EFFECT OF FISH FORTIFIED *AMARANTHUS CRUENTUS* AND *SOLANUM MACROCARPON* POWDER ON ANAEMIA AND VITAMIN A STATUS OF 4 TO 8 – YEAR – OLD SCHOOL CHILDREN IN KODZOBI, ADAKLU DISTRICT OF GHANA

BY

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DECLARATION

I Margaret Mary Tohouenou, author of this dissertation do hereby declare that, aside references to other people's work cited accordingly, this work has entirely resulted from my research supervised by Professor Matilda Steiner-Asiedu, Dr. Godfred Egbi and Dr. Agartha Ohemeng and has not been presented for another degree elsewhere.

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DEDICATION

To the glory of my faithful God

To my husband Codjo and my daughter Dziedzorm

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ABSTRACT

Background: Anaemia, vitamin A deficiency and infections are widespread among Ghanaian children of school-going age and are topics of public health concern. Several interventions including supplementation have been implemented over the years to improve the situation, however the problem persists. Globally, food-based approaches have been recognized as more sustainable in addressing micronutrient deficiencies. In Ghana there is a lack of knowledge on using a combination of fish and vegetables powder as a food-based intervention in combating anaemia and vitamin A deficiency.

Objective: The research sought to investigate the effect of consumption of fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder on anaemia and vitamin A status of 4 to 8-year old school children at Kodzobi in Adaklu District of the Volta Region of Ghana.

Methods: The study was a randomized controlled trial with data collection point at baseline and end line and a four-month nutrition intervention period. Three groups made up of 54 children each were identified to participate in the study through random sampling. Parental and participant background information was collected using questionnaires. Haemoglobin and serum retinol concentrations were determined using the heamocue haemoglobinometer and the high-performance liquid chromatography respectively. The Giemsa staining and the Kato-Katz techniques were employed to examine malaria-parasitaemia and soil-transmitted helminths infections respectively. Dietary data were collected using the 24-hour recall method and a food frequency questionnaire. Using WHO standard procedures, weight and height measurements were taken to assess participants' anthropometric nutritional status. Haematological indices and

dietary data were normally distributed so means ± standard deviations were used to present their summary values. Outcome measures of variables (haemoglobin, serum retinol, dietary nutrient intakes, Z - scores) were compared within groups using paired T-test for continuous variables and chi-square for categorical variables. Analysis of co-variance (ANCOVA) was used to test for differences in mean changes among the 3 groups and between paired groups to determine any significant differences. Binary logistic regression was used to determine factors associated with anaemia and vitamin A deficiency whilst adjusting for potential confounders.

Results: At baseline, the mean haemoglobin concentration of the two intervention groups A and B and the control group C were 12.0 ± 1.1 g/dl, 11.4 ± 1.0 g/dl and 11.6 ± 1.0 g/dl respectively. At end line, it was 12.4 ± 1.3 g/dl, 11.8 ±1.9 g/dl and 11.6 ± 1.3 g/dl for groups A, B and C respectively. A significant difference in mean haemoglobin concentration was recorded across the groups at p = 0.024 at post-intervention. At baseline, the prevalence of anaemia was 46.3 %, 51.9 % and 42.9 % for groups A, B and C respectively. At the end of the intervention period, the prevalence of anaemia reduced to 34.5 %, 45.2 % 41.2 % for intervention groups A, B and the control group C respectively. The difference in the prevalence of anaemia across the groups was significant at p = 0.036. The mean serum retinol concentrations of the three groups (A, B and C) at baseline were 23.1 ± 7.3 µg/dl, 22.3 ± 5.6 µg/dl and 22.8 ± 6.8 µg/dl respectively. At end line, it increased to 28.3 ± 8.8 µg/dl, 24.7 ± 6.5 µg/dl and 23.8 ± 6.5 µg/dl for groups A, B and C. A significant difference in serum retinol concentration was recorded across the groups at p = 0.044. Prevalence of vitamin A deficiency decreased from 31.5 % to 9.6 % for intervention group A; 29.6 % to 20.0 % for intervention group B and 35.2 % to 29.4 % for the control group C from baseline to end line respectively. Across the groups, differences

in vitamin A deficiency (VAD) at the beginning and end of the study were not significant. Malaria was prevalent among the participants at the baseline and at the end of the study. Malaria prevalence declined from baseline to end line within the three groups: group A 46.3 % to 13.5 %; group B 25.9 % to 10.0 % and group C from 38.9 % to 9.8 %, however, the decreases were not statistically significant. Only one participant was reported to have hookworm infestation. Serum retinol level was significantly associated with anaemia. Marital status and anaemia status were the factors significantly associated with vitamin A deficiency.

Conclusion: Anaemia and vitamin A deficiency are problems of public health significance in school-aged children in Kodzobi at Adaklu District. Fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder improved the haemoglobin concentration of participants significantly and so has the potential to reduce anaemia prevalence but not vitamin A status of the study participants.

LIST OF ACRONYMS

ACSMP	*Amaranthus cruentus* and *Solanum macrocarpon* Powder
AREDS	Ade-Related Eye Disease Study
ASH	American Society of Haematology
ANCOVA	Analysis of Covariance
AOAC	Association of Analytical Communities
BMIZ	Body Mass Index-for-age z score
CSIR	Center for Scientific and Industrial Research
CRP	C – Reactive Protein
CONSORT	Consolidated Standards of Reporting Trials
EAR	Estimated Average Requirement
FACSMP	Fish Fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder
FRI	Food Research Institute
G	Gram
GMSR	Ghana Micronutrient Survey Report
HAZ	Height-for-age Z Score
IDE	Iron Deficiency Erythropoiesis
IMH	Indonisia Ministry of Health
IQ	Intelligent Quotient
IRB	Institutional Review Board
IVACG	International Vitamin A Consultative Group
Kcal	Kilocalories
Kg	Kilogram
MFP	Meat Fish and Poultry

mg	Milligram
mg/dl	Milligram per Deciliter
MMN	Multiple Micronutrient
MOFP	Moringa Olifera Powder
NAR	Nutrient Adequacy Ratio
NIH	National Institute of Health
NMIMR	Noguchi Memorial Institute for Medical Research
^{o}C	Degree Celsius
OR	Odds Ratio
PHC	Population and Health Census
RBC	Red Blood Cells
RBP	Retinol-Binding Protein
RCT	Randomized Controlled Trial
RDA	Recommended Dietary Allowance
SR	Serum Retinol
SPSS	Statistical Package for Social Sciences
TS	Transferrin-iron Saturation
µg/dl	Microgram per Deciliter
UNSCN	United Nations Standing Committee on Nutrition
UV	Ultra violet
WAZ	Weight-for-age Z Score

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Over two billion persons worldwide are afflicted by micronutrient deficiencies. This translates into one in three people globally (FAO, IFAD, and WFP 2014). Paramount among these micronutrient challenges are anaemia and vitamin A deficiency. Anaemia has been defined as haemoglobin concentrations below 11.0 g/dl for children below five years and 11.5 g/dl for children between five and eleven years (WHO, 2001). For children four to eight years old, the World Health Organization considers serum retinol values less than 20 µg/dl as a deficiency in vitamin A or low serum retinol concentration (WHO, 2009).

Anaemia presents serious health consequences and influences the economic and social development of individuals and communities at large (Stevens *et al.*, 2013; Beacházar, 2013). Globally, anaemia affects 1.6 billion people (WHO, 2011). Forty-three percent of young children between 6 and 59 months as well as 25 % of those between 5 and 15 years are anaemic globally (De Benoist *et al*., 2008; Stevens *et al*., 2017). Out of the 25 % of children of school-going age with anaemia globally, forty percent of them can be found in under-developed nations (De Benoist *et al.*, 2008). Anaemia prevalence among toddlers and infants in Sub-Saharan Africa vary from one country to another. It is 42 % in Swaziland and 91 % in Burkina Faso (Magalhães and Clement, 2011). In Ghana, the latest demographic and health survey recorded anaemia prevalence of 66 % in children 0 to 59 months old (GSS, 2014). Studies among children have reported anaemia prevalence as an issue of public health importance in Ghana; 73 % among 2 to 10 years old (Egbi, 2012); 63 % among school children 5 to 12 years of age (Abdul-Razak *et al.*, 2012); 33 %

amongst school children from 6 to 12 years (Egbi *et al*., 2018); and 25 % among vegetarian children (Osei-Boadi *et al.,* 2012).

Multiple factors cause anaemia. The factors could be related to nutrition (deficiencies in vitamins, minerals and proteins); or not related to nutrition (infection and infestation) (Al-Zain, 2013). Poor health of pregnant women, poor breastfeeding practices, use of unsuitable breastmilk substitutes, the introduction of complementary foods before six months after delivery, unsafe drinking water and unsanitary conditions, are factors that may lead to the development of anaemia (Bain *et al*., 2013). However, in several developing countries, inadequate intake of dietary iron is considered the principal reason for the development of anaemia in children (Bain *et al.*, 2013). About half of all anaemia incidences are because of deficiency in iron (Lopez *et al.*, 2016); the absence of other nutrients like folate, vitamin B_{12} and vitamin A, and also inflammations, infections, infestations, bleeding and hereditory disorders (thalassaemia and sickle cell disease which affect erythrocytes) may account for the other half (Al-Zain, 2013). Other causes of anaemia include the intake of foods with anti-nutritional factors like oxalates which are found in leafy vegetables, tannins and polyphenols present in legumes, tea, and in coffee; phytates in whole grains and calcium salts found in milk and milk products; these anti-nutritional factors inhibit iron bioavailability thus affecting iron absorption (Woyengo and Nyachoti, 2013).

In Sub-Saharan Africa, deficiency in vitamin A is common in children especially those in low-income areas (Arlappa *et al.*, 2016). Nearly one hundred and ninety million infants and toddlers mostly living in South East Asia and Africa are vitamin A deficient (WHO, 2017). In Africa alone, preschoolers with low serum retinol level are 37 % in Eastern and

Southern Africa, West Africa accounts for 35 % and in Central part of Africa, 33 % (Arlappa *et al.*, 2016).

The first vitamin A status survey done in 1993 by the Ministry of Health Ghana, revealed 37.2 % of children in the Southern sector had low serum retinol levels (VAST, 1993). Subsequently, an observational study done in the Eastern part of Ghana with children 2 to 10 years (Egbi, 2012) reported vitamin A deficiency prevalence of 36 % among the study participants. In another Ghanaian study in the Volta Region, among school children 6 to 12 years, vitamin A deficiency prevalence declined from 95.1% at the beginning of the research to 77.5 % at the end of the study among participants (Alatiah, 2013).

Vitamin A supports rapid growth and helps combat infections (WHO, 2017). Insufficient intake leading to vitamin A deficiency causes visual impairment specifically night blindness which raises the risk of illness, childhood infection and in severe cases may lead to loss of life (WHO, 2017). The consequence of vitamin A deficiency is aggravated by poverty and infections (West, 2009). Some studies have reported episodes of severe diseases, poor immunization status, poor use of vitamin A supplements, inadequate maternal knowledge on the vitamin and its deficiency, and high birth order as determinants of vitamin A deficiency (Demissi *et al*., 2009; Gebreselassie *et al.*, 2013, Chhabra and Mishra, 2018). Additionally, poor maternal education, low socio-economic status, unsanitary practices, and stunting were significantly associated with vitamin A deficiency (Coles *et al.*, 2004; Laxmaiah, 2010). Further, inadequate dietary intake and poor pro-vitamin A bioavailability contribute to the development of the condition (Ramakrishnan, 2002).

Due to the devastating consequences of anaemia and vitamin A deficiency, specific interventions have been proposed. Key amongst them are supplementation, food fortification, food diversification and nutrition education. The most practical, feasible and sustainable alternatives for addressing micronutrient deficiencies in the population are food diversification and modification (Thompson and Amoroso, 2011). In Ghana, nutrition interventions have been focusing on nutrition education, food fortification, dietary modification, dietary diversification and behaviour change communication.

Vegetables and fruits are a rich source of vitamins and minerals phytonutrients (Muhanji *et al.,* 2011). Green leafy vegetables (GLV's) contain a collection of nutrients comprising iron, carotenoids, zinc, folate, vitamin C and phytonutrients (Kwenin *et al*., 2011). *Amaranthus cruentus* (amaranth) and *Solanum macrocarpon* (eggplant) are leafy vegetables that are less expensive to most rural folks, rich in beta-carotene, total carotenoid, iron and some B vitamins (Djuikwo *et al.,* 2011; Kwenin *et al.,* 2011; Acho *et al*., 2014), and are accepted and commonly consumed in Ghana. However, their availability is seasonal; in abundance mostly in the wet seasons but limited in supply in the dry season. Green leafy vegetables can be processed into powder and used to fortify stews and soups to increase their nutrient content during the dry periods when vegetables are out of season. Fortifying green leafy vegetables powder with fishmeal will ensure the availability of a factor (meat, fish, poultry – MFP factor). This factor has been demonstrated to improve the absorption of iron when consumed at the same time as other foods (Hoffman, 2017). Martinez -Torres and co-workers (1971) were the first scientists to report on enhancement of non-haem iron absorption using the MFP factor. They detected that consuming black beans and maize-based meals with veal liver, veal muscle or fish,

increased the non-haem iron absorption in human participants by 150% (Martinez -Torres *et al*., 1971).

Fish is a storehouse for protein. Fish contributes to the supply of retinol-binding proteins (RBP) which are carrier protein synthesized within the liver. Retinol-binding proteins transport retinol mobilized from liver stores. This process of vitamin A mobilization and delivery to peripheral tissues is highly regulated and controlled by processes that regulate the rate of retinol-binding protein production (D'Ambrosio *et al.*, 2011). The concentration of RBP falls when there is inadequate protein consumption (Mandaliya *et al.*, 2004). Both inflammation and infection may also affect the supply of RBP (Burke *et al*., 2018).

1.2 Problem statement

The best food source of heam iron and vitamin A are animal based. This is because vitamin A and iron obtained from animal food sources are bioavailable and readily absorbed compared to non-haem iron and pro-vitamin A carotenoids from plant sources. However, foods from animal sources are quite expensive and not affordable to many rural households as well as low-income households in urban settings. *Amaranthus cruentus* and *Solanum macrocarpon* on the other hand, are less costly but are endowed with appreciable amounts of minerals and vitamins including beta-carotene and non-haem iron; precursors of vitamin A and iron respectively. In 2011, the African Regional Workshop on promoting fruits and vegetables consumption for health, suggested 400 g or more of fruits and vegetables in a day for one person (PROFAV, 2011). This protects against diet-related chronic diseases (PROFAV, 2011) and ensures the supply of vitamins and minerals to the body. However, a huge gap has been reported to be present between the quantity

recommended and actual intake; and many people including children are not consuming the quantity of vegetables and fruits required (Pem and Jeewon, 2015).

Recommended daily allowances for vitamins and minerals like vitamin A and iron are difficulty to or not met at all by many rural households and low-income households in urban areas in the non-raining seasons when vegetables are unavailable (Faber and Laubscher, 2008; Appleton *et al.*, 2016). Deficiencies in iron and vitamin A are therefore pronounced in the season of scarcity of these food items.

Fresh GLV's could be processed by drying, crushing and milling into fine powder for consumption during the dry season to make micronutrients particularly precursors of iron and vitamin A more available to the human body for absorption. This powder could also be fortified with fishmeal such as anchovies to further enhance its nutrient content. When diets of cereals, legumes, and other food groups are eaten in combination with meat, fish or poultry, the MFP factor promotes the absorption of non-haem iron that is present in the food (Hoffman, 2017). The added fish powder could provide amino acids necessary for the formation of retinol-binding protein required in the transport of retinol from the liver stores to action sites in the body. It is based on this background that this study sought after investigating the effect of consumption of fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder on anaemia and vitamin A status of 4 to 8-year-old school children in the Kodzobi of Adaklu District of the Volta Region of Ghana.

1.3 Study questions

- Will the consumption of fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder increase the haemoglobin levels of study participants?

- Will the consumption of fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder increase the serum retinol levels of study participants?

1.4 Objectives

1.4.1 Main objective

To assess the effect of consumption of fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder on anaemia and vitamin A status of 4 to 8 – year – old school children in Kodzobi.

1.4.2 Specific objectives

1. To assess the haemoglobin concentration and prevalence of anaemia of study participants at baseline and end line.
2. To assess the serum retinol concentration and prevalence of low vitamin A level of study participants at baseline and end line.
3. To assess the dietary intakes of study participants at baseline and end line
4. To assess the anthropometric nutritional status of participants at baseline and end line
5. To assess the infection status of participants at baseline and end line.

1.4.3 Study hypotheses

1. Consumption of fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder will not increase haemoglobin concentration significantly to reduce the prevalence of anaemia among study participants.

2. Consumption of fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder will not increase serum retinol significantly to reduce the prevalence of anaemia among study participants.

CHAPTER TWO

2.0 LITERATURE REVIEW

Globally, micronutrient deficiencies affect the good health, nutritional wellbeing and development of many children. Many individuals world-wide are either silently or visibly affected by vitamin A, iron, or iodine deficiencies, which are the main micronutrient problems (United Nations Standing Committee on Nutrition, 2004; Darnton-Hill *et al.*, 2015). The human body requires micronutrients in small quantities; however, this is certainly not consistent with the nature and extent of damage their deficiencies can cause. Their effects pervade the biological, physical, socioeconomic and cultural aspects of humanity (UNICEF, 2012). Of concern in the current research are anaemia and vitamin A deficiency, which decrease learning and cognitive ability, impair growth, reduce working capacity, lessen the strength of the immune system making it less effective in combating infections, result in several pregnancy complications and blindness (Nordin *et al.*, 2013).

Over time, several strategies have been implemented to manage, control and even avert anaemia and vitamin A deficiency. The strategies comprise fortification, supplementation, and dietary diversification (Gibson, 2011). This review draws attention to the existing knowledge and gaps on vitamin A deficiency and anaemia in children as well as strategies and interventions for their prevention and management with an emphasis on food-based approaches.

2.1 Global and national prevalence of anaemia

Anaemia has been well explained as blood haemoglobin below established cut-off points. Thus, less than 11.0 g/dl, 11.5 g/dl or less than 12.0 g/dl in children between 6 and 59 months, 5 to 11 years, and 12 to 14 years respectively (WHO, 2009). Anaemia has been

reported as the most widespread nutritional disorder among children worldwide (Özdemir, 2015). The map in figure 2.1 below shows anaemia prevalence per region and per country. Majority of the African countries including Ghana, have high anaemia prevalence of severe public health significance.

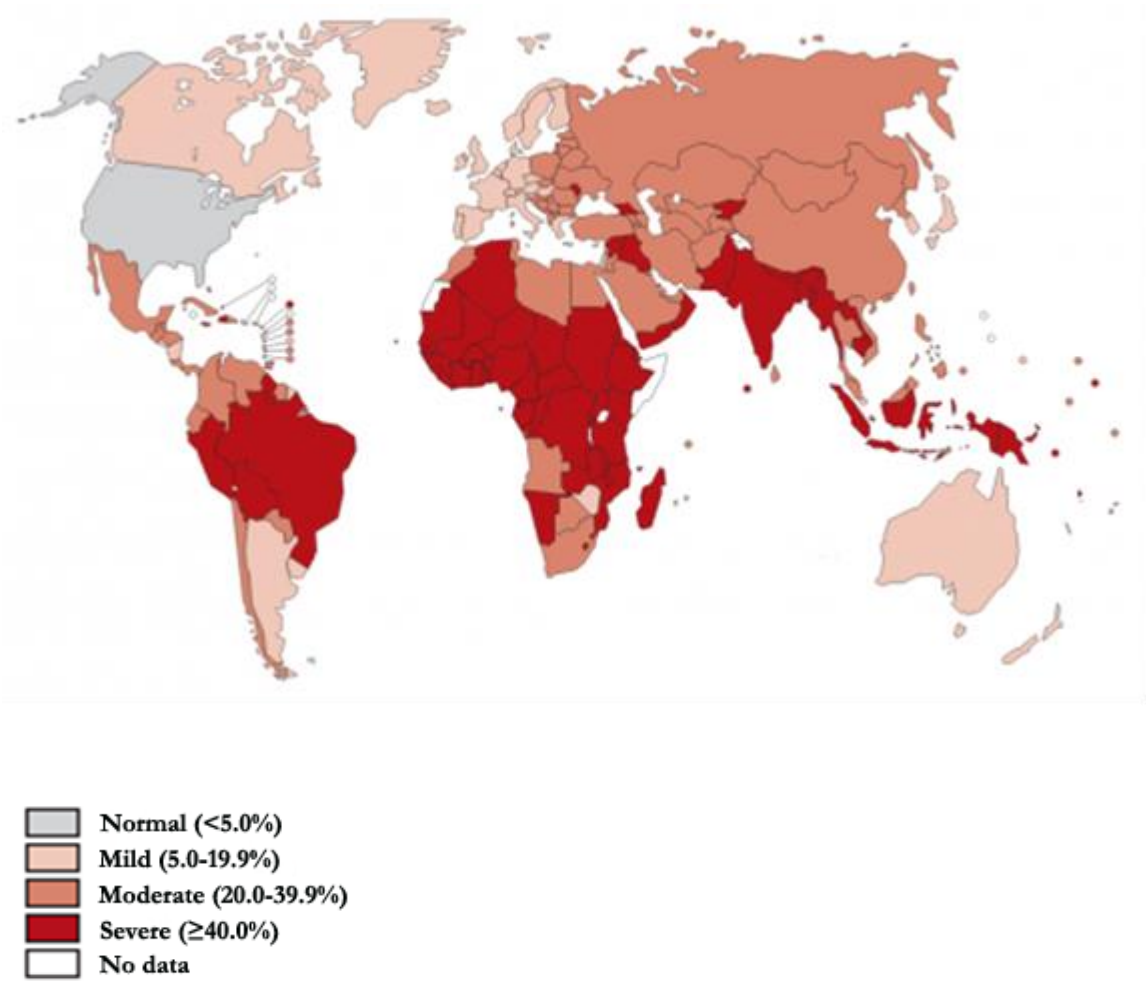


Figure 2.1: Prevalence of anaemia by severity per Region and Country (WHO, 2011)

The WHO projected that about 1.6 billion people in the world (one-fourth of the world's populace), have anaemia with the African region being the most affected" (WHO, 2011). In Sub-Saharan Africa, the prevalence of anaemia among preschoolers varies from one country to the other; it is forty-two percent in Swaziland and 91 % in Burkina Faso (Magalhães and Clements, 2011). Among school-aged children, the prevalence is 33 % in

Côte D'Ivoire, 81 % in Ethiopia and 85.5 % in rural Nigeria (Kokore *et al.,* 2013; Ayogu *et al.,* 2015; Mainasara *et al.,* 2017; Getaneh *et al.,* 2017).

