MORINGA OLEIFERA LEAF SUPPLEMENTATION ON VITAMIN A STATUS OF CHILDREN IN ADA-EAST DISTRICT OF GHANA

By

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DECLARATION

I declare that this thesis is the result of research work undertaken by me at the University of Ghana, that the work has not been accepted for the award of any degree elsewhere, and is not concurrently submitted for any degree other than the Doctor of Philosophy (PhD) degree of the University of Ghana.

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ABSTRACT

Background

Globally over 195 million pre-school children are vitamin A deficient, whilst in Ghana, it was estimated that 72 % of pre-school children are vitamin A deficient. Periodic administration of high dose vitamin A capsules is currently used as a prevention strategy, but this requires appropriate healthcare infrastructure and is donor dependent, hence may not be sustainable if donor support is withdrawn. Inadequate dietary intake of vitamin A-rich foods is a major etiological factor in vitamin A deficiency, so one other prevention strategy could be to promote the consumption of these foods. Plant-based foods such as orange-fleshed roots and tubers, fruits and dark green leafy vegetables like *Moringa oleifera* (*M. oleifera*) leaves are rich sources of pro-vitamin A carotenoids that could be beneficial to vulnerable populations in low socio-economic households who mostly derive their nutrition from plant sources.

M. oleifera leaves have been reported to improve retinol levels in rats. However, there are only anecdotal reports on the ability of *M. oleifera* leaves to improve retinol levels in humans. There is the need therefore to conduct evidence-based research to assess the effect of *M. oleifera* leaves on retinol levels in humans.

Objective

The objective of the study was to assess the efficacy of *M. oleifera* leaf supplementation to improve the vitamin A status of children in Ada-East district of Ghana.

Methods

The interventional study was preceded by an assessment of dietary uses of *M. oleifera* leaves in Ada-East district through a community survey of mothers/caregivers aged 19 years and above, and an acceptability test of M. *oleifera* leaf-fortified dishes by children in Ada-East district. Thereafter,

children aged 5-12 years were randomized to either receive or not receive dried *M. oleifera* leaves for 9 weeks in three local dishes. The Intervention group (n = 85) consumed *M. oleifera* leaf-fortified dishes at 0.2 g/kg body weight, three times a week, whilst the Control group (n = 85) consumed the same food without *M. oleifera* leaves. Vitamin A, haemoglobin, haematocrit, erythrocytes, mean corpuscular volume, kidney and liver chemistry, and infections were assessed at the beginning and end of study. Vitamin A was assessed by high performance liquid chromatography (HPLC). Red blood indices were assessed on EDTA whole blood samples in the laboratory using Sysmex KX - 21N, an automated haematology analyser. Kidney and liver chemistry were assessed with EliTech clinical systems kits.

Results

One hundred and eighty (79.7 %) of respondents use *M. oleifera* leaves as a food ingredient in the district, but on an irregular basis, while two hundred and twelve (93.8 %) said they would feed their children when requested to do so, because they learned the leaves would make the children strong. *M. oleifera* leaf-fortified dishes were also highly acceptable to the children. Leaf supplementation improved retinol levels in the Intervention group significantly ($P < 0.05$). Those with marginal vitamin A status (serum retinol < 0.7 µmol/l) at baseline showed significant increases at end of study. There was a significant positive association between vitamin A and haemoglobin in the Intervention group ($P < 0.05$), but not in the Control group. All markers of kidney and liver safety did not show any significant changes at end of study.

Conclusion

M. oleifera leaf supplementation was efficacious to improve the vitamin A status of children in Ada-East district of Ghana.

DEDICATION

THIS THESIS IS DEDICATED TO THE TRIUNE GOD – GOD THE FATHER, GOD THE SON AND GOD THE HOLY SPIRIT

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LIST OF ACRONYMS AND ABBREVIATIONS

ACC/SCN	Administrative Committee on Coordination/Subcommittee on Nutrition (United Nations)
ADRA	Adventist Development and Relief Agency
ALT/GPT	Alanine aminotransferase/glutamate pyruvate transaminase
ALP	Alkaline phosphatase
AST/GOT	Aspartate aminotransferase /glutamate oxaloacetate transaminase
AOAC	Association of Officials of Agricultural Chemistry
AAS	Atomic Absorption Spectrophotometry
CDE	Centre for Development of Enterprises
CHD	Child
CG	Control Group
CSIR	Council for Scientific and Industrial Research
dl	deciliter
DW	Dry Weight
EPO	Erythropoietin
EDTA	Ethylene diamine tetraacetic acid
FAO	Food and Agriculture Organization
GAMMA GT (GGT)	Gamma glutamyl transfrase

GDHS	Ghana Demographic and Health Survey
GS	Ghana Standard
GSS	Ghana Statistical Service
GSFP	Government School Feeding Programmme
g	gramme
GLV	Green Leafy Vegetable
HCT	Haematocrit
GHB	Haemoglobin
HPLC	High Performance Liquid Chromatography
HIV	Human Immunodeficiency Virus
ICN	International Conference of Nutrition
IU	International Unit
IVACG	International Vitamin A Consultative Group
IG	Intervention Group
MALPARA	Malaria parasite density
ug	microgramme
ul	microliter
µmol	micromole

mg	milligramme
mm	millimeter
MOH	Ministry of Health
MAG	Morringa Association of Ghana
NMIMR	Noguchi Memorial Institute for Medical Research
NUT	Nutrition
pg	picogramme
RBC	Red Blood Cells
RAR	Retinoic Acid Receptors
RBP	Retinol Binding Protein
RE	Retinol Equivalence
SDA	Seventh Day Adventist
TIBC	Total Iron Building Capacity
TS	Transferrin Saturation
UNICEF	United Nations International Children's Education Fund
UNU	United Nations University
VAD	Vitamin A deficiency
WHO	World Health Organization

CHAPTER ONE

1.0 Introduction

1.1 Background

Vitamin A deficiency (VAD) is a major public health problem worldwide, particularly in developing countries including Ghana (Aguayo and Baker, 2005). Globally, 5.2 million preschool children had clinical vitamin A deficiency (VAD), while 190 million had subclinical VAD (serum retinol < 0.70 µmol/l) (WHO, 2009). Ghana's Ministry of Health (MOH, 1997) and World Health Organisation (WHO, 2000) reported that 79.3 % of children under five years in some coastal communities surveyed in Ghana had sub-clinical vitamin A deficiency, whilst Rice *et al*., (2007) estimated that 72 % of chidren in Ghana were vitamin A deficient.

The consequences of VAD include adverse effects on growth, reproduction which embraces embrayonic development and spermatogenesis in males (Clagett-Dame and Knutson, 2011), and resistance to infection, with the most severe effect being xerophthalmia which could eventually lead to blindness (WHO**,** 1995). Although ocular symptoms and signs are the most specific indicators of VAD, they occur only after other tissues have impaired functions that are less specific and less easily assessed (Sommer, 1994). Sub-clinically, vitamin A deficiency affects the health of many preschool-age children and older children (Kassaye *et al*., 2001) and pregnant or lactating women (Samba *et al*., 2013). In young children, sub-clinical VAD, like clinical deficiency, increases the severity of some infections, particularly diarrhoea and measles, and the risk of dying (Beaton, 1993). The compromised integrity of the epithelium, together with the possible alteration in hormonal balance at severe levels of deficiency, impairs normal reproductive functions in animals (Ross and Gardner, 1994; Eskild and Hanson, 1994; Bates, 1983).

Of much significance also is the relationship between vitamin A and iron metabolism. A number of studies in both humans and animals have shown an interaction between vitamin A and iron metabolism. Mejia and Chew (1988) reported that vitamin A supplementation produced significant elevations in serum retinol, blood haemoglobin, haematocrit, erythrocytes (RBCs), serum iron and percent transferrin saturation (% TS), but had no effect on total iron binding capacity (TIBC) or serum ferritin. Their study further indicated that iron supplementation did not affect serum retinol, although it improved haematological indices. Other studies supported this observation (Hodges *et al*., 1978; Mejia *et al*., 1976; Bloem *et al*., 1989). In many developing countries where VAD rate is high together with high anaemia (Semba and Bloem, 2002), it is anticipated that interventions that will improve vitamin A status and eliminate VAD could also improve haematological parameters and prevent anaemia.

Large doses of vitamin A supplements were periodically administerd to children 0 – 59 months to reduce the consequences of VAD (Kandlakunta *et al*., 2008). However, supplement distribution is mainly funded by international organizations/donors (WHO/UNICEF/IVACG, 1988). Apart from being costly, the supplementation with vitamin A also needs appropriate health care infrastructure to ensure broad coverage (Jalal *et al*., 1998). Since inadequate dietary intake of vitamin A has been identified as a major etiological factor of VAD, particularly in low socioeconomic groups, promoting the consumption of vitamin A-rich foods is an important prevention strategy. Dietary diversification using provitamin A (α-carotene, β-carotene and β-cryptoxanthin) -rich roots and tubers, cereals, fruits and green leafy vegetables, which are found in tropical countries, could serve as an inexpensive way of improving vitamin A status of low income groups which are most often the populations at risk (Persson *et al*., 2001; Singh *et al*.,

2001). These foods include orange-fleshed sweet potatoes, yellow yam, yellow maize, carrots, many fruits including mangoes and dark green leafy vegetables such as amaranth and drumstick (*Moringa oleifera*) leaves. If even mild to moderate VAD is corrected at community level, it could lead to a reduction of 23 % mortality in young children (Persson *et al*., 2001). The Plan of Action of the International Conference of Nutrition (ICN) recommended the adoption of dietary based strategies for vitamin A deficiency control in low income countries (FAO/WHO, 1993).

Much interest has been generated in dark green leafy vegetables as a source of β-carotene for developing countries. Singh *et al*., (2001) reported that 95 % of the β-carotene in India comes from fruits and vegetables and 90 % of this is provided by green leafy vegetables (52 %) and fruits (38 %). Moreover, several workers have quantified carotenoid levels in green leafy vegetables (Aizawa and Inakuma, 2007; Raju *et al*., 2007; Bhaskarachary *et al*., 2006; Chanwitheesuk *et al*., 2005) and have reported that this class of food is a potential source of β-carotene that could be tapped for improving vitamin A status especially in low socio-economic groups.

Seasonal fluctuations in the availability of green leafy vegetables could, however, influence availability of provitamin A carotenoids from this food source *e.g*. during the rainy season, provitamin A-rich green leafy vegetables may be readily available, but during the lean season, accessibility may be low (Oyuri-Nawiri, 2011; Babu, 2000). Hence green leafy vegetables like *M. oleifera* that are available all year round could help in alleviating seasonal scarcity and ensure adequate supply of β-carotene and all the other provitamin A carotenoids (Glover-Amengor and

Mensah, 2012). Furthermore, those green leafy vegetables that would retain a high percentage of β-carotene on drying and storage could be processed for convenient use.

Moringa oleifera, a high β-carotene-containing, perennial plant, that retains a very high percentage of its β-carotene on drying and storage, has the potential for ensuring adequate supply of β-carotene in developing countries. Beta-carotene is the most important of the provitamin A carotenoids because it yields two molecules of vitamin A on hydrolysis as against one molecule by α-carotene and β-cryptoxanthin. Fuglie, (1999) reported β -carotene levels of 18.9 mg/100 g in dried *M. oleifera* leaves, whilst 31 mg/100 g (dry weight) was reported by Glover-Amengor and Mensah (2012). Nambiar and Seshadri (2001) reported 19.2 mg/100 g fresh weight while Seshadri *et al.,* (1997) also reported 17.4 mg of β-carotene in 100 g fresh leaves and further indicated that more than 50 % of this amount was finally retained after processing procedures and three months storage. Additionally, the authors indicated that dehydration did not affect the acceptability characteristics of the leaves. Glover-Amengor *et al.,* (2012) also reported that *M. oleifera* leaf powder retained 87.54 % of its β-carotene content after four months of storage.

Although *M. oleifera* is a perennial plant that produces leaves all year round, is easy to cultivate, fast growing and adapting (Luu *et al*., 2005; Palada and Chang, 2003), in areas where enough leaves could not be harvested in the lean season due to climatic factors, dried leaves could ensure adequate supply all year round to provide β-carotene for developing countries to meet their vitamin A needs (Oyuri-Nawiri, 2011). Additionally, dried leaves provide a convenient source that could reduce the burden of harvesting fresh leaves daily.

M. oleifera leaves and tender pods are used as vegetable in Haiti, India and other Asian countries, Senegal and many other tropical countries (Price 2000; Bhaskarachary *et al*., 1995; Kidmose *et al.*, 2006). In Ghana, *M. oleifera* has long been known in the Northern, Volta and parts of Greater Accra regions where it is used both as food and medicine (personal communication). The leaves are used in soups and in making sauces. In northern Ghana, *M. oleifera* leaves are also boiled, spiced, and eaten as vegetable balls by children (personal communication). The leaves are also used to treat fever.

M. oleifera has been promoted extensively in Ghana since 2001 by the Methodist Church, Ghana and Seventh Day Adventist (SDA) Church who embarked on nutrition education and processing of the leaves. From 2001-2006, Adventist Development and Relief Agency (ADRA) introduced *M. oleifera* as a backyard tree to farm households in thirty communities in all regions of Ghana except Western, as part of their food security programme. Households were educated on the nutritional benefits of *M. oleifera* leaves, and were also taught its use in food preparation. ADRA showed a number of television (TV) documentaries on this initiative. The Methodist church also encouraged all societies in her various dioceses to plant *M. oleifera* (personal communication). Furthermore, The Moringa Association of Ghana (MAG), Moringanews, France and the Centre for the Development of Enterprises (CDE) conducted a series of tests on *M. oleifera* leaves at the Council for Scientific and Industrial Research-Food Research Institute (CSIR-FRI) which guided the publication of a handbook on growing and processing *M. oleifera* leaves (de Saint Saveur and Broin, 2010), and also the development of standards by Ghana Standards Authority to guide processors and consumers of *M. oleifera* leaves (GS 998; GS IM, 12).

1.2 Problem Statement

At least 195 million preschool children suffer from clinical and subclinical vitamin A deficiency (VAD) globally (WHO, 2009). Vitamin A deficiency (VAD) adversely affects growth, reproduction and resistance to infection, and causes xerophthalmia which could eventually lead to blindness (WHO**,** 1995). With the support of international organizations (WHO/UNICEF/IVACG, 1988), large doses of vitamin A supplements are given to children under five years to reduce the serious consequences of VAD (Kandlakunta *et al.*, 2008). For sustainability it must be repeated routinely, and apart from being costly, it also needs appropriate health care infrastructure to ensure broad coverage (Jalal *et al.*, 1998). However, since inadequate dietary intake of vitamin A has been identified as a major etiological factor of VAD, particularly in low socio-economic groups, promoting the consumption of vitamin A-rich foods could help address VAD. Although animal source foods such as liver, glandular meat, fish oils and dairy products are good sources of pre-formed vitamin A, these are mostly not affordable to low socio-economic groups; likewise fortified foods such as vegetable oil and wheat flour. Provitamin A-rich foods which include green leafy vegetables and orange-fleshed fruits and, which are found in abundance in tropical countries are however cheap and could be affordable to low socio-economic groups. Thus consumption of provitamin A (α-carotene, β-carotene and β-cryptoxanthin) -rich fruits and green leafy vegetables such as *M. oleifera,* could serve as a cheap way of improving vitamin A status of low income groups.

The conceptual framework for *M. oleifera* leaf consumption is shown in Figure 1.

Vitamin A status of children could be improved by high-dose capsule supplementation and consumption of vitamin A fortified foods such as vegetable oil, and animal source foods.

Supplemenation is expensive in terms of personnel and health-care infrastructure, so it may not be sustainable if not backed by strong government policy. Food fortification and consumption of animal source foods could not benefit low socio-economic groups because of affordability. Provitamin A carotenoids which are obtained from plant sources such as *M. oleifera*, however, are easy to cultivate in the tropics due to favourable climatic conditions. Through community support by way of skills acquisition and nutrition education, they could be grown in backyards, and dried for convenience to ensure constant supply.

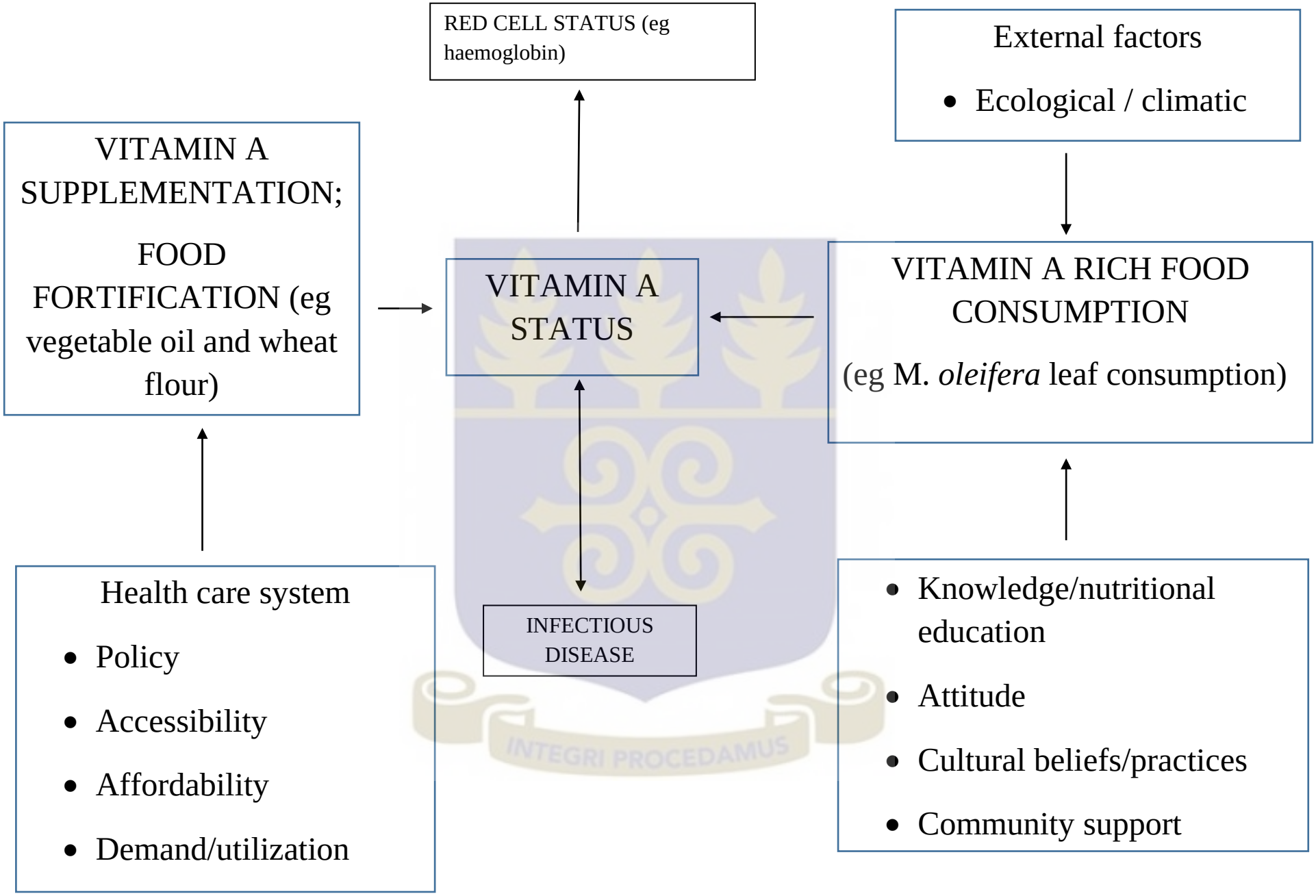

Figure 1: Conceptual Framework for *M. oleifera* leaf Consumption

1.3 Rationale

M. oleifera, a tropical, perennial green leafy vegetable has generated much interest due to its high β-carotene content. A number of scientific journals have reported the nutritional, pharmacological, as well as microbiological properties of the plant (Kasolo *et al*, 2010; Fahey, 2005; Khalaffala *et al.*, 2010). *M. oleifera* leaves have been reported to improve retinol and haemoglobin levels in laboratory animals (Nambiar and Seshadri, 2001), and its β-carotene has also been reported to be highly bioavailable *in vitro* (Yang *et al.*, 2006), but whether the leaves can improve retinol levels in humans is not yet clear, because there is inadequate scientific data on its effect in humans (Thurber and Fahey, 2009). Most of the reports on *M. oleifera's* potential for improving retinol and haemoglobin levels in humans are anecdotal (Fuglie, 1999; Fahey 2005), and therefore need to be substantiated scientifically. Although efficacious in animal studies, whether *M. oleifera* leaves could be efficacious in improving retinol and haemoglobin levels in humans needs to be investigated scientifically. There is, therefore, the need to conduct evidence-based research to generate data that will provide information on strategies to prevent micronutrient deficiencies using local plant-based foods. If found, to be efficacious in improving vitamin A status in humans, *M. oleifera* leaves would be a cheap source of vitamin A for tropical countries because it easily adapts in these countries with favourable climatic conditions for its production (Luu *et al.*, 2005).

Seasonal fluctuations in the availability of fruits and green leafy vegetables could influence availability of provitamin A carotenoids from these food sources *e.g.* during the rainy season, provitamin A-rich fruits and green leafy vegetables may be readily available, but during the lean season, accessibility may be low (Oyuri-Nawiri, 2011; Babu, 2000). Hence those green leafy

vegetables that are available all year round could help in alleviating seasonal scarcity and ensure adequate supply of β-carotene and all the other provitamin A carotenoids. Furthermore, those green leafy vegetables that retain a high percentage of β-carotene on drying and storage could be dried for convenient use. Thus *M. oleifera*, a high β-carotene-containing, perennial plant that retains over 50 % of its β-carotene on storage (Glover-Amengor *et al*., 2012; Nambiar and Seshadri, 2001), has the potential for ensuring adequate supply of β-carotene in developing countries. Rural households mostly derive their nutrition from plant food sources which include cereals, legumes and vegetables (Babu, 2000). Studies that will assess the effects of these foods in humans as well as dietary innovations like fortification of local staples with *M. oleifera* leaves will inform the populace on how to make food choices.

1.3.1 Hypothesis

H_0 There will be no significant difference in vitamin A levels between the Intervention and Control groups at end of study.

H_1: There will be a significant difference in vitamin A levels of children supplemented with *M. oleifera* leaves and those not supplemented in Ada-East district of Ghana.

1.4 Objectives

1.4.1 Main Objective

To assess the efficacy of *M. oleifera* leaf supplementation on vitamin A status of children in Ada-East district of Ghana.

Specific Objectives

1. To describe the dietary uses of *M. oleifera* leaves in Ada-East district
2. To assess the acceptability of *M. oleifera* leaf-fortified dishes by children.
3. To assess the vitamin A status of children, before and after *M. oleifera* leaf consumption
4. To assess effect of *M. oleifera* leaf consumption on the haematological status of children
5. To assess adverse events associated with the consumption of *M. oleifera* leaves.

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CHAPTER TWO

2.0 LITERATURE REVIEW

Globally, acute malnutrition is an important risk factor for illness and death, with young children and pregnant women being the most vulnerable (Muller *et al*., 2003; FAO, 2011). Eight hundred and forty-two (842) million people worldwide are affected by inadequate food supply to meet energy requirements, out of which 827 million are from developing countries (FAO, IFAD and WFP, 2013). Malnutrition plays a role in more than half of all deaths of children worldwide, and children who survive malnourished childhood are intellectually and physically less productive, and are more likely to suffer from chronic illness and disability (Millward and Jackson, 2004). Malnutrition could therefore have a great economic toll on society (Millward and Jackson, 2004).

One type of malnutrition, often referred to as "hidden hunger", is that due to micronutrient deficiency. Micronutrient deficiency is prevalent particularly in developing countries, where limited economic resources affect diversity in diets. Iron, vitamin A, iodine and zinc deficiencies are the main manifestations of micronutrient malnutrition (UNICEF/WHO, 2004**)**. Zinc deficiency affects about 2 billion people mainly young children and pregnant women; 1 billion have iron-deficiency anaemia, while vitamin A deficiency (VAD) affects some 250 million, in developing countries (Black, 2003; Kwena *et al.*, 2003). Other reports indicate that 39 % of children under five years, and 48 % of children 5-14 years in developing countries are anaemic (Zimmermann, 2007). Half of the anaemia is reported to be due to iron deficiency whilst the rest of the causes accounting for the other half include nutritional deficiencies (such as those of folate, B_{12}, vitamin A and riboflavin) and infections (such as malaria, hookworm, HIV and

tuberculosis), hemoglobinopathies and ethnic differences in normal haemoglobin distributions. Hence diets that contain adequate levels of iron and these other nutrients could be beneficial in anaemia prevention (Zimmermann, 2007).

Unfortunately, due to little diversification as a result of food insecurity, diets in populations in developing countries often lack adequate amounts of micronutrients. This leads to specific micronutrient deficiencies (Black, 2003; Millward and Jackson, 2004). This situation is further worsened by the high prevalence of bacterial and parasitic diseases in developing countries (Millward and Jackson, 2004; Dickson *et al.*, 2000; Smith and Haddad, 2000). Malnutrition also increases one's susceptibility to infections, and severity of infections (FAO, 2004; Brabin and Coulter, 2003; Muller *et al.*, 2003). Both vitamin A deficiency (VAD) and anaemia are major public health problems in Ghana, just like most developing countries. Ghana's Ministry of Health (MOH, 1997) reported that 72 % of children under 5 years had low plasma retinol while "The Ghana Demographic and Health Survey" (GSS *et al.*, 2008) indicated that 78 % of children under five years were anaemic.

Interventions to address anaemia and other micronutrient deficiencies included nutrition education programmes, fortification and supplementation, and cultivation of exotic vegetables. Most of these interventions could not, however, be sustained because they are expensive. A probable approach to addressing these problems could be identification and promotion of indigenous micronutrient-rich foods that are adapted to the environment and are easy and cheap to produce. Currently, many reports have indicated that indigenous green leafy vegetables including *M. oleifera*, have the potential for meeting a lot of micronutrient requirements of

developing countries (Anwar *et al*., 2007; Babu, 2000; Raju *et al*., 2007; Sheela *et al*., 2004; Agte *et al*., 2000; Fahey, 2005).

2.1 Vitamin A

2.1.1 Vitamin A in human metabolic processes

Vitamin A or retinol is a fat-soluble vitamin that performs several functions in humans. It is needed in small amounts by humans for growth and development and for normal visual system performance as well as for maintenance of epithelial cellular integrity, reproduction and immune function (Villamor and Fawzi, 2005; Stephensen, 2001). In its aldehyde form, retinal, it is needed by the retina of the eye for both dim light vision and daylight vision. When in its irreversibly oxidized form known as retinoic acid, it acts as a hormone –like growth factor for epithelial and other cells (Solomons, 2001). In human diet, vitamin A is provided mainly as retinyl esters of fatty acids (its storage form) which is converted to retinol in the small intestine or as provitamin A carotenoids (FAO/WHO, 2001; Rodriguez-Amaya and Kimura, 2004).

