

**UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES**

**IMPROVING VITAMIN A STATUS OF INFANTS IN THE UPPER
MANYA KROBO DISTRICT WITH COMPLEMENTARY FOODS
FORTIFIED WITH *MORINGA OLEIFERA* LEAF POWDER – A PILOT
STUDY**

**IRENE MARTEKUOR ASHLEY
(ID: 10276546)**

**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON, IN
PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF
MASTER OF SCIENCE IN DIETETICS DEGREE**

DEPARTMENT OF NUTRITION AND DIETETICS

DECEMBER, 2017

DECLARATION

I, Irene M Ashley, declare that with the exception of the references cited, all information in this document was obtained via research under the supervision of Dr. Gladys Peprah Boateng and Mrs. Laurene Boateng of the College of Health sciences, University of Ghana. This dissertation has never been presented in part or whole to any institution for the award of any degree or diploma.

Signature

.....

IRENE MARTEKUOR ASHLEY

DATE

(STUDENT)



Signature

.....

DR. GLADYS PEPRAH BOATENG

DATE

(SUPERVISOR)

Signature

.....

MRS. LAURENE BOATENG

DATE

(SUPERVISOR)

ABSTRACT

Background: Micronutrients are extremely important components of a high-quality diet and even though they are required in very small amounts, they are important in the building of healthy brains, bones and body. Micronutrient deficiency is a problem as its effects are irreversible. In Africa, vitamin A deficiency is reported to be the cause of about 6% of deaths of children under the age of 5 years, and a major public health issue in Ghana. Inadequate dietary intake of vitamin A-rich foods is a major cause of vitamin A deficiency, so one preventive strategy could be the promotion of the consumption of these foods. *Moringa oleifera* leave powder is rich in nutrients especially vitamin A. It is well accepted in infants and have proved successful in combatting malnutrition in infants. Therefore, there is a need to design a pilot study to test the efficacy of *Moringa oleifera* on serum retinol levels in infants.

Aim: To test the efficacy of *Moringa* Leaf Powder (MLP) in improving the serum vitamin A level of children in the complementary feeding age using secondary data.

Methods: A pilot study with three study arms was carved out. Infants were randomly assigned to receive one of the 3 study foods (*CF-35g*, *MCL-35g* and *M-5gS*) depending on the Child Welfare Centre (CWC) they were recruited from. One arm received a cereal-legume blend fortified with MLP named *MCL-35g*, a second arm received *Moringa oleifera* leaf powder as a supplement to be sprinkled on infant's usual foods (*MS-5g*) and a third arm received a cereal legume blend, without MLP (*CF-35g*) to serve as controls. The feeding intervention lasted for 6 weeks. Baseline data on infant and maternal characteristics, household characteristics, anthropometry, hemoglobin level and child morbidity occurrence, were collected. Serum vitamin A was the primary outcome measure and was determined at both baseline and endline using the icheck fluoro device. Morbidity occurrence, dietary intake and adherence to study foods were secondary outcome measures. Data on dietary

intake was taken at baseline and endline using a 2 day 24-hour recall. Adherence to study foods and morbidity occurrence were collected over the study duration.

Results: A total of 103 infant-mother pairs were recruited for the study but a total of 65 completed the study. All the infants in the study were vitamin A deficient at both baseline and endline when compared to the WHO threshold of 0.70 $\mu\text{mol/l}$. There was no significant difference in serum vitamin A concentration within and among the control group and the two experimental groups at both baseline and endline. However, there was a marginal increase in serum vitamin A concentrations in all three groups at endline. There was a significant difference ($p=0.026$) in the iron intake of infants at baseline. There was also a significant difference ($p<0.05$) in adherence to study foods between those in the *CF-35 g* group and *MCL-35 g* group but no significant difference between the two experimental groups.

Conclusion: Findings of this study showed that although there was a marginal increase in serum vitamin A concentrations for infants in all three groups, from baseline to end line, vitamin A levels of all infants were low when compared to the WHO threshold of 0.70 $\mu\text{mol/l}$. This implies that, vitamin A deficiency (VAD) remains a major public health issue in the study population and in Ghana for that matter.

As part of food diversification approaches to combat VAD, *Moringa Oleifera* leaf powder can be incorporated in complementary foods of infants in the developing world to improve their serum vitamin A levels and consequently their nutritional status.

DEDICATION

This work is dedicated to the glory of God for His mercies and guidance which enabled me to successfully complete the work. To Him I am forever grateful. Amen.

ACKNOWLEDGEMENTS

To God is the Glory. I am grateful to God Almighty for the wisdom and strength to carry out this research.

I would like to express my heartfelt appreciation and acknowledgement to Dr. Gladys Peprah Boateng, and Mrs. Laurene Boateng at the Department of Nutrition and Dietetics, School of Biomedical and Allied Health Sciences, University of Ghana who devoted their precious time to supervise this work by directing, vetting, correcting and advising. To you, I say I am very grateful. Again, I would like to acknowledge the relentless efforts they put in for this research to be completed.

To all those who helped me in one way or the other, I am very grateful.

Finally, I thank my parents and siblings for their moral support.

TABLE OF CONTENTS

DECLARATION	i
ABSTRACT.....	ii
DEDICATION.....	iv
ACKNOWLEDGEMENTS.....	v
TABLE OF CONTENTS.....	vi
LIST OF FIGURES	ix
LIST OF TABLES.....	x
ABBREVIATIONS	xi
CHAPTER ONE.....	1
1.0 INTRODUCTION	1
1.1 BACKGROUND.....	1
1.2 PROBLEM STATEMENT.....	4
1.3 AIM.....	4
1.4 SPECIFIC OBJECTIVES	4
CHAPTER TWO.....	5
2.0 LITERATURE REVIEW	5
2.1 INTRODUCTION	5
2.2 VITAMIN A IN HUMAN NUTRITION	6
2.2.1 Metabolism of vitamin A	8
2.2.2 Functions of vitamin A.....	8
2.2.3 Dietary sources of vitamin	9
2.3 PREVALENCE OF VITAMIN A DEFICIENCY (VAD).....	10
2.3.1 Causes of VAD.....	12
2.3.2 Consequencies of VAD	13
2.2.3 Combating VAD.....	15
2.4 MEASURING VITAMIN A STATUS	15
2.5 COMPLEMENTARY FEEDING IN THE DEVELOPING WORLD	16
2.6 THE <i>MORINGA OLEIFERA</i> PLANT	18
2.6.1 Nutritional potential of <i>Moringa Oleifera</i> in combatting VAD.....	18
2.7 PILOT STUDIES.....	20
CHAPTER THREE	22
3.0 METHODS	22

3.1 STUDY DESIGN.....	22
3.2 SAMPLE SIZE.....	22
3.3 STUDY SITE.....	22
3.4 STUDY POPULATION	22
3.31 Inclusion criteria.....	23
3.4.2 Exclusion criteria.....	23
3.5 STUDY PROCEDURE	23
3.6 PREPARARTION OF STUDY FOODS.....	23
3.6.1 Nutrient content of study foods.....	24
3.7. DATA COLLECTION	24
3.7.1 Baseline data collection.....	24
3.7.2 Infant and maternal chracteristics.....	24
3.7.3 Household characteristics	25
3.7.4 Anthropometry	25
3.7.5 Child morbidity occurrence.....	25
3.7.6 Biochemical data.....	25
3.7.7 Dietary data collection.....	26
3.7.8 Intervention.....	26
3.7.9 Outcome measures.....	27
3.8 DATA ANALYSIS.....	28
3.9 ETHICS.....	28
CHAPTER FOUR.....	29
4.0 RESULTS	29
4.1 FLOW OF INFANTS THROUGH THE STUDY	30
4.2 BACKGROUND CHARACTERISTICS OF STUDY PARTICIPANTS AT BASELINE	31
4.3 SERUM VITAMIN A CONCENTRATION AT BASELINE AND END LINE	33
4.4 COMPARISON OF INTAKE OF ENERGY AND SELECTED NUTRIENTS AT BASELINE AND ENDLINE.....	34
4.5 ADHERENCE TO STUDY FOODS	36
4.6 MORBIDITY OCCURRENCE AT ENDLINE OF THE STUDY	37
CHAPTER FIVE	38
5.0 DISCUSSION	38
5.1 BACKGROUND CHARACTERISTICS OF STUDY PARTICIPANTS AT BASELINE	38
5.1.1 Background information of infants.....	38

5.1.2 Background characteristics of mothers/caregivers	39
5.1.3 Household characteristics.....	39
5. 2 SERUM VITAMIN A CONCENTRATION AT BASELINE AND END LINE	40
5.3 ENERGY AND SELECTED NUTRIENT INTAKE.....	41
5.4 ADHERENCE TO STUDY FOODS	42
5.5 MORBIDITY OCCURRENCE DURING THE STUDY	42
5.6 LIMITATIONS.....	43
CONCLUSION AND RECOMMENDATIONS.....	44
REFERENCES	45
APPENDIXES	57

LIST OF FIGURES

- Figure 2.1** Schematic diagram of vitamin A metabolism
- Figure 2.2** Global extent of VAD as defined by prevalence of serum retinol
<0.70 μ mol/l in preschool children
- Figure 4.1** Flow of infants through the study
- Figure 4.2** Adherence to study foods

LIST OF TABLES

Table 2.1	Specific nutrient content in 100g of selected food items
Table 3.1	Nutrient content of study foods
Table 4.1	Background characteristics of study participants at baseline
Table 4.2	Differences in Vitamin A between baseline and end line for infants who completed the study
Table 4.3	Comparison of intake of energy and selected nutrients at baseline and endline
Table 4.4	Morbidity occurrence among infants during the study

LIST OF ABBREVIATIONS

CWC	Child Welfare Clinic
DALY	Disability Adjusted Life years
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organization
GDHS	Ghana Demographic and Health Survey
HPLC	High Performance Liquid Chromatography
JHS	Junior High School
MLP	Moringa Leaf Powder
NIH	National Institute of Health
RE	Retinol Equivalent
RDA	Recommended Daily Allowance
RBP	Retinol Binding Protein
UL	Upper Limit
UNICEF	United Nation International Children's Emergency Fund
UNHCR	United Nations High Commissioner for Refugees
VAD	Vitamin A Deficiency
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Malnutrition refers to deficiencies, excesses or imbalances in a person's intake of energy and/or nutrients and covers two main groups of conditions. Undernutrition which includes stunting (low height for age), wasting (low weight for height), underweight (low weight for age) and micronutrient deficiencies or insufficiencies on one hand and overweight and obesity on the other hand (WHO, 2016).

Globally, about 159 million children are stunted and 50 million are wasted (WHO, 2016). UNICEF also reports that, as at 2011, globally, about one quarter of all children below the age of 5 years (about 156 million children) are stunted. Forty percent (40%) of the children who are stunted are in Sub- Sahara Africa while 39% are in South Asia. Furthermore, South Asia and Sub- Saharan Africa are home to the highest number of children under five (5) years who are underweight, with 26%, which is 30 million of underweight children in Sub- Saharan Africa (UNICEF, 2011).

The 2014 Ghana Demographic and Healthy Survey (GDHS) report showed that, among Ghanaian children under 5 years, 19% were stunted, 5 percent were wasted and 11 percent were under weight. About 3 percent of children were overweight (heavy for their height) and micronutrient malnutrition was highly prevalent and persistent (GDHS, 2014).

Micronutrient malnutrition pertains to diseases that are caused by a dietary deficiency of vitamins or minerals. Vitamin A deficiency (VAD), iron deficiency anemia and iodine deficiency are the most common forms of micronutrient malnutrition (FAO, 2015). Serum retinol $<0.70 \mu\text{mol/l}$ and the prevalence of night blindness above various population-specific thresholds are used in classifying vitamin A deficiency in countries as a public health issue (WHO, 2009). Globally 5.2 million preschool age children are affected by night blindness

and 190 million preschool age children had low serum retinol concentrations and in Africa. Vitamin A deficiency is responsible for about 2% of child deaths under the age of 5 years (WHO, 2011).

