IMPACT OF MATERNAL DIETARY INTAKE ON BREAST MILK COMPOSITION AND INFANT NUTRITION STATUS AMONG LACTATING WOMEN IN NYERI COUNTY, KENYA

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H87/37658/2016

RESEARCH THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN FOOD, NUTRITION AND DIETETICS, IN THE SCHOOL OF PUBLIC HEALTH AND APPLIED HUMAN SCIENCES OF KENYATTA UNIVERSITY

AUGUST, 2020

DECLARATION

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

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DEDICATION

I am humbled to dedicate this work to my wife Esther, my children (Marlek and Sasha) and my parents (Mrs. Jenifer Wanjiku Kiboi and the late Mr. Sammy Kiboi Kahanya) for their love, encouragement and unequivocal support.

ACKNOWLEDGEMENT

I would like to give all glory to the Almighty God for His love and grace which has made this possible.

I am deeply grateful to Kenyatta University for giving me an opportunity to pursue a Doctor of Philosophy degree in Nutrition and Dietetics. I express my sincere and heartfelt gratitude to my supervisors, Prof. Judith Kimiywe and Dr. Peter Chege, for their guidance and scholarly contributions to this study. I further extend special thanks to Prof. Edelweiss, Dr. Robin and the entire fraternity of Northwest West University, Centre for Excellence in Nutrition for their valuable guidance in my Ph.D. journey. Profound gratitude also goes to Julia, Grace, Dan, Mike, Muriuki, Maina and Murigu for their help and support during the data collection process.

I acknowledge and thank the Kenya National Research Fund for funding this study. I also wish to express my appreciation to Jomo Kenyatta University of Agriculture and Technology for allowing me to analyse the study samples in the institution. I am thankful to Mr. David for his support during the sample analysis. I would also like to recognise my sisters Jedidah, Phyllis and Ann for their unending support and encouragement throughout my doctoral experience. Finally, my appreciation goes to the participants of this study without whom the research could not have succeeded.

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OPERATIONAL DEFINITION OF TERMS

Breast milk: Milk from the human breast.

Breast milk composition: This referred to carbohydrates (lactose), protein, fat, vitamin A, calcium, magnesium, iron and zinc content of breast milk from lactating mothers.

Dietary diversity: The number of different food groups consumed over a given reference period by the participants. In this study, the period was 24 hours.

Dietary intake: This referred to maternal dietary diversity, food consumption frequency, meal frequency and the intake of energy, macronutrients and micronutrients from the foods consumed by the lactating mothers included in this study.

Exclusive breastfeeding: This refers to feeding an infant for the first six months with breast milk alone without giving any other liquids or solids including water (with the exception of medicine if necessary) (WHO, 2003a).

Infant: This is a child who is less than one year in age. In this study, was a child below six months.

Lactation: The secretion of milk from the mammary glands of a female after childbirth to provide nourishment to a child.

Maternal factors: The lactating women age, parity, child sex, mode of delivery, morbidity status and nutrition status.

Nutritional status: The health status as influenced by food and nutrient intake. Body Mass Index (BMI), Mid Upper Arm Circumference (MUAC) and serum micronutrient levels assessed the lactating mothers' nutritional status. Length and weight. assessed the infants' nutrition status.

Participants: Referred to lactating mothers with infants less than six months included in the study.

True proteins: Total protein corrected for non-protein nitrogen (NPN) obtained by subtracting non-protein nitrogen from the total nitrogen.

ABBREVIATIONS AND ACRONYMS

AAP	**:**	American Academy of Paediatrics
AAS	**:**	Atomic Absorption Spectrophotometer
AOAC	**:**	Association of Official Analytical Chemists
BMI	**:**	Body Mass Index
CI	**:**	Confidence Interval
CS	**:**	Caesarean Section
DDS	**:**	Dietary Diversity Score
DHA	**:**	Docosahexaenoic Acid
EBF	**:**	Exclusive Breastfeeding
FAO	**:**	Food and Agriculture Organization
GOK	**:**	Government of Kenya
HPLC	**:**	High Pressure Liquid Chromatography
IDDS	**:**	Individual Dietary Diversity Score
JKUAT	**:**	Jomo Kenyatta University of Agriculture and Technology
KDHS	**:**	Kenya Demographic and Health Survey
KCal	**:**	Kilocalories
KNBS	**:**	Kenya National Bureau of Statistics
LAZ	**:**	Length for Age
LMICs	**:**	Low and Middle-Income Countries
MDD-W	**:**	Minimum Dietary Diversity for Women of Reproductive Age
MDGs	**:**	Millennium Development Goals
MOH	**:**	Ministry of Health
MUAC	**:**	Mid Upper Arm Circumference
MIYCN	**:**	Maternal, Infant and Young Child Nutrition
NIH	**:**	National Institute of Health
NPN	**:**	Non-Protein Nitrogen
PUFAs	**:**	Polyunsaturated Fatty Acids
RNI	**:**	Recommended Nutrient Intake
RPM	**:**	Revolutions Per Minute
SD	**:**	Standard Deviation
SPSS	**:**	Statistical Package for Social Sciences
TCA	**:**	Trichloroacetic acid

UNICEF	**:**	United Nations Children's Fund
UN	**:**	United Nations
US	**:**	United States
WAZ	**:**	Weight for Age
WDDS	**:**	Women Dietary Diversity Score
WHO	**:**	World Health Organization
WLZ	**:**	Weight for Length

ABSTRACT

Exclusive breastfeeding for the first six months of life, with continued breastfeeding up to 2 years after the introduction of complementary feeds, is considered as the standard norm for infant feeding. Although the benefits of breastfeeding have long been known, studies on the composition of human milk are still ongoing. Considering that breast milk is the sole source of the nutrition for the infant in the first six months of life, it is essential to have accurate and up to date data on its composition. The composition of human milk could vary according to many maternal factors such as diet, nutritional status, genetics and environmental exposures, among other factors. The amount of variability in human milk composition that could be attributed to maternal dietary intake remains largely unknown. In Africa, particularly in Kenya, there is scanty information available on the nutrient composition of breast milk and the factors that affect its composition. This study therefore investigated the association between maternal dietary intake with breast milk nutrient composition and their effect on the nutrition status of the infant (under six months). The longitudinal study was conducted in Nyeri County, Kenya. Participants included lactating mothers with infants under six months. The lactating mothers' dietary intake, nutrition status (BMI, MUAC and serum micronutrients) and that of their infants (recumbent length and weight) were assessed. Additionally, breast milk samples were collected and analyzed for selected nutrient content and correlated with both maternal dietary intake and the infant's nutritional status. The breast milk samples were analysed for energy, macronutrients and selected micronutrients (vitamin A, calcium, magnesium, zinc and iron). All the measurements were done at two time points (first and the fifth month of lactation) among a comprehensive sample of 104 mother-child dyads. Significant changes in the mean nutrient composition were noted between the first and fifth month of lactation (protein-0.96 g/dL and 0.85 g/dL, $p = 0.03$; vitamin A-22.48 and 31.61 µg/dL, p value, $p < 0.001$; iron-0.39 mg/L and 0.47 mg/L, $p = 0.02$). There was statistically significant ($p < 0.05$) relationship between mother energy intake with milk lactose ($r = 0.30$); carbohydrate intake with milk true proteins ($r = 0.24$); protein intake with milk true protein ($r = 0.44$) and milk iron ($r = 0.31$); fat intake with milk energy ($r = 0.29$), milk true protein ($r = 0.40$) and milk fat ($r = 0.35$); percent of energy from fat with milk energy ($r = 0.39$) and milk fat ($r = 0.45$). Furthermore, vitamin A intake with milk retinol ($r = 0.56$), calcium intake with milk zinc ($r = -0.32$) and iron ($r = -0.27$), zinc and iron ($r = -0.26$). For maternal nutrition status, hemoglobin (Hb) was correlated with both milk energy ($r = 0.30$) and true protein ($r = 0.44$). Serum retinol, magnesium and iron were correlated with milk retinol ($r = 0.34$), magnesium ($r = 0.29$) and iron ($r = 0.33$), respectively. Association between breast milk nutrient composition and infant nutrition status revealed that the milk true protein ($r = 0.58$) and retinol ($r = 0.32$) were positively correlated with weight for age and MUAC ($r = 0.31$) of the infant. Weight for length was also positively correlated with milk lactose ($r = 0.47$). No relationship was found between other maternal factors (age, parity, child sex and mode of delivery) and any of the selected breast milk nutrient content ($p > 0.05$). The study observes that breast milk nutrient composition is variable over the course of lactation. Further, both maternal dietary intake and nutrition status (serum micronutrient status) are associated with the nutrient composition of human milk. Moreover, breast milk nutrient composition is related to the infant nutrition status. Promoting adequate nutrient intake and optimal maternal nutritional status during lactation is essential to ensure adequate child growth and development.

CHAPTER ONE: INTRODUCTION

1.1 Background to the Study

Human milk is the ideal food for infants and therefore exclusive breastfeeding (EBF) for the first six months of life in human being, with continued breastfeeding along with appropriate complementary foods for two years of life or longer, is acknowledged as the standard norm for infant feeding (Pediatrics, 2012; World Health Organization (WHO), 2003a). Globally, evidence on the benefits of human milk in ensuring neonatal health and increasing child survival continues to accumulate and is now copious (Chapman & Nommsen-Rivers, 2012).

Human breast milk is exceptionally suited to the human infants, due to its nutritional content and other bioactive components that enhance child survival and optimal growth (Ballard & Morrow, 2013). Further, human milk feeding has over time been shown to reduce morbidity and mortality among infants (Quinn et al., 2012). Substantial evidence has additionally shown that breast milk improves cognitive, physical development and has long term health outcomes for infants such as reduced risk to diabetes, obesity, cardiovascular diseases, among other disorders (Isaacs et al., 2010).

Unlike infant formulas, human breast milk is reported to be an invariant fluid, which could fluctuate in micro and macronutrients composition longitudinally in the course of feeding. The reported variations in the human milk composition between individual women and populations could be a result of cultural differences such as diet, genetic make-up and other environmental factors (Bravi et al., 2016). Studies in different regions have reported that maternal dietary intake could be associated with breast milk composition and child nutritional outcomes (Allen, 2012; Daneel-Otterbech et al., 2005; Kiprop et al., 2016; Luxwolda et al., 2014; Nasser et al., 2010). Given that human milk is recommended as the only source of infant nourishment for their first six months after birth (Jara-Palacios et al., 2015; WHO, 2003a), and then as an important nutrient source till the child is two years, promoting the nutritional quality of human milk is of great public health concern (Chapman & Nommsen-Rivers, 2012).

In Kenya, several initiatives have been put in place to promote optimal Maternal, Infant and Young Child Nutrition (MIYCN) practices. These initiatives have resulted in improved feeding practices for children. According to Kenya Demographic and Health Survey (KDHS) of 2014, the vast majority (91%) of children were breastfed within one day of birth and the proportion of children younger than six months who were exclusively breastfed increased from 32% in 2008-09 KDHS to 61% in 2014 (KNBS & ICF International, 2015). However, despite these improvements in child feeding, the majority of these initiatives have only resulted in improved feeding practices for the children with minimal initiatives being done to the mothers in relation to improved macro and micronutrient intake.

If lactating mothers’ dietary intake is associated with breast milk composition, then maternal deficiencies can result in sub-optimal nutrient concentration in breast milk and negatively affect infant growth and health (Allen, 2012). For instance, lactating women in developing nations were reported by a study to have substantially low human milk ascorbic acid output than those in developed nations due to their dietary intake differences (Daneel-Otterbech et al., 2005). However, more research is needed to unravel the relationship between maternal dietary intake and breast milk composition

Given the importance of breast milk, many studies have been conducted in recent years to identify its components and the factors associated with them. This research in breast milk has continued to reveal the dynamic nature of its composition. Many factors that could affect the content of human milk among mothers have been reported including; the stage of lactation, lifestyle factors and maternal diet among others (Ares et al., 2016; Franca et al., 2010; Kent et al., 2006). However, a systematic review by Bravi et al. (2016) has indicated that the variability in human milk content attributed to dietary intake remains mostly unknown (Bravi et al., 2016). Investigating the association between maternal dietary intake and breast milk composition is thus important.

Additionally, for non-breastfed infants, human milk composition is used as the standard reference in decisions on the adequacy of breast milk substitutes (Butts et al., 2018). Therefore, understanding human milk composition and its determinants provide a crucial tool for optimizing infant feeding, especially for infants at a higher risk and also for comprehending the possible effect of maternal nutrition on human milk nutrients and other components. Similarly, this will contribute to the knowledge of the role of human breast milk in ensuring adequate health and appropriate infant growth.

Unfortunately, despite breast milk being regarded and recommended as the best source of nutrient for infants, its nutritional composition and its relationship to maternal dietary intake in developing countries has not been adequately investigated (Nakamori et al., 2009). Therefore, this study sought to contribute to this knowledge gap by determining the role of maternal dietary intake in breast milk composition and infant nutritional status in Nyeri County, Kenya.

1.2 Statement of the Problem

Malnutrition is the single greatest contributor to child mortality in Kenya at 53% (KNBS & ICF International, 2015; Ministry of Public Health and Sanitation, 2012). Unfortunately, malnutrition rates among Kenyan children are still high. According to the available data, 26% of the Kenyan children are stunted, 11% underweight and 4% wasted (KNBS & ICF International, 2015). According to WHO (2009), sub-optimal breastfeeding is associated with a high prevalence of child malnutrition and morbidity in many countries. Poor breast milk quality could contribute to the malnutrition.

The recommendation for exclusive breastfeeding until six months pre-supposes that breast milk will provide all the nutritional requirements (macronutrients and micronutrients) for the infant, both qualitatively and quantitatively. Until recently, it was widely conceived that nutrition deficiencies in breastfed infants was relatively uncommon (Nommsen-Rivers, 2012). WHO expert consultation on the optimal duration of breastfeeding acknowledged that data was inadequate to rule out the possibility of macro and micronutrient deficiencies among infants breastfed exclusively for six months (WHO, 2003b).

Based on the aforementioned, there have been efforts to understand better the composition and the determinants of human milk composition. More so, efforts to unravel the relationship between maternal dietary intake, micronutrient status and breast milk quality, especially among populations reported to have inadequate dietary intake are much needed as the information is scanty (Allen, 2005; Chapman & Nommsen-Rivers, 2012). This study therefore sought to establish the relationship between maternal dietary intake and breast milk composition among lactating mothers and its influence on the infant nutritional status.

Notably, and as reported by Czosnykowska-Łukacka et al. (2018), a workshop on human milk composition organized in 2017, by the National Institute of Health (NIH) and the United States (U.S) Department of Agriculture identified some gaps in the available human milk studies. One of the gaps identified was that the available human milk studies had small sample sizes. Secondly, the available data may not reflect the inter-individual variations that could occur between mothers across the world as most of the studies were from western countries. Therefore, studies of human milk composition in other regions and populations would fill this gap. This study reports on the breast milk composition of lactating mothers in central Kenya.

Additionally, and according to Su et al. (2010), the few studies investigating the association between maternal dietary intake and human milk composition have given inconsistent results and therefore more studies are needed to validate these findings. Moreover, in a systematic review, Bravi et al. (2016) indicated that data on the variability of human milk based on dietary intake is very scarce. Notwithstanding the different dietary practices among lactating mothers in Kenya, there is a paucity of data on the impact of maternal dietary intake on breast milk composition and infant nutritional status in Kenya. Therefore, this study contributes to addressing this gap by reporting on the association of maternal dietary intake and breast milk composition longitudinally among nursing mothers in Kenya.

Lastly, Ballard and Morrow (2013) noted that multi-population studies of human milk composition and its determinants are particularly needed to create a rigorous and comprehensive reference of nutrients and bioactive factors. Therefore, this study makes an empirical contribution to this noble course.

1.3 Purpose of the Study

The purpose of this study was to determine the impact of maternal dietary intake on the nutrient content of breast milk and infant (under six months) nutritional status among lactating women in Nyeri County, Kenya.

1.4 Objectives of the Study

1. To determine the dietary intake of mothers with infants under six months in Nyeri County, Kenya.
2. To assess the lactating mothers-child dyad nutritional status in Nyeri County, Kenya.
3. To determine the human milk nutrient composition of lactating mothers with infants under six months in Nyeri County, Kenya.
4. To establish the relationship between maternal dietary intakes, nutrition status and breast milk nutrient composition among lactating mothers with infants under six months in Nyeri County, Kenya.

5. To identify the predictors (maternal factors) of breast milk nutrient composition among lactating mothers with infants under six months in Nyeri County, Kenya.
6. To establish the relationship between breast milk nutrient composition and infant (under six months) nutrition status among lactating mothers in Nyeri County, Kenya.

1.5 Research Hypotheses

Ho$_1$: The lactating mothers' breast milk nutrient composition does not change significantly in the first five months of lactation.

Ho$_2$: There is no statistically significant association between the lactating mothers' dietary intake and their breast milk nutrient composition.

Ho$_3$: There is no statistically significant association between the lactating mothers' nutrition status and their breast milk nutrient composition.

Ho$_4$: There is no statistically significant association between the lactating mothers' breast milk nutrient composition and their infant nutrition status.

1.6 Significance of the Study

WHO supports human milk banking as a strategy for improving health outcomes and reducing health care cost particularly among high risk infants who lack access to their mother's breast milk (Ganapathy et al., 2012; Quigley & McGuire, 2014; WHO, 2008). This study will inform human milk banking programs on how to promote breast milk quality for banking by understanding the association between maternal dietary intake and breast milk composition. Furthermore, the available data from Kenyan women will provide a clear understanding of the human milk composition, which will be crucial for developing standards and policies for producing infant formulas and for updating the infant dietary reference intakes. Additionally, the human milk nutrient composition information may also be a useful marker of the population's nutritional status. The study will also provide empirical evidence in the growing body of literature on the role of dietary intake in breast milk nutrient composition and child nutritional status outcome.

1.7 Assumption of the Study

The study assumed that there was no major change in the dietary practices and intake of the study group during the study period.

1.8 Delimitations of the Study

The scope of this study was in Nyeri County and therefore can only be generalized in other areas with similar characteristics. Further, this study only considered a period of five months postpartum and therefore findings may not be generalizable to later postpartum periods.

1.9 Conceptual Framework

The conceptual framework (Figure 1.1) details the implications of current maternal dietary intake and related factors on the infant nutritional status. It highlights the factors that could be targeted to improve breast milk composition and infant nutritional status. The framework hypothesized that the participants' dietary intake could influence their breast milk composition either directly or indirectly through maternal nutrition status. Dietary intake influences maternal nutrient stores, which may potentially affect the composition of their breast milk. Figure 1.1 further demonstrates that extraneous factors socioeconomic and demographic characteristics of the participants affect their food access and intake. Factors such as lower education, low income and unemployment have been associated with food insecurity. These factors affect the extent to which an individual is physically and economically able to obtain nutritious foods.

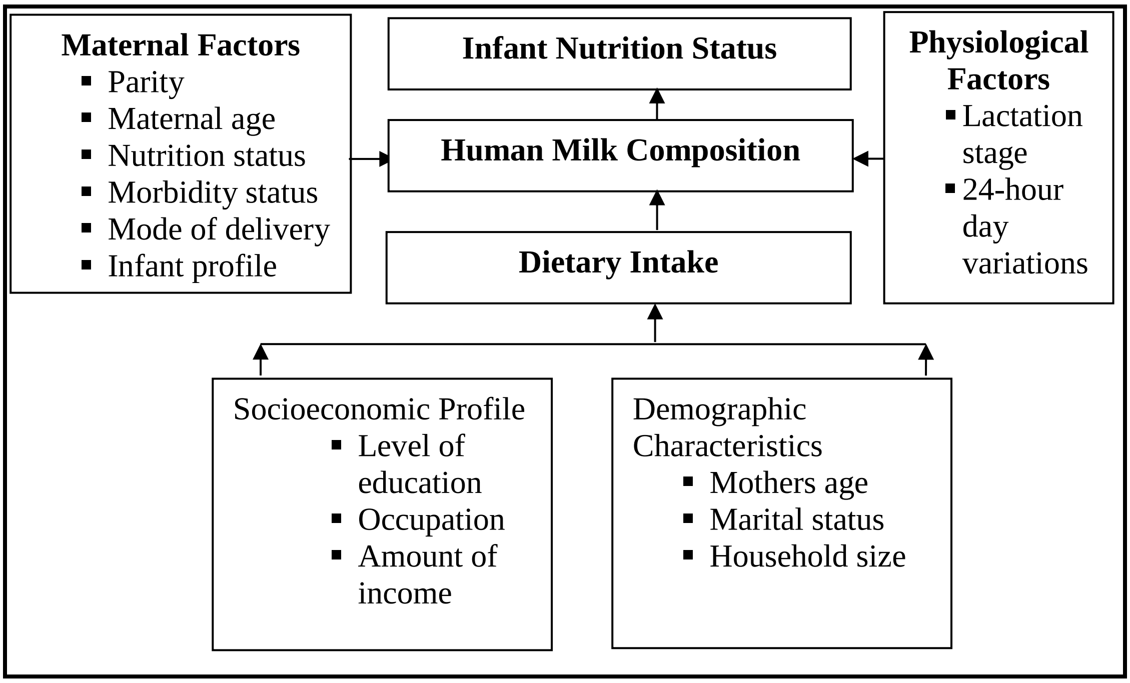

Figure 1.1: Conceptual framework on maternal, physiological and dietary factors that may influence human milk composition and child nutritional status
Source: Modified from UNICEF (2009)

Human milk composition may also be influenced by other maternal factors such as maternal age, parity, mode of delivery, morbidity status and infant characteristics. The mother's health may affect her breast milk composition. Morbidity may negatively affect the mother's dietary intake, nutrient stores, and nutrient utilization affecting her breast milk composition. The human milk composition could also change in response to the infant's age, sex and other characteristics. The infant needs could potentially impact on the quantity and quality of human milk. The human milk composition could also vary based on the time of the day as it may be influenced by dietary practices and body physiological processes.

Child growth and development is primarily dependent on their nutrient intake and utilization. Therefore, breast milk nutrient composition influences the child nutrition status outcome as hypothesized in the framework (Figure 1.1). Of note, the main focus of this study was on the impact of maternal dietary intake on breast milk composition and infant nutritional status.

CHAPTER TWO: LITERATURE REVIEW

2.1 Benefits of Human Breast Milk Feeding

Breastfeeding is considered the physiological norm for infant and child feeding (American Academy of Pediatrics [AAP], 2012; Labbok & Taylor, 2008). As such, breast milk has been endorsed as the optimal source of nutrition by many organizations (AAP, 2012; UNICEF et al., 2010; WHO, 2001). The benefits of breast milk feeding are compelling to both the mother and the baby (Ip et al., 2009). Interestingly, the benefits are said to extend the mother-child benefits to other positive health and economic changes (Murtagh & Moulton, 2011). Breast milk is known to have immunologic properties and nutrients for the baby (Eidelman & Schanler, 2012).

Infants who are exclusively breastfed for the optimal duration are significantly protected against major childhood illnesses (AAP, 2012). According to Ip et al. (2009) and UNICEF et al. (2010) feeding babies with breast milk has been associated with reduced risk of sudden infant death syndrome, asthma, respiratory infections, leukemia, gastroenteritis, obesity, type 1 and 2 diabetes among others. Additionally, research has shown that breast milk contributes to cognitive development in infants (Holme, Macarthur & Lancashire, 2010).

Moreover, there is strong evidence in literature that women who have breastfed have a decreased risk of breast and ovarian cancers, postpartum depression, urinary tract infection and diabetes type 2 (Godfrey & Lawrence, 2010; Ip et al., 2009; Levy et al., 2009). Additionally, breastfeeding enables a mother to gain her pre-pregnancy weight faster and she is also more likely to keep amenorrhea for a longer period (Kramer & Kakuma, 2012; WHO, 2012). Despite the numerous benefits of breast milk being well documented, disparities in breast milk nutrient composition among different populations have been reported. A factor which could influence breast milk nutrient composition is maternal dietary intake, however there is limited information reporting on its association with breast milk content. This study therefore assessed the role of maternal dietary intake on breast milk nutrient composition and infant nutritional outcome.

2.2 Maternal Dietary Intake during Lactation

Research evidence points out that the nutritional requirements during lactation are high than in any other stage of a woman's reproductive life (Kominiarek & Rajan, 2016; Selimoglu, 2013). According to Chen et al. (2012), a balanced and adequate diet among lactating mothers is crucial for meeting the physiologic needs of the mother and for the health, growth and development of the infant. Lactating women should therefore increase their energy and nutrient intakes in comparison with those who are not pregnant and lactating (Doran and Evers, 1997; Selimoglu, 2013; Sylvia & Mary 2002). The requirements are greater than any other stage of their life, since breast milk is required to supply an adequate amount of all the nutrients required for optimal infants' growth and development.

According to Allen (2012), Haileslassie et al. (2013), Hanson et al. (2015), Kominiarek and Rajan (2016), appropriate dietary practices among lactating women are considered key in achieving optimal lactation and sustaining it without depleting the mother's nutrient reserves leading to malnutrition. Of note, malnourished mothers are said to have reduced lactation performance contributing to the increased risk of child mortality (Demissie et al., 2003).

Inadequate maternal diet could lead to poor secretion of nutrients in breast milk which can have long term effects on the health of the child. This means that malnutrition and maternal deficiencies of nutrients could affect the nutritional status of the child and eventually compromise the overall growth and development of the child (Jones et al., 2010; Picciano, 2001). Notable, some studies have reported that maternal dietary intake could influence breast milk quantity production and nutrient content (Allen, 2012; Ballard & Morrow, 2013; Mosca & Giannì, 2017). To the contrary, some studies have reported that maternal dietary intake does not influence breast milk composition (Mohammad et al., 2009; Quinn et al., 2012).

Despite, nutrition of the lactating woman being reported by some studies to be vitally crucial in breast milk composition and production, while others showing no influence, there is inadequate research-based information to report on this subject conclusively. This is more particularly among lactating women in developing countries where poor dietary intakes have been reported. This study therefore sought to generate information on the role of maternal dietary intake on breast milk composition among lactating mothers with infants less than six months.

2.2.1 Factors affecting Dietary Intake

2.2.1.1 Socioeconomic and Demographic Factors

Past research has shown that dietary intake and nutritional status is affected by the socioeconomic and demographic factors (Arruda et al., 2014; Brinkman et al., 2010; Darmon & Drewnowski, 2008; Dunneram & Jeewon, 2013; Rashid et al 2011; Sekhampu, 2012). A study conducted in Germany reported that sex, age employment status, household size, and the level of education are key determinants to diet quality

(Thiele & Weiss, 2003). Households with higher income and resources are said to have a diet of higher nutritional quality. Silk et al. (2008), Darmon and Drewnowski (2008) observed that those individuals with lower education and income have a greater likelihood of having a poor-quality diet compared to those with a higher level of education and income. Since most of these studies have been conducted in developed countries, it is crucial to assess the association in a developing country like Kenya.

2.3 Phases of Lacto Genesis during Lactation

After delivery mothers produce the first fluid called colostrum, which is distinct in appearance, volume and composition. It's produced in low quantities in the first few days postpartum (Ballard & Morrow, 2013). The breast milk is rich in immunologic components for example secretory immunoglobulin (IgA), leukocytes, lactoferrin, as well as developmental factors such as epidermal growth factor (Castellote et al., 2011; Pang & Hartmann, 2007; Kulski & Hartmann, 1981). Reduced progesterone postpartum (first 4 days) leads to copious milk secretion. The stage is marked with changes in the milk volume and composition (Neville et al., 2001).

According to Ballard and Morrow (2013), transitional milk occurs after 5 days to 2 weeks postpartum, after which the breast milk is considered largely mature. After the 4th week the milk is considered fully mature. The substantial increase in milk volume is also accompanied by increased synthesis of all the nutritional components of mature milk such as lactose, protein, lipids, sodium, calcium, magnesium among others (Neville & Morton, 2001). Important to note, unlike infant formula with a standardized composition, breast milk composition is reported to change in the course of lactation, between mothers and populations. The changes could be influenced by genetic factors, environmental factors and maternal lifestyle, which could include dietary habits (Franca et al., 2010). These factors have however not been well investigated. This study therefore assessed the nutritional changes of breast milk composition among lactating mothers within five months postpartum.