In Ghana, anaemia remains a key public health challenge among preschoolers and children of school-going age. According to national surveys performed over the years, anaemia prevalence amongst children under five were 76 % in 2003, 78.4 % in 2008 and 65.7 % in 2014 (GSS, 2003; GSS, 2008; GSS, 2014). For school-going children 2 to 10 years old in Ghana, Egbi (2012) recorded a prevalence of 76 % in the Eastern Region. Another study also reported a prevalence of 63 % among children 5 to 12 years old (Abdul-Razak *et al.,* 2012). Anaemia is still a public health challenge in Ghana despite the downward trend recorded since 2003 among children under 5 years (GSS, 2003; GSS, 2008; GSS, 2014).

2.1.1 Causes of anaemia

Multiple factors cause anaemia. The factors may be attributed to the influence of both nutritional and non-nutrition causes (Obse *et al.,* 2013; Oguntibeju, 2015). Micronutrient deficiencies and parasitic infections are among the main contributors to the development of anaemia (Ngesa and Mwambi, 2014). Iron, vitamin B_{12}, folate and vitamin A deficiencies, which influence haemoglobin metabolism, are also leading causes of anaemia (Iannotti *et al.,* 2015). Among these micronutrients, deficiency in iron, which results from inadequate dietary intake and poor bioavailability, is the major cause of anaemia globally (Wirth *et al.,* 2017). Dietary iron is in two forms. Haem iron and non-haem iron. Haem iron is found in animal food including meat, poultry and fish. It is more bioavailable and is easily absorbed by the body. Non-haem iron on the other hand, is both plant and animal-based, has poor bioavailability and is poorly absorbed. Other vitamin deficiencies are associated including vitamin C deficiency, which contributes to iron deficiency and iron-deficiency anaemia due to impaired iron absorption (Ozsoylu and Aytekin, 2011).

Infections also contribute to the development of anaemia. It has been reported that intestinal parasitic infections particularly hookworm, *Trichuris trichiura*, and high prevalence of malaria are major causes of anaemia in school-age children (Righetti *et al.*, 2013; Udoidung *et al.*, 2015). Increased iron and blood loss in the gastrointestinal tract due to infections also causes anaemia. Malaria is linked to a continual decrease in the amount of haemoglobin, parasitized red blood cells destruction, shortening of the lifespan of non-parasitized red blood cells and decrease in the production of red blood cells in the bone marrow (McDevitt *et al.*, 2004; Njunda *et al.*, 2015).

Bleeding in the gastrointestinal tract, which may be due to peptic ulcers, haemorrhoids or colitis can also cause anaemia (Alexandrescu, 2009). Lack of the enzyme glucose-6-phosphate dehydrogenase causes impulsive breakdown of red blood cells faster than the body can replace. Deficiency of this enzyme causes haemolytic anaemia. Furthermore, haemoglobinopathy, which is a set of genetic haemoglobin disorders resulting in abnormal structure of one of the globin chains of the haemoglobin molecule, also causes anaemia, an example being sickle cell anaemia (Korenromp *et al.*, 2004). In many resource-poor nations, early cessation of exclusive breastfeeding, early introduction of complementary foods to infants, use of unsafe drinking water, unhygienic environment, poor health of pregnant women and poverty may also lead to the development of anaemia (WHO, 2001).

2.1.2 Signs and effects of anaemia

Oxygen is conveyed in the blood by haemoglobin to body tissues for their activities. Therefore, the first sign of anaemia is fatigue. This is due to the lack of an adequate supply of oxygen for physical activity. Other major symptoms of anaemia include weakness, pale skin, and shortness of breath, increased thirst, sweating, rapid breathing and lower leg cramps. Heart-associated symptoms such as irregular heart rhythms, an enlarged heart and

heart failure has also been recorded (NIH, 2016). Anaemia in children is associated with reduced cognitive development, low cognitive function, low immunity, low growth rate and high morbidity and mortality rates (Achouri *et al.*, 2015). A review of studies on adolescents and school-aged children reported that iron deficiency anaemia could influence intellectual function, motor performance and language learning (Ji *et al.*, 2017). This finding corroborates Baker's (2010) study where it was reported that iron deficiency escalates susceptibility to infections and affects cognitive and motor development in children.

2.1.3 Types of anaemia

Anaemia may be grouped into two main forms, nutritional and non-nutritional anaemia. Nutritional anaemia is caused by nutrient deficiencies, which include folate, iron, zinc, vitamin A, and vitamin B_{12}, deficiencies. Other types include sickle cell anaemia, anaemia of chronic disease, drug-induced immune haemolytic anaemia due to deficiency in glucose-6-phosphate dehydrogenase deficiency, idiopathic aplastic anaemia, idiopathic autoimmune haemolytic anaemia and secondary aplastic anaemia (Girerd-Barclay and Tiwari, 2002; Al Qahtani, 2018).

2.1.3.1 Nutritional anaemia

The main types of nutritional anaemia are iron deficiency anaemia and vitamin deficiency anaemia (mainly folate and vitamin B_{12} deficiencies), also referred to as megaloblastic anaemia. According to Semba and Bloem (2008) nutritional anaemia is caused by inadequate consumption of iron-rich foods and poor bioavailability of haemopoietic nutrients needed to meet the demands for the synthesis of haemoglobin and erythrocytes. The main nutrient involved in haemopoiesis is iron according to Camaschella and Nai (2015); minerals including copper, zinc, magnesium, cobalt, molybdenum; and vitamins,

especially folic acid, vitamin B_{12} and amino acids (Wiwanitkit, 2007) play a role in haematopoiesis.

The absorption of nutrients that promote haemopoiesis is affected by physiological and pathophysiological conditions. Helicobacter pylori, for instance, is known to reduced iron stores through several different mechanisms (Girerd-Barclay and Tiwari, 2002; Hudak, *et al.*, 2017). For school-aged children and other at-risk groups like infants and preschoolers, restricted access to different micronutrient-rich diets, can aggravate nutritional anaemia. Each micronutrient plays specific roles in the human body; however, multiple micronutrient deficiencies tend to exist, and the synergistic effect of these deficiencies is important in the development of nutritional anaemia in an individual.

Absence of healthy red blood cells due to deficiencies in certain vitamins, cause vitamin deficiency anaemia. Vitamins linked with the development of vitamin deficiency anaemia include vitamin B_{12}, vitamin A, folate, and vitamin C. Deficiencies of these vitamins occur when there is inadequate consumption of them from the diet, the body is not able to absorb them or when there are gastrointestinal problems.

Vitamin B_{12} (Cyanocobalamin)*:* Vitamin $B_{12,}$ a water-soluble vitamin is needed for the synthesis of red blood cells and deoxyribonucleic acid (DNA). Vitamin B_{12} deficiency occurs when there is a lack of consumption of animal-based food products. This includes meat, poultry, eggs, milk and cheese. Vegetarians who do not consume milk and eggs could be at risk of developing vitamin B_{12} deficiency. Anaemia due to vitamin B_{12} deficiency is called pernicious anaemia; and that due to folate deficiency is called folate deficiency anaemia (Adhikari *et al.*, 2016). A high prevalence of vitamin B_{12} deficiency has been recorded in Guatemala amongst infants and women as well as among school

children (Rogers *et al.*, 2003; Chebaya *et al*., 2017), possibly related to inadequate dietary intake.

Folate: Deficiency in folate can lead to folate deficiency anaemia where the red blood cells become unusually large or megaloblastic (McNeil *et al*., 2011). The production of DNA and cell divisions are affected when there is folate deficiency. When this happens, locations of rapid cell turn over like the bone marrow, are affected. Severe birth flaws also known as neural tube defects, therefore, occur during severe folate deficiency. Ensuring folic acid supplementation before pregnancy and early in pregnancy reduces the chance of delivering a baby with neural tube defect (Ami *et al*., 2016).

Folate deficiency and vitamin B_{12} anaemias are also known as megaloblastic anaemia where red blood cells become larger with heavier nuclear-to-cytoplasmic ratios compared to normoblast cells (Mahajan and Aundhakar, 2015). Megaloblastic anaemia development is often deceptive; most patients would generally show no symptoms of the deficiency because they have time to adjust to the marked fall in haemoglobin levels (Lanier *et al*., 2018; Válka *et al*., 2018).

Vitamin C: Citrus fruits including orange, lime, grapefruit, lemon and tangerines are great sources of vitamin C. The vitamin can contribute to nutritional anaemia when its deficiency occurs. This is because non-haem iron absorption is enhanced when the body has adequate stores of the vitamin (Okaka and Okaka, 2009). Vitamin C is reported to be positively linked with serum ferritin and appears to improve the correction of ferritin deficit and anaemia because of the beneficial influence of vitamin C on the absorption of iron (Fairweather-Tait *et al*., 2014; Gesquiere *et al*., 2014).

Vitamin A: Vitamin A plays a role in red blood cell development and helps improve the concentration of haemoglobin. It also increases the efficacy of iron supplementation just as it decreases infections (Kapil and Kapil, 2018). Therefore, its deficiency can cause anaemia (Kapil and Kapil, 2018). Three distinct biological mechanisms can be used to clarify the impact of Vitamin A status on iron deficiency anaemia. These are direct modulation of the production of red blood cells (erythropoiesis), increase resistance to infections leading to anaemia and Vitamin A's impact on iron absorption or organization of iron stored from tissues (Gebremedhin, 2014).

Through epidemiological surveys, populations with vitamin A deficiency, particularly those in developing countries, have also been reported to have a high prevalence of anaemia (Semba and Bloem, 2002). Anaemia prevalence has generally decreased with improvement in vitamin A status. Studies from Brazil, Mexico, Bangladesh, Pakistan, South Africa, Ethiopia and Malawi, for instance, have reported in same populations, relatively high prevalence of both vitamin A deficiency and anaemia (Food and Agriculture Organization *et al.,* 2017). This could be because food sources of vitamin A and nutrients that prevent nutritional anaemia are similar (Semba and Bloem, 2002; Costa *et al.,* 2013). Figure 2.2 below illustrates the metabolic pathway and vitamin A's role in red blood cell formation (erythropoiesis). The mechanism falls into three general classifications. The first is the modulation of erythropoiesis. This is followed by the modulation of immunity to infection also known as anaemia of infection and lastly iron metabolism modulation (Semba and Bloem, 2002). The fact that erythropoiesis and iron metabolism are modulated by infection could lead to some overlap between these mechanisms.

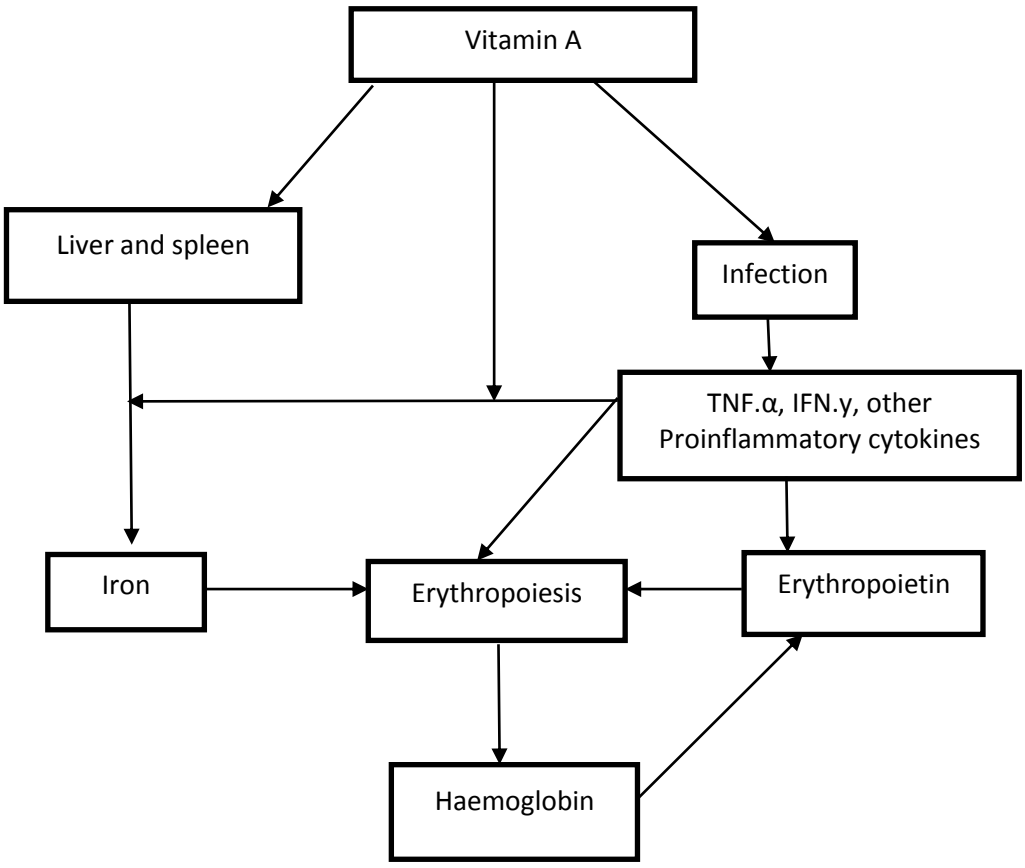


Figure 2.2: Conceptual model of Pathophysiology of vitamin A-induced anaemia

(Semba and Bloem, 2002)

The impact of improved vitamin A status on haemoglobin and anaemia through fortification and supplementation has been assessed by some intervention studies (Al-Mekhlafi *et al.*, 2013; Da Cunha *et al.*, 2018). A Community based trial in Indonesia, for instance, estimated the outcome of improved level of vitamin A on anaemia (Bhuta *et al.*, 2008). The mean haemoglobin concentration of participating children who received vitamin A fortified monosodium glutamate in the study was enhanced compared to the control groups (Bhuta *et al.*, 2008). In another study, supplementation with vitamin A in a controlled trial, improved the haemoglobin levels, haematocrit and serum iron levels of school children between 1 to 8 years (Jimenez *et al.*, 2010). There is evidence which confirms that vitamin A supplementation reduced the risk of anaemia by 26 %

and raised haemoglobin levels. This was reported for treated groups compared to the non-treated group in a meta-analysis of clinical trials (Da Cunha *et al.*, 2018). Other studies have suggested a much more increase in haemoglobin concentration when both iron and vitamin A supplements were administered. The influence of daily administration of iron and or vitamin A supplements has been reported in Tanzanian children. (Semba and Bloem, 2002). Mean haemoglobin level was increased to 13.5 g/l at 3 months for groups who were given vitamin A and to 22.1 g/l in those children who received both iron and vitamin A supplements (Semba and Bloem, 2002). Another study among women, on the other hand, reported a decrease in serum ferritin when both iron and vitamin A supplements were administered (Gebremedhin, 2014).

2.1.3.2 Iron and iron deficiency anaemia

When there is not enough iron in the body, iron deficiency occurs. The situation results in low levels of red blood cells (RBCs). The reason being that iron is required in building a protein in RBC's. This protein enables RBC's to transport oxygen around the body. The protein is called haemoglobin. There are three stages of iron deficiency. The first is the depletion of storage iron (Ferritin). This is followed by the exhaustion of transport iron (soluble transferrin receptor) and finally depletion of haemoglobin (anaemia). When iron requirements are higher than intake, depletion of iron in storage occurs. When this happens, iron stores are reduced. However, haemoglobin levels may stay in the normal range for a while. This means that being iron deficient does not necessarily make one anaemic. When iron transport is reduced in the body, iron-deficient erythropoiesis (IDE) occurs. Iron deficiency anaemia takes place when IDE coexists with low haemoglobin concentration (Turgeon *et al.*, 2016).

The haemoglobin molecule has iron as a key component. In the ferrous state, iron binds to a complex known as the protein protoporphyrin, to form haem. Thus, unavailability of iron leads to hypochromic microcytic anaemia due to low haem concentrations (Lazarte *et al.*, 2015). The most encountered micronutrient deficiency and significant contributor to anaemia in both adults and children is iron deficiency. It accounts for about 50 % of total anaemia cases (Stevens *et al.,* 2013). In many third world countries, anaemia resulting from a deficiency in iron is extremely common (Kishwar *et al.,* 2015). It prevents optimum cognitive and motor development and causes fatigue, which leads to low productivity (Balarajan *et al*., 2011; Thuret, 2017). Anaemia impairs growth in children and increases morbidity and death rates in susceptible populations (Grantham-McGregor, 2010).

2.1.4 Detection and diagnosis of iron deficiency anaemia

To establish iron deficiency anaemia, the haemoglobin test is often used (American Society of Haematology, 2017). Normal haemoglobin levels for children below five years is 11.0 g/dl and for those between five to eleven years is 11.5 g/dl (WHO, 2011). Test that determines the size and colour of red blood cells may be used to diagnose the condition. With this method, the colour of red blood cells become paler and the size become smaller than normal if iron deficiency anaemia is present (D'alessandro and Liumbruno, 2018). Haematocrit is additionally another test method which gauges the amount of platelet in the blood. Ferritin is also a method used to quantify the amount of iron stored in the body. Serum iron and iron-restricting limit are test methods utilized for distinguishing iron lack pallor. Serum ferritin makes a qualification between iron deficiency anaemia and anaemia of chronic disease, which is otherwise called anaemia of inflammatory response. It has been shown that taking iron supplements when iron deficiency anaemia has been

diagnosed can help manage the condition however, it is harmful to take iron supplements when anaemia of chronic disease is established (Johnson-Wembley and Graham, 2011).

2.1.5 Factors affecting bioavailability and absorption of iron

Bioavailability refers to the proportion of a nutrient that is absorbed from the diet and used for normal body functions (Fairweather-Tait and Sesmaisons, 2018). There are two types of iron in nourishments; haem iron and non-haem iron. Animal foods, for example, meat, fish and poultry contain both heam and non-haem iron. Forty percent of the iron found in animals is haem iron and the remaining 60% is comprised of non-haem iron. Plant foods contain only non-haem iron (Reddy *et al*., 2006). Haem iron is bioavailable and is effectively absorbed. Non-haem iron, however, has poor bioavailability (Young *et al.*, 2018). Research has reported between 20 % to 30 % absorption of haem iron from dietary sources contrasted with between 5 % to 15 % absorption of non-haem iron (Young *et al*., 2018).

The body's absorption of iron from plant-based foods is affected by the consumption of dietary inhibitors and enhancers. Vitamin C, meat, fish and poultry (MFP) factor, are examples of iron absorption enhancers. Inhibitors, on the other hand, include those present in whole grains like phytates, oxalates in vegetables and calcium found in salts and milk. These enhancers and inhibitors affect the absorption of iron when ingested during and shortly before or after a meal (Collings *et al*., 2013). For instance, adding orange juice that contains 40mg to 50mg vitamin C to a breakfast meal made up of bread, egg, and tea or coffee, has been shown to increase iron absorption from 3.7 % to 10.4 % (Horimoto and Lim, 2017). Vitamin C was also reported to be the reason behind the sevenfold upturn in iron absorption from maize porridge when pawpaw was added in the meal (Scrimshaw and Wallerstein, 2012). However, oxidation and heating destroy most of the vitamin C in

foods during storage and cooking (Gillooly *et al.*, 2007). Thus, the vitamin C content of raw food, may not be used precisely to forecast the extent of iron absorption improvement expected from processed food manufactured from the raw material.

Meat, fish and poultry (MFP) represent a factor in animal protein sources that increases non-haem iron absorption. A meal made with black beans recorded double iron uptake when 100 g of fish was added (Hallberg *et al.*, 2003). A similar effect was observed when the total amino acid content of the fish in its purified form (210 mg of cysteine, the amount present in 100 g fish) was added alone. The addition of cysteine to maize and soybean also enhanced the absorption of iron (Baech *et al.*, 2003; Hallberg *et al*., 2003).

Animal products are excellent sources of bioavailable haem iron; however, in many developing countries consumption of animal-based products is limited by cost and availability (Bruinsma, 2003). Many diets originating from Africa are usually not rich in vitamin C. The diets however often contain chelators that bind the mineral in the digestive tract preventing its absorption (Zimmermann *et al.*, 2005). In rural Tanzania for instance, evaluation of typical household consumption patterns indicated mainly grains and vegetable-based diets. These diets are very low in absorbable iron due to the presence of elevated levels of inhibitors like phytates and polyphenols (Tolentino and Friedman *et al.*, 2007).

2.1.6 Populations at-risk of anaemia

Infants and young children including those of school-going age, female adolescents and women have been reported to be the groups most at-risk of anaemia (Siva *et al*., 2016; Syed *et al*., 2016; WHO, 2015). The rapid growth of female adolescents coupled with the experience of first menstrual periods, which leads to iron losses, put adolescent girls at an

even greater risk of developing anaemia. Other at-risk groups include women of childbearing age, pregnant and lactating women. These also have elevated micronutrient requirements. People with low dietary ingestion of iron over a long period are also at risk of anaemia (Johnson-Wimbley and Graham, 2011).

The iron requirements of the foetus and baby throughout pregnancy and after delivery are dependent on the status of the pregnant woman and the mother. The probability that the growing foetus will be iron deficient is very high if the mother-to-be is deficient (Abu-Ouf and Jan, 2015). This is because iron is necessary for the normal growth and maturity of many organs in the growing foetus, particularly the haematopoietic ones. It is also very vital for the development and growth of the brain cells (Cao and Fleming, 2016). Women within the reproductive age should ideally have adequate stores of iron at conception. The reason being that it is more challenging to correct iron deficiency while pregnant when the requirements of mother and foetus are higher (Santos *et al.,* 2010). Compared to infants born to mothers with appreciable iron reserves, those born to deficient mothers have been reported to have poor brain function and a lower intelligence quotient (Milman, 2011; Jáuregui-Lobera, 2014).

