. S., Falk, M., Münger, A., Dohme-Meier, F., van Dorland, H. A., Bruckmaier, R. M., & Gross, J. J. (2017). Metabolic load in dairy cows kept in herbage-based feeding systems and suitability of potential markers for compromised well-being. *Journal of animal physiology and animal nutrition*, *101*(4), 767-778.

Zebeli, Q., Aschenbach, J. R., Tafaj, M., Boguhn, J., Ametaj, B. N., & Drochner, W. (2012). Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. *Journal of dairy science*, *95*(3), 1041-1056.

Zhang, J., Guan, F., Huang, S., Ma, Y., Wen, S., Jin, W., & Mao, S. (2024). Fermented soybean meal modified the rumen microbiota and increased the serum prolactin level in lactating Holstein cows. *Frontiers in Veterinary Science*, *11*, 1498639.

Zhang, J., Deng, L., Zhang, X., Cao, Y., Li, M., & Yao, J. (2023). Multiple essential amino acids regulate mammary metabolism and milk protein synthesis in lactating dairy cows. *Animal Feed Science and Technology*, *296*, 115557.

APPENDICES

Appendix I: ANOVA Analysis outputs

Table 1: Effect of parity on milk yield

Source of variation	d.f	s.s	m.s	v.r	F pr.
Parity	2	548.69	274.35	8.27	0.001
Residual	267	8854.43	33.16		
Total	269	9403.12			

Table 1 shows the ANOVA statistic that examined the effects of parity on milk production. The mean milk production under the different parities showed a progressive increase in milk and the statistic, $F(2, 267) = 8.27$, $p = 0.001$ indicates statistically significant differences in milk production based on parity levels.

Table 2: Effect of parity on serum Cortisol levels

Source of variation	d.f	s.s	m.s	v.r	F pr.
Parity	2	24054.4	12027.2	17.97	0.001
Residual	42	28107.4	669.2		
Total	44	52161.8			

Table 2 shows the ANOVA statistic that examined the effects of parity on the levels of cortisol hormones. As shown by the statistic, $F(2, 44) = 17.91$, $p = 0.001$ indicates statistically significant differences in the levels of cortisol hormones based on parity levels.

Table 3: Effect of parity on serum IGF-α1 levels

Source of variation	d.f	s.s	m.s	v.r	F pr.
Parity	2	9062	4531.	0.58	0.566
Residual	42	329897.	7855.		
Total	44	338959.			

Table 3 shows the ANOVA statistic that examined the effects of parity on the levels of **IGF-α1** hormones. As shown by the statistics, F (2, 44) = 0.58, p = 0.566 indicates no statistically significant differences in the levels of **IGF-α1** hormones based on parity levels.

Table 4: Effect of parity on serum prolactin levels

Source of variation	d.f	s.s	m.s	v.r	F pr.
Parity	2	4673.	2336.	0.26	0.769
Residual	44	371492.	8845.		
Total	44	376165.			

Table 4 shows the ANOVA statistic that examined the effects of parity on the levels of prolactin hormones. As shown by the statistics, F (2, 44) = 0.26, p = 0.796 indicates no statistically significant differences in the levels of prolactin hormones based on parity levels.

Table 2: Effect of parity on serum Oestrogen levels

Source of variation	d.f	s.s	m.s	v.r	F pr.
Parity	2	3.2100	1.6050	2.06	0.140
Residual	44	31.0911	0.7773		
Total	44	34.4702			

Table 5 shows the ANOVA statistic that examined the effects of parity on the levels of oestrogen hormones. As shown by the statistics, F (2, 44) = 2.06, p = 0.140indicates no statistically significant differences in the levels of oestrogen hormones based on parity levels.