2.1.2 Dietary sources

Vitamin A is found either preformed or as provitamin A carotenoids that could later be converted into the preformed state. In the preformed state, it is found in animal products such as liver, glandular meat, egg yolk, dairy products, fish-liver oils and human milk. It is also found in vitamin A fortified foods such as oil, flour, sugar, cereals, condiments and fats (Rodrigue-Amaya, 1997). Provitamin A carotenoids are found in yellow fruits and vegetables (e.g. pumpkins, squash, carrots, butternut, papaya, apricots, mangoes) and green leafy vegetables (e.g. amaranth, drumstick leaves (*M. oleifera*) and spinach (Sangeetha and Baskaran, 2010;

Rodriguez-Amaya and Kimura, 2004; Kanlakunta *et al*., 2008; Booth *et al*., 1992; Chandrashekhar and George, 1991). Red palm oil produced in many tropical and sub-tropical countries is also rich in provitamin A carotenoids (Zagre *et al*., 2003). Orange- fleshed sweet potatoes and yellow yams are also rich sources of provitamin A carotenoids (Bhaskarachary *et al*., 1995). Bioavailability of vitamin A from provitamin A foods tends to be less. Nevertheless, quantities retained after dehydration and cooking of some green leafy vegetables are high enough to provide sufficient amounts of retinol equivalents (RE/day) for all age brackets (Glover-Amengor *et al*., 2012; Oyugi-Nawiri, 2011; Seshadri *et a*l., 1997). Provitamin A foods are plant-based and are generally more affordable than animal source foods. This is the main reason why provitamin A foods are the chief source of vitamin A for developing countries, accounting for more than 80 % of their dietary vitamin A (Sangeetha and Baskaran, 2010).

2.1.3 Absorption and metabolism of preformed vitamin A and provitamin A carotenoids

Vitamin A in the diet consists of retinoids in animal tissues and provitamin A carotenoids from plants (Solomons, 2001). Beta (β)- carotene, the major provitamin A carotenoid yields two molecules of vitamin A on hydrolysis; all others (α-carotene and β-cryptoxanthin) yield one molecule of vitamin A and some other compound. Carotenoids in food are generally C_{40} compounds whilst retinoids are C_{20} compounds. The C_{20} retinoid in foods is mostly esterified to palmitic acid, but the C_{40} β–carotene is rarely esterified (Rodriguez-Amaya and Kimura, 2004; Solomons, 2001). Beta (β-) carotene is structurally made up of two molecules of vitamin A, so it is the most potent provitamin A carotenoid; it is also the most widely spread provitamin A carotenoid. Alpha (α-) carotene and β-cryptoxanthin, the other two provitamin A carotenoids exhibit only about 50 % vitamin A activity (Rodriguez-Amaya and Kimura, 2004).

Vitamin A is mainly ingested orally as retinyl palmitate which must be freed from its food matrix sequentially by mastication, emulsification and mixed miscelle formation through the action of bile salts (Solomons, 2001; Ong, 1994; Parker, 1996; Bloemhoff, 1991). The miscelles are transported into the intestinal cells, where the retinyl esters are moved across the mucosal membrane and hydrolysed within the cells. Retinol is re-esterified in the cells by intracellular binding proteins, retinol binding protein (RBP) into chylomicra which then enter the mesenteric lymphatic system and pass into systemic circulation (Solomons, 2001).

2.1.4 Factors affecting bioavailability of vitamin A

The food matrix in which provitamin A carotenoids are located is a major dietary factor that affects their bioavailability (Castenmiller and West, 1998). However once inside the intestinal wall, the provitamin A carotenoid may pass into the lymphatic circulatory system intact and retained until the cell is desquamated or it may be cleaved and converted to a retinoid, or metabolized into an inactive species. Mechanical homogenization or heat treatment enhance the bioavailability of carotenoids; ranges of 1-99 % absorption have been reported (Parker *et al*., 1999). The dietary fat required to enhance the bioavailability of carotenoids is 3-5 g per meal (van het Hof *et al*., 2000). Ong (1994) and Krinisky (1993) reviewed the bioconversion of provitamin A carotenoids to retinoids, and reported that β-carotene is cleaved to retinyl aldehyde via the 15-15-oxygenase enzyme in the intestine which leads to central cleavage with the formation of two molecules of retinyl aldehyde. The vitamin A status of the individual influences the bioconversion of plant carotenoids, and these have an inverse relationship which explains the

observation that one cannot reach toxicity levels with provitamin A carotenoid intake (Solomons, 2001; Wardlaw, 1999).

Vitamin A, packed in chylomicra aggregates of enterocyte lipoprotein, moves across the basement membrane of enterocyte into the mesenteric lymphatic system and via the thoracic duct into the systemic circulation. Liver cells (hepatocytes) take up newly absorbed retinyl esters which then follow the normal metabolism and degradation of chylomicra to their remnants (Ong, 1994). This process is faster in younger persons than in older persons. Transport of vitamin A from the hepatocytes may be to near-by fat storing stellate (Ito) cells or to the tissue sites where vitamin A is utilized in the body. This transport occurs primarily by way of shuttle of recently absorbed vitamin A from the liver as part of the retinol-binding protein-transthyretin complex (RBP-TTR**) (**Semba, 2001; Blomhoff *et al*., 1994**).**

2.1.5 Functions of vitamin A

The active forms of vitamin A perform 3 essential functions. These have to do with vision, cellular differentiation and immune response. Vitamin A functions in cones and rods of the retina of the eye for daylight and dim light vision. Retinoids also function in cellular differentiation. They regulate gene expression via nuclear retinoic acid receptors, RARs, based on proliferation and maturation of young cells (Solomons, 2001). They also play a very important role in reproductive tissues and mammalian embryogenesis that depend on RAR regulation of genetic expression (Solomons, 2001). Vitamin A further plays a crucial role in normal immune function and regulation. Vitamin A influences barrier immune function in the form of mucosal mucin secretion which is influenced by vitamin A dependent expression of synthetic enzymes for

glycosaminoglycans and epithelial glycoproteins (Ross, 1998). Another potentially important insight into vitamin A's role in immune function is its support of immunoglobulin A and the T helper cell 2 cytokine system in the small intestine (Semba, 1999).

2.2 Prevalence of VAD in the world

In the early 1990s, WHO estimated that about 3 million children had some form of xerophthalmia annually and, on the basis of blood levels, another 250 million were sub-clinically deficient (WHO, 1995). The actual number of sub-clinical deficiencies based on the prevalence of low serum levels of retinol, however, remains uncertain because of the confounding and poorly quantitative role of infections. Epidemiologic studies repeatedly report clustering of VAD that might probably result from concurrent occurrence of several risk factors (Katz, 1993).

Vitamin A deficiency can occur in individuals of any age, but it is more disabling and potentially fatal for children less than 6 years of age. Vitamin A deficiency related blindness is most prevalent in children under 3 years of age (Sommer, 1994). During this period there is high requirement for vitamin A to support early rapid growth and transition from breast-feeding to dependence on other dietary sources of the vitamin. Increased frequency of respiratory and gastrointestinal infections also necessitate high requirements for vitamin A. Increased mortality risk from concurrent infections affects children at least to 6 years of age and implicates both clinical and sub-clinical VAD (Beaton, 1993). Bitot's spots prevalence (i.e. white foamy patches on the conjunctiva) may be highest in school-age children, but may reflect past more than current history of VAD (Sommer, 1980).

Women of reproductive age are also vulnerable to VAD during pregnancy and lactation because they often suffer from night blindness (Blomhoff *et al.*, 1994; Christian, 1998) and frequently have low levels of vitamin A in their breast-milk (Miller *et al*., 2006; Radhika *et al*., 2003). Not all night blindness in pregnant women, however, responds to vitamin A (Christian, 1998). There is no clear indication in humans of a gender differential in vitamin A requirements during childhood (WHO, 1995). The additional vitamin A requirement in pregnant and lactating women is to support maternal and foetal tissue growth and lactation losses, which is not needed by other post-adolescent adults (National Academy Sciences, Food and Nutrition Board, Institute of Medicine. 1990).

2.3 Risk factors for VAD

About 90 % of ingested preformed vitamin A is absorbed, whereas the absorption efficiency of provitamin A carotenoids varies widely depending on the type of plant source and the fat content of the accompanying meal (Erdman, 1988). Increased dietary fat will likely improve the absorption of vitamin A from the diet. In VAD endemic areas, fluctuations in the incidence of VAD throughout the year reflect the balance between intake and need. Periods of general food shortage (and specific shortages in vitamin A–rich foods), peak incidence of common childhood infectious diseases (diarrhoea, respiratory infections, and measles), and periodic seasonal growth spurts affect the balance between intake and need. Seasonal food availability can influence VAD prevalence by directly influencing access to provitamin A sources; for example, the scarcity of yellow fruits and vegetables in the lean seasons followed by glut during harvest seasons (Marsh *et al*.**,** 1995). These seasonal variations are often reflected in seasonal growth spurts observed in children frequently following seasonal postharvest increases in energy and macronutrient

intakes. These increases, however, are usually obtained from staple grains (e.g., maize, rice) and tubers (e.g. cassava, light-coloured yams) which are not good sources of some micronutrients (e.g. vitamin A to support the growth spurt) (Sheela *et al*., 2004).

Culture-specific factors for feeding children, adolescents, pregnant and lactating women, food habits and taboos often affect consumption of potentially good food sources of vitamin A (e.g. eggs, mangoes and green leafy vegetables) (Guillermo and Garcia, 2013; Glover-Amengor *et al*., 2005; Johns *et al*., 1992; Zeitlan, 1992).

2.4 Consequences of VAD

2.4.1 Morbidity and mortality

The consequences of VAD are manifested differently in different tissues. In the eye, the symptoms and signs, together referred to as xerophthalmia, have until recently been the basis for estimating the global burden from the disease (Sommer, 1994). Ocular symptoms and signs are the most specific indicators of VAD, but they occur only after other tissues have impaired functions that are less specific and less easily assessed. The prevalence of ocular manifestations (i.e. xerophthalmia or clinical VAD) is now recognised to far underestimate the magnitude of the problem of functionally significant VAD. Sub-clinically deficient preschool-age children and perhaps older children and pregnant or lactating women have their health compromised. In young children, sub-clinical deficiency increases the severity of some infections, notably diarrhoea and measles, and the risk of dying (Beaton, 1993). The incidence (Barreto, 1994) and prevalence (Bhandari *et al*., 1994) of diarrhoea may also increase with sub-clinical VAD. Metaanalyses conducted by three independent groups using data from several randomised trials showed that community-based improvement of the vitamin A status of deficient children 6

months to 6 years of age reduces their risk of dying by 20–30 % on average (West, 2003; Beaton, 1993; Fawzi, 1993; Glasziou and Mackerras, 1993). Mortality in children due to blindness from keratomalacia or who have corneal disease is reported to be from 50 % to 90 % (Sommer, 1994; Menon and Vijayaraghavan, 1979) and measles mortality associated with VAD is increased up to 50 % (Hussey and Klein, 1990).

Infectious diseases depress circulating retinol and contribute to vitamin A depletion. Enteric infections may alter absorptive-surface area, compete for absorption-binding sites, and increase urinary loss (Alvarez, 1995; Solomons and Keusch, 1981; Feachem, 1987). Febrile systemic infections also increase urinary loss (Glasziou and Mackerras, 1993) and metabolic utilisation rates and may reduce apparent retinol stores if fever occurs frequently (Campos *et al*., 1987). In the presence of latent deficiency, disease occurrence is often associated with ocular signs (Curtale *et al*., 1995; Sommer and West, 1996). Measles virus infection in particular greatly affects vitamin A metabolism, adversely interfering with both efficiencies of utilisation and conservation (Hussey and Klein, 1990; Sommer and West, 1996; Foster and Yorston, 1992). Severe protein-energy malnutrition affects many aspects of vitamin A metabolism, and even when some retinyl ester stores remain, malnutrition, often coupled with infection, can prevent transport-protein synthesis, resulting in immobilization of existing vitamin A stores (van Jaarsveld *et al*., 2005; Thurber and Fahey, 2009).

Impairment of normal reproductive functions in animals occurs due to compromised integrity of the epithelium, together with the possible alteration in hormonal balance at severe levels of deficiency (Ross and Gardner, 1994; Eskild and Hanson, 1994; Bates, 1983). In animals and

humans, congenital anomalies can result if the foetus is exposed to severe deficiency or large excesses of vitamin A at critical periods early in gestation (first trimester) when foetal organs are being formed (Public Affairs Committee of the Teratology Society**,** 1987). Reproductive performance measured by infant outcomes in one community-based clinical intervention trial, however, was not influenced by vitamin A status (West, 1997).

The growth of children may be impaired by VAD. As VAD seldom occurs in isolation of other nutrient deficiencies that also affect growth and may be more limiting, interventions with vitamin A only have not consistently demonstrated improved growth in community studies (Underwood, 1994). A lack of vitamin A for example can affect iron metabolism when deficiencies of both nutrients exist particularly in environments prone to frequent infections (IVACG, 1998). Maximum haemoglobin improvement is obtained when iron and vitamin A deficiencies are corrected together (Suharno *et al*., 1993). Vitamin A deficiency influences the availability of storage iron for haematopoiesis (Suharno *et al*., 1993; Sijtsma, 1993).

2.5 Measurement of sub-clinical vitamin A deficiency

Several practical biochemical methods are used for estimating sub-clinical vitamin A status, but all have limitations (WHO/UNICEF, 1994; Underwood and Olson 1993; Underwood, 1990; Olson, 1992). Though each method is useful to identify deficient populations, none of these indicators is definitive or directly measures disease occurrence. The indicators of choice are listed in Table 1. These indicators are less specific to VAD than clinical eye signs and less sensitive for measuring sub-clinical vitamin A status. WHO recommends the measurement of at least two sub-clinical biochemical indicators, or one biochemical and a composite of non-

biochemical risk factors, both of which should point to deficiency in order to identify populations at high risk of VAD (WHO/UNICEF, 1994). Cut-off points given in Table 1 represent the consensus reached comparing populations with some evidence of VAD with those without VAD.

Table 1: Biochemical indicators of sub-clinical VAD in mothers and in children 6–71 months of age (WHO, 1996).

Indicator	**Cut-off to indicate deficiency**
Night blindness (24-71) months	≥ 1 % report of a history of nightblindness
Biochemical:	
Breast milk retinol	≤ 1.05 µmol/l (≤ 8µg/g milk fat)
Serum retinol	≤ 0.70 µmol/l
Relative dose response	≥ 20 %
Modified relative dose response	Ratio ≥ 0.06

The current biochemical indicator of choice, however, for population assessment is the distribution of serum retinol levels (de Pee and Dary, 2002). It is only at very low blood levels (< 0.35 µmol/l) that there is an association with corneal disease prevalence (Sommer and Muhilal, 1982). Blood levels between 0.35 and 0.70 µmol/l are likely to indicate sub-clinical deficiency, but sub-clinical deficiency may still be present at levels between 0.70 and 1.05 µmol/l and occasionally above 1.05 µmol/l (Flores, 1984). The prevalence of serum vitamin A values below 0.70 µmol/l is a generally accepted population cut-off for preschool-age children to indicate risk of inadequate vitamin A status (WHO/UNICEF, 1994**)** and above 1.05 µmol/l to indicate an

adequate status (Flores, 1991; Pilch, 1987). Clinical and sub-clinical infections can lower serum levels of vitamin A on average as much as 25 % independently of vitamin A intake (Christian *et al.*, 1998; Filteau *et al*., 1993).

Table 2: Available supply of vitamin A by WHO Region (ACC/SCN, 1993)

Region	Total (µg RE/day)	Animal sources (µg RE/day)	Vegetable sources (µg RE/day)
Africa	775	122	654 (84)[a]
Americas	814	295	519 (64)
Southeast Asia	431	53	378 (90)
Europe	738	271	467 (63)
Eastern Mediterranean	936	345	591 (63)
Western Pacific	997	216	781 (78)
Total	782	212	565 (72)

[a] Numbers in parenthesis indicate the percent of total retinol equivalents from carotenoid food sources

Table 3: Estimated mean requirement and safe level of intake for vitamin A (FAO/WHO, 1988)

Age Group	Mean Requirement (µg RE/day)	Recommended safe intake (µg RE/day)
Infants and Children		
0-6 months	180	375
7-12 months	190	400
1-3 years	200	400
4-6 years	200	450
7- years	250	500
Adolescents, 10-18 years	330-400	600

2.6 Toxicity

Vitamin A is fat soluble and can be stored, primarily in the liver, so routine consumption of large amounts of preformed vitamin A over a period of time can result in toxic symptoms, which may include liver damage, bone abnormalities and joint pain, headaches and vomiting, and skin desquamation (Russell, 2000; Wiegand *et al.*, 1998). Hypervitaminosis A may be due to abnormal transport and distribution of vitamin A and retinoids caused by overloading of the plasma transport mechanisms (Smith and Goodman, 1976). Hathcock (Hathcock, 1990; Hathcock, 1997) reported an association betwwen daily consumption of 7500 µg vitamin A taken for 6 years and liver cirrhosis. He indicated that very high single doses can also cause transient acute toxic symptoms that may include bulging fontanels in infants; headaches in older children and adults; and vomiting, diarrhoea, loss of appetite, and irritability in all age groups. Toxicity however, rarely occurs from ingestion of food sources of preformed vitamin A, unless there is frequent consumption of liver products. Toxicity from food sources of provitamin A carotenoids is not reported except for the cosmetic yellowing of skin (Wardlaw, 1999).

2.7 Vitamin A, haematological indices and infections

Several human observational and interventional studies have suggested a positive biological association between vitamin A and iron. This association is important in interventional programmes as nutritional anaemia and vitamin A deficiency are the most prevalent nutritional problems in developing countries (ACC/SCN, 1992). At least 50 % of the anaemia world-wide is due to dietary iron deficiency (ACC/SCN, 1992**)**, whilst both clinical and sub-clinical vitamin A deficiency have been estimated at 254 million (WHO, 1995).

In a study with human volunteers who ate a diet very low in vitamin A for 359 – 771 days (Hodges *et al.*, 1978), a gradual anaemia developed several months from the onset of the study that did not respond to medicinal iron. They also found a decline in serum iron, but not in total iron-binding capacity. All patients had a prompt and complete haematological recovery after repletion with β-carotene or vitamin A. In a review, Mejia *et al.*, (1997) found a positive correlation between serum-retinol and haemoglobin in children 5 - 12 years old. No correlation, however, was found between iron and serum-retinol in these children. Similarly, Hodges *et al.*, (1978) reviewed statistical correlations between vitamin A nutrition and anaemia in regions where iron intake was ≥ 14 mg/d whilst vitamin A intake was low or marginal. The data was collated on non-pregnant and non-lactating women aged between 15 – 41 years. They did not find any relationship between haemoglobin and iron intake but a strong relationship existed between serum vitamin A and haemoglobin.

Several other observational studies also indicated a positive association between serum retinol and haemoglobin (Bloem *et al.*, 1989; Suharno *et al.*, 1993). Bloem *et al.*, (1989) found a significant association between serum retinol, packed cell volume, serum iron, ferritin, transferrin and saturation of transferrin. They however, did not find any correlation between haemoglobin and serum retinol. In an interventional study too, the researchers found significant increases in levels of haemoglobin, packed cell volume and serum iron after vitamin A supplementation (Mohanram *et al.*, 1977). Mejia and Chew (1988) supplemented four groups of anaemic children for 2 months with vitamin A, Fe, vitamin A plus Fe or a placebo. They reported that vitamin A supplementation produced significant increases in serum retinol levels and iron, haemoglobin, packed cell volume, erythrocyte count and percentage transferrin saturation, but

had no effect on total iron-binding capacity or serum ferritin. Supplementation with vitamin A and iron together gave a higher level of serum iron. The results led them to conclude that the primary effect of vitamin A supplementation is an increase in serum iron levels.

Bloem *et al*., (1989) in a high-dose vitamin A trial in anaemic children in Thailand showed that there were increases in serum iron and transferrin saturation after 2 months supplementation but not after 4 months. They did not observe any significant change in haemoglobin levels. They concluded that seasonal variations in nutrient intake might have lead to an improvement in the nutritional intake of the control group as well, after the lean season. Zimmermann *et al*., (2006) measured the effect of vitamin A supplementation on haemoglobin, iron status and circulating erythropoietin (EPO) in children with poor iron and vitamin A status. They concluded that in children deficient in vitamin A and iron, vitamin A supplementation mobilised iron from existing stores to support increased erythropoiesis, an effect likely mediated by increases in circulating EPO. Walczyk *et al*., (2003), however, did not find any enhancing effect of vitamin A supplementation on iron absorption in humans in five studies using young adult human beings. They concluded that it is possible that the effect of vitamin A on iron could be observed in people with impaired vitamin A status.

Table 4: Haemoglobin and haematocrit cut-offs used to define anemia in people living at sea level (WHO/UNICEF/UNU (2001).

Age or sex group	Haemoglobin below g/dL	Haematocrit below (%)
Children 6 months to 5 years	11.0	33
Children 5–11 years	11.5	34
Children 12–13 years	12.0	36
Non-pregnant women	12.0	36
Pregnant women	11.0	33
Men	13.0	39

A number of factors may lead to reduction in haemoglobin levels, and these may include iron deficiency, malaria, hookworm and other helminthic infections, chronic diseases and other nutritional deficiencies (West *et al.*, 2007; Glover-Amengor *et al.*, 2005; Dreyfuss *et al.*, 2000; Brooker *et al.*, 1999). Although iron deficiency results from a depletion of the body's iron stores and plasma levels, iron deficient haematopoiesis could also occur if normal or elevated iron stores are sequestered as a result of inflammation from infection (Means, 2000; Semba *et al.*, 2001) or chronic disease (Cook, 2005). Several studies have shown that both symptomatic and asymptomatic malaria contribute to anaemia in African children (Artemis *et al.*, 2008). Malaria causes suppression of haematopoiesis and in addition, a *Plasmodium falciparum*-infected erythrocyte undergoes haemolysis easily (Caulfield *et al.*, 2004).

Associations have also been reported between xerophthalmia and several preceding acute illnesses including diarrhea, pneumonia and measles (Fawzi *et al.*, 2000; SanJoaquin and

Molyneux, 2009). Sommer *et al*., (1987) reported from a prospective study that children who had suffered from diarrhea or respiratory disease were more likely to develop xerophthalmia subsequently, suggesting that a reduction in serum retinol concentration may follow a variety of infections presumably mediated by a common host response. Several studies have also reported an association between malaria infection and low serum-retinol levels. A study in Malawi found that 92 % of 247 severe anaemia cases (mostly severe malarial anaemia) had low serum retinol levels as against 66 % of 262 controls (Calis *et al*., 2008). Filteau *et al*., (1993) and Stephensen *et al*., (1994) also reported an inverse relationship between serum retinol concentration and malaria parasite density. In a case control study conducted by Galan *et al*., (1990), they found that 37.5 % of 454 preschool children suffering from malaria had plasma retinol levels lower than 10 µg/dl. They found a significantly lower mean serum retinol levels in those suffering from malaria than in the control groups. Similar findings were reported by Raza *et al*., (2009), Davis *et al*., (1994) and Thurnham and Singkamani (1991; Campos *et al*., 1987). Ascaris lumbricoides infection has also been reported to affect serum retinol levels negatively (Jalal, 1991; Jalal *et al*., 1998; Caufield *et al*., 2004).

Vitamin A deficiency is important in anaemia because of the role vitamin A plays in the absorption, storage, release or transport of iron to the bone marrow and may therefore increase the risk of iron deficient erythropoiesis and anaemia (Hashizume *et al*., 2005). Vitamin A is also required for both innate and acquired immune response. It is required for maintaining the integrity of the epithelia and its deficiency is associated with alterations in the ocular, respiratory, gastrointestinal and genitourinary epithelial tissues (Semba and Bloem, 2002; Villamor and Fawzi, 2005). Retinoic acid receptor-modulated genes control the development of neutrophils in

the bone marrow, and T-cell immune competence can be affected by VAD at various levels, including lymphopoiesis (Villamor and Fawzi, 2005). Hence any intervention that controls for vitamin A deficiency, has the potential to control anaemia that is caused both by infection and malnutrition (West *et al*., 2007). Anaemia reduces growth rate and impairs cognitive performance. The Ghana Demographic and Health Survey (GSS *et al*., 2008) indicated that 78 % of children under five years were anaemic. Intake of poorly utilized cereals, lack of food diversification and ignorance of plant-based foods rich in iron and vitamin A could contribute to the causes of anaemia in Ghanaian children.

Table 5: Haemoglobin levels to diagnose anaemia at sea level (g/l) (FAO/WHO, 1992; WHO/ UNICEF/ UNU, 2001)

	Anaemia*			
Population	**Non-anaemia***	**Mild**	**Moderate**	**Severe**
Children 6-59 months of age	110 or higher	100-109	70-99	Lower than 70
Children 5-11 years of age	115 or higher	110-114	80-109	Lower than 80
Children 12-14 years of age	120 or higher	110-119	80-109	Lower than 80
Non-pregnant women (15 years of age and above)	120 or higher	110-119	80-109	Lower than 80
Pregnant women	110 or higher	100-109	70-99	Lower than 70
Men (15 years of age and above)	130 or higher	110-129	80-109	Lower than 80

* Haemoglobin in grammes per litre

2.8 *Moringa oleifera*

M. oleifera is a β –carotene rich, tropical green leafy vegetable that has been reported in many journals as a plant with a high potential for meeting the vitamin A needs of developing countries (Asaolu and Omotayo, 2007; Glover-Amengor and Mensah, 2012). In addition to vitamin A, (β –carotene), *M. oleifera* also contains vitamins B, C, E and minerals such as calcium, iron, and zinc, among others. It could therefore be referred to as a natural multivitamin (Fuglie, 1999). Reports indicate that *M. oleifera* is prescribed for anaemia in the Philippines (Mathur *et al*., 2005). Being a plant that readily establishes in tropical and sub-tropical climates (Selvam and Dhayala, 2005), it could be easily cultivated in backyards and around villages where it could be harvested at any time.