2.2 Vitamin A in nutrition and health

Vitamin A comprises a group of fat-soluble vitamins collectively called retinoids. These include retinal, retinol, and retinyl esters. Precursor varieties specifically pro vitamin A carotenoids are also included (Johnson and Russell, 2011). The vitamin plays key roles in the functioning of the immune system, visual acuity, cell communication, bone and tooth growth and mucus membrane synthesis (Solomons, 2006; Johnson and Russell, 2011). The role played by vitamin A during embryo growth and development in humans is very

crucial. It will be a challenge for the fertilized egg to develop into a foetus without vitamin A (Oruch and Pryme, 2012).

2.2.1 Types of vitamin A and their metabolism

In foods, vitamin A exists as pro-vitamin A carotenoids or retinol (pre-formed) and its esterified derivative known as retinyl (Johnson and Russell, 2011; Ross, 2006). Pre-formed vitamin A is promptly utilized by the human body. It is contained in animal foods like fish, liver oils, egg yolk, meat, milk and other milk-derived products. Enriched foods including fortified margarine, oils and cereals are also good sources of vitamin A (Saeterdal *et al*., 2012). Carotenoids are obtained from plants and can be converted to retinol. Fortified foods as well as green vegetables like spinach and amaranth; palm fruits, palm oil, and orange-fleshed potatoes contain considerable amounts of carotenoids (Harrison, 2012). Yellow and orange coloured vegetables and natural crops like pumpkins, squash, carrots, mangoes, apricots and papayas are endowed with carotenoids. Carotenoids exist as beta-carotene, alpha-carotene and beta-cryptoxanthin. These carotenoids can be converted to retinol with beta-carotene being the most significant carotenoid. Lycopene, zeaxanthin and lutein are different carotenoids that cannot be converted into vitamin A by the body. One microgram of physiologically accessible retinol is proportionate to 12 µg of beta-carotene, or 24 µg of alpha-carotene or beta-cryptoxanthin (NIH, 2016). With five conjugated carbon-carbon double bonds, retinoids typically comprise four isoprenoid units. The schematic chart in figure 2.3 below demonstrates the metabolism of vitamin A from dietary sources when ingested into the body.

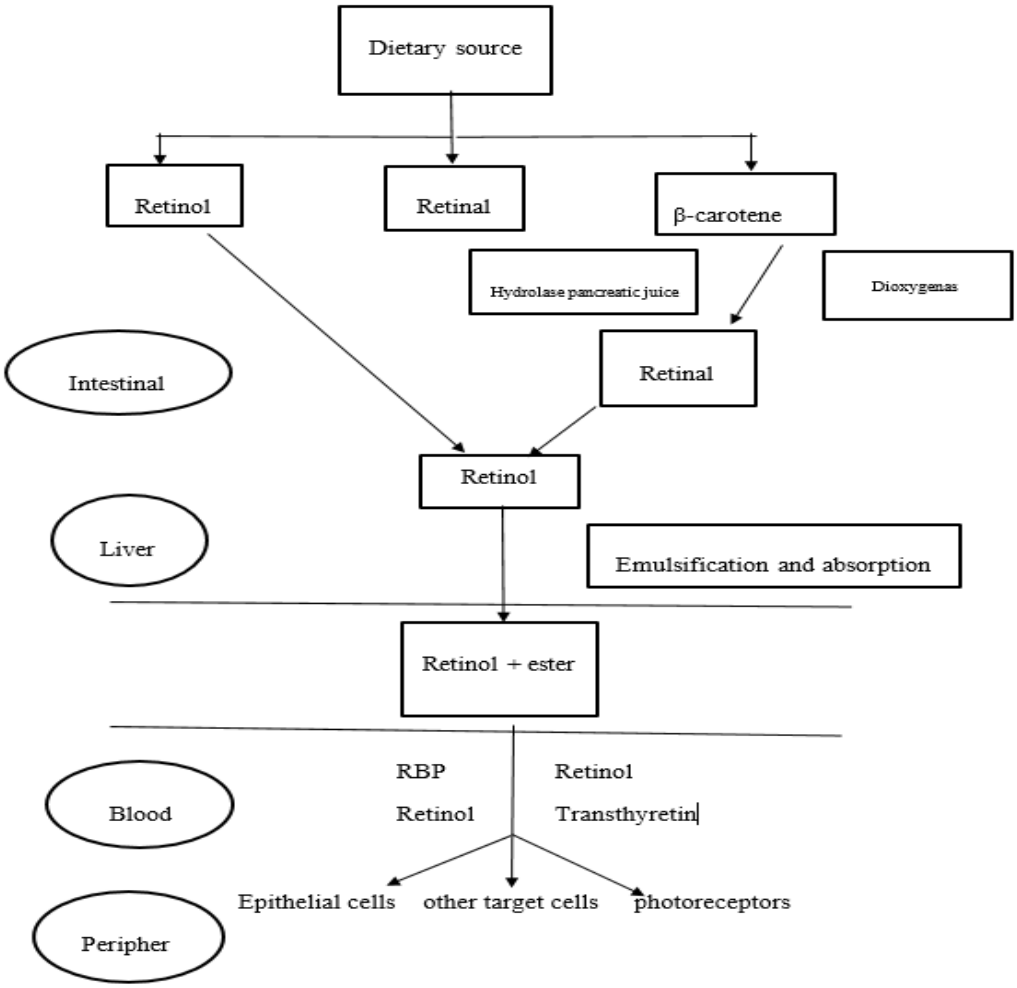


Figure 2.3: Schematic diagram of vitamin A metabolism (D'Ambrosio *et al.*, 2011)

Pro-vitamin A carotenoids consumed in plant-based foods are released from proteins in the stomach. In the intestinal mucosa, the carotenoids are sliced into molecules of retinaldehyde. The molecules are then metabolized to retinol and further esterified to retinyl esters (O'Byrne and Blaner, 2013). They are then transported to the blood through micelles in the lymphatic drainage of the intestine. From retinol and carotenoids, the retinyl esters formed are then transported to the liver as components of chylomicrons (Reboul, 2013). Fifty to eighty percent of vitamin A bound to retinol-binding protein, is stored in the liver. The 20 % to 50 % left are deposited as retinyl esters in the kidney, lungs and the adipose tissues (O'Byme and Blaner, 2013).

2.2.2 Causes of vitamin A deficiency

Inadequate consumption of vitamin A rich-foods from the diet and infections are the foremost cause of deficiency in vitamin A. In developing countries, the deficiency could be diagnosed during infancy, when colostrum and mature breastmilk fed do not contain enough of the vitamin (WHO, 2009). Inadequate consumption of colostrum could lead to a low blood level of vitamin A in newly born babies. Most mothers in low-income populations have low vitamin A concentration in their breast milk because they consume diets low in the vitamin. In Bangladesh, low consumption of vitamin A-rich foods was recorded to be the main determinant of vitamin A deficiency among different groups (Akhtar *et al.,* 2013). Women from low-income Regions, for instance, met only 31 % to 57 % of their recommended daily allowance (RDA) (Zeitlin *et al.,* 1992) whereas women in developed communities met 75 % to 90 % of their RDA. It was reported in a systematic review, that only 10 % to 30 % of African children were fed with foods that were rich sources of the vitamin (Aguayo, 2017). A study in Ethiopia corroborates the above, where 28.8 % of children between 6 and 23 months consumed foods rich in vitamin A (Jemberu *et al.,* 2017). Children in developed economies obtain most of their vitamin A from animal sources; under-privileged children from emerging nations, on the other hand depend on affordable plant sources which may have low vitamin A activity (West, 2002). For example, among toddlers in America, median consumption of animal-based vitamin A-rich foods was 404 µg, which exceeded their RDA of 300 µg by as much as 35 % (NHANES III, 2010). On the contrary, studies on children in some emerging countries like India, Kenya, Mexico and Egypt revealed that intake of vitamin A from animal-based foods was 33 µg, 50 µg, 119 µg, and 174 µg respectively, supplying 11 % to 58 % of the RDA (Ramakrishnan, 2002). More nutrition education will be needed to increase the

consumption of vegetables and foods from animal origin to increase the intake of vitamin A among populations in many developing countries.

Another key cause of vitamin A deficiency is infections, particularly diarrhoea and measles (WHO, 2009). It has been reported that helminths infestation and diarrhoea undermine the integrity, morphology and the performance of the intestinal absorptive mucosa, which may result in malabsorption of vitamin A (McKay *et al.*, 2017). Studies done in emerging countries have estimated that diarrhoea prevalence among children varies from 18 % to 29 % (Omelonye *et al.*, 2015; Thiam *et al.*, 2017; Getachew *et al.*, 2018). Consequently, an average child in a developing country may suffer diarrhoea about 36 to 70 days annually (Dalby-Panye and Elliott, 2011). Acute respiratory illness suffered by children in evolving countries alone is 25 % to 60 % (Clasen *et al.*, 2015). These illnesses aggravate vitamin A status by decreasing the quantity of food consumed due to anorexia, malabsorption and increasing food utilization through higher catabolism and urinary loss. It has been recorded that anorexia is a leading contributor to low dietary consumption during periods of diarrhoea among children, a situation under which dietary intake is best studied (Paintal and Aguayo, 2016). Compared to children still breastfeeding, reduction in food intake appears to be more profound among weaned children suffering from diarrhoea. For instance, diarrhoea resulted in a 20 % reduction in energy intake among Italian children not receiving breast milk (Grote *et al.*, 2015). Sivakumar and Reddy (1998) demonstrated virtually complete absorption of oily retinol by healthy children whereas there was a 30 % decrease in the absorption of the same oily retinol among children with respiratory infection and gastroenteritis. In a related study, the absorption of 100,000 IU vitamin A supplement was reduced by 22 % and 17 % when added to oral rehydration salt solution and water respectively in diarrhoeal children

(Agrawal and Agrawal, 2013). Apart from malabsorption, higher catabolism of vitamin A during infection may be another factor that leads to the development of VAD.

2.2.3 Consequence of vitamin A deficiency

Vitamin A deficiency causes xerophthalmia. It is the number one and most widely known sign in infants and pre-schoolers (Chander *et al*., 2013). From night blindness, the eye disorder aggravates to more severe clinical consequences including corneal scars, keratomalacia, and permanent blindness (Wodaye *et al*., 2016). Prevalence of xerophthalmia in urban India was about 6.5 % (Sinha *et al.,* 2011). Out of this, 0.2 % was attributed to night blindness and or bitot spots (Sinha *et al.,* 2011). In low-income communities such as Nagpur, the xerophthalmia prevalence was 8.7 % in children (Akhter *et al,* 2013). Annual reports suggested that about 250,000 to 500,000 children deficient in vitamin A ended up with blindness. Fifty percent of that number lose their lives in a year of becoming blind (Bourne *et al.,* 2017). In children, the risk of dying from diarrhoea and measles is high with Vitamin A deficiency (Imdad *et al.,* 2010, Imdad *et al.,* 2011, Awasthi *et al.,* 2013). It has been reported that 94,500 and 11,200 deaths due to diarrhoea and measles are attributable to deficiencies in vitamin A. This counts for 1.7 % of all deaths in children younger than 5 years in countries with low and middle income (Imdad *et al*., 2010). In Indonesia, children with vitamin A deficiency were shown to be two to three folds more prone to developing diarrhoeal and acute respiratory tract infections (IMH, 2017). These two diseases accounted for nearly half of childhood deaths in India (Chakravarty, 2000). For the growing foetus and the breastfeeding baby, consequences of a deficiency in vitamin A include increased infant morbidity and death, high risk of developing anaemia, slower infant growth, and growth retardation (Mwanri *et al.,* 2000).

2.2.4 Groups at-risk of vitamin A deficiency

Individuals most at risk of vitamin A deficiency include Infants, children, pregnant women, lactating mothers and individuals with cystic fibrosis. Lactating mothers and pregnant women require additional vitamin A to support foetal and infant growth. These groups of people also require the vitamin for tissue maintenance and to ensure their metabolic processes (McCauley *et al.,* 2015). High proportions of the groups with vitamin A deficiency live in emerging countries. Clinical vitamin A deficiency is rare among infants in industrialized populations; it takes place mostly in those whose small intestines do not function properly when it comes to absorbing nutrients (Mactier and Weaver, 2005). Nine million and eight hundred thousand pregnant women worldwide have been estimated to have xerophthalmia because of VAD (WHO, 2009). Liver stores and plasma concentration of retinol tend to be low at birth in preterm infants, and it usually remains low until the end of the first year (Darlow and Graham, 2016). It has also been reported that the risk of developing an eye disease, chronic lung, and gastrointestinal diseases is higher when vitamin A deficiency is reported (Mactier and Weaver, 2005). In infants and young children, VAD results in an increased risk of developing anaemia, slower growth and development and even death (Ahmed *et al*., 2017).

It has been reported that the vitamin content of breastmilk of mothers in the developed world is enough to sustain their babies in the first six months. However, women in developing countries mostly have insufficient vitamin A and also produce breast milk with inadequate vitamin A content; which is usually not enough during the first six months of exclusive breastfeeding to maintain adequate vitamin A store for the infant (Oliveira-Menegozzo *et al.,* 2010).

Majority of individuals with cystic fibrosis have difficulty in absorbing fat and so develop pancreatic insufficiency, which increases their risk of becoming vitamin A deficient (Bonfant *et al.*, 2014). About 15 % to 40 % of cystic fibrosis patients are vitamin A deficient (Bertolaso *et al.*, 2014; Rana *et al.*, 2014). Nonetheless, most of them have become vitamin A sufficient with pancreatic replacement treatments and better nutrition (Bonifant *et al.*, 2014).

2.2.5 Prevalence of Vitamin A deficiency (VAD)

Vitamin A deficiency assumes severe public health significance requiring intervention when at least 20 % of a population test to serum retinol concentration less than 20 µg/dl (WHO, 2011). Figure 2.4 below shows the global map indicating the prevalence of VAD among children with varying degrees of prevalence per country with most developing countries showing high prevalences.

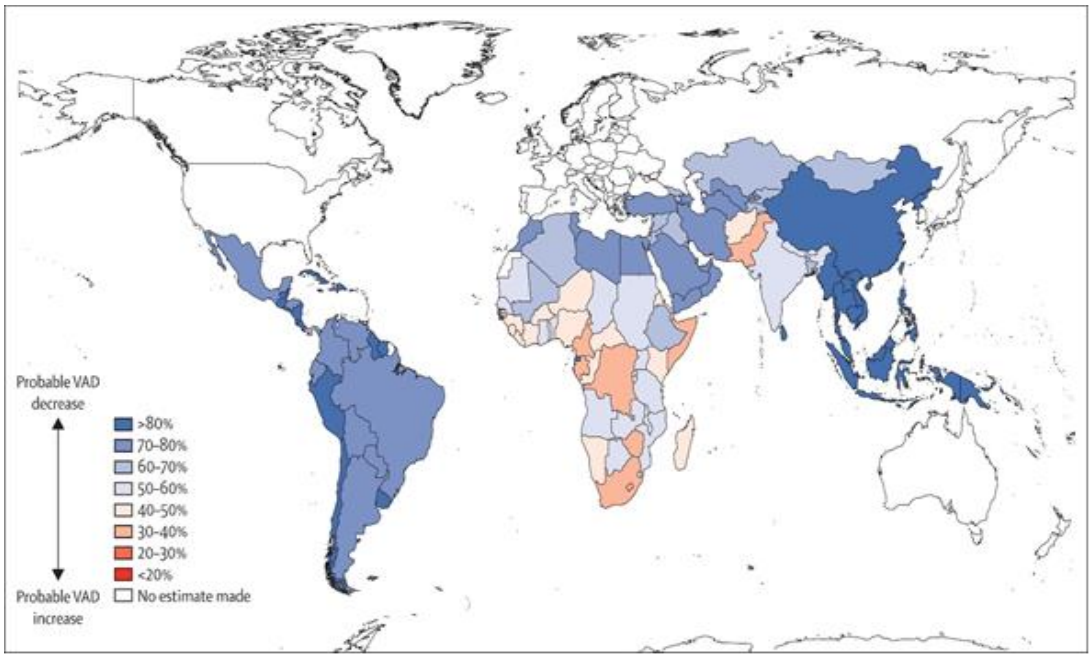


Figure 2.4: Vitamin A deficiency in children (UNICEF, 2017)

A well-nourished child will usually have adequate liver stores of vitamin A to maintain serum retinol levels between 10 mmol/1 and 14 mmol/1 (Olmedilla-Alonso *et al.*, 2011).

In assessing vitamin A deficiency status in populations, a commonly used and widely accepted method is using a cut-off point to assess the prevalence of low serum retinol concentrations (Sommer and Davidson, 2002). Values less than 0.70 mmol/l or 20 µg/dl are considered indicative of a deficiency in children. For pre-school aged children, it was estimated that, 140 – 250 million were at risk of vitamin A deficiency (ACC & SCN, 2000). This included 3 million who had clinical signs every year. Two years later, West (2002) reported that 127 million preschoolers were vitamin A deficient worldwide. Twenty five percent of that figure lived mostly in developing countries (West, 2002). Subsequent estimates recorded that 13 million children globally were xerophthalmic (Underwood, 2004). A few years later, the WHO reported that 190 million preschool children around the world had serum retinol concentration below 0.70 mmol/l (WHO, 2009). In the Southern Asian Regions, approximately 44 % to 50 % of preschool children were severely vitamin A deficient (WHO, 2009). WHO's Regional estimates in 2011 recounted Africa to be housing the highest proportion (2 %) of preschool children affected by night blindness compared to 0.5 % children in South - East Asia (WHO, 2011). The number of children with night blindness in Africa amount to about half of the number of children with the disease in the world (WHO, 2011). Globally, the prevalence of vitamin A deficiency is a problem of public health importance with Sub-Saharan Africa having the highest rate of 48 % followed by South Asia at 44 % (UNICEF, 2013). In Africa, the country with the greatest number of children (6.7 million) with vitamin A deficiency is Ethiopia (UNICEF, 2013). In West Africa, the prevalence of vitamin A deficiency in children vaies from 30% in Nigeria to 75% in Sierra Leone (Diedhiou, 2018). Forty percent of preschoolers in Cameroon are vitamin A deficient (Engle-Stone *et al*., 2017). In the industrialized world, however, vitamin A deficiency is almost non-existent (UNICEF, 2013).

In Ghana, the vitamin A supplementation trial in 1993 (VAST, 1993) reported 65 % severe vitamin A deficiency in children in the northern part of the country. Four years later, the Ministry of Health's vitamin A prevalence survey (1997) revealed that 37.2 % of children in southern Ghana had low serum levels of vitamin A. Later, WHO (2009) estimated that 2.5 million children under five years in Ghana were vitamin A deficient. Subsequently, a cross-sectional study conducted among school children 2 to 10 years in the Eastern Region of Ghana indicated a vitamin A deficiency prevalence of 36 % (Egbi, 2012). A few years on, an intervention study among 6 to 12 years old school children in the Volta Region of Ghana recorded a prevalence of 95.1 % at pre-intervention and 77.5 % at the end of the study period (Egbi *et al.*, 2018). Generally, 20 % of children in Ghana are vitamin A deficient. The prevalence is higher (31 %) for children in the northern belt but among children living in wealthier households, the prevalence is lower at 9 % (GMSR, 2017).

2.3. Prevention and control of anaemia and vitamin A deficiency

In children, anaemia and vitamin A deficiency contribute to unfavourable health outcomes; these may include inadequate growth, immune incompetence, poor mental and physical development, and poor reproductive outcomes (Ramakrishnan, 2002; Baily *et al*., 2015). Approaches to stop and regulate micronutrient deficiencies are therefore very essential, especially in developing countries. Three key strategies that may be used separately or together to prevent and or control micronutrient deficiencies have been proffered. These are long-term dietary diversification, medium-term food fortification and the short-term micronutrient supplementation. In combination with other public health interventions like parasitic disease control and infection control, these three strategies have been successfully deployed in many regions and countries. However, the sustainability of

many supplementation programmes has been of concern in many developing countries due to the high operational cost involved. Food-based approaches (which involve dietary quality improvement through food fortification, food diversification and or dietary modification), nutrition education and bio fortification are being practised. These represent the most desirable, sustainable and the most financially prudent methods for preventing micronutrient malnutrition (Nair *et al.,* 2016).

2.3.1 Micronutrient supplementation

Supplementation, which entails giving supplements or capsules to vulnerable groups at risk of micronutrient deficiencies, should be regarded as one of several methods in the prevention, management and control of micronutrient deficiency (Baltussen *et al.,* 2004). Supplementation has been employed in many countries and proved beneficial. In India for instance, an intervention study recorded an improvement in short-term memory of 6 to 10-year-old children who received iodine, folate, iron, vitamins B_6 and B_{12} supplements (Muthayya *et al.,* 2009). Similarly, a review by Eilander and partners (2010) revealed a possible association between micronutrients supplementation and academic performance and the ability to reason and think conceptually. Iron supplementation, for example, had a modest effect on mental development, predominantly on intelligent quotient (IQ) scores in children above seven years of age. Similar results were obtained among children who had iron deficiency anaemia (Szajewska *et al.,* 2010). A significant reduction in macular degeneration was also observed in a clinical trial that assessed the effect of supplementation on eye disease (AREDS, 2006). Furthermore, Sommer (2008) found that Indonesian children older than 6 months who received bi-annual vitamin A supplements were significantly less likely to die compared to those who were not given supplements. The influence of maternal vitamin A supplementation on maternal health has also been

studied. In Bangladesh for instance, weekly supplementation of mothers with preformed vitamin A or β-carotene was associated with a significant reduction in maternal mortality (West *et al.*, 2011). Supplementation of neonates in South East Asia with vitamin A in the first month of life, led to a 21 % reduction in neonatal mortality (Bhutta *et al.*, 2008). There was no impact however, of the intervention on neonatal mortality when similar studies were conducted in Africa (Benn *et al.*, 2008; Lund *et al.*, 2014).

For children living in poverty, multiple micronutrient deficiencies may be common, thus, the correction of a single deficiency may not be enough. In such situations, multiple micronutrients (MMN) supplementation may be considered. A supplementation trial on 6 to 9-year-olds that involved the use of multiple micronutrients, revealed a positive effect of supplementation on growth (Rameshwar *et al.*, 2006). Similarly, interventions with MMN in Peru proved effective in reducing the prevalence of anaemia (Munayco *et al*., 2013; Zavaleta *et al*., 2013).