Moringa Oleifera leaves have been used to combat malnutrition, especially among infants and nursing mothers (Doerr and Cameron, 2005). The leaves of *Moringa Oleifera* are rich in minerals like calcium, potassium, zinc, magnesium, iron and copper (Kasolo et al, 2010). Beta-carotene, B vitamins such as folic acid, pyridoxine and nicotinic acid, vitamins C, D and E are also present in *Moringa Oleifera* leaves (Mbikay, 2012). *Moringa* leaf powder is prepared by drying the leaves in the shade. Drying them in the sun will cause loss of vitamin A. The flakey dried leaves are pounded, sifted and stored. The Moringa Leave Powder (MLP) can then be included in a baby's food, added to soups, and to vegetables to improve nutritional quality but not change the taste (Price, 2007).

A study conducted in Senegal using *Moringa Oleifera* leaf powder to supplement the meals of children and women showed that “adding one rounded tablespoon (8 g) of *Moringa* leaf powder satisfied about 14% of the protein, 40% of the calcium, 23% of the iron and nearly all the vitamin A needs for a child aged 1-3” (Fuglie, 2005). Furthermore, a study by Glover-Amengor et al (2015) in Ghana, to test the micronutrient composition of *Moringa Oleifera* leaf fortified dishes in children, showed that *Moringa Oleifera* leaves that they sampled contained all the micronutrients tested (β-carotene, Copper (Cu), Zinc (Zn), Magnesium (Mn), and Iron (Fe)) which implies that *Moringa Oleifera* leaves could be used to fortify diets with micronutrients and have the potential of serving as a less expensive β-carotene and mineral source in the diets especially in children with marginal vitamin A status and iron deficiency anemia. An acceptability trial by Boateng et al (2017) showed that *Moringa Oleifera* leaf powder, when used in the diet of infants is well accepted by infants. Therefore, incorporating pulverized *Moringe Oleifera* leaves in infants' food could diversify food intake,

and will ensure food security and reduce some micronutrient deficiency diseases (Nwosu et al, 2014).

1.2 PROBLEM STATEMENT

The National Nutrition Policy for Ghana report (2013), indicates that 70% of children under five years have various forms of vitamin A deficiency. According to UNICEF (2015), the main interventions for the expulsion of micronutrient deficiencies, including VAD are supplementation, either single multiple or micronutrients, fortification of foods for a whole population (flour, oil, salt, sugar) or fortification of specific foods, including home-fortification. Also, the promotion of desirable feeding & diversified diets, that is satisfactory breastfeeding (early initiation of breastfeeding, exclusive breastfeeding for 6 months and continued breastfeeding for up to 2 years), consumption of micronutrient-rich foods, including animal source foods and public health measures which includes infection control, hygiene and immunization.

Moringa Oleifera leaves are a rich source of provitamin A or beta-carotene. Fresh *Moringa Oleifera* leaves contain almost 7-8 mg of beta-carotene per 100 g, while the leaf powder contains twice that amount in 100 g (Marcu, 2005).

A research conducted by Kouevi (2013) which investigated the nutritional properties of infant weaning foods developed in West Africa found that most complementary food blends were low in energy, vitamin A, vitamin C, iron and calcium. However, there was a significant increase in all nutrients particularly vitamin A complementary food blends with *Moringa Oleifera* leaves. However, studies that have tested the efficacy of *Moringa Oleifera* leaf powder in improving Vitamin A levels in infants in the complementary feeding age, when used as a supplement in their diet are lacking.

1.3 SIGNIFICANCE OF STUDY

Infants and young children are a nutritionally vulnerable group of a population as they are at a crucial stage of the growing process (Custodio et al, 2008). Findings of this study will contribute to the body of literature on nutritional interventions aimed at combatting VAD among infants and young children and will further help with refining procedures and identifying modifications needed for the design of a larger study.

1.4 AIM OF THE STUDY

To test the efficacy of *Moringa* Leaf Powder in improving the serum vitamin A level of children in the complementary feeding age.

1.5 SPECIFIC OBJECTIVES

1. To compare the serum retinol level of the children pre-and post-intervention using the i-check fluoro device.
2. To assess the energy and selected nutrient intakes of infants pre-and post-intervention.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction to Undernutrition

Undernutrition indicates inadequate intake of energy and nutrients to fit an individual's needs to sustain good health (Maleta, 2006). Undernutrition encompasses underweight, stunting, wasting and deficiency in vitamins and minerals (UNICEF, 2006). Micronutrient undernutrition is particularly of greater concern as its deleterious effects pervade the biological, physical, socioeconomic and cultural aspects of humanity (WHO, 2000).

Micronutrients are extremely important constituent of a high-quality diet and have a very great effect on health. Even though they are needed in minuscule amounts, they are important in the building of healthy brains, bones and body. Deficiency of micronutrients are often regarded as hidden hunger since they develop gradually overtime and their destructive effect is not seen until the damage has been done. This has led to many children suffering from stunted growth, cognitive delays, weakened immunity and diseases (UNICEF, 2015). Micronutrient malnutrition can occur even if the person is getting enough energy (UNHCR, 2003).

According to Black et al (2008), micronutrient deficiencies are now recognized as an important contributor to the global burden of disease and it is a problem as its effects are irreversible (Barret & Bevis, 2013).

The Ghana Demographic and Health Survey (2014) states that, in Ghana, the predominant levels of micronutrient deficiency levels related to anemia, vitamin A, and iodine are

considered high and of major public health significance by World Health Organization standards.

Vitamin A is of great relevance to human existence as this is noticeable through the consequences of its deficiency (Kapil and Bhavna, 2002).

2.2 VITAMIN A IN HUMAN NUTRITION

Vitamin A is a fat-soluble vitamin that is intrinsic in many foods (NIH, 2016). It is also a generic term for many other related compounds. The various forms of vitamin A are, “Provitamin A: β -carotene, Vitamin A1: Retinol (Vitamin A alcohol), Vitamin A2: 3-Dehydro-retinol, Vitamin A Aldehyde: Retinal, Vitamin A Acid: Retinoic acid, Vitamin A Ester: Retinyl ester, and Neo Vitamin A: Stereoisomer of Vitamin A1 which has 70–80% of biological activity of Vitamin A1”. Retinol and retinal are usually called preformed vitamin A (NIH, 2016 Bennisir et al, 2010). In plants, there is a group of red and yellow pigments known as carotenoids, some of which can be converted to vitamin A. In their unconverted state, they have no vitamin activity and the most important is the red pigment β -carotene. It is very abundant in carrots and occurs also in many other plants’ (Wiseman, 2004). Pre-formed vitamin A (retinol) or vitamin A precursors (carotenoids) must be obtained from the diet of an individual (NIH, 2006).

Vitamin A is insoluble in water, but soluble in ethanol (alcohol) and freely soluble in organic solvents including fats and oils. In solution, retinoids and carotenoids can experience slow conversion by light, heat, and iodine through cis–trans isomerism of the side-chain double bonds (Combs Jr., 2008)

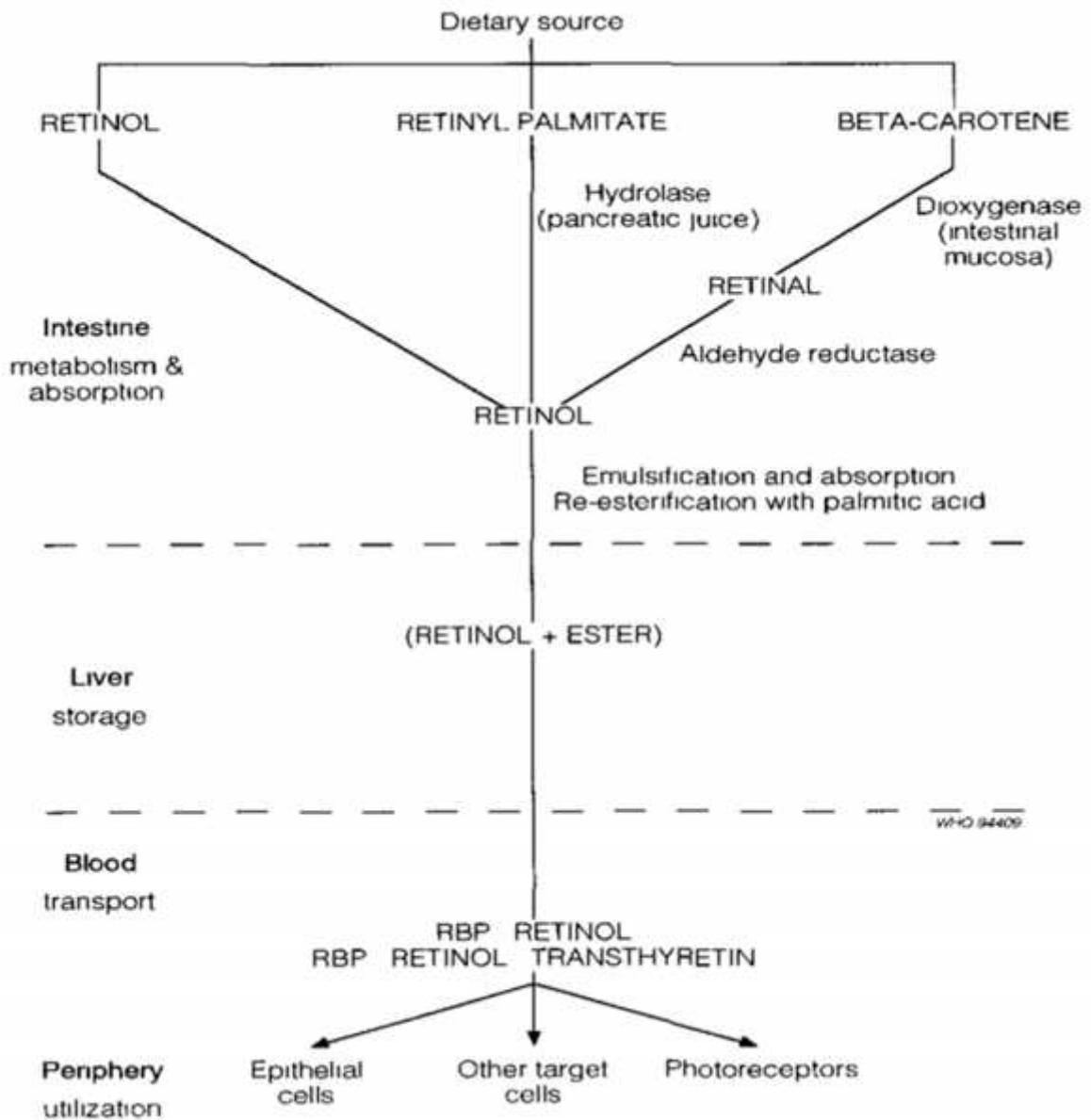
In nutrition, the primary unit of biologic activity for vitamin A is 1 μg of all trans retinol. To show both preformed vitamin A and provitamin A carotenoids in foods as a lone nutritive

value, the retinol equivalent (RE) was formed. One μg RE is equal to 1 μg of all-trans retinol, to 6 μg of all-trans 13-carotene, or to 12 μg of other provitamin A carotenoids in foods. However, the bioavailability of carotenoids changes greatly, based on their physical state in foods (Olson, 1964)

The recommended dietary allowance (RDA) and upper level (UL) values for vitamin A for infants 0–12 months are 400 $\mu\text{g}/\text{day}$ and 600 $\mu\text{g}/\text{day}$ respectively. The upper limit for children 1–3 years 300 $\mu\text{g}/\text{day}$ (RDA) and 600 $\mu\text{g}/\text{day}$ (UL) and for children 4-8 years, the RDA is 400 $\mu\text{g}/\text{day}$ and UL is 900 $\mu\text{g}/\text{day}$ (Ross & Harrison, 2007).

2.2.1 Vitamin A Metabolism

Figure 2.1: Schematic diagram of vitamin A metabolism



Source: Sommer, 1995

2.2.2 Functions of Vitamin A

Vitamin A is needed for the regular functioning of the visual system, preservation of cell function, for growth, epithelial integrity, production of red blood cells, immunity, reproduction, for the linings of the lungs, intestine and urinary tract, and bone (Mayor-Wilson et al, 2011)

The function of Vitamin A according to WHO & FAO (2004) in the body is in two folds. Vitamin A's first functions is in the visual cycle in the retina of the eye and secondly in all body tissues where it functions systemically at maintaining the increase and soundness of cells. Therefore, vitamin A deficiency affects epithelial cells differentiation and growth throughout the body.