Figure 2: *Moringa oleifera* plant

2.8.1 Uses of *M. oleifera*

M. oleifera leaves and tender pods are used as vegetable in Haiti, India (Price, 2000), Senegal and many other tropical countries; the plant is also used as forage for livestock in the tropics (Luu *et al*., 2005; Foidl *et al*., 1999). The leaves are pounded and used for cleaning utensils and walls. The wood yields a blue dye that is used in the textile industry while the wood pulp is used for paper production (Fuglie, 1999). In folk medicine (Anwar *et al*., 2007; Goyal *et al*., 2007; Fahey, 2005; Moorthy *et al*., 2002), *M. oleifera* flowers, leaves and roots are used as remedies for many ailments including tumors. Leaves are ground and used as poultice on sores, rubbed on the temple for headaches, whilst the root juice is applied externally as a counter-irritant (Fahey, 2005). Its root, bark and leaves are taken as infusions, teas and decoctions to promote digestion (Fahey, 2005). *M. oleifera* seed oil is applied externally to cure skin diseases, and in the Philippines *M. oleifera* is prescribed for anaemia (Fuglie, 1999). In Senegal and Benin, *M. oleifera* leaves are dispensed as powder at health facilities to treat moderate malnutrition in children (Kasolo *et al*., 2010). Verma *et al*., (2009) assessed the antioxidant properties of *M. oleifera* in vivo and in vitro and concluded that *M. oleifera* leaf possessed high phenolic content and potent antioxidant properties, whilst Sulaiman *et al*., (2008) investigated its anti-inflammatory effect in a rat model, and found it to have anti-inflammatory properties.

2.8.2 Nutrient Content of *M. oleifera*

Several workers have evaluated the nutritional composition of *M. oleifera* leaves. Fuglie (1999) reported β –carotene levels of 18.9 mg/100 g in dried *M. oleifera* leaf powder whilst Glover-Amengor and Mensah, (2012) reported 31 mg/100 g (dry weight (DW)). Protein levels in the dry leaves were found to range from 17.01 % to 30.29 % (Glover-Amengor and Mensah, 2012;

Moyo *et al*., 2011; Ogbe and Affiku, 2011; Jongrungruangchok *et al*., 2010; Oduro *et al*., 2008 and Fuglie, 1999). It, however, has low fat and carbohydrate levels. The plant also has high calcium and iron content but it is low in phosphorus and oxalates (Fuglie, 1999). *M. oleifera* leaves are also rich in the sulphur- containing amino acids methionine and cystine that are often not found in abundance. The leaves have negligible amounts of tannins whilst trypsin and amylase inhibitors, lectins, cyanogenicglucosides and glucosinolates were not detected in the leaves (Yang *et al*., 2006; Makkar and Becker, 1997; Asaolu and Omotayo, 2007). Anaysis of *M. oleifera* pods, fresh (raw) leaves and, dried and milled leaves (leaf powder) have shown them to contain the following per 100 g of edible portion (Fuglie, 1999) as shown in Table 6.

Table 6: Nutrient content of *M. oleifera* pods, fresh (raw) leaves and leaf powder (per 100 g of edible portion, Fuglie, 1999).

Parameter	Pods	Leaves	Leaf Powder
Moisture (%)	86.9	75.0	7.5
Calories	26.0	92.0	205.0
Protein (g)	2.5	6.7	27.1
Fat (g)	0.1	1.7	2.7
Carbohydrate (g)	3.7	13.4	38.2
Fiber (g)	4.8	0.9	19.2
Ca (mg)	30.0	440.0	2003.0
Mg (mg)	24.0	24.0	368.0
P (mg)	110.0	70.0	204.0
K (mg)	259.0	259.0	1324.0
Fe (mg)	5.3	7	28.2
Vitamin A - B carotene (mg)	0.1	6.8	18.9
Vitamin B -choline (mg)	423.0	423.0	-
Vitamin B_1 -thiamin (mg)	0.05	0.21	2.64
Vitamin B_2 -riboflavin (mg)	0.07	0.05	20.5
Vitamin B_3 -nicotinic acid (mg)	0.2	0.8	8.2
Vitamin C -ascorbic acid (mg)	120.0	220.0	17.3
Vitamin E -tocopherol acetate (mg)	-	-	113.0
Cu (mg)	3.1	1.1	0.57
Selenium (mg)	-	-	0.09
Zinc (mg)			3.29
Sulfur (mg)	137	137	870
Phytate (g)		2.1	

Table 7: *M. oleifera* leaves compared to common foods (Fuglie, 1999)

Foods (Values per 100g edible portion)					
Nutrient	*M. oleifera* Leaves	Carrots	Oranges	Cow's Milk	Bananas
Vitamin A	6780 µg retinol eq.	1890 µg retinol eq.			
Vitamin C	220 mg		30 mg		
Calcium	440 mg			120 mg	
Potassium	259 mg				88 mg
Protein	6.7 gm			3.2 gm	

M. oleifera leaves are compared to some common food sources of micronutrients (Table 7). (www.treesforlife.org/moringa/book). From Table 7, it could be seen that *M. oleifera* leaves could serve as an alternative source of micronutrients to carrots, oranges, *etc*. in tropical countries.

Table 8: Comparison of the nutritional composition of spirulina powder and *M. oleifera* leaf powder (de Saint Saveur and Broin, 2010)

Element	***Spirulina platensis***	***Moringa oleifera***
Moisture (%)	3	7
Digestible proteins (g)	40	20-26
Potassium (mg)	1400	800-1800
Calcium (mg)	700	1600-2200
Phosphorus (mg)	800	200-600
Magnesium (mg)	400	350-500
Iron (mg)	100	18-28
Vitamin A (µg retinol eq.)	7000	4000-8000
Vitamin C (mg)	0	15-100
Riboflavin (µg)	3500	8800
Nicotinamide (µg)	4000	10400

It could be seen from Table 8 that *M. oleifera* leaves contain micronutrient levels that compare favourably to the levels in spirulina, a micronutrient-rich weed. In addition, they contain quite a high level of vitamin C which is missing in spirulina.

2.8.3 Bioavailability of β-carotene from *M. oleifera*

Yang *et al.*, (2006) demonstrated that total carotene and β-carotene retention was 46-63 % during pressure cooking of *M. oleifera* leaves without oil whilst the addition of oil resulted in 76-99 % retention. Nambiar and Seshadri (2001) also reported that administration of both fresh and dehydrated *M. oleifera l*eaves improved retinol levels significantly over the baseline levels in a rat model. There was also observed improved growth parameters in the *M. oleifera* fed rats as against those fed on synthetic vitamin A. The synthetic vitamin A-fed group, however, showed higher retinol levels over the *M. oleifera* groups. This led to the conclusion that in developing countries, provitamin A containing plants such as *M. oleifera* could help in solving vitamin A deficiency problems.

Nambiar *et al.*, (2003**)** assessed the feasibility and acceptability of introducing dehydrated *M. oleifera* leaves as a source of vitamin A among 40 pre-school children in an integrated child development programme. They incorporated into food, 5-7 g dehydrated leaves /100 g product which was fed to children 1-5 years for one month. Although this study did not assess the vitamin A status of the children, they observed high compliance and concluded that it was feasible to introduce *M. oleifera* leaves into the feeding programme and for a longer duration.

2.9 Botany

M. oleifera is one of about thirteen species in the *Moringaceae* family. Native to the southern foothills of the Himalayas in northwestern India, *M. oleifera* is now grown in Central and South America, Africa, and other tropical, sub-tropical and semi-arid regions of the world. *M. oleifera* has many useful parts (Babu, 2000; de Saint Saveur and Broin, 2010). It is a perennial, short,

slender and deciduous tree that grows to about 10m in height, with a brittle stem and drooping branches. *M. oleifera* leaves are 30-60 cm long with leaflets that are many and 1.3-2 cm long and 0.3-0.6 cm wide; leaves are also feathery, pale green and, compound. Its stem bark is corky whilst the main root is thick. *M. oleifera* produces creamy flowers. Its fruits are pod-like and are brownish when dry. The fruits split lengthwise into three to release seeds that have three papery wings. The pods are usually 30-120 cm long (Ramachandran *et al*., 1980).

2.10 Agronomy

The *M. oleifera* plant can be propagated either by seeds or by cuttings (mature or brown wood) (Selvam and Dhayala, 2005). Seeds could be sown directly in the field, in seedbeds or in containers, but direct sowing is the most desirable because the seeds establish very well, and this method also eliminates the burden of transplanting from seedbeds (de Saint Saveur and Broin, 2010). It produces leaves throughout the year. *M. oleifera* grows very well in tropical and sub-tropical climates at a height of 0-2000 metres and temperature of 25-35 ^{o}C (Luu *et al.*, 2005) and, -1 ^{o}C - 3 ^{o}C (Palada and Chang, 2003). The desired soil type for its cultivation is loamy, sandy or sandy-loam and a rainfall range of 250 mm-2000 mm. The required pH range is 5-9 (slightly acidic to slightly alkaline) (Selvam and Dhayala, 2005). *M. oleifera* trees flower and fruit annually and even twice a year in some regions, so seeds are available annually for propagation (Goyal *et al*., 2007). The plant is reported to be tolerant to bacteria, drought, fungus and mycobacteria (organisms that cause plant diseases in the tropics), and could therefore be easily cultivated in the tropics (de Saint Saveur and Broin, 2010). *M. oleifera* could, therefore, be a readily available backyard tree in homes where it will provide nutrient - rich leaves for household nutrition (Luu *et al.*, 2005; de Saint Saveur and Broin, 2010).

CHAPTER THREE

3.0 Methods

3.1 Study area

The study was conducted in Ada-East district of Ghana. The Ada-East district lies in the coastal savannah area and is in the eastern part of Greater Accra region and stretches from Hwakpo along the Accra - Aflao road to Kewunor. It is boarded on the north by North Tongu district, on the west by Ada-West and on the east by South Tongu district. To the south is the Gulf of Guinea. The district has a total land area of 463 square kilometers. According to the Population and Housing Census of 2010 (PHC, 2010), the population of Ada-East district was 71,671 people, with 37,659 (52.6 %) being females, and 34, 012 (47.4 %) being males. Ada-Foah, the district capital is about 22 kilometers off the Accra - Aflao road. There are 43 primary schools and 31 kindergartens in the district. Predominantly, the indigenous people in Ada-East district are farmers and fishermen. Most of them being peasant farmers, engage directly in the production of onions, pepper, tomatoes and cassava. Majority of the youth are diverting into the cultivation of onions on a large scale. This has intensified the sprinkler system of irrigation farming in the district (Ada-East District Assembly, 2013).

Temperatures are high in the district throughout the year and range from 23 ^{o}C to 28 ^{o}C with a maximum of 33 ^{o}C during the very hot periods. It experiences high rainfall during the rainy seasons (March to September) with an average of about 750 mm (Ada-East District Assembly, 2013). The district is a malaria endemic area. Other common diseases in the area are diarrhoeal diseases. Immunisation coverage in the district is above 80 %; vitamin A supplementation coverage is also above 80 % (District Health Annual Report, 2012).

Figure 3: Map of Ada-East district

3.2 Study design

The study is made up of three parts: Assessment of the uses of *M. oleifera* leaf as a food ingredient in Ada-East district (Part I); Acceptability and feasibility study of *M. oleifera* leaf-fortified dishes (Part II) and Supplementation of children with *M. oleifera* leaf powder (Part III). All Parts of the study were undertaken in one study area called Ada-East district.

Ada-East district was chosen for the study because it was one of the coastal districts of Ghana for which 79.3 % of children under five years had been reported to be vitamin A deficient (WHO, 2000). Study Part I involved data collection on the dietary uses of *M. oleifera* leaf in the district, as well as other vegetables, fruits and staples. Part II involved tasting various *M. oleifera* leaf-fortied dishes by school children to determine their acceptability, while Part III was an assessment of serum retinol levels, haematological indices and, liver and kidney chemistry in school children supplemented with dried *M. oleifera* leaves.

3.2.1 Study Part I design

The study was a cross-sectional one in which data was collected on dietary uses of *M. oleifera* leaves in Ada-East district. Percentages were generated from the data and presented as bar charts.

3.2.2 Variables measured

Dietary uses of *M. oleifera* leaves in Ada-East district. Other vegetables and fruits eaten in the district and their frequency of consumption. Staples eaten and their frequency of consumption.

3.2.3 Sampling

3.2.3.1 Study population

The study subjects were mothers/caregivers, aged 19 years and above, selected from the study area.

3.2.3.2 Inclusion and exclusion criteria

Eligible candidates were mothers/caregivers of households who resided permanently in the community. Visitors in households were excluded.

3.2.3.3 Sample size

The most important indicator that was measured was uses of *M. oleifera* leaf as a food ingredient in the district. It was estimated that this proportion would be 15% (Ghana Nutrition Survey, 2012).

$n > (Z_{\alpha/2})^2 \, p \, (1-p) / d^2$

- $Z_{\alpha/2} = 1.96$
- p = proportion of interest = 15% (Ghana Nutrition Survey, 2012).
- d^2 = margin of error = 5%

Based on this assumption, a sample size of 197 was calculated (Kirkwood and Sterne 2003).

3.2.3.4 Sampling methods/procedure

Sampling was done by simple randomization. List of communities in the district and household numbers were obtained from District Health Administration (DHA) and Ghana Statistical Service. There are 83 communities in the district, and eight (8) of these were selected by simple random method. Sheets of paper were cut and the names of the commuinties written on them. These were folded, mixed thoroughly in a box and drawn one by one. For each selected

community, the household numbers were written on pieces of paper, starting with one until the last household number for that community. The pieces of paper were folded individually and tightly, put into a box and mixed thoroughly. Samples were drawn one at a time from the box until the number required for that community was attained. The pieces of paper were unfolded, and the households whose number have been picked were interviewed. Probability proportionate to size (PPS) was used to determine the number of households for each selected community.

3.2.4 Data collection technique/method and Tools/instruments

3.2.4.1 Ethical clearance

Ethical clearance was obtained from the Institutional Review Board (IRB) of the Noguchi Memorial Institute for Medical Research (NMIMR). A consent form was developed that clearly spelt out the purpose for which the data was being collected; it elaborated on the benefits of the results of the study to the community, and further indicated that the study will not infringe on the privacy of the respondent. The form was read and explained in the local language (Ada) after which those willing to participate appended their signature or thumb-printed. Before commencement of study in the district, permission was sought from the district assembly, Ghana Health Service and Ghana Education Service. Introductory letters from the Department of Epidemiology and Disease Control, School of Public Health, University of Ghana, were presented to these stakeholders. The chiefs of the selected communities granted permission for the study to be carried out. Permission was also sought from household heads before commencement of the interview.

3.2.4.2 Training of interviewers

Ten university, polytechnic and SHS graduates were selected for training in questionnaires administration. Selected interviewers were those who could speak both English and the local (Ada) language. The questions were read and explained to them. They were asked to administer the questionnaires on each other in both English and the local language after which all ambiguities were corrected.

3.2.4.3 Pre-testing

The questionnaires were pretested on twenty women in Ada-East district who did not constitute part of the final sample, by three interviewers.

3.2.4.4 Data collection

Structured questionnaires were used for data collection. The questionnaires were developed and pre-tested before the main survey work. The questionnaires were administered by trained personnel who were able to translate the questions into the local language.

a) Interview - vegetables and fruit intake

Respondents were asked to mention all the vegetables and fruits consumed in their locality. They were then asked if they knew *M. oleifera* and were further asked to identify *M. oleifera* from a number of green leafy vegetables presented. The vegetables were cocoyam (*Xanthosoma sagitifolium*) leaves, garden eggs (*Solanum melongena*), okro (*Abelmoschus esculentus*), fresh/dried pepper (*Capsicum annum*), onions (*Allium cepa*) (large/small), tomatoes (*Lycopersicum esculentus*), amaranth (*Amaranthus hybridus*), tossa (*Corchorus olitorius*), African spinach, (*Talinum traingulare*), leafy eggplant (*Solanum marcrocarpon),* and drumstick

(*M. oleifera).* Respondents were further asked to give the local name of *M. oleifera*. They were also asked to indicate the preferred form in which they would like to use *M. oleifera* as a food ingredient and also indicate any other uses of *M. oleifera* leaves apart from dietary use.

b) Interview - staples intake

Respondents were asked to indicate the types of staples they consume in the district from a list of staples provided. These included maize, rice, cassava, yam and plantain.

3.2.4.5 Quality control

The interviewers were trained and the questionnaires were pretested before administration to ensure that each interviewer understood what was to be done. Each interviewer was allocated specific households by the researcher. After collection, the data were cross-checked in the field and any necessary corrections made before leaving the field. In addition, the researcher randomly picked 5 questionnaires and re-administered them in the households to ensure that the data were correctly collected.

3.2.5. Data processing and analysis

Data were entered into Excel spreadsheet, and same was used for the analysis. Data were reported as percentages and presented as bar charts.

3.2.6 Limitations of study

Communities described as hard to reach or 'overseas' areas were not accessible during the time of data collection

Study Part II design

The study was a cross-sectional one in which children tasted *M. oleifera* leaf-fortified dishes for their acceptability.

3.3.1 Variables measured

Taste

3.3.2 Sampling

3.3.2.1 Study population

These were children aged 4-12 years enrolled in the school used for the acceptability testing.

3.3.2.2 Inclusion and exclusion criteria

School children aged 4-12 years, present in school on the day of testing. In addition, the child's caregiver would have given his/her ascent for participation in the study. Children aged more than 12 years were excluded.

3.3.2.3 Sample size

The sample size was estimated, based on a feasibility study conducted by Nambiar *et al*., (2003). Thirty-eight (38) children were estimated to taste the dishes, and each child was to taste three (3) dishes.

3.3.2.4 Sampling methods/procedure

The school for the study was purposively selected in a village called Luhuese because it had a kindergarten and was also participating in the Ghana Government School Feeding Programme

(GSFP). Class registers were pooled together from kindergarten to class three to obtain a sampling frame. All eligible children (aged 4-12 years), present in school on the day of testing and whose caregivers had given ascent to their participation (N=101) were listed. Since the sample size was thirty-eight (38), 38 "Yes" and 63 "No" were written on pieces of paper, folded well and mixed thoroughly in a box. All children who picked "Yes" were admitted into the study, while "No" was rejected.

The various dishes were coded with three digit random numbers generated with Excel. The children's names were written, but each was given an alphabetical code only known to the research team. Given that each child will taste three (3) dishes only, the number of children that tasted each dish was generated with Excel Stat.

3.3.3. Data collection technique/method and Tools/instruments

3.3.3.1 Ethical clearance

Ethical clearance was obtained from the Institutional Review Board (IRB) of the Noguchi Memorial Institute for Medical Research (NMIMR). An ascent form was developed that clearly spelt out the purpose of the study; it elaborated on the benefits of the results of the study to the community and further indicated that the study will not be harmful to the participants. It also stated that the privacy of the respondent will be respected. Before commencement of study in the district, introductory letters from the Department of Epidemiology, School of Public Health, University of Ghana, Legon, were presented to the district assembly, Ghana Health Service and Ghana Education Service.

After the chief of the selected community granted permission for the study to be carried out, the form was read and explained to the teachers in the school, who also explained it to the parents, and those willing to allow their children to participate, appended their signature or thumb-printed.

3.3.3.2 Data collection

Score sheets (smileys) were used to assess acceptability of *M. oleifera* leaf-fortified dishes using a 5-point hedonic scale (Appendix 21).

***a) M. oleifera* leaf preparation**

Leaves were harvested from a field in the premises of the Methodist church at Dansoman in Accra. About forty kilogrammes of fresh leaves were collected per replication. The leaves were transported to the processing hall of Council for Scientific and Industrial Research-Food Research Institute (CSIR-FRI) where they were washed first in potable water, then in 1 % saline for three minutes to reduce the microbial load, and again in potable water (FDGS 998). These leaves were then thinly spread on aluminium trays and dried between 35 ^{o}C - 55 ^{o}C in a solar dryer for 5 hours. Leaves were sampled randomly from the field for three times at two weeks intervals (three replications.). Moisture, pH, mineral and β-carotene levels were determined in the dried leaves (Appendix 2). The replicates were thoroughly mixed together and then milled with a locally fabricated stainless steel hammer mill (0.8 mm particle size); the milled product was put in clean polythene bags and kept at room temperature (27 ^{o}C -28 ^{o}C), in a wooden cupboard.

b) **Incorporation of *M. oleifera* leaf powder into various local dishes**

Various local dishes that included *ofam* (baked ripe plantain-roasted maize meal blend), beans with *gari* (roasted cassava grits), *waakye (*boiled red cowpea and rice); groundnut soup, porridge (composite white maize, groundnut and white cowpea meal), *nkontomire* (cocoyam leaves) sauce, *jollof* rice and *apapransa* (boiled red cowpea in roasted white maize meal) were standardized, fortified with *M oleifera* leaf powder at three levels in the test kitchen of CSIR-Food Research Institute (CSIR-FRI) (Appendix 20) The dishes, eight in all, were then individually assessed in-house for sensory attributes of taste, colour, texture, appearance, flavour, aftertaste and overall acceptability on a 9-point hedonic scale by ten semi-trained, adult panelists (Appendix 1). After in-house acceptability testing, 2 g and 3 g levels were selected for child acceptability test as a multiple range analysis of the sensory attributes showed that increased concentration of *M. oleifera* leaf powder significantly affected the colour of some of the dishes, and in some cases, appearance, taste, aftertaste and flavor (Table 9). The dishes were up-scaled to provide the needed quantity for acceptability testing. After child acceptability and feasibility tests, three dishes – porridge, *waakye* and groundnut soup were selected for the efficacy trial. These were dishes most preferred by the children (Table 12). These dishes were then fortified with *M. oleifera* leaf powder at 3 g/100 g and their micronutrient levels were assessed. Micronutrient levels of non-fortified dishes were also assessed (Table 11).

c) **Acceptability test by school children**

Thirty-eight (38) children, aged 4-12 years assessed eight (8) dishes (but nine samples) comprising both sweet (porridge and *ofam*) and salty dishes (*waakye*, *jollof* rice, *apapransa*, beans with *gari*, groundnut soup and *nkontomire* sauce (fortified at 2 g/100 g and also 3 g/100 g),

for general acceptability using score sheets with facial expressions (smileys) depicting a 5-point hedonic scale of "Like Extremely" to "Dislike Extremely". In the exception of *nkontomire* sauce which was fortified at 2 g/100 g and 3 g/100 g, all other dishes were fortified at 2 g/100 g product only. It was a school based study, so the children, assisted by the research team, assessed the dishes in their class-rooms at a village called Luhuese in Ada-East district (Table 12). One sample was served at a time, and about 2 g food was given to a child. Each child tasted three (3) dishes. Hot food was transported to the school in food warmers, so the samples were hot at the time of serving.

d) **Feasibility test by school children**

After the acceptability study, the children were further supplemented for two weeks with *M. oleifera* leaf-fortified *waakye,* groundnut *soup or jollof* rice. Each child received 2 g dried leaves three times a week. The meals formed part of the regular menu served the children in this school which was one of the schools participating in the Government of Ghana's School Feeding Programme (GSFP). The leaves were incorporated into about 150 g food, the normal portion size served each child, to assess the feasibility of introducing dried leaves into the school menu (Nambiar *et al*., 2003). The supplementation trial was assessed by consumption of the portions allocated to the children, left-overs (plate waste, no plate waste) and presence in school during the intervention period.

3.3.3.3 Quality control

Composite maize-meal was milled with a hardened steel hammer mill (Jacobson Machinery Works, Minneapolis, USA. Model 160B) with a 250µm mesh sieve, to prevent the introduction

of heavy metals usually introduced into food samples when attrition mill is used. Kitchen spoons and knives used were made of stainless steel. *M. oleifera* leaves were milled with stainless steel hammer-mill. Samples were weighed using precision scales to ensure that the right quantities were used.

3.3.4 Data processing and analysis

Statistical analysis was done using Statistical Package for Social Scientists (SPSS, 2012) version 21.0. Analysis of Variance (ANOVA) and a multiple range test (Duncan test) were conducted at a level of significance of $P < 0.05$ to test for significant differences between means of nutrients in fortified and non-fortified dishes. For the child acceptability test, a generalized linear model (GLM) was used for the calculations. Analysis of Variance (ANOVA) was used to determine if a difference existed among the sample scores, and Fisher's test (least significance test) was used to determine how different the various samples were from each other.

3.4 Study Part III design

The study was an interventional (longitudinal or prospective) one in which children **5-12** years were randomized to receive or not receive *M. oleifera* leaf supplementation over a 9-week period.

3.4.1 Variables measured

Vitamin A, haemoglobin (HGB or Hb), red blood cells or erythrocytes (RBC), haematocrit (HCT), mean corpuscular volume (MCV), Alanine transaminase (ALT), Aspartate

aminotransferase (AST), Gamma-glutamyltransferase (GGT), Alkaline phosphatase (ALP), Direct bilirubin, Total bilirubin, urea, creatinine, sodium, potassium and chloride.

3.4.2 Sampling

3.4.2.1 Study population

Children aged 4-12 years enrolled in the selected schools for the intervention.

3.4.2.2 Inclusion and exclusion criteria

Eligible children recruited into the interventional study were those who (a) were **4-12** years of age (b) resided in the study area (c) did not weigh more than 26kg.

3.4.2.3 Sample size

The difference between mean haemoglobin levels in the treatment and control groups that would give detection of statistical significance ($P < 0.05$) was 0.25 g/dl. It was also assumed that the standard deviations of the distributions of haemoglobin levels would be similar in both groups and was 0.45 g/dl. It was also estimated that there will be an 80 % follow-up rate, hence the sample size was 82 for each of the 2 arms with 90 % power and alpha (significance level) = 0.05.

- Difference between Hb levels in the two groups to be detected $\mu_1 - \mu_0 = 0.25$ g/dl;
- Standard deviations (ð) of Hb in each group assumed to be similar = 0.45 g/dl in each group.
- Significant level = v= 5 % ($P < 0.05$); power = u= 90 % ; follow-up = 80 %
- Sample size: $(u+v)^2 (ð_1^2 + ð_0^2)/(\mu_1 - \mu_0)^2$
- u = 1.28; v = 1.96

- Sample size of 82 for each arm (Kirkwood and Sterne, 2003).

A total of 164 children thus needed to be recruited for the trial (Kirkwood and Sterne, 2003)

3.4.2.4 Sampling methods/procedure

Subjects were selected by simple random sampling method. A sampling frame was obtained by pooling together the class registers from kindergarten to class three. Children who met the eligibility criteria were selected (N= 300). It was desired to recruit 170 children into the study, so 170 "Yes" and 130 "No" were written on pieces of paper, mixed thoroughly in a box and the children were asked to pick one each. Those who picked 'Yes" were recruited into the study. To recruit children into the Intervention and Control groups, "I" and "C" tokens were written, and the process of picking tokens was repeated. The flow chart for the intervention is shown in Figure 4.