Vitamin A supplementation may probably be the most well-known intervention practised clinically for treatment and in public health as a preventive measure. Currently, the World Health Organization recommends it for countries where vitamin A deficiency is a public health issue (WHO, 2011). In low-income countries however, national supplementation programs may be too expensive, difficult to implement for logistical reasons, and in some cases, very challenging to achieve effective coverage of the entire population. Other issues with supplementation, particularly with iron are the unpleasant side effects, poor compliance to treatment regimen mostly due to insufficient health and nutrition education and threat of overload if the supplementation regimen is not followed (Gereklioglu *et al*., 2016).

2.3.2 Food fortification

Food-based interventions are the most viable strategies for increasing the micronutrient consumption and status of various age groups in a population. These include food fortification and dietary diversification. To overcome and prevent malnutrition and micronutrient deficiencies, food-based interventions focus on food; be it in its natural state, processed or fortified form, or in combination, as a primary means of improving the nutrient content of diets. Food-based approaches also impact the availability, absorption and the body's utilization of micronutrient-rich foods through the production and consumption of nutrient-rich improved varieties of crops (Allen, 2006).

Compared to other food sources, green leafy vegetables (GLV) are less expensive, available during the raining season and rich sources of micronutrients like iron, carotenoids and folate. Using green leafy vegetables in the fresh state, dried or powdered form as fortificants is one way of ensuring micronutrient intake. According to Odunlade *et al.*, (2017), African eggplant and amaranth resulted in notable increases in iron, zinc and calcium content when used to fortify wheat bread. Fortification of marshmallow with green spinach and tomato for instance, increased the iron content of the marshmallow (Yudhistira *et al.,* 2017). In Nigeria, a cereal gruel customarily known as Ogi is used both as a complementary food for infants and a family cereal (Arise *et al.,* 2014). The incorporation of *Moringa oleifera* powder (MOFP) into ogi considerably improved the nutrient content of the cereal gruel (Arise *et al.,* 2014; Abioye and Aka, 2015; Adewumi *et al.,* 2018), increasing the vitamin A presence by around 15 folds (Olorode *et al.,* 2013). Different supplement constituents like protein, calcium, iron and phosphorus likewise improved altogether because of the *Moringa olifera* powder added (Abioye and Aka, 2015). Prepared and consumed in Nigeria and many other West African countries, Amala is another well-known staple. Though a low nutritional value staple, (Jimoh and

Olatidoye, 2009; Abiodun and Akinoso, 2014), amala has been fortified with different fortificants like distillers spent grain, soybean flour and *Moringa oleifera* leaf powder (Jimoh and Olatidoye, 2009; Awoyale *et al*., 2010; Karim *et al*., 2013, Karim *et al*., 2015) to improve its nutritional value. The fortification of amala with 10% of *Moringa olifera* leaf powder (MOLP) for instance, increased the protein content by nearly 48% (Karim *et al.*, 2013). Also, the content of iron, magnesium, calcium, potassium and sodium of the fortified variant improved subsequently by the addition of MOLP (Karim *et al.*, 2013). It has been reported that *Moringa oleifera* leaf consumption would ensure adequate reserves of iron and β-carotene that could become available for conversion when needed by the body. Consumption of 10 g of dried *Moringa oleifera* leaves a day, for instance, could provide 50 % to 100 % of the vitamin A needs of people of different age groups and about 30% of the iron needs of children between 1 and 12 years old (De Saveur *et al.*, 2010).

2.3.3 Diversification and modification of the diet

Dietary diversification approach is a set of interventions that enhance food consumption pattern by improving access, availability and use of foods with high nutrient content and micronutrient bioavailability over some time. It comprises an increase in the consumption of vegetables, fruits and animal foods (Gibson and Anderson, 2009; Gibson, 2014). Many rural diets are mostly composed of cereals, legumes, starchy roots and or tubers with limited or no addition of meat, dairy, fruits and or vegetables. Dietary diversification will thus increase the quantity and variety of micronutrient-rich foods in the diet there by decreasing micronutrient deficiencies (Gibson, 2014; Nair *et al.*, 2016). This is generally achieved through social and behaviour change activities that employ good and modern agricultural practices, improved food selection and enhanced household methods for preparing indigenous meals. Compared to micronutrient supplementation, dietary

diversification could be more economically feasible and thus sustainable and more culturally acceptable especially when it is backed with nutrition education.

2.3.4 Biofortification

Biofortification or biological fortification is a procedure for breeding crops. It is the development of crops that by harvest have accumulated higher amounts of one or more micronutrient than the conventional crops. Cost- effective analysis has demonstrated that biofortification is considerably cheaper than either fortification or supplementation approaches to tackle vitamin A deficiency, for example, in several countries (Asare-Marfo *et al.,* 2014). Several research reports have indicated that it is a promising, and sustainable technique of delivering micronutrients to a population that has limited access to diverse diets especially in developing countries (Greiner, 2017; Garg *et al*., 2018; Lockyer *et al.,* 2018). The Food and Agriculture Organization of the United Nations has estimated that around 792.5 million people across the world are malnourished, out of which 780 million people live in developing countries such as Africa (McGuire *et al.*, 2015). The generation of biofortified food crops with improved nutrient contents such as increases in iron, zinc, selenium, and provitamin A are providing adequate levels of these and other such micronutrients that are frequently lacking in the diets of many malnourished people.

Plant breeding has been used to grow high yielding genotypes of indigenous plants impervious to heat, and rich in iron, zinc and carotenoids (Zuma *et al.,* 2018). New varieties of oat grains, cassava roots, and orange-fleshed potatoes with improved zinc, beta-carotene and iron are only a few examples of crops created by the strategy (Burri, 2011; Garg *et al*., 2018). Methionine and cysteine and sulfur-containing amino acids that enhance the absorption of zinc and non-haem iron in maize, have also been produced

through plant breeding (Li *et al*., 2017). HarvestPlus and International Potato Centre (CIP) have developed and released several varieties of orange sweet potato with high vitamin A content. Consumption of the HarvestPlus orange sweet potato had a significant effect on household food and nutritional security in Sub Saharan Africa (Ricachenevsky *et al.,* 2018). A study of 120 Bangladeshi women with low vitamin A status reported a significant difference in plasma β- carotene levels between those consuming orange sweet potato (fried or boiled) as snacks and those consuming white sweet potato (Jamil *et al.,* 2012). Also, the median vitamin A intake among children who consumed orange sweet potato was 426 µg RAE/day compared to 56 µg RAE/day in those who did not consume orange sweet potatoes and serum retinol was significantly higher in the intervention group as well (0.74 compared to 0.67 µmol/l) (Low *et al.,* 2007).

2.3.5 Home gardening

Home gardening that focuses on cultivating micronutrient-rich vegetables and fruits, is a long-term strategy that can contribute to combating anaemia, vitamin A and other micronutrient deficiencies with public health implications in poorly resourced developing areas (Faber and Laurie, 2010). Home garden crops become more readily available and affordable when cultivated at the household (backyard garden) or in the community. Indigenous green leafy vegetables such as *Amaranthus cruentus* and *Solanum macrocarpon*, can make available to households uninterrupted access to foods rich in iron and pro-vitamin A carotenoids. It has been debated that home gardening is an inherently effective intervention, and most households can practice it when they have access to land and other agricultural inputs (Schreinemachers *et al.,* 2016). Furthermore, home gardens are cost-effective, sustainable, easy to practice, culturally acceptable and have the potential for being used as an income generation activity while improving household

micronutrient intake (Berti *et al.,* 2014). A home or community gardening project implemented in the KwaZulu-Natal Province, for instance, recorded almost 50% of the children in the area to be vitamin A deficient at baseline (Oelofse, 1999). Project evaluation at end line showed a higher vitamin A rich food intake, increase in vitamin status (children aged 2 – 5 years) and a positive impact on maternal knowledge regarding vitamin A food sources, dietary vitamin A intake and nutrition (Oelofse, 1999). Introducing nutrition knowledge and behavioural change communication components would ensure that gardening activities translate into improved dietary quality, and nutrient intake (Ruel, 2001). Dietary modification through the successful change in behaviours that ensure adequate dietary intake, together with ensuring the availability of supply, would contribute to making the strategy more affordable and sustainable over time (FAO and WHO, 2014).

2.4. Enhancing nutrient bioavailability

Plant foods are major staples which are largely consumed, consumption of animal foods, however, are often low because of economic and or religious concerns in many developing countries. Several local vegetables have been consumed by communities mainly as supplements to conventional foods and to contribute to improving health and nutrition (Addis *et al.,* 2013). This is because vegetables are less expensive and are recognized as very good sources of protective nutrients (Nnamami *et al.,* 2009); vitamins and minerals and enhance the taste and colour of many rural meals (Adenipenkun and Oyetunji, 2010). Vegetables can, therefore, help overcome micronutrient malnutrition. However, some dietary and host-related factors such as the nature of food matrix, the chemical form of the nutrient, nutrient interactions and other organic components within the food, pretreatment

during food processing and or preparation, can impact the bioavailability of certain nutrients in vegetables (Platel and Srinivasan, 2016).

Absorption of micronutrients is probably greatly affected by the food matrix. The bioavailability of pro-vitamin A and folate, for instance, could reduce because they could be trapped in the insoluble plant matrix (Gregory, 2012). Studies have shown that pure beta-carotene dissolved in oil was better absorbed compared to the carotene in raw carrots or lycopene in fresh tomato juice (Harrison, 2012). The reason is that the addition of a small quantity of oil to the fat-soluble carotenoids, enhance the bioavailability of the carotenoids (Harrison, 2012). An example of a surface area effect is seen in folate containing foods. Thus, folate in whole spinach leaves was less bioavailable compared to folate in chopped spinach leaves which had more of its surface area exposed (Riso *et al.,* 2004).

Some household food preparation methods and practices can help reduce the adverse effect of some organic components in plant foods on nutrient bioavailability. Thermal processing for instance, can improve the bioavailability of some vitamin precursors like carotenoids, and vitamins such as thiamin, vitamin B_6 and niacin by releasing them from entrapment in plant matrix (Gregory, 2012). Contrasted with raw forms, greater increases in total beta-carotene and lycopene have been reported after the consumption of cooked carrots, boiled spinach and cooked tomatoes (Kelekeb *et al.,* 2017). This could be attributed to the disruption of plant cell wall making the carotenoids all the more promptly available for absorption in the intestinal lumen (Harrison, 2012).

While boiling reduces oxalate content in plant-based foods, it also enhances calcium absorption. Non-haem iron absorption in some legumes could be enhanced by germination and malting which reduce polyphenol content in legumes (Gibson *et al,* 2006).

Germinating and malting again induce hydrolysis of phytate, hence increasing iron, zinc, calcium and magnesium absorption. Germination and malting can cause a change in consistency, which could facilitate starch digestion and increase non-haem iron absorption (Gibson *et al.*, 2006).

2.4.1 Role of vegetables in nutrition and health

Vegetables are a key component of the human diet, always regarded as food and eaten as complements to staples (Liu, 2013). As a rich source of micronutrients (vitamins and minerals), vegetables play an important role in human nutrition and health. Vegetables serve as a store house for pro-vitamin A carotenoids, vitamin C, thiamine, niacin, pyridoxine, folic acid and minerals such as iron; and dietary fibre (Oguntibeju *et al.*, 2013). To protect against diet-related diseases, the World Health Organization has recommended an intake of at least 400 g of vegetables and fruits per person every day (WHO, 2018). However, this recommendation is not being adhered to in most African households. In Sub-Saharan Africa, estimates of fruits and vegetable consumption are between 70 g and 312 g per person in a day. A figure below the minimum 400 g recommended for one day or 146 kg for an individual in a year (Ruel *et al.*, 2005). Results from the World Health Survey conducted in 52 countries indicated that Ghana had the lowest proportion of vegetable and fruit intake among the surveyed countries (Amo-Adjei & Kumi-Kyereme., 2014). The consumption rate of fruits and vegetables was about 36.6 % among men and 38.0 % among women in Ghana (Amo-Adjei and Kumi-Kyereme, 2014). Pakistan, on the other hand, recorded higher consumption rates of 99.2 % and 99.3 % for males and females respectively (Amo-Adjei and Kumi-Kyereme, 2014).

Vegetables and fruits contain numerous bioactive and potentially anticarcinogenic substances including carotenes, dithiolthiones, flavonoids, indoles, isothiocyanates,

phenols, folic acid and vitamins C and E (Malin *et al.*, 2003). It has been established that low consumption of fruits and vegetables constitute a risk factor for the development of chronic diseases such as cancer, coronary heart disease (CHD), stroke and cataract (Boeing *et al*., 2012). It has also been reported that 20 % of most types of cancers can be prevented with frequent consumption of vegetables (Oguntibeju *et al.*, 2013). Frequent consumption of vegetables also keeps the brain young and slows the mental decline associated with growing old (Morris *et al.*, 2006).

Further research that analyzed the relationship between utilization of vegetable and coronary illness, affirmed a connection between fibre consumption from vegetables and coronary illness (Slavin and Lloyd, 2012). Report from a meta-investigation showed a backwards relationship between vegetable utilization and the event of a stroke. This supports the suggestion that vegetable consumption can prevent cardiovascular diseases (Daucher *et al.*, 2006; He *et al*., 2006). High vegetable intake is also associated with a healthy eating habit (Hu *et al*., 2000) and is inversely associated with consumption of fatty foods (Tucker *et al*., 2005). It has been recorded that the risk of developing a stroke is decreased among those with a high intake of vegetables. Consuming 600 g of vegetables every day could lessen the worldwide burden of stroke by 19 % and decline the danger of coronary heart disease by 31 % (Lock *et al*., 2005; He *et al.*, 2006).

Consumption of vegetables can influence anaemia status positively. For Iron Deficiency Anaemia (IDA), fruits and vegetables exert the beneficial effects mainly through their high non-heme iron and ascorbic acid content (citrus fruits) which contributes to greater bioavailability of iron by functioning as a stimulating factor in its absorption (Bothwell *et al.*, 1989). According to Ghose and Yaya (2018), women who consumed less than five servings of fruits and vegetables had higher odds of suffering from severe and moderate

anaemia compared to those who consumed five servings or more. In a related study, the consumption of *Gnetum africanum* (Eru), a green leafy vegetable was able to account for the higher levels of adjusted haemoglobin in women in forest communities (Tata *et al.*, 2019).

The low frequency of vegetable consumption was associated with lower plasma concentrations of carotenoids (Augusto *et al.*, 2015). Furthermore, the occurrence of nutritional deficiencies in children with low fruits and vegetable consumption was twice as high as in children with usual fruits and vegetable consumption (Augusto *et al.*, 2015). Some cohort studies have proposed that consuming vegetables, especially carotenoids and vitamin C rich vegetables resulted in a reduction in the severity of cataracts (Christen *et al.*, 2005; Oguntibeju *et al.*, 2013). In the United States, high broccoli and spinach intake have been accounted for to be related to reduction in cataract among men (Chan *et al*., 2014).

2.4.2 Solanum macrocarpon

Solanum macrocarpon, popularly called the African eggplant or gboma (as it is known locally), is a vegetable of the Solanaceae family. It is a tropical widespread plant with over 1000 species worldwide and about 100 indigenous species in Africa. It originated from West Africa but is now widely distributed in Central and East Africa and grows in the Caribbean, South America and some parts of East Asia (Oboh *et al.*, 2005). Being a small sticky tropical perennial plant with alternate leaf pattern, it can grow to a height of 1 - 1.5 meters. The egg plant's leaves are hairy with stellate hair on both sides. The leaves are also oval and lobed with a wavy margin. *Solanum macrocarpon* is widely cultivated for food, as an ornamental plant or for medicinal purposes. All parts of the plant are used for food. While the fruits are eaten raw or cooked and eaten with a carbohydrate base such as

rice; the leaves are used for stews and vegetable soups. In Ghana, the root of *Solanum macrocarpon* is used to prevent bronchitis, treat itches, body aches and asthma. The seeds are also used to treat toothache and the root for the treatment of wounds. In other parts of the world, juice extracted from the vegetable, is used for the treatment of rheumatism, gout, angina and as a sedative (Bonsu *et al.*, 2002). *Solanum macrocarpon* contains vitamins like pro-vitamin A carotenoids, niacin, thiamine, riboflavin, ascorbic acid, calciferol and tocopherol. According to Usunobun and Igwe (2016), *Solanum macrocarpon* is rich in retinol (42 mg/100 g) ascorbic acid (2.5 mg/100 g), thiamin (0.032 mg/100 g), iron (31.41 mg/100 g) and riboflavin (0.033 mg/100g). This corroborates earlier findings by Dougnon *et al.*, (2012) who reported 3530 mg/kg vitamin A, 251 mg/kg iron, 220 mg/kg zinc and 92.58 % ash.

2.4.3 Amaranthus cruentus

Amaranthus cruentus though a vegetable has clusters of pink flowers. It yields the nutritious staple amaranthus grain. It is one of three Amaranthus species cultivated as a grain source, the other two being *Amaranthus hypochondriacus* and *Amaranthus caudatus*. *Amaranthus cruentus* has different names in different languages. In Ghana the Ewes call it 'Aleefu', the English, on the other hand, have named it blood amaranth, red amaranth and purple amaranth. It is used for treating constipation, anaemia, kidney complaints in young children and lactating mothers. The roots are boiled with honey as a laxative for infants and its water extract is used to treat pains in the limbs, as a tape worm expellant and for wound dressing (Schippers, 2002). The leaves and tender stems are cooked or fried in oil and mixed with meat, fish, groundnut and palm oil. This is eaten with the main dish of cereals or tubers. The powdered dry leaves are used in sauces during the dry season (Grubbens and Denton, 2004).

Amaranthus cruentus is a rich source of carotene (5716 µg/100 g), iron (8.9 mg/100 g) and ascorbic acid (64 mg/100 g) (Alegbejo, 2013). According to Ogunka-Nnoka and Ekrika (2018), the vegetable showed high carbohydrates (27.7 %), fibre (22.9 %), and ash (18.8 %) content when analyzed. *Amaranthus cruentus* is also a great source of vitamins and amino acids including vitamins A, B_6, C, and B_{12}; lysine (9.5 g/100 g), phenylalanine (6.7 g/100 g), leucine (6.6 g/100 g), and arginine (5.7 g/100 g) were the predominant essential amino acids (Ogunka-Nnoka and Ekrika, 2018). Figures 2.5 and 2.6 below give pictorial evidence of the two green leafy vegetables.

Figure 2.5: Amaranthus cruentus

Figure 2.6: Solanum macrocarpon

2.5 Role of fish in nutrition

Fish has been reported to be the most important animal-source food in the diets of over one billion people worldwide (Tacon and Metian, 2009). The quantity of fish consumed vary widely from one region to another. In the Oceania Region, consumption of fish is about twenty-five percent per capita, in Latin America and the Caribbean, it is about ten kilograms per capita (Food and Agriculture Organization, 2018). A significant difference in consumption exists between industrialized countries (27.4 kg per capita) and low-income food deficient developing countries (10.3 kg per capita). Considerable variations still exist in fish consumption even within the same country with the rich consuming significantly more fish than the poor (Thilsted *et al.*, 2014).

Fish is a key source of essential nutrients to humans, both macro and micronutrients. Globally, about 17 % of animal protein intake is obtained from fish (Food and Agriculture Oeganization, 2018). In many countries, specifically West African coastal countries, the dependence on fish as an animal protein source exceeds 50 % (Food and Agriculture

Organization, 2018). In Ghana, Sierra Leone and Senegal, for instance, the percentage of dietary protein obtained from fish were 55 %, 72 % and 43 % respectively (Food and Agriculture Organization, 2012). The contribution of fish to total protein intake is higher in Asia and some of the small Island states. It has been reported that in Maldives, Cambodia, Bangladesh, Indonesia and Sri Lanka, the contribution of fish to total protein intakes was 70 %, 60 %, 57 % 54 % and 55 % respectively (Food and Agriculture Organization, 2012).

Fish has become the source of a range of micronutrients in the diets of the poor. Especially for small-sized species which are usually eaten whole with the fish head, gut and bones. These fishes such as anchovies are excellent sources of many essential nutrients needed by the body. The nutrients include iron, calcium, iodine, selenium, zinc, phosphorus, potassium, thiamin, riboflavin, niacin as well as vitamins A and D from fatty species. Vitamin A and calcium from small-sized fish such as mola (*Amblypharyngodon mola*), are more readily available and easily absorbed by the body (Roos *et al.,* 2007). Besides, fatty fish contains more vitamin A than lean species (Thilsted *et al.,* 2014).

In the rural part of northern Bangladesh, the intake of small-sized fish during the peak fish production season, contributed about 32 % of calcium and 40 % of vitamin A recommendations of an average household (Thilsted *et al.,* 2014). Similarly, the consumption of a traditional daily meal prepared with an iron-rich small-sized fish known as changwa plieng or *Esomus longimanus*, provided about 45 % of the daily iron requirement for Colombian women (Kawarazuka and Bene, 2011). For large fishes, the bones, guts and organs are usually not consumed, as a result, the micronutrient content is much lower compared to small fishes (for example anchovies) which are consumed whole. It has been demonstrated in children that, the daily iron and zinc requirements can be

obtained from consuming only 20 g of trey changwa plieng fish (Kawarazuka and Bene, 2011). The mola fish is reported to have an excess of 2500 µg retinol activity equivalent (RAE) of vitamin A in 100 g of the fish. This means that, based on a recommended daily allowance (RDA) of 500 µg for a child, consuming 140 g of this fish will have enough to cover its need for vitamin A in one week (Roos *et al.*, 2007).

Small-sized fish like anchovies, fresh, smoked or dried when consumed whole with its bones, viscera and organs in combination with plant-based foods could enhance the nutrient content of the diet (Sankar *et al.*, 2013). When prepared as fish flour, they can be used to enrich vegetable powders to enhance the bioavailability and absorption of micronutrients. Fish flour or powder has also been used to enrich local cereal-based porridges used for infant and young child feeding in many rural settings (Abeshu *et al.*, 2016). In Malawian menu for instance, plant-based relish eaten two times in a day are supplemented with 100 g of fish powder, with each serving containing about 24 g of the dried small-sized fish powder prepared whole with the bones. This supplementation resulted in an increased intake of zinc and iron by 15 % and 14 % respectively daily (Gibson and Hotz, 2001).

CHAPTER THREE

3.0 METHODOLOGY

3.1. Study design

This study was a randomized controlled trial with a baseline and an end line design and a four-month nutrition intervention. It involved three groups: two experimental groups (fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder – FACSMP plus school lunch group; *Amaranthus cruentus* and *Solanum macrocarpon* powder – ACSMP plus school lunch group) and a control (school lunch only) group.