2.4 Nutrient Content of Human Breast Milk and its Relationship to Maternal Dietary Intake

Human breast milk contains many nutrients that are essential for child growth and development (WHO, 2012). The nutritional quality of the breast milk may originate from lactocytes synthesis, maternal stores and dietary sources (Ballard & Morrow, 2013). Literature shows that breast milk is composed of 10 % solids and 90% water. The solid components are crucial for energy and growth while the water is important to maintain babies' hydration (Lawrence & Lawrence, 2005).

2.4.1 Macronutrients Composition of Human Breast Milk

The macronutrient composition may change in the whole course of lactation. The major components of solid breast milk are carbohydrate, protein and fats (Lawrence & Lawrence, 2005; Riordan, 2005). According to Ballard and Morrow (2013) the mean macronutrient content of mature breast milk is estimated to be 6.7 - 7.8 g/dL for lactose, 0.9 - 1.2 g/dL for protein and 3.2 – 3.6 g/dL for fat. Additionally, the energy content is estimated to be approximately 65 – 70 kcal/dL. The protein content of breast milk has been shown to vary at each stage of lactation ranging from 1.4 - 1.6 g/100 mL during early lactation, 0.8 - 1.0 g/100 mL by 3-4 months of lactation and 0.7 - 0.8 g/100 mL after 6 months of lactation (Jensen, 1995). The protein profile of breast milk also evolves to fulfil the nutritional needs of the infant for its growth and development. The two major breast milk proteins are whey and casein (Liao et al., 2011; Gao et al., 2012).

Some studies though minimal have reported the relationship between the protein quality of maternal diet and breast milk content (Linbald & Rahimtoola, 1974; Ogechi & Irene, 2013). A study has reported that poorly nourished mothers in Pakistan secreted milk low in methionine and cysteine and attributed it to poor protein quality in the diet of the mothers they studied. Since minimal data exist, further studies are needed to validate the relationship between breast milk protein content and maternal dietary intake.

In regard to fat and fatty acid composition, human milk is marked by high content of palmitic and oleic acids. It is also reported to be the most dynamic macronutrient of human milk (Ballard & Morrow, 2013; Saarela et al., 2005). Human milk fat is the major source of energy, essential fatty acids, and fat-soluble vitamins for breastfed infants (Koletzo et al., 2001). The content of polyunsaturated fatty acids (PUFAs) in breast milk is, by and large, reported to be a reflection of the maternal dietary intake (Jensen & Lapillonne, 2009). A study by Lauritzen et al. (2002) showed a maximal increase of decosahexaenoic acid (DHA) in breast milk after 10 hours of fish fat intake and that the effect had almost disappeared after 24 hours.

Additionally, North America women with low DHA dietary intake have been reported to have low DHA milk content (Valentine et al., 2010). Unfortunately, there is scarcity of literature in regard to the association between maternal dietary intake and breast milk macronutrient composition and particularly among African countries. This study therefore helps fill this gap by assessing this association among lactating women in Kenya.

2.4.2 Micronutrient Composition of Human Breast Milk

Cognizant of the aforementioned components, breast milk is said to be rich in vital vitamins and minerals. Ballard and Morrow (2013) have discussed that many micronutrients may vary depending on maternal dietary intake. Since maternal diet may not always be optimal, multivitamin supplements have been recommended in some cases (Allen, 2012; Greer, 2001). According to Allen (1994) some micronutrient such as thiamine, riboflavin, vitamin B-6, vitamin B-12, choline, retinol, vitamin D, selenium, and iodine are of great public health interest since their secretion into breast milk is rapidly and substantially reduced by maternal depletion. As of such, adequate maternal diets or supplementation with these nutrients could improve breast milk concentrations and ultimately enhance infant nutritional status.

Some studies have documented positive correlation between maternal dietary intake and breast milk micronutrient composition (Allen, 2009; Antonakou et al., 2011; Kodentsova & Vrzhesinskaya, 2006; Valent et al., 2011). Contrary to these findings, other studies have reported that there is no correlation between maternal dietary intake and the breast milk content (Bianchi et al., 1999; Lim et al., 1998). Inadequate literature exists to conclusively report the association between maternal dietary intake and breast milk micronutrients concentration (Bravi et al., 2016). More studies are therefore needed especially in African countries where such data is minimal. To add this much needed literature, this study sought to establish the relationship between maternal dietary intake of selected micronutrients and its respective concentration in lactating mothers' breast milk.

2.5 Maternal Nutrition Status and Human Breast Milk Composition

The nutritional status of lactating mothers is critical as it may affect breast milk nutrient composition. Pregnant and lactating women are considered vulnerable to poor nutritional status due to higher nutrient requirements (Mecacci et al., 2015). Poor maternal nutrition over this period risks depletion of the mother's own nutrient stores resulting to poor maternal health and sub-optimal growth of the child. Notably, maternal malnutrition (under and over-nutrition) have been reported as one of the main public health challenges particularly in developing countries (Black et al., 2013). Further, Haileslassie et al. (2013) have indicated that information on nutritional status and its associated factors among lactating women is urgently needed. This information is crucial for initiating and prioritizing programs geared towards improving maternal and child nutrition.

To sustain the production of adequate quantity and quality breast milk, lactating women are reported to have higher requirements of energy, macronutrients, and micronutrients (Kominiarek & Rajan, 2016). However, the information available on the influence of maternal nutritional status and breast milk nutrient content is scarce and diverse. Some studies have reported that lactating women body composition and anthropometric measurements have no association with breast milk nutrient content (Domellöf et al., 2004; Dias & Nakhawa, 2016; Kothari et al., 2017; Örün et al., 2012; Quinn et al., 2012; Yalcin et al., 2015). For example, Domellöf et al. (2004) reported that breast milk mineral content was independent of maternal mineral status.

Conversely, Allen (2005) reported that maternal nutritional status and dietary intake strongly affect the amount of nutrients secreted in the breast milk. Similarly, a study conducted in Russia revealed that maternal riboflavin deficiency resulted in low breast milk concentration of the vitamin (Kodentsova & Vrzhesinskaya, 2006). Furthermore, Dritsakou et al. (2017) and Fujimori et al. (2015) reported that women with higher BMI levels had higher fat and energy content in their breast milk. This indicates that maternal nutritional status could influence breast milk content. According to Yalcin et al. (2015) further studies are required to explicitly clarify the impact of maternal nutritional status on breast milk composition. There is no data in Kenya regarding the effect of maternal nutritional status on the composition of human milk.

2.6 Human Breast Milk Composition and its Relationship to Infant Nutrition Status

The nutritional status of a population, particularly children, is an important indicator of the quality of life in a society (Oliveira et al., 2013). Additionally, nutrition is an important component of children’s health and nutritional status (Ma et al., 2014). Breast milk contains all the desired nutrients that could have a major impact on an infant’s nutritional status. Breast milk feeding for children is therefore considered a pivotal factor for appropriate growth and survival of children (Islam et al., 2013; WHO, 2003a). To attain optimal infant growth and development, the global recommendations is that the infants need to be breastfed exclusively in their first six months after birth (WHO, 2008).

In early infancy, nutritional vulnerability can occur if breast milk offered to the infants does not meet their nutritional requirements. A growing amount of evidence points that malnutrition at early infancy leads to irreversible impaired growth and development of the infant that could make the infant not to realize his or her full potential later in life (Barker & Thornburg, 2013; WHO, 2003b). Human milk being the only source of nourishment for the infant, it is recommended that the infant be exclusively breastfed for the first six months of their life (WHO, 2001).

Until recently, it was widely believed that nutritional deficiencies in breastfed infants were relatively rare. According to WHO (2002) expert consultation on the optimal duration of exclusive breastfeeding, there is insufficient data to rule out to the possibility of micronutrient deficiencies even among infants who are exclusively breastfed for six months. With that regard, promoting human milk quality has become of great public health concern (Chapman & Nommsen-Rivers, 2012).

Some studies have pointed out that that sub-optimal maternal dietary intake may lead to sub-optimal breast milk nutrient concentration which might adversely affect the nutritional status of breastfed children (Ettang et al., 2004; Chapman & Nommsen-Rivers, 2012; Kiprop et al., 2016). According to Ballard and Morrow (2013), some human milk components could be modified through dietary intakes or exposures to optimize child growth and health. Nevertheless, the information remains largely scanty. This study sought to increase the scientific evidence by assessing breast milk nutrient composition and its relation to infant nutritional status in Nyeri County, Kenya.

2.7 Predictors of Human Breast Milk Composition

Maternal factors which may influence breast milk composition include; maternal dietary intake during both the gestation and lactation, stage of lactation, infant characteristics, maternal morbidity and mother's nutritional status (Chapman and Nommsen-Rivers, 2012; Kiprop et al., 2016; Novak and Innis, 2011; Powe et al., 2010). Concerning maternal morbidity, some studies have reported that morbidity during lactation could influence breast milk composition. For example, Amaral et al. (2019) reported that there was a lower concentration of lactose and fat in the breast milk of women with diabetes mellitus. Further, higher levels of total protein, sodium and potassium were reported in the breast milk of hypertensive mothers (Massmann et al., 2013). On the contrary, there was no significant difference in breast milk sodium and potassium levels between hypertensive and non-hypertensive mothers (Sírio et al., 2007). As evidenced by the inconsistencies in the findings above, the influence of maternal morbidity on the nutritional composition of human milk is still unclear and more research is thus needed.

Mode of delivery could also be a predictor of breastmilk composition. The mode of delivery was reported to have a strong influence on the microbiota content of breast milk (Toscano et al., 2017). Furthermore, a study by Dizdar et al. (2014) reported that vaginal delivery is associated with higher colostrum protein content. However, there is scanty information reporting on the association between mode of delivery and breastmilk composition particularly mature breast milk.

Studies have also cited maternal age and infant sex as potential determinants of breast milk composition (Dritsakou et al., 2017; Powe et al., 2010). Nevertheless, there is a paucity of information on the effect of maternal age and infant characteristics on breast milk composition. Due to minimal studies on breast milk composition, information on the predictors of breast milk composition is unclear and is being identified. The knowledge of these predictors will be very crucial in promoting interventions geared toward ensuring optimal infant health and development through modification of breast milk composition.

2.8 Summary of Literature Review

Based on the importance of breast milk in ensuring child survival, the need to optimize its quality becomes extremely indispensable. It is regarded that maternal dietary intake could influence breast milk nutrient composition either directly or indirectly. However, the level of maternal dietary influence on human milk nutrient concentration remains largely unknown. Furthermore, reviewed literature reveals that the available information in regard to this topic is scanty. Additionally, the scarce information available has been reported to be diversified and limited to certain populations. The literature also revealed that most of the literature available does not show the African situation.

Evidently, much more needs to be known about the nutrient composition of breast milk in different population groups with a range of nutritional status and dietary practices. Furthermore, information on the influence of maternal dietary intake on breast milk composition is still very minimal. Reference values additionally need to be developed based on concentrations in the breast milk of well-nourished women. These gaps in our information about the nutrient content of breast milk, especially in populations with reported poor dietary intake and nutritional status, indicate that this should be a research priority. With that consideration, this study therefore sought to determine the impact of maternal dietary intake on breast milk composition. The study also assessed the influence of breast milk composition on the infant nutritional status in Nyeri County.

CHAPTER THREE: METHODOLOGY

3.1 Study Design

A longitudinal study was conducted to establish the impact of maternal dietary intake on breast milk composition and infant nutritional status for a period of five months. The study adopted repeated measures design. The design allowed the detection of within-person change over time and also gave a higher statistical power for making inferences (Guo et al., 2013). Data was collected on the mother-child dyad at the 1st and 5th month of the infant life. Based on this, a comparison of breast milk nutrient composition among the lactating mothers based on their respective dietary intake was achieved.

3.2 Study Variables

The dependent variables were breast milk composition and infant nutritional status. The independent variables were the maternal factors which included; dietary intake, maternal nutrition status, parity, mode of delivery, stage of lactation, demographic and socioeconomic factors.

3.3 Study Area

The study was conducted in a rural setting in Kieni sub-county, Nyeri County. According to KDHS 2014, over one-quarter of children under five years are stunted or short for their age. Stunting is more common in rural areas than in urban areas (29% versus 20%) (KNBS & ICF International, 2015). Kieni sub-county is an Arid and Semi-Arid Land (ASAL) with a reported stressed food security situation due to unreliable rainfall and frequent droughts. This could therefore be affecting the dietary intake, breast milk composition and infant nutritional status of lactating women residing in this area.

According to Kenya National Drought Management Authority (NDMA) drought early warning bulletin for January 2017, the number of children at risk of malnutrition in Kieni Sub County increased by 40 percent as compared to 2011-2015 short term averages of 2.5 percent (GOK, 2017). Nyeri County is one of the 47 counties in Kenya and covers an area of 3,337.2 Km^2. The county is located between longitudes 360 38" and 370 20" East and between the equator and latitude 00 38" South. According to the Kenya Population Census (2009) the county has an approximated population of 693,558 people. Crop farming, dairy farming, trade and tourism are the main county economic activities (KNBS & ICF Macro, 2010).

3.4 Target Population of the Study

The target population were lactating women with infants under six months in Nyeri County.

3.4.1 Inclusion Criteria

The study included all lactating mothers with children under six months and who consented to participate. Only mothers who had a normal, uncomplicated pregnancy, given birth to a healthy singleton child and who had the intention of exclusively breastfeeding for 6 months were included in this study. Moreover, the lactating mothers had to have resided in the study area for not less than one year prior to the period of the study. This enabled them to have an experience of the whole food security cycle.

3.4.2 Exclusion Criteria

Chronically ill mothers or infants (identified from medical records and verified by a medical personnel) were excluded from the study. Mothers who had delivered a pre-term infant (< 37 weeks of gestation) or with infants who required neonatal care were also excluded. Other exclusion criteria included the lack of signed consent for participation in the research.

3.5 Sample Size Determination

The calculation of sample size gave a sample of 109 lactating mothers. This was computed using the OpenEpi software version 3.01 (Dean, 2013) based on the following considerations: 80% power of the test, an alpha of 0.05 and assuming a 20% change in breast milk nutrient content over the 5 months study period (Chan, 2003 & Dean, 2013). The software gave a sample size of 99 but 10% of the calculated sample size was added to cater for any drop out in the course of the study. A total of 109 lactating mothers were sampled, however, due to withdrawal and loss of follow up, this study collected and reported data on 104 mothers (Figure 3.1).

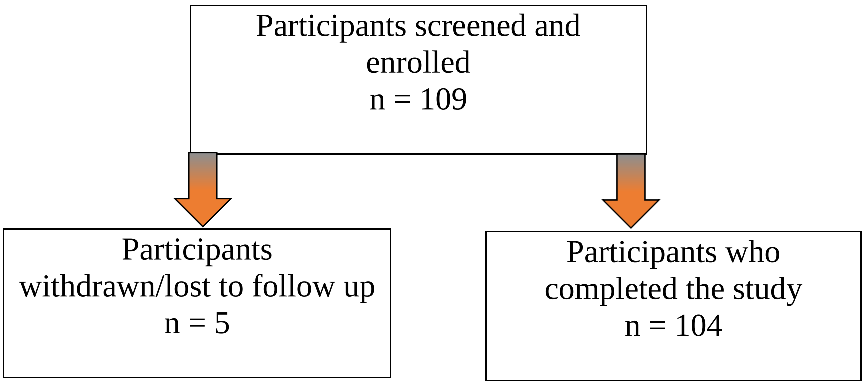

Figure 3.1: Flow chart showing study participants recruitment.

3.6 Sampling Technique of the Study

Purposive sampling method was used to select the study area (Nyeri County, Kieni sub-county). Comprehensive sampling method was used to select the participants who met the inclusion criteria as they visited the sub-county maternal and child health clinics for their usual antenatal care (Figure 3.6). The mothers were recruited for the study in the third trimester of their pregnancy as they attended the antenatal clinic.

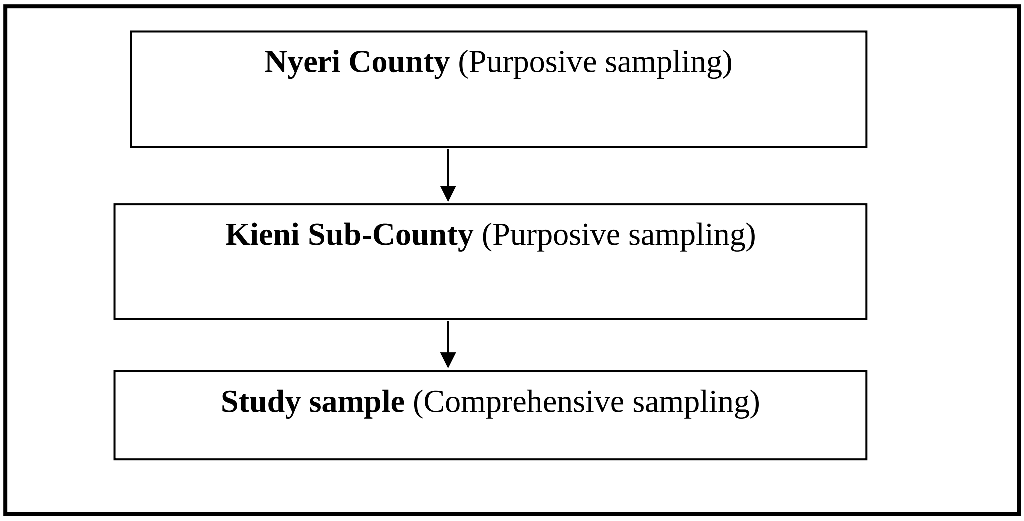

Figure 3.2: Flow chart on the sampling procedure

3.7 Recruitment of Study Participants

Recruitment was done by the researcher and assisted by the nutritionists attached to the maternal and child health clinics. Health facilities with maternal and child health clinic were purposively selected. Comprehensive enrolment was done with respect to the study inclusion criteria until the desired sample size was achieved. After delivery, the mothers were followed to their household where the study data and breast milk sample collection was conducted. Data collection was done one month after the delivery of the participants (to get mature breast milk), where an in-depth survey was conducted to obtain information on demographic, socioeconomic, morbidity, dietary intake, breast milk nutrient content and mother-child dyads nutritional status.

3.8 Data Collection Tools

A researcher administered questionnaire was used to collect data at the 1st and 5th month of the child's life. Information such as the age of participants, mode of delivery, parity, infant sex, monthly household income, occupation, level of education, household size, household assets, dietary intakes, morbidity pattern, breastfeeding practices (frequency & duration) and nutritional status were collected. Information on dietary intake was obtained using multiple 24-hour dietary recalls (two weekdays and one weekend day) and food frequency questionnaires. Seca infatometer and salter scale were used to assess children nutritional status while a standard adult non-stretchable Mid Upper Arm Circumference (MUAC) tape (Cogill, 2003), bathroom scale and stadiometer were used to assess the mothers' nutritional status. Equipment such as portable freezers, micro vials, clean containers, aluminium foil were used for transporting and storing serum and expressed breast milk.

3.9 Pretesting of the Data Collection Tools

Pretesting of the tools was conducted on 11 mothers (10% of expected sample size) living in the study sub-county to validate the research instruments and study procedures. The pretesting involved simulating the formal data collection process on a small scale to assess the ability of the tools to collect the desired data and to identify practical problems with regard to data collection instruments and methodology. Participants involved in the pretest were from the same study sub-county as the main study to ensure similar characteristics with the main study sample.

The participants who were involved in the pretesting were not sampled in the main study data collection. Of note, to avoid contamination of any form to the sampling pool of subjects involved in the study, the pretesting participants were drawn from a significantly distant health facility from the facilities involved in the main study. The tools were tested to assess whether they would collect the information required. This was carried out to assess the length, clarity, consistency, wording and content of the tools. Appropriate adjustments were thereafter made.

3.9.1 Validity of the Data Collection Tools

The data collection tools were evaluated and validated by a panel of nutrition professionals/experts.

3.9.2 Reliability of the Data Collection Tools

The Test-retest method was used to test the reproducibility of results among 10% of the study sample size. The study questionnaire was administered twice on the same participants within 7 days and the correlation coefficient determined. A correlation coefficient of 0.94 was established. A correlation coefficient of above 0.70 is considered acceptable (Cronbach & Shavelson, 2004; Mukaka, 2012).

3.10 Recruitment and Training of the Study Research Assistants

Six research assistants who had at least a Bachelor of Science degree in Food, Nutrition and Dietetics were recruited in this study. In addition, the research assistants must have had at least 4 months experience in nutritional field surveys. They were trained for 7 days on the objectives, study procedures, use of data collection tools and on interviewing skills. The training entailed the use of lectures, demonstrations, role plays and discussions.

3.11 Data Collection Procedures

3.11.1 Human Breast Milk Sample Collection

Breast milk was collected by manual expression by the mothers. The breast milk samples were transported using a portable freezer (-20^{o} C) to Jomo Kenyatta University of Agriculture and Technology (JKUAT) where they were stored at -80^{o} C prior to analysis (the samples were analysed at JKUAT Food Science Laboratory). The breast milk samples were collected four weeks after delivery when the milk is considered fully mature and thereafter at the 5th month of the infant life. To allow for comparable samples, mothers were requested to hand express the milk from their breast and the hindmilk collected. The process was repeated within a span of two weeks to reduce within day and day to day variations.

All the samples were collected in the morning (0800-1030) since this time has been shown to be most representative of 24-hour milk averages (Ruel et al., 1997). The samples were frozen and stored at -80^{o} C until the biochemical analyses were done at one point in time. A pooled total of 50 mL at each of the two stages of lactation was collected from each mother and mixed thoroughly before analyses. The samples were analysed immediately after thawing. In this study, carbohydrates (lactose), protein, fat, retinol, calcium, magnesium, iron and zinc were considered.

3.11.2 Nutrient Intake of the Participants

Maternal nutrient intake information was obtained using multiple 24-hour dietary recalls (two weekdays and one weekend day). All information about all foods consumed by the participant was captured. The process involved asking the participants to list all the foods they had consumed in the previous 24 hours from the day of the interview. The assessments were done at two terms; 1st and 5th month of their infant age.

3.11.3 Dietary Diversity of the Participants

Participants Individual Dietary Diversity Scores (IDDS) was determined based on 24-hour dietary recalls. Dietary diversity assessment is an important nutritional assessment as it is a proxy measurement of nutrient adequacy especially micronutrient adequacy. The information collected from the 24-hour recalls was used to assess dietary diversity score of each study subject. In this study, 10 food groups as indicated in the global dietary diversity questionnaire to measure women dietary diversity were considered (FAO & FHI, 2016).

The 10 food groups included: grains, white roots and tubers, and plantains; pulses (beans, lentils and peas); nuts and seeds; dairy; meat, fish and poultry; eggs; dark green leafy vegetables; other vitamin A rich fruits and vegetables; other vegetables and other fruits. Women who had consumed food from at least 5 food groups were considered to have attained the minimum dietary diversity for women (MDD-W) and had a higher likelihood of micronutrient adequacy (FAO & FHI, 2016).

3.11.4 Food Frequency of the Participants

Food frequency assessment helped to determine the habitual dietary intake of the participants. To determine the food intake frequency, a pretested food frequency questionnaire was used in assessing the participants household food intake and availability. In this study, a 7-day food frequency questionnaire was used. The food frequency questionnaire included a list of all commonly consumed food items as was identified from the community.

3.11.5 Breastfeeding Practices (Frequency and Duration)

To estimate the amount of breast milk consumed by the child, the participant was required to state the average frequency of breastfeeding per day and the average duration per feeding in minutes. A feed lasting for 10 minutes or more was assumed to be 100 mL in volume (10 mL per minute) and a proportion of this was computed if the feed was of a shorter duration (Emmett, 2000 & Özden et al., 2015).

3.11.6 Mother Child Dyad Morbidity Status

Illness affects dietary intake. Morbidity patterns were established by asking the women to recall any disease incidence in the previous two weeks prior to the study. The type, frequency, duration and manifestation of illness was established. The participants were also asked to state whether the illness affected their dietary practices. Infant morbidity pattern was obtained from the caregivers and medical reports.

3.11.7 Mother Child Dyad Nutrition status

3.11.7.1 Anthropometric Measurements

Child weight and recumbent length were used to assess the infants' nutrition status. Weight was measured to the nearest 1 g using a Seca electronic baby scale while the length was measured using a Seca infantometer and recorded to the nearest 0.1 cm. The lactating women MUAC (mid upper arm circumference) of their less active arm without clothes was also measured. The measurement was also recorded to the nearest

0.1 cm. Their weight and height were also measured and their respective BMI calculated [weight (kg)/height (m^2)]. To improve on accuracy, all the measurements were taken thrice and the average computed.

3.11.7.2 Biochemical Measurements

To establish the effect of maternal blood micronutrient status on breast milk composition of the lactating mothers, blood micronutrient status (retinol, calcium, magnesium, iron and zinc) was assessed. Blood samples were collected using standard methods of blood collection. Venous blood (5 mL) was obtained in the morning using disposable syringes by a qualified medical technologist. The blood samples once collected were covered using aluminium foil to protect them from light. Serum was obtained by whole blood centrifugation at 2500 rpm, for 10 minutes and at a room temperature of 4^{o} C. This was done within a time frame of one hour after blood collection at a health facility. The serum was thereafter aliquoted into cryovials, properly labeled and stored at –20° C by use of portable freezers to allow for transportation to the Jomo Kenyatta University of Agriculture and Technology laboratory (JKUAT). Once in the JKUAT laboratory, the serum samples were stored at -80^{o} C until the final biochemical analysis was conducted.

3.12 Breast Milk Sample Analysis

For accuracy purposes all the sample tests were carried out in triplicate and the average calculated.

3.12.1 Lactose Content Determination

Breast milk samples (5 g) were refluxed with 20 mL of 96% (v/v) ethanol for 1 hour at 100º C. The extract was then filtered and the residue washed with 80% v/v ethanol. The filtrate was evaporated to dryness in a rotary vacuum evaporator at 60º C and the dry residue was reconstituted with 10 mL distilled water and defatted using diethyl ether. The sample was micro-filtered with 0.45 µm filter and 20 µl injected into the High-Pressure Liquid Chromatography-HPLC (Shimadzu LC-20A) equipped with a refractive index detector (Shimadzu RID-10A). The mobile phase consisted of acetonitrile: water (75:25) at a flow rate of 0.8 mL/min on NH2P column of (250 mm x 4.6 mm x 5 ul). Quantification was done using standard solutions of lactose at the concentration range of 0.5-1% g/100 mL.

3.12.2 Proteins Content Determination

Protein was determined using the semi-micro Kjeldahl method. The breast milk was allowed to thaw, warmed to 38±1º C and mixed thoroughly. Immediately a weighed sample (5 mL) was put into the Kjeldahl digestion flask. All the weights were recorded to the nearest 0.0001 g. Then 40 mL 15% Trichloroacetic acid (TCA) solution was added to the flask. Of note, TCA solution was used to precipitate the protein and remove the Non-Protein Nitrogen (NPN). The mixture was then swirled and the precipitate allowed to settle for about 5 minutes. The mixture was then poured from the Kjeldahl flask through filter paper (Whatman No. 1, 15 cm, N-free; or equivalent) and the filtrate collected.

A pump dispenser was used to add 10 mL of 15% TCA to Kjeldahl flask and used to rinse any precipitate on the neck of the flask down into the bulb. The mixture was then swirled then poured from Kjeldahl flask through the same filter paper, and the filtrate added to the previously collected. The process was repeated till the entire filtrate which was clear and free from particulate matter was collected. Wearing TCA-resistant gloves and with a lot of care not to lose any precipitate, the filter paper was picked and dropped into the Kjeldahl flask. Boiling chips, 5 g potassium sulphate, 0.5 g of copper sulphate and 15 mL of sulphuric acid were then added. The mixture was heated in a fume hood till the digest colour turned blue/green. This signified the end of the digestion process.