Two adjacent coastal communities, Ocanseykope and Anyakpor that had kindergartens, were purposively (not participating in the Government of Ghana's School Feeding Programme), selected for the study which started in May and ended in July, spanning the duration of a normal school term. Ocanseykope had a total of 86 pupils in the kindergarten and 288 in the primary school while Anyakpor had 80 and 246 respectively. Coastal communities were chosen for the intervention because nutritionally, they were regarded as more vulnerable by the district health administration (District Health Report, 2011). Since the schools were not participating in the School Feeding Programme, the meals for the delivery of *M. oleifera* leaf powder were completely researcher managed.

3.4.3 Data collection technique/method and Tools/instruments

3.4.3.1 Ethical clearance

The study was conducted according to the Helsinki declaration, but with a modification, and ethical clearance was obtained from the Institutional Review Board (IRB) of the Noguchi Memorial Institute for Medical Research (NMIMR). The ascent form developed for obtaining ethical clearance clearly spelt out the purpose of the study; it elaborated on the benefits of the results of the study to the community, and further indicated that it was not a harmful study. It also stated that the privacy of the respondent will be respected. Participants were also informed that they were at liberty to withdraw at any time that they no longer felt comfortable participating. The headteachers and staff of the selected schools were met, and the rationale for the study was explained to them. The ascent form was also read and explained to the teachers. The headteachers then introduced the investigators to the chiefs and opinion leaders of the communities in which the selected schools resided. After permission was obtained from the community leaders, the headteachers called Parent Teacher Association (PTA) meetings where the parents and guardians were sensitized about the study. The form was read and explained to the parents; those willing to allow their children to participate in the study appended their signature or thumb-printed.

3.4.3.2 Training of interviewers

Ten university, polytechnic and SHS graduates were selected for training in questionnaires administration. Selected interviewers were those who could speak both English and the local (Ada) language. The questions were read and explained to them. They were asked to administer

the questionnaires on each other in both English and the local language after which all ambiguities were corrected.

3.4.3.3 Pre-testing

The questionnaires were pretested on twenty-five caregivers at a village called Luhuese in Ada-East district.

3.4.3.4 Data collection

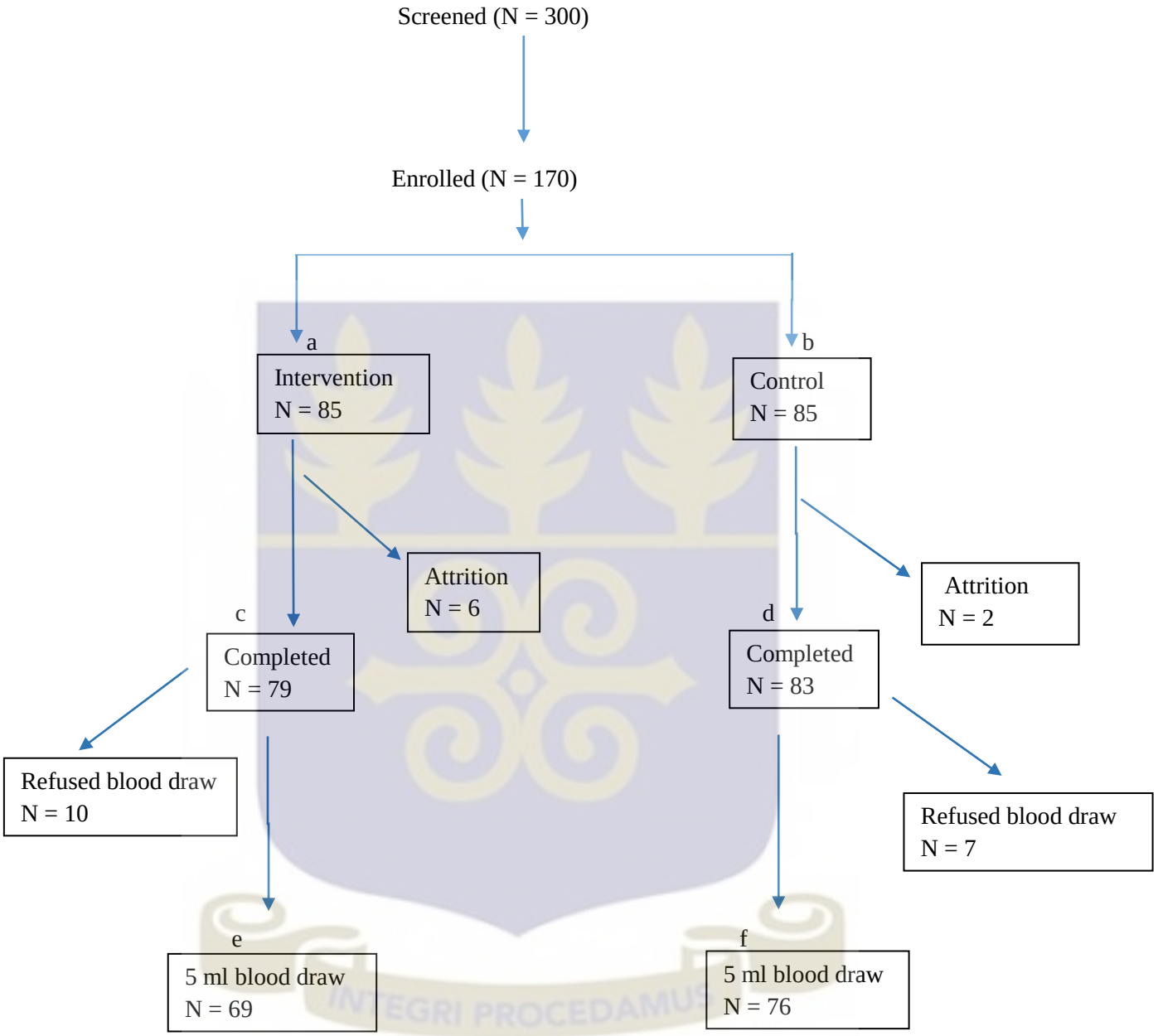

Figure 4: Flow chart for the interventional study.

3.4.3.4.1 Demography and food consumption

Structured questionnaires were administered to mothers and other caregivers to collect data on the demography of the children. Food frequency data was also collected for three non-consecutive times over the study period at 3-week intervals. The interviews were conducted in the homes of the children. Older children were allowed to answer the questions alongside their caregivers. This provided information on the food consumption of the children that was used as a guide in assessing the effect of *M. oleifera* leaf supplementation.

Figure 5: Children drinking porridge at Ocansykope

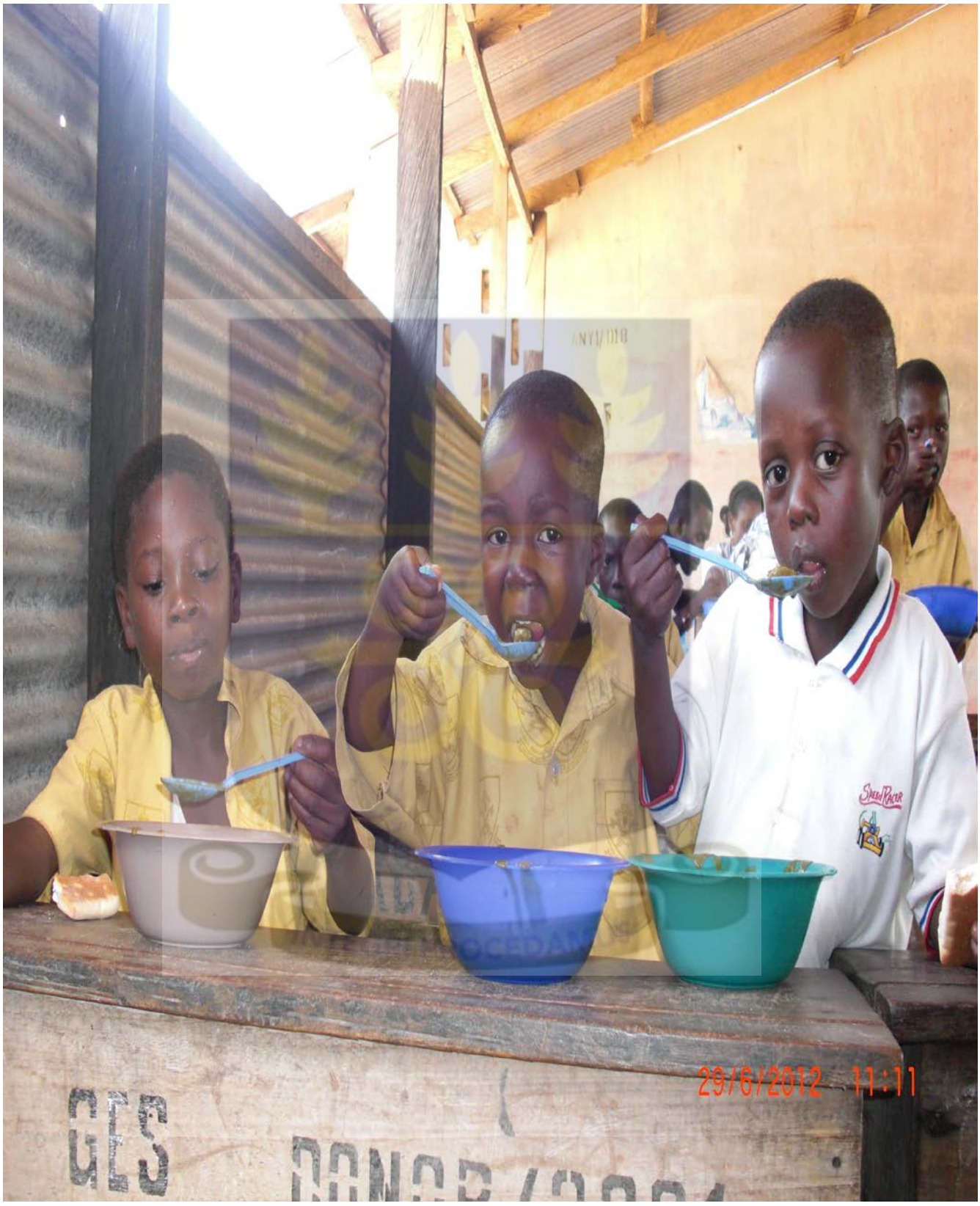

Figure 6: Children drinking porridge at Anyakpor

3.4.3.4.2 Feeding

Befor commencement of feeding, 5 ml venous blood was drawn from each child for laboratory analysis. Three dishes, based on the outcome of the acceptability study, were selected for the main intervention. These were fortified porridge (a sweetened dish), *waakye* and groundnut soup (salty dishes). The children were supplemented three times in a week for nine (9) weeks. Porridge was served with doughnut or biscuits, twice a week alternated with either *waakye* or groundnut soup with rice balls or *gari*. There was no feeding at the weekends. The groundnut soup was prepared from whole peanut butter to provide oil which enhances the absorption of β-carotene. Similarly, the composite flour used in porridge preparation contained peanuts to provide both protein and oil, and the gravy used in serving *waakye* was made with coconut oil and smoked herring (Appendices 20 and 22).

The weight of the pupils ranged from 12 kg to 25.4 kg with a mean weight of 21.1 kg (Tanita electronic scale BWB-800). The meal was designed to give 0.2 g leaf powder /kg body weight. A ninety (90) ml ladle and a fifteen (15) ml spoon were used to give the children portions of food in proportion to their body weight (These measures were weighed to determine the weight of food). Feeding bowls and spoons were provided for the children, and these were cleaned thoroughly after each session by the research team and stored away. The meal was used as a vehicle for the delivery of *M. oleifera* leaf powder. The study was not blinded due to the intense green colour of *M. oleifera* leaves which made blinding difficult (van Jaarsveld *et al.*, 2005). Serving of food started at 11:30 a.m. *i.e.* just before the lunch break. The Control group received an equal amount of non-*M. oleifera* leaf-fortified food. Each child was given a code. Sheets were used to record compliance. Separate sheets were given to the Intervention and Control groups. Each child's name was called out during the feeding sessions and ticked to indicate presence or

absence. Compliance was defined as the number of days that a child received and ate all the portion, expressed as a percentage of the total number of days of feeding. The food was prepared in the community by the research team and served to the children on the same day. Hot food was transported to the schools in food warmers. The research team did the serving and supervised the consumption with the assistance of the class teachers. The children were encouraged to eat the food, but not forced. At the end of the 9-week trial, 5 ml of venous blood was collected from each child (a day after the last feeding) by venipuncture (van Jaarsveld *et al*., 2005; de Pee and West, 1995) and all tests carried out at baseline were repeated.

Figure 7: A phlebotomist collecting blood samples from children

Figure 8: Serum retinol determination by HPLC.

3.4.3.4.3 Laboratory Examinations

a) Vitamin A (serum retinol)

i) Serum preparation from blood

Five (5) ml of blood was drawn from each child by venipunture by phlebotomists from Ada-East district hospital. Serum was prepared from 4.5 ml whole venous blood, while 0.5 ml was used for haematological measurements. Blood samples on separator gels were put on ice in an ice chest until end of sampling; they were transported to Ada-East district hospital. Sample collection and transport to the district hospital took approximately three hours. The gel in the separator pulls down the bigger cells and traps them at the bottom, thus facilitating faster separation of serum. After coagulation, the samples were spun in a centrifuge at 5,000 rpm for 5 minutes in a dark room. The serum was collected into eppendorf tubes and transported in an ice chest from Ada-East district hospital to Noguchi Memorial Institute for Medical Research (NMIMR) immediately, where it was stored at -80^{o}C until assessment. Serum retinol was assessed by high performance liquid chromatography (HPLC) at baseline and end of study. The frozen serum was allowed to thaw at room temperature in a wooden cupboard in a dark room to prevent direct exposure to light, to which retinol is sensitive.

ii) Serum retinol determination

Materials and reagents

Retinol standard (Sigma), retinyl acetate (Sigma) (for internal standard), hexane, methanol (HPLC grade) and eppendorf tubes (2 ml).

Equipment

Multiple vortexer, Shimadzu HPLC (Shimadzu SPD – 6A detector; Shimadzu LC – 6A pump and Shimadzu CR-5A printer) and Hitachi spectrophotometer (U – 1100).

Retinol standard curve

A small amount of the retinol standard was dissolved in methanol. Its absorbance was read using the Hitachi spectrophotometer at 325 nm (Appendix 13**).** The absorbance should not exceed 0.800. A concentrated solution was further diluted. Different volumes of the retinol standard solution (5 µl – 25 µl) were then injected on a reverse phase HPLC at 325 nm. An internal control was used to set the equipment every time before start of determination. These absorbance values were used to plot a standard curve.

Preparation of internal standard

A small quantity of retinyl acetate was dissolved in a volume of methanol and its absorbance (ABS) was read on the spectrophotometer. The absorbance (ABS) was not allowed to exceed 0.5. If it did, the solution was further diluted with methanol until the absorbance was 0.5 or less. This solution served as the internal standard.

Preparation of serum for HPLC analysis

One hundred and twenty (120) µl of serum was placed into a 2 ml eppendorf tube containing an equal volume of the methanol-retinyl acetate solution (internal standard), mixed with a vortex mixer for 30 s. This denatured the retinol binding protein (RBP), which transports retinol in serum, thus releasing retinol into the organic-solvent extraction medium. Five hundred (500) µl

of hexane was then added and it was mixed again with a vortex mixer for 2 min. The hexane was added to solubilise all the fat-soluble components. The mixture was centrifuged at 10,000 rpm for 2 min. Two hundred and fifty (250) µl of the supernatant was removed into a vial and evaporated slowly and carefully under nitrogen gas to dryness. The residue was reconstituted with 120 µl of methanol. This was mixed with a vortex mixer and 20 µl of the reconstituted solution was injected onto the HPLC column (Shimadzu model, Japan) with methanol as the mobile phase. Concentrations were read on the standard curve generated from the exogenous retinol.

b) Haematological parameters

Whole blood samples (0.5 ml) were used for the haematological measurements at baseline, and at end of study. Blood was drawn with vacutainer and butterfly needles. The blood was put into EDTA tubes, mixed gently by tilting the tube 8 – 10 times, to prevent coagulation; these were kept in racks, and later transported to the laboratory at Ada-East district hospital immediately after sampling, where the haematological indices were read. Red cell indices were measured from EDTA whole blood (WB) samples in the laboratory using Sysmex KX – 21 N (KOBE, JAPAN), an automated haematology analyser. Parameters measured were haemoglobin (HGB), red blood cells (RBCs), haematocrit (HCT), mean corpuscular volume (MCV).

c) Kidney function

This was assessed by measuring creatinine and urea using the methods of First, (2003), Tietz, (1995), Butler, (1975) and Newman, (2001) as outlined in ELITech clinical systems kits (ELITech Group, France), as well as sodium (Na), potassium (K), and chloride (Cl) levels in sera

(Keogh, 2010) of subjects at baseline and end of study. The tests were carried out at Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana.

d) Liver function

Liver function was assessed by measuring Alanine transaminase (ALT), Aspartate aminotransferase (AST), Total and Direct bilirubin, Gamma-glutamyltransferase (GGT), Alkaline phosphatase (ALP) and albumin levels in sera of study subjects at baseline and end of study, by the methods of Schreiber, (2003), Tietz, (1995), Sherwin and Thompson, (2003), Dufour, (2010) and Wu, (2006) as outlined in ELITech clinical systems kits (ELITech Group, France). The tests were carried out at Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana.

3.4.4 Quality control

All equipment used for data collection were calibrated before use. Sterile syringes, needles, and blood-collection tubes (EDTA and serum separator tubes) were used during blood drawing sessions. Each child was given a code that was known to the research team only. All field procedures were carried out as quickly as possible, and throughout, serum samples were protected from direct light. Blood samples for retinol determination were put on ice immediately after collection to prevent deterioration, and transported to the Ada district hospital immediately after field work for serum separation. Separation was done in a dark room after which samples were transported on ice the same day to NMIMR and stored at -80^{o}C until retinol determination. Kidney and liver chemistry were also measured at NMIMR using fractions of the sera. All haematological indices were measured in one laboratory (Ada-East district hospital) on the day

of collection, to reduce error and variability, likewise, malaria parasitaemia and hookworm. Samples collected into EDTA tubes were kept in racks, and transported to the laboratory immediately after field work, for analysis. Data for each child was printed out on an A4 sheet after determination using an Epson printer connected to the haemoanalyser. The data was checked immediately ensure that codes were correctly entered on the print-out. Personnel were trained before baseline data collection to create uniformity and ensure that the correct data was collected, and the collected data was thoroughly checked in the field to ensure that all data was complete. Serving ladles and spoons were weighed carefully to ensure that the right quantity of food was delivered to each child.

3.4.5 Data processing and analysis

3.4.5.1 Proximate and micronutrient levels

Data were entered into Excel and the means and standard deviations were calculated. Statistical package for social scientists (SPSS, 2012) version 21.0 was used to analyse the data using ANOVA followed by Duncan test to determine significant differences ($P < 0.05$). Means with the same superscript are not significantly different.

3.4.5.2 Haematological and biochemical indices

Data were analysed using SPSS (SPSS, 2012) version 21.0. Means, standard deviations and frequencies were generated. Statistical analysis included baseline data comparisons between groups for differences in haematological and biochemical variables by using independent-sample t-tests. For children in the Control and Intervention groups who completed the study, Fishers' and chi-square test (for the percentages) and paired t-tests (for the continuous variables) were

used to determine whether changes from baseline to end of study were statistically significant. Changes in variables were calculated for both the Control and Intervention groups and comparisons of the difference between the changes in the Intervention group with that in the Control group were made using independent-sample t-tests.

Difference-in-differences (DD) models were used to estimate the effects of the intervention diet. The basic DD model compares the difference in outcomes over time for a Control group to the difference in outcomes during the same period for the Intervention group subject to the intervention diet during the study period. The model includes all the subjects who were in the Intervention group once the subject ate the intervention diet for an X (threshold) number of times, thus taking care of compliance. The advantage of this design is that the change for the Control group picks up any naturally occurring changes, whereas the Intervention group change reflects both the (same) naturally occurring change and the effect of the intervention diet (Ghosh *et al.*, 2010), thereby allowing for an accurate measurement of the effect of the intervention diet. A P value < 0.05 was used to identify statistical significance.

CHAPTER FOUR

4.0 Results

4.1. Study Part 1

4.1.1 Dietary uses of *M. oleifera* leaves in Ada-East district

M. oleifera leaves are used to prepare various local dishes in Ada-East district (Figure 9). One hundred and eighty-two (81 %) of the respondents gave its local name as *Kpokpotsoba/Nyabawesi* whilst twenty-four (10.6 %) of the respondents only knew it as Moringa.

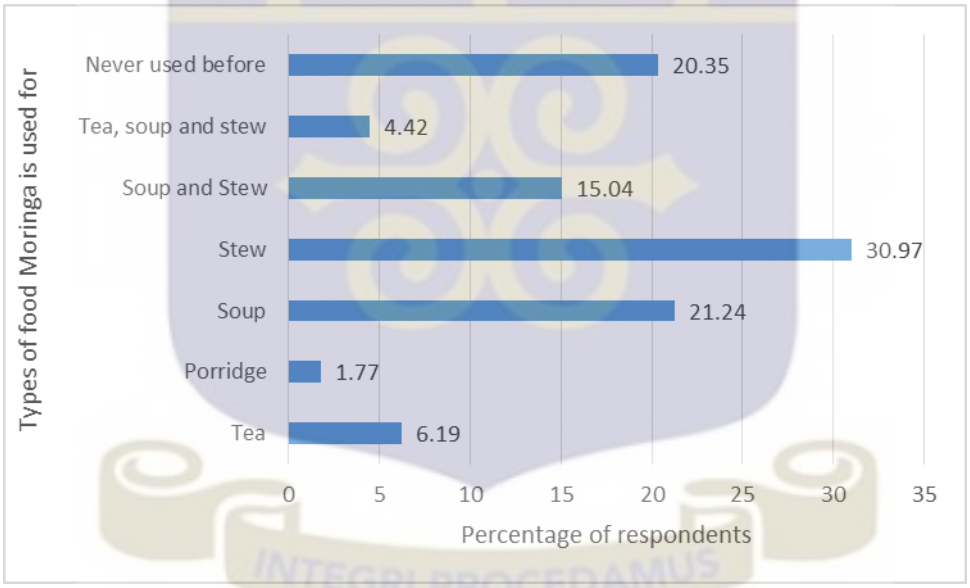

Figure 9: Types of food prepared with *M. oleifera* leaves.

One hundred and eighty (79.7 %) of the respondents use *M. oleifera* leaves as food. The type of food it is used for include “tea” or infusion, soup and stew. Seventy (31 %) of the respondents use it for preparing stew only. Forty-eight (21 %) of the respondents use it for preparing soup

only. Thirty-four (15 %) of the respondents use it for preparing both stew and soup. Forty-six (20%) of the respondents had never used it to prepare food.

One hundred and fifty-two (67.3 %) of the respondents said *M. oleifera* leaves are fed to children in their locality with sixty (26.5 %) of respondents saying children are not fed *M. oleifera* leaves in the locality. One hundred and forty-four (63.7 %) of the respondents said children from 6 months – 5 years could be fed *M. oleifera* leaves in their locality. Most of the respondents, however, could not tell the quantity of leaves to be fed to children (Appendix 27). Some of them said 1 cup (450 g magarine tin size), others said 1, 2 and three bowls (six magarine tins/bowl). Ninety –eight (43.4 %) said they actually fed their children with *M. oleifera* leaves once in a while, twenty-eight (12.4 %) said they fed the children once a week with the leaves, whilst seventy-two (31.9 %) did not feed their children with *M. oleifera* leaves. Two hundred and twelve (93.8 %) of respondents however, said they would feed their children with the leaves when asked to do so, because they had learned from neigbours and friends that it gives strength, it is a food and good for children. In addition, it has medicinal value and is good for children. Four (1.8 %) said it is bitter and another four (1.8 %) said they don't know how to use it.

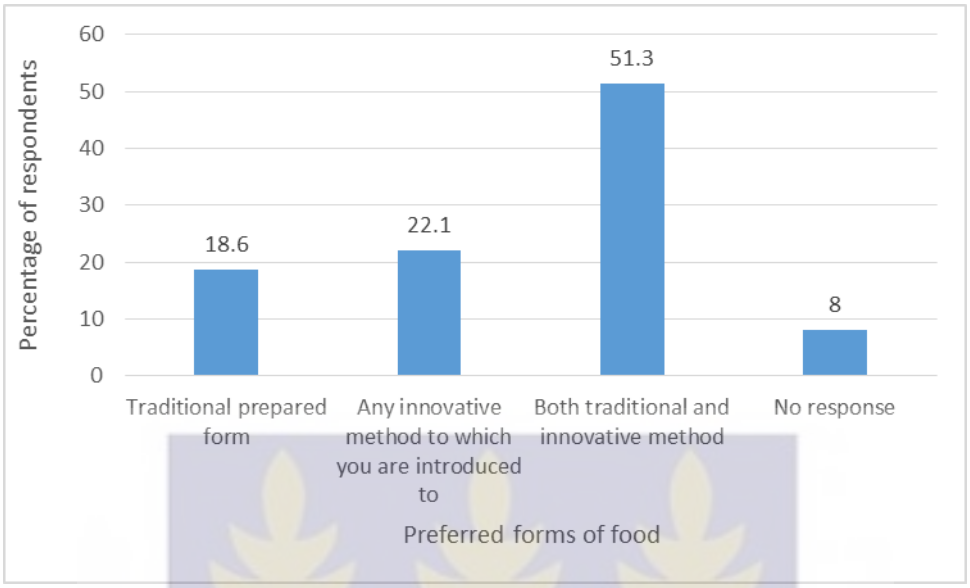

Figure 10: Preferred forms of *M. oleifera* leaf consumption

The preferred forms of *M. oleifera* leaf consumption are shown in Figure 10. One hundred and sixteen (51.3 %) stated that they would prefer to use both the traditional form (i.e. the form in which it is normally consumed in the district which included stew, soup and infusion ('tea'), and any innovative method they are introduced to. Fifty (22.1 %) of the respondents preferred only any innovative method they are introduced to, whilst forty-two (18.6 %) of the respondents preferred the traditional form only. Eighteen (8.0 %) of the respondents did not answer this question.

Apart from using *M. oleifera* leaves as food, one hundred and eighty (80 %) of the respondents do not have any alternative use for the plant. Twenty-eight (12 %) of the respondents use it as medicine whilst eighteen (8 %) of the respondents use it as animal feed as shown in Figure 11.