Vitamin A functions in the eye when retinol is transported to the retina through circulation and accumulates in retinal pigment epithelial cells. Retinol is esterified to form a retinyl ester, which can be stored. When the retinyl esters are needed, they are broken apart and isomerized. 11-cisretinol is formed, which can be oxidized to form 11-cis-retinal. The latter can be moved across the interphotoreceptor matrix to the rod cell to bind to a protein called opsin to form the visual pigment, rhodopsin (visual purple). The function of vitamin A in vision is based on this. Rod cells with rhodopsin are able detect very small amounts of light, which makes them important for night vision. Therefore, inadequate retinol accessible to the retina results in impaired dark adaptation, known as night blindness (Bennasir et al, 2010 & Ball, 2004)

Vitamin A provides immunity for the body by functioning as an anti-infective vitamin, and is needed for the usual functioning of the immune system. Retinol and its metabolites are needed to keep the integrity and work of the skin and mucosal cells as they work as a wall and serves as the initial defense of the body against infection (Semba, 2001).

Retinoic acid works in limb development and formation of the heart, eyes, and ears and it has also been found to control the gene for growth hormone during embryonic development. However, both an excess and deficiency in vitamin A can cause birth defects. Furthermore, stem cells, which are precursor cells from which red blood cells like all blood cells are derived are dependent on retinoids for normal differentiation into red blood cells. Vitamin A also eases the movement of iron from storage sites to the developing red blood cell for incorporation into hemoglobin, which is the oxygen carrier in red blood cells (Bennasir et al, 2010).

2.2.3 Dietary Sources of Vitamin A

A nursing infant can meet his/her vitamin A requirement if the mother's diet has adequate vitamin A rich foods (Monte & Guigliani). During the time of complementary feeding, meeting micronutrient needs seems to be the biggest challenge. Good food sources of vitamin A include liver, leafy green vegetables, milk, eggs, cheese and some orange or red fruits and the amounts required for infants 6-11 months is 1-50g/d because breast milk is a rich source of vitamin A. However, in areas where VAD is endemic, greater intakes of complementary foods rich in vitamin A is advisable (WHO, 1998).

Also, tomatoes, carrots, spinach, papayas, mangoes, corn, and sweet potatoes are good sources of vitamin A. Beta-carotene and other carotenoids are found in green leafy vegetables (Biscontini, 2007). Since the liver is the organ that stores the vitamins in the body, the liver of meat animals is a rich source of vitamin A (Ball, 2004). Foods that have been processed which may include sugar, cereals, condiments, fats, and oils are fortified with preformed vitamin A. Red palm oil is especially rich in provitamin A and some native plants may be uniquely rich sources of provitamin A. Foods having provitamin A carotenoids tend to have little biologically available vitamin A but are cheaper than animal products. This is why

carotenoids supply a lot of the vitamin A action in the diets of economically disadvantaged populations (WHO & FAO, 2004).

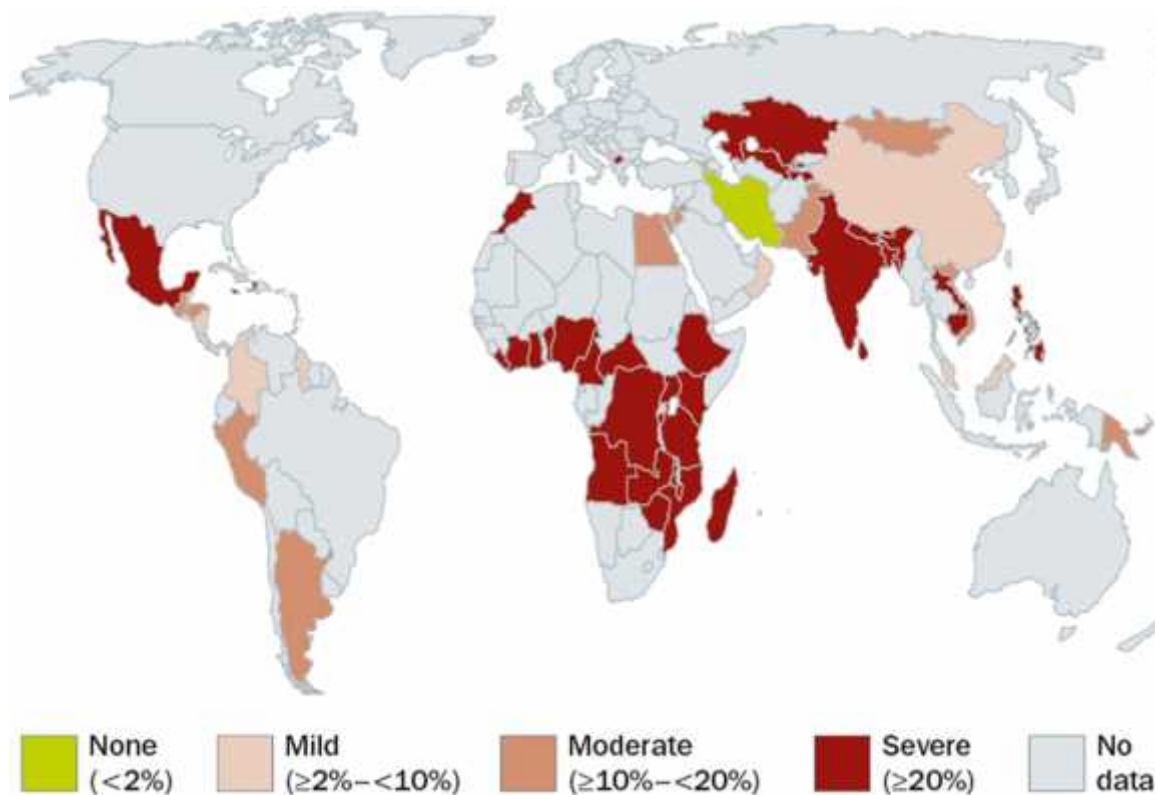
2.3 PREVALENCE OF VITAMIN A DEFICIENCY (VAD)

Vitamin A deficiency is a systemic disease that affects cells and organs throughout the body (Sommer, 1995). VAD has been “classified by the World Health Organization as a severe public health problem among Ghanaian children ages 6-59 months”. Inadequate intake of either pre-formed vitamin A or vitamin A precursors results in VAD (WHO, 2012). When very less vitamin A is present in the food for an extended period of time, vitamin A deficiency (VAD) occurs. Since vitamin A is fat soluble, excess of it can be deposited in the liver. Therefore, VAD will come about when the stores of Vitamin A in the body are depleted and not directly when there is no Vitamin A in the food but when the storage in the body has been depleted (FAO/WHO 2002). Since Vitamin A is needed in the retina of the eyes for acclimatization to darkness, a deficiency will lead to night blindness. Also, since vitamin A is essential in all body tissues to sustain growth and health of cells, VAD will affect the epithelial cells which will lead to the debilitation of the immune system and to irreparable blindness due to eye injury (Lorch, 2005).

Globally, 21% of children have vitamin A deficiency and suffer high rates of death from diarrhea, measles, and malaria. About 800,000 deaths in children and women of reproductive age are attributable to VAD which accounts for 1.8% of the global burden of disease (Black, 2003). VAD also increases the severity of infections such as measles and diarrhea disease in children and slows recovery from illness (GDHS, 2014). “Between 1997 and 2001 the number of child deaths in Ghana due to VAD will total 49,000 and has an immense impact on morbidity levels. VAD accounts for close to 5% of clinic attendances and 18% of hospital

admissions of pre-school children”. Thus, improving nutrition would lead to significant savings for the country and will have a huge impact on reducing morbidity, particularly in children under five years (Academy for Educational Development, 2003).

Figure 2.2: Global extent of VAD as defined by prevalence of serum retinol $<0.70\mu\text{mol/l}$ in preschool children



Source: WHO, (2009). Global Prevalence of vitamin A in populations at risk 1995 – 2005

2.3.1 Causes of VAD

The type and quantity of vitamin and provitamin (primarily β -carotene) eaten, and the absorptive, transport, and storage volume and metabolic requirements of the individual are

used in determining the cause of vitamin A deficiency although its cause is quite complex (Sommer, 1994).

Populations that take in a lot of their vitamin A needs from provitamin carotenoid sources and has the lowest dietary fat available usually have VAD being common. Close to 90% of ingested preformed vitamin A only is absorbed. However, the efficiency of absorption of provitamin A carotenoids varies widely, and is dependent on the type of plant source the amount of fat in the meal its eaten with. Foods in season can also influence the prevalence of VAD by influencing access to provitamin A sources. Food practices and taboos usually prevent the intake of possible good food sources of vitamin A. This is a common factor in the cultural practices in and for feeding children, adolescents, and pregnant and lactating women, which can be a contributing factor to VAD (WHO & FAO, 2004).

Another major factor that contributes to VAD among children in developing countries is infection. Diarrhea and helminths infestation for instance can undermine the integrity, morphology and the performance of the intestinal absorptive mucosa all of which may result in malabsorption of vitamin A (Stephensen, 2001).

2.3.2 Consequences of VAD

Blindness and an increased risk of death due to VAD are two of the most well-known adverse outcomes closely connected with vitamin A deficiency in preschool-age children (Sommer 1982; Sommer and West 1996). According to Gilbert and Foster (2001) recent estimates shows that vitamin A deficiency leading to corneal scarring remains one of the most common of childhood blindness in developing countries that can be preventable.

VAD can contribute directly and indirectly to death and appears to influence morbidity patterns especially in developing countries. Adverse health effects associated with VAD include blindness, which can be partial or total blindness (keratomalacia) and occurs when

VAD is prolonged and leads to ulcerations. About 190 million children under five (33.3% of the preschool age population) are estimated to be vitamin A deficient, with about 5.2 million affected by night blindness by WHO (2009). Other adverse health effects of VAD are impaired cognitive function; impaired physical work capacity; morbidity (incidence and/or severity) due to diarrhoea, measles, acute respiratory infections, malaria and other infectious diseases; cause-specific mortality related to these diseases; and all-cause mortality. In pregnant women, likely outcomes of VAD are associated with fetal loss, low birth weight, preterm birth, all-cause infant mortality, maternal morbidity and maternal mortality (Rice et al, 2004, WHO 1995, 2009)

2.3.3 Combatting VAD

Supplementation with regular administration of pharmacological preparations of vitamin A in capsules to groups at risk, mainly to young children and food based approaches in combating VAD are the basic strategies that have been designed (Lorch, 2005). Although food-based approaches may be thought of as competitive or distracting, the supplementation approach has failed to enhance vitamin A status in the young child. Unlike supplementation, the food based approach reaches every member of the community and its safe, sustainable and with a wide range of benefits than just improving vitamin A status. Furthermore, a wide variety of indigenous foods have been validated to improve vitamin A status in short term trials (Greiner, 2013).

Borlaug (2007) argues that low-income populations usually have monotonous diets with most of the energy coming from starchy staple foods. Therefore, variations in diet can then be achieved through promoting increased production and consumption of a range of nutritious foods, many of which will lead to improving vitamin A status. Additionally, most of the foods used in food-based approaches to combat VAD are perishable and thus grown locally

in the low-income countries where malnutrition exists. Most of the people suffering from hunger and living in farming or pastoral households in Africa and Asia thus could be double beneficiaries and will not need to purchase manufactured nutrients as it benefits only a few large companies. Self-sufficiency and food security are advances in food based approaches unlike other approaches.

2.4 MEASURING VITAMIN A STATUS

Current methods available for measuring vitamin A status are biological, functional and histological methods and biochemical methods. Xerophthalmia, night blindness, conjunctival impression cytology, and dark adaptometry form part of the biological, functional and histological methods. Whereas biochemically, serum retinol concentrations, breast milk retinol concentrations, relative dose response and modified relative dose response tests, deuterated retinol dilution assay either using the 20-d equilibration time or the shortened 3-d relationship are used. Xerophthalmia classification used to be the traditional method by which populations were classified as having VAD. Night blindness and dark adaptometry are currently suggested as population assessment methods. In areas where the signs of VAD is not severe enough, eye signs and function tests are still used. Whereas in populations that are at risk of vitamin A deficiency, serum and breast milk retinol concentrations are used (Tanumihardjo, 2004).