The digest was cooled, transferred to a 100 mL volumetric flask and topped up to the mark with deionized water. Blank digestion with the catalyst was also made. 10 mL of the diluted digest was transferred into the distilling flask and washed with distilled water. 15 mL of 40% NaOH was added and this also washed with distilled water. Distillation was done to a volume of about 60 mL distillate. The distillate was titrated using 0.02 N HCL to orange colour of the mixed indicator, which signified the endpoint.

Calculations

Total Protein Nitrogen = $(V_1 - V_2) * N * F * 100 / (V * 100/S)$

Where: V_1 is the titre for sample in mL, V_2 is titre for blank in mL; N = normality of standard HCL (0.02); f = factor of standard HCL solution; V = volume of diluted digest taken for distillation (10 mL); S = weight of sample taken for distillation.

Total Protein = Total Protein Nitrogen* protein factor (6.38) (FAO, 2003)

Notable, in human milk, about 20-25% of nitrogen is Non-Protein Nitrogen (NPN). For this reason, the total protein nitrogen was obtained by removing the NPN from the total nitrogen (Ballard & Morrow, 2013; Wu et al., 2018). The non-protein nitrogen compounds include creatinine, urea, uric acid and nucleotides (Ballard & Morrow, 2013).

3.12.3 Fats Content Determination

Lipid fraction was extracted and determined from the breast milk as recommended by the Association of Official Analytical Chemists International (AOAC) Official Method 989.05 (AOAC, 2005) with slight modifications. The breast milk was thawed in a water bath heated to 38^{o} C. The milk was then vortexed for 1 minute. After vortexing, 10 g of the breast milk (to the nearest 0.0001 g) were weighed into clean and dry round bottomed flasks. The dry cork stoppers used during the sample weighing process were replaced with a wet cork stoppers which has had previously been soaked in water for six hours. NH_4OH (1.5 mL) was then added to the flasks and mixed thoroughly. This was done to neutralize any acid present and to dissolve casein in the milk. Additionally, 3 drops of phenolphthalein indicator were added which were to help sharpen the visual appearance of the interface between the ether and aqueous layers during the extraction.

After that, 10 mL of ethyl alcohol was added and a stopper with cork put to each flask. They were then shaken for 30 seconds. After shaking, 25 mL ethyl ether was added, stoppers with cork put, and the flasks shaken thoroughly for 1 minute. Pressure was released by loosening the stopper. Petroleum ether (25 mL) was then added, stopper with cork put and the shaking repeated for 1 minute. The flasks were then centrifuged at about 600 rpm for at least 45 seconds to obtain a clean separation of aqueous (bright pink) and ether phases. Ether solutions were decanted into clean weighed flasks. The solvents were then completely evaporated on a hot plate at 100^{o} C. The extracted fat plus the weighing flasks were then dried to a constant weight in a forced air oven at 100±1^{o} C for 45 minutes. The flasks were then removed from the oven and placed in a desiccator and allowed to cool to room temperature. The weight of each flask plus fat was taken to the nearest 0.0001 g.

Reagent blanks: The breast milk samples were replaced with 10 mL of distilled water and the test run as normal. The weight of any dry residue collected was recorded and used in the calculation.

Calculations

$$\text{Percent fat} = \frac{[(\text{wt flask + fat}) - (\text{wt flask})] - (\text{average wt blank residue})}{(\text{wt of breast milk})} \times 100$$

wt = weight

3.12.4 Energy Content Determination

In this study, energy in the breast milk was calculated from the proximate composition of macronutrients (carbohydrates, proteins and fats) through the application of energy conversion factors. The study adopted the United Nations (UN’s) FAO published energy conversion factor for milk and milk products. The factors are; 3.87, 4.27 and 8.79 for carbohydrates, proteins and fats, respectively (FAO, 2003). The macronutrient (carbohydrates, proteins and fats) were measured in grams per 100 mL of milk.

3.12.5 Retinol Content Determination

Retinol was analyzed by HPLC using a modification of the method by Zahar and Smith (1990). Triplicate breast milk samples (0.5 g) were homogenized with 4 mL of 95% ethanol and 1 mL of 50% KOH. The mixtures were saponified by heating in a 70° C water bath for 15 minutes and then cooled in an ice bath. Fat-soluble vitamins were extracted with 1 mL hexane containing 0.2% BHT, and a 1 mL aliquot of the hexane layer was evaporated under nitrogen. Saponification, extraction, and evaporation procedures were performed under yellow light. Samples were then reconstituted with 0.25 mL ethanol containing 0.1% BHT.

A Shimadzu 20 A series liquid chromatograph equipped with a (250 mmx 4.6 mm x5 ul) stainless steel ODS reversed-phase column was used to quantify α retinol as measures of vitamins A. The mobile phase was 95:5 methanol: water, for separation at a flow rate of 1 mL/min and the injection volume was 20 ul. The retinol was monitored at 325 nm wavelength on a UV-VIS detector (Shimadzu SPD 20 A). External standards were compared to sample extracts for the determination of vitamin concentrations.

3.12.6 Mineral Content Determination

The determination of minerals was done by dry ashing and atomic absorption spectrophotometer (AAS), according to AOAC (1995). In this study, the minerals that were determined were calcium, magnesium, zinc and iron. Acid washed mineral-free containers were used to store the samples. A 5 g sample was transferred into a previously weighed porcelain crucible which was evaporated to dryness in an electric furnace with low power. The crucibles with the samples were then placed on a hot plate under a fume hood and slowly the temperatures were increased until smoking ceased and the samples became thoroughly charred. They were then put into a muffle furnace and the temperatures increased gradually to 250^{o} C and heated for 1 hour. The temperatures were then increased to between 550^{o} C and incinerated to complete ashing for 18 hours. The temperatures were then decreased to 300^{o} C. The crucibles were removed and cooled at room temperatures. The resulting white ash was then weighed.

The ash of each crucible was then transferred quantitatively into 100 mL beaker using 20 mL of 1N-HCl. The solution was then heated at between 80- 90° C on a hot plate for 5 minutes. It was then transferred to a 100 mL volumetric flask and filled up to the mark with 1 N-HCl and properly mixed. Filtration was then done and the filtrate transferred to a well labelled polythene bottle. Of note, for calcium determination, lanthanum chloride as a releasing agent was added to achieve 0.5% in the sample solution. Suitable standard solutions were prepared, their absorbance's measured and calibration curves prepared. Concentrations of the minerals in the samples were determined by reading absorbances against calibration curves prepared from standard stock solutions of each mineral. The mineral detection wavelengths were; 422.7 nm, 285.2 nm, 248.3 nm and 213.9 nm for calcium, magnesium, iron and zinc, respectively.

3.13 Statistical Analysis and Data Presentation

Data from 24-hour dietary recalls was analysed using Nutri-survey version 2007 software to determine the amount of nutrients taken by each participant. This was compared with recommended nutrient intakes to assess for adequacy in consumption.

With regards to dietary diversity, all women with an intake of 5 and above food groups were considered to have reached the threshold for micronutrient adequacy. Infant weight for length z-score (WLZ), length for age z-score (LAZ) and weight for age z-score (WAZ) were calculated according to WHO growth reference standards (WHO, 2006). As recommended by the growth reference standards, the infants' nutrition status was categorized as; overweight and obese (≥ 2 z-scores), normal (≥ -2 to < 2 z-scores), moderately malnourished (< -2 to ≥ -3 z-scores), and severely malnourished (< -3 z-scores) (WHO, 2006).

According to WHO guideline, maternal Body Mass Index (BMI) was classified into four categories which included; underweight (< 18.5 kg/m^2), healthy (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obese (≥ 30 kg/m^2). Further, mothers with a MUAC value of less than 23 cm were considered undernourished (UNICEF, 2009; Ververs et al., 2013). The participants' dietary nutrient intake and nutrition status were correlated with their respective breast milk nutrient content.

Statistical Package for Social Sciences (SPSS) version 22 for windows was used to analyse data. Data was checked for normality using the Shapiro-Wilk test. Where data was skewed log transformations were carried out. For normally distributed continuous data, the Student t-test was used to check for mean differences based on the different groups, repeated breast milk measurements, repeated nutrition status measurements and also based on other maternal factors. For continuous skewed data, Wilcoxon Signed-Rank test and Mann-Whitney U test were used. Descriptive statistics like frequency, means, median ranges, standard deviations and variance are used to describe the research study population.

Pearson's moment correlation and Spearman's Rank correlation coefficient depending on data normality were used to test for correlation between the participants' nutrient intake and their respective breast milk nutrient composition. They were also used to determine which other variables were associated with the breast milk nutrient composition. The general linear model with repeated measures and mixed effects was used to establish the relationship between maternal factors and breast milk nutrient composition. In all the statistical analysis, a p-values of less than 0.05 was deemed as statistically significant.

3.14 Ethical and Logistical Considerations

Approval to carry out the study was sought from Kenyatta University Graduate School. Ethical clearance and the research permit were obtained from the Ethical Review Committee of Kenyatta University and from the Kenya National Commission for Science, Technology and Innovation (NACOSTI), respectively. Moreover, permission was sought from the County Director of Health Services. Informed signed consent was obtained from the study participants before the start of the interviews. A discussion of risks and possible benefits of the study was provided to the participants and their families. Consent forms described the study procedures and possible benefits and risks as required prior to starting of the study.

3.15 Care and Protection of the Study Participants

The research procedure was adequately explained to the participants. This was to assure them that no risk was associated with the study. Once the recruitment was conducted at the health facility, the mothers were followed to their household after delivery where the interviews, breast milk and serum samples collection were conducted in order to ensure privacy. Further, to ensure confidentiality, participant’s names were not used. Instead, codes were used to help identify the participants. The participants were also informed of their right to withdraw from the study at any time without penalty.

CHAPTER FOUR: RESULTS

4.1 Study Response Rate

The response rate of the study was calculated from the minimum required sample size without adjustment for non-response. The minimum expected sample size was 99 and after non-response adjustments the expected sample size of 109 was derived. A total of 109 participants (mother-infant dyads) were selected for this study at baseline, of which 5 participants were withdrawn/lost to follow up and hence were not included in the study. Therefore, a response rate of 100% of the required sample size and 95.4% of the expected sample size was reported in this study.

4.2 Description of the Study Population

4.2.1 Demographic Characteristics of the Participants

Demographic characteristics of the mothers who participated in this study are presented in Table 4.1. The mean maternal age was 28.83 (± 6.47 SD) years. The mothers' age ranged from 18 to 44 years and with the majority (49%) being in the age category of 25-34 years. In regards to religious affiliations, almost all (98.1%) of the mothers were Christians and the majority (82.7%) were married. Almost two thirds (63.5%) of the mothers' households had between 2 and 4 household members. More than half (58.7%) of the participants had one or two children in their households. Among the children, majority (68.3%) of the households had only one child below the age of 5 years.

With regards to the mode of delivery, majority (76%) of the mothers delivered vaginally. Ninety-eight mothers (94.2%) delivered at a health facility. In respect to the mothers' parity, the study observed that a significant proportion (90.4%) of the mothers were multiparous (Table 4.1).

Table 4.1: Demographic characteristics of the participants

Characteristic	N = 104 n	%
Age group (years)		
≤ 24	31	29.8
25-34	51	49.0*
35-44	22	21.2
Mean age 28.83 (± 6.47 SD)		
Marital status		
Married	86	82.7*
Single	16	15.4
Divorced/separated	2	1.9
Religion		
Christian	102	98.1*
Muslim	2	1.9
Household size		
2-4	66	63.5*
5-6	26	25.0
7-8	12	11.5
Mean household size 4.18 (± 1.50 SD)		
Number of children (household)		
1-2	61	58.7*
3-4	38	36.5
5-6	5	4.8
Mean 2.37 (± 1.22 SD)		
Number of children < 5 years (household)		
1	71	68.3*
2	33	31.7
Mean 1.32 (± 0.47 SD)		
Mode of delivery (index child)		
Caesarean section	25	24.0
Vaginal delivery	79	76.0*
Place of delivery (index child)		
Hospital/health facility	98	94.2*
Home delivery	6	5.8
Mothers' parity		
Primiparous	10	9.6
Multiparous	94	90.4*

* Majority

4.2.2 Socio-economic Characteristics of the Participants

The socio-economic characteristics of the participants are presented in Table 4.2.

Table 4.2: Socioeconomic characteristics of the participants

	N = 104	
Characteristic	**n**	**%**
Mother education status (highest attained)		
No formal education	4	3.8
Primary	39	37.5
Secondary	43	41.3*
Tertiary	18	17.3
Mothers' occupation status		
Unemployed	34	32.7*
Casual labourer	9	8.7
Farmer (own farm)	25	24.0
Self-employed/business	29	27.9
Employed (salaried)	7	6.7
Household head education status		
Primary	25	24.0
Secondary	59	56.7*
Tertiary	20	19.2
Household head occupation status		
Unemployed	2	1.9
Casual labourer	29	27.9
Farmer (own farm)	17	16.3
Self-employed	31	29.8*
Employed (salaried)	25	24.0
Average household monthly income		
≤ Ksh 10000	38	36.5*
Ksh 10001-20000	27	26.0
Ksh 20001-30000	24	23.1
> Ksh 30000	15	14.4
Main source of household income		
Casual labour	29	27.9
Farming proceeds	19	18.3
Own business	31	29.8*
Employed	25	24.0
Main source of household food		
Own production	36	34.6
Purchased	53	51.0*
Own production and purchased	15	14.4
Livestock ownership		
Households that owned livestock	68	65.4*
Land ownership		
Households that owned land	83	79.8*

* Majority

A total of forty-three mothers (41.3%) had attained secondary education as their highest level of education. About 37.5% had primary education. At the time of data collection, the highest proportion (32.7%) of the mothers were unemployed/housewives with 24% reporting to be working on their farms. All (100%) the household heads had some formal education with the higher proportion (56.7%) reporting secondary level as their highest level of education. The highest proportion (29.8%) of the household heads were self-employed.

Pertaining to household average monthly income, slightly more than a third (36.5%) reported to be earning an average monthly income of less than or equal to Ksh 10000. Income was assessed as a combined income of both parents. With respect to the source of household income, the highest proportion (29.8%) of the participants reported that the money came from self-owned businesses. This study also assessed the main source of household foods.

The study noted that more than half (51%) of the participants were majorly purchasing their food. About 34% expressed that they largely relied on their farm production as their main source of food. Of note, the majority (65.4%) of the participants stated that their household owned some livestock. As shown in Table 4.2, the majority (79.8%) of the households were reported to own land.

4.2.3 Infants' Characteristics

Slightly more than half (55.8%) of the children were males. The study further found that the mean birth weight of the infants was 3032 (± 514 SD) grams.

4.3 Maternal Dietary Intake and Breastfeeding Practices

4.3.1 Mothers' Breastfeeding Practices

Timely initiation of breastfeeding is vital for the survival of infants. In this study, 89.4% of the mothers reported initiating breastfeeding within 1 hour of birth (Table 4.3). Moreover, the majority (92.3%) of the mothers indicated that they had fed colostrum/first milk to their infants. The study also observed that most (68.3%) of the mothers had received breastfeeding education/support which was provided by healthcare providers during pregnancy and/or after the delivery.

Table 4.3: Baseline characteristics of the mothers' breastfeeding practices

	N = 104	
Characteristic	**n**	**%**
Number of mothers who reported timely initiation of breastfeeding within 1 hour of birth	93	89.4
Number of infant breastfed on colostrum after birth	96	92.3
Number of mothers who reported receiving breastfeeding support	71	68.3
Number of infant breastfed in the previous 24 hours	104	100
Number of times the child was breastfed in the previous 24 hours		
1-5	9	8.7
6-10	61	58.7
11-15	26	25.0
16-20	8	7.7
Mean 10.25 (± 3.704 SD)		
Average duration (in minutes) of each breastfeeding session in the previous 24 hours		
1-10	54	51.9
11-20	33	31.7
21-30	17	16.3
Mean 13.99 (± 8.61 SD)		

Twenty-four hours prior to the interview, all (100%) mothers had breastfed their index child at the two time points. Additionally, the study also assessed the frequency and the average duration (per session) of breastfeeding per day among the mothers.

As reflected in Table 4.3, more than half (58.7%) of the mothers reported to be breastfeeding their infants for between 6-10 times per day. With regards to the average duration per session, slightly more than half (51.9%) stated that the breastfeeding session lasted for between 1-10 minutes.

4.3.2 Mothers' Dietary Intake

In this study, dietary intake of the mothers was assessed using four indicators namely; dietary diversity score, food frequency, meal frequency and nutrient intake. The assessment was done at both 1st month and 5th month after delivery.

4.3.2.1 Dietary Diversity of the Lactating Mothers

Dietary diversity of the mothers was based on repeated 24-hour recalls. As recommended by global dietary diversity questionnaire to measure women dietary diversity score (WDDS), the study considered 10 food groups (FAO & FHI, 2016). The considered food groups included; grains, white roots and tubers, and plantains; pulses (beans, lentils and peas); nuts and seeds; dairy; meat, fish and poultry; eggs; dark green leafy vegetables; other vitamin A rich fruits and vegetables; other vegetables and other fruits. Women who had consumed at least 5 food groups were considered to have attained the minimum acceptable dietary diversity (MDD-W) for a higher likelihood of micronutrient adequacy.

The mean dietary diversity score of the lactating mothers was 5.32 (± 1.05 SD) and 5.16 (± 1.08 SD) at 1st month and 5th month, respectively. Out of the 10 food groups, the minimum dietary diversity score among the lactating mothers was two food groups at both 1st and 5th month. The maximum food groups consumed was 8 and 9 at

1st and 5th month, respectively. Table 4.4 displays the percentage of consumption of each food groups at the different times of assessment. Starchy staples, other vegetables, dairy and pulses were the major and dominant food groups consumed by most of the participants at both times of assessment (1st and 5th month of lactation). Nonetheless, nuts and animal source foods in the exception of dairy were least consumed. Worthwhile noting, only 33.7% and 16.3% of the study participants reported the consumption of meat and eggs at month one of lactation, respectively. The consumption was noted to decline at month five for both meat (26%) and eggs (13.5%).

Table 4.4: Dietary diversity of the lactating mothers

N = 104		**Month of lactation**					
		1st month			**5th month**		
	Food group	**n**	**%**	**95% C. I**	**n**	**%**	**95% C. I**
1.	Grains, white roots and tubers and plantains	103	99.0	96.8-100	101	97.1	92.9-100
2.	Pulses (beans, lentils and peas)	76	73.1	64.4-79.8	73	70.2	61.5-79.8
3.	Nuts and seeds	2	1.9	0.0-5.8	7	6.7	1.9-11.5
4.	Dairy	93	89.4	82.7-95.5	94	90.4	83.3-97.1
5.	Meat, poultry and Fish	35	33.7	21.8-47.7	27	26.0	16.7-34.3
6.	Eggs	17	16.3	8.3-24.4	14	13.5	7.7-20.5
7.	Dark green leafy vegetables	73	70.2	63.1-79.8	77	74.0	64.1-81.7
8.	Other vitamin A rich fruits and vegetables	30	28.8	20.8-36.9	24	23.1	15.4-32.1
9.	Other vegetables	96	92.3	86.2-98.1	93	89.4	84.3-94.6
10.	Other fruits	29	27.9	20.8-37.8	27	26.0	18.9-34.9

4.3.2.1.1 Minimum Dietary Diversity of the Lactating Mothers

The prevalence of lactating mothers meeting the minimum dietary diversity score (≥5 food groups) was 79.8% [95% CI: (72.1, 86.5)] at first month and 73.1% [95% CI: (65.4, 80.6)] at the fifth month of lactation (Figure 4.1).

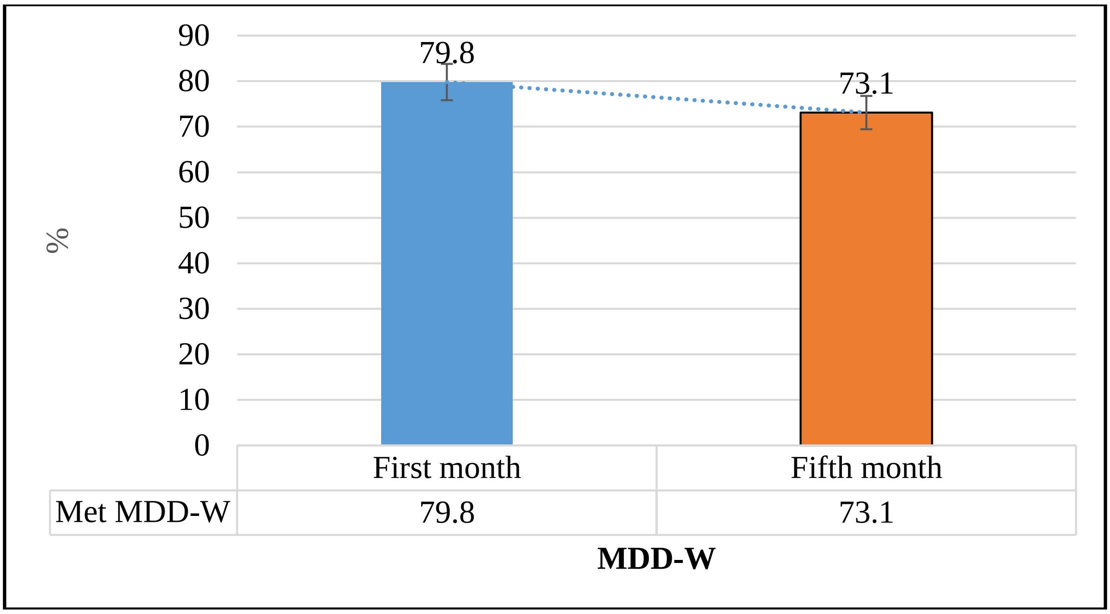

Figure 4.1: Prevalence of lactating mothers meeting the minimum dietary diversity for women (MDD-W)

4.3.2.2 Food Frequency of the Lactating Mothers

The food frequency of the participants was determined using a reference period of seven days. Commonly consumed foods in the community were listed and their consumption in the previous 7 days established (Table 4.5). In this study, consumption of a food for more than three times in a week was considered to be a regular food intake. As reflected in Table 4.5, cereal-based foods were most commonly consumed. Of note, maize meal was consumed by everyone in the study group. The least consumed foods were mangoes, pawpaw, organ meat, cassava and groundnuts in both 1st and 5th month of the mothers' lactation. The mean intake (days) of the majority of foods was not significantly different in the two stages of lactation as shown in Table 5.

Table 4.5: Proportion of the lactating mothers consuming various foods (7 days recall)

N = 104	Month of lactation						
	1st month			5th month			
Food group	**n**	**%**	**Mean intake(days)**	**n**	**%**	**Mean intake(days)**	**P value (t-test)**
Cereals							
Maize	94	90.4	2.15	90	86.5	1.97	0.377
Maize flour	104	100	3.71	104	100	4.13	0.145
Rice	99	95.2	3.07	96	92.3	2.88	0.525
Sorghum	85	81.7	5.70	73	70.2	4.42	0.008*
Cassava	18	17.3	0.97	13	12.5	0.57	0.208
Millet	90	86.5	5.88	80	76.9	4.61	0.004*
Chapati/bread/cakes	103	99.0	3.89	93	89.4	3.21	0.033*
Raw bananas	71	68.3	1.75	73	70.8	1.57	0.461
Vegetables and tubers							
Kales	92	88.5	3.56	98	94.2	3.78	0.469
Cabbage	96	92.3	3.62	85	81.7	3.08	0.070
Spinach	91	87.5	3.70	83	79.8	3.26	0.203
Amaranth(terere)	40	38.5	1.03	30	28.8	0.67	0.129
Carrots	88	84.6	3.59	73	70.2	2.77	0.022*
Tomatoes	98	94.2	6.07	90	86.5	5.40	0.058
Pumpkin	56	53.8	1.28	65	62.5	1.31	0.894
Sweet potatoes	57	54.8	1.47	48	46.2	1.13	0.197
Irish potatoes	89	85.6	3.36	96	92.3	3.64	0.342
Pulses/nuts/seeds							
Beans	96	92.3	2.79	99	95.2	2.59	0.968
Peas	47	45.2	0.96	59	56.7	1.21	0.808
Green grams	81	77.9	1.90	70	67.3	1.57	0.071
Lentils	31	29.8	0.60	37	35.6	0.64	0.856
Ground nuts	6	5.77	0.15	15	14.4	0.30	0.342
Milk and milk products							
Milk/Yoghurt	95	91.3	6.32	97	93.3	6.02	0.087
Eggs	80	76.9	2.25	70	67.3	1.94	0.454
Meat							
Organ meat	30	28.8	0.44	27	26.0	0.34	0.367
Other meat	93	89.4	2.08	84	80.8	1.73	0.109
Fruits							
Avocado	84	80.8	2.66	75	72.1	2.24	0.426
Mangoes	18	17.3	0.55	35	33.7	0.85	0.977
Pawpaw	26	26.9	0.45	23	22.1	0.35	0.093
Passion fruit	51	49.0	1.30	55	52.9	1.32	0.786
Ripe bananas	93	89.4	3.39	82	78.8	2.78	0.945
Oranges	85	81.7	2.88	77	74.0	2.16	0.496
Watermelon	57	54.8	1.38	51	49.0	1.09	0.629

***** Means significantly different

4.3.2.3 Meal Frequency of the Lactating Mothers

The quality of an individual diet is dependent on meal frequency and food groups contained in the diet. This study assessed the meal frequency of the lactating mothers. Both the main meals and snacks were considered during the computation of each participant meal frequency. The majority of the study participants had a meal frequency of 5 meals (34.6%) at 1st month and 4 meals (31.7%) at 5th month (Table 4.6).

Table 4.6: Meal frequency of the lactating mothers

N = 104	**Month of lactation**						
	1st month			**5th month**			
Number of meals per day	**n**	**%**	**95% C. I**	**n**	**%**	**95% C. I**	**P value**
2	0	0	0-0	10	9.6	3.5-14.7	
3	17	16.3	9.3-24.0	28	26.9	17.9-36.5	
4	22	21.2	14.1-27.9	33	31.7*	22.7-41.0	
5	36	34.6*	24.0-43.6	26	25.0	17.3-34.0	
6	29	27.9	20.2-35.9	7	6.7	2.9-10.9	
< 3	0	0	0-0	10	9.6	4.5-15.7	
≥ 3	104	100	100-100	94	90.4	84.3-95.5	
Mean (SD)	4.74 ± 1.04			3.92 ± 1.09			<0.001**

*majority, **paired t-test

4.3.2.4 Dietary Intake of Energy and Selected Nutrients by the Lactating Mothers

The dietary assessment for energy and nutrients intake was determined using multiple 24-hour dietary recalls and the average computed. The assessment was done at both the 1st and 5th month of lactation. The selected nutrients by the study included; carbohydrates, proteins, fats, vitamin A, calcium, magnesium, zinc, and iron. The intake of each participant was compared with the Recommended Nutrient Intake (RNI) to determine the proportion of study participants meeting their nutrient requirements. The summary of the lactating mothers' energy and nutrient intake is presented in Table 4.7.