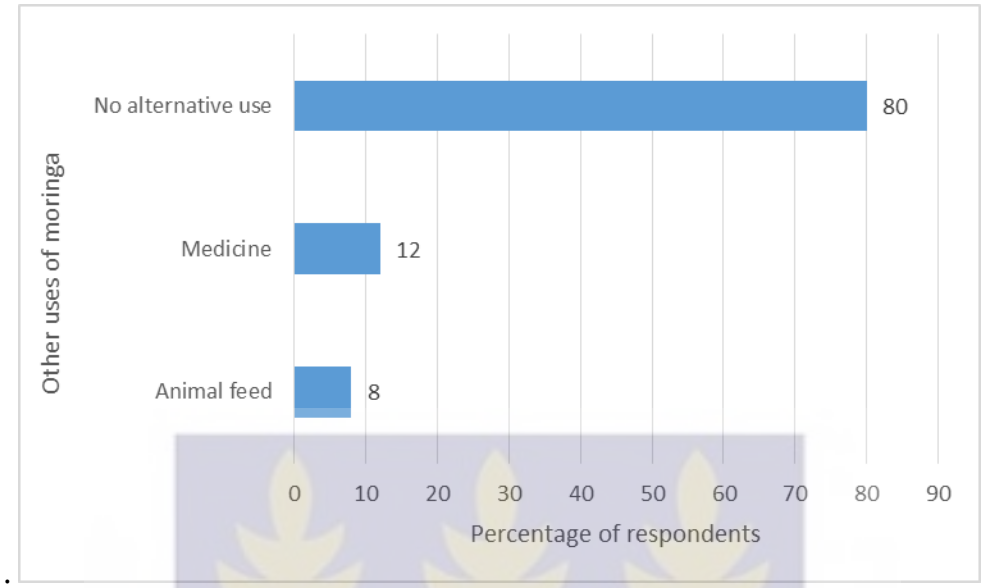

Figure 11: Non-dietary uses of *M. oleifera* leaves in the district

4.1.2 Other vegetables and fruit consumption

The vegetables consumed in the district included garden eggs, tomatoes, pepper, onions, okro and green leafy vegetables which were cocoyam leaves, African spinach, leafy eggplant, and amaranth. All the respondents (two hundred and twenty-six (100 %)) consumed tomatoes, pepper and onions on daily basis. Garden eggs, cocoyam leaves and okro were also consumed by all respondents, but not on regular basis. One hundred and sixty (70.8 %) consumed tossa, one hundred and twenty-eight (56.6 %) consumed African spinach, one hundred and twelve (49.6 %) consumed leafy eggplant, whilst fifty-two (23 %) consumed amaranth. The frequency of consumption of these vegetables were once a week, three times a week and ocassionally, or when available. Survey of the local market also showed that these vegetables are sold on market days which are held twice a week. However, with the exception of onions, okro, tomatoes and pepper, all other vegetables are not cultivated in the district. Tossa, amaranth and leafy eggplant are brought in by traders from neighbouring South Tongu and Akatsi districts on market days.

Cocoyam leaves are brought by traders from Accra. Fruits consumed in the district included bananas, oranges, pineapple, mangoes and watermelon.

4.1.3 Staples intake

The basic diet of the communities comprised carbohydrate staples (cassava, maize and rice) which were prepared into *kenkey* or *banku* and eaten with fried fish. Boiled rice, yam, cocoyam and plantain were also consumed occasionally. Cowpea and peanuts were also important sources of protein, in addition to fish, in the district. Children ate the same food as the adults. On the average, people ate two main meals a day.

4.2 Study Part II

4.2.1 Acceptability and feasibility studies

4.2.1.1 Acceptability test by school children

The children's acceptability test rated porridge as the most preferred dish among all the others. Moreover, all the other dishes were also highly acceptable to the children (Table 12). Porridge, beans with *gari*, groundnut soup, *waakye*, *nkontomire* sauce (2 g/100 g) had mean scores ranging from 4.64 to 5 implying that they were liked extremely, while all the remaining dishes had mean values indicating that they were also liked very much.

Results of a multiple range analysis for attributes of *M. oleifera* leaf fortified dishes by an in-house semi-trained adult judges is shown in Table 9. Increasing concentrations of *M. oleifera* leaf powder significantly affected the appearance, taste, aftertaste and overall acceptability of some of the dishes. There were no differences in overall acceptability of porridge with increasing concentration of *M. oleifera* leaf powder. Concentration did not have any significant effect on

colour at 2g and 3g levels, but a significant difference existed at 5g fortification level. With *Nkontomire* sauce which is another green leafy vegetable, although there was a significant difference between the colour at 2g and 3g levels, there was no significant difference between 3g and 5g levels.

Table 9: Multiple range analysis for attributes of *M. oleifera* leaf fortified dishes by adult judges

Food Sample	Appearance	Colour	Texture	Flavour	Taste	Aftertaste	Overall Acceptability
Porridge 3g	4.00±0.88^{c}	7.10±0.97^{a}	6.50±0.82^{ab}	7.00±0.95^{a}	6.30±1.17^{a}	6.40±1.07^{ab}	6.40±1.35^{a}
Porridge 2g	5.00±1.06^{b}	7.70±1.03^{a}	7.20±1.15^{a}	7.00±1.45^{a}	7.10±1.69^{a}	7.20±1.25^{a}	7.00±1.35^{a}
Porridge 5g	6.00±1.10^{a}	5.90±1.03^{b}	5.80±1.06^{b}	6.70±1.20^{a}	6.10±1.20^{a}	5.90±1.52^{b}	5.90±1.25^{a}
Ofam 2g	6.80±1.23^{a}	6.60±1.26^{a}	6.80±1.32^{a}	7.10±1.10^{a}	6.80±1.62^{a}	6.80±1.32^{a}	7.10±1.52^{a}
Ofam 3g	6.60±1.07^{a}	6.30±1.06^{ab}	7.20±1.03^{a}	6.90±1.37^{a}	7.00±1.15^{a}	7.20±1.03^{a}	7.10±1.10^{a}
Ofam 5g	5.90±0.88^{a}	5.30±1.25^{b}	6.20±1.23^{a}	6.00±1.41^{a}	6.40±1.26^{a}	6.30±0.95^{a}	6.20±1.03^{a}
Beans with *gari* 2g	7.30±0.95^{a}	7.60±0.70^{a}	7.00±0.82^{a}	6.60±1.35^{a}	5.80±1.40^{a}	6.10±1.52^{a}	6.60±1.43^{a}
Beans with *gari* 3g	7.20±0.95^{a}	7.30±1.06^{a}	6.70±1.06^{a}	6.20±0.92^{a}	5.10±1.91^{a}	4.60±1.65^{a}	5.10±1.79^{a}
Beans with *gari* 5g	6.90±1.37^{a}	7.30±1.34^{a}	6.50±1.08^{a}	5.80±1.48^{a}	5.10±1.97^{a}	4.80±1.99^{a}	5.30±1.95^{a}

Apapransa 2g	7.38±1.00[a]	7.63±0.88[a]	7.38±0.88[a]	6.75±1.39[a]	6.50±1.42[a]	6.63±1.69[a]	6.78±1.48[a]
Apapransa 3g	4.55±2.13[b]	4.56±2.19[b]	5.22±1.48[b]	5.22±2.11[a]	4.67±1.41[b]	4.22±1.48[b]	4.33±1.58[b]
Apapransa 5g	3.56±1.67[b]	3.89±1.90[b]	6.22±1.39[ab]	5.33±1.80[a]	4.33±1.58[b]	4.33±1.32[b]	4.22±1.20[b]
Nkontomire sauce 2g	7.60±0.52[a]	7.50±0.53[a]	6.90±0.74[a]	6.90±1.20[a]	6.90±1.60[a]	6.80±1.40[a]	7.20±1.55[a]
Nkontomire sauce 3g	6.70±0.67[b]	6.80±0.63[b]	6.50±1.27[a]	5.90±1.29[ab]	4.50±1.43[b]	4.80±1.14[b]	5.70±1.18[b]
Nkontomire sauce 5g	6.50±0.71[b]	6.50±0.71[b]	6.60±0.84[a]	5.70±1.25[b]	4.20±0.79[b]	4.60±1.58[b]	5.50±1.06[b]
Groundnut soup 3g	7.33±0.87[a]	7.33±1.00[a]	6.67±1.00[a]	7.33±1.22[a]	6.67±1.41[a]	6.44±1.13[a]	6.78±0.97[ab]
Groundnut soup 2g	7.22±1.09[a]	7.56±0.73[a]	7.00±1.81[a]	7.11±0.93[a]	6.67±1.58[a]	6.22±1.48[a]	7.99±0.87[a]
Groundnut soup 5g	6.11±1.83[a]	6.00±1.80[b]	6.44±1.81[a]	6.00±2.06[a]	5.56±2.19[a]	5.67±2.06[a]	5.44±2.07[b]
Waakye 2g	6.67±1.50[a]	6.44±1.42[a]	7.00±1.41[a]	6.89±1.05[a]	7.00±1.12[a]	6.56±1.42[a]	7.00±1.73[a]
Waakye 3g	4.89±2.15[b]	4.56±2.01[b]	6.11±1.76[a]	5.33±1.58[b]	6.00±1.87[ab]	5.56±2.01[ab]	5.89±1.76[ab]
Waakye 5g	4.56±1.59[b]	3.89±1.69[b]	6.11±1.83[a]	5.11±1.62[b]	4.56±1.42[b]	4.44±1.51[b]	4.56±1.42[b]
Jollof rice 2g	6.11±1.62[a]	6.44±1.33[a]	6.67±1.12[a]	6.11±1.54[a]	6.44±1.51[a]	5.78±1.99[a]	6.22±1.64[a]
Jollof rice 5g	3.78±1.79[b]	3.78±2.11[b]	5.44±2.01[a]	5.33±2.45[a]	5.56±1.67[ab]	5.00±2.06[a]	5.78±1.92[ab]
Jollof rice	2.67±2.06[b]	2.78±2.05[b]	6.33±1.80[a]	4.44±2.65[a]	3.89±2.09[b]	3.00±2.00[b]	3.78±2.64[b]

3g

ANOVA was used to determine if a difference existed among the sample attributes and Fisher's test (least significance test) was used to determine how different the various samples were from each other. Means with the same superscript are not significantly different (P < 0.05)

The β -carotene, copper, zinc, iron and manganese levels in dried *M. oleifera* leaves are shown in Table 10. The Table shows that *M. oleifera* leaves contained various levels of the micronutrients β-carotene, Cu, Zn, Mn, and Fe.

Table 10: Mineral and β-carotene content of dried *M. oleifera* leaves.

Replicate	Sample	Cu (mg/100g)	Fe (mg/100g)	Mn (mg/100g)	Zn (mg/100g)	β -carotene (mg/100g)	Moisture (%)	pH
1	*Moringa leaves*	0.40±0.18^{a}	19.49±2.99^{a}	5.20±0.21^{a}	5.38±0.25^{a}	20.79±0.01^{a}	7.61±0.07^{a}	5.34±0.01^{b}
2	*Moringa leaves*	0.37±0.09^{a}	22.20±11.91^{b}	5.67±4.56^{ab}	6.14±0.23^{b}	20.15±0.00^{b}	7.58±0.02^{a}	5.32±0.04^{b}
3	*Moringa leaves*	0.32±0.04^{b}	21.20±2.43^{bc}	6.54±1.84^{b}	8.85±0.23^{c}	23.31±0.01^{c}	7.73±0.04^{a}	5.35±0.01^{b}
	Mean of Reps	0.36±0.04	20.96±1.37	5.80±0.68	6.79±1.82	21.42±1.67	7.64±0.08	5.34±0.02

Cu = Copper; Fe = Iron; Mn = Manganese; Zn = Zinc. 1, 2, 3 are replications of sample. Means with the same superscript are not significantly different (P < 0.05).

Levels of micronutrients in the fortified and non-fortified dishes are shown in Table 11. Fortification significantly increased levels of all the micronutrients except Zn (P<0.05). Fortified porridge for example contained 1.28mg/100g β-carotene as against 0.02mg/100g in the non-fortified dish. Similarly, fortified *waakye* contained 2.35mg/100g iron as against 0.96mg/100g in the non-fortified dish. For *waakye*, the fortification resulted in a significant increase in all the

micronutrients. While fortification of soup led to a significant increase in levels of Fe and Mn, there was no change in Zn levels.

Table 11: Mineral and β-carotene content of dishes

Sample	Food	Cu (mg/100g)	Fe (mg/100g)	Mn (mg/100g)	Zn (mg/100g)	β-carotene (mg/100g)
1	Porridge	0.09±0.11^{a}	0.36±0.49^{d}	0.15±0.17^{b}	0.40±0.46^{f}	0.02±0.00^{a}
2	Porridge	0.15±0.13^{c}	2.13±1.64^{g}	0.26±0.08^{d}	0.39±0.21^{f}	1.28±0.04^{c}
1	*Waakye*	0.10±0.03^{ab}	0.96±1.28^{e}	0.10±0.01^{a}	0.56±0.62^{g}	0.08±0.05^{a}
2	*Waakye*	0.14±0.22^{bc}	2.35±3.83^{g}	0.62±0.31^{e}	0.78±0.69^{h}	0.94±0.51^{bc}
1	Soup	0.10±0.13^{ab}	1.54±0.69^{f}	0.20±0.04^{c}	0.40±0.36^{f}	0.34±0.06^{ab}
2	Soup	0.10±0.26^{ab}	2.62±2.43^{g}	0.26±0.30^{d}	0.43±0.16^{f}	2.98±0.39^{d}

Cu = Copper; Fe = Iron; Mn = Manganese; Zn = Zinc. 1 = Non-fortified; 2 = M. oleifera leaf fortified. Dishes were fortified at 3g/100g product. Means with the same superscript are not significantly different (P < 0.05).

Table 12: Acceptability of *M. oleifera* leaf-fortified dishes

Dish	Mean + S.D	N
Porridge (2 g/100 g)	5.0 ± 0^{a}	13
Waakye (2 g/100 g)	4.88 ± 0.33^{a}	12
Nkontomire sauce (2 g/100 g)	4.80 ± 0.42^{ab}	12
Groundnut soup (2 g/100 g)	4.71 ± 0.83^{ab}	13
Beans and *gari* (2 g/100 g)	4.64 ± 0.50^{ab}	13
Apranpransa (2 g/100 g)	4.40 ± 0.97^{ab}	13
Jollof rice(2 g/100 g)	4.36 ± 0.92^{ab}	12
Ofam (2 g/100 g)	4.18 ± 1.33^{b}	13

Nkontomire sauce (3 g/100 g)	1.50	1.43[c]	13

ANOVA was used to determine if a difference existed among the sample scores, and Fisher's test (least significance test) was used to determine how different the various samples were from each other. P-value was 0.0026; Thus P < 0.05. Means with the same superscript are not significantly different (P < 0.05).

4.2.1.2 Feasibility test by school children

In the feasibility study, the children ate all their portions during the two-week intervention period, and there were no plate wastes either. Compliance was 100 %. This implied that *M. oleifera* leaf-fortified dishes - *waakye*, groundnut soup and *jollof* rice were acceptable to the children during the two-week fortification of their school (GSFP) menu.

4.3 Study Part III

4.3.1 Background characteristics, baseline and posttest parameters

4.3.1.1 Background characteristics of study subjects and caregivers

The study group comprised ninety (52.95 %) males and eighty (47.1 %) females in the age range 5-12 years. Eighty-five (50.0 %)) of the children were in the age bracket 8-10 years. The caregivers' age ranged from 25 years to 50+ years. One hundred and thirty-six (80 %) of them were married. One hundred and sixty-three (95.9 %) of them were Christians, and the majority (153 (90 %)) had no formal education. Their main occupation was farming (130 (76.5 %)). Most of them were ethnic Adas (168 (98.8 %)) and all the participants (170 (100 %)) were in the lowest tertile of wealth index. Whereas all caregivers (170 (100 %)) did not know what vitamin A was, most of them (141 (82.8 %)) knew about anaemia, and gave its symptoms as weakness, pale sole, palm and eyes (Appendix 24). One hundred and sixty-six (97.6 %) of the children slept

under a mosquito net. (Table 14). The people were quite similar in terms of occupation, water sources and sanitation (Table 15). They all drank from the same well with the majority disposing of their waste in the open (124 (72.9 %)), and 161 (94.7 %) defaecating in the open (Table 15). The food frequency of study subjects is shown in Table 13. Foods consumed were *banku*, kenkey, *waakye*, *koose*, roasted groundnuts, okro soup, fried fish, fresh "pepper", palmnut soup, beans with *gari.*The main vegetables consumed were pepper, onions and tomatoes which were eaten as "pepper".

Table 13: Food frequency of subjects during the study period

Food Group	Food Items	Number	Percentage
Cereal and grains	maize, rice	170	100
Roots, tubers and other starchy foods	Cassava, yam, sweet potatoes (white)	170	100
Legumes, nuts, and seeds	Cowpea, groundnut	91	55.3
Vegetables	Pepper, tomatoes, onions	170	100
Fruits		-	-
Animal foods and animal products	Meat (beef), fish, oyster	72	42.3
Fats and oil	Palmnut, palm oil, groundnut oil	39	23.0

Foods consumed were banku, kenkey, waakye, koose, roasted groundnuts, okro soup, fried fish, fresh "pepper", palmnut soup, beans and *gari.* The main vegetables consumed were pepper, onions and tomatoes which were eaten as "pepper".

4.3.1.2 *M. oleifera* leaf consumption

Mean compliance (the number of days that a child received and ate all the portion, expressed as a percentage of the total number of days of feeding) was 85 % in the Intervention group and 82 % in the Control group. There were no left-overs after consumption. Seventy-nine (79) out of 85 children in the Intervention group completed the study with an attrition of six (6). In the Control group, however, eighty-three (83) children completed the study with an attrition of two (2) (Figure 4).

4.3.1.3 Baseline vitamin A and haematological parameters

Baseline values for all the parameters studied - vitamin A, haematology, kidney and liver chemistry - were not significantly different between the Intervention and Control groups (Table 16-18). Sex and age of the children also did not show any significant difference in baseline values (Appendices 6 and 7).

At baseline 53 (76.8 %) of children in the Intervention group were anaemic as against 62 (81.6 %) in the Control group. Those with normal haemoglobin levels were 16 (23.2 %) in the Intervention group and 14 (18.4 %) in the Control group. The mean Hb was 10.63 g/dl in Intervention group, and 10.56 g/dl in Control group with an overall mean Hb of 10.59 g/dl. The WHO haemoglobin cut-off for children 5-11 years is 11.5 g/dl while that for children 12 years is 12.0 g/dl (Tables 4 and 5). Presence of infections (Strongyloides stercoralis, Ascaris lumbricoides, Intestinal flagellates, Taenia, Trichuris trichuris and Hymenolepsis nana) had a significant effect on haemoglobin (HGB) and erythrocyte (RBC) levels of the children (Appendix 25).

Table 14: Background characteristics and infection profile of children

Characteristic	Intervention Group (n=69) n (%)	Control Group (n=76) n (%)	Total (n=145) n (%)
Sex of child			
Male	50 (58.8)	40 (47.1)	90 (52.9)
Female	35 (41.2)	45 (52.9)	80 (47.1)
Child's age (years)			
5-7	33 (38.8)	33 (38.8)	66 (38.8)
8-10	44 (51.8)	41 (48.2)	85 (50.0)
11-12	8 (9.4)	11 (12.9)	19 (11.2)
Protection of house with mosquito net			
Yes	8 (9.4)	6 (7.1)	14 (8.2)
No	77 (90.6)	79 (92.9)	156 (91.8)
Sleep under bed-net			
Yes	85 (100.0)	81 (95.3)	166 (97.6)
No	0 (0.0)	4 (4.7)	4 (2.4)
Malaria parasites			
Present	19 (22.4)	14 (16.5)	33 (19.4)
No mps	66 (77.6)	71 (83.5)	137 (80.6)
Other infections			
Yes	7 (8.2)	5 (5.9)	12 (7.1)
No	78 (91.8)	80 (94.1)	158 (92.9)

Table 15: Background characteristics, water and sanitation management of caregivers

Characteristic	Intervention Group n (%)	Control Group n (%)	Total n (%)
Caregiver's age			
25-29	10 (11.8)	7 (8.3)	15 (8.8)
30-34	11 (12.9)	18 (21.2)	29 (17.1)
35-39	23 (27.1)	21 (24.7)	44 (25.9)
40-44	14 (16.5)	11 (12.9)	25 (14.7)
45-49	11 (12.9)	13 (15.3)	24 (14.1)
50+	16 (18.8)	15 (17.6)	31 (18.2)
Marital status			
Single	12 (14.1)	18 (21.2)	30 (17.6)
Married	70 (82.4)	66 (77.6)	136 (80.0)
Divorced /Widowed	3 (3.5)	1 (1.2)	4 (2.4)
Religion			
Christianity	83 (97.6)	80 (94.1)	163 (95.9)
Islam	1 (1.2)	4 (4.7)	5 (2.9)
Traditional	1 (1.2)	1 (1.2)	2 (1.2)
Formal education			
No education	77 (90.6)	76 (89.4)	153 (90.0)
Middle school/JSS	8 (9.4)	9 (10.9)	17 (10.0)
Occupation			
Unemployed	4 (4.7)	5 (5.9)	9 (5.3)
Trader	11 (12.9)	15 (17.6)	26 (15.3)
Farmer	67 (78.8)	63 (74.1)	130 (76.5)
Student	3 (3.5)	2 (2.4)	5 (2.9)
Ethnicity			
Ada	84 (98.8)	84 (98.8)	168 (98.8)

Ewe	1 (1.2)	1 (1.2)	2 (1.2)
Water supply management			
Self	82 (96.5)	81 (95.3)	163 (95.9)
Community operated	3 (3.5)	4 (4.7)	7 (4.1)
Household refuse disposal			
Refuse dump	62 (72.9)	61 (71.8)	123 (72.4)
Burned	20 (23.5)	22 (25.9)	42 (24.7)
Buried	3 (3.5)	2 (2.4)	5 (2.9)
Toilet type			
Pit latrine	2 (2.4)	3 (3.5)	5 (2.9)
Public toilet	2 (2.4)	2 (2.4)	4 (2.4)
Bush	81 (95.3)	80 (1.2)	161 (94.7)

Table 16: Vitamin A and haematological indices of children 5-12 years at baseline

Haematological indices	Intervention Group Mean ± SD	Control Group Mean ± SD	Total Mean ± SD	*P-value*
Vitamin A (µmol/l)	0.69 ± 0.27	0.77 ± 0.31	0.73 ± 0.29	0.053
RBC x 10^4/µl	4.21 ± 0.43	4.15 ± 0.44	4.18 ± 0.44	0.370
HGB (g/dL)	10.63 ± 0.97	10.56 ± 1.20	10.59 ± 1.08	0.664
HCT (%)	32.89 ± 2.76	32.59 ± 2.89	32.74 ± 2.82	0.485
MCV (fL)	78.38 ± 5.52	79.02 ± 5.83	78.70 ± 5.67	0.460

RBC= Red blood cells; HGB= Haemoglobin; HCT= Haematocrit; MCV= Mean corpuscular volume

Table 17: Kidney indices of children 5-12 years at baseline

kidney indices	Intervention Group Mean ± SD	Control Group Mean ± SD	Total Mean ± SD	*P-value*
Urea	2.69 ± 0.31	2.35 ± 0.11	2.52 ± 0.16	0.316
Creatinine	51.51 ± 3.23	59.39 ± 5.75	55.43 ± 3.29	0.232
Sodium	136.68 ± 0.78	136.58 ± 2.20	136.63 ± 1.15	0.966
Potassium	3.78 ± 0.05	3.80 ± 0.05	3.79 ± 0.04	0.830
Chloride	101.49 ± 0.64	101.54 ± 1.19	101.51 ± 0.67	0.973

Table 18: Liver indices of children 5-12 years at baseline

Liver indices	Intervention Group Mean ± SD	Control Group Mean ± SD	Total Mean ± SD	*P-value*
ALT	20.05 ± 1.27	17.76 ± 1.24	18.92 ± 0.89	0.200
AST	35.24 ± 5.12	29.13 ± 0.75	32.20 ± 2.61	0.242
ALP	602.93 ± 18.25	613.39 ± 15.29	608.16 ± 11.87	0.661
Albumin	42.65 ± 0.44	43.03 ± 0.40	42.84 ± 0.30	0.518
Total Protein	86.70 ± 9.18	79.79 ± 1.17	83.27 ± 4.65	0.459
GGT	15.29 ± .72	18.33 ± 3.35	16.80 ± 1.70	0.374
Direct bilirubin	3.63 ± 1.07	3.20 ± 0.30	3.42 ± 0.57	0.706
Total bilirubin	6.71 ± 0.98	7.12 ± 0.98	6.91 ± 0.69	0.767

*ALT= Alanine Aminotransferase; AST= Aspartate Aminotransferase; ALP= Alkaline phosphatase; GGT= G*amma-glutamyl transferase

4.3.1.4 Vitamin A and haematological indices of study subjects with time

There was a significant increase in vitamin A level in the Intervention group at end of study, whilst the level in the Control group showed a slight, non-significant decrease at end of study. The difference in mean change between the Intervention group and the Control group was also significant (Table 19). In the Intervention group, the number of vitamin A deficient children decreased from 53.6 % at baseline to 13.0 % at end of study, whereas in the Control group, it increased from 46.7 % to 52.6 %. There were no significant changes, however, in haematological indices in both Intervention and Control groups (Table 22). Vitamin A correlated positively and significantly with haemoglobin (HGB or Hb), haematocrit (HCT) in the Intervention group (Table 20a), but there was no significant correlation in the Control group (Table 20b). At cut – offs of both < 0.7 µmol/L and < 1.05 µmol/L to define vitamin A deficient status, there were significant improvements in the vitamin A status of the children after *M. oleifera* leaf consumption (Table 21). The indicators of kidney and liver function did not show any significant changes in posttest values over baseline, except AST which showed a positive change in the Control group (Tables 23 and 24).