Biochemically two indicators are currently recommended for deciding whether VAD is a public health issue are serum retinol and serum retinol-binding protein (RBP). Since the body's stores of vitamin A are reduced, serum retinol concentration mirrors an individual's vitamin A status. This is because serum retinol concentration is homeostatically controlled and will not drop until body stores are significantly compromised. Also, liver stores of

vitamin A when extremely depleted or very high are reflected by serum retinol levels. Therefore, in children serum retinol values are most often measured. High-performance liquid chromatography (HPLC) is the method suggested for the measurement of low serum retinol concentrations because the measurement requires high precision (de Pee & Dary, 2002, & WHO, 2011).

Therefore, vitamin A deficiency among preschool-age children, is defined in relation to low serum retinol concentrations among children in the 0–4-year age. A child is vitamin A deficient when he/she has serum retinol concentration $<0.70\text{mmol/l}$ and is vitamin A sufficient when he/she has serum retinol concentration $\geq 0.70\text{mmol/l}$ (Rice et al, 2004)

Aglago et al, (2015), reiterates that at the population level, serum retinol is the most commonly recommended indicator to test vitamin A status and the most widely used method for measuring retinol concentrations is high-performance liquid chromatography (HPLC). However, simple and rapid test kits such as ICHECK Fluoro ® are obtainable to assess vitamin A status during and after nutritional interventions.

The ICHECK Fluoro ® is a new kit that can be used for the analysis of vitamin A concentrations in both fortified foods and biological fluids (Schweigert, 2011). The ICHECK Fluoro ® kit uses a mobile analyzing module and seems reliable and affordable as compared to laboratory-based methods, especially in developing countries. “Analysis by ICHECK includes three major steps: injection of the sample into a reagent solution vial (IEX Mila ®), separation by vigorous manual mixing, and measurement in the portable device chamber”. Furthermore, a research by Aglago et al, (2015) to compared the ICHECK Fluoro kit to High-performance liquid chromatography in assessing serum retinol showed that ICHECK measurement provided good correlations with HPLC for serum concentrations and that it may

offer dependable mean for testing serum retinol measurements done with no significant time delay.

2.5 COMPLEMENTARY FEEDING IN THE DEVELOPING WORLD

Complementary feeding is the process that starts when breast milk alone is no longer enough to provide the nutritional requirements of infants, and therefore other foods and liquids are needed including the breast milk (WHO, 2016). The advantages of optimal complementary feeding (timely, adequate, appropriate and safe) includes little risk of anemia, the child is less likely to die, less diarrhea and respiratory infections, averting overweight/obesity, child gets optimal growth, less risk of micronutrient deficiencies, prevention of stunting and acute malnutrition, cognitive development is improved, and better psychosocial development (UNICEF, 2012)

A WHO report in 2001 on complementary feeding stated that, infants enter a vulnerable period when they are transitioning gradually to family meals when from six months onwards the breast milk alone is not enough to make up all their nutritional needs. Therefore, “foods for complementary feeding need to be more nutrient-rich than the foods typically consumed by other age groups in the household” (Dewey & Vitta, 2013).

Sufficient nutrition throughout infancy and early childhood is basic to the growth of each child’s complete human potential (Dewey, 2001). Consuming different range of nutrient-dense foods in addition to breast milk is the best way for young children to get necessary micronutrients in their diets. However, in many parts of the world, children’s diet does not contain enough micronutrients and deficiencies have thus become widespread as the nutrient demands of young children increase and this puts them in the at-risk population of micronutrient deficiencies (UNICEF, 2015 and Das et al, 2013).

A good complementary food should be rich in energy, protein and micronutrients (like vitamin A) and should be available and affordable (WHO, 2009). Complementary foods in developing countries are adequate in energy and protein but deficient in micronutrients such as iron and vitamin A. For instance, the traditional complementary food that is usually fed to infants in Ghana, is fermented maize porridge, which has low energy is not nutrient dense (Lartey et al, 1999). Furthermore, a comparison of actual nutrient densities of complementary foods with the target nutrient densities by Dawey (2013), showed that protein was generally adequate but several micronutrients were not.

Daelmens et al (2009) suggests that effective methods of improving the quality of complementary foods include increasing diet diversity and using fortified food products.

2.6 THE MORINGA OLEIFERA PLANT

There are about 13 species of moringa trees in the family Moringaceae and *Moringa Oleifera* is the most best known and widely distributed (Anwar et al, 2006). The leaves, fruit, flowers and immature pods of the *Moringa Oleifera* are edible and are considered as immensely nutritive in many countries (Valdez-Solana, 2015; Anwar and Bhangar, 2003; Anwar et al., 2005). The pods are eaten as green beans and valuable in treating digestive problems and in preventing colon cancer. The flowers are used for teas or eaten and the seeds are eaten as peas when still green and when seeds mature, oil is extracted from it (Price, 2000 & Gopalakrishnan et al, 2016).

2.6.1 Nutritional potential of *Moringa Oleifera* leaves in combatting VAD

Moringa leaves in particular have been reported to be a “rich source of β -carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants; and thus, enhance the shelf-life of fat containing foods due to the presence of various types of

antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids” (Dillard and German, 2000; Siddhuraju and Becker, 2003).

Vitamin A content of *Moringa oleifera* leaves reported by Babu (2000) was “3767 IU per 100 g edible portion” and from a publication by Kuhnlein (2003) in Niger also reported *Moringa* as containing “5880 µg beta-carotene per 100 g edible portion”.

Table 2.2: Specific nutrient content in mcg per 100g of selected foods

Nutrients	Common foods, mcg	Fresh leaves, mcg	Dried leaves, mcg
Vitamin A	Carrots 1.8	6.8	18.9
Calcium	Milk 120	440	2003
Potassium	Bananas 88	259	1324
Protein	Yoghurt 3.1	6.7	27.1
Vitamin C	Orange 30	220	17.3

Source: *Moringa Oleifera* (www.info@dolcas-biotech.com, 2008).

A research by Abioye, (2015) revealed that fortification of yellow maize ogi (maize gruel) with *Moringa Oleifera* leaf powder was possible, as proximate analysis showed an increase in nutritional content and it thus enhanced the nutritional status of the populace especially the children and adult who consume this meal for breakfast.

Kouevi (2013), reports that the mean value of beta-carotene found in *Moringa* leaf powder is 17.6mg per100g with a Vitamin A content of 3.15mg per100g. This shows that 15g of *Moringa* leaf powder will contains 0.47mg of Vitamin A and this amount gives almost 80% of the daily requirement for an infant of the age 6 to 12 months.

Another research in Burkina Faso to test the impact of *Moringa Oleifera* leaf powder in enhancing the nutritional status of the vulnerable population, including children, revealed that Moringa can be used as supplement to fight malnutrition (Zongo et al, 2013)

Furthermore, another research done in Malawi on the introduction of *Moringa* to combat vitamin A deficiency in the country showed that Moringa was superior in providing vitamin A as compared to all commonly eaten vegetables in Malawi and that it is useful to use indigenous foods in designing interventions as its provides a more sustainable approach and also improves the nutritional status of the population (Babu, 2004).

In Chad, the fortification of local infant flours with *Moringa oleifera* leave powder and pulps of *Parkia biglobosa* showed an increase in all minerals, vitamins, proteins and carbohydrates with the increase in vitamin A and calcium being the strongest. The study concluded that *Moringa oleifera* can be used to fight used to fight malnutrition and fortifying complementary foods with local plant with rich sources of protein and micronutrients can effectively combat malnutrition due to micronutrient deficiency (Kayalto et al 2016)

2.7 PILOT STUDIES

A pilot study is a small version of a large study which is also known as a feasibility study, although there are suggestions that pilot studies are different from feasibility studies. Pilot studies are also used in testing research instruments like questionnaires. To have a good study design, pilot studies are very significant. Pilot studies increases the possibility of having a good study design although it may not be the reason for having a successful study (van Teijlingen & Hundley, 2001). Pilot studies can be conducted for both quantitative and qualitative studies (Thanbane et al, 2010).

Conducting a pilot study before a main study gives the researcher, an opportunity to properly train his/her research team for the larger study (Leon et al, 2011). Another advantage of a pilot study is that it allows for the primary testing of a hypothesis which leads to testing accurate and exact hypothesis in the main study. This may cause a hypothesis to be changed, a new one may be developed or some may be dropped. Pilot studies allows a researcher to get some thoughts, clues and ways of getting clearer results in the larger study. Pilot studies gives the researcher the permission to thoroughly check how to analyze data from the study and how the data analyzed will be useful. This will inform the researcher on a better way to collect data for efficient analysis in the main work. Furthermore, pilot studies can reduce unforeseen problems and help redesign and overcome any difficult area revealed. It also saves time and money and it allows a researcher to have enough data to decide whether to continue with the main study or not. Finally, a pilot study gives the researcher the chance to try out other measures and the choose the one that will give the clearest results in the main study (Woken, 2013).

Problems that may arise from pilot studies include, the possibility of making inaccurate assumptions and predictions based on the data from the pilot study. Also, there may be problems with contamination. In this case, the data from the pilot study is added to the main study of the participants in the pilot study are added to the participants in the main study (van Teijlingen & Hundley, 2001)

Given the benefits of pilot studies, this study was designed to test the efficacy of *Moringa* Leaf Powder in improving the serum vitamin A level of children in the complementary feeding age.

CHAPTER THREE

3.0 METHODS

3.1 Study Design

A pilot study was coined out

3.2 Sample size

A sample size of 35 infants per study arm was conveniently chosen for the purpose of this pilot study. This is because, according to the Central Limit Theorem, the rule of thumb in selecting a sample size is $n \geq 30$, which is approximately normally distributed, regardless of the population being sampled from (Sullivan, 2016). An attrition rate of 15% was used, giving a sample size of 35 infants per study arm.

3.3 Study Population

The study population included infants under two years of age attending weight monitoring sessions at the Child Welfare Clinics in the Upper Manya Krobo District. According to the District Health Directorate (2012) of the district, agriculture is the main economic activity of the people of the district, employing about 80% of the population, most of whom are subsistence farmers with very few commercial ones.

3.3.1 Inclusion Criteria

Infants who were aged 8 to 12 months, at the time of the study; were still being breastfed; had no congenital abnormalities and whose mothers planned to stay at the study site for the duration of the study were included in the study.

3.3.2 Exclusion Criteria

Infants who were known to have any known intolerances to any of the ingredients of the study foods, were excluded from the study.

3.5 Study Procedure

Study participants were screened for eligibility. For those who met the inclusion criteria, written informed consent was obtained from parents and/or caregivers, after which infants were randomly assigned to receive one of the 3 study foods (*CF-35g*, *MCL-35g* or *MS-5g*) depending on the CWC they were recruited from. One arm received a cereal-legume blend fortified with (MLP) named *MCL-35g*, a second arm received MLP as a supplement to be sprinkled on infant's usual foods (*MS-5g*) and a third arm received a cereal legume blend without MLP (*CF-35g*) to serve as controls.

Mothers and caregivers of recruited infants were visited at home by trained field assistants to verify eligibility, to explain the study protocol in detail, and to obtain written informed consent. Study foods were delivered to the infants twice a week. Cooking demonstrations were carried out to teach mothers how to prepare study foods. Mothers were instructed to feed foods 2-3 times daily, 7 days a week. The feeding intervention lasted for 6 weeks. During the period, each infant received a total of 4 follow-up home visits.

3.6 Preparation of Study Foods

The preparation and packaging of both *MCL-35g* and *MS-5g* were carried out at the Nutrition Unit of the Food Research Institute of the Centre for Scientific and Industrial Research (CSIR-FRI), Ghana. *MCL-35g*, (maize 60%, soybean 25% and MLP 15%), was formulated based on the cereal-legume ratios used for the preparation of Weanimix TM (Lartey et al., 1999), with some of the ingredients being partly substituted with 15% of *Moringa oleifera* leaf powder as a fortificant. *MCL-35g* was packaged in 35 g sachets, with each sachet containing 5 g of MLP. The energy, protein, iron and vitamin A composition of *MCL-35g* and *MS-5g* were determined. *MS-5g* (*Moringa oleifera* leaf powder) was packaged in 5 g sachets and sprinkled on infants' usual foods. The 5-g daily supply of MLP added 1.13 mg of iron and 24.8 µg/RE of vitamin A to infants' complementary diets. These levels translated into 10% and 7%, respectively, of the Recommended Nutrient Intakes (RNIs) for iron and vitamin A for infants aged 6 – 23 months according to World Health Organization. (1998). Due to concerns about acceptability of foods that are fortified with higher levels of MLP, the 5 g daily dose of MLP incorporated in infants' complementary foods, was chosen. (Oyeyinka & Oyeyinka, 2016).