3.2 Study site

The study was conducted at Kodzobi a community in Adaklu District, one of the twenty-five administrative districts in the Volta Region of Ghana. Adaklu – Waya is the administrative capital of the district, positioned geographically in the centre of the District. The district shares boundaries to the east with Ho municipal assembly, to the south with Agotime-Ziope District, to the north with Akatsi District and to the west with Ketu North District. It covers a total land area of 800.8 m^2 with mainly savanna vegetation consisting of patches of forests. The inhabitants are Ewes with a total population of 36,391; 49 % males and 51 % females respectively (GSS, 2012). Agriculture is the principal economic activity in the District, and it employs about 78 % of the labour force (GSS, 2012). Cereals and legumes, for example, maize, cowpea, groundnut, rice; tubers including cassava, sweet potatoes, and vegetables (tomatoes, garden eggs, pepper, aleefu, gboma, okro) are the crops generally cultivated by the farmers in the area.

Established in 1969, the Adaklu Kodzobi District Assembly Basic School is made up of a creche, kindergarten, primary and junior high sections, with a total population of six

hundred and fifty-eight school children. The school serves communities such as Adaklu Kodzobi and Adaklu Ando. The population of school children within the age range of 4 to 8 years was two hundred and eight-four.

3.3 Study population

Male and female children 4 – 8 years old, enrolled in Adaklu Kodzobi District Assemble School and participating in the Ghana School Feeding Programme (GSFP) constituted the study population. This was to ensure that their feeding could be easily supervised and monitored throughout the study period. Children 4 to 8 years were chosen because they are a vulnerable group and severe micronutrient deficiencies among them lead to growth faltering with an adverse effect on cognitive development (Rivera, 2003).

3.3.1 Inclusion criteria

- School children 4 – 8 years old.
- School children taking part in the Ghana school feeding programme.
- School children who do not exhibit adverse reaction (allergic reaction) upon consumption of the two vegetables (*Amaranthus cruentus* and *Solanum macrocarpon)* and fish.

Information on an adverse reaction to the consumption of fish and or *Amaranthus cruentus* and *Solanum macrocarpon* based foods were sought from parents or caregivers.

3.3.2 Exclusion criteria

- School children with serum retinol concentration below 10 µg/dl or with severe vitamin A deficiency and or severe anaemia (haemoglobin concentration below 7.5 g/dl).

- School children who were allergic to fish and or *Amaranthus cruentus* and *Solanum macrocarpon.*
- School children on supplements (iron and or vitamin A supplements).

Children found with severe anaemia and severe vitamin A deficiency (haemoglobin concentration < 7.5 g/dl; serum retinol concentration < 10 µg/dl) at baseline were referred by the Medical Officer on the study team to the Ho Regional Hospital (TRAFALGAR) for treatment. They were also excluded from participating in the intervention study.

3.4 Sample size and sampling

A total of 67 children was estimated per arm of the study based on 80 % statistical power, 95 % confidence interval, a standardized effect size of 0.5, and a mean and standard deviation from a previous study (Egbi *et al.,* 2014), (Browner *et al*., 2001). Assuming a 10 % attrition rate, the samples size was made up to 85 participants per arm of the study giving a total of 256 participants.

The list of Ghana school feeding programme schools (GSFPS) were obtained from the Adaklu District Education Service. There were six (6) schools and Adaklu Kodzobi Basic District Assembly (DA) School was selected using simple random sampling. Pieces of paper with the name of a school written on each were folded, mixed thoroughly in a box and one school was picked.

Class registers were pooled together from kindergarten to class four to obtain a sampling frame. Two hundred and eighty (280) eligible children within the study's age group (4 to 8 years), whose parents or care givers consented to the study and were present in school on the day of baseline data collection were listed. To obtain the sample size of two hundred and fifty-six (256), pieces of paper with two hundred and fifty-six (256) "YES" and twenty-four (24) "NO" written on them were folded tightly, mixed in a box and drawn by

the participants. All children who selected "YES" were enlisted and those who picked "NO" were rejected.

At the beginning of the baseline data collection, 55 children of the estimated 256 could not be included because 5 were hospitalized 10 relocated and 40 refused. The refusals were based on religious and cultural reasons such as taboo on the donation of biological samples. Thirty-nine children were found with severe malaria and/or severe anaemia (Children with haemoglobin <7.5 g/dl) after baseline screening so they were excluded from the study and referred by the medical officer on the study team to the Ho Regional Hospital for treatment.

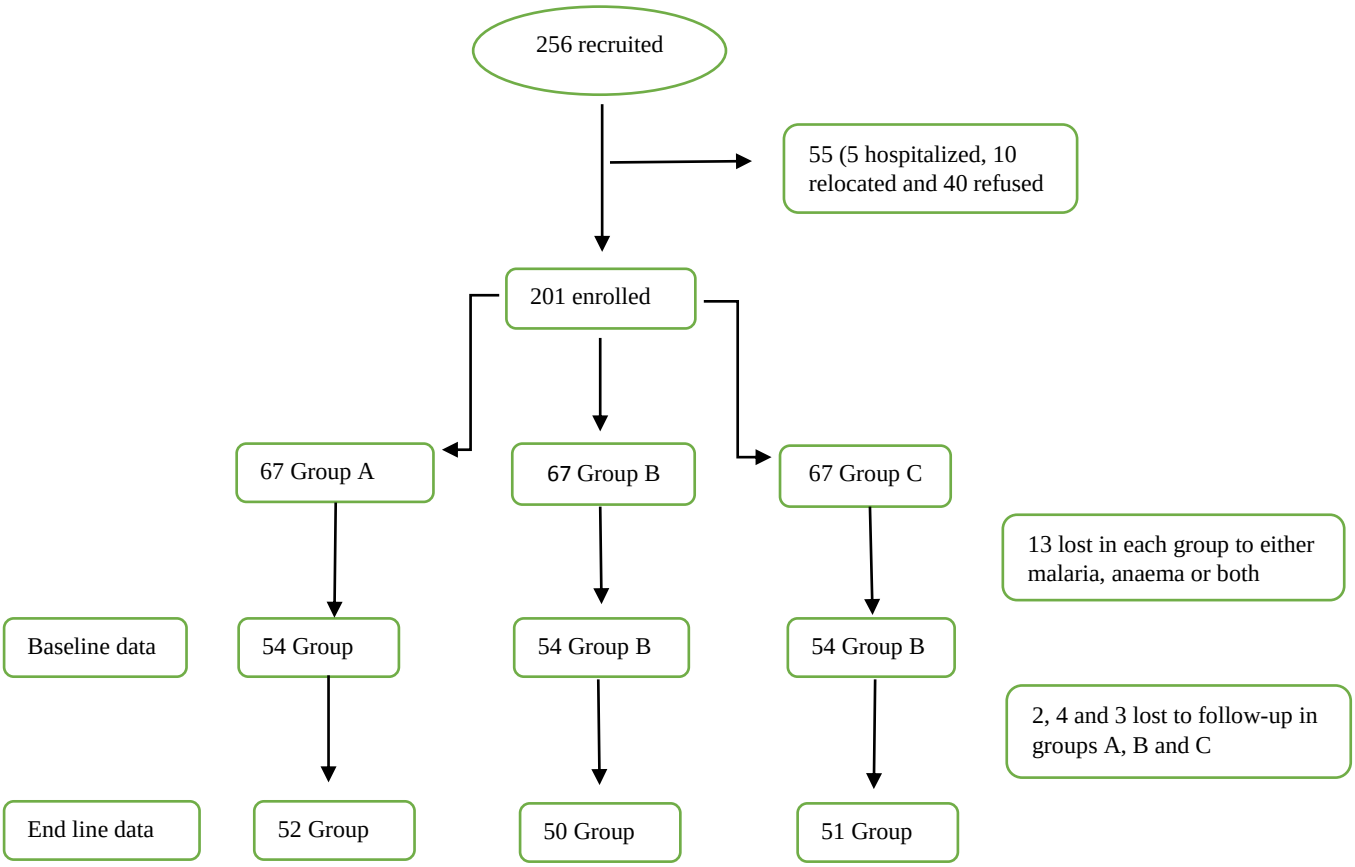


Figure 3.1: Consolidated Standards of Reporting Trials (CONSORT) diagram showing a sampling flow chart from eligible participants through randomization and follow-up.

3.5 Data collection and measurements

The language used in developing the questionnaires was English. The questionnaires were then translated into the "Ewe" dialect, which is the local dialect of the people of Kodzobi. Twelve research assistants who could speak both English and Ewe were recruited from the local community and trained in the translation and administration of the questionnaires. The questionnaires were read and explained to them. Under the supervision of the principal investigator, the research assistants administered the questionnaires on each other before they were administered to parents or care givers at home. Difficulties with the translation of questions from English into "Ewe" comprising mainly of clarification of language and rephrasing of sentences were also resolved before data collection commenced.

Socio-demographic, dietary and anthropometric were gathered at baseline. Biological (stool and blood) samples were also collected to derive haematological and parasitological data at baseline. At end line, dietary and anthropometric as well as stool and blood samples for haematological and parasitological analysis were obtained from participants. Dietary data (24-hour recall; food frequency questionnaire) and socio-demographic data were collected through administered questionnaires. A total of 4 ml blood sample was collected from each study participant; 2 ml each at the beginning and 2 ml at the end of the study respectively.

A pretested questionnaire (Appendix B) made up of seven sections was used for data collection. Section A gathered socio-demographic data. Section B solicited information from parents and caregivers on participants' morbidity and section C was used to assess the water and sanitation situation of households. While sections D and E assessed the cultural practices pertaining in the community, sections F and G, used the food frequency

questionnaire and the 24-hour dietary recall to elicit information on the types, forms and frequency of food consumption especially vitamin A and iron-rich foods among the participants. The details of each method used are elaborated below.

3.5.1 Background data

Using questionnaire and interviews, information was gathered from parents and care givers on household characteristics and socio-demographics of the community. Household characteristics consisted of fathers' and mothers' age, marital status, education, occupation, income and family size. Information on socio-demographics collected included sources of water, sanitary facilities, market, health care facilities, postal and communication facilities as well as National Health Insurance Scheme membership.

3.5.2 Anthropometric measurements

3.5.2.1 Weight measurement

The weight of participants was taken in triplicates to the nearest 0.1 kg using the Precision Health Scale UC - 300 (from AN and D Company Limited, Higashi-Ikebukuro, Toshima-Ku, Tokyo, Japan). The World Health Organization standard procedures for weight measurement was followed (WHO, 2006). The average of the triplicate measurement was considered as the actual weight of each participant. Wearing light clothing, the weight measurements were taken one hour before lunch to ensure accuracy. The weighing scale was also calibrated using an object of known weight before usage.

3.5.2.2 Height measurement

The height of each child was measured with a stadiometer. Height measurements were taken to the nearest 0.1 cm in triplicates using a stadiometer. The mean of the three readings was considered as the actual height of study participants. Participants stood straight with the head in a position such that the frankfurt plane was horizontal. With the

arms hanging loosely by the sides, the palms facing the thighs, the feet together, the knees straight, and shoulder blades, heels and buttocks in contact with the stadiometer via the vertical surface, the height measurements were taken for each child (WHO, 2006).

3.5.3. Dietary assessment

3.5.3.1 Food frequency questionnaire

Food Frequency Questionnaire (FFQ) was used to evaluate the intake frequency of consumption of diets that belong to the various food groups, especially, iron, pre and pro-vitamin A containing foods. The diets taken in, belonging to the various food categories (meat and meat products, poultry, milk and milk products, grains, vegetables, roots, tubers, nuts, fats and oils) were recorded. The number of times each food group was eaten by each participating child was additionally logged. For easy recollection, photographs of the food groups were made accessible to participants.

3.5.3.2 24-Hour dietary recall

The 24-dietary recall method was used to assess the dietary intake of study participants on two non-consecutive days, one weekday and one weekend to obtain a typical days' intake. The method estimated the food consumption of the participating children within the preceding 24 hours as was recalled from memory. The interviews were conducted in an open, pleasant, friendly and tactful manner. A structured questionnaire was used to obtain information from each participant on all foods eaten at home, at school and in other places aided by specific probing questions. Primary caregivers of participating children were interviewed along with their children. Detailed information concerning the description of each food (whether fresh, boiled, smoked or steamed) were also collected from respondents.

Market and household surveys were done before the recall to identify commonly used utensils and measures with their corresponding volumes. Prices of cooked food purchased out of home, as well as estimates of portion sizes in household measures consumed were recorded. The sensitive electronic scale was also used to weigh and estimate quantities of purchase foods. To help participants estimate portion sizes of foods consumed, measuring aids such as ladles, cups, spoons, and household utensil were made available to them.

Total amounts of foods consumed were computed manually. Protein, fat, iron, vitamin C, carotenoids and beta-carotene were determined using the Research to Improve Infant Nutrition and Growth (RIING) Project Nutrient Database (RIING Project, unpublished).

The total quantities of nutrients taken in were then compared with Recommended Dietary Allowances (RDAs) and Estimated Average Requirements (EAR). The estimated average requirement details the amount of nutrient that equals the requirement of half of the healthy individuals in a life stage and sex (Gibson, 2005). Nutrient Adequacy Ratio (NAR) which measures the ratio of nutrient intake to the estimated average requirement for a specific nutrient was then calculated as follows:

$$\text{NAR (Nutrient Adequacy ratio)} = \frac{\text{Actual nutrient in the diet}}{\text{Estimated Average requirements}}$$

It was important to calculate the Nutrient adequacy ratio to helped determine whether participants in the current study fulfilled their nutrient requirements or not. It also helped determine the risk of deficiency of the nutrients of interest to the current study, particularly iron, beta-carotene, vitamin C, and protein, with respect to their low or high intakes.

3.5.4 Biochemical data

3.5.4.1 Haemoglobin concentration determination

Haemoglobin concentration (Hb) of each participant was determined in duplicates, immediately after blood sample collection, with the Haemocue Hemoglobinometer – Hb 201 (HemoCue AB Angelhom, Sweden). The manufacturer's instructions were strictly adhered to when determining the haemoglobin concentrations. The mean Hb concentration of the duplicates represented the actual Hb concentration of each participating child.

3.5.4.2 Retinol determination

The current research focused on serum retinol to determine the vitamin A status of the participating children. A certified phlebotomist gathered 4 ml of fasting venous blood by venipuncture from each child (2 ml each beginning and end of the study). The blood samples collected were transported on ice packs to the Volta Regional Hospital's (TRAFALGAR) laboratory. After centrifuging at 2,500 rpm for 15 minutes, aliquots of serum from each sample was pipetted into 1.5ml Eppendorf tubes and transported on ice to the Noguchi Memorial Institute for Medical Research (NMIMR) and put in storage at -80 ºC until examined.

The NMIMR retinol analysis protocol was used to analyze for retinol from each serum samples collected at both base and end line (NMIMR, 1997). Frozen serum samples were thawed gently and completely in the dark. One hundred and twenty microliters of serum were pipetted into 1.5 ml Eppendorf tubes and 120 µl of methanol added. The mixture was vortexed at a speed of 9 x 1000 shakes for 30 seconds. Five hundred microliters of hexane were added to the mixture and shaken for 120 seconds. The supernatant (250 µl) was then pipetted into a clean Eppendorf tube and the content evaporated to dryness with nitrogen

gas. The dried residue was reconstituted with 120 µl methanol (High-performance liquid chromatography – HPLC grade) and vortexed for 15 seconds. The reconstituted sample was transferred into clean vials. All vials with their contents were placed into an Agilent HPLC rack and the loaded rack placed in its position in the HPLC rack chamber. One milligram of retinol standard (from Sigma Aldrich) in ethanol with 0.1 % (w/v) Butylated Hydroxytoluene (BHT) was separated in 1ml methanol. Six successive dilutions in the range of 0.016 – 0.50 mg/ml were made from the 1mg/ml stock with 500 µl of methanol. One hundred and twenty microliters of each dilution of the reference vitamin A standard was injected into the HPLC system. To establish a standard calibration curve, the resultant peaks were plotted against their respective concentrations. Ten microliters of each sample extracted were automatically injected into the HPLC operating system and retinol concentrations determined accordingly. Before running serum of each batch, 120 µl aliquot of standard retinol was infused into the HPLC as a control check. For a retention time of 6 minutes, the operation wavelength was 350 nm at a flow rate of 1ml every minute. Respective peak areas were used to determine the serum retinol concentrations from the calibration curve.

3.5.5 Parasitological examinations

3.5.5.1 Malaria parasites

Thick and thin blood smears from the blood sample taken from each participant was prepared on clean, labelled and grease-free microscope slides in duplicates. Each slide was labelled with the date and participant's identification number. The blood films were then allowed to air-dry with the slide in a horizontal position.

3.5.5.2 Staining of malaria parasites – Giemsa staining technique

The Giemsa staining technique (Cheesbrough, 1998) was to determine malaria parasites. Freshly prepared 3 % Giemsa stain (50 ml of saline, pH 7.1 - 7.2 plus 1.5 ml Giemsa stain) was used to stain all the dried blood films. The films were then allowed to stain at room temperature for 30 minutes, washed under running tap water and then air-dried. The prepared samples were then transported to the Noguchi Memorial Institute of Medical Research (NMIMR) laboratory and viewed under the microscope (Olympus BH2 Microscope, Japan) at 100 x magnification. To determine parasite density (parasites per µl), malaria parasites were counted against 200 white blood cells (WBCs) and the number multiplied by each individual's white blood cells.

3.5.5.3 Faecal examination

3.5.5.3.1 Direct faecal smears – saline and iodine wet mount preparations

Sterile stool sample collectors with spoons were distributed to participants' parents or caregiver at home. The method for collecting stool sample (s) from participants was demonstrated to each caregiver and advised to adhere to it. A container of mashed Banku mixed with red palm oil was used as the demonstration stool. A spatula was used to collect enough quantity of the demonstration faeces and carefully placed in the stool container. Each participant provided about 2 g to 3 g teaspoon size of stool in a sterilized stool container. The stools were collected before they took their breakfast. Upon reception of the stool samples by the parasitologist, they were transported on ice to Noguchi Memorial Institute of Medical Research (NMIMR) laboratory for preparation and examination. A small portion (2 g) of each participant's faeces was picked with a matchstick and added to a drop of saline. A similar portion was added to a drop of iodine. The faeces were mixed with the drops (saline and iodine respectively) to form suspensions. Each drop was

covered with a cover-slip making sure no air bubbles were trapped. The preparations were then examined under the microscope (Olympus BH2 Microscope, Japan) at 40 x magnification to identify the presence of worms.

3.5.5.3.2 The standard Kato-Katz technique

Following the standard Kato-Katz method (Cheesbrough, 1998), a sample of stool was picked with a spatula and passed through a fine-mesh sieve (105 µm mesh size) to loosen the specimen and remove fibres. This was done by placing the sieve on top of the stool specimen on a hard-flat surface and scrapping the top side with the spatula. The sieved specimen was then transferred into a circular hole in a card template placed onto a clean slide. The hole in the template was filled with the specimen and the surface flattened to obtain a standard mass of 50 mg. The template was then removed and a circular cellophane cover slip (22 mm in diameter), previously impregnated with 50 % (v/v) glycerol in water containing 3 % methylene blue solution placed on top. The sample applied slide was then turned upside down and passed gently but firmly against a hard surface to spread the stool specimen uniformly under the cellophane. The prepared slide was left for about an hour in the open at room temperature to clear. They were examined microscopically for parasite cysts and ova at x100 to x 400 magnification.

3.6.0 Preparation for intervention

All study participants; control and the two experimental groups were given treated mosquito bed nets after the baseline data collection. They were advised to sleep under the treated mosquito nets and wash their hands before and after eating and after visiting the toilet. This was to minimize malaria and soil-transmitted helminths infections respectively. Except for providing FACSMP and ACSMP to the experimental groups all other services

or treatments provided by the project applied to all participating children in the three (3) groups:

Group A: fed with diet A - school launch plus fish fortified *Amaranthus cruentus and Solanum macrocarpon* powder.

Group B: fed with diet B - school lunch plus *Amaranthus cruentus* and *Solanum macrocarpon* powder

Group C: fed with diet C - only the school lunch.

3.6.1 *Amaranthus cruentus* and *Solanum macrocarpon* powder (ACSMP) preparation

Amaranthus cruentus (amaranth) and Solanum macrocarpon (eggplant) leaves were chosen for the composite vegetable powder preparation because of their rich beta-carotene; total carotenoid and iron content (Acho *et al.*, 2014; Djuikwo *et al.*, 2011; Kwenin *et al.*, 2011). The two vegetables complement each other in terms of the two key nutrients (iron and beta carotene) of interest to this study. *Amaranthus cruentus* has a higher iron content compared to *Solanum macrocarpon*; *Solanum macrocarpon,* on the other hand, has a higher beta carotene content than *Amaranthus cruentus* (Ejoh *et al.*, 2019). Also, the two vegetables are well known and consumed by members of the study community. They were purchased from three identified vegetable farmers engaged in urban vegetable farming. Each green leafy vegetable specimen was washed in clean water, rinsed with 1% saline water for 3 minutes to remove pesticide residues and then washed under running tap water. The leaves were then dried in a locally manufactured mechanical oven at Food Research Institute (FRI) of the Center Scientific and Industrial Research (CSIR) at 45 ^{o}C for six hours. The dried *Solanum macrocarpon* and *Amaranthus cruentus* leaves were separately milled with Philips kitchen blender into a fine powder. One

hundred and sixty grams of each vegetable powder was weighed, mixed thoroughly with a cake mixer and packaged into transparent airtight polyethylene bags. The batch packs of ACSMP were packed in a large polyethylene bag with water absorbents and stored in cardboard boxes until they were ready for use.

Smoked anchovies accepted and consumed in the research community was selected and used as a fortificant. Anchovies were chosen because they are oily fish and a good source of haem iron and protein (Wu *et al.,* 2012). Haem iron to help make the non-haem iron in green leafy vegetables more bioavailable for absorption, and proteins to provide retinol-binding protein (RBP) for transportation of retinol in the blood and the oil to enhance the absorption of β-carotene from the green leafy vegetables powder. The smoked anchovies were milled into fine powder and mixed with the composite *Amaranthus cruentus* and *Solanum macrocarpon* powder (ACSMP). The fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder (FACSMP) made up of 300 g of the vegetables powder and 36 g fish powder, was then packed and sealed in airtight plastic sachets with water absorbents until ready for use.