Table 19: Vitamin A and haematological indices of children according to study group with time

Indices	Intervention Group				Control Group				Differences in mean changes at end of study
	Baseline Mean ± SD	Posttest Mean ± SD	Change	*P-value*	Baseline Mean ± SD	Posttest Mean ± SD	Change	*P-value*	
Vitamin A (µmol/L)	0.69 ± 0.33	1.03 ± 0.32	0.33 ± 0.25	<0.001**	0.77 ± 0.32	0.71 ± 0.28	-0.06 ± 0.22	0.094	0.39*
RBC x 10^4/µl	4.18 ± 0.44	4.27 ± 0.44	0.08 ± 0.59	0.250	4.16 ± 0.42	4.28 ± 0.36	0.12 ± 0.54	0.052	-0.04#
HGB (g/dL)	10.64 ± 1.00	10.65 ± 1.02	0.01 ± 1.29	0.963	10.57 ± 1.21	10.82 ± 1.10	0.26 ± 1.46	0.131	-0.25#
HCT (%)	32.73 ± 2.86	32.73 ± 2.51	0.00 ± 3.34	0.994	32.57 ± 2.81	33.01 ± 2.90	0.44 ± 3.83	0.324	-0.43#
MCV (fL)	78.59 ± 5.77	77.09 ± 5.21	-1.50 ± 7.65	0.109	78.93 ± 5.57	77.33 ± 5.63	-1.59 ± 7.25	0.060	0.10#

*RBC= Red blood cells; HGB = haemoglobin; MCV= Mean corpuscular volume; HCT = haematocrit **Differences significant within-group change comparison at P < 0.05; *Significant differences were observed in difference in changes between the Intervention and Control groups at P < 0.05; #No significant differences were observed in difference in changes between the Intervention and Control groups at P < 0.05.*

Table 20a: Bivariate correlation between vitamin A and haematological indices among children in the Intervention group

	VITA	RBC	HGB	MCV	HCT
RBC	0.185				
HGB	0.280*	0.555#			
HCT	0.325#	0.739#	0.870#	0.870#	
MCV	0.097	-0.680#	0.104		-0.017

*#Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed); VITA= Vitamin A; RBC= Red blood cells (x 10^4/µl); HGB= Haemoglobin; HCT= Haematocrit; MCV= Mean corpuscular volume (fL).*

Table 20b: Bivariate correlation between vitamin A and haematological indices among children in the Control group

	VITA	RBC	HGB	MCV	HCT
RBC	-0.014				
HGB	0.036	0.502#			
HCT	0.054	0.639#	0.888#	0.888#	
MCV	0.077	-0.423#	0.447#		0.427#

#Correlation is significant at the 0.01 level (2-tailed); No significance at P < 0.05. VITA= Vitamin A; RBC= Red blood cells (x 10^4/µl); HGB= Haemoglobin; HCT= Haematocrit; MCV= Mean corpuscular volume (fL).

Table 21: Effect of *M. oleifera* leaf consumption on vitamin A status of children 5-12 years

Vitamin A (µmol/L)	Intervention Group			
	Baseline Mean ± SD	**Posttest** Mean ± SD	**Change**	***P-value***
< 0.7	0.45 ± 0.16	0.94 ± 0.29	0.49 ± 0.29	< 0.001*
> 0.7	0.91 ± 0.20	1.13 ± 0.34	0.22 ± 0.30	
< 1.05	0.61 ± 0.23	0.99 ± 0.29	0.38 ± 0.32	< 0.001*
> 1.05	1.21 ± 0.22	1.39 ± 0.48	0.18 ± 0.37	

Table 22: Vitamin A and haematological status of school-aged children by study group

Indices	Intervention Group		Control Group	
	Baseline n (%)	Posttest n (%)	Baseline n (%)	Posttest n (%)
Vitamin A (µmol/L)				
< 0.7	37 (53.6)	9 (13.0)	35 (46.7)	40 (52.6)
< 1.05	63 (91.3)	39 (43.5)	64 (85.3)	74 (97.4)
RBC x 10^4/µl				
Low	24 (34.8)	20 (29.0)	28 (36.8)	17 (22.4)
Normal	42 (60.9)	46 (66.7)	46 (60.5)	58 (76.3)
High	3 (4.3)	3 (4.3)	2 (2.6)	1 (1.3)
HGB (g/dL)				
Anaemia	53 (76.8)	56 (81.2)	62 (81.6)	59 (77.6)
Normal	16 (23.2)	13 (18.8)	14 (18.4)	17 (22.4)
HCT (%)				
Low	63 (91.3)	67 (97.1)	72 (94.7)	74 (97.4)
Normal	6 (8.7)	2 (2.9)	4 (5.3)	2 (2.6)
MCV (fL)				
Low	37 (53.6)	48 (69.6)	40 (52.6)	48 (63.2)
Normal	32 (46.4)	21 (30.4)	36 (47.4)	28 (36.8)

RBC= Red blood cells; HGB= Haemoglobin, HCT= Haematocrit; MCV= Mean corpuscular volume; < 0.7 µmol/l; < 1.05 µmol/l = cut-offs to define vitamin A deficiency.

Table 23: Kidney function indices of participants according to study group with time

Haematological indices	Intervention Group				Control Group				Differences in mean changes at end of study
	Baseline Mean ± SD	Posttest Mean ± SD	Change	*P-value*[#]	Baseline Mean ± SD	Posttest Mean ± SD	Change	*P-value*[#]	
Urea	2.78 ± 0.38	2.31 ± 0.10	-0.47 ± 0.39	0.241	2.43 ± 0.11	2.43 ± 0.10	0.00 ± 0.12	0.991	-0.47[#]
Creatinine	49.89 ± 3.86	53.45 ± 1.93	3.57 ± 4.24	0.403	60.22 ± 6.20	50.80 ± 1.57	-9.42 ± 6.48	0.150	12.99[#]
Sodium	136.39 ± 0.92	138.02 ± 1.90	1.64 ± 2.00	0.415	138.51 ± 1.74	136.69 ± 2.38	1.82 ± 3.00	0.546	-0.19[#]
Potassium	3.70 ± 0.04	3.81 ± 0.08	0.11 ± 0.08	0.193	3.78 ± 0.06	3.81 ± 0.07	0.03 ± 0.09	0.717	0.08[#]
Chloride	101.02 ± 0.74	101.51 ± 1.34	0.49 ± 1.46	0.736	101.58 ± 1.29	102.06 ± 1.09	0.48 ± 1.80	0.789	0.01[#]

#No significant differences were observed in difference in changes between the intervention and control groups at $P < 0.05$

Table 24: Liver function indices of participants according to study group and with time

Haematological indices	Intervention Group				Control Group				Differences in mean changes at end of study
	Baseline Mean ± SD	Posttest Mean ± SD	Change	*P-value*[#]	Baseline Mean ± SD	Posttest Mean ± SD	Change	*P-value*	
ALT	18.83 ± 1.30	17.66 ± 1.44	-1.17 ± 1.65	0.481	16.89 ± 1.21	18.85 ± 1.08	1.96 ± 1.37	0.157	-3.13[#]
AST	35.78 ± 6.15	30.95 ± 1.60+	-4.84 ± 6.21	0.439	28.94 ± 0.76	31.46 ± 1.06	2.52 ± 1.18	0.036*	-7.35[#]
ALP	585.71 ± 18.38	616.46 ± 19.18	30.75 ± 22.00	0.167	602.58 ± 14.71	640.13 ± 17.25	37.55 ± 20.06	0.065	-6.81[#]
Albumin	42.50 ± 0.49	42.98 ± 0.36	0.48 ± 0.64	0.459	42.84 ± 0.41	42.83 ± 0.48	-0.01 ± 0.57	0.989	0.48[#]
Total Protein	88.12 ± 11.04	81.31 ± 0.87	-6.81 ± 11.17	0.544	79.99 ± 1.26	81.11 ± 1.32	1.12 ± 1.70	0.513	-7.93[#]
GGT	15.08 ± 0.82	15.30 ± 0.68	0.22 ± 0.91	0.806	18.60 ± 3.66	15.63 ± 0.84	-2.97 ± 3.84	0.441	3.20[#]
Direct bilirubin	3.73 ± 1.29	2.22 ± 0.24	-1.51 ± 1.32	0.256	3.16 ± 0.32	5.10 ± 1.48	1.94 ± 1.49	0.196	-3.45[#]
Total bilirubin	6.05 ± 1.06	7.31 ± 0.77	1.26 ± 1.37	0.361	6.97 ± 1.03	8.17 ± 0.93	1.20 ± 1.43	0.405	0.06[#]

*ALT= Alanine Aminotransferase; AST= Aspartate Aminotransferase; ALP= Alkaline phosphatase; GGT= G*amma-glutamyl transferase; **Differences significant within-group change comparison at P < 0.05; #No significant differences were observed in difference in changes between the intervention and control groups at P < 0.05*

The efficacy of *M. oleifera* leaf supplementation on Vitamin A deficiency in the current study is shown in Table 25.

Table 25: Efficacy of M. oleifera leaf supplementation on Vitamin A deficiency.

	Vitamin A Deficiency (VAD)		
VAD	Yes	No	Total
Intervention	9	60	69
Control	40	36	76
Total	49	96	145

Risk ratio (RR) = (9/69)/(40/76)

= 0.13/0.53

=0.246

Efficacy = 1 - RR

=1-0.246

= 0.754

= 75.4% (Kirkwood and Sterne, 2003)

CHAPTER FIVE

5.0 Discussion

5.1 Dietary uses of *M. oleifera* leaves

More than 50 % of the people surveyed ate *M. oleifera* leaves in one form or the other. It could be found planted in several households in the district. However, the leaves were not consumed regularly in the district. They were eaten once in a while. More than half of the respondents (51.3 %) would accept any innovations in *M. oleifera* leaf recipes in addition to their traditional uses. In addition, 212 (93.8 %) of mothers/caregivers would be willing to feed *M. oleifera* leaves to their children when requested to do so, because they learnt it is nutritious and good for children. Moreover, ninety-eight (43.4 %) actually fed their children with *M. oleifera* leaves once in a while and twenty-eight (12.4 %) fed their children once a week (Appendix 27),

Consumption of other β-carotene-rich foods in the district is not high and their availability is seasonal. Only fifty-two (23 %) of the respondents eat amaranth which is a β-carotene-rich leafy vegetable. *M. oleifera l*eaves, being known and used in the district as a food ingredient could serve as a good source of β-carotene for the district. Being already known but used sparingly in the district, nutrition education and promotion could enhance its uptake and regular use.

Tomato, pepper and onions are consumed normally as part of all salty foods in the community. However, onions and pepper are used as spices, so although they contain some level of β-carotene, they may contribute only small amounts to the diet of the people. Mangoes are also consumed, but mango production is seasonal (two seasons per year), and it is hardly available during the lean season (Ada-East District Assembly, 2013).

5.2 Acceptability of *M. oleifera*-fortified dishes

The *M. oleifera* leaves sampled contained all the micronutrients tested (β –carotene, Cu, Zn, Mn and Fe) in amounts comparable to the findings of other workers (Glover-Amengor and Mensah, 2012; Moyo *et al.*, 2011; Ogbe and Affiku, 2011; Seshadri and Nambiar 2003; Fuglie, 1999). These levels were reflected in the fortified dishes which showed significantly higher levels of micronutrients than the non-fortified dishes (Table 11). Leaf-fortified porridge (Porridge 2), for example, contained 0.15 mg/100 g Cu, 0.26 mg/100 g Mn, and 2.13 mg/100 g Fe. The non-fortified dishes however, contained 0.09 mg/100 g Cu, 0.15 mg/100 g Mn and 0.36 mg/100 g Fe respectively. The implication of these findings is that *M. oleifera* leaves could be used to increase the levels of Cu, Fe, Mn, Zn and β –carotene of diets.

Porridge can be instantly prepared with cereal/legume composite flour and fed to children, hence its preference over other dishes further enhances its use as a vehicle for *M. oleifera* leaf delivery. Interestingly, when supplemented for two weeks, the children still found the fortified dishes acceptable, and ate their portions over the supplementation period. Nambiar *et al.*, (2003) also assessed the feasibility and acceptability of introducing dehydrated *M. oleifera* leaves as a source of vitamin A among 40 pre-school children in an integrated child development programme. They also found that the fortified dishes (5-7 g/100 g product) were highly acceptable to the children. Typically, a child aged 5 years could consume 200 g of porridge (personal communication), so if fortified at 2 g/100 g, then 4 g of dried *M. oleifera* leaves becomes available to that child. Hence if fed twice a day with *M. oleifera* leaf-fortified porridge, 8 g dried leaves would be available to the child.

The implication of these results for food-to food fortification is that *M. oleifera* leaves have the potential to serve as a less expensive β-carotene and mineral source in the diets of children in Ghana and other tropical countries where these vegetables grow and adapt easily and where children often have marginal vitamin A status (Luu *et al*., 2005; Singh *et al*., 2001), and also suffer from iron deficiency anaemia in addition to other mineral deficiencies.

The study also showed other *M. oleifera* leaf-fortified dishes such as *waakye*, groundnut soup and *jollof* rice were also highly acceptable to the children during the two-week fortification of their school (GSFP) menu, and they (100 %) ate their portions of food during the period. This is significant because the plant could be grown in backyards for household use. Also, the GSFP could include *M. oleifera* leaves for enriching the diets of the children.

Although β-carotene is fat soluble, it does not pose any toxicity challenges, because β-carotene is stored safely and only converted to vitamin A when the body's vitamin A stores are depleted (Wardlaw, 1999). *M. oleifera* leaf consumption would thus ensure adequate reserves of β-carotene that could become available for conversion when needed by the body. Ten (10) grammes of dried *M. oleifera* leaves a day could provide 50-100 % of the vitamin A needs of all categories of age brackets and about 30 % of the iron needs of children between 1 and 12 years (de Saint Saveur and Broin 2010; Food and Nutrition Board, 2005). All the other nutrients including protein would also become available to the child (Kouevi, 2013). Zinc levels though not adequate to meet the RDI of 8 mg per day, could supplement other dietary sources.

5.3 Vitamin A, haematological indices and infections

5.3.1 Serum vitamin A

There was a significant increase in vitamin A levels in children in the Intervention group ($P < 0.05$), whilst those in the Control group experienced a slight but non-significant decrease in serum vitamin A levels (Table 19). In addition, 37 (53.6 %) of children were vitamin A deficient at baseline whilst 9 (13 %) were deficient at end of study in the Intervention group (serum retinol < 0.70 µmol/l). However, in the Control group, 35 (46.7%) and 40 (52.6 %) children were vitamin A deficient at baseline and end of study espectively (Table 22). This implies that subclinical vitamin A deficiency existed in the study groups at baseline and that *M. oleifera* leaf consumption improved serum retinol levels in the Intervention group.

Furthermore, in the Intervention group, those children who were vitamin A deficient at baseline (serum retinol < 0.70 µmol/l), significantly improved more in vitamin A status, whilst there was not much change in the serum retinol levels of those children who had retinol levels above 0.70 µmol/l (Table 21). Those with serum vitamin A < 0.70 µmol/l at baseline had an average value of 0.45 µmol/l, but at end of study, this value rose to 0.94 µmol/l, resulting in a change of 0.49 µmol/l, whereas in those with vitamin A level > 0.70 µmol/l at baseline, the change was from 0.91µmol/l to 1.13 µmol/l, resulting in a non-significant change of 0.22 µmol/l ($P < 0.001$). These findings were similar to those obtained by Ullah *et al.*, (2011), Lala and Reddy, (1970) and Zagre *et al*., (2003) who supplemented green leafy vegetables and red palm oil respectively. They found increases in serum retinol after supplementation and also observed that there was higher absorption in those with lower levels of serum retinol.

Agte *et al*., (2006), Persson *et al*., (2001) and Tyssandier *et al*., (2002) also reported that β-carotene levels increased when children were supplemented with dark green leafy vegetables and other carotenoid-rich foods respectively. In a well-controlled study, Jalal, (1991) found an increase in serum retinol when he supplemented red sweet potato and dark green leafy vegetables. Similarly, Vuong *et al*., (2002) reported increases in plasma retinol level in children when they supplemented the fruit *Momordica cochinchinensis* (*gac*) while van Jaarsveld *et al*., (2005) reported an increase in vitamin A status of primary school children supplemented with orange-fleshed sweet potato using modified-relative-dose-response (MRDR) test. The implication of these findings is that in low socio-economic countries where vitamin A status is mostly marginal (Luu *et al*., 2005; Singh *et al*., 2001), plant-based β-carotene-rich foods including green leafy vegetables could serve as alternative sources of vitamin A to children in place of high-dose capsule supplementation which is expensive in terms of health infrastructure and personnel, and animal source foods which are also unaffordable to low income groups.

Although de Pee and West (1995) did not find any significant improvement in serum retinol when they supplemented dark green vegetables in breastfeeding women, but only had significant levels in those women supplemented with β-carotene enriched wafer, they found a significant increase in serum β-carotene levels, implying that β-carotene in the leaves was absorbed by study participants, similar to the findings of Persson *et al*., (2001) and Tyssandier *et al*., (2002). In addition, all the women supplemented had serum retinol levels above 0.70 µmol/l, (0.89 µmol/l in the vegetable group, 0.84 µmol/l in the enriched wafer group and 0.81 µmol/l in the control wafer group) at baseline. This meant that although marginal, the women were not vitamin A deficient at baseline (cut-off < 70 µmol/l), so bioconversion of β-carotene to vitamin A in these

women could be low as these two processes have an inverse relationship with each other (Solomons, 2001; Wardlaw, 1999), and it is more enhanced in people with very low vitamin A status (Lala and Reddy, (1970) and Zagre *et al*., (2003).

Several dietary factors affect the absorption of carotenoids from foods and the major one is the type of food matrix in which the carotenoids are located. Dietary fat also plays an important role in carotenoid absorption (van het Hof *et al*., 2000; Solomons, 2001). Disruption of the food matrix through mechanical homogenization, and also heat treatment enhance the absorption of the carotenoids (van het Hof *et al*., 2000; Parker *et al*., 1999). Yang *et al*., (2006) reported a 46-63 % retention of total carotene and β-carotene from *M. oleifera* leaves during pressure cooking without oil whilst the addition of oil resulted in 76-99 % retention. In the current study, powdered leaf rather than the whole leaf was used, and all the dishes were cooked for five more minutes after the addition of *M. oleifera* leaf powder to enhance β-carotene absorption. Secondly, all the three dishes used in the current study contained some amount of oil to enhance nutrient absorption. The composite flour used for porridge contained peanut which is an oil seed (48 % oil). The gravy used in serving *waakye* was prepared with coconut oil, whilst the soup was made from peanut butter. A calculated efficacy 75.4 % for *M. oleifera* leaf supplementation on vitamin A deficiency in the current study indicates the potential of *M. oleifera* leaves in fighting vitamin A deficiency.

Dehydrated *M. oleifera* leaves contain about 25 mg/100 g β-carotene. Hence if there is 50 % absorption of β-carotene, then about 12.5 mg β-carotene becomes available for every 100 g leaf consumed. Someone consuming 10 g of dehydrated *M. oleifera* leaves daily would have

available to him or her, about (12.5/100) x 10 g β-carotene. This gives a value of 1.25 mg β-carotene/day

1 µg retinol = 1 RE

1 µg β-carotene = 0.167 RE (0.167 µg retinol)

So, for 1.25 mg (1250 µg) β-carotene: (1250 x 0.167 µg = 208.75 RE (208.75 µg retinol) would be available on consumption of 10 g *M. oleifera* leaf powder daily. The estimated mean requirement and safe level of intake for vitamin A is shown in Table 3 (FAO/WHO, 1988). Children 4-6 years require 200 RE/day; those 7 years require 250 RE/day, while adolescents, (10-18 years) require 330 - 400 RE/day. Thus *M. oleifera* leaf consumption has a potential for meeting vitamin A needs, and 10 g dried leaves would theoretically meet the requirements of children 4-6 years (de Saint Saveur and Broin, 2010).

Hence if well promoted, *M. oleifera* leaves could serve as a cheap source of vitamin A for tropical countries where the plant grows, and whose nutrition is mostly plant based. Daily consumption of the leaves will not pose any problem of vitamin A toxicity as opposed to ingestion of high doses of preformed vitamin A, because β-carotene absorbed from the leaves is stored, and is only converted to vitamin A in response to the body's need. Moreover, all other nutrients such as protein, the B-vitamins and minerals present in *M. oleifera* leaves will be available to the consumer (Table 6).

5.3.2 Haematological indices

There was no significant change in the haematological indices (RBC, HGB, HCT and MCV), both in the Intervention and Control groups at the end of study (Table 19). Mean haemoglobin was 10.64 g/dl at baseline and 10.65 g/dl at end of study in the Intervention group, with the

Control group having values of 10.57 g/dl and 10.82 g/dl at baseline and end of study respectively. However, vitamin A correlated positively and significantly with haemoglobin and haematocrit ($P < 0.05$) (Table 20a). RBC also correlated positively with HGB and HCT ($P < 0.01$). This is expected because one major role of vitamin A in the body is the mobilization of iron from its stores for haematopoiesis (Hashizume *et al*., 2005). Correlation of vitamin A with the haematological indices confirms this important role of vitamin A in the body (Hashizume *et al*., 2005). Hence in the presence of adequate iron stores, improved vitamin A status would theoretically improve haematological indices. There was no correlation between vitamin A and haemoglobin in the Control group (Table 20b).

The haemoglobin level of an individual could be determined by both nutritional and non-nutritional factors, and these may include iron deficiency, malaria, hookworm and other helminthic infections, chronic diseases and other nutritional deficiencies (West *et al*., 2007; Glover-Amengor *et al*., 2005; Dreyfus *et al*., 2000). There were no hookworm infections among the children studied. Malaria parasitaemia though present in both the Intervention and Control groups at baseline and end of study, did not cause any significant changes in the haematological indices (Appendix 26). At baseline, children with malaria parasitaemia had significantly low levels of vitamin A in both Intervention and Control groups, but at end of study, the differences were not significant in both arms of study (Appendix 26). A *P. falciparum* infected erythrocyte is changed and easily undergoes haemolysis (Caufield *et al*., 2004). Malaria also causes suppression of haematopoiesis, because it leads to low serum retinol levels, and since retinol facilitates haematopoiesis by mobilizing iron from its storage organs, reduced levels would hamper haematopoiesis. Moreover iron deficient erythropoiesis could also occur if normal or

elevated iron stores are sequestered as a result of inflammation from infection (Means, 2000; Semba *et al*., 2001). Infections generally depress circulating retinol and contribute to vitamin A depletion (Glasziou and Mackerras, 1993; Solomons and Keusch, 1981). Christian *et al.*, (1998) and Filteau *et al*., (1993) reported that clinical and sub-clinical infections can lower serum vitamin A levels by as much as 25 % on the average independently of vitamin A intake.

5.4 Kidney and Liver Function

5.4.1 Kidney Function

The results of kidney function indices of children according to study group with time are presented in Table 23. *e.g*. the mean urea level in the Intervention group at baseline was 2.78 ± 0.38; that in the Control group was 2.43 ± 0.11. At end of study, values obtained were 2.31 ± 0.10 for Intervention group and 2.43 ± 0.10 for Control group. Similarly all the other parameters are presented with the changes that occurred between baseline and end of study values. There were no significant changes in parameters between the Intervention and Control groups, and within groups (baseline and end of study). All values fell within the recommended safety limits for kidney chemistry. *M. oleifera* leaf consumption then could be described as safe, and not injurious to the kidneys.

The biosynthesis of urea (a major metabolite of protein catabolism) from ammonia is exclusively carried out by hepatic enzymes. More than 90 % of urea is excreted through the kidneys, with the remainder being excreted through the gastrointestinal tract or skin. Blood urea concentrations can be increased by numerous factors such as increased protein catabolism, as in haemorrhage

into gastrointestinal tract, shock, some chronic liver diseases or acute or chronic renal diseases and renal obstruction to urine flow (Newman and Price, 2001; Tietz, 1995; First, 2003).

Creatinine is the spontaneous waste product of creatine metabolism. The serum creatinine rate turns to remain constant; when a high serum creatinine rate is observed (associated with a high urea rate), it corresponds to a decrease in renal glomerular filtration (FGR). Creatinine is thus an excellent marker of renal function. (Tietz, 1995; Burtler, 1975). The determination of urea rate is used together with the determination of creatinine rate to determine renal function.

Proteins constitute about 6 % of the blood plasma volume with all the ions in the blood – sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), chloride (Cl) phosphate (PO_3^{3-}) and sulphate (SO_4^{3-}) occupying the remaining 94 %. (Preuss, 2001). Healthy kidneys protect the circulating concentrations of proteins by preventing renal losses. Thus renal regulation of fluids and solutes creates homeostatis in the body. High levels of sodium lead to thirst, and this requires the consumption of water to quench the thirst. This water then dilutes the blood plasma, and hence needs to be excreted to maintain the homeostatis in the body (Preuss, 2001). Extra work then needs to be done by the heart and kidney to pump out the excess water, resulting in a rise in blood pressure in the process. Sodium is coupled to chloride in the performance of its role. Excretion of high levels of the ions then meant a disturbance of the body's homeostatis that might exert pressure on the kidney.

In kidney chemistry, reference values are set for these various parameters that determine kidney function i.e. urea, creatinine, potassium, sodium and chloride ions. In the current study, all the

values fell within the recommended limits both at baseline and end of study (Tietz, 1995; Burtler, 1975); moreover, there were no significant differences between baseline and end of study values, suggesting that *M. oleifera* leaf consumption did not affect these markers for kidney safety in the children. It implies that *M. oleifera* leaf consumption did not have any adverse effect on kidney function. Ugwu *et al.*, (2013) similarly found that *M. oleifera* leaf extract did not have any adverse effect on serum creatinine level in mice.

5.4.2 Liver Function

Liver function indices of children according to study group with time are presented in Table 24. For example, AST levels were 35.78 ± 6.15 and 30.95 ± 1.60 at baseline and end of study respectively in the Intervention group, while in the Control group, the values were 28.94 ± 0.76 at baseline and 31.46 ± 1.06 at end of study. Changes between baseline and end of study values are also presented. No significant changes were observed between Intervention and Control groups and within groups (baseline and end of study). All values fell within the recommended safety limits for liver chemistry. *M. oleifera* leaf consumption then could be described as safe, causing no injury to the liver.

A number of enzymes act in several processes that are involved with the liver, and changes in levels of these enzymes are markers for liver function. The enzymes include Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Gamma-glutamyltransferase (GGT) and Alkaline phosphatase (ALP). Bilirubin, a by-product of haemoglobin metabolism that is derived from the heme moiety of haemoglobin is also a good marker for liver function. Approximately 80-85 % of the bilirubin produced is derived from the heme moiety of the

haemoglobin released from aging erythrocytes in the reticuloendothelial cells. Bilirubin, bound to albumin, is transported into the liver where it is rapidly conjugated with glucuronide to increase its solubility. Then it is excreted into billiary canaliculi, and hydrolyzed in the gastrointestinal tract. Unconjugated serum bilirubin concentration increases in case of overproduction of bilirubin (acute or chronic haemolytic anemias) and in case of disorders of bilirubin metabolism and transport defects (impaired uptake by liver cells). Reduced excretion due to hepatocellular damage, hepatitis, cirrhosis and obstruction to the flow of bile (most often produced by gallstones or by tumours) induce an important elevation of conjugated bilirubin (Schreiber, 2003; Sherwin and Thompson, 2003).