Table 3.1: Nutrient content of study foods

	<i>MCL-35g</i>	<i>MS-5g</i>	<i>CF-35g</i>
Daily ration g/day	35	5	35
Energy (kcal)	144.64	10.25	142.15
Protein (g/100g)	6.40	1.38	5.88
Iron (mg/100g)	3.78	1.13	2.53
Vitamin A (μ g RE/100g)	51.95	24.82	0.27

3.7 DATA COLLECTION

3.7.1 Baseline Data

Following the recruitment of study participants into the study, the following baseline data were collected;

Infant age, birth order; age of mother/caregiver, educational level of mother, total number of members in household, main household water source

3.7.2 Anthropometry

Measurements of infant weight and length and maternal weight and height were taken. Infant weight and length measurements were taken in accordance with World Health Organization guidelines (WHO, 2016). Infants were weighed naked on a digital infant scale (model 1583, Tanita, Quick Medical, Snoqualmie, WA) to the nearest 100 g. Recumbent length was measured with an infantometer (model 447, Infantronic Digital Infantometer; Quick Medical) to the nearest 0.1 cm. Two trained field assistants were responsible for taking measurements - whilst one ensured that the top of the infant's head gently touched the head board, the other ensured that the child's legs were fully extended before taking the reading to ensure accurate results.

3.7.3 *Child morbidity occurrence*

Information on diarrhea, defined as 3 or more watery stools per day (WHO, 2017) symptoms of respiratory infections cough, (tachypnea/dyspnea, lower chest wall in-drawing and fever) in the past 1 month was collected by care giver interview at baseline and during the bi-weekly visits. Further questions regarding symptoms (fever, chills, loss of appetite) suggestive of malaria was asked.

3.7.4 *Biochemical data*

1 ml of blood was drawn from each infant by a qualified phlebotomist. The blood was stored in EDTA bottles (to prevent coagulation of blood samples) wrapped with aluminium foil and stored in an ice chest, and taken to the Asesewa Government Hospital laboratory where the icheck device was set up. The aluminium foil was to prevent sunlight (UV light) from coming into contact with the blood.

Vitamin A analysis - In the laboratory, 0.5 ml of blood was drawn from the EDTA bottle and injected into a reagent vial. The reagent vial was then shaken vigorously for 10 seconds and allowed to stand for 5 minutes after which the reading was taken with the icheck fluoro device manufactured by Bioanalyt (GERMANY). The i-check analysis was done the same day as the blood samples were taken.

Hemoglobin analysis - Another 0.5ml of blood was drawn from the EDTA bottles and hemoglobin concentration was measured using an automated haematology analyser by Sysmex KX – 21 N (KOBE, JAPAN)

3.7.6 *Dietary data collection*

Two-day 24-hour dietary recalls of all foods and drinks consumed by infants were collected from mothers or caregivers by trained research assistants at baseline. Containers of common baby foods, and household utensils (e.g. bowls, cups, and spoons) were used to estimate amounts consumed. Additional questions to prompt mothers/primary caregivers about easily forgotten foods, such as snacks and drinks and special dietary issues (e.g. foods that child does not eat or purportedly reacts to) were also asked.

3.7.7 Intervention

For infants who received *MCL-35g* and *CF-35g*, each mother was given two 250 g sachets (placed in a clean transparent plastic container with a firmly closing lid) for use within a 2-week period. Approximately 35g of the flours, equivalent to 2 heaped tablespoons was used to prepare meals for infants each day. An extra 10g package of food was added to the plastic container for each child just in case other individuals within or outside the home will want to taste the food. For the third study arm, fourteen 5g sachets of *MS-5g* (*Moringa Oleifera* leaf powder) was supplied to infants in their homes every two weeks. One sachet (5g) was sprinkled on child's usual complementary food everyday whilst the food was very hot or freshly taken off the fire. Mothers were given plastic bowls with spoons to feed their infants and a cake of soap to wash their hands before preparation of the foods. Mothers were taught how to prepare porridge from *the MCL-35g* flour as well as how to use the flour to prepare other local meals such as 'banku/akple', 'aprapransa' and 'kakro'. Trained field workers demonstrated the preparation of the recipes. Mothers were instructed to feed the study foods 2-3 times/day to infants. Each mother was instructed to continue breastfeeding as well as feeding their infants with other complementary foods aside the study foods. All study foods were supplied to infants without charge. Each mother/infant pair were paid a total of 4 visits (biweekly) by a field worker over the 6 weeks feeding duration. The purpose of the visit was

to monitor how much food the infants were eating, to obtain mothers' feedback on their experiences with the use of the food as well as to monitor infant morbidity using a questionnaire.

3.7.8 Outcome measures

Serum vitamin A level was the primary outcome measure of the study after 6 weeks of feeding with complementary foods that incorporated MLP. Serum vitamin A measurements were taken at baseline and 6th week. Morbidity occurrence, dietary intake and adherence to study foods were secondary outcome measures. A record of infant's dietary intake (both study and non-study foods) were collected by 24-hour recalls for 2 days (1 weekday and 1 weekend day), at baseline and at 6th week. Morbidity data (diarrhoea, symptoms of respiratory infections, and fever) was collected at baseline and then every 2 weeks, during the time of the home visits when project foods were delivered to the mothers/caregivers. Adherence to study foods was determined as the amount of study food that disappeared over the study duration. Study foods that were left at the time of the biweekly visits were weighed and added together over the study duration and expressed as a percentage of the total study foods given to each group.

3.8 DATA ANALYSIS

Data collected was analyzed using IBM Statistical Package for Social Sciences (SPSS) version 20 (IBM, USA). Analysis of covariance was used to determine any significant differences between the 3 study groups in blood retinol concentrations at end line. Paired t-test was used to examine within group differences in blood retinol concentrations from baseline to endline.

Dietary intakes collected from the two day 24-hour recall were converted to energy and nutrients using a food database (RIING food composition database, Nutrition Department, University of Ghana) and ESHA-F-pro software.

3.9 ETHICAL CLEARANCE

Approval for the study was obtained from the Institutional Review Board of the Noguchi Memorial Institute for Medical Research, and the Ghana Health Service Ethical Review Committee. Written permission to carry out the study in the Upper Manya Krobo District was obtained from the District Health Administration of the Ghana Health Service (GHS).

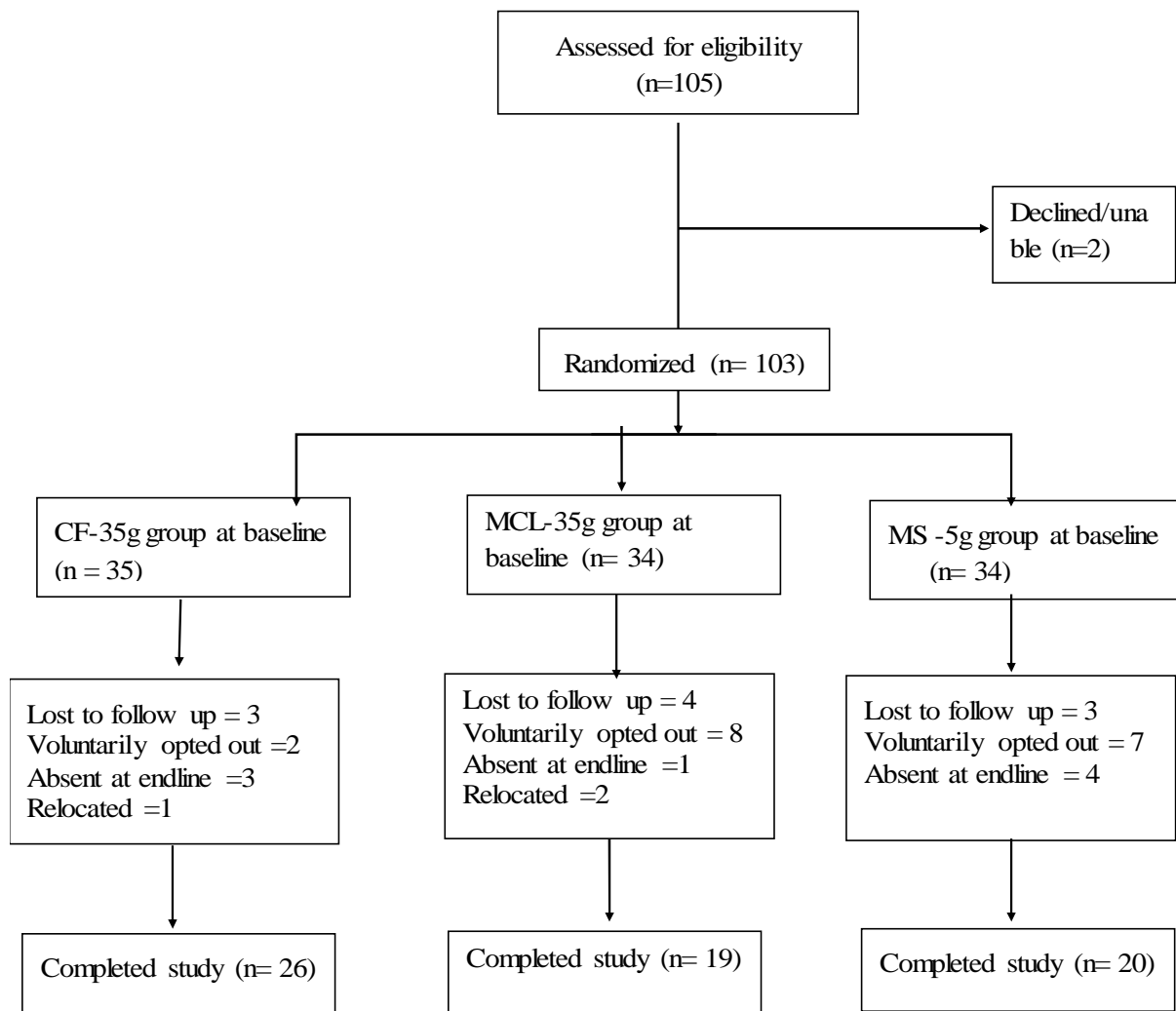
Informed consent was obtained from the parents/caregivers of each infant using a written form (translated into Krobo, the native language of the study area) that described the study and consent process. Information obtained from the participants was kept strictly confidential.

CHAPTER FOUR

4.0 RESULTS

4.1 FLOW OF INFANTS THROUGH THE STUDY

Figure 2 is a flow diagram that displays the progress of all participants through the study. A total of 105 infant-mother pairs were conveniently chosen and assessed for eligibility. Two of them declined to being part of the study. One hundred and three infant-mother pairs were then randomly placed in one of the three study arms. At baseline, there were 35 infant-mother pairs for the *CF-35g* group, 34 in the *MCL-35g* group and 34 in the *MS-5g* group. During the study, some of the study participants were lost to follow up, some voluntarily opted out, others were absent at endline while others relocated from the study area. Therefore, at endline, 26 infant-mother pairs completed the study in the *CF-35g* group, 19 in the *MCL-35g* group and 20 in the *MS-5g* group. A total of 65 infant- mother pairs completed the study.



CF-35g- control, MCL-35g – Moringa with Weanimix, MS-5g – Moringa Sprinkled

Figure 4.1: Flow of infants through the study

4.2 BACKGROUND INFORMATION OF STUDY PARTICIPANTS AT BASELINE

Table 4.1 represents the background information of infants, mothers/caregivers and on the household.

Majority (62.9% for *CF-35g*, 58.8 for *MCL-35g* and 73.5% for *MS-5g*) of the infants in the study were males. The mean age of infants in all the groups were 9.0 ± 1.4 and a mean height of 68.20 ± 5.69 cm. The mean weight of infants in the *CF-35g* and the *MCL-35g* group was 7.98 ± 1.07 kg and 7.87 ± 0.97 kg respectively and that of the *MS-5g* group was 8.89 ± 6.31 kg, being the highest.