Table 4.7: Nutrient intake of energy and selected nutrients among the participants

N = 104		**Month of lactation**				
		1st month		**5th month**		
Energy and Selected Nutrient	**Reference value**	**Mean (SD)**	**% meeting RNI**	**Mean (SD)**	**% meeting RNI**	**P value[a]**
Energy (kcal)	2555	2792.88 ± 688.95	61.5	2618.96 ± 591. 28	49.0	<0.001*
Carbohydrates (g)	175	557.22 ± 245.77	100	541..33 ± 227.83	100	0.450
Carbohydrate (%)[b]	45-65	67.20 ± 5.06	30.8	67.55 ± 5.72	33.7	0.572
Protein (g)	71	87.36 ± 27.65	66.3	84.13 ± 26.25	61.5	0.076
Protein (%)[b]	10-15	11.12 ± 2.26	76.9	10.90 ± 2.68	53.8	0.926
Fat (g)	90	80.09 ± 27.75	26.9	74.49 ± 22.35	21.2	0.011*
Fat (%)[b]	20-30	21.78 ± 3.75	83.7	21.35 ± 4.31	79.8	0.412
Vitamin A (µg)	850	1483.50 ± 1834.15	61.5	1391 ± 1782. 80	63.5	0.859
Calcium (mg)	1000[c]	807.57 ± 389.13	23.1	817.91 ± 373.76	26.0	0.444
Magnesium (mg)	270	681.42 ± 219.33	98.1	636.75 ± 203.76	97.1	0.005*
Zinc (mg)	9.5[d]	8.12 ± 4.58	32.7	7.99 ± 4.45	30.8	0.630
Iron (mg)	10[e]	45.69 ± 44.83	90.4	48.56 ± 53.10	96.2	0.290

RNI adapted from *FAO/WHO 1981; FAO/WHO 2001; WHO/FAO 2004*

[a]P-values for the differences in the energy and nutrient intake between 1st and 5th month of lactation; two-tailed paired Student's *t*-test. [b]Expressed as a percentage of total energy intake. [c]Based on a low animal protein intake. [d]Based on a moderate bioavailability level. [e]Based on a 15% bioavailability level. *significantly different

Analysis of mean energy and the selected nutrient intake of the women revealed that the women exceeded the requirements for energy, carbohydrates, vitamin A, magnesium and iron at both stages of lactation (1st and 5th month). The proportion of lactating mothers with dietary intake below the recommended intake varied between the nutrients. The study observed that more than half (> 50%) of the mothers did not meet the recommended nutrient intake for fats, calcium and zinc in both stages of lactation.

Furthermore, more than half (61.5%) of the participants met the recommended energy intake in the first month of lactation which reduced to slightly below half (49.0%) at the fifth month postpartum. Almost all (> 90%) of the participants met the recommended nutrient intake for magnesium and iron. Interestingly, all the mothers (100%) met their carbohydrate requirements at the two stages of lactation considered by the study. The mean intake of all the selected nutrients was not significantly different at the two stages of lactation in the exception of fat (p value = 0.011) and magnesium (p value = 0.005). Energy intake was also noted to be significantly different between the 1st and the 5th month of lactation (p value < 0.001).

4.4 Mother-Child Dyad Nutrition Status

4.4.1 Infants' Nutrition Status

The nutritional status of the infants was determined by weight-for-length, length-for-age, weight-for-age and their morbidity profile. The anthropometric measurements were used to establish the level of under nutrition among the infants.

4.4.1.1 Prevalence of Wasting (weight-for-length)

The mean z-score for boys was 0.92 (± 2.19 SD), while for girls was 1.36 (± 2.69 SD), at month one postpartum. At month five, the mean z-score for boys was 0.05 (± 1.74 SD), while for girls was 1.03 (± 1.99 SD). The mean z-score of boys and girls was not significantly different at 1st month (t-test, p value = 0.358), but became significantly different at 5th month post-delivery (t-test, p value = 0.009). The mean z-score for all the infants was 1.11 (± 2.42 SD) and 0.48 (± 1.91 SD) at 1st and 5th month, respectively. The burden of wasting (global acute malnutrition) among the study infants was 6.7% and 8.7% at 1st and 5th months, respectively (Table 4.8).

Table 4.8: Prevalence of wasting

	N = 104					
	1st month			**5th month**		
Nutrition status	**n**	**%**	**C.I**	**n**	**%**	**C.I**
Normal	97	93.3	88.1-97.1	95	91.3	85.6-96.2
Moderate	4	3.8	0.2-7.5	5	4.8	0.7-8.9
Severe	3	2.9	0.3-6.1	4	3.8	0.2-7.5
Global acute malnutrition	7	6.7	1.9-11.5	9	8.7	3.1-14.1

4.4.1.2 Prevalence of Stunting (length-for-age)

The mean z-score for all the infants was 1.20 (± 1.78 SD) and -0.53 (± 1.43 SD) at 1st and 5th month, respectively. The mean z-score for boys was 0.91 (± 1.85 SD), while for girls was 1.58 (± 1.63 SD), at month one postpartum. At the fifth post-delivery, the mean z-score for boys was -0.52 (± 1.52 SD), while for girls was -0.53 (± 1.33 SD). The means between boys and girls at both points of assessment were not significantly different (t-test, p value > 0.05). Table 4.9 shows the prevalence of stunting among the children.

Table 4.9: Prevalence of stunting

	N = 104					
	1st month			5th month		
Nutrition status	**n**	**%**	**C.I**	**n**	**%**	**C.I**
Normal	101	97.1	93.3-100	94	90.4	83.7-95.5
Moderate	3	2.9	0.3-6.1	7	6.7	1.9-11.5
Severe	0	0	0.0-0.0	3	2.9	0.3-6.1
Global acute malnutrition	3	2.9	0.3-6.1	10	9.6	3.9-15.3

4.4.1.3 Prevalence of Underweight (weight-for-age)

This study observed a mean z-score of 2.18 (± 1.74 SD) and -0.04 (± 1.19 SD) at 1st and 5th month, respectively among all the children. Girls had a higher z-score of 2.81 (± 1.58 SD) than boys [1.68 (± 1.70 SD)], at month one postpartum. At the fifth post-delivery, the mean z-score for boys was -0.36 (± 1.23 SD), while for girls was 0.37 (± 1.00 SD). The means between boys and girls were significantly different at both month one (t-test, p value = 0.01) and fifth month (t-test, p value = 0.01) postpartum. As demonstrated in Table 4.10, there was no child who was underweight in first-month post-delivery.

Table 4.10: Prevalence of underweight

	N = 104					
	1st month			5th month		
Nutrition status	**n**	**%**	**C.I**	**n**	**%**	**C.I**
Normal	104	100	100-100	98	94.2	89.4-98.1
Moderate	0	0	0.0-0.0	6	5.8	1.3-10.3
Severe	0	0	0.0-0.0	0	0	0.0-0.0
Global acute malnutrition	0	0	0.0-0.0	6	5.8	1.3-10.3

4.4.1.4 Infants' Morbidity Profile

This study determined the morbidity status of the infant in a period of two weeks prior to the interviews at both points of assessment. Child morbidity data were acquired through the examination of the children's clinical records and from the reports by the children's mothers. At one-month post-partum, 28.8% of the children were reported to be unwell. The prevalence of morbidity increased to 36.5% in the fifth month of lactation. During both stages of lactation, respiratory infections and gastrointestinal disorders were the most common among the infants (Table. 4.11). Other morbidities included fever without cough, eye and skin infection. Most of those who had sick children reported seeking medical attention. The study further observed that majority of the children caregivers who sought medical attention sought it from government hospitals.

Table 4.11: Infants' morbidity profile

	N = 104			
	1st month		**5th month**	
Characteristic	**n**	**%**	**n**	**%**
Number of sick infants within the previous two weeks	30	28.8	38	36.5
Type of morbidity among the sick infants (multiple responses)	N = 30		N = 38	
Respiratory infection (fever and cough)	21	70.0	23	60.5
Fever without cough	4	13.3	7	18.4
Diarhoea	4	13.3	5	13.2
Vomiting	2	6.7	4	10.5
Stomachache	1	3.3	5	13.2
Eye infection	2	6.7	1	2.6
Skin infection	0	0	3	7.9
Total	34	113.3	48	126.3
Number of mothers who sought medical attention	25	83.3	29	76.3
Type of facility where medical attention was sought	N = 25		N = 29	
Government hospital	20	80.0	18	62.1
Private hospital	4	16.0	8	27.6
Chemist	1	4.0	3	10.3
Total	25	100	29	100

4.4.2 Mothers' Nutrition Status

Nutrition status of the mothers was determined using anthropometric measurements (BMI and MUAC), biochemical analysis and their morbidity profile.

4.4.2.1 Anthropometric Measurements

Most of the mothers had normal ranges of body mass index at the first (n = 47, 45.2 %) and fifth (n = 54, 51.9 %) month postpartum. Seven (6.7%) and sixteen (15.4%) of the mothers had a BMI less than 18.5 kg/m^2 (chronically energy deficient) at the first and the fifth month of lactation, respectively. The rest of the women had a BMI greater than or equal to 25 kg/m^2 (over weight and obese) as presented in Table 4.12. With regards to MUAC, the majority (> 90 %) of the mothers had a MUAC value of ≥ 23 cm at both stages of lactation. Furthermore, this study observed statistically significant differences (paired t-test) between MUAC, weight and BMI values in the first and fifth month of lactation (MUAC; p-value < 0.001, weight; p-value < 0.001 and BMI; p value = 0.002).

Table 4.12: Anthropometric status of the lactating women

	N = 104				
	1st month		**5th month**		
Characteristic	**n**	**%**	**n**	**%**	**P value**
Maternal BMI					
Underweight	7	6.7	16	15.4	
Normal	47	45.2	54	51.9	
Overweight	34	32.7	22	21.2	
Obese	16	15.4	12	11.5	
Mean weight (kg)	64.66±15.11^a	-	59.92±14.22^a	-	< 0.001^b
Mean height (cm)	161.29±7.82^a	-	161.29±7.82^a	-	-
Mean BMI (kg/m^2)	24.88 ± 5.38^a	-	23.02 ± 5.42^a	-	0.002^b
MUAC					
< 23 cm	2	1.9	5	4.8	
≥ 23 cm	102	98.1	99	95.2	
Mean MUAC (cm)	29.28±3.83^a	-	28.53±3.78^a	-	<0.001^b

^{a}Data expressed as mean ± standard deviation (SD); ^{b} means significantly different

4.4.2.2 Biochemical Measurements

The study also assessed hemoglobin and serum nutrient status (serum retinol, calcium, magnesium, zinc and iron) of the mothers. The mean serum nutrient profile of the lactating mothers is shown in Table 4.13. There was no difference in the mean concentration of selected parameters between the mothers at first and fifth month postpartum except for iron.

Table 4.13: Serum status of the lactating women

Biochemical variable	Reference values[b]	N = 104 Month of lactation 1st month mean	5th month mean	P value
Retinol (µg /dL)	20-80	25.47±19.19	29.77±16.15	0.080
Calcium (mg/ dL)	8.2–10.4	9.67 ± 5.46	10.06 ± 3.38	0.459
Magnesium (mg/dL)	1.5-2.5	2.24 ± 0.90	2.42 ± 1.39	0.244
Zinc (µg /dL)	50-150	85.40 ± 34.85	93.03 ± 36.31	0.146
Iron (µg /dL)	60-150	88.88 ± 37.09	101.72 ± 46.07	0.038[a]
Hemoglobin (g/dL)	12-16	13.17 ± 1.57	13.21± 1.42	0.855

Data expressed as mean ± standard deviation (SD), [a] means significantly different (t-test), [b]reference values-WHO (2004) & Khoushabi et al. (2016).

4.4.2.3 Mothers' Morbidity Profile

Based on hemoglobin levels, the prevalence of anemia among the mothers was established. The prevalence of anemia (Hb <12.0 g/dL; WHO, 2001) was found among 15.4 % and 12.5 % at 1st and 5th month post-delivery, respectively (Figure 4.2).

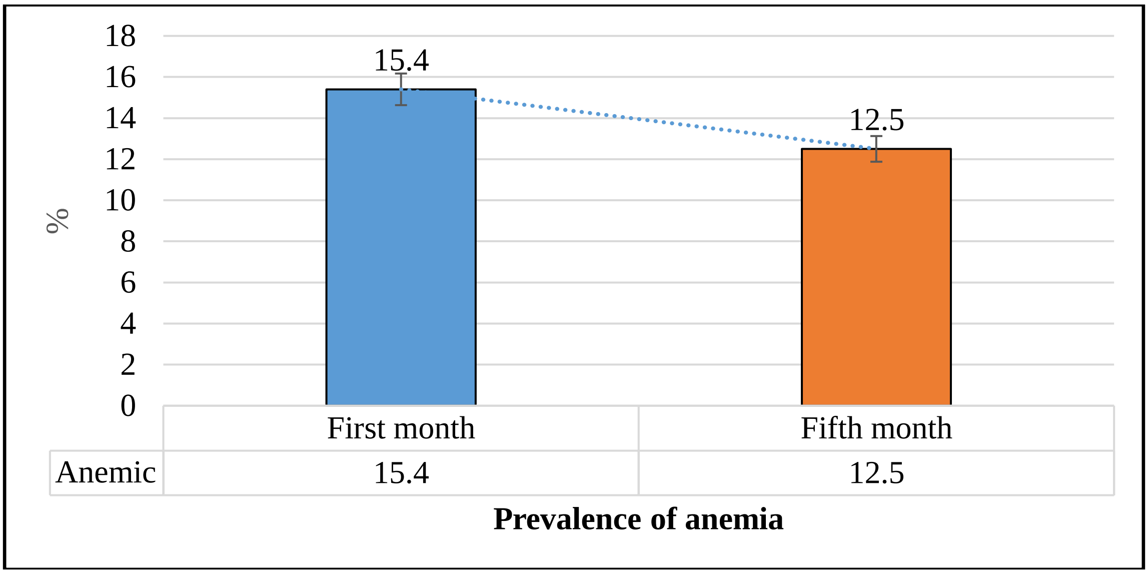

Figure 4.2: Prevalence of anemia among the lactating mothers

Furthermore, any other morbidity incidence among the mothers was assessed. A reference period of two weeks prior to the day of the interview was considered by this study. Data on the morbidity incidence among the mothers was gathered from the mother's self-reports and examination of their clinical records. This study observed that 23.1% (1st month) and 19.2% (5th month) of the mothers were unwell two week preceding the interview day. The most reported morbidities among the mothers were respiratory infection (cough with fever) and gastrointestinal problems. Most of the mothers sought medical attention, with the majority seeking it at government hospitals. Table 4.14 shows the mothers' morbidity profile.

Table 4.14: Mothers' morbidity profile

	N = 104			
	1st month		**5th month**	
Characteristic	**n**	**%**	**n**	**%**
Number of sick mothers within the previous two weeks	24	23.1	20	19.2
Type of morbidity among the sick mothers (multiple responses)	N = 24		N = 20	
Respiratory infection (fever and cough)	9	37.5	8	40.0
Fever without cough	7	29.2	5	25.0
Gastrointestinal problems	8	33.3	8	40.0
Toothache	1	4.2	0	0.0
Obstetric complications	4	16.7	3	15.0
Total	29	120.8	24	120.0
Number of mothers who sought medical attention	18	75.0	12	60.0
Type of facility where medical attention was sought	N = 18		N = 12	
Government hospital	11	61.1	8	66.7
Private hospital	4	22.2	2	16.7
Chemist	3	16.7	2	16.7
Total	18	100	12	100

4.5 Breast Milk Composition of the Lactating Mothers

Where necessary, the human milk specific gravity of 1.031 g/mL was used for unit conversion (Wu et al., 2018). The lactating mothers' milk energy and nutrient profile are detailed in Table 4.15. The mean values in the two stages of lactation are also presented. Downward trends for energy and all the other nutrients were observed except for vitamin A and iron which had an upward trend. The study further observed that average milk concentration at the two stages of lactation for proteins, vitamin A and iron was significantly different (Table 4.15). The mean concentration of energy and the other selected nutrients was not significantly different at the first and fifth month postpartum among the mothers.

Table 4.15: Energy and nutrient profile of the lactating women breast milk

		N = 104 Month of lactation		
Nutrient	**Units**	**Month 1**	**Month 5**	**P value**
Energy	Kcal/dL	66.36 ± 21.45	63.20 ± 19.87	0.284
Carbohydrate (Lactose)	g/dL	6.94 ± 1.40	7.08 ± 1.48	0.413
Proteins	g/dL	0.96 ± 0.31	0.85 ± 0.25	0.029*
Fats	g/dL	3.88 ± 2.33	3.50 ± 2.13	0.231
Vitamin A(retinol)	µg/dL	22.48 ± 23.51	31.61 ± 21.97	0.004*
Calcium	mg/dL	28.64 ± 17.87	27.39 ± 18.09	0.626
Magnesium	mg/L	19.16 ± 17.79	15.92 ± 13.04	0.128
Zinc	mg/L	0.63 ± 0.51	0.51 ± 0.45	0.090
Iron	mg/L	0.39 ± 0.40	0.47 ± 0.32	0.015*

P value; paired student t-test/Wilcoxon Signed Rank test, * statistically significant

4.6 Association between Dietary Intakes and Breast Milk Nutrient Composition of the Lactating Mothers

4.6.1 Association between Maternal Nutrient Intake and Breast Milk Nutrient Composition of the Lactating Mothers

Table 4.16 presents correlation coefficients (r) between the mothers' nutrient intake and their milk macronutrient content. This study observed positive correlations between the mothers' energy intake and their respective breast milk lactose content. Table 4.16 further shows that the lactating mothers carbohydrates intakes were positively correlated with their breast milk content of the true proteins. Similar correlations were also established between the mothers' milk true proteins and their respective intake of proteins and fats. Furthermore, percent of energy from carbohydrates correlated with the mothers' breast milk energy content. Moreover, the lactating mother's fat intake and percentage of energy contribution from fat were positively correlated with the mothers' breast milk fat content. This study did not observe any statistically significant association between the mothers' micronutrient intakes and their respective breast milk macronutrient contents.

Table 4.16: Correlations between Breast Milk Nutrient Content (Macronutrients) and the Mothers' Dietary Intake

Energy and Nutrient Intake by the Mothers	**Month of Lactation**	**(N = 104) Breast milk macronutrient composition**			
		Energy[a]	**Lactose[b]**	**True protein[b]**	**Fat[b]**
Energy (kcal)	1	0.036	0.304*	0.042	0.003
	5	-0.067	-0.030	-0.069	-0.059
Carbohydrates (g)	1	0.004	0.115	0.241*	-0.031
	5	0.070	0.019	0.106	0.061
Percent of energy from carbohydrates	1	-0.048	0.127	0.006	-0.077
	5	0.047	0.107	-0.007	0.004
Proteins (g)	1	0.021	-0.008	0.444*	0.039
	5	-0.021	-0.162	0.134	0.022
Percent of energy from proteins	1	0.003	-0.135	-0.073	0.044
	5	-0.085	-0.176	0.012	-0.035
Fat (g)	1	0.291*	0.075	0.396*	0.346*
	5	0.118	-0.080	0.174	0.308*
Percent of energy from fats	1	0.391*	-0.092	0.009	0.451*
	5	0.101	-0.104	0.022	0.115
Vitamin A (µg)	1	-0.017	0.094	0.021	0.008
	5	0.122	0.054	0.017	0.093
Calcium (mg)	1	-0.057	0.067	0.020	-0.050
	5	0.076	-0.086	0.072	0.088
Magnesium (mg)	1	-0.034	0.112	-0.042	-0.034
	5	-0.128	-0.131	-0.102	-0.091
Zinc (mg)	1	0.122	-0.173	0.189	0.160
	5	0.009	-0.120	-0.016	0.024
Iron (mg)	1	-0.119	0.132	0.084	0.159
	5	-0.052	0.046	-0.037	-0.054

*- correlation is significant (2-tailed), **[a]**- energy is presented in kcal/dL, **[b]**- nutrient is presented in g/dL

To further explore the association between maternal dietary intake and breast milk nutrient composition, this study also examined the correlation between the mothers' intake of the selected nutrients and the micronutrient content of their breast milk (Table 4.17).

Table 4.17: Correlations between Breast Milk Nutrient Content (Micronutrients) and the Mothers' Dietary Intake

Energy and Nutrient Intake by the Mothers	**Month of Lactation**	**(N = 104) Breast milk micronutrient composition**				
		Retinol[a]	**Calcium[a]**	**Magnesium[a]**	**Zinc[a]**	**Iron[a]**
Energy (kcal)	1	0.176	0.062	0.041	-0.126	-0.125
	5	0.180	0.126	-0.072	-0.070	-0.067
Carbohydrates (g)	1	0.100	0.089	-0.025	-0.108	-0.190
	5	0.038	0.076	0.097	-0.120	-0.139
% of energy from carbohydrates	1	0.135	0.122	-0.090	0.157	0.063
	5	0.020	0.008	0.076	-0.083	-0.098
Proteins (g)	1	0.095	-0.008	-0.033	-0.154	0.310*
	5	0.023	0.135	0.029	-0.080	0.178
% of energy from proteins	1	0.109	-0.044	-0.076	-0.047	0.024
	5	0.031	0.009	-0.074	0.012	0.010
Fat (g)	1	0.134	-0.164	0.025	-0.163	-0.003
	5	0.038	0.001	0.068	-0.115	-0.040
% energy from fats	1	0.024	-0.177	0.040	-0.130	-0.050
	5	0.020	-0.009	-0.086	0.058	0.122
Vitamin A (µg)	1	0.434*	-0.135	-0.115	-0.109	-0.040
	5	0.558*	0.066	0.027	0.025	0.022
Calcium (mg)	1	0.096	0.007	-0.066	-0.316*	-0.270*
	5	0.123	0.013	0.022	-0.103	0.001
Magnesium (mg)	1	0.122	0.039	-0.056	-0.157	-0.073
	5	0.134	0.135	0.057	-0.051	-0.067
Zinc (mg)	1	0.129	0.014	0.008	-0.042	-0.261*
	5	0.017	0.116	0.155	-0.068	-0.083
Iron (mg)	1	0.124	0.080	-0.010	-0.042	0.021
	5	0.020	0.099	0.111	0.007	-0.060

*- correlation is significant (2-tailed), [a]- nutrient is presented in mg/dL

Significant associations were observed between the mother protein intake and the iron content of the breast milk. In both time points (1st and 5th month postpartum), significant positive correlations were observed between the mothers' vitamin A intake and the retinol levels of their breast milk. Important to note, the mothers' calcium intake was negatively correlated with zinc and iron content of their breast milk. Similarly, zinc intake was negatively correlated with breast milk iron content (Table 4.17).

4.6.2 Association between Dietary Diversity, Meal Frequency and Breast Milk Nutrient Composition of the Lactating Mothers

Table 4.18 shows that there were no correlations between the mothers' dietary diversity and meal frequency with the energy and macronutrient composition of their breast milk.

Table 4.18: Correlations between Breast Milk Nutrient Content (Macronutrients) and the Mothers' Dietary Intake (dietary diversity & meal frequency)

Mothers Dietary Practices	Month of Lactation	(N = 104) Breast milk macronutrient composition			
		Energy[a]	Lactose[b]	True protein[b]	Fat[b]
Dietary Diversity Score-WDDS	1	-0.086	-0.075	0.131	-0.046
	5	0.166	0.117	-0.013	0.128
Meal frequency	1	-0.056	-0.003	-0.018	-0.003
	5	-0.001	-0.156	0.134	0.060

[a]- energy is presented in kcal/dL, [b]- nutrient is presented in g/dL

However, the mothers' dietary diversity and meal frequency were correlated with their respective breast milk micronutrients content (Table 4.19). The study observed significant correlations between the mother's dietary practices (dietary diversity and meal frequency) and their milk retinol composition ($p < 0.05$). As dietary diversity of the mothers increased, their breast milk retinol levels also increased ($r = 0.409$). A similar observation was noted with respect to meal frequency of the mothers ($r = 0.327$). The significant correlations were observed in the fifth month of lactation among the mothers.

Table 4.19: Correlations between Breast Milk Nutrient Content (Micronutrients) and the Mothers' Dietary Practices

Mothers Dietary Practices	Month of Lactation	(N = 104) Breast milk micronutrient composition				
		Retinol[a]	Calcium[a]	Magnesium[a]	Zinc[a]	Iron[a]
Dietary Diversity Score-WDDS	1	0.162	0.008	-0.028	0.077	-0.049
	5	0.409*	0.025	0.009	-0.032	-0.072
Meal frequency	1	0.165	-0.170	-0.053	-0.023	-0.072
	5	0.327*	-0.054	-0.048	-0.098	0.007

*- correlation is significant (2-tailed) [a]- nutrient is presented in mg/dL

4.7 Association between Maternal Nutrition Status and Breast Milk Nutrient Composition

The study also assessed the relationship between the mothers' nutritional status and their respective breast milk nutrient composition. This study did not observe significant correlations between all the selected nutrition status parameters and the breast milk macronutrient content save for the mothers' hemoglobin levels. The mothers' hemoglobin levels were positively correlated with both their breast milk energy and true protein content (Table 4.20).

Table 4.20: Correlations between Breast Milk Nutrient Content (Macronutrients) and the Mothers' Nutritional Status

Mothers Nutritional Status	**Month of Lactation**	**(N = 104) Breast milk macronutrient composition**			
		Energy[a]	**Lactose[b]**	**True protein[b]**	**Fat[b]**
Retinol (mg/dL)	1	-0.052	0.105	0.027	-0.017
	5	0.084	0.009	0.029	0.048
Calcium (mg/dL)	1	0.067	-0.020	0.036	0.077
	5	-0.113	0.037	-0.130	-0.120
Magnesium (mg/dL)	1	-0.067	0.089	-0.004	-0.108
	5	-0.186	-0.063	-0.039	-0.171
Zinc (mg/dL)	1	-0.192	-0.076	0.070	-0.178
	5	-0.101	0.001	0.069	-0.093
Iron (mg/dL)	1	-0.031	-0.077	-0.064	0.006
	5	-0.015	-0.041	-0.017	-0.005
Hb (g/dL)	1	0.303*	0.164	0.103	0.154
	5	0.039	-0.024	0.439*	-0.048
MUAC (cm)	1	-0.088	-0.066	-0.055	-0.039
	5	0.068	0.102	-0.003	0.051
BMI (kg/m^2)	1	-0.078	0.054	0.045	-0.089
	5	0.049	0.139	0.089	0.020

*- correlation is significant (2-tailed), **a**- energy is presented in kcal/dL, **b**- nutrient is presented in g/dL

Correlations between the mothers' nutrition status and their milk micronutrient status are shown in Table 4.21. Positive correlations were revealed between the mothers' blood serum retinol levels and their milk retinol content at the 5th month postpartum. Similarly, the mothers' serum magnesium was positively correlated with their breast milk magnesium content at the 5th months after delivery. Furthermore, the study observed positive correlations between the mothers' serum iron levels and their breast milk iron content.

Table 4.21: Correlations between Breast Milk Nutrient Content (Micronutrients) and the Mothers' Nutritional Status

Nutrition status of the Mothers	**Month of Lactation**	**(N = 104) Breast milk micronutrient composition**				
		Retinol[a]	**Calcium[a]**	**Magnesium[a]**	**Zinc[a]**	**Iron[a]**
Retinol (mg/dL)	1	-0.002	0.019	-0.077	-0.036	-0.084
	5	0.335*	0.057	0.031	0.082	0.031
Calcium (mg/dL)	1	0.161	-0.089	-0.107	-0.143	-0.081
	5	0.148	0.114	-0.020	0.011	0.103
Magnesium (mg/dL)	1	0.030	-0.051	-0.116	-0.105	0.054
	5	-0.105	0.035	0.294*	0.023	0.172
Zinc (mg/dL)	1	-0.077	-0.040	-0.015	-0.054	-0.039
	5	0.192	0.140	-0.065	0.093	0.009
Iron (mg/dL)	1	0.102	0.111	-0.120	0.120	0.331*
	5	-0.019	-0.022	-0.161	-0.022	0.144
Hb (g/dL)	1	0.082	0.041	0.041	-0.200	-0004
	5	-0.052	0.094	-0.024	0.058	-0.106
MUAC (cm)	1	0.074	0.017	0.001	-0.170	-0.095
	5	-0.165	-0.059	0.008	-0.034	-0.035
BMI (kg/m^2)	1	0.001	0.108	0.012	-0.049	-0.071
	5	-0.191	-0.025	0.065	-0.037	-0.036

*- correlation is significant (2-tailed), [a]- nutrient is presented in mg/dL

4.8 Association between other Maternal Factors and Breast Milk Nutrient Composition

4.8.1 Association between Maternal Age, Parity, Mode of delivery, Infant Sex and Breast Milk Nutrient Composition

This study established the relationship between the breast milk nutrient composition and the mothers' age, parity, mode of delivery and infant sex using bivariate analysis. General linear model with repeated measures and with mixed effects was used. According to the results of the present study, there was no statistically significant relationship between the selected nutrient milk composition and the mothers' age, parity, mode of delivery and infant sex. The results of the between-subject effect are shown in Table 4.22.