Aspartate aminotransferase (AST) also known as glutamate oxaloacetate transaminase (GOT) is a transaminase that catalyses the transfer of the amino group of L-aspartate to α–ketoglutarate to give L-glutamate. AST is widely distributed in the body, but the highest levels are found in heart, liver, skeletal muscles and kidneys. Damages to cells of these tissues induce AST increase in serum. Liver cell necrosis or injury of any cause, for example intake of alcohol, and administration of drug induce moderate AST elevation (Sherwin and Thompson, 2003).

Alanine aminotransferase (ALT) also known as glutamate pyruvate transaminase (GPT) is another transaminase. ALT catalyses the transfer of the amino group of L-alanine to alpha-ketoglutarate to give L-glutamate. The highest levels are found in the liver and the kidneys, and in smaller amounts in heart and skeletal muscle. ALT concentration is increased when hepatic cells are damaged (liver cell necrosis or injury of any cause). Intake of alcohol, delirium tremens, and administration of various drugs induce slight or moderate elevation of ALT. Measurement of

both AST and ALT have some value in distinguishing hepatitis from other parenchymal lesions (Wu, 2006; Tietz, 1995).

Gamma-glutamyltransferase (GGT) is a membrane-localized peptidase mainly present in kidneys, pancreas, liver and prostate. This enzyme plays a significant role in glutathione metabolism and takes part in the transport of amino acids into the cells. The rise of GGT activity, often isolated (earlier and longer increase compared to other enzymes), is one of the most sensitive indicators of an affection of the liver or bile ducts (Dufour, 2010).

Alkaline phosphatase (ALP) corresponds to a group of phosphatases that display maximum activity at alkaline pH. ALP is widely distributed in liver, osteoblasts, intestinal epithelium, kidneys, and placenta. The rate of ALP rises physiologically for children and teenagers during periods of active growth, as well as for women during the third trimester of pregnancy (Wu, 2006).

Total Protein

Most plasmatic proteins are synthesized by the liver except immunoglobulins. In human plasma, albumin accounts for 50 – 60 % of total proteins. For a normal plasmatic volume, abnormal total protein rates only occur in the event of disorder affecting the concentration of albumin or immunoglobulins. Thus severe proteinic insufficiency (malabsorption, maldigestion, dietary insufficiency), renal and hepatic diseases result in hypoproteinemia. If total protein concentration is lower than 40 g/l, oedemas can be observed (Preuss, 2001).

Albumin

Albumin, synthesized primarily by the liver, represents 50 – 60 % of total serum proteins. Due to its small size and its high plasma concentration, albumin is the major protein component of most extravascular body fluid including urine. Albumin's primary function is the maintenance of colloidal osmotic pressure in both extravascular and vascular spaces, with continuous equilibration. Increased levels of albumin are present only in acute dehydration. Hypoalbuminemia is seen in a multitude of diseases such as acute and chronic inflammation, hepatic insufficiency and increased loss.

All the markers of liver function determined in this study fell within the recommended safety limits except for ALP which had elevated values, but this is in line with literature (Wu, 2006; Tietz, 1995) as the value of this enzyme rises physiologically for children and teenagers during periods of active growth; there were no significant differences between baseline and end of study values either, implying that *M. oleifera* leaf consumption did not cause any significant changes in the markers of liver function studied. Ugwu *et al.,* (2013) also did not find any adverse effect on bilirubin levels in mice supplemented with ethanolic *M. oleifera* leaf extract. Similarly, a controlled study conducted at School of Pharmacy, University of Ghana showed that *M. oleifera* leaf was non-toxic to rats (Asiedu-Gyekye *et al*., 2014), confirming the safety of *M. oleifera* leaf consumption.

CHAPTER SIX

6.0 Conclusions and Recommendations

6.1 Conclusions

1. *M. oleifera* leaf is used as a food ingredient in Ada-East district of Ghana.
2. *M. oleifera* leaf-fortified dishes were acceptable to children in Ada-East district of Ghana.
3. *M. oleifera* leaf supplementation improved the vitamin A status of children in Ada-East district of Ghana.
4. *M. oleifera* leaf supplementation did not increase haemoglobin levels in the children in this study, but there was a significant positive association between vitamin A and haemoglobin in the Intervention group.
5. There was no adverse event associated with *M. oleifera* leaf consumption as all markers of both kidney and liver safety *(*Alanine transaminase (ALT), Aspartate aminotransferase (AST), Gamma-glutamyltransferase (GGT), Alkaline Phosphatase (ALP), Direct bilirubin, Total bilirubin, urea, creatinine, sodium, potassium and chloride) did not change significantly after *M. oleifera* leaf consumption. Moreover, all values fell within permitted safety levels at end of study.
6. The null hypothesis (no significant difference) is rejected, while the study failed to reject the alternative hypothesis of significant difference between vitamin A levels in the Intervention and Control groups at end of study.

6.2 Recommendations

It is recommended that:

- ✓ *M. oleifera* leaf is introduced as food into the Government of Ghana School Feeding Programme (GSFP) in Ada-East district. However, a further study is recommended to assess the extra cost it will add to the feeding budget.
- ✓ Ada-East District Assembly educates the communities on the nutritional benefits of *M. oleifera* leaves, and sets processes in place for training mothers and caregivers in processing, preservation, storage and preparation of various dishes and snacks from *M. oleifera* leaves.
- ✓ The District Health Administration introduces *M. oleifera* leaf-fortified porridge as complementary food for infants.
- ✓ Further work is done on *M. oleifera* leaf supplementation and haemoglobin and other indicators of iron status.

CHAPTER SEVEN

7.0 References

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APPENDICES

Appendix 1: In-house consumer acceptability of dishes

ANOVA

Sample	ATTRIBUTES	MEANS	*P value*
Ofam	Appearance	6.433333	0.1616
	Colour	6.066667	0.0548
	Texture	6.733333	0.1907
	Flavour	6.666667	0.1517
	Taste	6.733333	0.6097
	Aftertaste	6.766667	0.2111
	Overall Acceptability	6.800000	0.1911
Porridge	Appearance	6.827586	0.0036
	Colour	6.413793	0.0373
	Texture	6.827586	0.7692
	Flavour	6.413793	0.3355
	Taste	6.413793	0.2123
	Aftertaste	6.344828	0.3244
	Overall Acceptability	6.13793	0.3139
Beans and *gari*	Appearance	7.133333	0.7291
	Colour	7.400000	0.7694
	Texture	6.733333	0.5336
	Flavour	6.200000	0.3844
	Taste	5.333333	0.6024
	Aftertaste	5.166667	0.1287
	Overall Acceptability	5.666667	0.1302
Nkotomire sauce	Appearance	6.933333	0.0014

	Colour	6.933333	0.0043
	Texture	6.666667	0.6402
	Flavour	6.166667	0.0880
	Taste	5.200000	0.0001
	Aftertaste	5.400000	0.0022
	Overall Acceptability	6.133333	0.0117
Groundnut soup	Appearance	6.888889	0.1194
	Colour	6.962963	0.0317
	Texture	6.703704	0.6619
	Flavour	6.814815	0.1464
	Taste	6.296296	0.3193
	Aftertaste	6.111111	0.5779
	Overall Acceptability	6.407407	0.0586
Apapransa	Appearance	5.076923	0.0004
	Colour	5.269231	0.0006
	Texture	6.230769	0.0092
	Flavour	5.730769	0.1878
	Taste	5.115385	0.0156
	Aftertaste	5.000000	0.0047
	Overall Acceptability	5.84615	0.1972
Jollof rice	Appearance	4.185185	0.0018
	Colour	4.333333	0.0010
	Texture	6.148148	0.3007
	Flavour	5.296296	0.3128
	Taste	5.296296	0.0173
	Aftertaste	4.592593	0.0212
	Overall Acceptability	5.259259	0.0488

Waakye	Appearance	5.370370	0.0396
	Colour	4.962963	0.0122
	Texture	6.407407	0.4443
	Flavour	5.777778	0.0300
	Taste	5.851852	0.0076
	Aftertaste	5.518519	0.0425
	Overall Acceptability	5.814815	0.0156

There are significant differences among attributes with $p < 0.05$

Appendix 2: Nutrient Determination in Food Samples

Beta carotene was determined in the *M. oleifera* leaf powder as well as the fortified foods used in the intervention. Iron, zinc, copper and manganese levels were also determined. In addition, proximate values of carbohydrate, fat, protein and ash were determined in the food samples Beta-carotene was determined by spectrophotometry (Karnjanaurpagnyl *et al.*, 2010). The *M. oleifera* leaf extract was filtered slowly through anhydrous sodium sulphate sitted on glass micro fibre filter paper at the base of a funnel to remove chlorophyll from it.

i) For proximate values, moisture was determined using AOAC 925.10 (1990); ash by AOAC 923.03 (2000); fat by AOAC 920.39C (2000); protein by AOAC 984.13 (1990) whilst carbohydrate including fibre was determined by difference between all the proximate values, and energy was determined by Atwater Factor (A16).

i) Iron, zinc, copper and manganese were determined by Atomic Absorption Spectrophotometry. The dry ashing method was used for Atomic Absorption Spectrophotometry (AAS) analysis (AOAC 2005). For each sample 3g was ashed in a muffle

furnace at 550ºC for 8 hours followed by acid digestion. Buck Scientific 210VGP Flame Atomic Absorption Spectrophotometer (Buck Scientific, Inc. East Norwalk, USA) was used to read the absorbance at appropriate wavelengths, of the metals of interest in the sample solution. Reference samples for validation of results were obtained from FAPAS (Food Analysis Performance Assessment Scheme), UK and National Institue of Science and Technology, USA (for vitamin A by HPLC).

Appendix 3: Reference values for haematological indices

Haematological indices	Reference range
[1]White Blood Cells (WBC)	3.5-11.0 (K/µL)
[1]Red Blood Cells (RBC)	4.0-5.0 (M/µL)
[2]Haemoglobin level (Hb)	12.0-18.0 (g/dl)
[2]Haematocrit (HCT)	37.0-51.0 (%)
[2]Mean corpuscular volume	80.0-97.0 (fL)
[2]Mean corpuscular Haemoglobin	26.0-32.0 (pg)
[2]Mean corpuscular Haemoglobin Concentration	31.0-36.0 (g/dl)

[1]http://www.mayoclinic.com
[2]Reference values are those provided by the Ghana Health Service

Appendix 4: Reference values for biochemical indices

Biochemical indices	Reference range
[1]Total Serum Protein	6.6-8.7 (g/dl)
[1]Serum Albumin	3.8-5.3 (g/dl)
[1]Serum Globulin	2.0-4.8 (g/dl)

[1]**Serum iron**	37-145 (µg/dl)
[2]**Serum ferritin**	15-150 (µg/l)

[1]Reference values are those provided by the manufacturer of the reagents (Human Gesellschaft Biochemical und Diagnostica, Germany); [2]Reference values (WHO, 2011)

Appendix 5: Classification of anaemia

Level of anaemia	**Haemoglobin Cut offs (g/dl)**
Normal/non anaemic	≥11.0
Any form of anaemia	<11.0

Source: Ghana demographic and health survey (2008)

Appendix 6: Vitamin A and haematological indices of children 5-12 years by sex at baseline

Haematological indices	**Intervention**			**Control**		
	Sex		***p-value#***			***p-value#***
	Male	**Female**		**Male**	**Female**	
Vitamin A (µmol/L)						
< 0.7	20 (29.0)	17 (24.6)	0.938	19 (25.3)	16 (21.3)	0.646
< 1.05	35 (50.7)	28 (40.6)	0.405	34 (45.3)	30 (40.0)	0.346
RBC x 10^4/µl						
Low	12 (17.4)	12 (17.4)	0.144	14 (18.4)	14 (18.4)	0.999
Normal	25 (36.2)	17 (24.6)		24 (31.6)	22 (28.9)	
High	0 (0.0)	3 (4.3)		1 (1.3)	1 (1.3)	
HGB (g/dL)						
Anaemia	30 (43.5)	23 (33.3)	0.404	30 (39.5)	32 (42.1)	0.378
Normal	7 (10.1)	9 (13.0)		9 (11.8)	5 (6.6)	

HCT (%)						
Low	35 (50.7)	28 (40.6)	0.405	37 (48.7)	35 (46.1)	0.999
Normal	2 (2.9)	4 (5.8)		2(2.6)	2(2.6)	
MCV (fL)						
Low	24 (34.8)	13 (18.8)	0.055	19 (25.0)	21 (27.6)	0.501
Normal	13 (40.6)	19 (27.5)		20 (26.3)	16 (21.1)	

RBC= Red blood cells; HGB= Haemoglobin; HCT= Haematocrit; MCV= Mean corpuscular volume; #No statistically significant differences were observed at $p < 0.05$ (Fisher's exact test). Normal/non anaemic = **Hb** ≥11.0; any form of anaemia = Hb <11.0

Appendix 7: Vitamin A and haematological indices of children 5-12 years by age at baseline

Haematological indices	**Intervention (n=69)**				**Control (n=76)**			
	Age (years)			***p-value***	**Age (years)**			***p-value***
	5-7	**8-10**	**11-12**		**5-7**	**8-10**	**11-12**	
Vitamin A								
< 0.7	13 (18.8)	21 (30.4)	3 (4.3)	0.879	17 (22.7)	13 (17.3)	5 (6.7)	0.231
< 1.05	21 (30.4)	35 (50.7)	7 (10.1)	0.865	26 (34.7)	30 (40.0)	8 (10.7)	0.301
RBC x 10^4/µl								
Low	9 (13.0)	11 (15.9)	4 (5.8)	0.330	12 (15.8)	13 (17.1)	3 (3.9)	0.388
Normal	12 (17.4)	27 (39.1)	3 (4.3)		14 (18.4)	25 (32.9)	7 (9.2)	
High	2 (2.9)	1 (1.4)	0 (0.0)		2 (2.6)	0 (0.0)	0 (0.0)	
HGB (g/dL)								
Anaemia	19 (27.5)	27 (39.1)	7 (10.1)	0.187	27 (35.5)	28 (36.8)	7 (9.2)	0.021*
Normal	4 (5.8)	12 (17.4)	0 (0.0)		1 (1.3)	10 (13.2)	3 (3.9)	
HCT (%)								
Low	20 (29.0)	36 (52.2)	7 (10.1)	0.826	28 (36.8)	35 (46.1)	9 (11.8)	0.246

Normal	3 (4.3)	3 (4.3)	0 (0.0)		0 (0.0)	3 (3.9)	1 (1.3)	
MCV (fL)								
Low	13 (18.8)	20 (29.0)	4 (5.8)	0.938	16 (21.1)	21 (27.6)	3 (3.9)	0.349
Normal	10 (14.5)	19 (27.5)	3 (4.3)		12 (15.8)	17 (22.4)	7 (9.2)	

RBC= Red blood cells; HGB= Haemoglobin; HCT= Haematocrit; MCV= Mean corpuscular volume; *Differences significant at $p < 0.05$ (Fisher's exact test).

Appendix 8: Malaria and infection indices of school-aged children 5-12 years by sex

Haematological indices	**Intervention (**			**Control**		
	Sex		***P-value*#**	**Sex**		***P-value*#**
	Male	**Female**		**Male**	**Female**	
	Baseline					
Anaemia						
Severe				0 (0.0)	3 (3.9)	0.180
Moderate	24 (34.8)	17 (24.6)	0.598	23 (30.3)	19 (25.0)	
Mild	6 (8.7)	6 (8.7)		7 (9.2)	10 (13.2)	
Normal	7 (10.1)	9 (13.0)		9 (11.8)	5 (6.6)	
Malaria parasite						
No MPS	29 (42.0)	26 (37.7)	0.542	34 (44.7)	30 (39.5)	0.341
Symptomatic	8 (11.6)	5 (7.2)		4 (5.3)	7 (9.2)	
Max. parasitimia	0 (0.0)	1 (1.4)				
Infections						
Absent	33 (47.8)	25 (36.2)	0.324	36 (47.4)	33 (43.4)	0.708
Present	4 (5.8)	7 (10.1)		3 (3.9)	4 (5.3)	
	Posttest					

Anaemia						
Severe						
Moderate	21 (30.4)	16 (23.2)	0.196	18 (23.7)	21 (27.6)	0.597
Mild	12 (17.4)	7 (10.1)		12 (15.8)	8 (10.5)	
Normal	4 (5.8)	9 (13.0)		9 (11.8)	8 (10.5)	
Malaria parasite						
No MPS	29 (42.0)	18 (26.1)	0.071	27 (35.5)	27 (35.5)	0.803
Symptomatic	8 (11.6)	12 (17.4)		12 (15.8)	10 (13.2)	
Max. parasitimia	0 (0.0)	2 (2.9)				
Infections						
Absent	33 (47.8)	25 (36.2)	0.324	36 (47.4)	33 (43.4)	0.708
Present	4 (5.8)	7 (10.1)		3 (3.9)	4 (5.3)	

#No statistically significant differences were observed at $p < 0.05$ (Fisher's exact test)

Appendix 9: Malaria and infection indices of school-aged children 5-12 years by age

Haematological indices	**Intervention**				**Control**			
	Age (years)			***P-value#***	**Age (years)**			***P-value***
	5-7	**8-10**	**11-12**		**5-7**	**8-10**	**11-12**	
	Baseline							
Anaemia								
Severe	0 (0.0)	0 (0.0)	0 (0.0)	0.411	1 (1.3)	2 (2.6)	0 (0.0)	0.155
Moderate	15 (21.7)	20 (29.0)	6 (8.7)		18 (23.7)	18 (23.7)	6 (7.9)	
Mild	4 (5.8)	7 (10.1)	1 (1.4)		8 (10.5)	8 (10.5)	1 (1.3)	
Normal	4 (5.8)	12 (17.4)	0 (0.0)		1 (1.3)	10 (13.2)	3 (3.9)	
Malaria parasite								
No MPS	19 (27.5)	30 (43.5)	6 (8.7)	0.566	24 (31.6)	32 (42.1)	8 (10.5)	0.908

Symptomatic	3 (4.3)	9 (13.0)	1 (1.4)		4 (5.3)	5 (6.6)	2 (2.6)	
Max. parasite	1 (1.4)	0 (0.0)	0 (0.0)		0 (0.0)	1 (1.3)	0 (0.0)	
Infections								
Absent	19 (27.5)	34 (49.3)	5 (7.2)	0.389	26 (34.2)	33 (43.4)	9 (11.8)	0.872
Present	4 (5.8)	5 (7.2)	2 (2.9)		2 (2.6)	5 (6.6)	1 (1.3)	
				Posttest				
Anaemia								
Moderate	14 (20.3)	18 (26.1)	5 (7.2)	0.622	18 (23.7)	19 (25.0)	2 (2.6)	0.036*
Mild	5 (7.2)	12 (17.4)	2 (2.9)		8 (10.5)	9 (11.8)	3 (3.9)	
Normal	4 (5.8)	9 (13.0)	0 (0.0)		2 (2.6)	10 (13.2)	5 (6.6)	
Malaria parasite								
No MPS	18 (26.1)	25 (36.2)	4 (5.8)	0.529	18 (23.7)	28 (36.8)	8 (10.5)	0.604
Symptomatic	4 (5.8)	13 (18.8)	3 (4.3)		10 (13.2)	10 (13.2)	2 (2.6)	
Max. parasite	1 (1.4)	0 (0.0)	0 (0.0)					
Infections								
Absent	22 (31.9)	31 (44.9)	5 (7.2)	0.123	26 (34.2)	33 (43.4)	10 (13.2)	0.635
Present	1 (1.4)	8 (11.6)	2 (2.9)		2 (2.6)	5 (6.6)	0 (0.0)	

#No statistically significant differences were observed at *P <0.05* (Fisher's exact test); *Differences significant at *P <0.05* (Fisher's exact test)

Appendix 10: Effect of malaria parasitaemia on haematological indices

Haematological indices	**Intervention**		***P-value***	**Control**		***P-value***
	Malaria parasitemia	**No malaria parasites seen**		**Malaria parasitemia**	**No malaria parasites seen**	
			Baseline			

RBC x 10^4/µl	4.30 ± 0.42	4.15 ± o.45	0.275	4.12 ± 0.39	4.16 ± 0.43	0.729
HGB (g/dL)	11.08 ± 1.14	10.53 ± 0.94	0.065	10.81 ± 1.03	10.52 ± 1.25	0.460
HCT (%)	33.89 ± 3.59	32.43 ± 2.60	0.088	32.93 ± 2.17	32.50 ± 2.92	0.638
MCV (fL)	78.99 ± 6.37	78.49 ± 5.67	0.775	80.35 ± 6.13	78.66 ± 5.47	0.338
			Posttest			
RBC x 10^4/µl	4.21 ± 0.49	4.30 ± 0.42	0.437	4.20 ± 0.30	4.31 ± 0.38	0.204
HGB (g/dL)	10.36 ± 1.00	10.79 ± 1.01	0.100	10.45 ± 1.13	10.97 ± 1.07	0.062
HCT (%)	32.08 ± 2.41	33.06 ± 2.52	0.127	32.13 ± 2.61	33.36 ± 2.96	0.093
MCV (fL)	76.72 ± 5.62	77.28 ± 5.05	0.678	76.76 ± 5.90	77.57 ± 5.56	0.573

#No statistically significant differences were observed at $p < 0.05$ (Fisher's exact test); *Differences significant at $p < 0.05$ (Fisher's exact test)

Appendix 11: Effect of malaria parasitemia on Vitamin A and haematological indices

Indices	Intervention)		*P-value*	Control		*P-value*	Intervention)		*P-value*	Control		*P-value*
	MAL	NMAL		MAL	NMAL		MAL	NMAL		MAL	NMAL	
	Baseline						Endline					
Vitamin A (µmol/L)	0.62 ± 0.26	0.83 ±0.34	0.014*	0.58 ± 0.28	0.81 ± 0.32	0.024*	1.05 ± 0.28	1.02 ± 0.35	0.758	0.68 ± 0.22	0.74 ± 0.19	0.228
RBC x 10^4/µl	4.30 ± 0.42	4.15 ± 0.45	0.275	4.12 ± 0.39	4.16 ± 0.43	0.729	4.21 ± 0.49	4.30 ± 0.42	0.437	4.20 ± 0.30	4.31 ± 0.38	0.204
HGB (g/dL)	11.08 ± 1.14	10.53 ± 0.94	0.065	10.81 ± 1.03	10.52 ± 1.25	0.460	10.36 ± 1.00	10.79 ± 1.01	0.100	10.45 ± 1.13	10.97 ± 1.07	0.062
HCT (%)	33.89 ± 3.59	32.43 ± 2.60	0.088	32.93 ± 2.17	32.50 ± 2.92	0.638	32.08 ± 2.41	33.06 ± 2.52	0.127	32.13 ± 2.61	33.36 ± 2.96	0.093
MCV (fL)	78.99 ± 6.37	78.49 ± 5.67	0.775	80.35 ± 6.13	78.66 ± 5.47	0.338	76.72 ± 5.62	77.28 ± 5.05	0.678	76.76 ± 5.90	77.57 ± 5.56	0.573

RBC= Red blood cells; HGB= Haemoglobin; HCT= Haematocrit; MCV= Mean corpuscular volume; MAL= Malaria; NMAL= No malaria; *Statistically significant differences were observed at $p < 0.05$.

Appendix 12: Kidney function indices of school-aged children 5-12 years by age

Kidney function indices	Intervention				Control			
	Age (years)			*p-value*[#]	Age (years)			*p-value*[#]
	5-7	8-10	11-12		5-7	8-10	11-12	
Baseline								
urea								
Low	4 (6.2)	3 (4.7)	0 (0.0)	0.680	2 (2.7)	9 (12.3)	0 (0.0)	0.092
Normal	18 (28.1)	31 (48.4)	7 (10.9)		26 (35.6)	28 (38.4)	8 (11.0)	
High	0 (0.0)	1 (1.6)	0 (0.0)		0 (0.0)	0 (0.0)	0 (0.0)	
creatinine								
Low	17 (27.0)	32 (50.8)	7 (10.9)	0.415	23 (31.9)	32 (44.4)	7 (9.7)	0.939
Normal	4 (6.2)	3 (4.7)	1 (1.6)		4 (5.6)	5 (5.6)	1 (1.4)	
Posttest								
urea								
Low	4 (6.2)	5 (7.8)	0 (0.0)	0.590	5 (6.8)	7 (9.6)	0 (0.0)	0.600
Normal	18 (28.1)	30 (46.9)	7 (10.9)		23 (31.5)	30 (41.1)	8 (11.0)	
creatinine								
Low	21 (32.8)	32 (50.0)	6 (9.4)	0.651	28 (38.4)	36 (49.3)	7 (9.6)	0.209
Normal	1 (1.6)	3 (4.7)	1 (1.6)		0 (0.0)	1 (1.4)	1 (1.4)	

#No statistically significant differences were observed at $P < 0.05$ (Fisher's exact test)

Appendix 13: Calculation of absorbance for retinol assessment

$$ng/\mu l = \frac{ABS \times 10{,}000}{}$$

Appendix 14: Reference values for Kidney Function

Kidney indice	Limits	
urea N	**1.6**	**7.2**
creatinine	**81.4**	**141.2**
sodium		
potassium		
chloride		

Appendix 15: Reference values for Liver Function

Liver indice	**Limits**	
ALT	**11.4**	**53.0**
AST	**18.0**	**65.0**
ALP	**5.0**	**270.0**
Albumin	**35.0**	**68.0**
Total Protein	**50.0**	**90.0**
GGT	**0.0**	**50.0**

Direct bilirubin	**0.0**	**12.0**
Total bilirubin	**1.7**	**30.8**

Appendix 16: Formulae for Calculation of Carbohydrate and Energy

Carbohydrate = 100 – (Proximate values of fat and protein)

Energy = (Protein x 4) + (Fat x 9) + (Carbohydrate x 4)

Appendix 17: Questionnaire for Survey

TO BE ADMINISTERD TO MOTHERS/CAREGIVERS OF HOUSEHOLDS

School of Public Health

University of Ghana

Legon

***MORINGA OLEIFERA* LEAF SUPPLEMENTATION AND VITAMIN A STATUS OF CHILDREN IN ADA-EAST DISTRICT OF GHANA**

Community:

Name of Interviewer:

Name of House:

House Number:

Date:

A. Vegetables

1. What vegetables do you often consume?

   Cocoyam leaves (kontomire)

   Garden eggs

   Okro

   Pepper (fresh or dried)

   Onions (large/small)

   Tomatoes (fresh)

   Amaranthus

   Ademe

   Bokorbokor (Yevu-gboma)

   Gboma

   Moringa oleifera leaves

   Other vegetables

2. How often do you consume these vegetables?

   a. [ ] daily b. [ ]once/week c. [ ] three times/week d. [ ] other (specify)

3. What is your local name for *M. oleifera*?
4. How is *M. oleifera* leaves prepared for use as food in your locality?
   a. [ ]in soup (specify type of soup) b. [ ]in stew (specify and describe)

   c. [ ] other (specify)

5. Is *M. oleifera* fed to children in your locality? a.Yes[ ] b. No [ ]
   From what age can children be fed with *M. oleifera* in your locality?
   a. [ ]0 – 6 months b. [ ] 6months – 5 years c. [ ]other (specify)
6. What quantity could you use at a time? (Describe)
7. How often do you feed it to children?
   a. [ ]daily b. [ ]once/week c. [ ]three times/week d. [ ]other (specify)
8. Have you ever fed your child with *M. oleifera* leaves? a.Yes[ ] b. No [ ]
9. Would you like to feed your child with *M. oleifera* leaves if requested to do so?
   a.Yes[ ] b. No [ ]
   If no, why?
   If yes, why?
   In what form would you prefer to use *M. oleifera* leaves?
   a. [ ]traditionally prepared form b. [ ] any innovative method to which you are introduced
   Give reasons for your answer

   Apart from food use, what else do you use *M. oleifera* leaves for in your locality?