Results on birth order showed that the mean birth order of infants in the study was averagely

3. The mean hemoglobin concentration of the infants for the three groups was 10g/dl.

The mean ages of the mothers of children in the three groups was between 25 and 27 years.

Results showed that majority of the mothers/caregivers had been educated up to the Junior Secondary School level (JHS). *MCL-35g* group had the highest number of mothers who had completed JHS.

The mean house hold size for the groups was about 6 for the experimental groups and the control group. Results on the household characteristics also revealed that the source of water used by majority of the participants was public tape, boreholes and wells with the rest being other water sources like streams, ponds and rivers.

For all three groups, participants reported to have had very few morbidity symptoms.

However, fever and nasal discharge were the highest morbidity symptom reported in the *MS-5g* group representing 50% of the participants for each symptom.

Table 4.1: Background characteristics of study participants at baseline

CATEGORIES	STUDY ARMS		
	<i>CF-35g</i>	<i>MCL-35g</i>	<i>MS-5g</i>
	(n=35)	(n=34)	(n=34)
Infant sex, Male (%)	62.9	58.8	73.5
Infant age (months) (Mean ± SD)	9.0±1.3	9.1±1.4	9.3±1.4
Infant Height (cm)	68.11±2.84	68.51±2.78	68.00±11.45
Infant weight (kg)	7.98±1.07	7.87±0.97	8.89±6.31
Hb concentration of infants (g/dl) (Mean± SD)	10.40 ± 1.13	10.36 ± 1.36	10.17±1.30
Birth order (Mean ±SD)	2.71±2.15	3.09 ±2.07	2.88±1.90
Mother's age (years) (Mean ±SD)	26.77±9.34	27.68±7.66	25.82±6.45
Mother's education (%) Primary - JHS	82.9	91.2	73.5
Number of people in household (Mean ±SD)	6.80±2.41	6.32±3.23	6.18±2.36
Use public water (%) (Public tap/borehole/well)	74.3	44.1	35.3
Vomiting	5.7	20.6	8.8
Diarrhoea	28.6	11.8	26.5
Cough	28.6	29.4	32.4
Nasal discharge	45.7	26.5	50
Fever	40	38.2	50

CF-35g- control, *MCL-35g* – *Moringa* with *Weanimix*, *MS-5g* – *Moringa* Sprinkled

4.3 SERUM VITAMIN A CONCENTRATION AT BASELINE AND END LINE

Table 4.2 represents the mean serum vitamin A concentrations of participants at baseline and at end line. At baseline, the *CF-3g* had the least mean serum vitamin A concentration (0.45 μ mol/l) of the three, with the *MS-5g* group having the highest (0.57 μ mol/l). At endline, the *CF-35g* group still had the least (0.55 μ mol/l) mean serum vitamin A concentration and *MS-5g* group also still had the highest (0.65 μ mol/l) serum vitamin A concentration.

There was an increase in serum vitamin A concentration for all three groups at end line. However, there was also no statistically significant difference between and within the three groups at baseline and at endline.

Table 4.2: Concentration of Vitamin A at baseline and end line

Group	N	Vitamin A	Mean \pmSD (μmol/l)
<i>CF -35g</i>	35	Base line	0.45 \pm 0.16
	26	End line	0.55 \pm 0.16
<i>MCL -35g</i>	34	Base line	0.52 \pm 0.18
	19	End line	0.63 \pm 0.38
<i>MS -5g</i>	34	Base line	0.57 \pm 0.20
	20	End line	0.65 \pm 0.20

CF-35g- control, *MCL-35g* – *Moringa* with *Weanimix*, *MS-5g* – *Moringa* Sprinkled

4.4 COMPARISON OF INTAKE OF ENERGY AND SELECTED NUTRIENTS INTAKES AT BASELINE AND ENDLINE

Table 4.3 presents the baseline and endline intakes of energy and selected nutrient by the study participants. Results are from a 2 day 24 hour recall taken at baseline and end line. The results of an analysis of variance test showed no significant difference among the three groups for energy and almost all of the selected nutrients at baseline and endline for all the days that the recalls were taken. However, a significant difference ($p = 0.026$) was seen among the three groups for their dietary iron intake at baseline.

Difference between vitamin a for mcl -35g although it was statistically significant $p=$

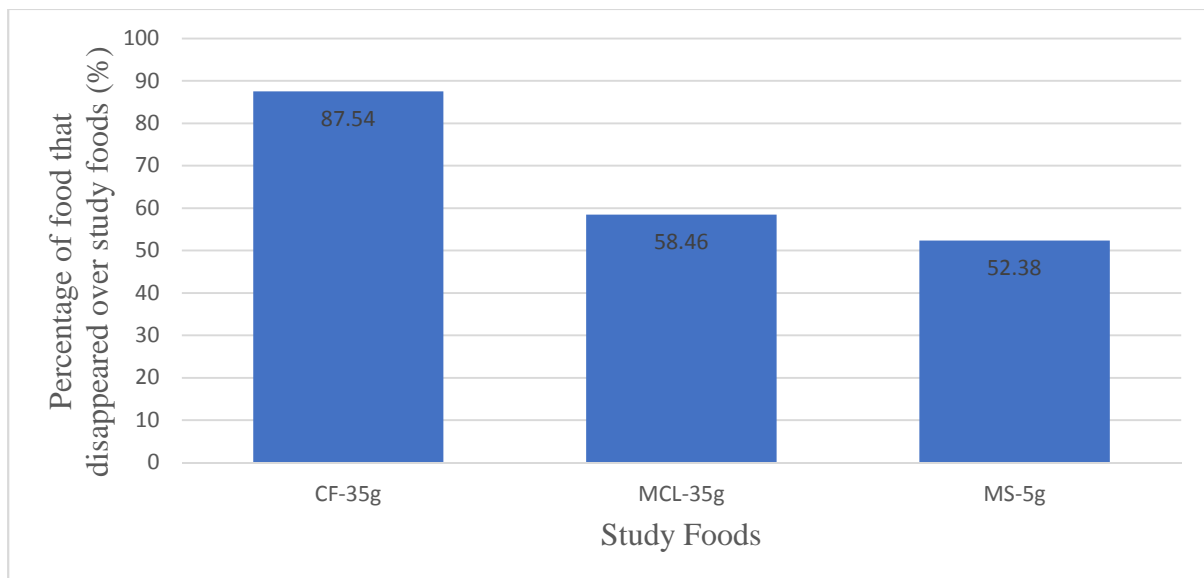
Table 4.3: Comparison of intake of energy and selected nutrients at baseline and endline

Energy and selected nutrients	CF-35g		MCL-35g		MS-5g	
	<i>Baseline n=35</i>	<i>Endline n=26</i>	<i>Baseline n= 34</i>	<i>Endline n= 19</i>	<i>Baseline n= 34</i>	<i>Endline n=20</i>
Energy (kcal)	356±244	570±324	426±249	554±221	448±253	598±399
Protein (g)	11.97±16.99	15.38±8.91	11.27±7.29	18.88±8.96	11.63±7.27	17.28±10.75
Carbohydrate (g)	73.71±111.32	94.38±56.67	73.81±36.71	82.76±32.45	70.74±42.52	95.48±71.99
Fat (g)	10.58±10.93	16.35±14.42	14.71±14.75	17.54±10.68	9.83±10.83	32.865±59.51
Iron (mg)	5.075±4.47*	8.43±5.82	8.78±4.56*	11.46±6.58	6.135±3.88*	9.145±7.36
Vitamin A (µg/RE)	280.87±635.73	642.38±944.29	703.035±809.99*	634.03±619.59*	632.33±715.66	896.98±770.70

CF-35g- control, *MCL-35g* – *Moringa* with *Weanimix*, *MS-5g* – *Moringa* Sprinkled; Values reported as mean and standard deviations

4.5 ADHERENCE TO STUDY FOODS

Figure 4.2 shows the disappearance of study foods over the study duration. The *CF-35g* had the highest disappearance rate (87.54%) with the *MS-5g* group having the least (52.38%). There was a significant difference ($p < 0.05$) between the *CF-35g* group and the *MCL-35g* group for adherence. However, there was no significant difference between the two experimental groups. This shows that infants in the *CF-35g* group which is the control group adhered more to the study foods than infants in the other group the experimental groups.



CF-35g- control, *MCL-35g* – *Moringa* with *Weanimix*, *MS-5g* – *Moringa* as Sprinkled,

$p < 0.05$

Figure 4.2: Adherence to study foods

4.6 MORBIDITY OCCURRENCE AT ENDLINE

Table 4 shows the occurrence of morbidity among the infants who participated in the study over the study duration. Generally, relatively low morbidity was reported by mother/caregivers over the study duration. The highest (50%) morbidity occurrence was seen in the *MCL -35g* group where half (n=16) of the infants experienced nasal discharge.

Table 4:4: Morbidity occurrence among infants at endline

Morbidity	<i>CF-35g</i> n=33	<i>MCL-35g</i> n= 32	<i>MS-5g</i> n= 29
Vomiting (%)	8.6	8.8	8.8
Diarrhoea (%)	22.9	8.8	26.5
Cough (%)	22.9	38.2	11.8
Nasal Discharge (%)	28.6	50	8.8
Fever (%)	28.6	44.1	32.4

CF-35g- control, *MCL-35g* – *Moringa* with *Weanimix*, *MS-5g* – *Moringa* as Sprinkles

CHAPTER FIVE

5.0 DISCUSSION

5.1 BACKGROUND CHARACTERISTICS OF STUDY PARTICIPANTS

5.1.1 Back ground Characteristic of Infants

A total of 103 mother and infant pairs took part in the study; however, 65 of them completed the study. Results showed no significant differences in the background characteristics of participants in the control group and the intervention groups. Background data collected showed that there were more males infants in each of the groups than females. Sommer (1995), on the causes of VAD, alluded to the fact that, boys have normally been found to be at higher risk of mild xerophthalmia (a consequence of VAD) than girls during the preschool and early school-age years; but there is less evidence in relation to gender and severe xerophthalmia. According to Darton-Hill (spelling) et al (2005) there is proof to insinuate that commonly, vitamin A deficiency is reported to be up to 10 times more regular in males than in females.

The average weight and length of infants in the study were all within the normal range for infants in the complementary feeding age according to WHO growth charts (WHO, 2016).

The average haemoglobin concentration (10.40 ± 1.13 for *CF-35g*, 10.36 ± 1.36 for *MCL-35g* and 10.17 ± 1.30 for *MS-5g*) respectively of infants in each of the three study groups was also within the normal range (9.5-13g/dl) for infants (Merrit, 2017).

In this study, the birth order of infants was 3. However, a study by Sarvar et al (2017) to clinically assess micronutrient deficiencies in children aged 1-5 years showed that children with higher birth order showed higher prevalence of micronutrient deficiencies.

5.1.2 Background Characteristics of Mothers/Caregivers

In this study, the educational level of majority of the mothers was Junior High School. It is accepted that the educational status of a mother plays a major role on the nutritional status of children. A study conducted to determine the demographic and health-related risk factors of subclinical VAD, categorising education as 'illiterate' and 'literate' showed no association with subclinical vitamin A deficiency among the children (Demisie et al, 2009). However, Desirè (2012) alludes to the fact that one of the causes of VAD in Ghana is because, many Ghanaians have received little education and may not comprehend the importance of vitamin A consumption. A research also by Sommer (1995), showed that parents of cases (children with VAD) were less educated than parents in the control group.

5.1.3 Household Characteristics

The average household size of participants in the study was 6. The mean household size according to the Ghana Demographic and Statistical Survey (2014), is 3.5, which means household sizes of participants were quite large.

The most common source of water for most of the participants in this study was from boreholes or wells with only one person from each group having public tap their water source. de Queiroz et al (2013), found poor water sources as an associate factor for children in urban areas being susceptible to VAD and further reiterates that, according to WHO, a water supply that is not sufficient for drinking, personal hygiene and for growing food is part of the factors associated with malnutrition and that includes VAD. This therefore explains the risk of VAD in association with poor indoor plumbing in a home. Intestinal parasites which may be gotten from poor water

sources can reduce the absorption of vitamin A and in some instances, be associated with clinical VAD (McLaren & Frigg, 2001).