Appendix II: Datasheet for Hormone levels in cows

Table 1: Serum Cortisol hormone levels(ng.ml^{-1})

	Elfam Farm			**Elso Farm**			**Betan Farm**		
	Parity 2	Parity 3	Parity 4	Parity 2	Parity 3	Parity 4	Parity 2	Parity 3	Parity 4
Day 1	210.50	150.52	125.23	191.38	149.33	110.19	201.94	142.30	120.36
Day 8	190.08	122.64	110.04	168.18	136.65	100.04	180.13	129.65	111.04
Day 15	139.90	117.34	87.23	124.90	101.85	98.23	128.00	107.79	94.54
Day 22	126.80	103.82	86.62	101.85	99.31	77.57	114.34	92.97	80.70
Day 29	123.95	100.05	62.42	100.76	71.77	68.14	112.36	83.10	63.10

Table 2: Serum Prolactin hormone levels(ng.ml^{-1})

	Elfam Farm			Elso Farm			Betan Farm		
	Parity 2	Parity 3	Parity 4	Parity 2	Parity 3	Parity 4	Parity 2	Parity 3	Parity 4
Day 1	56	105	175	67	96	127	61	86	116
Day 8	149	169	201	86	184	187	77.5	168	213
Day 15	166	205	260	177	218	204	168.5	185	216.5
Day 22	294	289	358	342	272	324	300.5	318	316
Day 29	320	326	392	369	319	408	308.5	344.5	400

Table 3: Serum Oestrogen hormone levels(ng.ml^{-1})

	Elfam Farm			**Elso Farm**			**Betan Farm**		
	Parity 2	Parity 3	Parity 4	Parity 2	Parity 3	Parity 4	Parity 2	Parity 3	Parity 4
Day 1	1.32	1.03	1.08	1.48	1.07	1.19	1.42	1.05	1.14
Day 8	2.38	1.86	1.94	2.68	1.74	2.01	2.56	1.79	1.98
Day 15	2.67	2.11	2.08	2.71	2.11	2.16	2.7	2.06	2.12
Day 22	3.36	2.86	2.71	3.46	3.08	2.86	3.41	3.04	2.79
Day 29	4.0	2.94	3.06	4.12	3.42	3.24	4.08	4.08	3.15

Table 4: Serum **IGF-α1** hormone levels(ng.ml^{-1})

	Elfam Farm			Elso Farm			Betan Farm		
	Parity 2	Parity 3	Parity 4	Parity 2	Parity 3	Parity 4	Parity 2	Parity 3	Parity 4
Day 1	132	102	69	140	133	94	128	110	80
Day 8	152	120	86	166	145	142	140	130	98
Day 15	194	186	168	340	300	200	166	201	170
Day 22	232	230	205	378	360	260	245	236	210
Day 29	320	295	254	400	381	324	380	305	262

Appendix III: Datasheet for Weight gain/loss

Table 1: Daily weight(Kgs) for the cows during experiment period

	Elfam Farm			Elso Farm			Betan Farm		
Day	Parity 2	Parity 3	Parity 4	Parity 2	Parity 3	Parity 4	Parity 2	Parity 3	Parity 4
1	570	599	633	606	574	689	572	590	660
2	568	598	633	606	573.5	688.5	569	586	658
3	566	598	630	605	573	688	568	586	658
4	566	598	629	604.5	572.2	687.5	566	584	656
5	565	597	629	603	571.4	687	564	584	656
6	565	595	628	601.6	570	686.4	562	584	654
7	563	595	626	599.5	569.5	685.5	560	580	654
8	562	593	625	598	569	684.2	558	580	653.5
9	560	592	623	597	568	683.2	554	579	650
10	558	590	618	596	567	681.5	552	578	648.5
11	558	589	617	594.5	566	680.5	550	575	646
12	557	588	617	592.5	565.5	679.5	549	575	645
13	556	586	616	591	563.5	678	552	570	643
14	555	585	615	590	563	677	555	570	640
15	554	584	613	589	562.2	676.5	556	568	638
16	553	583	612	587.5	561	675.4	558	568	636
17	552	580	611	586.4	560	674.5	560	570	635
18	554	579	610	585.2	558	673.2	562	574	635
19	554	578	609	584.5	557	671.5	563	574	636
20	554	580	609	584	556.5	676	564	576	640
21	555	582	610	582.6	556	676.5	564	577	641
22	555	586	612	581.4	555.4	677	565	578	642
23	555	587	615	580	556.5	677.5	565	578	644
24	556	588	616	581.5	558.5	678	566	578	644
25	556	590	617	583.5	560.5	678.5	567	579	644
26	557	590	618	583	564	679	568	580	650
27	557	592	620	586.5	565	680	568	581	650
28	557	592	620	588.6	566	680	568	582	652
29	557	592	623	589.7	567	682	570	584	652
30	557	593	625	586.7	568	683	570	585	654