Table 4.22: Association between Maternal Factors (Age, Parity, Child sex and Mode of delivery) and Breast Milk Nutrient Composition

Milk nutrient content	**(N = 104) Between subject effect p value**			
	Mother age	**Parity**	**Child sex**	**Mode of delivery**
Energy (kcal/dL)	0.581	0.284	0.619	0.837
Lactose (g/dL)	0.612	0.157	0.274	0.110
True protein (g/dL)	0.609	0.996	0.051	0.318
Fat (g/dL)	0.671	0.533	0.972	0.714
Retinol (mg/dL)	0.816	0.715	0.607	0.660
Calcium (mg/dL)	0.874	0.990	0.890	0.067
Magnesium (mg/dL)	0.518	0.869	0.056	0.716
Zinc (mg/dL)	0.425	0.651	0.451	0.499
Iron (mg/dL)	0.457	0.766	0.767	0.074

4.8.2 Association between Maternal Morbidity Profile and Breast Milk Nutrient Composition

Table 4.23 presents the association between maternal morbidity status and the breast milk nutritional composition. The study found that the mothers who were sick two weeks prior to the date of the interview had a relatively lower breast milk nutrient composition as compared to those who were not sick. This was observed in almost all of the nutrients. However, the study did not find any significant difference in the breast milk composition of the mothers based on their morbidity profile (sick/not sick) two weeks prior to the date of the interviews (Table 4.23).

Table 4.23: Association between Maternal Morbidity Profile and Breast Milk Nutrient Composition

Milk nutrient content	Month of Lactation	Nutrient mean		P value
		sick	not sick	
Energy (kcal/dL)	1	63.50	67.21	0.459
	5	64.95	62.95	0.798
Lactose (g/dL)	1	6.78	6.99	0.523
	5	7.02	7.31	0.443
True protein (g/dL)	1	0.93	0.94	0.947
	5	0.80	0.86	0.393
Fat (g/dL)	1	3.63	3.95	0.560
	5	3.53	3.50	0.941
Retinol (mg/dL)	1	14.90	24.75	0.072
	5	32.60	31.38	0.824
Calcium (mg/dL)	1	28.48	28.69	0.960
	5	27.03	28.87	0.685
Magnesium (mg/dL)	1	15.37	20.30	0.236
	5	13.20	16.57	0.301
Zinc (mg/dL)	1	0.49	0.67	0.131
	5	0.60	0.49	0.339
Iron (mg/dL)	1	0.39	0.40	0.934
	5	0.46	0.49	0.679

Mann Whitney U-test/ Student t-test

4.9 Predictors of Maternal Breast Milk Nutrient Composition among the Lactating Mothers

Based on the associations above, maternal dietary diversity, meal frequency, nutrient intake (energy, carbohydrates, proteins, fats, vitamin A, calcium and zinc), maternal hemoglobin levels and serum micronutrient status (retinol, magnesium and iron) were the factors associated with the mothers' breast milk nutrient composition. The other maternal factors (mothers age, parity, child sex, mode of delivery) were not important predictors of the mothers' breast milk nutrient composition (p value > 0.05).

4.10 Association between Maternal Breast Milk Nutrient Composition and Infant Nutrition Status

The children nutritional status (weight-for-length, length-for-age, weight-for-age and MUAC) were correlated with the mothers' breast milk nutrient composition. The true protein content of the mother's milk was positively correlated with both weight for age and MUAC of the child. The study also found a positive correlation between the mothers' milk lactose content and the weight for length scores of the children (Table 4.24).

Table 4.24: Association between Maternal Breast Milk Nutrient Composition (Macronutrients) and the Infants' Nutrition Status

Child Nutrition Status	**Month of Lactation**	**(N = 104) Breast milk macronutrient composition**			
		Energy[a]	**Lactose[b]**	**True protein[b]**	**Fat[b]**
WAZ	1	-0.005	0.122	0.576*	-0.059
	5	0.068	0.036	0.017	0.060
LAZ	1	0.008	0.135	0.046	-0.087
	5	0.174	0.024	0.117	0.165
WLZ	1	0.012	0.465*	0.127	0.005
	5	-0.066	0.189	-0.002	-0.063
MUAC	1	0.059	0.041	0.189	0.056
	5	0.123	0.052	0.309*	0.157

*- correlation is significant (2-tailed), [a]- nutrient is presented in mg/dL

Moreover, the study observed a significant positive correlation between child weight for age and both breast milk retinol and calcium levels. Milk iron was also correlated with the infant weight for length z-scores. Furthermore, the study established that there were statistically significant correlations between the children MUAC and the respective mothers' breast milk content of retinol and iron (Table 4.25).

Table 4.25: Association between Maternal Breast Milk Nutrient Composition (Micronutrients) and the Infants' Nutrition Status

Child Nutrition status	**Month of Lactation**	**Breast milk micronutrient composition (N = 104)**				
		Retinol[a]	**Calcium[a]**	**Magnesium[a]**	**Zinc[a]**	**Iron[a]**
WAZ	1	0.059	0.158	0.044	-0.005	0.035
	5	0.317*	0.307*	-0.003	0.065	0.173
LAZ	1	-0.104	-0.063	-0.076	-0.032	0.091
	5	0.021	-0.010	-0.064	-0.089	-0.081
WLZ	1	0.113	0.055	0.051	0.054	0.171
	5	0.131	-0.099	0.100	0.084	0.317*
MUAC	1	0.305*	0.052	0.057	0.118	0.183
	5	0.047	0.048	-0.014	0.057	0.321*

*- correlation is significant (2-tailed), [a]- nutrient is presented in mg/dL

CHAPTER FIVE: DISCUSSION

5.1 Maternal Dietary Practices and Intake

5.1.1 Mothers' Breastfeeding Practices

To save children's lives and to ensure a good supply of breast milk by the mother, the World Health Organization (WHO) encourages early initiation of breastfeeding within the first hour after birth (WHO, 2018). The prevalence of timely initiation of breastfeeding (89.4%) observed in this study was higher than that reported in Sudan (87.2%) by Hassan et al. (2018), Saudi Arabia (43.6%) by Ahmed & Salih (2019) and Nepal (66.4 %) by Adhikari et al. (2014). Further, the findings of a meta-analysis conducted in Ethiopia revealed a pooled prevalence of 61.4%. The relatively high prevalence observed in this study could be due to variations in culture and also due to the fact that more than two thirds (68.3%) of this study participants reported receiving breastfeeding support from a health facility. According to WHO (2003a), the rate of 89.4% would be recognised as good.

This study also observed that almost all the mothers fed their children colostrum within the first three days after delivery. Majority of the mothers (83.5%) of a study conducted in Nepal had also fed colostrum to their children (Bhandari et al., 2019). Lower rates (46.3%) have been observed in another study conducted in India (Cacodkar et al., 2016). The differences could have been influenced by disparities in maternal breastfeeding knowledge. Majority of participants in this study reported receiving breastfeeding support after delivery and therefore could have been educated and advised to give their children colostrum. Colostrum is the first milk that is very crucial in protecting infants against infections. The milk is rich in immunoglobulin G and other immune-protective compounds, which have a great role in disease resistance among infants (Abie & Goshu, 2019). Additionally, it has positive effect in prevention of childhood malnutrition (Conneely et al., 2014; Teshome et al., 2009).

5.1.2 Dietary Intake of the Lactating Mothers

The current study determined the lactating mothers' dietary intakes by assessing their dietary diversity, meal frequency, food frequency and nutrient intake at the two points of lactation (1st and 5th month).

5.1.2.1 Dietary Diversity of the Lactating Mothers

Dietary diversity is considered as a proxy indicator of maternal nutrient adequacy. Promotion of diverse diets is one of the several approaches to improving micronutrient nutrition for women, particularly among pregnant and lactating women (FAO, 2016). Out of the 10 food groups considered in this study, the average DDS score was above 5 food groups at both the first (5.32) and the fifth (5.16) month of lactation. The finding of this study is higher than that reported in other countries; Nepal (3.9), Ethiopia (4.5) and South Africa (3.5) (Boke & Geremew, 2018; Chakona & Shackleton, 2017; Henjum et al., 2015). Further, a study conducted among lactating mothers in Nairobi Kenya reported a mean dietary diversity score of 4.3 (Ongosi et al., 2014).

The findings also revealed that about 20.2% of the participants in the first month and 26.9% in the fifth month postpartum had low dietary diversity (< 5 food groups). These women did not achieve the minimum dietary diversity and thus were more likely to have inadequate micronutrient intake. The prevalence of low dietary diversity observed in this study was relatively lower compared to a study conducted in

Ethiopia which had a prevalence of 52.2 % (Boke & Geremew, 2018) and another conducted in South Africa with a prevalence of 75% (Chakona & Shackleton, 2017). These differences could be explained by the difference in the food security status of the study areas. Additionally, the difference could be due to differences in maternal nutrition knowledge among the study participants which could influence dietary habits.

The results from the present study showed that the women's diet was predominantly cereal-based. This finding is consistent with those reported in other studies conducted in Africa countries which have reported that cereals and starchy foods especially maize-based foods are the most consumed in the continent (Chakona & Shackleton, 2017; Desta et al., 2019; Gitagia et al., 2019; Weldehaweria et al., 2016). The high consumption of cereals-based foods could be attributed to the high intake of porridge and githeri (meal of maize and legumes, mostly beans mixed and boiled together) by the lactating mothers at the two stages of lactation. The porridge was predominantly made with maize flour, sorghum and millet.

Meat, nuts and fruits were minimally consumed, a finding consistent with those of other studies conducted in Bangladesh, Vietnam, Ethiopia, Kenya, Nigeria and South Africa who had similar observations (Chakona & Shackleton, 2017; Nguyen et al., 2013; Ogechi, 2014; Ongosi et al., 2014). Due to the low intake of fruits and vegetables, these populations are at a high risk of micronutrient inadequacy. Low consumption of meat and diet low in animal products implies that the lactating women could be affected by anemia. Some studies have documented that there could be some relationship between micronutrient intake and breast milk nutrient content and consequently poor micronutrient intake could affect the breast milk quality (Duda et al., 2009; Grilo et al., 2015).

5.1.2.2 Meal frequency of the Lactating Mothers

Lactating mothers require an adequate diet in order to achieve optimal lactation and sustain it without depleting their nutrient stores. To achieve this, it is recommended that all lactating women should take at least two additional meals (Haileslassie et al., 2013). Despite this recommendation, about 6.7% of the present study participants had an average meal frequency of two meals in the fifth month post-delivery. This low meal frequency could affect their nutrient intake, nutrition status, breast milk quantity and quality and eventually child growth and development.

5.1.2.3 Nutrient intake of the Lactating Mothers

In this study, the mean intake of all the selected nutrients was above the recommended nutrient intake except for fats, calcium and zinc. This could be attributed to the fact that the majority of the participants had achieved the minimum acceptable dietary diversity. Notable, at the first month post-partum, only a few participants had met the recommended nutrient intake of calcium and zinc. This could be due to the minimal consumption of animal-based foods as observed in this study. Other studies from other countries have reported sub-optimal diet quality and nutrient intake among lactating mothers (Moran et al., 2013; Tavares et al., 2013).

5.2 Mother-Child Dyad Nutritional Status

5.2.1 Infants' Nutritional Status

Undernutrition among children less than 5 years is still a public health concern in most developing countries (UNICEF, 2009). Despite substantial achievements in maternal and child health over the past two decades, Kenya still faces significant rates of child malnutrition (KNBS & ICF Macro, 2015). In this study, 6.7% and 8.7% of the children had global acute malnutrition (wasting) at the first and the fifth month, respectively. Wasting reflects a recent period of inadequate nutrient intake or a recent episode of illness. This malnutrition could be due to suboptimal breastfeeding practices or due to low breast milk nutrient content of the mothers.

The prevalence of stunting was relatively higher in the fifth month (9.6%) in comparison with the first month (2.9%). Stunting is a condition that results from prolonged inadequate intake of nutrients or recurrent episodes of illnesses. Of note, 5.8% of the children were underweight in the fifth month post-partum. There was no underweight among the children at one month post-delivery. Weight-for-age (underweight) is a general indicator of a child's nutritional status. This index shows that the child may have suffered from chronic malnutrition (stunting) or acute malnutrition (wasting). However, this index does not distinguish between those conditions.

In comparison with other studies, a study conducted in Tanzania reported a relatively higher prevalence of malnutrition, where 30.1%, 21. 6% and 16.0% of children below six months were stunted, underweight and wasted, respectively (Mgongo et al., 2017). In another study conducted in Ethiopia, stunting and wasting were reported among

13.6% and 15.5% of children below six months, respectively (Amare et al., 2019). In northern Ghana, 13.2%, 18.9% and 15.4% of children between 0-5 months were found to be stunted, underweight and wasted, respectively (Glover-Amengor et al., 2016). The differences observed in these studies could be due to differences in breast milk composition and breastfeeding practices of the lactating mothers.

Higher malnutrition rates were observed in the fifth month as compared to the first month post-partum. The possible reason could be due to poor breast milk quality as lactation progresses or due to poor breastfeeding practices among the mothers as the child grows. This is possible since much attention is usually given to a child immediately after birth than in later growth stages. Moreover, among the indexes as presented in this study, there were significant differences in the mean z-scores (weight for length and weight for age) of boys and girls except for stunting (length for age). Boys had a lower z-score than girls.

Some studies have also shown that child sex is a predictor for child nutritional status with boys being more likely to be malnourished than girls. The possible reason according to these studies, is that boys need more energy and nutrients for growth than girls (Asfaw et al., 2015; Ramli et al., 2009). On the contrary, another study conducted in India reported that females were more likely to be stunted and wasted than males. The differences were attributed to gender inequality that affected practices within a household (Patel et al., 2013).

In this study, it was observed that respiratory infections and diarrhoea were the most common type of infections among the children. In agreement with this finding, a report by UNICEF (2006) indicated that five infectious diseases (pneumonia, diarrhoea, malaria, measles, and AIDS) accounted for more than one-half of all deaths in children aged below 5 years, most of whom are undernourished. Morbidity among children could affect optimal breastfeeding, eventually leading to malnutrition. It is, therefore, of prime importance for the mothers to seek immediate medical attention once a child is detected to be unwell.

5.2.2 Mothers' Nutritional Status

This study assessed the lactating women nutritional status based on both anthropometric (BMI and MUAC) and biochemical parameters (hemoglobin and serum micronutrient levels). Based on anthropometry a significant proportion had a higher BMI than the recommended normal ranges of 18.5-24.9 kg/m^2. A high prevalence (52.4%) of maternal overweight and obesity have also been observed in other studies (Portela et al., 2015). This could be possible since most of the mothers might not have regained their pre-pregnancy weight during early lactation. As noted by Koletzko et al. (2019), breastfeeding women should consume a balanced diet providing adequate nutrient intakes and promoting the reduction of post-partum weight retention.

In the present study, a few mothers (first month- 6.7% and fifth month- 15.4%) were reported to be underweight and with low MUAC values (first month- 1.9% and fifth month- 4.8%). The sub-optimal nutrition status (underweight) among the mothers could be due to poor dietary intake or presence of disease episodes during the pre-pregnancy period or during pregnancy, resulting in malnutrition.

Based on the biochemical parameters, serum iron levels were significantly different in the two points of lactation (first month- 88.88 µg/dL and fifth month- 101.72 µg/dL). This could be due to dietary habits and physiological status of the lactating mothers. Iron levels requirements are relatively lower during lactation as compared to during pregnancy. This could have resulted in lowered iron serum levels immediately after delivery (first month) as compared to later lactation (fifth month). Anemia prevalence was at 15.4% and 12.5% at the first and fifth month post-delivery, respectively. Consistent with this study, a secondary cross-sectional analysis of data in Ethiopia revealed a pooled anemia prevalence of 22.1% among lactating mothers (Lakew et al., 2015). Other studies conducted in different countries have shown a relatively high (63% and 60.3%) prevalence of anemia among lactating mothers (Siddiqui et al., 2017; Zhao et al., 2014). The differences could be due to different dietary habits by the different participants of the studies.

This study also observed that 23.1% (1^{st} month) and 19.2% (5^{th} month) of the mothers were unwell two weeks preceding the interview day. Sickness during lactation could affect the mother’s food intake, breast milk quality and breastfeeding practices subsequently affecting the nutritional status of the infant. It is thus important that mothers seek immediate medical attention when they feel unwell.

5.3 Breast Milk Composition of the Lactating Mothers

The present study measured the macronutrient and micronutrient contents in breast milk longitudinally among the lactating mothers. Measurements were taken at 1^{st} and 5^{th} month post-partum.

5.3.1 Energy and Macronutrient Composition of the Lactating Mothers' Breast Milk

Lactose, a β-disaccharide consisting of glucose and galactose, is the predominant sugar in human milk. Human milk has a higher concentration of lactose than any other species reflecting higher nutritional requirements for human beings (Mosca & Giannì, 2017). As reported by previous studies, this study confirmed that breast milk macronutrient content has wide variations (Chang et al., 2015; Kent et al., 2006). The lactose content of mature breast milk is estimated to range from 6.7 to 7.8 g/dL in the first year of lactation (Ballard & Morrow, 2013).

The mean lactose content observed in this study (month one- 6.9 g/dL and month five-7.1 g/dL) was in agreement with the estimated ranges. Other studies have also documented lactose concentrations that are comparable to this study. A mean lactose concentration of 7.1 g/dL was reported by two studies conducted among Chinese and Korean mothers (Chang et al., 2015; Yang et al., 2014). The concentration reported in this study was also nearly equal to the ones reported among Latvian (6.5 g/dL), Egypt (7.4 g/dL) and Japanese women (6.4 g/dL) (Aumeistere et al., 2017; Soliman et al., 2014; Yamawaki et al., 2005). The small deviations in the results could be due to differences in individual factors, sampling and analytic methodologies. Notably, the concentration of human milk lactose is said to be the least variable and has been reported to vary from 6.3-8.1 g/100 mL (Miller et al., 2013).

In agreement with the current research, the concentration of lactose concentration in this study did not differ significantly during the two stages of lactation (1^{st} month- 6.94 g/100 mL, 5^{th} month- 7.08 g/100 mL) that were focused by this study. Similar findings were documented by Saarela et al. (2005) and Shi et al. (2011) who reported in their studies that there were no significant changes in mother's milk lactose content during the first six months of lactation.

Human milk proteins provide nutritional, immunological and hormonal support to the growing infants. The protein content of mature milk observed in this study (month one- 1.0 g/dL and month five- 0.9 g/dL) was comparable to that of Chinese mothers (0.9 g/100 mL), Australian mothers (1.0 g/100 mL) and Nigerian mothers (1.1 g/100 mL) (Khan et al., 2013; Ogechi & Irene, 2013; Yang et al., 2014). However, results from studies conducted among Korean (1.4 g/100 mL) and Egypt (1.3 g/100 mL) mothers showed a relatively higher protein level compared to this study (Chang et al., 2015; Soliman et al., 2014). The difference could be due to the non-inclusion of the non-protein nitrogenous substances in the current study, which account for approximately 25% of the total nitrogen. These non-protein nitrogenous substances include urea, uric acid, amino acids, creatine, creatinine and nucleotides (Ballard & Morrow, 2013).

This study also observed a significant difference in the protein level at the 1st and 5th month of lactation. This denotes that the stage of lactation is a predictor of human breast milk protein content. A similar decrease in protein content as lactation progress has been observed by other studies (Bauer & Gerss, 2011; Gidrewicz & Fenton, 2014; Grote et al., 2016).

The mean fat content in the breast milk of the current study participants (3.9 g/100 mL in 1st month and 3.5 g/100 mL in 5th month post-delivery) was comparable to findings of other studies conducted in New Zealand (3.8 g/100 mL), China (3.4 g/100 mL), Japan (3.6 g/100 mL), Poland (3.5 g/100 mL), Korea (3.0 g/100 mL), France (3.5 g/100 mL), Egypt (3.5 g/100 mL) and the United States (3.2 g/100 mL) (Butts et al., 2018; Bzikowska et al., 2018; Chang et al., 2015; Léké et al., 2019; Morita et al., 2012; Soliman et al., 2014; Wojcik et al., 2009; Yang et al., 2014). Some of the minimal divergences between the findings may relate to differences inherent to the method of milk collection, time of collection, dietary habits and storage conditions. It is reported that fat is the most variable macronutrient in human milk. Factors such as time of collection (morning or evening) and type of milk (fore or hindmilk), frequency of feeding and maternal diet could cause such variability (Keikha et al., 2017; Mitoulas et al., 2002).

The mean energy content of the breast milk observed in the present study was 66.4 kcal/100 mL in the first month and 63.2 kcal/100 mL in the fifth month postpartum. The energy content reported in the current study was consistent with values reported by Bzikowska et al. (2018) who reported an energy value of 65.9 kcal/100 mL at first month and 61.2 kcal/100 mL at the fifth month postpartum. Zielinska et al. (2019) and Soliman et al. (2014) reported an energy value of 69.5 kcal/100 mL and 66.01 kcal/100 mL, respectively, in the first month of lactation. Another study by Czosnykowska-Łukacka et al. (2018) reported an average energy of 65.8 kcal/100 mL among mothers with infants 1-12 months, which agreed with the present study.

Contrary to these studies, a relatively higher mean energy (76.56 kcal/100 mL) was reported among lactating mothers in France (Léké et al., 2019). The difference could be due to the breast milk sampling period, as their breast milk samples were collected during the neonate period which included transitional milk while this study only considered mature breast milk. Further, a relatively lower mean energy (56.7 kcal/100 mL) was observed among breast milk samples of mothers from Brazil (Abranches et al., 2014). The difference could be explained by the variations in the breast milk macronutrient contents. The participants in that study had low breast milk fat content. Breast milk fat content is the major contributor to the overall energy content of human milk (Cooper et al., 2013; Vieira et al., 2011).

Of note, there was no significant difference in the mean energy at the first and the fifth month postpartum. This observation was consistent with a study conducted by Bzikowska et al. (2018). The study reported that there was no statistically significant difference in the energy content of lactating mother's milk at their 1^{st}, 3^{rd} and 6^{th} month of lactation. These observations suggest that energy content of human breast milk from 1-6 month post-delivery does not vary based on the stage of lactation.

5.3.2 Micronutrient Composition of the Lactating Mothers' Breast Milk

The mean concentration of vitamin A observed in the present study was 22.5 μg/100 mL and 31.6 μg/100 mL at the first and fifth month, respectively. There was a significant difference in the means at the two stages of lactation. The differences indicate that the stage of lactation is an important determinant of breast milk vitamin A content. The mean concentrations from the present study were slightly lower or higher than those reported in other studies. A study conducted among mothers in South Korea reported breast milk retinol levels of 36.4 μg/100 mL at the fifth month of lactation which was consistent with this study (Kim et al., 2017). Two other studies documented a slightly higher concentration of 59.8 μg/100 mL and 57.1 μg/100 mL

(Daniels et al., 2019; Duda et al., 2009). Considerable higher levels (81.5 μg/100 mL) in comparison with this study were reported among Turkish lactating mothers (Tokuşoğlu et al., 2008). On the other end, a study conducted by Hailu et al. (2016) reported a lower mean of 12.9 μg/100 mL. The differences could be due to differences in dietary intakes among the populations or due to methodological factors, such as time of milk collection, stage of lactation and sample storage conditions.

Calcium mean of 28.6 mg/100 mL and 27.4 mg/100 mL in the first and the fifth month, respectively, was observed in this study. These means are comparable with that reported in the literature by other studies from different populations. For example, Butts et al. (2018) reported a mean of 27.5 mg/100 mL, 29.1 mg/100 mL and 30.9 mg/100 mL in three different ethnic groups of New Zealand. Another study by Vítolo et al. (2004) reported a mean calcium concentration of 28.0 mg/100 mL. Further, Kim et al. (2017) reported a breast milk calcium concentration of 29.8 mg/100 mL at the first month of lactation and 27.1 mg/100 mL at the 5th month of lactation.

The lactating mother’s breast milk magnesium mean was 1.9 mg/100 mL and 1.5 mg/100 mL at the two stages of lactation. Other studies have reported relatively similar or slightly different values. Butts et al. (2018) in their study reported a mean of 3.08 mg/100 mL, Mastroeni et al. (2006) reported a mean concentration of 2.9 mg/100 mL while Daniels et al. (2019) reported a mean of 3.0 mg/100 mL in the mature milk of the participating mothers. Additionally, a study conducted among South Korean mothers documented a concentration of 2.9 mg/100 mL and 3.0 mg/100 mL in the first and the fifth month, respectively (Kim et al., 2017). The differences could be due to the analytic methods used or maternal factors influence on the milk composition.

In the present study, there were no statistically significant differences in the breast milk magnesium means observed based on the mother's stage of lactation. Dorea (2000) and Vítolo et al. (2004) also indicated in their studies that magnesium concentration in human milk was constant throughout lactation. Based on this finding, the stage of lactation is therefore not a predictor of breast milk magnesium content among lactating mothers at the first and the fifth month of lactation.

A downward trend was observed in the mothers' breast milk zinc concentration (first month- 0.6 mg/100 mL^{-1} and fifth month- 0.5 mg/100 mL^{-1}). Similar trends have been observed in other studies where statistically significant differences were noted based on the stages of lactation (Yalcin et al., 2015). A systematic review by Yang et al. (2014) support this observation and indicates that zinc concentration in breast milk decreases rapidly as the stage of lactation progresses.

Comparable, slightly lower or higher zinc content level was observed in mature breast milk of lactating mothers from Latvia (0.1 mg/100 mL^{-1}), Iran (0.1 mg/100 mL), Indonesia (0.1 mg/100 mL), United States (0.2 mg/100 mL), and Tehran (0.3 mg/100 mL) (Aumeistere et al., 2018; Daniels et al., 2019; Hannan et al., 2009; Khaghani et al., 2010; Taravati Javad et al., 2018). Other studies conducted in Brazil, Sweden and Libya reported a zinc concentration of 0.4 mg/100 mL, 0.3 mg/100 mL and 0.5 mg/100 mL, respectively (Andrade et al., 2014; Björklund et al., 2012; Hannan et al., 2005). The differences could be due to the analytic methodologies used, sampling time (morning-afternoon-night or fore milk-hind milk), stage of lactation, dietary habits and other environmental factors. Noteworthy, zinc is essential for the normal functioning of the body and its deficiency can lead to retardation in child growth and development. However, when taken in excess, the metal can be harmful to the body (Soetan et al., 2010; Winiarska-Mieczan, 2014).

The iron content of breast milk is often characterized as low. However, the iron amount provided by human breast milk is adequate to prevent iron deficiency anemia for at least the first six months of child life. Longitudinal analysis of breast milk composition in this study showed an iron concentration of 0.39 mg/L and 0.47 mg/L during the first and the fifth month postpartum. Other previous studies have reported similar or close values for the iron concentration of breast milk, ranging from 0.2 to 0.7 mg/L (Andrade et al., 2014; Ejezie et al., 2011; C & Sv, 2016; Daniels et al., 2019; Fernández-Sánchez et al., 2012; Silvestre et al., 2001). The differences reported by these studies may be due to differences in sampling procedures, analytical procedures, inter-individual variability, as well as the stage of lactation.

Furthermore, a difference was found in the concentration of the breast milk iron content at the first and the fifth month of lactation. A significant increase in the concentration was observed between the 1^{st} and the 5^{th} month of lactation. The change in concentration could be possible since the demands for iron is relatively lower during lactation as compared to during pregnancy. Immediately after delivery, some mother could have lower iron levels which could affect the amount available for milk production. This finding is however inconsistent with a previous study in which breast-milk concentrations of iron decreased significantly between 1st and 3rd month of lactation (C & Sv, 2016).