5.2 SERUM VITAMIN A LEVELS BEFORE AND AFTER INTERVENTION

Comparing the mean serum concentrations of all infants in the study to the WHO threshold for classifying VAD biochemically ($<0.70 \mu\text{mol/l}$) revealed that, all the children in the study were vitamin A deficient at baseline and at endline. The mean serum vitamin A in all three groups increased marginally at endline, however the increase in the two experimental groups was approaching the WHO threshold of $0.70 \mu\text{mol/l}$. Results from previous studies support the findings of this study. Glover-Amengor (2015), showed that MLP can improve the vitamin A status of children. Studies by Ullah et al (2011) and Lala & Reddy (1970), revealed that serum retinol increased in majority of subjects after they were fed with dark green leafy vegetables. Results from a study by Perssons (2001), showed substantial increase in serum vitamin A after children were fed for six weeks with dark green leafy vegetables. Babu, (2000) also reiterates the fact that *Moringa* ranks high in providing vitamin A compared to other vegetables in a study in Malawi and advocates the usefulness of indigenous knowledge on local foods in planning rural nutrition interventions. This supports the fact that food based approaches can be used in combatting VAD (Greiner, 2013).

5.3 ENERGY AND SELECTED NUTRIENTS INTAKE BEFORE AND AFTER INTERVENTION

Results from this study showed an increase in energy and nutrient intakes of infants at end line. The increase was seen in the treatment groups as well as the control groups. Although, there was no significant difference in energy and the nutrient selected for this study, previous studies on the effect of moringa on its ability to increase the nutritional content of complementary foods have been established. A study by Zongo et al (2013) in Burkina Faso on rehabilitation of clinically and severely malnourished children with *Moringa*, concluded that *Moringa* is loaded with nutritional potential and can be used as dietary supplement to help fight malnutrition. Again, Andrew's (2010) study proved that adding moringa to a child's food on daily basis can facilitate rapid recovery from malnutrition. Kouevi (2013) also reported, after proximate analysis on African weaning meals with *Moringa* at various percentages and without *Moringa* showed that the implementation of *Moringa* had a positive relation with the general quality of the weaning meals.

For this study, there was a significant difference in iron intake as it was lower than the average requirement of iron for infants in the complementary feeding age (WHO, 1998). This suggests that the diet of the children was low in iron. Also, iron, appeared to be inadequate in the diet of young children aged 9 months to 2 years in a research by Heath et al (2002) and this supports the finding of this study. Insufficient iron in the diet of infants can predispose them to iron deficiency which can lead to anemia (UNICEF, 2017). Anemia in infants can be related to retarded physical growth, reduction in resistance to infections and slow development of learning abilities (FAO, 2017).

5.4 ADHERENCE TO STUDY FOODS

A major obstacle in studies involving dietary modification is lack of participant adherence to prescribed diet (Moreira, 2011). A study by Hall (2005) showed that participants usually adhere to diets when foods are prepared and provided at the clinical site or home delivered. In this study adherence to study foods was high in the *CF-35g* group when compared to *MCL-35g* and *MS-5g* groups. *CF-35g* was well accepted in the study population compared to foods with MLP.

5.5 MORBIDITY OCCURRENCE DURING THE STUDY

Results on morbidity throughout the study showed that, there were few reports on morbidity both at baseline and end line. The highest however was nasal discharge at endline. It occurred in half (n=16) of the infants in the *MCL-35g* group. According to Zamani (2011), nasal discharge occurs when a tissue called the mucosa which produces mucus to protect the nose is irritated. Common cold is the most typical cause for nasal discharge. Allergies and bacterial infections are also known to cause nasal discharge in children.

A study by Andrew (2010), observed a high prevalence of diarrhea, acute respiratory infection and malaria at the baseline. However, there was a reduction in the treatment group whose diet was supplemented with *Moringa* leaf powder as compared to the control group. The difference in morbidity was attributed to the supplementation with moringa leave powder in the diet of the children due to the vitamin A, zinc and other micronutrient in moringa and has been shown to reduce both the prevalence of diarrhea and acute respiratory infection.

However, in this study, morbidity occurrence was generally low for all groups over the study period.

5.6 LIMITATIONS

The study had some limitations. The sample size at endline was small as some of the participants of the study were lost to follow up, others voluntarily dropped out, while others relocated from the study area. Also, the measurement of morbidity occurrence and adherence over study period was not objective. Measurement of morbidity occurrence was based on reports from mothers/caregivers. The measurement of adherence was based on the disappearance rate of study foods given to study participants. The amount that disappeared may not be the actual quantities consumed by infants in the study.

CONCLUSION AND RECOMMENDATION

CONCLUSION

Findings of this study showed that although there was a marginal increase in serum vitamin A concentrations for infants in all three groups, from baseline to end line, vitamin A levels of all infants were low when compared to the WHO threshold of $0.70\mu\text{mol/l}$. This implies that, VAD remains a major public health issue in the study population and in Ghana for that matter.

As part of food diversification approaches to combat VAD, *Moringa Oleifera* leaf powder can be incorporated in complementary foods of infants in the developing world to improve their serum vitamin A levels and consequently their nutritional status.

RECOMMENDATIONS

1. The efficacy of MLP in improving vitamin status of infants in the complementary feeding age needs to be ascertained in a well-designed larger trial which will last for a longer period.
2. In designing the larger study, a larger sample size higher than what will be estimated should be used to account for the possible high attrition.
3. More objective methods should also be employed in the measurement of adherence and morbidity occurrence in the larger study to draw better conclusions.

REFERENCES

- Abioye, V.F. (2015) 'Proximate composition and sensory properties of *Moringa* fortified yellow maize-ogi' *African Journal of Food Science Research*, Vol. 3 (1), pp. 155-159,
- Academy for Educational Development (2003). Nutrition in Ghana: Investing now for the year 2020-1255 Twenty-Third St. / Washington, DC 20037.
- Aglago, K. E., Imane, M., Kaoutar, B., Khalid, K., Asmaa1, H., Mehdi A, A., Haloui N., and Aguenou Hassan A., (2015) 'Comparison of ICHECK Fluoro Kit with High performance Liquid Chromatography for the Assessment of Serum Retinol' *African Health Sciences* Vol 15 Issue 2, June 2015
- Andrew A., (2010) 'Effect of *Moringa Oleifera* Leaf Powder supplement to improve nutritional status of severely malnourished children aged 6-24 months in Arusha Region'. Sokoine University of Agric, Morogoro, Tanzania
- Anwar, F., Latif, S., Ashraf M., and Gilani A.H (2007). '*Moringa oleifera*: A Food Plant with Multiple Medicinal Uses'. *Phytotherapy. Research.* 21, 17–25 (2007)
- Babu S.C. (2000) 'Rural nutrition interventions with indigenous plant foods – a case study of vitamin A deficiency in Malawi Suresh' International Food Policy Research Institute. 2033 K Street, NW. Washington, DC 20006.

- Barrett C B., and Bevis, L E M., (2013), 'The Micronutrient Deficiencies Challenge in African Food Systems' *The Fight Against Hunger and Malnutrition: The Role of Food, Agriculture, and Targeted Policies* Oxford University Press
- Bennasir, H., Sridhar, S., and Abdel-Razek, T.T. (2010) 'VITAMIN A ... From Physiology to Disease Prevention' *International Journal of Pharmaceutical Sciences Review and Research* Volume 1, Issue 1, March – April 2010; Article 014
- Black R.E, Allen., L. H., Bhutta, Z.H., Caulfield, L.E., de Onis, M., Ezzati, M., Mathers, C., and Rivera, J. (2008) 'Maternal and child malnutrition: global and regional exposures and health consequences' *The Lancet* Volume 371, No. 9608, p243–260
- Boateng L, Nyarko R, Asante M, & Steiner-Asiedu M. 'Acceptability of Complementary Foods That Incorporate Moringa Oleifera Leaf Powder Among Infants and Their Caregivers'. *Food and Nutrition Bulletin*, 2017; 0379572117708656.
- Borlaug, N. (2007) 'Feeding a hungry world' *Science*, 318(5849): 359.
- Combs Jr G. (2008) 'The Vitamins: Fundamental Aspects in Nutrition and Health' Third Edition
- Custodio E, Descalzo MA, Roche J, Sánchez I, Molina L, Lwanga M, Bernis C, Villamor E and A Baylin (2008) 'Nutritional status and its correlates in Equatorial Guinea preschool children: Results from a nationally representative survey' *Food and Nutrition Bulletin* 29: 49-58.
- Das JK, Salam RA, Kumar R, Bhutta ZA. (2013)' Micronutrient Fortification of Food and its Impact on Woman and Child Health: A Systematic Review' *System Review* 2(1):1.

- Daelmans, B., N. Mangasaryan, J. and Martines, J. (2009) ‘Strengthening actions to improve feeding of infants and young children 6 to 23 months of age: summary of a recent World Health Organization/UNICEF technical meeting’ *Food and Nutrition Bulletin* 30: S236–S238.
- Darton-Hill, I., Webb, P., WJ Harvey, PWJ., Hunt, JM., Dalmiya, N., Chopra, M., Ball, MJ., Bloem, MW., and de Benoist B. (2005) ‘Micronutrient deficiencies and gender: social and economic’ *American Journal of Clinical Nutrition* 2005;81(suppl):1198S–1205S.
- de Pee S., & Dary, O. (2002) ‘Biochemical Indicators of Vitamin A Deficiency: Serum Retinol and Serum Retinol Binding Protein’ *The Journal of Nutrition* American Society for Nutritional Sciences.
- de Queiroz, D., de Azevedo Paiva, A., Pedraza, D.F., Lins da Cunha, M.A., Esteves G.H., de Luna J.G., and da Silva Diniz A. (2013) ‘Vitamin A deficiency and associated factors in children in urban areas’ *Rev Saúde Pública* 2013;47(2)
- Demissie, T., Ali, A., Mekonnen Y., Haider J., and Umeta M. (2009) ‘Demographic and Health-related Risk Factors of Subclinical Vitamin A Deficiency in Ethiopia’ *Journal for Health and Population Nutrition* 27(5):666-673 ISSN 1606-0997
- Desirée C M., (2012), ‘Vitamin A Deficiency among Young Ghanaian Children: A Cause and Effect Analysis and Possible Solution’ *Global Medical Brigades*
- Dewey K.G., & Vitta B.S. (2013) ‘Strategies for ensuring adequate nutrient intake for infants and young children during the period of complementary feeding’ *Alive & Thrive* Issue 7, Pages 1-14

- Dillard CJ, German JB. (2000). 'Phytochemicals: nutraceuticals and human health: A review.' *Journal of Science Food and Agriculture* 80: 1744–1756.
- District Health Directorate. Upper Manya Krobo 2012 Half Year Report. Asewewa, Eastern Region. 2012.
- Doerr, B. & Cameron, L. (2005) 'Moringa Leaf Powder. *ECHO* (Educational Concerns for Hunger Organization) Technical Notes. Available: echo@echonet.org.
- Fuglie, L., (2005). 'The *Moringa* Tree – A local solution to Malnutrition?' (<http://www.moringanews.org/documents/Nutrition.pdf>) Assessed: September 2017
- Ghana Statistical Service (2008). Ghana Demographic and Health Survey. Ghana Statistical Service Ghana Health Service Accra, Ghana ICF Macro Calverton, Maryland, U.S.A.
- Ghana Demographic and Health Survey (2014). Ghana Demographic and Health Survey. Ghana Statistical Service Ghana Health Service Accra, Ghana ICF Macro Calverton, Maryland , U.S.A.
- Glover-Amengor M., (2015). '*Moringa Oleifera* Leaf supplementation on vitamin A Status of children in Ada-East District of Ghana' *Food Science and Nutrition*, vol 5, Issue 2, pages 317-323
- Gopalakrishnanb, L., Doriyaa, K., Kumara, D.S. (2016). '*Moringa oleifera*: A review on nutritive importance and its medicinal application'. *Food Science and Human Wellness* 5 (2016) 49–56
- Greiner T. (2013) 'Vitamin A: Moving the food-based approach forward' FAO & WHO