Appendix IV: Datasheet for Milk yield in Experimental cows

Table 1: Daily milk yield (Kgs) for the animals during experiment period

	Elfam Farm			Elso Farm			Betan Farm		
Day	Parity 2	Parity 3	Parity 4	Parity 2	Parity 3	Parity 4	Parity 2	Parity 3	Parity 4
1	7	11	12.2	18	18	19	7	13	16
2	9	13	19.4	19.5	18	21	8	13	18
3	10.4	15	26.4	18.5	19	20.5	10	14	17
4	10	15	27.5	18.5	19	21	12	13	15.5
5	9.4	16.1	25.8	20.6	20	21	16	13.5	16.5
6	8	24	26.1	19.5	20	20.5	18	12.5	17.5
7	8	23.2	27.6	20.5	26	21.5	18	14.5	18.5
8	14.6	22	25.9	18	25	21.5	18.5	16	19.5
9	14.6	21.1	26.8	19.5	20	22	17.5	18.5	20
10	13.8	20.4	26.9	20.5	24	23	17	17.5	22
11	15.1	19.6	27.3	21.5	25	23.5	18.5	17	24
12	14.6	20.4	27.1	20.5	26	24.5	19.5	16	24.5
13	13.8	22.4	29.9	26	26	25	19	16	23.5
14	15.5	20.7	27.2	28	26	26.5	20	20	20.5
15	16.4	18.8	26.3	26	26	26	20.5	22	22.5
16	18	20.1	30.6	28	26	26	24	24	23
17	19	20.6	30.9	28	26	27	23	23.5	24
18	20	19.7	28.3	29	28	28	22.5	26.5	26
19	21	20.1	26.8	31	28	29	25	27	25.5
20	23	21.4	27.5	32	28	28.5	26	28.5	26.5
21	25	20.9	25.4	30.5	28	27.5	25.5	25	28.5
22	26	19.5	26.1	31.5	28	29	26.5	25	24.5
23	28	20.3	26.1	29.5	28	30.5	24.5	26.5	24.5
24	26	21.1	26.2	32.5	30	31	26.5	25	26
25	25	19.8	22.1	30.3	31	32	25	28.5	27.5
26	28	19.7	25.4	33	34	32.5	26.5	28	25.5
27	29	17.5	26.1	32.5	34	33	27	28	26.5
28	28	20.5	23.8	30.5	36	32.5	28	26.5	26.5
29	29	22.5	22.2	30.5	36	35	25	26.5	27.3
30	29	23.5	22.6	31	36	35	26	27	26.3

Appendix V: Datasheet for Feed Intake

Table 1: Daily Feed intake (Kgs) for the animals during experiment period

	Elfam Farm			Elso Farm			Betan Farm		
Day	Parity 2	Parity 3	Parity 4	Parity 2	Parity 3	Parity 4	Parity 2	Parity 3	Parity 4
1	16	31	21	16	10	14.5	12	10	15
2	20	32.1	22	16	12	14.7	14	12	15
3	23	26	24	17	14	15	14	11	16
4	25	31	24	22	14	15	14	12	21
5	26	31	26	22	16	18	15	13	23
6	29	33.7	26	26	20	22	16	15	25
7	30.3	34	26	27	21	24	16	20	26
8	31	32	28	27	21	26	18	20	26
9	34	34	29	29	22	26	20	20	28
10	29	34	30	29	20	27	26	22	28
11	31.8	30.5	31	30	25	28	28	24	30
12	33	29	32	30	27	28	28	26	29
13	32	33	32	31	27	28	28	26	30
14	32	33	33	32	26	28	29	27	30
15	28	32	33	32	27	29	29	28	31
16	29.9	30	33	32	28	29	29	29	31
17	31	31	32	32	28	30	30	29	31
18	30	32	31	32	28	30	30	28	32
19	29	33	32	32	28	31	29	30	32
20	31	34	33	34	30	32	28	30	33
21	32.4	33.4	34	34	30	33	31	30	34
22	29	33.5	34	34	30	34	32	32	34
23	26.4	34.5	32	36	32	34	32	34	35
24	31	35	31	38	32	34	32	33	36
25	35	34.5	33	38	32	34	33	35	36
26	34	35.5	34	39	33	35	33	35	37
27	32	36	34	39	33	35	34	35	38
28	36.5	37	35	39	35	36	34	35	39
29	36	37	33	39	35	39	35	36	39
30	36	37	32	39	35	39	35	36	39