3.6.2 The intervention diets:

The three diets used for the intervention were:

Diet A: School feeding meal plus fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder (FACSMP)

Diet B: School lunch plus *Amaranthus cruentus* and *Solanum macrocarpon* powder (ACSMP)

Diet C: School lunch

Either fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder or *Amaranthus cruentus* and *Solanum macrocarpon* was added to school lunch and their effects evaluated after participants were fed.

3.6.3 Acceptability trial by school children

An acceptability trial was conducted on two consecutive days with children 4 to 8 years old. This was to assess the acceptability of the powders and to give room for the identification of any adverse reaction to the intervention diet. Two groups (A and B), made up of 10 children in each group participated in the acceptability trial. Group A received school lunch plus fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder (FACSMP) and group B received school lunch plus *Amaranthus cruentus* and *Solanum macrocarpon* powder (ACSMP).

Each child in group A received 100 g beans stew, 20 g gari, 10 g ACSMP fortified with 1.2 g fish powder on day one. On the second day, children in group A were given 100 g beans stew, 20 g gari and 5 g of ACSMP fortified with 0.6 g fish powder. Children in group B received the same type and quantity of meal as group A on each of the two days. However, their stews contained 10 g of ACSMP on day one and 5 g of ACSMP on day two.

Eighty-five percent of the children in both groups failed to consume at least 50 % of their ration on day one. On the second day however, children in both groups consumed at least 70 % of the meals they were served with. Based on the trial results and to ensure compliance, the study decided to use 5 g of the vegetable powders and 0.6 g of fish powder in the study diets. There was no report of any illness or adverse reaction attributed to the trial diet or perceived by parents or caregivers.

3.6.4 Meals

Intervention diets were prepared by a caterer assigned by the Ghana School feeding programme under the supervision of the principal investigator and a trained research assistant. Three stews or soup of the same base were prepared each day concurrently; the regular stew or soup with no vegetable powder; the regular stew or soup with FACSMP and the regular stew or soup with ACSMP. The menu used each week is shown in table 3.1 below.

Table 3.1: Menu for the school feeding programme per week

Weekday	Menu
Monday	Beans stew with Gari
Tuesday	Tomato stew with Rice
Wednesday	Groundnut soup with Banku
Thursday	Tomato stew with Rice
Friday	Beans stew with Gari

3.6.4.1 Recipes for stews and soup

Below are the ingredients and recipes used in preparing the two stews (tomato and beans stews) and soup (groundnut soup) every week:

3.6.4.1.1 Tomato Stew preparation

Ingredients used:

Blended fresh tomato	600 g
Groundnut oil	400 g
Blended onion	50 g
Iodized salt	20 g
Powdered pepper	5 g

The groundnut oil was added to three different hot aluminum - cooking pots on fire labelled A, B and C, followed by blended onion, powdered pepper and blended fresh tomato paste in that order. The mixture in each pot was allowed to simmer with periodic stirring for about 30 minutes. Iodized salt was then added and allowed to simmer for about 5 minutes. When stews were cooked, FACSMP or ACSMP was added to each pot (labelled A or B), mixed thoroughly for about 60 seconds, taken off the stove and served. Tomato stew was prepared and consumed two times in a week and served with Rice on Tuesdays and Thursdays. Each participant received an average of 50 g of stew.

3.6.4.1.2 Bean Stew preparation

Ingredients used

Cowpea	3000 g
Blended fresh tomato	600 g
Groundnut oil	400 g
Blended onion	50 g
Iodized salt	20 g
Powdered pepper	5 g

Three sets of 3000 g of dry cowpea were weighed, rinsed with water and added to boiling water in each of three aluminum cooking pots (labelled A, B and C) and boiled to softness. The cooked cowpea was then added to prepared tomato stew. The mixture was allowed to simmer for about 20 minutes with regular stirring. Two of the cooked stews labelled A and B had either ACSM or FACSMP added, stirred thoroughly until evenly mixed and allowed to simmer for about 60 seconds and taken off the stove. Each participant in each of the three study groups was served an average of 100 g of beans stew which was served twice in a week on Mondays and Fridays (Table 3.1).

3.6.4.1.3 Groundnut Soup preparation

Ingredients used:

Groundnut paste	400 g
Blended fresh tomatoes	600 g
Blended onion	50 g
Iodized salt	20 g
Powdered pepper	5 g

To each of the three cooking pots, blended fresh tomato, blended onion, powdered pepper and water were added and allowed to cook for about 30 minutes. The groundnut paste was then reconstituted with water and poured into the boiling mixture and allowed to cook for about 45 minutes. Salt was then added and allowed to boil for 5 minutes. When the soup was cooked of either FACSMP or ACSMP was added to pots A and B respectively and mixed thoroughly for 60 seconds. Each participant in each of the three groups received an average of 100 g of soup which was served on Wednesdays.

With the guidance and supervision of the principal investigator, teachers and research field staff, children in each of the three groups received their meals at a separate location and in different coloured plates (red, blue and yellow) to discourage sharing of food among groups. Red, blue and yellow coloured badges were given to the interventions and control groups respectively for easy identification (Group A-red; group B-Blue, Group C-yellow). Participating children were fed five times every week during school lunch for four consecutive months.

3.7 Analyses of intervention and control diets

The nutritional composition of the intervention diets (school lunch plus fish fortified *Amarathus cruentus* and *Solanum macrocarpon* powder; school lunch plus *Amarathus cruentus* and *Solanum macrocarpon* powder, and school lunch) were analyzed. Moisture and protein content of the diets were determined using the oven and Kjeldahl methods respectively. It was necessary to determine the moisture content because moisture could influence the stability and the quality of the powders and the diets. High moisture content could lead to microbial growth and spoilage and shorten shelf life (Pomeranz and Melon, 1994). Iron and fat were estimated using the standard method of analysis (AOAC, 2010). It was important to know the fat content of the diets because fat facilitates the utilization of carotenoids and helps determine serum beta-carotene or retinol responses following ingestion of meals containing carotene and fat sources. Total carotenoids and beta-carotene were determined using a spectrophotometer and the High-Performance Liquid Chromatography respectively.

3.7.1 Determination of moisture

The moisture contents were examined using the method described by AOAC, (2010); method 930.15). Three clean dry moisture dishes were weighed, and the weights recorded. Two grams of each of the three diet samples were weighed in duplicates into the dry moisture dishes and placed in an air oven (Fisher Science Isotemp Oven, model 655F, Chicago, USA) at 105 ºC. The food samples and dishes were weighed at intervals until constant weights were obtained. Moisture was analysed to determine its level in the *Amaranthus cruentus* and *Solarnum macrocarpon* powder, fish fortified *Amaranthus cruentus* and *Solarnum macrocarpon* powder as well as in the intervention and control

diets. Giving that moisture affects the keeping quality and causes food spoilage through microbial growth.

3.7.2 Determination of Ash

The total ash content of the intervention diets and the powders (*Amaranthus cruentus* and *Solanum macrocarpon* powder; fish fortified *amaranthus cruentus* and *Solanum macrocarpon* powder) was analyzed using the electrical heat furnace (Fisher Isotemp Muffle Furnace, model 186A, USA). This was done to be able to determine the mineral content (iron, zinc) of the powders and the diets. Two grams of each diet sample was weighed into a previously weighed porcelain crucible. The samples were incinerated in the muffle furnace for 6 hours. The incinerated samples were cooled in a desiccator. The porcelain crucibles with the ashes were weighed.

3.7.3 Determination of protein

The protein content of the two powders (*Amaranthus cruentus* and *Solanum macrocarpon* powder; fish fortified *amaranthus cruentus* and *Solanum macrocarpon* powder), as well as each intervention diet sample was determined by Kjeldahl digestion method using AOAC standard procedure (AOAC, 2010). Using the nitrogen - protein conversion factor (6.25), the amount of protein in each diet sample was calculated.

3.7.4 Determination of fat

The fat content of the diets and the powders used in the current study were determined for the following reasons: to know the quantity of fat; and to ensure the presence of fat in the diet which is needed to facilitate the absorption of beta carotene by the digestive system given that beta-carotene is fat-soluble. The fat contents of the diet samples and the powders (*Amaranthus cruentus* and *Solanum macrocarpon* powder; fish fortified *amaranthus cruentus* and *Solanum macrocarpon* powder) were determined using the

soxhlet extraction method as indicated by the methodology of Pearson (Pearson, 1976). Three porous cellulose thimbles were used as containers for three samples. The thimbles were then set in an extraction chamber, which was suspended over a flask containing petroleum ether at 40 ºC – 60 ºC as a solvent and beneath a condenser. The cup was heated and the liquid vapourized and moved into the sample. The extraction chamber was structured in a way that when the liquid encompassing the sample exceeds a specific limit, it overflows and trickles back into the flask cup. Toward the finish of the extraction, a procedure, which takes about three hours, the flask containing the liquid and lipid, was removed. The liquid in the flask was vapourized and the fat was weighed.

3.7.5 Mineral content determination

Mineral ash obtained was used to determine iron and zinc content for each diet sample. The Atomic Absorption Spectrophotometry (Perkin Elmer Atomic Absorption Flame Emission Spectrophotometer Model 3110) at the Ecological laboratory, University of Ghana, Legon was used. Each of the three diet samples was digested by treating the samples with a ternary acid mixture made from concentrated nitric acid, sulphuric acid and per chloric acid.

Approximately (0.1 - 1.0 g) of each sample was weighed into 125 ml Erlenmeyer flask. About 10ml of the ternary mixture was added. The content was mixed and heated at a low to medium heat on a hot plate under perchloric acid fume. Heating continued for thirty minutes until white dense fumes from the sulphuric acid appeared. The mixture was allowed to cool, and 50 ml of distilled water added and boiled at medium heat. The mixture was again allowed to cool at room temperature and filtered with a wash bottle into 100 ml Pyrex volumetric flask. The solution was then stored for the heavy metal

determination using Perkin-Elmer Analyst 400. The iron content was read at wavelength 248.33 and zinc at 213.86. The minerals present were calculated as below:

$$\% \text{ Mineral (e.g. Iron)} = \frac{AAS\ reading}{1000} \times \frac{100}{1000} \times \frac{100}{\text{weight of samples}}$$

3.7.6 Determination of carotenoids

3.7.6.1 Extraction of total carotenoids

Using the Harvest Plus method, carotenoids were determined (Rodriguez-Amaya and Kimura, 2004). Carotenoids are photosensitive and so are easily degraded under UV light. Thus, the extraction process was carried out with minimum light in the laboratory. Duplicates of each of the three diet samples were weighed. Each was transferred into a mortar and a small amount of anhydrous sodium sulphate added as a desiccant. To allow for effective grinding, pyrogalol was added. The pyrogallol was added before extraction to prevent the oxidation of the active compounds. Extraction was done with 50 % cold acetone. Whatman number 4 filter paper was then used to filter the mixture and the residue re-extracted under the same conditions until the extraction solvent became colourless. A separating funnel was mounted with the support of a retort stand containing 20 ml petroleum ether. The filtrate was poured in the separating funnel followed by the addition of 300 ml of distilled water. This was separated into two layers; the aqueous layer and the organic layer. The aqueous layer beneath the organic layer was discarded by opening the separating funnel tap. Distilled water was then carefully introduced into the separating funnel with the aid of a wash bottle with the water trickling down the walls of the funnel. The organic layer was dried by passing it through anhydrous sodium sulphate into the newly labelled falcon tube. An equal volume of 20 % methanolic KOH was used to

saponify the carotenoid solution and kept overnight at room temperature (25 ºC) after partitioning in the petroleum ether.

3.7.6.2 Determination of total carotenoid content

The Harvestplus Method as done by Rodriguez-Amaya and Kimura (2004) was followed. The organic layer obtained from the petroleum ether extraction was used. Using Shimadzu UV- 120 - 02 spectrophotometer with a wavelength of 450 nm the absorbance was read in triplicate. The formula used to calculate the total carotenoid is shown below:

Total Carotenoid content (µg/g) = $\frac{Absorbance \times Total\ volume\ of\ extract\ (ml) \times 10^4}{Sample\ weigh\ t\ (g) \times Absorbancecoef ficient\ of\ \beta{-}carotene\ (2592)}$

Total carotenoid content (µg/100g) = Total carotenoid content (µg/g) ×100

3.7.7 Beta-carotene standards preparation

Standard beta-carotene was obtained from Caramore (UK). A stock solution of beta-carotene was prepared by taking 1 µg in 1 ml of methanol. Two times serial dilution was done to different known concentrations of 1, 0.5, 0.25, 0.125, and 0.0625 µ/ml. Each standard solution was injected into the HPLC system installed at the Nutrition department laboratory at Noguchi, using Shimadzu Prominent Reverse Phase HPLC consisting of a binary solvent system. (LC – 20 AB), a degasser (DGU – 20 A3), an auto sampler (SIL – 20 ACHT), a column temperature controller (CTO – 10 ASVT) and a photo diode array detector (SPD - M20A). HPLC condition used included a C18 reverse-phase column (diameter 5 µm, length × width, and 250 mm ×4.6 mm). To calibrate the HPLC, the mobile phase (a mixture of hexane and methanol in the ratio 1:9 respectively) was run at a flow rate of 0.8 ml per minutes. The wavelength was fixed at 475 nm. The column temperature was maintained at 40 ºC. With the injector in a load mode, each standard solution (20 µl) of beta carotene was injected. The standard beta carotene peak was

achieved at a retention time of 13.5 minutes. To obtain a straight line, the concentrations of the beta-carotene standards were plotted against the peak areas.

3.7.7.1 Sample assay

Each sample of beta-carotene extract was used for HPLC assay as standard. Twenty micro liters of each sample extract (20 µl) was measured using a micro liter pipette and injected into the HPLC system. The peak was identified by the detector and quantified comparing the retention time of the sample to the retention time of the standard.

Table 3.2: Nutrition composition of control and intervention diets as consumed/ 100 g

Stew/Soup	Group	Moisture	Ash	Protein	Iron	Fat	B-carotene
TS+FACSMP	A	67.8±0.2	33.6±2.0	5.6±0.2	7.9±1.0	10.3±0.3	411±15.1
TS+ACSMP	B	68.4±0.4	32.2±2.4	5.2±0.1	7.7±0.9	10.1±0.1	403±15.2
TS	C	70.7±0.2	30.1±1.5	4.6±0.5	3.0±0.2	9.9±0.5	264±4.6
BS+FACSMP	A	62.3±1.2	17.8±1.3	9.2±1.6	11.2±1.3	10.5±0.6	412±22.1
BS+ACSMP	B	62.3±2.0	17.2±1.2	8.9±1.4	10.5±1.3	9.0±0.1	400±16.8
BS	C	63.7±1.0	15.5±1.3	8.5±1.7	10.2±1.1	10.0±1.1	243±5.7
GS+FACSMP	A	71.4±1.4	26.2±1.4	6.7±0.8	5.8±0.4	16.0±0.8	396±19.1
GS+ACSMP	B	72.2±1.0	25,2±1.9	6.3±0.6	4.9±0.5	15.0±0.3	388±16.8
GS	C	77.5±1.2	21.3±2.7	5.7±0.2	3.8±0.1	15.8±0.2	267 ± 6.1

TS = Tomato stew; BS = Beans stew; GS = Ground nut soup. FACSMP = Fish fortified *Amaranthus cruentus Solanum macrocarpon* powder; ACSMP = *Amaranthus cruentus* and *Solanum macrocarpon* powder

3.8 Quality assurance

To assure the quality of the data collected, all Research assistants were oriented on the objectives of the study, their roles and the importance they should attach to the performance of their duties as Research assistants. They were also introduced to the content of the questionnaires particularly their approach to asking questions and to ensure

that correct data was collected. All completed questionnaires were reviewed to ensure consistency and reliability of all responses provided.

Data collected from participants was protected and kept safe under lock and key from unauthorized persons at the Nutrition Unit of the Noguchi Memorial Institute for Medical Research.

Qualified phlebotomist and parasitologist were employed to collect and analyze biochemical samples. All equipment used for the anthropometric measurements and biochemical determinations were calibrated regularly during the entire duration of the study using known weights and laboratory reference materials to authenticate the quality of results.

3.9 Data analyses

Data collected on each participant at the beginning and the end of the research was entered twice into the computer with Epi Info™ version 7 software. The data was then cleaned and exported to the Statistical Package for Social Sciences (SPSS version 22) for analyses. The Research to Improve Infant Nutrition and Growth (RIING) Project Nutrient Database available at the Department of Nutrition and Food Science, University of Ghana (RIING Project, unpublished) was used to convert the 24-hour dietary recall information to energy and nutrients. Age-specific cut-off points for haemoglobin (Hb) concentration were used to identify participants with anaemia (Hb concentrations less than 11.0 g/dl and less than 11.5 g/dl for children less than 5 years and children between 5 and 11 years respectively) (WHO, 2011). Serum retinol concentrations less than 20 µg/dl or less than 0.70 µmol/l) was considered as low serum retinol concentration (vitamin A deficiency) (WHO, 2011). Information on age and sex obtained from participants as well as measurements on height,

weight, were used to calculate Z-score nutritional indices. Participating children were categorized as underweight, stunted or wasted if their calculated weight-for-age, height-for-age, and body mass index-for-age z-scores, was less than or equal to negative two (-2) standard deviations. All measured variables were checked for normality. Summary data were presented as proportions or as means with plus or minus standard deviations. Outcome measures of continuous variables (haemoglobin, serum retinol, dietary nutrient intakes, Z - scores) were compared within-group using paired T-test or chi-square. Analysis of co-variance (ANCOVA) was employed to test for differences in the mean changes among the three groups to determine any significant differences. Binary logistic regression was used to determine factors associated with anaemia and low serum retinol concentration whilst adjusting for potential confounders. Significant differences were set at a level of $p < 0.05$.

3.10 Ethical clearance

Upon request, the Institutional Review Board (IRB) of Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon provided ethical approval (clearance number: IORG 0000908) for this research to be carried out (Appendix M). The District Director of the Ghana Education Service, the chief and elders of the community and the Head teacher of the selected school (Kodzobi District Assembly Primary School) gave consent for the research to be performed. Parents, caregivers and their children participating in the study gave their approval by signature or thumb print (after the details of the research had been explained to them) to be part of the study.

CHAPTER FOUR

4.0 RESULTS

This chapter show cases the results of the effects of fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder on anaemia and vitamin A status of the study participants. The study participants were in three groups: Intervention group A (participants fed with Diet A: school lunch plus fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder); Intervention group B (participants fed with Diet B: school lunch plus *Amaranthus cruentus* and *Solanum macrocarpon* powder) and group C, the control group (participants fed with Diet C: school lunch only).

4.1 Background characteristics of study participants

The main source of drinking water for most households (93.8 %) that participated in the study was tap water. Forty-two percent of households used Kumasi Ventilated Improved Pit (KVIP) as a place of convenience. The majority (84.0 %) of participants were Christians with traditional religious believers in the minority. Most participants (83.0 %) were Ewes with few (2.4 %) Hausas (Table 4.1).

The study participants were between 4 to 8 years old with the mean age of the participants in groups A, B and C being: 6.8 ± 2.6, 7.2 ± 2.9 and 6.6 ± 3.5 years respectively (Table 4.1). Majority of the participants in each group were males (Table 4.1). Most participants in each group were registered members of the Ghana National Health Insurance Scheme (Table 4.1).

Table 4.1: Background characteristics of participants per study group

Variable	Group A n = 54	Group B n = 54	Group C n = 54
Child age (mean ± SD)	6.8 ± 2.6	7.2 ± 2.9	6.6 ± 3.5
Sex n (%)			
Boys	31 (57.4)	28 (51.9)	30 (55.6)
Girls	23 (42.6)	26 (48.2)	24 (44.4)
Child NHIS status n (%)			
Registered	50 (92.6)	52 (96.3)	48 (90.7)
Not registered	4 (7.4)	2 (3.7)	6 (11.1)
Type of toilet facility n (%)			
KVIP	22 (40.7)	23 (42.6)	24 (44.4)
Water closet	8 (14.8)	12 (22.2)	9 (16.7)
Free range	8 (14.8)	9 (16.7)	11 (20.4)
Pit laterine	16 (29.6)	10 (18.5)	10 (18.5)
Religon n (%)			
Chriatian	43 (79.6)	45 (83.3)	49. (90.7)
Islam	8 (14.8)	8 (14.8)	2 (3.8)
Traditional	3 (5.6)	1 (1.9)	3 (5.6)
Dailect n (%)			
Ewe	46 (85.2)	48 (88.9)	41 (75.9)
Twi	7 (13.0)	3 (5.6)	5 (9.3)
Housa	1 (1.8)	1 (1.8)	2 (3.7)
other	0 (0.0)	2 (3.8)	6 (11.1)
Source of drinking water n (%)			
Tap	50 (92.6)	52 (96.3)	50 (92.6)
Bore hole	4 (7.4)	1 (1.8)	2 (3.7)
Well	0 (0.0)	1 (1.8)	2 (3.7)

Sample size = 162. n (%): number (percentage); mean ± SD-standard deviation

4.2 Household characteristics

Majority of both parents across the three groups were thirty years old or below and most of them had completed secondary level of education and were mostly farmers. Few

households earned above five hundred Ghana cedis as monthly income and majority of them were married. Household size ranged between 3 and 12 with most households having between 3 and 6 members. (Table 4.2).