Since statistically significant changes in human milk nutrient composition at the first and the fifth month of lactation have been observed, the null hypothesis that stated that; **Ho_1**: The breast milk nutrient composition of the lactating mothers does not change significantly in the first five months of lactation was therefore rejected. The observation implies that human milk is not a static, invariant fluid but varies based on the stage of lactation.

5.4 Association between Dietary Intake and Breast Milk Nutrient Composition of the Lactating Mothers

This study showed that maternal dietary intake influences breast milk composition of lactating mothers. This study reported an association between breast milk energy content and the fat intake of the mothers. The association could be possible since the study found a positive correlation between the mothers' fat intake and the fat content of their milk. With that note, the association could be so considering that fat is the major source of energy in breast milk. The study data also revealed that energy intake positively correlated with breast milk lactose content. From a search of literature, this study did not find any study with a similar observation. Studies by Bzikowska et al. (2018) and Butts et al. (2018) did not observe any correlation between the mothers' carbohydrate intake, energy intake and their breast milk composition.

Furthermore, this study found evidence of an association between the mothers' breast milk proteins and their nutrient intake of carbohydrates, proteins and fat. This observation was in agreement with that of Hascoët et al. (2019), who reported a positive correlation between carbohydrate intake and breast milk proteins. The few studies linking diet and breast milk proteins have not shown consistent results. For example, a randomized controlled trial by Metcalfe et al. (2016) reported that maternal egg ingestion was positively associated with breast milk ovalbumin concentration.

Moreover, and as reported by Bravi et al. (2016), a study conducted in Sweden reported higher breast milk protein content among mothers who had high protein intake. These studies highlight the potential of maternal protein intake towards improving the breast milk protein content. According to Mosca and Giannì (2017), increasing evidence indicates that the quantity and the quality of protein in human milk play a crucial role in modulating infant growth and body composition (Mosca & Giannì, 2017). Others studies, however, have not documented any association between maternal dietary intake and breast milk protein content (Bzikowska et al., 2018; Ogechi & Irene, 2013).

This study observed correlations between maternal dietary fat intake and breast milk fat content. The observation denotes that immediate maternal fat intake influences breast milk fat content. Consistent with this study, recent studies by Kim et al. (2017) and Jiang et al. (2016) reported that the levels of fatty acids in breast milk highly correlated with maternal dietary intake. Another study by Butts et al. (2018) found positive associations between maternal dietary intake of poly-unsaturated and mono-unsaturated fats and their breast milk concentration of omega 6, Poly-unsaturated

Fatty Acids (PUFAs) and linoleic acids. Similarly, maternal intake of saturated fats positively correlated with the concentration of trans-fatty acids in their breast milk.

Kiprop et al. (2016), in their study, also indicated that breast milk fatty acids could be influenced by the immediate maternal diet as opposed to body depots. Further and in line with these findings, a study by Lauritzen et al. (2002) showed breast milk increase in docosahexaenoic acids (DHA) after 10 hours of fish fat intake and the effect had almost disappeared after 24 hours. Besides, a systematic review by Yang et al. (2018) has reported that fat which is the primary source of energy in breast milk, is affected by maternal dietary intake. However and inconsistent with the current study findings, others studies did not find any association between maternal dietary intake and their respective breast milk fat content (Boniglia et al., 2003; Bzikowska et al., 2018; Ogechi & Irene, 2013). The inconsistencies observed in these studies could be due to inter-individual variability and differences in methodologies particularly milk sampling, sample sizes and milk samples analytic procedures.

With regards, to breast milk micronutrient contents, the present study found significant correlations between the mother protein intake and the iron content of the breast milk. This study, however, did not find any study reporting on the association between protein intake and human breast milk iron content. The mothers' vitamin A intakes were statistically correlated with their breast milk retinol contents. A few studies investigating the association between human breast milk retinol content and the mothers' vitamin A intake have documented inconsistent findings. For example and in agreement with this study, Duda et al. (2009) and Daniels et al. (2019) reported that there were significant correlations of the human milk vitamin A content and its intake among the mothers. Kim et al. (2017) also reported higher breast milk retinol concentration among supplement users than non-users.

Two other previous studies reported that single-dose maternal supplementation of vitamin A during the early postpartum period increased breast milk concentration of vitamin A and reduced the incidence of child illness (Basu et al., 2003; Roy et al., 1997). These studies suggest that enhancing maternal dietary vitamin A intake could enhance the concentrations of retinol in breast milk, ultimately improving the health outcomes of breastfeeding infants. Additionally, a study by Grilo et al. (2015) demonstrated that supplementation with vitamin A in postpartum resulted in a significant increase in the retinol concentration in human milk. In contrast with these studies, Hailu et al. (2016) and Palmer et al. (2016) in their studies did not find any correlation between maternal vitamin A intake and human milk content of the vitamin. The differences could be due to variability in population characteristics.

The mothers' calcium intake was negatively correlated with zinc and iron content of their breast milk. Similarly, zinc intake was negatively correlated with breast milk iron content. High calcium intake could inhibit the absorption of iron and zinc as reported by some studies (Thompson et al., 2010; Walczyk et al., 2014; Wood & Zheng, 1997). This could eventually affect breast milk iron and zinc content. Conversely, a study conducted in Latvia reported that human milk zinc content was unaffected by the maternal dietary intake (Aumeistere et al., 2018). Furthermore, some studies have reported that the concentration of several minerals including calcium, magnesium, iron and zinc are tightly regulated in human milk and that they are largely unaffected by maternal diet (Butts et al., 2018; Montalbetti et al., 2014;

Vítolo et al., 2004). The differences could be as a result of differences in methodological approaches and population characteristics. However, it is needful to appreciate that this study did not find any direct correlation between the selected minerals (calcium, magnesium, zinc and iron) intake and their respective milk concentration among the mothers.

Since statistically significant correlations between maternal dietary intake and breast milk nutrient composition have been observed, the null hypothesis that stated that; **Ho_2**: There is no statistically significant association between the lactating mothers' dietary intake and their breast milk nutrient composition is therefore rejected. This denotes that maternal dietary intake is an important predictor of human milk nutrient composition.

5.5 Association between Maternal Nutrition Status and Breast Milk Nutrient Composition of the Lactating Mothers

5.5.1 Association between Maternal Nutrition Status and Breast Milk Macronutrient Composition of the Lactating Mothers

This study did not observe significant correlations between all the selected nutrition status parameters (retinol, calcium, magnesium, zinc, iron, hemoglobin, MUAC and BMI) and the breast milk macronutrient content save for the mothers' hemoglobin levels. The mothers' hemoglobin levels were positively correlated with their breast milk energy and true protein content. Hemoglobin is made of proteins and therefore high protein levels could result in better hemoglobin levels. Similarly, increased serum protein levels would be used for milk protein synthesis hence the association. Higher milk protein would subsequently increase the energy content of the breastmilk.

A study conducted by Bzikowska et al. (2018) made similar observations with regards to protein. The study reported positive correlations between human breast milk total protein content and the mother’s fat-free mass (kg) and muscles (kg).

In the present study, no association between maternal BMI and breast milk composition was observed at any time point. This is in agreement with the study by Hascoët et al. (2019) where no significant association between the mothers BMI (pre-pregnancy) and the breast milk content was observed. On the contrary, other previous studies based on maternal BMI have reported a positive relationship between BMI and human milk fat concentration (Bzikowska et al., 2018; Chang et al., 2015; Quinn et al., 2012; Yang et al., 2014). For instance, Chang et al. (2015) reported positive correlations between breast milk lipid content and the mothers BMI. Interestingly, Quinn et al. (2012) reported an inverse relationship where between the mothers BMI and the sugar concentration of their breast milk. More research among different populations is needed to unravel this relationship.

Carbohydrates (lactose) in human milk is reported to be the least variable macronutrient (Miller et al., 2013). In support of the argument, this study did not find any association between maternal nutrition status and breast milk lactose concentration. This finding is in line with another study conducted by Kugananthan et al. (2017), who indicated that lactose concentration was not affected by maternal adiposity profiles. Given that lactose is essential in maintaining a constant osmotic pressure in milk, maternal nutritional status may not be expected to have a significant effect on the breast milk lactose concentration (Martin et al., 2016). However, Chang et al. (2015) in their study, reported that maternal BMI was inversely correlated with lactose concentration.

5.5.2 Association between Maternal Nutrition Status and Breast Milk Micronutrient Composition of the Lactating Mothers

Positive correlations were revealed between the mother's blood serum retinol levels and milk retinol content. Likewise, a study conducted in Ethiopia reported a significant positive correlation between the concentration of retinol in breast milk and the respective mother's plasma retinol concentration (Hailu et al., 2016). Positive association between breast milk vitamin A content and serum retinol content has also been reported among Brazilian mothers (Mello-Neto et al., 2009). This study finding denotes that maternal serum retinol levels is an important predictor of human milk retinol content.

In the present study, correlations were also found between the mothers' serum magnesium and their breast milk magnesium. A similar correlation was also observed between the mothers' serum iron levels and their breast milk iron content. In agreement with this study, a recent study reported that maternal iron status during pregnancy affects the amount of iron during lactation (Hampel et al., 2018). Conversely, Hailu et al. (2016), Koreti and Prasad (2014) and Shashiraj et al. (2006) reported that there was no association between maternal iron status and breast milk iron levels.

Moreover, Domellöf et al. (2004) and Nakamori et al. (2009) reported that iron, zinc and copper concentrations are independent of maternal mineral status. In contrast, Dumrongwongsiri et al. (2015) reported that maternal plasma zinc status was associated with breast milk zinc content and plasma zinc status of infants. Important to note, this study did not find any study reporting on maternal serum magnesium status and its respective breast milk concentration.

Since statistically significant associations between maternal nutrition status and their respective breast milk nutrient composition have been observed, this study null hypothesis that stated that; **Ho_3**: There is no statistically significant association between the lactating mothers' nutrition status and their breast milk nutrient composition is therefore rejected. This suggests that maternal nutrition status is a critical determinant of human milk composition.

5.6 Association between other Maternal Factors and Breast Milk Nutrient Composition

5.6.1 Association between Mothers Age, Parity, Child Sex, Mode of Delivery and Breast Milk Nutrient Composition

There were no statistically significant associations between the selected nutrient milk composition and the mother's age, parity, child sex and mode of delivery. Consistent with this study, Hascoët et al. (2019), in their study, did not find any association between mother's age and breast milk composition. However, a study investigating the effects of advanced age on human milk macronutrient composition reported that carbohydrate content in mature breast milk was higher among older mothers. In the study, milk carbohydrate content was significantly correlated with maternal age (Lubetzky et al., 2015). Another study conducted by Bachour et al. (2012) reported that there was an increase in lipid concentration in the breast milk of mothers over 35 years of age. Furthermore, a study by Argov-Argaman et al. (2017) reported low total breast milk fat content among younger mothers as compared to the older ones. The study methodologies and population differences could have contributed to the different observations.

The current study found no association between mothers' parity and breast milk nutrient content. In contrast with this observation, Bachour et al. (2012) in their study reported that an increase in parity led to an increase in breast milk lipid concentration. Another study reported that multi-parity was associated with a decrease in the human milk fat, carbohydrate and energy contents (Léké et al., 2019). Despite the current study not finding any relationship between mothers' parity and breast milk nutrient content, it must be appreciated that mothers breastfeeding practices could be affected by the mother's parity and ultimately affect the infant nutrition status. It has been reported that women who have breastfed previously have significantly different breastfeeding practices than the primiparous (Hackman et al., 2015; Kitano et al., 2015). Additionally, a meta-analysis by Cohen et al. (2018) revealed a positive relationship between multi-parity and continuation of breastfeeding. These observations could be due to the fact that previous mothers could be having considerable experience and exposure to child-rearing.

The present study found no association between mothers' infant sex and breast milk nutrient content. In agreement with the current study, a study conducted among Filipino mothers did not find any association between infant sex and human milk composition (Quinn, 2013). Conversely, a study conducted among American mothers reported that mothers of male infants produced higher energy content breast milk

(Powe et al., 2010). Another study reported that there was a statistically significant difference in the mean concentration of breast milk lipids among mothers based on the sex of their infants. Mothers with male infants had higher lipid concentration (Amin et al., 2018). Other researchers have also reported that human milk energy content was higher among mothers with female infants (Fujita et al., 2012; Hahn et al., 2017). However, these studies were conducted in different contexts, had different analytic methods and reported several limitations which could have contributed to the disparity in the findings.

No associations were found between the mother's mode of delivery and the breast milk nutrient content. Contrary to this study, a study by Dizdar et al. (2014) and Aleali et al. (2018) reported that vaginal delivery is associated with higher protein content than CS delivery. The difference with this current study could be because one of the studies (Dizdar et al., 2014), considered colostrum while the other one (Aleali et al., 2018) considered mothers with preterm babies. The present study considered mature breast milk and mothers who had full-term babies.

5.6.2 Association between Maternal Morbidity Profile and Breast Milk Nutrient Composition

This study found that the mothers who were sick two weeks prior to the date of the interview had a relatively lower breast milk nutrient composition as compared to those who were not sick in almost all nutrients. This could be attributed to the fact that morbidity could affect food intake, nutrient utilization and the body physiological functions, which could affect the breast milk synthesis and production process. The differences in this study were however not significantly different. Despite this, the study notes that a reference period of two weeks could be short to have considerable effects.

A recent study observed changes in human milk fatty acid composition among lactating mothers based on their maternal health. The study reported that capric acid and lauric acid were lower, while palmitic acid was higher in unwell mothers (Gardner et al., 2017). Further, differences in breast milk lipid composition have been reported among mothers with diseases that affect fat metabolism such as diabetes, hypobetalipoproteinemia, cystic fibrosis, among others (Hamosh & Bitman, 1992). Allergic diseases have also been reported to alter fatty acid profiles and eicosanoids (Laiho et al., 2003). From the aforementioned, the mothers' health may affect human milk composition but more research with a longer reference period is needed to conclude on the association.

5.7 Association between Maternal Breast Milk Nutrient Composition and Infant Nutrition Status

The contribution of human milk composition to infant growth is sparse (Eriksen et al., 2018). This study assessed the association between maternal breast milk nutrient content and infant nutrition status. The protein content of the mother's milk was positively correlated with both weight for age and MUAC of the child. This could be attributed to the fact that protein plays an important role in growth and development of a child. Inadequate protein intake is associated with low weight for age (underweight). The study also found a positive correlation between the mothers' milk lactose content and the weight for length scores of the children. This could be possible since acute low energy intake within a short period of time is associated with low weight for length (wasting) which represent acute malnutrition

This study found significant positive correlation between child weight for age and both breast milk retinol and calcium levels. Furthermore, the study established that there were statistically significant correlations between the children MUAC and the respective mothers' breast milk content of retinol and iron. This could be attributed to the role of vitamin A and iron in growth and development. Increasing vitamin A and iron content of breast milk can thus promote growth among infants. Calcium pivotal role in child bone and teeth development cannot be underestimated. Breast milk iron was also correlated with the infant weight for length z-scores. McCarthy et al. (2018) reported that serum iron status was associated with weight and length of children. This reveals the important role of iron in child growth and development.

There are few studies investigating the association between human milk nutrient concentration and the infant nutrition status. Some of the available studies report that there could be a significant association between the mother's breast milk composition and infant growth. For example, a study conducted by Nikniaz et al. (2009) reported that WAZ (weight for age) of infants whose mothers breast milk had higher lipid content was significantly higher than those whose mothers had lower breast milk lipid content. Another study conducted in Guatemala reported that minerals and trace elements in human breast milk were associated with infant anthropometric outcomes within the first 6 months (Li et al., 2016).

Further, a study conducted by Prentice et al. (2016) revealed positive correlations between the mother breast milk carbohydrate content and the infant weight, BMI and adiposity gains. Bernard et al. (2017) also reported that the mothers breast milk poly-unsaturated fatty acids (PUFAs) was associated with the infant's intelligence. This study findings denote that breast milk composition is a significant predictor of an infant's nutrition status, especially during the critical period of exclusive breastfeeding. However, it must be stressed that an infant's nutrition status could be influenced by other factors such as feeding practices and health status.

Since statistically significant correlations between the lactating mothers breast milk nutrient composition and their infant nutrition status have been observed, the null hypothesis that stated that; **Ho_4:** There is no statistically significant association between the lactating mothers' breast milk nutrient composition and their infant nutrition status is therefore rejected. This indicates that promoting human milk breast milk nutrient content leads to improved child growth and development.

CHAPTER SIX: SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary of the Findings

Most of the participants were young and in the age category of 25-34 years. Almost all of the participants were Christians. Additionally, the majority of the mothers were married and with the majority of their households reporting a mean household size of 4.18 (± 1.50 SD). The highest percentages of the participants had a vaginal delivery and delivered in a health facility. The study also observed that a significant proportion of the participants were multiparous. Overall, the study observed that most of the mothers had initiated breastfeeding within 1 hour of birth and had given their children colostrum.

Majority of the mothers met the minimum acceptable dietary diversity (≥ 5 food groups) and with a mean dietary diversity score of 5.32 (± 1.05 SD) and 5.16 (± 1.08 SD) at 1st month and 5th month, respectively. Starchy staples, other vegetables, dairy and pulses were the major and dominant food groups consumed by most of the participants at both times of assessment (1st and 5th month of lactation). Generally, nuts and animal source foods in the exception of dairy were least consumed. The results further indicated that the mothers' meal frequency declined from the first to the fifth month postpartum.

More than half of the mothers did not meet the recommended nutrient intake for fats, calcium and zinc at both stages of lactation. The study further revealed that the mean intake of all the selected nutrients was not significantly different at the two stages of lactation in the exception of fat and magnesium. Energy intake was also noted to be significantly different between the two stages of lactation.

The burden of wasting (global acute malnutrition) among the study infants was 6.7% in the first month and 8.7% in the 5th month of lactation. Prevalence of stunting was 2.9% and 9.6% in the first and the fifth month of lactation, respectively. Underweight was not found among the infant during the first month post-delivery but was observed among 5.8% in the fifth month post-partum. Results further revealed that some children were unwell (1st month post-partum-28.8% and 36.5% at the 5th month of lactation). Respiratory infections and gastrointestinal disorders were the most common morbidities among the infants

Moreover, the present study revealed that most of the mothers had normal ranges of body mass index and MUAC in the first and the fifth month postpartum. This study further observed that 23.1% (1st month) and 19.2% (5th month) of the mothers were unwell two weeks preceding the interview day. The most reported morbidities among the mothers were respiratory infection (cough with fever) and gastrointestinal problems.

With regards to breast milk composition, downward trends for energy and all the other selected nutrients were observed except for vitamin A and iron, which had an upward trend. The study further observed that average breast milk concentrations at the two stages of lactation for proteins, vitamin A and iron were significantly different.

This study observed associations between maternal dietary intake and breast milk nutrient composition. The mothers' dietary intake (dietary diversity and meal frequency) were correlated with their milk micronutrient composition. Additionally, maternal dietary intake of energy, carbohydrates, fat, percent of energy contribution from fats, and proteins were significantly associated with breast milk macronutrient content. Maternal macronutrient intake (protein) and breast milk micronutrient content (iron) were correlated. Further, the mother dietary micronutrient intake (vitamin A, calcium and zinc) was also associated with the micronutrient profile of their breast milk (retinol, zinc and iron).

No associations were observed between maternal anthropometric measurements (BMI & MUAC) and breast milk nutrient composition. However, maternal nutrition status (serum micronutrients) was associated with some breast milk micronutrient content (retinol, magnesium and iron). The mothers' Hb levels were also positively correlated with both their breast milk energy and true protein content. Associations between maternal factors (age, parity and mode of delivery) and breast milk nutrient composition did not find any significant association.

This study did not find any significant difference in the breast milk composition of the mothers based on their morbidity profile (sick/not sick) two weeks before the date of interviews. Of note, findings of this study revealed significant associations between the mother's breast milk composition and their infant nutritional status. The children's nutrition status (weight-for-length, length-for-age, weight-for-age and MUAC) were correlated with the mother's breast milk composition (protein, lactose, calcium, retinol and iron).

6.2 Conclusion of the Study

The study has demonstrated that dietary intake (dietary diversity and meal frequency) among the lactating women was above average as per the recommendations. However, their diets were observed to be predominantly cereal-based. Due to diversified diets, the majority of the mothers met the recommended nutrient intakes. With regards to nutrition status, the majority of the participants had an optimal nutrition status but a significant proportion of the participants had a higher BMI than recommended.

This study revealed that human milk nutrient composition is variable. The variations imply that human milk is not a static, invariant fluid but can vary based on the stage of lactation. The study also showed that both maternal dietary intake and nutrition status (serum micronutrient status) are important predictors of human breast milk nutrient content. The findings further reveal that malnutrition can occur even among exclusively breastfed children and that breast milk nutrient content is positively correlated with an infant's nutritional status. Promoting maternal dietary intake and optimal nutrition status can therefore improve breast milk quality (nutrient concentration), leading to improved child growth and development.

6.3 Strength and Limitation of the Study

The strength of this study was the application of repeated measures design, the number of samples used (relatively larger) and the use of advanced techniques to assess breast milk nutrient composition. These minimized possible errors in the breast milk nutrient composition analysis. Larger sample size is critical to cover significant variations inherent to demographic factors and thus allow for valid statistical analysis.

The limitation of this study was the constraints associated with multiple measurements such as standardizations of the time for the breast milk collection and the type of milk collected. Since all the samples were collected in the morning, diurnal variations in the nutrient profile of human milk could not be ruled out.

6.4 Recommendations of the Study

This study has the following recommendations;

6.4.1 Recommendation for Policy

Results have demonstrated that maternal dietary intake and nutrition status influences the mother's breast milk composition and ultimately the child's nutrition status. Based on these findings, policies supporting maternal and child health should continue being enacted and promoted. Such policies include protection of the rights of working breastfeeding mothers and providing them with a conducive environment to breastfeed or express breast milk. Further, policies to enhance access to adequate healthy foods and attaining optimal nutrition status in the context of women's general reproductive health are recommended.

6.4.2 Recommendation for Practice

Since the study has revealed that maternal dietary intake and nutrition status influences breast milk composition, it is crucial that health professionals such as nutritionists and dieticians among others, educate lactating mothers on the importance and strategies of achieving adequate dietary intake and optimal maternal nutrition status. The ministry of health and other stakeholders should also promote and support appropriate breastfeeding practices. The promotion and support are crucial since the findings have demonstrated the critical role of breast milk in achieving optimal child growth and development. Further, the government should initiate new and upscale existing interventions geared toward improving maternal and child nutrition.

6.4.3 Recommendation for Further Research

Since human milk is recommended as the sole source of nutrition for young infants, more data on human milk composition is needed. Breast milk content information among different and region-specific populations is needed in order to make a comprehensive reference point of human milk nutrient composition. The information is essential because different populations have different environmental exposures which could affect human milk composition.

Furthermore, due to the breast milk nutrient variability observed among the study participants, it will be valuable to establish the association of human breast milk composition with other environmental and perinatal factors in addition to nutritional factors. These factors include smoking, genetic background, health conditions, among others. Additionally, apart from the nutritive value, human milk provides other bioactive components that are crucial for infant growth and development. With that consideration, this study strongly recommended human milk research to identify these bioactive components and the identification of the factors associated with them.

This study only considered the early lactation period (below six months postpartum). It would also be vital to assess the human nutrient profile of other lactation period, particularly between 6-23 months. Since this is the period where complementary feeding alongside breastfeeding is recommended, that information would be necessary for guiding the type and the quantity of complementary foods to be added based on the child's needs and the breast milk content. All these research efforts to unravel the dynamics of human breast milk composition will contribute towards optimal growth, development and health of infants.

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APPENDICES

Appendix A: Letter of Introduction and Consent

Dear Participant,

My name is WILLY KAHANYA KIBOI from Kenyatta University pursuing a Doctor of Philosophy (PhD) degree in Foods, Nutrition and Dietetics. I am undertaking a study on the impact of maternal dietary intake on breast milk composition and infant nutritional status among lactating women in Nyeri County, Kenya. This study will inform the government and other stakeholders on the impact of maternal dietary intake on breast milk content and infant nutrition status. I will explain to you about the research and you may thereafter decide to participate in the research or decline.

Procedure to be followed

My research assistants and I would like to ask you some questions about yourself and your household. In addition, we will also take a sample of your breast milk for analysis. Moreover, we will assess your nutritional status through mid-upper arm circumference (MUAC), BMI and serum micronutrient status (5 mL blood). We shall also assess the nutritional status of your child by measuring his/her weight and length. Though your views are very important, you have the right to refuse participation in the study. You will get the same care and treatment whether you agree to join the study or not. The interviews and nutrition assessments will take about 1 hour and your cooperation is highly appreciated.

Possible benefits

The benefits from this study may not be directly anticipated but the findings may be useful to the relevant stakeholder in initiating interventions geared toward improving maternal and young child health. You will also benefit from understanding your current dietary practices and your nutritional status in addition to that of your child. If found to have a problem, advice on appropriate action will be provided.

Possible Risks/Discomfort

There are no foreseen risks associated with the study; however, some questions to be asked might make you uncomfortable. If this happens you may refuse to answer the questions and you are at liberty to stop the interview at any time.

Compensation

Your participation to the study is voluntary and thus you are not entitled to any form of payment.

Community consideration

All those participants with poor nutritional status and that of their children will be given appropriate advice. Moreover, on completion of the study, the result finding will be disseminated to the relevant community stakeholders for appropriate actions.

Care and protection of the study participants

The research procedure will adequately be explained to the participants. This will assure them that no risk is associated with the study. Once recruitment is conducted at the health facility, the mothers will be followed to their household where the interviews, breast milk and serum samples collection will be conducted in order to ensure privacy. Further, to ensure confidentiality, participants' names will not be used. Instead, codes will be used to help identify the participant. The participants will also be informed of their right to withdraw from the study at any time without penalty.

Confidentiality

Whatever information you provide will be treated with utmost confidentiality and will not be used for any other purpose other than the purpose of the study.

If you have any questions you may contact;

Willy Kiboi	or	Kenyatta University, Ethical Review Committee
P.O BOX 43844		P.O BOX 43844
Nairobi, Kenya		Nairobi, Kenya
Tel: +254 722653111		Tel: +254 20 8710901
Email: kiboiwilly@yahoo.com		Email: chairman.kuerc@ku.ac.ke or secretary.kuerc@ku.ac.ke

Participant's statement

The above information regarding my participation in the study is clear to me and I voluntarily agree to participate.

Participant Signature/ Thumb print--

Date: --

Interviewer's statement

I certify that, I the undersigned have explained the purpose and procedure, the potential benefits and possible risks associated with participating in this study to the above individual.