Appendix VI: Pictorial representation of the experiments

Researcher observing Feed preparation in Elso Farm

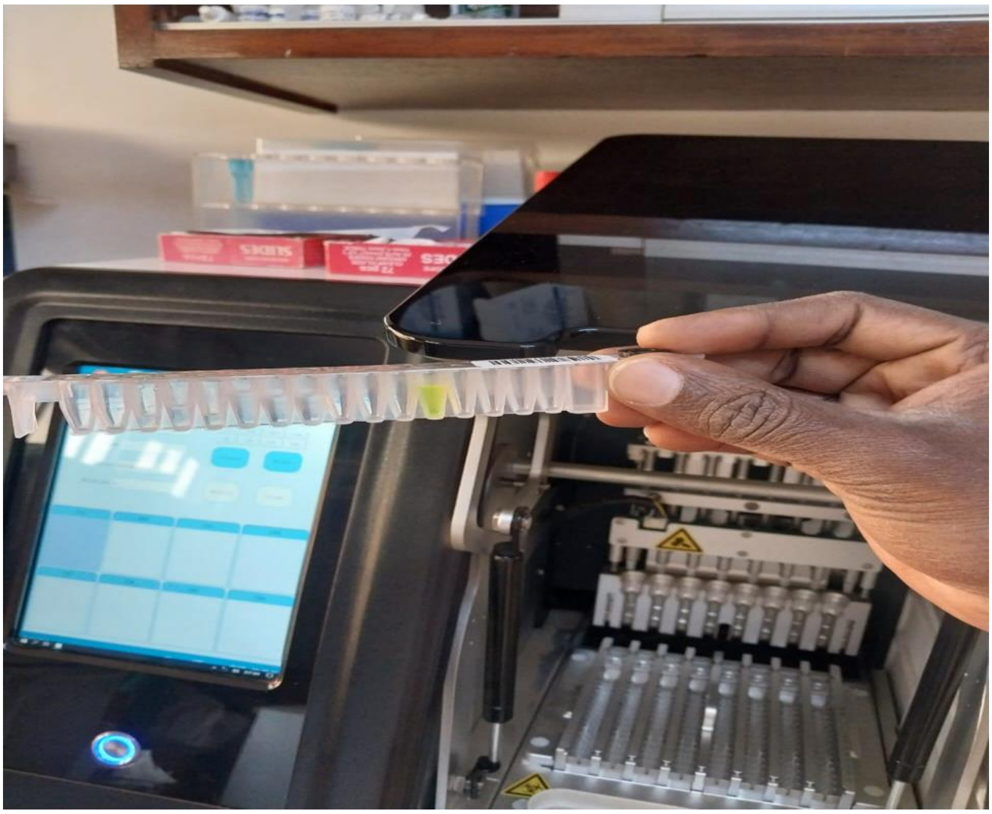
A caption of the feed Laboratory instruments in Nairobi Annex Laboratory

Researcher taking body weight measurement of an experimental cow in Betan farm

Researcher taking body weight measurement of an experimental cow in Elfam

Researcher drawing blood from jugular vein of an experimental cow in Elfam

Researcher drawing blood from jugular vein of an experimental cow in Elso Farm



Procedures in Nairobi Annex Laboratory; Cortisol Check -1 test kit

Appendix VII: Similarity Report

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