B. Fruits

Banana
Orange/tangerine
Pineapple
Mango
Avocado pear
Water melon
Coconut

10. How often do you consume these fruits?

[ ] daily b. [ ]once/week c. [ ]three times/week d. [ ] other (specify)

C. Staples

Which staples do you consume?

1. **Starchy Staples**

Cassava
Cocoyam
Plantain
Yam
Cassava dough
Gari
Kokonte
Others

2. **Cereals**

Maize Millet
Sorghum/guinea corn
Rice local
Rice-imported
Others

3. **Pulses and Nuts**

Beans
Groundnuts
Palm-nuts
Cola-nuts
Others

Is the children's food different from that of adults?

a. [ ]Yes b. [ ] No

Which of these staples do you use more often to feed the children?

a. [ ]Cereals b. [ ]Beans and Nuts c. [ ] Starchy roots

Mention some of the foods you give to the children

D. Water and Sanitation

Water

What is the main source of water for your household?

a. [ ]borehole b. [ ]well c. [ ]river/stream d. [ ] Ghana Water Company

e. [ ]other (specify)

How far is the source from your dwelling?

a. [ ]Less than 1km b. [ ]more than 1km

For what purposes do you use this water?

a. [ ]Drinking b. [ ]General use c. [ ] both

1. How regular is your source of water supply

a. [ ]All year round b. [ ]seasonal

2. If seasonal, how do you make up for the lean season?
   a. [ ]Tanker service b. [ ]sachet water c. [ ] other (specify)
3. How much water does your household use in a day?
   Litres ……..
   Gallons……..
   Bucket (No.34)……..

4. How is the water supply operated and managed?
   a. [ ]Self
   b. [ ]Community operated and managed
   c. [ ]Community Water sanitation agency
   d. [ ]Ghana Water Company Limited

5. Does the household pay a regular bill for this water supply?
   a. [ ]Yes b. [ ] No

Waste Disposal

1. How does your household dispose of refuse?
   a. [ ]Collected b. [ ] Refuse Dump c. [ ] Burned d. [ ] Buried e. [ ]other (specify)
2. How much does the household pay for refuse disposal?
3. How far is the dump from your residence?
   [ ]less than 1km b. [ ]more than 1km
4. What type of toilet does the household use?
5. [ ]Flush toilet b. [ ]Pit latrine c. [ ]KVIP d. [ ] Public toilet e. [ ]Bucket f. [ ] Bush
6. How much does the household pay for using the toilet?
7. What is the main material used for constructing the toilet?
   a. [ ]mud/mud bricks b . [ ]wood/bamboo c. [ ] cement/sandcrete blocks d. [ ]other (specify)
8. How far is the toilet from your house?
   [ ]less than 1km b. [ ]more than 1km

E. Bed-net Use

1. Is your house protected with a mosquito proof net?
   a. [ ]Yes b. [ ] No.
2. Do you sleep under a bed-net?
3. a. [ ]Yes b. [ ]No.
   If no, why?

If yes, why?

Demography

Age of mother/caregiver [ ] 15-19 yrs [ ] 20-24 yrs [ ] 25-29 yrs [ ] 30-34 yrs [ ] 35-39 yrs [ ] 40-44 yrs [ ] 45-49 yrs [ ] 50 yrs+

Appendix 18: Questionnaire for Demographic Data Collection

TO BE ADMINISTERD TO PARENTS/CARE-GIVERS OF STUDY SUBJECTS

School of Public Health

University of Ghana

Legon

***MORINGA OLEIFERA* LEAF SUPPLEMENTATION AND VITAMIN A STATUS OF CHILDREN IN ADA-EAST DISTRICT OF GHANA**

Community:

Name of Interviewer:

Child's Code:

Date:

A. Parent/Guardian's information common diseases

1. What types of diseases are common in your community?
2. Have you ever heard of vitamin A deficiency; anaemia
   [ ] Yes [ ] No
3. If yes, through whom?
   [ ] family member [ ] neighbour [ ] health worker
4. How would you identify anyone with these conditions?
   [ ] [ ] [ ] [ ] [ ]

B. Demography

Age of parent/guardian [ ] 15-19 yrs [ ] 20-24 yrs [ ] 25-29 yrs [ ] 30-34 yrs [ ] 35-39 yrs [ ] 40-44 yrs [ ] 45-49 yrs [ ] 50 yrs+

Age of child : …….

Marital status of parent/guardian [ ] single [ ] married [ ] divorced

Religion [ ] Christianity [ ] Islam [ ] Traditional

Education [ ] no education [ ] middle school/JSS [ ] SHS [ ] vocational/technical [ ] tertiary (university, polytechnic)

Occupation [ ] unemployed [ ] trader [ ] farmer [ ] other (specify)

Ethnicity [ ] Ada [ ] Ewe [ ] Other (specify)

Appendix 19: Food Frequency Questionnaire

***MORINGA OLEIFERA* LEAF SUPPLEMENTATION AND VITAMIN A STATUS OF CHILDREN IN ADA-EAST DISTRICT OF GHANA**

Child's Code**:** Respondent:

Interviewer: Community:

Date:

1. Please ask about and write down the kinds of foods eaten from the food groups in the past week.

Food group	**Code**	**Examples**	**On how many days did you consume these foods during the previous week (0-7)?**
Cereals and Derivative products	FFQ01	Maize, kenkey, tom brown, porridge, banku, akple, millet, sorghum, tuo-zaafi bread, noodles, bofloat corn flakes, other	
Roots, Tubers, and other starchy foods	FFQ02	Cassava, sweet potato, yam, water yam, plantain, cocoyam, gari, yakeyake, tapioca, konkonte, fufu, other	
Legumes, Nuts and Seeds	FFQ03	Beans, cowpeas, cowpea and gari, red-red, bambara, waakye, koose , akla, groundnuts, peas, lentils, nuts, seeds,	

		other	
Vegetables	FFQ04	*Gboma, ademe, aleefu,* tomato, onion, garden egg, okro, kontomire, bokoboko, cassava leaves, garlic, lettuce, cabbage, *M. oleifera*	
Fruits	FFQ05	Mangoes, pawpaw, guava, banana, orange, pineapple, pear watermelon, lemon, lime, apple, other	
Flesh meat	FFQ06	Goat, beef, lamb, mutton, chicken, duck, rabbit, wild game, guinea fowl, squirrel, sausage, pork, canned meat, other	
Organ meat	FFQ07	Liver, kidney, heart, intestine, gizzard lungs, blood-based foods, other	

Fish	FFQ08	Tilapia (fresh/smoked), mudfish, mackerel, tuna, anchovies, woevi, sardine, salmon, kaako, koobi, other	
Shellfish	FFQ09	Tortoise/turtle, lobster, crabs, prawns, snails, other	
Eggs	FFQ10		
Milk and milk products	FFQ11	Tinned milk, milk powder, wagashi curd, yoghurt, , fan milk, cheese, other	
Oils and fats	FFQ12	Coconut oil, palm oil, fats, kennel oil, soy oil, groundnut oil, butter, margarine, other	
Spices, condiments, seasonings	FFQ13	cayenne pepper, *kpakposhitor*, green pepper, sweet pepper, black pepper, soy sauce, momoni, iodated salt, raw salt, *dawadawa*, ginger, garlic, Royco cube,	

		Maggie cube monosodium glutamate (MSG), other	
Sweets, confectionery	FFQ14	Biscuits, candy, ice-cream, chocolates, cakes, doughnuts, other	
Beverages	FFQ15	Cocoa, coffee, tea, concentrates, alcoholic beverages, milo drink, chocolim, other	

End of interview. Thank subject.

Checked by:

Appendix 20: Standardised dishes used in *M. oleifera* leaf fortification

***a. Ofam*:**

Plantain (ripe and soft)	30g
Roasted white maize meal	4g
Palm oil	60ml
Salt	0.6g

Method:

The ripe plantain was mashed until very smooth. A soft mixture was made by adding roasted white maize meal. Salt and red palm oil were added and the mixture was thoroughly mixed with a wooden ladle. One (1)g, 2g or 3g *M. oleifera* leaf powder was added to 50g product. This was then baked in a cake-tin at 180^{o}C for 90min.

***b. Porridge*:**

Maize-cowpea-groundnut composite flour	30g
Water	264ml
Salt	0.15g
Sugar	11.8g

The porridge was prepared by adding a slurry of the composite flour to boiling water on fire. The mixture was stirred continuously to prevent lump formation. When done 2g, 3g or 5g *M. oleifera* leaf powder was added per 100g product and cooked further for 5 minutes.

c. Waakye

Beans (red) 140g

Rice 70g

Water (240ml) 240ml

Fresh tomatoes 8g

Tin tomatoes 15g

Fresh pepper 18g

Onions 10g

Salt 5.8g

Cooking (vegetable oil) 40ml

Method: The cowpea was boiled in water until soft; rice was added and cooked until soft. Gravy was made with the remaining ingredients. One (1)g, 2g or 3g leaf powder was added to 50g product and allowed to cook further for 5 min. The *waakye* was served with the gravy.

d. Bean stew

Beans (white) 140g

Rice 70g

Fresh tomatoes 6g

Tin tomatoes 10g

Fresh pepper 17g

Onions 7g

Salt 5.8g

Water (240g)	240ml
Red palm oil	50ml

Method: The beans (white cowpea) was cooked in water until soft; Gravy was made with red palm oil and the remaining ingredients. The cooked cowpea was then added to the gravy; 2g 3g or 5g leaf powder was added to 50g product and allowed to cook further for 5 min.

***e. Jollof rice*:**

Rice	**3 cups**
Fresh tomatoes	10g
Tin tomatoes	12g
Fresh pepper	17g
Onions	8g
Salt	5.8g
Water	**5 cups**
Cooking (vegetable oil)	40ml

Method: Gravy was prepared with the ingredients. Water was added, followed by rice, and cooked until done. One (1)g, 2g or 3g leaf powder was added per 50g product.

f. ***Nkontomire* stew**:

Fresh cocoyam leaves	200g
Herring (smoked and powdered)	20g
Fresh tomatoes	6g
Tin tomatoes	10g
Fresh pepper	17g
Onions	7g

Fresh ginger	8g
Salt	5.8g
Red palm oil	
50ml	

Method: Gravy was made with red palm oil and all other ingredients except cocoyam leaves; the cocoyam leaves were then steamed, mashed and added to the gravy. Two (2)g, 3g or 5g leaf powder was added per 50g product and allowed to cook further for 5 min. The stew was taken with boiled yam and boiled ripe plantain.

g. Apapransa

Composite flour	**25g**
Herring (smoked and powdered)	20g
Fresh tomatoes	6g
Tin tomatoes	15g
Fresh pepper	18g
Onions	8g
Fresh ginger	8g
Salt	5.8g
Water	150ml
(150g)	
Red palm oil	50ml

Method: Gravy was made with red palm oil, the vegetables and fish powder; water were added and the mixture brought to the boil; The composite flour was then added with continuous stirring to prevent formation of lumps; 1g, 2g or 3g leaf powder was added per 50g product and allowed to cook further for 5 min.

h. Groundnut soup

Groundnut paste:	75g
Horse mackerel (smoked and powdered)	22g
Fresh tomatoes	10g
Tin tomatoes	12g

Fresh pepper	18g
Onions	8g
Fresh ginger	8g
Salt	6 g
Water (150g)	150ml

Method: Water was added to the paste to make a slurry. The slurry was brought to the boil; all other ingredients were then added and cooked for 30min. Two (2)g, 3g or 5g *M. oleifera* leaf powder was added per 100g product, further cooked for 5min and served with rice balls (*omotuo*).

Appendix 21: Score sheets for dish evaluation

A. Sensory Test

Name:

Dish:

Date:

Please assess theses samples and rate them under the listed attributes using the scale below. Remember to rinse your mouth with the water provided before moving on to the next sample. Thank you.

Scale	**9**	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**
Interpretation	Like Extremely	Like Very Much	Like Moderately	Like Slightly	Neither Like nor Dislike	Dislike Slightly	Dislike Moderately	Dislike Very Much	Dislike Extremely

Sample Code	Appearance	Colour	Texture	Flavour	Taste	Afterta

Comments:

……

……

Acceptability Test

Name:

Class:

Date:

Sample Code	Like Extremely 	Like 	Neither Like nor Dislike 	Dislike 	Dislike Extremely 

"Simley" used for child acceptability test

Appendix 22: Composition of Composite Maize Meal

The composite flour was made from 60kg maize; 2.8 kg cowpea (white); 2.8 kg peanut

All the grains were roasted until light brown. They were milled with a hard steel hammer mill and sifted using a 500 micron mesh. This composite flour was stored in a clean stainless steel bowl and was used in porridge preparation.

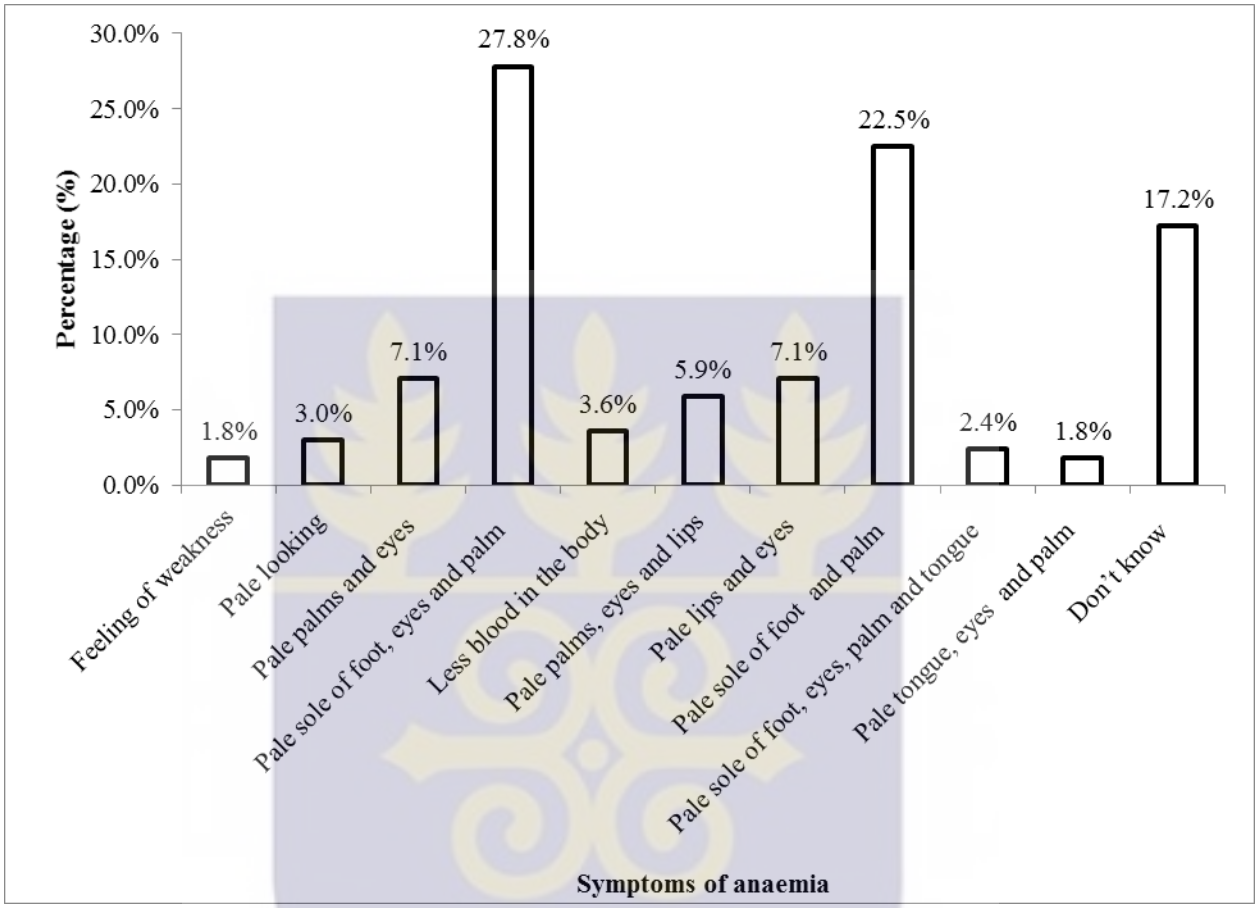
Appendix 23: Proximate analysis of dishes

Sample	Food	Protein (g/100g)	Fat (g/100g)	Moisture (g/100g)	Ash (g/100g)	Carbohydrate (Including fibre) (g/100g)	Energy (Kcal/100g)
1	Porridge	2.72 ± 0.10^{a}	0.94 ± 0.18^{d}	84.47 ± 0.07^{a}	0.45 ± 0.01^{a}	11.65 ± 0.64^{b}	66.55 ± 1.34^{a}
2	Porridge	2.63 ± 0.05^{a}	1.21 ± 0.15^{d}	80.62 ± 0.01^{b}	0.73 ± 0.01^{b}	14.80 ± 0.14^{c}	80.70 ± 0.57^{b}
1	*Waakye*	6.67 ± 0.20^{c}	3.89 ± 0.15^{f}	69.05 ± 0.13^{c}	1.31 ± 0.00^{c}	19.05 ± 0.49^{d}	137.85 ± 0.21^{d}
2	*Waakye*	7.09 ± 0.05^{c}	2.70 ± 0.27^{e}	68.61 ± 0.05^{d}	1.54 ± 0.02^{d}	20.05 ± 0.35^{d}	132.85 ± 1.20^{e}
1	Soup	4.84 ± 0.47^{b}	5.32 ± 0.07^{g}	85.73 ± 0.04^{e}	0.85 ± 0.06^{e}	3.20 ± 0.42^{a}	80.40 ± 0.42^{b}
2	Soup	6.81 ± 0.19^{c}	5.39 ± 0.27^{g}	82.36 ± 0.02^{f}	1.21 ± 0.02^{f}	4.25 ± 0.49^{a}	92.80 ± 1.13^{c}

1 = Non-fortified; 2 = M. oleifera leaf fortified. Means with the same superscript are not significantly different (P < 0.05).

Protein level was the same in both fortified and non-fortified porridge and *waakye*, but it was significantly different in the groundnut soup.

Appendix 24: **Caregiver's knowledge about anaemia**



Appendix 25: Univariate relationships of child and caregiver characteristics and haematological indicators

Characteristic	n (%)	Vitamin A µmol/L (SD)	HGB g/dL (SD)	RBC x 10^4/µl (SD)	HCT % (SD)	MC fL
Sex of child						
Male	90 (52.9)	0.70 (0.27)	10.54 (1.05)	4.17 (0.39)	32.52 (2.58)	78.
Female	80 (47.1)	0.76 (0.32)	10.66 (1.14)	4.20 (0.48)	32.99 (3.07)	79.
Malaria in children						
Yes	33 (19.4)	0.72 (0.30)	10.78 (1.14)	4.18 (0.43)	33.14 (3.00)	79.
No	137 (80.6)	0.73 (0.29)	10.55 (1.08)	4.18 (0.44)	32.64 (2.78)	78.
Other infections						
Yes	12 (7.1)	0.78 (0.35)	9.86 (1.26)*	3.88 (0.44)*	31.25 (3.87)	80.
No	158 (92.9)	0.72 (0.28)	10.65 (1.06)	4.21 (0.43)	32.85 (2.71)	78.
Child's age (years)						
5-7	2 (1.2)	0.67 (0.27)*	10.40 (1.06)	4.17 (0.48)	32.53 (2.98)	78.
8-10	15 (8.8)	0.78 (0.31)	10.73 (1.12)	4.22 (0.42)	32.90 (2.84)	78.
11-12	29 (17.1)	0.69 (0.24)	10.69 (0.99)	4.05 (0.30)	32.64 (2.20)	80.
Formal education						
No education	153 (90.0)	0.72 (0.29)	10.58 (1.09)	4.19 (0.42)	32.70 (2.83)	78.
Middle school/JHS	17 (10.0)	0.79 (0.33)	10.72 (1.11)	4.17 (0.58)	33.12 (2.77)	80.
Occupation						
Unemployed	9 (5.3)	0.72 (0.17)	10.74 (0.77)	4.15 (0.32)	32.94 (2.19)	79.
Trader	26 (15.3)	0.74 (0.35)	10.67 (1.38)	4.16 (0.48)	32.63 (2.79)	79.
Farmer	130 (76.5)	0.72 (0.27)	10.58 (1.07)	4.20 (0.43)	32.74 (2.88)	78.
Student	5 (2.9)	0.96 (0.54)	10.22 (0.53)	3.84 (0.28)	32.84 (3.33)	83.
Refuse disposal						
Refuse dump	124 (72.9)	0.70 (0.26)	10.63 (1.10)	4.20 (0.46)	32.80 (2.86)	78.
Burned	41 (24.1)	0.80 (0.34)	10.42 (1.06)	4.09 (0.35)	32.28 (2.69)	79.

Buried	5 (2.9)	0.77 (0.52)	11.16 (0.95)	4.43 (0.26)	35.04 (2.19)	79.
Toilet facility						
Pit latrine	5 (2.9)	1.01 (0.60)	11.10 (0.62)	4.20 (0.38)	33.68 (2.42)	80.
Public toilet	4 (2.4)	0.87 (0.07)	10.13 (0.62)	3.80 (0.15)	30.80 (1.31)	81.
Bush	161 (94.7)	0.72 (0.28)	10.60 (1.10)	4.19 (0.44)	32.76 (2.85)	78.
Sleep under a bed-net						
Yes	166 (97.6)	0.72 (0.29)	10.59 (1.10)	4.18 (0.43)	32.71 (2.85)	78.
No	4 (2.4)	0.89 (0.19)	10.90 (0.54)	4.49 (0.60)	33.90 (1.20)	80.

RBC= Red blood cells; HGB= Haemoglobin; HCT= Haematocrit; MCV= Mean corpuscular volume; *significant at $P < 0.05$. Other infections included Strongyloides stercoralis, Ascaris lumbricoides, Intestinal flagellates, Taenia, Trichuris trichuris and H. nana.

Appendix 26: Parasitology

Thick and thin blood smears were prepared from all children at baseline and at end of trial. These were Giemsa-stained (WHO, 1991), read and scored by trained malaria microscopists at Ada-East district hospital. Stool samples from the children were also analysed at baseline and end of trial for intestinal worms at Ada-East district hospital. Samples were analysed using the direct smear method (Cheesbrough, 1998). Parasite density was determined on the basis of the number of parasites per 200 white blood cells (WBCs) on a thick blood film assuming a total WBC count of 8000/µl.

Malaria and infection indices of school-aged children 5-12 years

Indicators	Intervention		Control	
	Baseline n (%)	Posttest n (%)	Baseline n (%)	Posttest n (%)
Malaria parasite				
No MPS	55 (79.7)	47 (68.1)	64 (84.2)	54 (71.1)
Symptomatic	13 (18.8)	20 (29.0)	11 (14.5)	22 (28.9)
Max. parasitimia	1 (1.4)	2 (2.9)	1 (1.3)	0 (0.0)
Infections				
Absent	58 (84.1)	58 (84.1)	68 (89.5)	69 (90.8)
Present	11 (15.9)	11 (15.9)	8 (10.5)	7 (9.2)

Malaria parasite density in participants according to study group and with time

Haematological indices	Intervention				Control				Differences in mean change between IG and CG[#]
	Baseline	Posttest	Change	*P-value*[#]	Baseline	Posttest	Change	*P value*	
MALPARA	419.83 ± 199.23	711.94 ±	292.12 ±	0.407	459.09 ± 190.60	418.41 ±	-40.68 ±	0.846	332.80

274.87	350.12	135.31	208.54

IG= Intervention group; CG= Control group; MALPARA= Malaria parasite density; *Differences significant within-group change comparison at $P < 0.05$; #No significant differences were observed in difference in changes between the Intervention and Control groups at $P < 0.05$

Appendix 27: *M. oleifera* leaf consumption by childen in Ada-East district

Is *Moringa oleifera* leaf fed to children in your locality?

	Frequency	Percent
Yes	152	67.3
No	60	26.5
Do not know	14	6.2
Total	226	100

At what age can children be fed with *M. oleifera* ileaves n your locality?

	Frequency	Percent
6 months – 5 years	144	63.7
13 years and above	6	2.7
Old people	4	1.8
Don't know	72	31.9
Total	226	100

What quantity could you use at a time? Describe

Quantity	Frequency	Percent
1 cup	6	2.7

1 table spoon powdered moringa	14	6.3
1 bowl	4	1.8
2 bowls	50	22
3 bowls	2	0.9
2 ladles	6	2.7
Handful	2	0.9
1 branch	16	7.2
3 branches	4	1.8
Can't tell	50	22
NA	72	31.9
Total	226	100

How often do you feed it to children?

	Frequency	Percent
Daily	18	8.0
Once per week	28	12.4
3 times per week	10	4.4
Once a while	98	43.4
Do not use	72	31.9
Total	226	100

Have you ever fed your children with *M. oleifera* leaves?

	Frequency	Percent

Yes	154	68.1
No	72	31.9
Total	226	100

Would you like to feed with *M. oleifera* leaves if requested to do so?

	Frequency	Percent
Yes	212	93.8
No	14	6.2
Total	226	100

If yes why and if no, why?

Reason	Frequency	Percent
Gives strength	6	2.7
Source of food	8	3.6
healthy	28	12.4
Nutritional quality/value	74	32.8
Do not want to use it	6	2.7
Medicinal value	92	40.7
Good for children	2	0.9
It is bitter	4	1.8
People say its good	2	0.9
Do not know how to use it	4	1.8
Total	226	100

Source of information on *M. oleifera*

Source	Frequency	Percent
Neigbours	156	69
Friends	70	31
Total	226	100