Interviewer Name ---

Signature--

Date: --

Appendix B: Researcher Administered Questionnaire

Impact of maternal dietary intake on breast milk composition and infant (under six months) nutritional status among lactating women in Nyeri County, Kenya

SECTIONS

1. SECTIONA: Demographic and socio-economic characteristics
2. SECTIONB: Child data and dietary practices
3. SECTIONC: Health information/morbidity status
4. SECTIOND: Mother Dietary diversity and dietary intake
5. SECTIONE: Food frequency
6. SECTION F: Anthropometric measurement

Checked by: --

ADMINISTRATIVE DETAILS

County ________________________ Location _________________________________

Questionnaire Number |___||___||___| Date of Interview __|___|/|___|___|/|___|___|

Interviewer's name____

Participant Code |___||___||___|

SECTION A: DEMOGRAPHIC AND SOCIO-ECONOMIC CHARACTERISTICS		
Part A: Participant Details		
Code		**Value/ choice**
	Participant details	
A1.	Age of the mother (in completed years). Confirm from ANC card_________	
A2.	Parity of the mother (Confirm from ANC card) ………_________	
A3.	Current child mode of delivery 1 = Normal delivery [ ] 2 = Caesarean section (CS) [ ]	
A4	Current child place of delivery 1 = Hospital/ Health facility [ ] 2 = Home [ ] 3 = Traditional birth attendant's home [ ] 4 = Other (specify) [ ] ----------------	
A5.	How many children do you have (Alive) _________	
	How many of your children are below 5 years of age? _________	
A6.	Marital status 1 = Married [ ] 2 = Single [ ] 3 = Divorced/Separated [ ] 4 = Widow/Widowed [ ]	
A7.	Religion of the mother 1 = Christian [ ] 2 = Muslim [ ] 3 = Hindu [ ] 4 = Tradition African [ ] 5 = None [ ] 6 = Other (specify) [ ] ----------------	
A8.	What is your highest level of education reached? 1 = No formal education [ ] 2 = Pre-school [ ] 3 = Primary school [ ] 4 = Secondary school [ ]	

	5 = College (certificate and diploma) [ ] 6 = University (degree and above) [ ]	
A9.	What is your occupation? 1= House wife/unemployed [ ] 2= Casual labourer [ ] 3 = Farmer(own farm) [ ] 4 = Self-employed/ business [ ] 5 = Employed (salaried) [ ] 6 = Other (specify) [ ] ----------------	
A10.	What is your husband's occupation? **(If A6 above is 1=Married)** 1= Unemployed [ ] 2= Casual labourer [ ] 3 = Farmer(own farm) [ ] 4 = Self-employed/ business [ ] 5 = Employed (salaried) [ ] 6 = Other (specify) [ ] ----------------	
	Household composition and characteristics	
A11.	How many people live in your household (members living together and sharing the same pot --------------_________	
A12.	Who is the household head 1= Participant [ ] 2= Husband [ ] 3= Child/ other relatives [ ] 4= Others (Specify) [ ] ----------------	
A13.	Gender of HH head 1= Male [ ] 2= Female [ ]	
A14	Highest education level attained by the household head 1 = No formal education [ ] 2 = Pre-school [ ] 3 = Primary school [ ] 4 = Secondary school [ ] 5 = College (certificate and diploma) [ ] 6 = University (degree and above) [ ]	
A15.	What is the house head occupation? 1= Unemployed [ ] 2= Casual labourer [ ] 3 = Farmer(own farm) [ ] 4 = Self-employed/ business [ ] 5 = Employed (salaried) [ ] 6 = Other (specify) [ ] ----------------	
A16.	What is the household main source of income? 1= Donation [ ] 2= Casual labourer [ ] 3 = Farming [ ] 4 = own business [ ] 5 = Employed (salaried) [ ] 6 = Other (specify) [ ] ----------------	
A17.	Please indicate the average household income per month------------ _______	

A18.	In the average household income stated above, approximately how much goes to food expenditure per month? _______	
A19.	Who majorly determines how family income will be used? 1= Participant [ ] 2= Husband [ ] 3= Others (specify) [ ] ----------------	
A20.	Main source of household food 1= Own production [ ] 2= Purchased [ ] 3= Own production and purchased [ ] 4= Donations [ ] 5= Others(specify) [ ] ----------------	
A21.	Type of living house: 1 = Rented house [ ] 2 = Own house [ ] 3 = Others(specify) [ ] -------- --------	
A22.	What is your main source of lighting? 1= Kerosene [ ] 2= Candle [ ] 3= Solar [ ] 4= Electricity [ ] 5= Other (specify) [ ] ----------------	
A23.	What is your main source of cooking fuel? 1 = Firewood [ ] 2 = Charcoal [ ] 3 = Kerosene [ ] 4 = Gas [ ] 5 = Electricity [ ] 6 = Other (specify) [ ] ----------------	
A24.	Does the household have land? 1 = Yes [ ] 2 = No [ ]	
A25.	Does the household own any livestock? 1 = Yes [ ] 2 = No [ ]	
	SECTION B: CHILD DATA AND DIETARY PRACTICES	
B1.	Child date of birth (**confirm from MCH card)**___|___|/|___|___|/|___|___| **D| D| /|M| M| /|Y| Y|**	
B2.	How old is your Child? (Age in completed months/days) _________________[**Confirm from MCH card**]|___|/|___|___| **|M| /|D_ |_D |**	
B3.	Sex of child 1 = Male [ ] 2 = Female [ ]	
B4.	How soon after birth did you put *[child]* on the breast? 1= Immediately (within 30 minutes) [ ] 2 = 30 min- 1 hour after delivery [ ] 3=Between 2-12 hours after delivery [ ]	

	4= More than 12 hours [ ]	
B5.	During the first 3 days after delivery, did you give *[Name]* the fluid/liquid that came from your breasts? 1 = Yes [ ] 2 = No [ ]	
B6.	Have you received any other information on breastfeeding? 1 = Yes [ ] 2 = No [ ]	
B7.	If yes, what type of information? _________	
B8.	Yesterday during the day or at night, was *your child* breast fed or given breast milk from you? 1 = Yes [ ] 2 = No [ ]	
B9.	How many times did you breastfeed in yesterday day and night _________	
B10	What was the average duration of each breastfeeding session in minutes _________	
B11.	Average frequency of breastfeeding per day (mother opinion) _________	
B12.	Average duration per feeding in minutes (mother opinion) _________	
B13.	If not breast fed yesterday, why was the child not breast fed 1= Mother was unwell [ ] 2=Baby was sick [ ] 3=I was at work [ ] 4=others (specify) [ ]	
SECTION C: HEALTH INFORMATION (MORBIDITY STATUS)		
	MOTHER	
C1.	Have you (mother) suffered from any disease for the last two weeks 1 = Yes [ ] 2 = No [ ]	

C2.	If yes, type of illness	Duration in days	
	1.		
	2.		
	3.		
	4.		
	5.		

C3.	Did you seek medical attention 1 = Yes [ ] 2 = No [ ]	
C4.	If yes, where? *(More than one response possible)* 1 = Government hospital [ ] 2 = Private hospital [ ] 3 = Traditional medicine [ ] 4 = Pharmacy/shop [ ] 5 = Others (specify) -------- [ ]	

	CHILD		
	QUESTION	RESPONSES	
C5.	Has the child been sick in the past 2 weeks?	1 = Yes [ ] 2 = No [ ]	
C6.	If Yes which? *(More than one response possible)*	1 = Diarrhoea [ ] 2 = Vomiting [ ] 3 = Fever with chills like malaria [ ] 4 = Fever, cough, difficulty in breathing [ ] 5= Intestinal parasites [ ] 6 = Measles [ ] 7 = Eye infections [ ] 8 = Skin infections [ ] 9 = Accident [ ] 10 = Malnutrition [ ] 11 = Stomachache [ ] 12 = Tooth ache [ ] 13 = Other (Specify) -------- ---- [ ]	
C7.	When the child was sick did you seek assistance?	1 = Yes [ ] 2 = No [ ]	
C8.	If YES Where? *(More than one response possible)*	If yes, where 1 = Government hospital [ ] 2 = Private hospital [ ] 3 = Traditional medicine [ ] 4 = Pharmacy/shop [ ] 5 = others (specify) [ ] ------- ---------	
C9.	Are you taking any supplement?	1 = Yes [ ] 2 = No [ ]	
C10.	If Yes, which ones	--------	

SECTION D: Dietary Intake/Diversity

Recording form for 24hr recall of foods and drinks consumed by the participant

Establish the drink/foods the participant consumed in the previous day starting from the time she woke up to the time she went to sleep whether at home or outside the home. Establish the meals taken per day, ingredients and the amount in the meals, the volume of the food cooked the volume of the food taken and calculate the amount of ingredients consumed in grams by the participant?

Confirm if the day was usual or unusual

1= usual day

2= unusual

If unusual, explain why

1=Celebration 2=Religious activity 3=Little food in household 4=Other (specify)

24-Hour dietary recall

								Official use
Time/ Meal	Type of the dish/Food name	Ingredients	HH measure	Amt in Grams	Vol cooked	Vol served	Vol taken	Amt taken grams
Breakfast								
Snack 1								

Lunch								

Snack 2								
Supper								

Snack 3								

3.2 DIETARY DIVERSITY QUESTIONNAIRE

Fill in the food groups based on the 24-hour recall information recorded above. For any food groups not mentioned, ask the respondent if a food item from this group was consumed.

Question number	Food group	Examples	YES=1 NO=0
1	CEREALS	Corn/maize, rice, wheat, sorghum, millet or any other grains or foods made from these (e.g. bread, noodles, porridge or other grain products) + *insert local foods e.g. ugali, nshima, porridge or pastes*	
2	WHITE ROOTS AND TUBERS	White potatoes, white yam, white cassava, or other foods made from roots	
3	VITAMIN A RICH VEGETABLES AND TUBERS	Pumpkin, carrot, squash, or sweet potato that are orange inside + *other locally available vitamin A rich vegetables (e.g. red sweet pepper)*	
4	DARK GREEN LEAFY VEGETABLES	Dark green/leafy vegetables, including wild forms + *locally available vitamin A rich leaves such as amaranth, cassava leaves, kale, spinach*	
5	OTHER VEGETABLES	Other vegetables (e.g. tomato, onion, eggplant) + *other locally available vegetables*	
6	VITAMIN A RICH FRUITS	Ripe mango, cantaloupe, apricot (fresh or dried), ripe papaya, dried peach, and 100% fruit juice made from these + *other locally available vitamin A rich fruits*	
7	OTHER FRUITS	Other fruits, including wild fruits and 100% fruit juice made from these	
8	ORGAN MEAT	Liver, kidney, heart or other organ meats or blood-based foods	
9	FLESH MEATS	Beef, pork, lamb, goat, rabbit, game, chicken, duck, other birds, insects	
10	EGGS	Eggs from chicken, duck, guinea fowl or any other egg	
11	FISH AND SEAFOOD	Fresh or dried fish or shellfish	

12	LEGUMES, NUTS AND SEEDS	Dried beans, dried peas, lentils, nuts, seeds or foods made from these (e.g. hummus, peanut butter)	
13	MILK AND MILK PRODUCTS	Milk, cheese, yogurt or other milk products	
14	OILS AND FATS	Oil, fats or butter added to food or used for cooking	
15	SWEETS	Sugar, honey, sweetened soda or sweetened juice drinks, sugary foods such as chocolates, candies, cookies and cakes	
16	SPICES, CONDIMENTS, BEVERAGES	Spices (black pepper, salt), condiments (soy sauce, hot sauce), coffee, tea, alcoholic beverages	
Individual level	Did you eat anything (meal or snack) OUTSIDE the home yesterday?		

Aggregation to construct Minimum Dietary Diversity for Women of Reproductive Age **(MDD-W)**

Groups/items/rows on questionnaire	**10 food groups in MDD-W**	**1 = Yes 0 = No**
A. Foods made from grains B. White roots and tubers and plantains	1. Grains, white roots and tubers, and plantains	
C. Pulses (beans, peas and lentils)	2. Pulses (beans, peas and lentils)	
D. Nuts and seeds	3. Nuts and seeds	
E. Milk and milk products	4. Dairy	
F. Organ meat G. Meat and poultry H. Fish and seafood	5. Meat, poultry and fish	
I. Eggs	6. Eggs	
J. Dark green leafy vegetables	7. Dark green leafy vegetables	
K. Vitamin A-rich vegetables, roots and tubers L. Vitamin A-rich fruits	8. Other vitamin A-rich fruits and vegetables	
M. Other vegetables	9. Other vegetables	
N. Other fruits	10. Other fruits	

NB: In MDD-W Sweets, sugars, spices, condiments and beverages are not considered

TOTAL DDS SCORE

NUMBER OF MEALS CONSUMED PER DAY

SECTION E: FOOD FREQUENCY

State the frequency of consumption of the selected food items by the mother per week

Food Item	**Freq/ Week**	**Food Item**	**Freq /Week**
Maize		Eggs	
Maize flour		Liver	
Rice		Kidney/heart	
Sorghum		Fish	
Cassava		Poultry	
Millet		Meat (other meat)	
Chapatti/bread/cakes		Avocado	
Raw bananas		Mangoes	
Irish potatoes		Pawpaw's	
Kales		Passion fruits	
Cabbage		Ripe bananas	
Spinach		Oranges	
Amaranth (terere)		Guava	
Carrots		Water melon	
Tomatoes		Herbs	
Pumpkins		Supplements	
Yellow Sweet potatoes		Other (specify)	
Beans			
Peas			
Green gram*(Ndengu)*			
Lentils (Kamande)			
Groundnuts			
Milk			
Yogurt			

SECTION F: Nutritional status: Anthropometric Measurement

MOTHER

		First reading	Second reading	Average
F1	MUAC			
F2	Weight			
F3	Height			

NB: Weight of the mother in Kg to the nearest 0.1kg and height in centimeters to the nearest 0.1cm**.**

MOTHERS HB g/dl

CHILD

		First reading	Second reading	Average
F4	MUAC			
F5	Weight			
F6	Length			

NB: Weight of the infant in Kg to the nearest 0.1kg and length in centimeters to the nearest 0.1cm**.**

APPENDIX A: BARUA YA UTANGULIZI NA IDHINI

Mpendwa Mshiriki,

Jina langu ni WILLY KAHANYA KIBOI kutoka Chuo Kikuu cha Kenyatta, idara ya chakula na lishe bora. Mimi nafanya utafiti juu ya uhusiano wa ulaji wa chakula kwa muundo wa maziwa ya mama na hali ya lishe ya watoto wachanga miongoni mwa wanawake wanaonyonyesha katika kata ya Nyeri County, Kenya. Utafiti huu utasaidia kujulisha serikali na wadau wengine kuhusu uhusioano wa ulaji wa chakula kwa utungaji wa maziwa ya mama. Pia utafiti huu utajulisha kuhusu matokeo inayowezekana kwa kuimarisha lishe ya mama wanaonyonyesha. Nitakuelezea kuhusu utafiti huu na baada ya hapo unaweza kuamua kushiriki katika utafiti au kukataa.

Utaratibu utakao fuatwa

Mimi na wasaidizi wa utafiti wangu tungependa kukuliza baadhi ya maswali kuhusu wewe mwenyewe na kaya yako. Aidha, tutachukua sampuli ya maziwa yako kwa ajili ya uchambuzi. Pia, tutachukua kipimo cha BMI (uzito na urefu) na MUAC itakayochukuliwa katika mzingo wa katikati juu ya mkononi chini ya kazi . Zaidi ya hayo, tutaangalia hali yako ya virutubisho kwa kutoa mililita tano (5) ya damu na kuifanyia uchambuzi. Sisi pia tuta tathmini hali ya lishe ya mtoto wako kwa kupima uzito na urefu wake. Ingawa maoni yako ni muhimu sana, una haki ya kukataa kushiriki katika utafiti. Wewe utapata huduma na matibabu sawa kama utakubaliana kujiunga na utafiti au la. Dodoso itachukua takribani saa moja, na ushirikiano wako unathaminiwa sana.

Faida zinazoweza kupatikana

Faida kutokana na utafiti huu huwenda si wa moja kwa moja, lakini matokeo yanaweza kuwa muhimu kwa wadau husika katika kuanzisha hatua zinazolenga kuboresha afya ya mama na mtoto. Pia utafaidika na kuelewa hali yako ya lishe ya sasa na ile ya mtoto wako. Ikiwa kutapatikana kuwa na tatizo , ushauri juu ya hatua inayofaa itatolewa.

Hatari / usumbufu unaowezekana

Hakuna hatari linaonekana kuhusishwa na utafiti huu; Hata hivyo baadhi ya maswali ya kuulizwa yanaweza kukupa wasiwasi. Kama hali hii itatokea, unaweza kukataa kujibu maswali hayo na uko na uhuru wa kusimamisha mahojiano wakati wowote.

Fidia

Ushiriki wako kwa utafiti huu ni wa hiari na hivyo hakuna aina yoyote ya malipo.

Masuala ya Jamii

Wale watakaoshiriki na kupatikana na lishe duni nay a watoto wao, watapewa ushauri unayofaa. Aidha, utafiti utakapokamilika, matokeo itasambazwa kwa wadau husika katika jamii kwa ajili ya kuchukua hatua zinazofaa.

Huduma naulinzi wa washiriki wa utafiti

Utaratibu wa utafiti utaelezewa vya kutosha kwa washiriki. Hii itawahakikishia kwamba hakuna hatari inayohusishwa na utafiti huu. Baada ya kusajuliwa kushiriki katika kituo cha afya, washiriki watafuatwa kwa kaya zao ambapo mahojiano yatafanyika na pia kuchukuliwa kwa sampuli ya maziwa na damu ili kuhakikisha faragha. Zaidi, ili kuhakikisha usiri, majina ya mshishiriki hayatatumiwa. Badala yake, nambari zitatumika kusaidia kutambua mshiriki. Pia washiriki watajulishwa kuhusu haki yao ya kujiondoa katika utafiti wakati wowote bila adhabu.

Usiri

Habari yoyote utakayotoa itachukuliwa kwa usiri mkumbwa na haitatumika kwa madhumuni mengine yoyote zaidi ya madhumuni ya utafiti.

Kama una maswali yoyote unaweza kuwasiliana na;

Willy Kiboi
SLP 43844
Nairobi, Kenya
Simu +254 722653111

ama

Chuo Kikuu cha Kenyatta
SLP 43844
Nairobi, Kenya
Simu: +254 20 8710901

Taarifa ya Mshiriki

Habari iliyopo hapo juu kuhusu ushiriki wangu katika utafiti ni wazi na mimi kwa hiari yangu nakubali kushiriki.

Saini ama alama ya dole gumba---

Tarehe--

Taarifa ya Mhoji

Ninathibitisha kwamba, mimi niliyetia sahihi hapa nilielezea mshiriki madhumuni, utaratibu, faida na uwezekano wa hatari zinazohusiana na kushiriki katika utafiti huu.

Jina La Mhoji---

Saini---

Tarehe ---

APPENDIX B: DODOSO LINALOSIMAMIWA NA MTAFITI

Uhusiano wa ulaji wa chackula kwa utungaji wa maziwa na hali ya lishe ya watoto wachanga kati ya wanawake wanaonyonyesha kati kata ya Nyeri, Kenya

SECTIONS

7. SEHEMU A: Demografia na hali ya jamii ya kiuchumi
8. SEHEMU B: Takwimu na desturi ya lishe ya mtoto
9. SEHEMU C: Habari za afya
10. SEHEMU D: Malazi tofauti na ulaji wa chakula wa mama
11. SEHEMU E: Marudio ya chakula
12. SEHEMU F: Kipimo cha Anthropometric

Imethibitishwa na: ---

MAELEZO YA UTAWALA

Kata _________________________ Eneo ____________________________________

Nambari ya dodoso |___||___||___| Tarehe ya mahojiano |___|___|/|___|___|/|___|___|

Jina la mhoji ____

Nambari ya mhojiwa |___||___||___|

SEHEMU YA A: DEMOGRAPHIA NA HALI YA KIUCHUMI YA JAMII		
Sehemu A: Maelezo ya mshiriki		
Alama		**Chaguo**
	Maelezo ya mshiriki	
A1.	Umri wa mama katika miaka iliyokamilika (Thibitisha kutoka kwa kadi ya ANC)________	
A2.	Idadi ya mimba ya mama (Thibitisha kutoka kwa kadi ya ANC) ________	
A3.	Jinsi ulivyojifungua motto wa sasa 1 = Kawaida [ ] 2 = Upasuaji (CS) [ ]	
A4	Mahalu ulijifungulia motto wa sasa 1 = Hospitali/kitu cha afya [ ] 2 = Nymbani [ ] 3 = Mkunga wa nyumbani [ ] 4 = Nyingine yeyote (Taja) [ ] -----------	
A5.	Una watoto wangapi (walio hai) ________	
	Una watoto wangapi walio chini ya miaka tano? ________	
A6.	Hali ya ndoa 1 = Oleka [ ] 2 = Asiyeoleka kamwe [ ] 3 = Talikiwa/Tengana [ ] 4 = Mjane [ ]	
A7.	Dini ya mama 1 = Mkristo [ ] 2 = Muislamu [ ] 3 = Hindu [ ] 4 = Mtamanduni wa kiafrica [ ] 5 = Hakuna [ ] 6 = Nyingine yeyote (Taja) [ ] ----------------	
A8.	Kiwango chako cha juu cha elimu? 1 = Hakuna elimu yeyote [ ] 2 = Kabla ya shule ya msingi [ ] 3 = Shule ya msingi [ ] 4 = Shule ya sekondari [ ]	

	5 = Chuo (cheti na stashahada [ ] 6 = Chuo kikuu(Shahada) [ ]	
A9.	Kazi ya mhojiwa? 1= mama wa nyumba/asiye na kazi [ ] 2= Mfanyikazi wa kawaida [ ] 3 = Mkulima (shamba lake) [ ] 4 = Kujiajiri/ biashara [ ] 5 = Ajira (mshahara) [ ] 6 = Nyingine yeyote (Taja) [ ] ----------------	
A10.	Kazi ya mume wako? **(kama A6 ni 1=Oleka)** 1= Asiye na kazi [ ] 2= Mfanyikazi wa kawaida [ ] 3 = Mkulima (shamba lake) [ ] 4 = Kujiajiri/ Biashara [ ] 5 = Ajira (mshahara) [ ] 6 = Nyingine yeyote (Taja) [ ] ----------------	
	Muundo na sifa za kaya	
A11.	Watu wangapi wanaishi katika kaya yako (Wanaoishi pamoja na kushirikiana sufuria moja ya chakula) _--------------________	
A12.	Nani mkuu wa kaya 1= Mhojiwa [ ] 2= Mume [ ] 3= Mtoto wako/ jamaa [ ] 4= Nyingine yeyote (Taja) [ ] ----------------	
A13.	Jinsia ya mkuu wa kaya (ruka kama mhojiwa ni mkuu wa kaya) 1= Kiume [ ] 2= Kike [ ]	
A14	Kiwango chako cha juu cha elimu (mkuu wa kaya) 1 = Hakuna elimu yeyote [ ] 2 = Kabla ya shule ya msingi [ ] 3 = Shule ya msingi [ ] 4 = Shule ya sekondari [ ] 5 = Chuo (cheti na stashahada [ ] 6 = Chuo kikuu(Shahada) [ ]	
A15.	Kazi ya mkuu wa kaya? 1= Asiye na kazi [ ] 2= Mfanyikazi wa kawaida [ ] 3 = Mkulima (shamba lake) [ ] 4 = Kujiajiri/ Biashara [ ] 5 = Ajira (mshahara) [ ] 6 = Nyingine yeyote (Taja) [ ] ----------------	
A16.	Nini chanzo kikuu cha mapato ya kaya 1= Donation [ ] 2= Kazi ya kawaida [ ] 3 = Ukulima [ ] 4 = Biashara [ ] 5 = Ajira (mshahara) [ ] 6 = Nyingine yeyote (Taja) [ ] ----------------	
A17.	Tafadhali onyesha kiwango cha mapato ya kaya katika	

	mwezi______	
A18.	Katika wastani wa mapato ya kaya yaliyotajwa hapo juu, takriban kiasi gani kinachoendelea kwa matumizi ya chakula kwa mwezi _______	
A19.	Ni nani anayeamua jinsi mapato ya familia yatatumika 1= Mhojia [ ] 2= Mume [ ] 3= Wengine (Taja) [ ] ----------------	
A20.	Chanzo kikuu cha chakula cha nyumbani 1= Uzalishaji wenyewe [ ] 2= Kununua [ ] 3= Uzalishaji na kununua [ ] 4= Misaada [ ] 5= Nyingine yeyote (Taja) [ ] ----------------	
A21.	Aina ya nyumba ya kuishi: 1 = Nyumba iliyopangwa[ ] 2 = Nyumba yangu [ ] 3 = Nyingine (taja) [ ] -------- --------	
A22.	Je! Ni nyenzo yako kuu ya mwangaza? 1= Mafuta taa [ ] 2= Mshumaa [ ] 3= Taa la shinikizo [ ] 4= Umeme [ ] 5= Nyingine (taja) [ ] ----------------	
A23.	Nini nyenzo kuu ya nishati ya upishi? 1 = Kuni [ ] 2 = Makaa [ ] 3 = Mafuta taa [ ] 4 = Gesi [ ] 5 = Umeme [ ] 6 = Nyingine (taja) [ ] ----------------	
A24.	Je, kaya inamiliki shamba?? 1 = Ndiyo [ ] 2 = La [ ]	
A25.	Je, kaya inamiliki mifugo yoyoye ?? 1 = Ndiyo [ ] 2 = La [ ]	
	SEHEMU YA B: TAKWIMU ZA MTOTO NA HALI YA MALAZI	
B1.	Tarehe ya kuzaliwa kwa mtoto (**thibitisha na kadi**) ___|___|/|___|___|/|___|___| **D**| **D**| /|**M**| **M**| /|**Y**| **Y**|	
B2.	Umri wa mtoto? (Umri katika miezi / siku kamili) _________________[**thibitisha na kadi**]|___|/|___|___| |**M**| /|**D_** |**_D** |	
B3.	Jinsia ya mtoto 1 = Kiume [ ] 2 = Kike [ ]	
B4.	Je, baada ya kujifungua ulikaa mda mgani kabla ya	

	kumunyonyesha mtoto? 1= Mara moja (ndani ya dakika 30) [ ] 2 = Kati ya dakika 30-1 (saa moja) baada ya kujifungua [ ] 3=Kati ya masaa 2-12 baada ya kuzaa [ ] 4= zaidi ya masaa 12 [ ]	
B5.	Katika siku tatu za kwanza baada ya kujifungua, je! Ulipa [Jina] maziwa / maji yaliyotoka kwa matiti yako? 1 = Ndiyo [ ] 2 = La [ ]	
B6.	Je! Umepokea maelezo mengine juu ya kunyonyesha? 1 = Ndiyo [ ] 2 = La [ ]	
B7.	Ikiwa ndiyo, ni aina gani ya habari? _________	
B8.	Jana wakati wa mchana au usiku, je, mtoto wako alinyonyeshwa au alipewa maziwa ya mama kutoka kwako? 1 = Ndiyo [ ] 2 = La [ ]	
B9.	Jana mchana na usiku ulinyonyesha mtoto mara ngapi? _________	
B10	Kwa wastani, kila kikao cha kunyonyesha kilichukua muda mgani kwa dakika_________	
B11.	Mara ya kunyonyesha mtoto (wastani) kwa siku (maoni ya mama) _________	
B12.	Muda kwa dakika (wastani) wa kunyonyesha kwa kila kikao (maoni ya mama)------------______	
B13.	Ikiwa mtoto hakunyonyeshwa jana, sababu ya kutomnyonyesha? 1= Mama alikuwa mgonjwa [ ] 2=Mtoto alikuwa mgonjwa [ ] 3=Nilikuwa kazi [ ] 4=Nyingine (taja) [ ]	
SEHEMU YA C: HABARI ZA AFYA (CHATI CHA MARADHI)		
	MAMA	
C1.	Je, umepata ugojwa wowote kwa wiki mbili zilizopita? 1 = Ndiyo [2 = La [ ]	

C2.	Kama ndiyo, aina ya ugongwa	Muda wa ugonjwa kwa siku	
	1.		
	2.		
	3.		
	4.		
	5.		
C3.	Je, ulitafuta matibabu? 1 = Ndiyo [ ] 2 = La [ ]		
C4.	Kama ndiyo, wapi? *(Zaidi ya jibu moja linawezekana)* 1 = Hospitali ya serikali [ ] 2 = Hospitali ya kibinafsi [ ] 3 = Dawa za jadi [ ]		

	4 = Duka la dawa [ ] 5 = Nyingine(taja) -------- [ ]		
	MTOTO		
	SWALI	JIBU	
C5.	Je, mtoto amekuwa mgonjwa wiki mbili zilizopita?	1 = Ndiyo [ ] 2 = La [ ]	
C6.	Ikiwa ndiyo wapi? *(Zaidi ya jibu moja linawezekana)*	1 = Kuhara [ ] 2 = Kutapika [ ] 3 = Homa na baridi kama malaria[ ] 4 = Homa, kikohozi, shida katika kupumua [ ] 5= Vimelea vya tumbo [ ] 6 = Surua [ ] 7 = Mambukizo ya jicho [ ] 8 = Maambukizo ya ngozi [ ] 9 = Ajali [ ] 10 = Utapi mlo [ ] 11 = Maumivu ya tumbo [ ] 12 = Maumivu ya meno [ ] 13 = Nyingine (taja) --------- ---- [ ]	
C7.	Mtoto alipokuwa mgonjwa ulitafuta matibabu?	1 = Ndiyo [ ] 2 = La [ ]	
C8.	Kama ndiyo, wapi? *(zaidi ya jibu moja linawezekana)*	1 = Hospitali ya serikali [ ] 2 = Hospitali ya kibinafsi [ ] 3 = Dawa za jadi [ ] 4 = Duka la dawa [ ] 5 = Nyingine(taja) -------- [ ]	
C9.	Je, unatumia virutubisho vya ziada ya chakula/matone ya kuongeza lishe?	1 = Ndiyo [ ] 2 = La [ ]	
C10.	Ikiwa ndiyo, gani	--------	

SECTION YA D: ULAJI/TOFAUTI YA MALAZI

Fomu ya kurekodi vyakula na vinywanji vilivyotumiwa masaa 24 iliyopita na mhojiwa.