Table 4.2: Household characteristics per study group

Variable	Group A n = 54 n (%)	Group B n = 54 n (%)	Group C n = 54 n (%)
Mother's age (years)			
≤ 20 - 30	48 (88.9)	45 (83.3)	48 (88.9)
31 - 40	1 (1.9)	6 (11.1)	1 (4.3)
> 40	5 (9.3)	3 (5.6)	5 (9.3)
Father's age (years)			
≤ 20 - 30	35 (64.8)	40 (74.1)	33 (82.5)
31 - 40	7 (12.9)	10 (18.5)	6 (11.1)
> 40	12 (22.2)	4 (7.4)	15 (27.8)
Mother's education			
Basic	15 (27.8)	14 (25.9)	14 (25.9)
Secondary	39 (72.2)	40 (74.1)	40 (74.1)
Father's education			
Basic	7 (13.0)	5 (9.3)	0 (0.0)
Secondary	47 (87.0)	49 (90.7)	54 (100)
Mother's occupation			
Farmer	35 (64.8)	34 (63.0)	40 (74.1)
Other	19 (35.2)	20 (37.1)	14 (25.9)
Father's occupation			
Farmer	38 (70.4)	38 (70.4)	36 (66.7)
Other	16 (29.6)	16 (29.6)	18 (33.3)
Mother's marital status			
Married	52 (96.3)	50 (92.6)	45 (82.3)
Not married	2 (3.7)	4 (7.4)	9 (16.7)
Father's marital status			
Married	50 (92.6)	48 (88.9)	48 (88.9)
Unmarried	4 (7.4)	6 (11.1)	6 (11.1)
Household monthly income (GHC)			
≤ 100 – 299	18 (33.3)	23 (42.6)	12 (22.2)
300 – 499	32 (59.3)	29 (53.7)	37 (68.5)
≥ 500	4 (7.4)	2 (3.7)	5 (9.3)
Household size			
3 – 6	34 (63.0)	31 (57.0)	32 (59.3)
7 – 12	20 (37.0)	23 (42.6)	22 (40.7)

n = number of children; n (%) = number (percentage); GHC = Ghana Cedis

4.3 Baseline characteristics of study participants

The mean haemoglobin concentration at baseline ranged from a low of 11.4 ± 1.0 to a high of 12.00 ± 1.1 g/dl (Table 4.3). Group B recorded the least mean haemoglobin concentration (11.4 ± 1.0 g/dl). The mean serum retinol concentration ranged from 22.3 ± 6.8 to 23.1 ± 7.3 g/dl with intervention group A recording the highest mean serum retinol concentration (Table 4.3). Prevalence of anaemia was highest among study participants in intervention group B (51.9 %) and the control group (group C) recorded the highest (35.2 %) vitamin A deficiency prevalence (serum retinol concentration < 20 µg/dl) at start of the study (Table 4.3).

Mean intake of protein ranged from a low of 15.6 ± 3.4 g among intervention group B to a high of 16.8 ± 2.8 g among children in intervention group A at baseline. Among the three groups, the control group C recorded the highest fat intake (21.6 ± 4.1 g) with intervention group B recording the least iron intake (4.3 ± 1.3 mg). The mean intake of beta-carotene ranged from 203.4 ± 33.3 µg to 214.6 ± 26.8 µg, with group C recording the highest mean vitamin C intake (25.7 ± 4.8 mg) at baseline (Table 4.3).

Malaria prevalence was more among participants in intervention group A than participants in intervention group B. Hookworm infestation was present in only one participant in study group B, none of the participants in the other two groups, (group A and C) was infested with hookworm (Table 4.3).

The mean weight of participants ranged from 21.5 ± 3.7 g to 23.0 ± 2.9 g with intervention group A recording the highest mean weight and mean height. Mean z scores of anthropometric indicators of malnutrition are also presented in Table 4.3. Participants in the control group (group C) had the highest prevalence of stunting followed by children in

intervention groups B and then A. Prevalence of wasting ranged from 3.7 % for participants in intervention group A to 5.5 % among participants in intervention group B (Table 4.3).

Table 4.3: Baseline characteristics of study participants

Variable	Group A n = 54	Group B n = 54	Group C n = 54	Pvalue
Mean haemoglobin concentration (m±sd)	12.0 ± 1.1	11.4 ± 1.0	11.6 ± 1.0	0.176
Mean serum retinol concentration (m±sd)	23.1 ± 7.3	22.3 ± 5.6	22.8 ± 6.8	0.889
Anaemia n (%)	25 (46.3)	28 (51.9)	23 (42.6)	0.056
Low serum retinol level n (%)	17 (31.5)	16 (29.6)	19 (35.2)	0.982
Protein intake (m±sd)	16.8 ± 2.8	15.6 ± 3.4	16.4 ± 3.1	0.626
Fat intake (m±sd)	19.5 ± 3.6	20.4 ± 3.4	21.6 ± 4.1	0.323
Iron intake (m±sd)	5.3 ± 1.9	4.3 ± 1.3	4.8 ± 1.6	0.137
β-carotene intake (m±sd)	203.4 ± 33.3	206.3 ± 19.8	212.6 ± 26.8	0.081
Vitamin C intake (m±sd)	24.0 ±7.4	26.3 ± 5.4	25.7 ± 4.8	0.129
Malaria parasitaemia n (%)	25 (46.3)	14 (25.9)	21 (38.9)	0.257
Hookworm infestation n (%)	0 (0.0)	1 (1.9)	0 (0.0)	0.128
Weight (m±sd)	22.9 ± 2.9	21.5 ± 3.7	21.7 ± 3.6	0.114
Height (m±sd)	121.2 ± 2.9	117.8 ± 8.4	118.9 ± 9.0	0.051
Weight-for-age z score	-0.27 ± 0.9	-0.55 ± 1.0	-0.76 ± 9.6	0.134
Height-for-age z score	-0.24 ± 1.2	-0.65 ± 1.1	-0.83 ± 1.2	0.116
Body-mass-index score	-0.14 ± 0.8	-0.25 ± 1.1	-0.31 ± 1.2	0.752
Underweight n (%)	4 (7.4)	5 (9.2)	5 (9.2)	0.089
Stunting n (%)	3 (5.5)	3 (5.5)	4 (7.4)	0.923
Wasting n (%).	2 (3.7)	3 (5.5)	2 (3.7)	0.686

n = number of children. n (%) = number and percentage. (m±sd) = Mean ± standard deviation; Unit of measure: - haemoglobin concentration = g/dl; serum retinol concentration = µg/dl; Protein and Fat intake = g/100g; iron and vitamin C intake = mg/100g; β-carotene intake = µg/100g; Weight = kg; Height = cm.

4.4. Prevalence of anaemia, vitamin A deficiency and malaria at baseline and end line

The prevalence of anaemia (haemoglobin concentration < 11.0 g/dl or 11.5 g/dl depending on child's age) reduced from baseline to end line among all the three study groups (Table 4.4). Intervention group A recorded the highest reduction (11.8 %) followed by group B (6.7 %) and then the control group C (1.4 %) respectively. The changes in the prevalence of anaemia within groups A and B from baseline to end line were significant at p = 0.036 and p = 0.042 respectively. The highest prevalence of anaemia at both baseline (51.9 %) and end line (45.2 %) was recorded among participants in intervention group B and the least at the end line among children in intervention group A (34.5 %). A significant difference in the prevalence of anaemia was recorded across groups at end line at p = 0.036.

The prevalence of vitamin A deficiency (< 20 µg/dl) reduced from 31.5 % at baseline to 9.6 % at end line among participants in intervention group A; from 29.6 % to 20.0 % among participants in intervention group B and from 35.2 % to 29.4 % among participants in the control group (Table 4.4). The highest change in prevalence of vitamin A deficiency (21.9 %) was recorded by intervention group A and the least (5.7 %) by intervention group C. Groups A and B recorded significant differences in the prevalence of vitamin A deficiency from baseline to end line at p = 0.001 and p = 0.045 respectively. Across the groups, no significant difference was observed in the prevalence of vitamin A deficiency at the end line.

All three groups recorded a decline in malaria prevalence from baseline to end line with intervention group A (32.8 %) recording the highest decrease followed by the control group C (29.1 %) and then group B (15.9 %) respectively (Table 4.4). However, the

decreases in prevalence from baseline to end line among children within all the three groups were not significant.

Table 4.4: Prevalence of anaemia, vitamin A deficiency and malaria parasitaemia at baseline and end line

Variable	Group A	Group B	Group C	P-value*
	n (%)	n (%)	n (%)	
Anaemia				
Baseline	25 (46.3)	28 (51.9)	23 (42.6)	0.056
End line	20 (34.5)	24 (45.2)	21 (41.2)	0.036
Difference	-5 (11.8)	-4 (6.7)	-2 (1.4)	
P-value**	0.036	0.042	0.108	
Low serum retinol				
Baseline	17 (31.5)	16 (29.6)	19 (35.2)	0.089
End line	5 (9.6)	10 (20.0)	15 (29.4)	0.051
Difference	-12 (21.9)	-6 (9.6)	-4 (5.7)	
P-value**	0.001	0.045	0.083	
Malaria parasitaemia				
Baseline	25 (46.3)	14 (25.9)	21 (38.9)	0.257
End line	7 (13.5)	5 (10.0)	5 (9.8)	0.925
Difference	-18 (32.8)	-9 (15.9)	-16 (29.1)	
P-value**	0.064	0.379	0.067	
Hookworm				
Baseline	0 (0.0)	1 (1.9)	0 (0.0)	0.128
End line	0 (0.0)	0 (0.0)	0 (0.0)	0.325
Difference	0 (0.0)	1 (1.9)	0 (0.0)	

Cut off values: Haemoglobin = 11.0 g/dl for children 0 -59 months and 11.5 g/dl for children 5 to 11 years old. Vitamin A deficiency = < 20 µg/dl for children 4 to 8 years old. n (%) = number (percentage). Number of participants in each group at base line = 54; at end line – group A = 52, group B = 50, group C = 51. P*-value = between groups. P-value ** = within group.VAD = vitamin A deficiency.

4.5. Comparison of mean values of measured outcomes at end line

There was no significant difference across the three groups in their weight-for-age z scores and BMI-z scores at the end of study (Table 4.5). There were however differences in mean haemoglobin concentration (p = 0.024), mean serum retinol concentration (p = 0.044), mean weight (p = 0.001) and mean height (p = 0.002). In the pairwise comparisons, children in intervention group A had significantly greater mean weight (+ 3.9 g; p = 0.006)

and mean height (+ 8.7 cm; p = 0.001) than children in group B. Participating children in intervention group A also recorded significantly higher mean haemoglobin concentration (+ 0.8 g/dl; p = 0.030), mean serum retinol concentration (+ 4.5 µg/dl; p = 0.021) and mean weight and height (+ 3.2 g; p = 0.011) and (+ 7.6 cm; p = 0.000) respectively compared to their counterparts in group C. Difference in height-for-age z score (p = 0.037) was significant between group B and C (Table 4.5).

Table 4.5: Comparison of mean values of measured outcomes at the end line

				Comparison of groups					
	Group A	Group B	Group C	A and B		A and C		B and C	
	n = 52	n = 50	n = 51						
Variable	(mean SD)	(mean ± SD)	(mean ± SD)	P	D (95 % CI)	P	D (95 % CI)	P	D (95 % CI)	P
HC	12.4±1.3	11.8±1.9	11.6±1.3	0.024	0.6 (-0.2, 1.0)	0.209	0.8 (-0.6, 1.3)	0.030	0.2 (-0.3, 0.9)	0.375
SRC	28.3±8.8	24.7±6.5	23.8±6.5	0.044	3.6 (-1.1, 5.9)	0.178	4.5 (1.6, 7.4)	0.021	0.9 (-1.8, 5.1)	0.346
Weight	24.5±3.8	20.6±4.7	21.3±4.4	0.001	3.9 (1.4, 2.4)	0.006	3.2 (1.3, 2.3)	0.011	0.7 (-1.1, 0.9)	0.788
Height	126.6±9.1	117.9±10.2	118.8±11.1	0.002	8.7 (1.2, 4.5)	0.001	7.6 (2.1, 5.3)	0.000	0.9 (-0.7, 2.5)	0.278
WAZ	-0.4±0.9	-0.6±1.0	-0.9±1.0	0.104	0.2 (-0.1, 0.3)	0.516	0.5 (-0.1, 0.3)	0.512	0.3 (0.07, 0.4)	0.190
HAZ	-0.2±1.1	-0.5±1.14	0.9±1.1	0.053	0.3 (-0.1, 0.4)	0.385	0.7 (-0.1, 0.4)	0.218	0.4 (0.02,0.6)	0.057
BMIZ	-0.3±0.8	-0.5±0.9	-0.5±1.0	0.714	0.0 (-0.2, 0.3)	0.636	0.2 (-0.2, 0.2)	0.854	0.1 (-0.2,0.2)	0.763

Group 1 received school lunch plus fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder. Group B received school lunch plus *Amaranthus cruentus* and *Solanum macrocarpon* powder. Group C received a school launch. HC (g/dl) = haemoglobin concentration. SRC (µg/dl) = serum retinol concentration. Weight (g). Height (cm). WAZ = weight-for-age *z* score; HAZ = height-for-age *z* score; BMIZ = BMI *z* score.
P = p value.
D = mean difference.
CI = Confidence interval.

4.6 Weekly consumption of the food groups by study participants per week at the baseline

Majority of the study participants consumed cereals, legumes, roots and tubers regularly (Table 4.6). Fish was the main animal-based protein source consumed but not meat and poultry. Consumption of fruits, fat and oil and dairy products was absent for most of the participating children and this cut across all the three groups. Dark green leafy vegetable consumption was also mostly limited to once or twice in a week (Table 4.6).

Table 4.6: Weekly consumption of the food groups by study participants at the baseline

Group	**Study Group A**								
	Group A n = 54			**Group B n = 54**			**Group C n = 54**		
Consumption frequency	**None n (%)**	**1 – 2 n (%)**	**≥ 3 n (%)**	**None n (%)**	**1 – 2 n (%)**	**≥ 3 n (%)**	**None n (%)**	**1 – 2 n (%)**	**≥ 3 n (%)**
MPF*	0 (0.0)	3 (5.6)	51 (94.0)	0 (0.0)	0 (0.0)	54 (100.0)	0 (0.0)	1 (2.0)	53 (98.0)
Fat and oil	29 (54)	23 (42.6)	2 (4.0)	31 (57)	19 (35.2)	4 (7.0)	36 (67.0)	18 (33.3)	0 (0.0)
Roots & tubers	8 (14.8)	23 (42.6)	23 (42.6)	0 (0.0)	35 (64.8)	19 (35.2)	5 (9.0)	35 (64.8)	14 (26.0)
Cereals	0 (0.0)	0 (0.0)	54 (100.0)	0 (0.0)	0 (0.0)	54 (100.0)	0 (0.0)	6 (0.0)	48 (89.0)
Legumes	12 (22.0)	29 (53.7)	13 (24.0)	9 (17.0)	33 (61.1)	12 (39.0)	13 (14.0)	31 (57.0)	10 (19.0)
Fruits	40 (74.0)	14 (25.9)	0 (0.0)	36 (67.0)	16 (29.6)	2 (4.0)	42 (77.8)	11 (20.4)	1 (1.9)
DGLV	10 (18.5)	36 (66.7)	8 (14.8)	3 (5.6)	29 (53.7)	22 (40.7)	16 (29.6)	30 (55.6)	8 (18.5)
Other Vegetables	0 (0.0)	19 (35.2)	35 (64.8)	0 (0.0)	7 (13.0)	47 (87.0)	0 (0.0)	4 (7.4)	50 (92.6)
Dairy products	54 (100.0)	0 (0.0)	0 (0.0)	53 (98.1)	1 (1.9)	0 (0.0)	54 (100.0)	0 (0.0)	0 (0.0)

*Consumption of fish. DGLV = Dark green leafy vegetables. n = number of participants; n (%) = number and percentage

4.7 Nutrient intake per study group and pairwise comparisons at endline

In the total sample, the mean changes in dietary intake of iron, protein and β-carotene across the three groups at end line were significant at $p = 0.032$, $p = 0.008$ and $p = 0.001$ respectively. Comparing intake of paired groups, significant differences in mean intakes were also recorded between groups A and C for iron intake; A and B for protein intake and between groups A and C; and B and C respectively in terms of beta-carotene intake (Table 4.7).

Table 4.7: Mean nutrient intake per study group and pairwise comparisons at the endline

				Comparison of groups					
	Group A	Group B	Group C	A and B		A and C		B and C	
Nutrient	**n = 52 (mean ± SD)**	**n = 50 (mean ± SD)**	**n = 51 (mean ± SD)**	**P**	**D (95% CI)**	**P**	**D (95% CI**	**P**	**D (95% CI)**	**P**
Iron	7.5±2.3	6.03±2.2	6.1±1.6	0.032	1.5 (-0.1, 1.7)	0.065	1.4 (0.3, 0.9)	0.011	-0.1 (-0.5, 1.1)	0.504
Protein	18.6±2.9	15.7±3.5	16.2±3.3	0.008	2.9 (1.4, 2.2)	0.005	2.4 (0.3, 2.0)	0.081	-0.5 (-1.0, 1.0)	0.700
Fat	42.6±17.1	43.8±16.6	42.7±12.6	0.056	-1.2 (0.2, 0.2)	0.054	-0.1 (-0.2, 0.2)	0.600	1.1 (-0.4, 0.4)	0.405
Vitamin C	25.9 ± 6.6	26.5 ± 5.9	23.6 ± 6.9	0.068	-0.6 (-3.7, 2.7)	0.912	2.3 (-874, 5.5)	0.203	2.9 (-0.358, 6.1)	0.093
β - C	714.9±66.8	711.8±16.6	516.7±33.9	0.001	3.1(-95.8,102.2)	0.997	198(99.7, 296.8)	0.001	195(95.6, 294.6)	0.001

Group 1 received school lunch plus fish fortified *Amaranthus cruentu* and *Solanum macrocarpon* powder. Group B received school lunch plus *Amaranthus cruentu* and *Solanum macrocarpon* powder. Group C received a school launch. n = number of participants.
P = P values
D= mean difference.
CI = confidence interval
Unit of measure: Protein (g); Fat (g); Iron (mg); β-C = beta-carotene (µg).

4.8. Nutrient adequacy ratio distribution by study groups at baseline and end line

The Nutrient Adequacy Ratio (NAR) distribution of study groups at baseline and end line for protein, iron, beta-carotene and vitamin C intakes are presented in Table 4.8. At baseline, 74 %, 54 % and 35 % of participants in intervention groups A, B and control group C met NAR > 1 for iron. By the end line, the percentage with NAR > 1 for iron increased to 100 %, 80 % and 59 % respectively. Percentage of children in all three groups that met NAR > 1 for vitamin A intake, increased from baseline to end line (Table 4.8). Most of the children, however, did not meet NAR > 1 for vitamin C at both baseline and end line.

Table 4.8: Nutrient adequacy ratio distribution by study groups at baseline and end line

	Study groups						
	Group A		Group B		Group C		
Nutrient	NI	NAR ≥ 1	NI	NAR ≥ 1	NI	NAR ≥ 1	
Protein	Mean ± SD	n (%)	Mean ± SD	n (%)	Mean ± SD	n (%)	P-value
Baseline	16.8 ± 2.8	42 (77.8)	15.6 ± 3.4	41 (75.9)	16.4 ± 3.1	44 (81.5)	0.626
End line	18.6 ± 2.9	48 (92.3)	15.7 ± 3.5	47 (94.0)	16.2 ± 3.3	50 (98.0)	0.008
Difference	1.8 ± 0.1	6 (11.5)	0.1 ± 0.1	6 (12.0)	0.2 ± 0.2	6 (11.8)	
Iron							
Baseline	5.3 ± 1.9	40 (74.1)	4.3 ±1.3	29 (53.7)	4.8 ± 1.6	19 (35.2)	0.137
End line	7.5 ± 2.3	52 (100.0)	6.0 ± 2.2	40 (80.0)	6.1 ± 1.6	30 (58.8)	0.032
Difference	2.2 ± 0.4	12 (25.9)	1.7 ± 0.9	11 (36.3)	1.3 ± 0.0	11 (23.6)	
β-carotene							
Baseline	203.4 ± 33.3	20 (37.0)	206.3 ± 20	24 (44.4)	212.6 ± 27	22 (40.7)	0.081
End line	714.8 ± 66.8	50 (96.0)	711.8 ± 54	49 (98.8)	516.7 ± 34	21 (41.2)	0.001
Difference	511.4 ± 33.5	30 (59.3)	505.5 ± 34	24 (54.4)	304.1 ± 7	-1 (0.05)	
Vitamin C							
Baseline	24.0 ± 7.4	8 (14.8)	26.3 ± 5.4	12 (22.2)	25.7 ± 4.8	11 (20.4)	0.129
End line	25.9 ± 6.6	8 (15.4)	26.5 ± 5.9	10 (20.0)	23.6 ± 6.9	12 (23.5)	0.068
Difference	1.9 ± 0.8	0 (0.6)	0.2 ± 0.1	2 (02.2)	2.1 ± 2.1	1 (3.1)	

NI = Nutrient intake
n (%) = number (percentage)
Unit of measure: Protein (g); Fat (g); Iron (mg); β-C = beta-carotene (µg).
NAR (nutrient adequacy ratio) was calculated as the ratio of an individual's intake to the EAR for a nutrient
EAR (Estimated Average Requirement) is the intake that meets the estimated needs of half the individuals in a group.
EAR for 4 to 8 – year - olds: Protein 0.76 g//kg/body weight/day; Iron 4.1 mg/day; vitamin A 275 µg/day; vitamin C 22 mg/day. Number of participants at base line per group: A = 54, B = 54, C = 54. Number of participants at end line per group: A = 52; B = 50; C = 51.

4.9 Prevalence of stunting underweight and wasting at base and end line

All three groups recorded slight increases in the prevalence of stunting from beginning to the end of the study period; however, the increases were not statistically significant. (Table 4.9). The highest prevalence of stunting was reported among the control group C at base line and end line, the least was reported at baseline among participant in

intervention group A (Table 4.9). The prevalence of underweight increased slightly from baseline to end line within study groups A and B (from 7.4 % to 7.6 % group A and 9.2 % to 12.0 % group B), however, it decreased among participants within study group C (9.2 % to 7.8 %) (Table 4.9). The differences were however not statistically significant.

Table 4. 9: Prevalence of stunting, underweight and wasting per study groups at baseline and end line

Nutritional status	Group A	Group B	Group C	
	n (%)	n (%)	n (%)	P-value
Stunting (HAZ < - 2SD)				
Baseline	3 (5.5)	3 (5.5)	4 (7.4)	0.058
End line	3 (5.7)	3 (6.0)	4 (7.8)	0.051
Difference	0 (0.2)	0 (1.5)	0 (0.4)	
Underweight (WAZ < - 2 SD)				
Baseline	4 (7.4)	5 (9.2)	5 (9.2)	0.121
End line	4 (7.6)	6 (12.0)	4 (7.8)	0.114
Difference	0 (0.2)	1 (2.8)	1 (1.2)	
Wasting (BAZ < - 2 SD)				
Baseline	2 (3.7)	3 (5.5)	2 (3.7)	0.784
End line	2 (3.8)	3 (6.0)	2 (3.9)	0.798
Difference	0 (0.1)	0 (1.5)	0 (0.2)	

n (%) = number (percentage). WAZ is weight –for-age Z score; HAZ is height-for-age Z score; BMIZ is body-mass index-for-age Z score. Number of participants in each group at baseline = 54; End line: group A = 52, group B = 50, group C = 51.