- Hall D.M., (2005) 'Dietary adherence in well-controlled feeding studies' *Journal of the American Dietetic Association*, 105(8):1285-8
- Heath, A.L.M., Tuttle, C.R., Simons, M.S.L., Cleghorn C.L., and Parnell W.R. (2002) 'Longitudinal study of diet and iron deficiency anaemia in infants during the first two years of life' *Asia Pacific Journal of Clinical Nutrition* 11(4): 251–257
- Kapil U. and Bhavna A. (2002) 'Adverse effects of poor micronutrient status during childhood and adolescence' *International Life Sciences Institute* 2:84–90
- Kasolo, J.N., Bimenya, G.S., Ojok, L., Ochieng, J., Ogwal-okeng, J.W. (2010) 'Phytochemicals and uses of *Moringa Oleifera* leaves in Ugandan rural communities' *Journal of Medicinal Plants Research*. 4: 753–757
- Kayalto, B., Zongo C., Otchom B, Kam L., and Traore AS. (2016) 'Local and improved infant flours in vitamin A and iron, using powdered *Moringa oleifera* leaves and *Parkia biglobosa*'s pulps powder. *International Journal of Scientific and Research Publications*, Volume 6, Issue 4, April 2016 27, ISSN 2250-3153
- Kouevi, KK. (2013) 'A study on *Moringa Oleifera* leaves as a supplement to West African weaning foods' Hamburg University of Applied Science.
- Kuhnlein, H.V. (2003) 'Micronutrient nutrition and traditional food system in indigenous people' *Food, Nutrition and Agric* 2003 32:33-39
- Lala, V.R., and Reddy, V. (1970) 'Absorption of β -Carotene from Green Leafy Vegetables in Undernourished Children' *The American Journal of Clinical Nutrition* Vol. 23, No. I, pp. 110-113

Sullivan, L. (2016) 'Central Limit Theorem' *The Role of Probability* Page 12

Lartey, A., Manu, A., Brown, K.H., Peerson, J.M., and Dewey K.G., (1999) 'A randomized, community-based trial of the effects of improved, centrally processed complementary foods on growth and micronutrient status of Ghanaian infants from 6 to 12 months of age1–3.' *The American Journal of Nutrition* 70:391–404.

Lorch, A., (2005) 'Vitamin A deficiency: diverse causes, diverse solutions' *Greenpeace International*

Maleta, K., (2006). 'Undernutrition' *Malawi Medical Journal* 18 (4): 189-205

Marcu, M G., (2005). 'Miracle Tree', *KOS Health Publications*, #251, 91011. April 2005

Mayo-Wilson E., Imdad, A., Herzer, K., Yawar Yakoob M.Y., Bhutta Z. A. (2011) 'Vitamin A supplements for preventing mortality, illness, and blindness in children aged under 5: systematic review and meta-analysis' *British Medical Journal* 2011;343: d5094 doi: 10.1136/bmj. d5094

Mbikay, M. (2012) 'Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycemia and dyslipidemia: A Review, Front' *Pharmacology & Therapeutics* 3: 1–12.

McLaren, D. S., & Frigg, M., (2001), 'Sight and Life Manual on Vitamin A Deficiency Disorders (VADD)' Task Force Sight and Life 4002 Basel, Switzerland.

<http://www.sightandlife.org>

Merrit, BY. (2017) 'Hemoglobin Concentration (Hb): Reference range and Interpretation'

[Internet]. Cited 2017. Nov 20. Available from <http://emedicine.medscape.com/article/2085614-overview#a1>

- Nadimina, Hadjub, V., As'adc, S., Bucharid A. (2015) 'The Extract of Moringa Leaf Has an Equivalent Effect to Iron Folic Acid in Increasing Hemoglobin Levels of Pregnant Women: A randomized Control Study in the Coastal Area of Makassar' *International Journal of Sciences: Basic and Applied Research (IJSBAR)*.
- NIH. (2006) 'Dietary Supplement Fact Sheet: Vitamin A and Carotenoids' Retrieved from <http://ods.od.nih.gov/factsheets/VitaminA-HealthProfessional/>
- Nwosu O., Nnam N, Ibeziako N and Maduforo A., (2014) 'Analysis of the Nutrient Content of Infant Complementary Food Fortificant-*Moringa oleifera* Leaves with the Commonly Consumed Local Infants Foods in Nigeria: *Zea mays* and *Glycine max*' *International Journal of TROPICAL DISEASE & Health 4(10): 1111-1122*
- Olson, J. A., (1964) 'The biosynthesis and metabolism of carotenoids and retinol (vitamin A)' *Journal of Lipid Research, ASBMB*.
- Oyeyinka, A.T., and Oyeyinka, S.A. (2016) '*Moringa oleifera* as a food fortificant: recent trends and prospects'. *Journal of the Saudi Society of Agricultural Sciences*.
<http://dx.doi.org/10.1016/j.jssas.2016.02.002..>
- Persson V., Ahmed F., Gebre-Medhin M., and Greiner T. (2001) 'Increase in serum beta-carotene following dark green leafy vegetable supplementation in Mebendazole-treated school in Bangladesh' *European Journal of Clinical Nutrition* Jan;55(1):1-9
- Price. M. (2007) The *Moringa* Tree. *ECHO* (Educational Concerns for Hunger Organization) Technical Notes.
- Rice A. L., West Jr. K. P. and Black R.E. (2004) 'Comparative Quantification of Health Risks; Sexual and Reproductive Health' Chapter 4, Pg. 212. World Health Organization.

- Rockwood, J.L., Anderson, B.G., Casamatta, D.A. (2003) 'Potential uses of *Moringa oleifera* and an Examination of Antibiotic Efficacy Conferred by *M. oleifera* Seed and Leaf Extracts using Crude Extraction Techniques available to under-served Indigenous Populations' *International Journal Phyto therapy Research* 3 (2013) 61–71.
- Ross C., and Harrison E.L., 'Vitamin A: Nutritional Aspects of Retinoids and Carotenoids' *Handbook on Vitamins*, (4):1-40
- Sarvar, R., and Ban,t D.D. (2017) 'Clinical assessment of micronutrient deficiencies among children (1- 5 years) enrolled in Anganwadis of old Hubli slums, Karnataka, India' *International Journal of Community Medicine and Public Health* Feb;4(2):598-602
<http://www.ijcmph.com>
- Schweigert F.J., Frey S.K, Mothes R., Dary O., Juarez P, Lascano V. (2011) 'A new test kit's potential for the rapid analysis of vitamin A in human and cow milk' *Sight and Life* 2011; 25 (3)18-22
- Semba R.D. (2001) 'Impact of vitamin A on immunity and infection in developing countries' *Preventive Nutrition: The Comprehensive Guide for Health Professionals*. (2) 329-346.
- Siddhuraju P, Becker K. 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agro-climatic origins of drumstick tree (*Moringa oleifera* Lam.). *Journal of Agriculture and Food Chemistry* 15: 2144–2155.
- Sommer A. (1989) 'New Imperatives for an Old Vitamin A' *Journal for Nutrition* 119, 96–10
- Sommer A. (1995) 'Vitamin A deficiency and its Consequences: A Field Guide to Detection and Control' 3rd edition

Sommer A. (2008) 'Vitamin A Deficiency and Clinical Disease: An Historical Overview' *Journal for Nutrition* 138: 1835–1839

Sommer A. and West K. P. (1996) 'Vitamin A deficiency: Health, Survival and Vision' New York: Oxford University Press.

Stephensen C. B. (2001) 'Vitamin A, infection, and immune Function' *Annual Review of Nutrition* 21:167–92

Statistical Package for Social Scientists (SPSS, 2012) version 21.0. SPSS 21 for Windows, Chicago. Illinois, USA.

Tanumihardjo, S. A (2004) 'Assessing Vitamin A Status: Past, Present and Future' *The Journal of Nutrition* American Society for Nutritional Sciences.

Teijlingen, van E. R. and Hundley V. (2001). 'The importance of pilot studies' *Social Research Update* Issue 35. University of Surrey.

Thabane, L., Ma J., Chu, R., Cheng, J., Ismaila¹, A., Rios L.P., Robson, R., Thabane M., Giangregorio, L., and Goldsmith, C. (2010). 'A tutorial on pilot studies: the what, why and how' *Medical Research Methodology* 2010, 10:1

Ullah, N., Khan, A., Khan, F.A., Khurram, M., Hussan, M., Umar, Khayam S., Amin, M. and Hussain, J. (2011) 'Composition and Isolation of Beta Carotene from Different Vegetables and Their Effect on Human Serum Retinal Level' *Middle-East Journal of Scientific Research* 9 (4): 496-502

United Nations High Commissioner for Refugees (2003) 'Micronutrient Malnutrition – Detection, Measurement and Intervention: A Training Package for Field Staff '

United Nations Children's Fund (2006) 'A Report Card on Nutrition' New York City

United Nations Children's Fund, (2011) 'Consequences of micronutrient deficiencies in Africa, Tanzania'

United Nations Children's Fund, (2017) 'Improving Breastfeeding, Complementary foods and Feeding Practices' New York City

Valdez-Solana, M.A., Mejía-García, V.Y., Téllez-Valencia, A., García-Arenas, G., Salas-Pacheco, J., Alba-Romero, J.J., and Sierra-Campos E., (2015) 'Nutritional Content and Elemental and Phytochemical Analyses of *Moringa oleifera* Grown in Mexico'. *Journal of Chemistry* Volume 2015, Article ID 860381, 9 pages

Wiseman, G., (2004) 'Nutrition and Health' Taylor & Francis Inc, New York

Woken M.D. (2013) 'Advantages of a Pilot Study' Center for Teaching and Learning, University of Illinois.

WHO (1998) 'Complementary Feeding of Young Children in Developing Countries: A review of current scientific knowledge' World Health Organization, Geneva, Switzerland

World Health Organization (2003) 'Infant and Young Child Feeding, A tool for assessing national practices, policies and programs' Geneva 2003

World Health Organization and Food and Agriculture Organization of the United Nations (2004) 'Vitamin and Mineral Requirements in Human Nutrition' Second edition

World Health Organization. (2009) 'Global Prevalence of Vitamin A Deficiency in Populations at Risk 1995–2005: WHO Global Database on Vitamin A Deficiency' Geneva, Switzerland: WHO.

World Health Organization (2009) 'Global Database on Vitamin A Deficiency' Geneva, World Health Organization, 2009.

World Health Organization (2011) 'Guideline: Vitamin A supplementation in infants and children 6–59 months of age' Geneva, World Health Organization.

World Health Organization (2011) 'Complementary feeding Report of the global consultation Summary of guiding principles' Geneva, World Health Organization.

World Health Organization (2011) 'Serum retinol concentrations for determining the prevalence of vitamin A deficiency in populations' Vitamin and Mineral Nutrition Information System. Geneva, World Health Organization, (WHO/NMH/NHD/MNM/11.3) (<http://www.who.int/vmnis/indicators/retinol.pdf>)

World Health Organization (2013) 'Essential nutrition actions: improving maternal, newborn, infant and young child health and nutrition' Geneva, Switzerland

World Health Organization (2016) a. 'Appropriate complementary feeding'. World Health Organization, Geneva

World Health Organization (2016) b 'What is Malnutrition?' World Health Organization, Geneva

World Health Organization (2017), 'Micronutrient Deficiencies' World Health Organization, Geneva

World Health Organization (2017), 'Appropriate Complementary Feeding' World Health Organization, Geneva

World Health Organization (1997) 'WHO Global Database on Child Growth and Malnutrition' World Health Organization, Geneva

World Health Organization (2009) 'Global database of Vitamin A deficiency' Geneva, WHO, 2009.

World Health Organization (2017) 'Diarrhoeal Disease' World Health Organization, Geneva

World Nutrition Development (2012) 'Malnutrition. Vitamin A Let food be our medicine' Volume 5, Number 12, December 2014

Zamani, AR., (2009), Runny Nose in the Child Care Setting. Health and Safety Notes, California Child Care Health Program

Zongo, U., Zoungrana S.L., Savadogo A., and Traoré, A.S (2013). 'Nutritional and Clinical Rehabilitation of Severely Malnourished Children with *Moringa oleifera Lam.* Leaf Powder in Ouagadougou (Burkina Faso)' *Food and Nutrition Sciences*, 2013, 4, 991-997