Uliza vyakula na vinywanji mhojiwa alizotumia siku iliyopita kuanzia alipoamka asubuhi hadi alipolala usiku kwa nyumba yake au nje ya nyumba yake. Uliza chakula iliyotumiwa kwa siku, viungo na kiasi katika milo, kiasi cha chakula kupikwa, kiasi cha chakula kilicholiwa na uhesabu kiasi cha viungo zilizotumiwa katika gramu na mhojiwa?

Thibitisha kama siku ilikuwa ya kawaida au siyo ya kawaida

1= siku ya kawaida

2= siku isiyo ya kawaida

Kama si ya kawaida, elezea kwa nini?

1=Sherehe 2=Shughuli ya kidini 3=Chakula kidogo katika kaya 4=Nyingine (Taja)

Kumbuko ya chakula kilicholiwa katika masaa 24 iliyopita

								Matumizi rasmi
Wakati/ Chakula	Aina ya chakula/Jina ya chakula	Viungo	Kipimo cha kaya	Kiasi katika gramu	Kiasi kupikwa	Kiasi pakuliwa	Kiasi kuliwa	Kiasi kuliwa katika gramu
Kifungua kinywa								
Kitafunio 1								

Chakula cha mchana								

Kitafunio 2								
Karamu								

Kitafunio 3								

DODOSO LA MALAZI TOFAUTI

Jaza makundi ya chakula kulingana na taarifa ya malazi kumbuka iliyoandikwa hapo juu. Kwa makundi yoyote ya chakula yenye haikutajwa, uliza mhojiwa kama kuna bidhaa ya chakula kutoka kundi hilo alikula.

mbari ya swali	Kundi la chakula	Mifano	Ndiyo=1 La=0
1	NAFAKA	mkate, tambi (supagetti), biskuti, mandazi, samosa au vyakula vingine vyovyote vilivyotengenezwa kutoka kwa mtama, wimbi, mahindi, mchele au ngano, kwa mfano: ugali, uji n.k.	
2	VIAZI VYEUPE NA VYAKULA VYA MIZIZI	viazi vya mboga, viazi vitamu vyeupe, nduma, mhogo au vyakula vilivyo tengenezwa kutokana na mizizi	
3	MBOGA NA VIAZI VILIVYO NAWINGI WA VITAMINI A	matango, karoti, viazi tamu vya manjano + vyakula vingine vipatikanvyo vyenye wingi wa vitamini A	
4	MBOGA ZA MAJANI ZENYE WINGI WA RANGI YA KIJANI KIBICHI	Sukuma wiki, mchicha, mnavu, mkunde, kigwada, bwere, mchunga, mchicha, mzungi, majani ya matango + mboga zingine zozote za majani yenye wingi wa rangi ya kijani kibichi	
5	MBOGA ZINGINE	Nyanya, kitunguu maji, hoho, biringanya, kabichi, tunguja, pilipili, mamumunye, n.k.	
6	MATUNDA YENYE WINGI WA VITAMINI A	Maembe yaliyoiva, paipai n.k.	
7	MATUNDA MENGINE	Machungwa, ndimu, ndizi, mapera, nanasi, matikiti, passion, kunazi, pepeta, vitoria, mkwaju, chenza, madafu, matunda ya damu (tree tomato), zabibu, avocado (parachichi) n.k.	
8	NYAMA YA VIUNGO	Maini, figo, moyo au nyama ya chombo nyingine au vyakula damu	
9	NYAMA	Nyama ya ng'ombe, nguruwe, kondoo, mbuzi, sungura, nyama ya mwituni, nyama ya kuku au bata au ndege wengine, nyama ya wadudu kama kumbikumbi, panzi, nyama ya mamba n.k.	

10	MAYAI	Mayai ya kuku, bata, ndege n.k.	
11	SAMAKI NA DAGAA	Samaki asiyekaushwa au aliyekaushwa, omena, papa, simu, kamba, ngege, mbuta, kweza, kaa n.k.	
12	BOROHOA, NJUGU AU KOROSHO NA MBEGU	Maharagwe, ufuta, mbaazi, kunde, ndengu, pojo, soya, njugu mawe, minji, njahi, au vyakula vingine vitokanavyo na hivi vilivyotajwa	
13	MAZIWA NA VYAKULA VYOTE VINAVYOTOKA KWA MAZIWA	Maziwa, maziwa lala, cheese, yogurt n.k.	
14	MAFUTA	Mafuta ya kupikia, mafuta ya samaki, mafuta ya nguruwe, siagi, mafuta ya nasi n.k.	
15	VYAKULA VYA SUKARI	Sukari, asali, soda yenye sukari, sukari nguru, kaimati, miwa, chokoleti, peremende, icecream, barafu (ice)	
16	VIUNGO, CHAI AU KAHAWA NA VILEO	Viungo kama vile tangawizi, mdalasini, binzari, dhania, kitunguu saumu, karafuu, iliki n.k.; kahawa, chai; vileo kama chang'aa, mnazi, matingasi/busaa, mukoma n.k.	
Ngazi ya kibinafsi	Je, ulikula kitu chochote (mlo au vitafunio) nje ya nyumba jana?		

Mkusanyiko wa kujenga kiwango cha chini cha malazi tofauti ya wanawake wa Umri wa Uzazi (MDD-W)

Vikundi / vitu / safu kwenye dodoso	**Makundi ya chakula kumi katika MDD-W**	**1 = Ndiyo 0 = La**
A. Nafaka B. viazi vyeupe na vyakula vya mizizi	1. Nafaka, viazi vyeupe na vyakula vya mizizi	
C. Kunde (maharagwe, mbaazi na ndengu)	2. Kunde (maharagwe, mbaazi na ndengu)	
D. Mbegu	3. Mbegu	
E. Maziwa na bidhaa za maziwa	4. Maziwa na bidhaa za maziwa	
F. Nyama ya viungo G. Nyama zingine na kuku H. Samaki na chakula cha baharini	5. Nyama, kuku na samaki	
I. Mayai	6. Mayai	
J. Mboga za majani zenye wingi wa rangi ya kijani kibichi	7. Mboga za majani zenye wingi wa rangi ya kijani kibichi	
K. Mboga zenye wingi wa vitamini A, viazi na mizizi L. Matunda zenye wingi wa vitamini A	8. Matunda na mboga zingine zenye wingi wa vitamini A	
M. Mboga zingine	9. Mboga zingine	
N. Matunda mengine	10. Matunda mengine	

Kumbuka: Katika mkusanyiko za MDD-W, peremende, sukari, viungo na vinywaji havizingatiwi

JUMLA YA ALAMA ZA DDS

IDADI YA MILO ILIYOLIWA KWA SIKU

SEHEMU YA E: MARUDIO YA CHAKULA

Eleza marudio ya vyakula (mama) vifutavyo vilivyochaguliwa kwa wiki

Chakula	Marudio kwa wiki	Chakula	Marudio kwa wiki
Mahindi		Mayai	
Unga ya mahndi		Maini	
Mchele		Figo/moyo	
Mtama		Samaki	
Mihogo		Kuku	
Wimbi		Nyama (nyama nyingine)	
Chapati/mkate/keki		Parachichi/ovakado	
Ndizi mbichi		Maembe	
Viazi		Papai	
Sukuma wiki		Matunda ya karakara (Passion)	
Kabichi		Ndizi iliyoiva	
Mchicha		Machungwa	
terere		Mapera	
Karoti		Tikiti maji	
Nyanya		Mimea ya asili	
Malenge		Matone ya virutumbisho	
Viazi vitamu za majano		Nyingine (taja)	
Maharagwe			
Mbaazi			
Ndengu			
Kamande			
Njugu karanga			
Maziwa			
Maziwa mgando			

SEHEMU YA F: HALI YA LISHE; VIPIMO

MOTHER

		Somo la kwanza	Somo la pili	Wastani
F1	MUAC			
F2	Uzito			
F3	Urefu			

Kumbuka: Uzito wa mama katika kilo na urefu katika sentimita

MAMA (HB g/dL)

MTOTO

		Somo la kwanza	Somo la pili	Wastani
F4	MUAC			
F5	Uzito			
F6	Urefu			

Kumbuka: Uzito wa mtoto katika kilo na urefu katika sentimita

Appendix C: Research Permit and Authorization

KENYATTA UNIVERSITY
GRADUATE SCHOOL

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

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 810901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School

DATE: 31st October, 2017

TO: Mr. Kiboi W. Kahanya
C/o Department of Foods, Nutrition & Dietetics
KENYATTA UNIVERSITY

REF: H87/37658/16

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that the Graduate School Board at its meeting 18th October, 2017 approved your Ph.D. Research Proposal entitled **"Impact of Maternal Dietary Intake on Breast Milk Composition and Infant (0-6 months) Nutritional Status among Lactating Women in Nyeri County, Kenya".**

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed supervision Tracking Forms per semester. The form has been developed to replace the progress Report Forms. The Supervision Tracking Forms are available at the University's Website under Graduate School webpage downloads.

By copy of this letter, the Registrar (Academic) is hereby requested to grant you substantive registration for your Ph.D. studies.

Thank you.

KENYATTA UNIVERSITY OFFICE OF DEAN
- 1 NOV 2017
GRADUATE SCHOOL 00100 NAIROBI

REUBEN MURIUKI
FOR: DEAN, GRADUATE SCHOOL

c.c. Chairman, Department of Foods, Nutrition & Dietetics

Supervisors:

1. Prof. Judith Kimiywe
   Department of Foods, Nutrition & Dietetics
   KENYATTA UNIVERSITY

2. Dr. Peter Chege
   Department of Foods, Nutrition & Dietetics
   KENYATTA UNIVERSITY

RM/cao

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Fax: 8711242/8711575
Email: chairman.kuerc@ku.ac.ke
secretary.kuerc@ku.ac.ke

Website: www.ku.ac.ke

P. O. Box 43844-00100
Nairobi.

Tel: 8710901/12

Our Ref: **KU/ERC/ Re- Review Appr./VOL.1** (56) Date: 29th January, 2018.

Kiboi Willy Kahanya
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

Dear Willy,

APPLICATION NUMBER- PKU/767/I835 'IMPACT OF MATERNAL DIETARY INTAKE ON BREAST MILK COMPOSITION AND INFANT (0-6 MONTHS) NUTRITIONAL STATUS AMONG LACTATING WOMEN IN NYERI COUNTY KENYA."

1. **IDENTIFICATION OF PROTOCOL**

The application before the Committee is with a research topic **PKU/767/I835** 'Impact of Maternal Dietary Intake On Breast Milk Composition And Infant (0-6 Months) Nutritional status Among Lactating Women In Nyeri County, Kenya" received on 8th November, 2017 and deliberated on the 16th of January, 2018 and received on 24th January 2018 for re review.

APPLICANT

Kiboi Willy Kahanya

2. **SITE**

Nyeri County. Kenya

3. **DECISION**

The Committee has considered the research protocol in accordance with the Kenyatta University Research Policy (Section 7.2.1.3) and the Kenyatta University Review Committee Guidelines **AND APPROVED that the research may proceed for a period of ONE year from 26th January 2018.**

ADVICE/CONDITIONS

You must include a Clinician in the Study and include an elaboration of Community benefits.

i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.
ii. Serious and unexpected adverse events related to the conduct of the study are reported to this committee immediately they occur.
iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.
iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above.
If you accept the decision reached and advice and conditions given please sign in the space Provided below and return to KU-ERC a copy of the letter.

KENYATTA UNIVERSITY OFFICE OF THE CHAIRMAN 29 JAN 2018 ETHICS REVIEW COMMITTEE NAIROBI

DR. TITUS KAHIGA.
CHAIRMAN ETHICS REVIEW COMMITTEE

I ....WILLY KAHANYA KIBOI.... accept the advice given and will fulfill the conditions therein.

Signature........................ Dated this day of... ....31/01....................2018.

C.c. DVC Research Innovation and Outreach

THIS IS TO CERTIFY THAT:
MR. WILLY KAHANYA KIBOI
of KENYATTA UNIVERSITY, 0-10100
NYERI, has been permitted to conduct
research in *Nyeri County*

on the topic: *IMPACT OF MATERNAL DIETARY INTAKE ON BREAST MILK COMPOSITION AND INFANT (0-6 MONTHS) NUTRITIONAL STATUS AMONG LACTATING WOMEN IN NYERI COUNTY, KENYA*

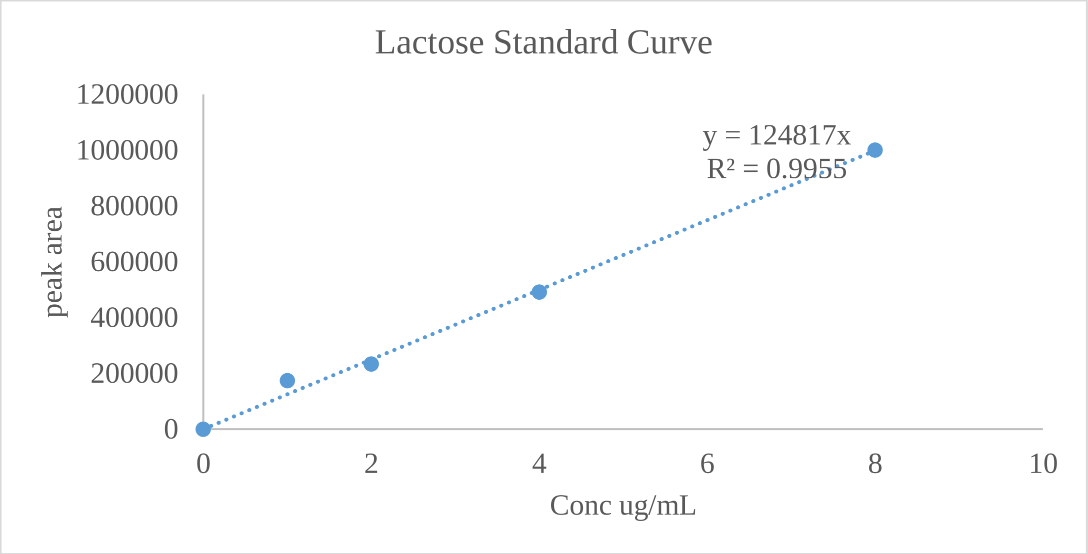
for the period ending:
20th February,2019

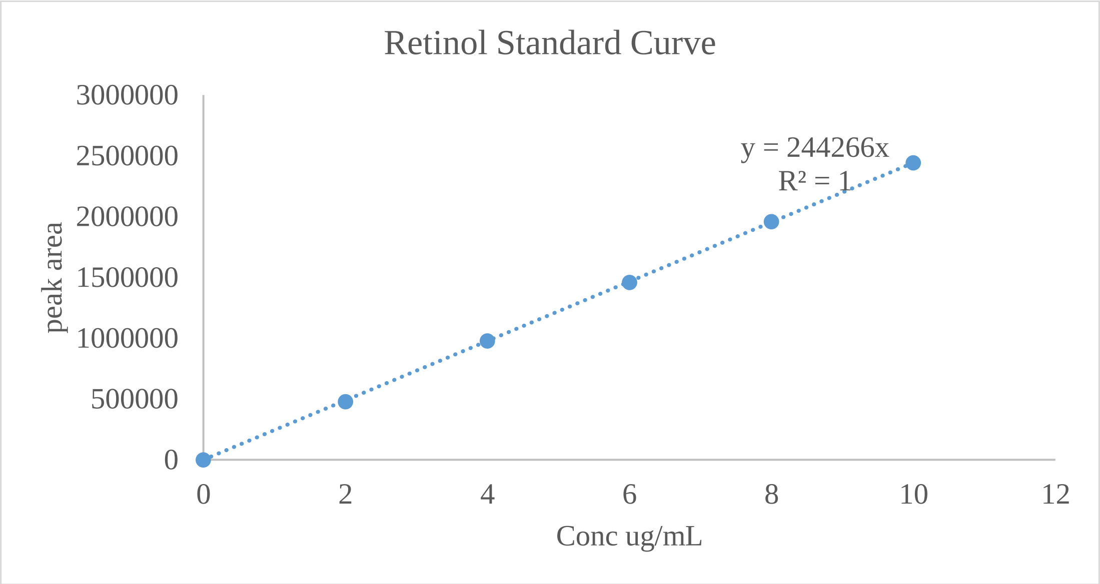
....
Applicant's Signature

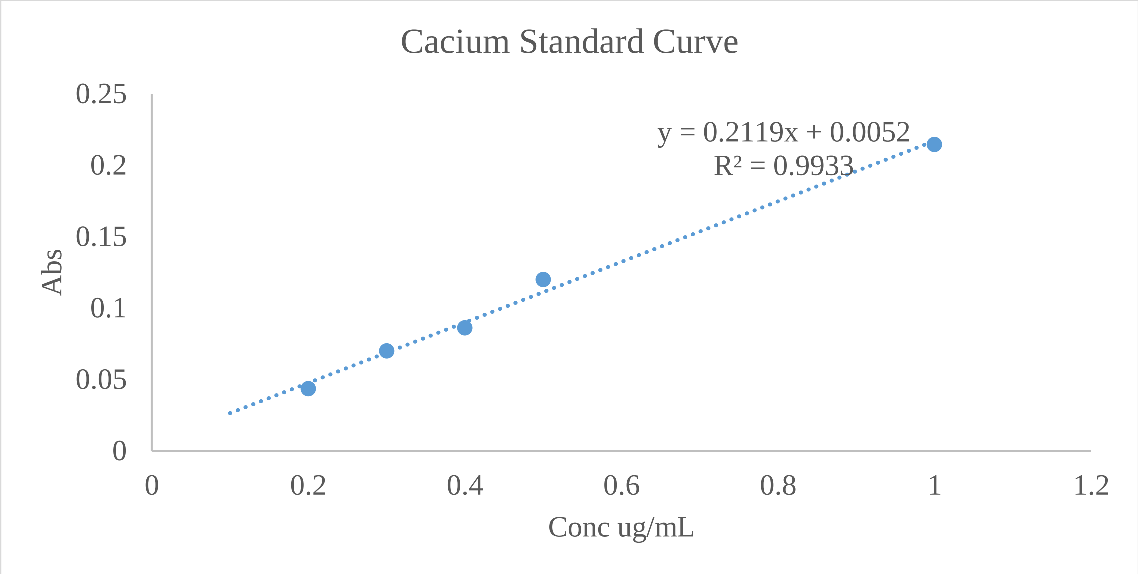
Permit No : NACOSTI/P/18/9607/21284
Date Of Issue : 20th February,2018
Fee Recieved :Ksh 2000

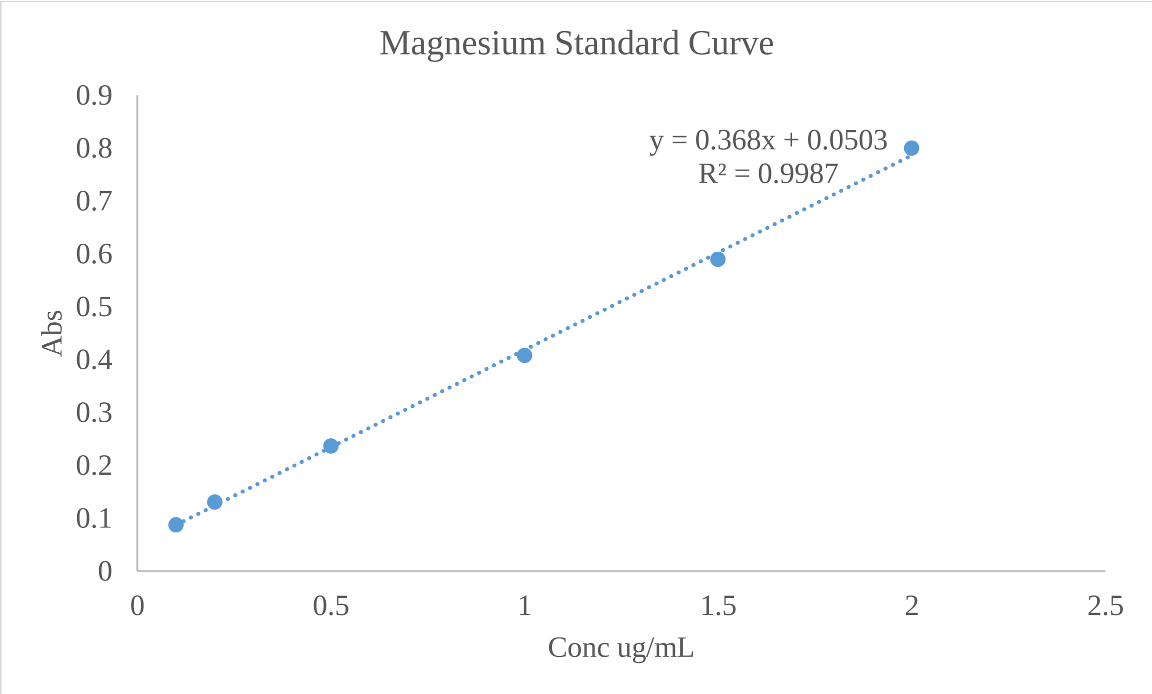
GP Kalerwa
....
Director General
National Commission for Science, Technology & Innovation

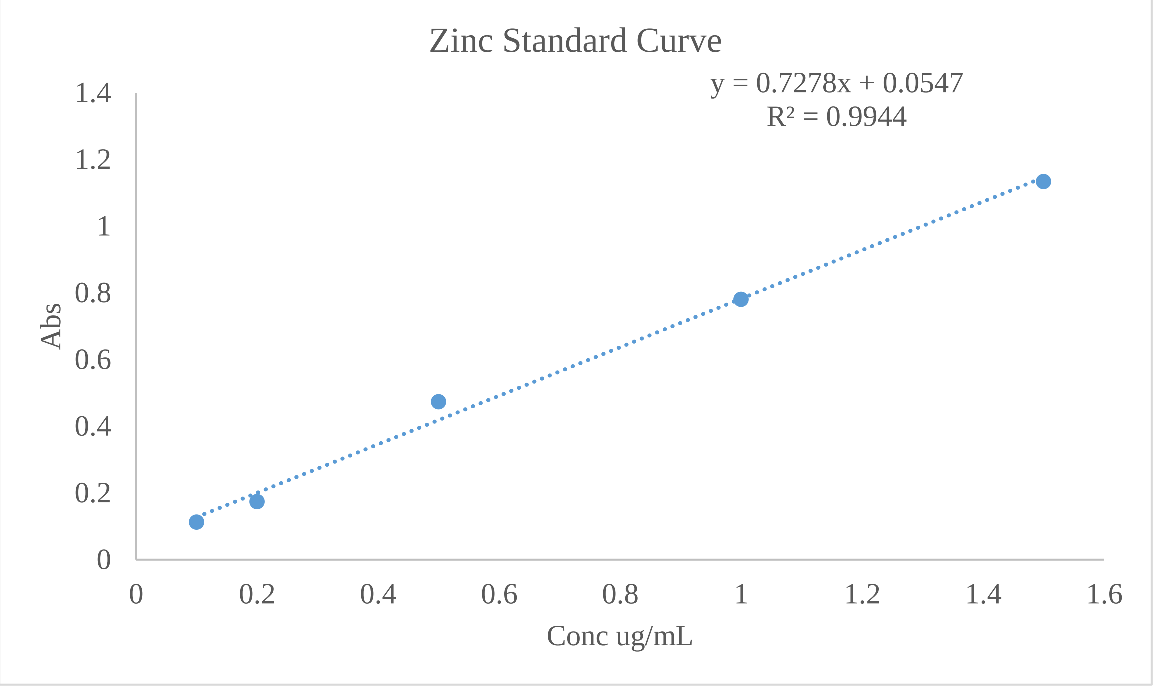
Appendix D: Sample Analysis Calibration Curves

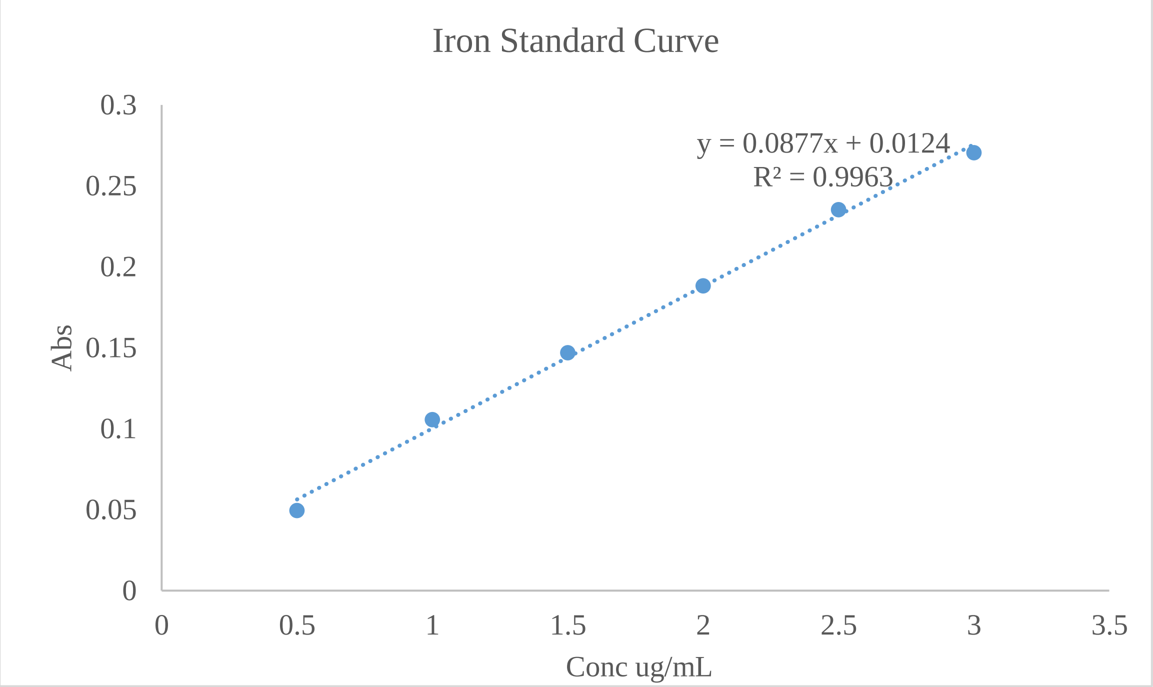





Cacium Standard Curve
y = 0.2119x + 0.0052
R² = 0.9933
Abs
0.25
0.2
0.15
0.1
0.05
0
0
0.2
0.4
0.6
0.8
1
1.2
Conc ug/mL


Magnesium Standard Curve
y = 0.368x + 0.0503
R² = 0.9987
Abs
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.5
1
1.5
2
2.5
Conc ug/mL


Zinc Standard Curve
y = 0.7278x + 0.0547
R² = 0.9944
Abs
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
Conc ug/mL


Iron Standard Curve
y = 0.0877x + 0.0124
R² = 0.9963
Abs
0.3
0.25
0.2
0.15
0.1
0.05
0
0
0.5
1
1.5
2
2.5
3
3.5
Conc ug/mL