4.10 Biochemical characteristics of study participants with anaemia at baseline and endline

Table 4.10 depicts the biochemical indices of participants who were mildly anaemic at the pre-intervention stage of the study and how they fared at post-intervention. All three study groups had some of their participants recording haemoglobin concentrations below the threshold of 11.0 g/dl or 11.5 g/dl depending on age group (group A: 10.5 ± 0.9 g/dl; group B: 10.5 ± 0.7 g/dl; group C: 10.7 ± 0.7 g/dl) at base line. At the end of the intervention period, the mean haemoglobin concentration for participants in the

intervention group A increased to 12.1 ± 0.6 g/dl. The other groups (groups B and C), still recorded mean haemoglobin concentrations below the threshold of 11.5 g/dl at the end of the study. Out of the 25, 28 and 23 study participants who had mild to moderate anaemia at pre-intervention in groups A, B and C, 13, 13 and 14 remained anaemic at post-intervention giving a prevalence of 52.0 %, 46.6 % and 60.7 % respectively (Table 4.10). Out of the three groups, intervention group A recorded the most change in serum retinol concentration at the end of the intervention period, a change that was statistically significant at p = 024 (Table 4.10). The number of participants who had malaria at the beginning of the study declined among all the three groups by the time the study ended.

Table 4.10: Biochemical characteristics of study participants with anaemia at baseline and endline

Variable	Group A n= 25	Group B n = 28	Group C n = 23	P-value*
Mean haemoglobin concentration g/dl (mean ± SD)				
Baseline	10.5 ± 0.9	10.5 ± 0.7	10.7 ± 0.7	0.055
End line	12.2 ± 0.6	11.4 ± 0.9	11.0 ± 1.3	0.698
Difference	1.7 ± 0.2	0.9 ± 0.2	0.3 ± 0.6	
p-value**	0.042	0.147	0.856	
Prevalence of anaemia (Haemoglobin concentration <11.5 g/dl) n (%)				
Baseline	25 (100.0)	28 (100.0)	23 (100.0)	0.129
End line	13 (52.0)	13 (46.6)	14 (60.7)	0.722
Difference	-12 (48.0)	-15 (53.4)	-9 (39.3)	
p-value**	0.035	0.042	0.052	
Mean serum retinol concentration µg/dl (mean ± SD)				
Baseline	24.1 ± 8.8	21.0 ± 5.8	23.0 ± 5.1	0.486
End line	29.8 ± 11.8	23.8 ± 7.7	23.2 ± 7.3	0.184
Difference	5.7 ± 3.0	2.8 ± 1.9	0.2 ± 2.2	
p-value**	0.024	0.087	0.247	
Prevalence of VAD (serum retinol concentration < 20 µg/dl) n (%)				
Baseline	7 (100.0)	10 (100.0)	7 (100.0)	0.806
End line	3 (42.9)	9 (90.0)	9 (128.6)	0.722
Difference	-4 (57.1)	-1 (10.0)	2 (28.6)	
p-value**	0.093	0.136	0.101	
Prevalence of malaria n (%)				
Baseline	9 (100.0)	9 (100.0)	12 (100.0)	0.423
End line	3 (33.3)	2 (22.2)	3 (25.0)	0.231
Difference	-6 (66.7)	-7 (77.8)	-9 (75.0)	
p-value**	0.062	0.059	0.52	

n = number of participants.
P* = p value among 3 groups
P** = p value within group difference
VAD = vitamin A deficiency

4.11 Factors associated with anaemia status among study participants

Factors with the tendency to predict anaemia in the current study are displayed in Table 4.11. Serum retinol level was the factor significantly associated with anaemia. The study group, maternal marital status, father's occupation and malaria status were not significantly associated with anaemia (Table 4.11). Participating children with normal

serum retinol status had 85 % (OR = 0.145; 95 % CI: 0.038 – 0.554; p = 0.005) less risk of being anaemic compared to those with low serum retinol concentration status.

Table 4.11: Factors associated with anaemia status among study participants

Factor	Odds ratio	95 % CI	P-value
Study group			
Group A	1	Reference	
Group B	1.572	0.213 – 5.385	0.351
Group C	2.377	0.311 – 8.266	0.182
Sex			
Female	1.272	1.102 – 6.580	0.624
Male	1	Reference	
Serum retinol concentration			
Normal serum retinol level	0.145	0.038 – 0.554	0.005*
Low serum retinol level	1	Reference	
Mother's marital status			
Divorced/unmarried	1.426	0.149 – 13.667	0.759
Married	1	Reference	
Father's occupation			
Farmer	1.888	0.704 – 5.064	0.207
Other	1	Reference	
Mother's educational level			
Basic	0.796	0.240 – 2.640	0.710
Secondary	1	Reference	
Father's educational level			
Basic	1.365	0.206 – 9.049	0.747
Malaria status			
Malaria absent	0.889	0.204 – 3.878	0.875
Malaria present	1	Reference	
Age	1.299	0.763 – 1.554	0.640

Child age was adjusted for OR (Odds ratio). * indicates values significant at p<0.05. (r) Reference category; (CI) Confidence Interval. Cox & Snell R^2 = 0.186. Negelkerke R^2 = 0.253.

4.12 Factors associated with low serum retinol level among the participants

Table 4.12 shows factors that tended to be associated with vitamin A deficiency (VAD) or low serum retinol level. While the absence of anaemia and maternal marital standing were significantly associated with VAD, father's monthly income, and the study group a child belonged to were not significantly associated with VAD (Table 4.12).

Children whose mothers were married had about 94% less risk of becoming vitamin A deficient (OR = 0.056; 95 % CI 0.004 – 0.024; p = 0.036) than those of single mothers. Furthermore, participating children who had no anaemia had about 84 % (OR = 0.164; 95 % CI 0.045 – 0.598; p = 0.006) reduced risk of becoming vitamin A deficient compared to those with anaemia (Table 4.12).

Table 4.12: Factors associated with serum retinol level among study participants

Factor	Odds ratio	95 % CI	P-value
Group			
Group A	1	Reference	
Group B	1.294	0.311 – 5.385	0.723
Group C	1.604	0.311 – 8.266	0.572
Anaemia status			
Anaemia absent	0.164	0.045 – 0.598	0.006*
Anaemia present (r)	1	Reference	
Mother's educational level			
Basic	1.34	0.275 – 6.538	0.716
Secondary	1	Reference	
Father's educational level			
Basic	0.977	0.081 – 11.856	0.986
Secondary	1	Reference	
Mother's marital status			
Married	0.056	0.004 – 0.024	0.036*
Unmarried (r)	1	Reference	
Father's monthly income			
< GH₵500.00	1.019	0.152 – 6.810	0.985
≥ GH₵500.00 (r)	1	Reference	
Age	1.089	0.704 – 1.686	0.702

Child age was adjusted for. OR Odds ratio. * indicates values significant at $p<0.05$. (r) Reference category; (CI) Confidence Interval. GH₵ = Ghana Cedi. Cox & Shell $R^2 = 0.200$. Nagelkerke $R^2 = 0.320$.

CHAPTER FIVE

5.0 DISCUSSIONS

This dietary intervention study was done to establish the effect that fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder (FACSMP) had on the anaemia and vitamin A status of school pupils aged four to eight years old living at Kodzobi, a community in the Adaklu District of the Volta Region, Ghana. It was necessary to carry out this study to investigate how feasible it was to use fish fortified vegetables powder as supplements, providing solution to anaemia and vitamin A deficiency among children in low-income communities. This segment of the thesis discusses the study findings of household characteristics, both haemoglobin, and serum retinol levels, anaemia and vitamin A deficiency, prevalence of malaria and soil helminth infestation, nutrients intake and anthropometric nutritional status.

5.1 Background characteristics

Data from the current study showed that most of the parents across the three groups attained at least a basic level of formal education with most parents having junior secondary level education (Table 4.2). Their level of educational attainment could influence the nutritional status and health of their children. The educational level could also help parents understand the purpose of the study, making it easier for them to allow their children to participate. This is because having the ability to read could aid literate parents to make informed nutrition and health choices for their families (Carbone and Zoellner, 2012). Learned parents and care givers are also able to challenge beliefs and taboos that prevent their children from consuming nutritious foods (McNamara and Wood, 2019) such as snails, lobsters and even eggs that positively contribute to their nutrition and that of their children.

The current study found no association between parental educational achievement and the status of anaemia and vitamin A of study participants. However, previous research demonstrated the presence of a positive relationship between mother's education and malnutrition. In Ethiopia, Getaneh and co (2017) showed that low maternal educational level was associated with anaemia status of school children. A similar observation was made in the North-western part of Ethiopia, where mother's literacy was associated with the level of anaemia in five to fifteen-year-old pupils (Birhanu *et al*., 2018). Maternal level of education associating with anaemia in the Ethiopian studies was because most of the mothers who participated in the study had little or no education. In the current study, however, the majority of the mothers had had at least junior secondary level of education. With very low literacy rate among women in Ethiopia (28.9 %) compared to women in Ghana (71.4 %), it was not surprising that most mothers in the Ethiopaan studies could not read and write (World Bank, 2017). This may have prevented them from making the right nutritional choices for their children thus affecting their anaemia status. There is one study that indicated that children of educated mothers suffer from little malnutrition, in the form of underweight, wasting, stunting and micronutrients deficiencies (Abuya *et al.,* 2012). It was reported that a mother's high educational achievement affects the health and nutrition of both the mother and her children in a positive manner (Mondal *et al.,* 2014). Ngesa and Mwambi (2014) showed by their research that a mother's educational attainment protected her children from anaemia. They established that mothers with secondary level education or more had their children at a lower risk of developing anaemia compared to those at the other extreme. This could be because education equips mothers with enough knowledge for providing sound health and nutrition to children, as it gives them also the opportunity to explore the labour market to deliver quality economic and social services to their families (Ngnie-Teta *et al.,* 2007; Legason *et al.,* 2017).

Despite their educational background, most households in the current study earned less than 500 Ghana Cedis as monthly income. An amount that was below the prevailing basic wage for workers in Ghana. This study was not able to show that any association existed between household monthly income at the end of the month and anaemia and between monthly income and low vitamin A levels. Nevertheless, some findings that suggest the existence of an association between household income and anaemia; and household income and vitamin A deficiency (Legason *et al*., 2017). Legason and co reported a declined scenario of the risk of developing anaemia in Ugandan children, with increased household income (Legason *et al.*, 2017). Their result was also in agreement with another study that demonstrated that children from the richest families, compared with children from the poorest homes had one and half times higher odds of childhood anaemia (Habte *et al.*, 2013). In a systematic review, it was shown that school-aged children with parents from the lower socio-economic strata were quite vulnerable to anaemia than those who had parents in the medium socioeconomic status category (Iglesias *et al.*, 2019). Probably the socioeconomic situation of a household could be the index of measure of getting enough food supply, adequate health delivery, deworming programmes, adequate hygiene and sanitation conditions, and availability of quality water resource. These are primary predictors of childhood malnutrition, which include anaemia and vitamin A deficiency.

Majority of mothers and fathers were between 20 and 30 years old (Table 4.2). This gave the indication that; most parents belonged to the highly energetic age group, capable of working and providing socially, economically and nutritionally for their families. There was no association between the age of mother or father and anaemia or vitamin A status of participants in the current study. On the contrary, in other related studies maternal age was demonstrated to have a negative association with childhood anaemia. In studying the risk

factors of mothers associated with childhood anaemia in Ethiopians, a negative association was reported between maternal age and childhood anaemia (Habte *et al*., 2013). First-time mothers who were twenty-seven years and below, had a greater risk of delivering children with moderate or severe anaemia (Finley *et al.*, 2011). Also, early motherhood was established to provide a higher risk of anaemia in children (Najati and Gojazadeh, 2010). The increased risk of anaemia in children of younger mothers suggests that young mothers are less established to provide the nutrient requirements of their children and at the same time perform the duties of motherhood (Legason *et al.*, 2017).

Majority of households in this current study were made up of between 3 to 6 members. Households with seven or more members were in the minority (Table 4.2). The current study found no association between household size and anaemia or vitamin A deficiency. However, large household size is largely regarded as a risk factor for malnutrition among infants and children residing in low-income countries (Ajao *et al.*, 2010). This could be because having more dependents to cater for, probably reduces household per capita calorie intake, as food intake may decrease with increase in family size, where many people would have to share a plate of food.

5.2 Anaemia and its related factors

Data collected and analyzed at the beginning of the study indicated that the mean haemoglobin concentration ranged from 11.4 ± 1.0 g/dl to 12.0 ± 1.1 g/dl among children in groups B (fed with diet B) and A (fed with diet A). Haemoglobin concentration of 11.4 g/dl was within WHO cut off point that defines anaemia for children under five years but slightly lower than 11.5 g/dl for those five to 11 years old. Compared to an observational cross-sectional study carried out in the Brong Ahafo Region among children of school-going age, the haemoglobin concentration recorded in the current study was high

(VanBuskirk *et al*., 2014). The current study findings corroborated results of baseline studies done with school-aged children in the Eastern, Volta and Central Regions of Ghana (Osei-Boadi *et al.,* 2012; Egbi *et al.,* 2014; Egbi *et al.,* 2018).

Prevalence of anaemia at baseline ranged from 42.6 % to 51.9 % among study participants. These were lower compared to the prevalence of cross-sectional studies conducted among school children in Mozambique (54 %), Tanzania (57 %), Mali and Nigeria (58 %), Sudan (88.3 %) and Ghana (73.1 %), (Atimati and Abiodun, 2013; Ewusi *et al.,* 2014; Eltayeb *et al.,* 2016; Osei-Boadi *et al.,* 2012).

The prevalence of anaemia across the three groups at baseline and end line were higher than reported for other African countries such as Kenya (28.8 – 35.3 %), Malawi (12 %) and Cape Verde (23.8 %) (Pullan *et al.,* 2013; Ngesa and Mwambi, 2014; Semedo *et al.,* 2014). According to WHO and UNICEF, (2004) anaemia is of public health significance if the prevalence is above five percent. It is classified to be of mild, moderate or severe public health significance when it ranges from 5 % – 19.9 %, 20 % – 39.9 %, and greater or equal to 40 % respectively (WHO, 2001). Therefore, the prevalence of 42.6 % to 51.9 % recorded in the present study at baseline, is of severe public health importance.

Factors that cause anaemia are diverse. They are mostly nutritional and non- nutritional factors (Obse *et al.,* 2013; Oguntibeju, 2015). The anaemia prevalence recorded during the beginning and end of the present study was probably because of the low consumption of iron and vitamin A-rich foods, which include animals (fish, poultry and other meat products), dark green leafy vegetables and fruits at the household level. Most participants (73 %) did not consume any fruit in a week (Table 4.6). Fruits are good sources of vitamin C, which enhance non-haem iron bioavailability and absorption. Vitamin C improves the bioavailability of iron from plant food sources for absorption. Consumption of fruits or

fruit juices (at least two servings per day) was largely associated with anaemia (Ferreira *et al.,* 2016). The haem iron content of animal foods (fish, poultry, meat and meat products) also help with iron absorption from the plant foods consumed with it, however, participants mostly did not consume animal-based foods at all, or their consumption frequency was low (Table 4.6). In Nigeria, the low consumption of animal foods in Ayogu *et al.,* (2016) study was attributed to costs, cultural beliefs and ignorance even though findings of the current study did not corroborate that result. Furthermore, the frequency of intake of green leafy vegetables in the current study was also quite low. It is generally known that leafy vegetables such as *Amarathus cruentus* and *Solanum macrocarpon* are rich in micronutrients including iron, folate and beta-carotene. These micronutrients play very important roles in blood formation and their frequent consumption could contribute to the reduction of anaemia in the participants. It has been reported that green leafy vegetables contributed 19 – 39 % of dietary iron intake in the diets of rural South Africans (Faber *et al.,* 2007). Their result is in line with findings of this very study where results from proximate analysis of the *Amaranthus cruentus* and *Solanum macrocarpon powder* used as supplements contained 78 mg of iron per 100 g of the powder. This iron content translated into about 38 % of the established daily requirement for four to eight-year-old children (Appendix C and I).

Cereals, legumes, tubers and roots were the plant foods available and most consumed by the participants and members of their household (Table 4.6). These foods consist of anti-nutritional factors that could affect the bioavailability of some micronutrients. These anti-nutritional materials (polyphenols, phytic acid in whole grains, oxalates and tannins) contained in plant foods, are capable of hindering the availability of non-haem iron (Natesh *et al.,* 2017; Bora, 2014). Thus, the frequent consumption of plant staple foods in

the current study may have partly resulted in the observed anaemia level recorded among the study participants. Consumption of plant-based food such as green leafy vegetables together with fish, meat, poultry and or citrus fruits have however, been demonstrated to enhance the bioavailability and absorption of non-haem iron (Hurrel, 1997; Conrad *et al.,* 1999; Beck *et al.,* 2014). Contrary to findings of this study, Mesfin *et al.,* (2015) established that children who consumed legumes less frequently had a greater risk of anaemia (APR, 1.069; 95 % CI, 1.022 – 1.118).

The high prevalence of malaria (46.3 % group A; 25.9 % B and 38.9% for control group C) could also have led to the high level of anaemia observed at the beginning of the study (Table 4.4). The existence of malaria parasitaemia among the three groups at baseline was low compared to reports by Oladeinde (2012) in Nigeria, and Egbi and co (2014) in Ghana. They recorded malaria parasitaemia prevalence of 67 % and 89 % respectively among school children (Oladeinde, 2012; Egbi *et al*., 2014). Nevertheless, the level of malaria infection reported currently represents a fundamental health problem and could have negatively influenced the health and quality of life of the children. Several research findings documented malarial infection as one of the major causes of anaemia (Tolentino *et al.,* 2007; De Mast *et al.,* 2010; Marcelline *et al.,* 2016). Malaria causes anaemia by enhancing iron loss in the intestinal tract, by decreasing the amount of haemoglobin, by increasing breakdown of parasitized erythrocytes, by shortening duration of non-parasitized erythrocytes and by further reducing the synthesis of erythrocyte cells in the bone marrow (McDevitt *et al.,* 2004; Njunda *et al.,* 2015). The presence of high level of malarial infection has been a major cause of low haemoglobin concentration in pupils (Glinz *et al.,* 2015; Udoidung *et al.,* 2015). In an earlier study, the risk of having haemoglobin concentration below the accepted cut-off points was one and half times (95

% CI = 0.8, 2.9) greater among malaria positive children than those who were malaria negative (Legason *et al.*, 2017). On the contrary, malaria parasitaemia was not significantly associated with anaemia according to the current findings being presented. Profuse gastrointestinal tract bleeding, as a result of peptic ulcers and or hemorrhoids can cause anaemia (Alexandrescu, 2009) even though this was not investigated in the present study. Malaria prevalence among the three groups decreased significantly from baseline to end line. The reduction in the prevalence of malaria from baseline to end line could be attributed to the use of insecticide-treated bed nets given to every participant after baseline data collection. From observation about 80 % of participants were using the insecticide-treated nets and that could account for the reduction in malaria prevalence within each group from baseline to end line.

The haem iron present in the fish of intervention diet A, served as a non-haem iron enhancer that helped make the non-haem iron present in the *Amaranthus cruentus* and *Solanum macrocarpon* powder more bioavailable for absorption and blood formation in the bone marrow (erythropoiesis). This is a process which occurs when the oxygen level in the blood becomes low. This triggers the kidney to produce the hormone erythropoietin into the blood. The hormone then fuels bone marrow synthesis of erythrocytes. Meat, fish and poultry contain readily bioavailable heam iron in addition to MFP factor that enhances the absorption of iron from plant food consumed together with animal foods. Pairing non-haem iron containing foods like vegetables with haem iron-rich foods like fish, promotes the absorption of non-haem iron (Zijp *et al.*, 2000). Huh *et al.*, (2014), demonstrated an enhancement in iron absorption from maize and soybeans when fish was added. Martinez-Torres and Layrisse (1971) were the first to observe a twofold upsurge in the absorption of iron when fish was included as a component of black beans meal. The decrease in anaemia

prevalence among participants in experimental group A from baseline to end and between intervention group A and the control group C at the end line of the present study could be due to the MFP factor in the FACSMP.

Participants that were mildly anaemic (haemoglobin concentration between 10.0 and 10.9 g/dl for children less than 5 years; between 11.0 and 11.4 g/dl for children 5 – 11 years) in each group at baseline were followed through to the last day of the study to determine whether their haemoglobin levels and anaemia status improved or worsened. The mildly anaemic children in all the three groups improved at varying degrees on their haemoglobin concentration and reduced anaemia prevalence from baseline to end line (Table 10). Children whose diets were supplemented with FACSMP (group A) recorded the most improvement in haemoglobin concentration (1.7 ± 0.2 g/dl) and the greatest reduction in anaemia prevalence followed by participating children fed with ACSMP - group B (0.9 ± 0.2 g/dl; 53 %) and then children fed with school lunch only (control group C) (0.3 ± 0.6 g/dl; 39 %). Compared to pupils who were non-anaemic at baseline (Appendix D), those with anaemia improved their haemoglobin concentration levels more at the end. This agrees with studies that anaemic people improve much more their haemoglobin status during supplementation than those with normal haemoglobin concentration who may either maintain status or become worse (Gretchan *et al*., 2013).

Low serum retinol concentration was observed to associate significantly with anaemia after adjusting for age (Table 4.11). Participating children with normal serum retinol status had about 85 % (OR = 0.145; 95 % CI: 0.038 – 0.544; p = 0.005) less risk of developing anaemia compared to those with low serum retinol status. In the current study, child sex had no significant association with anaemia, however, in a similar intervention study, child sex was associated with anaemia with the risk of males having anaemia being lower

(OR=0.2, CI 0.1-0.5, p=0.002) than females (Egbi *et al*., 2014). The results of Ngesa and Nwambi, (2014) showed that male children had a greater risk of being anaemic than females. Observations from some other African countries including Malawi (Akhwale *et al*., 2004; Kreamer & Zimmerman, 2007), indicated that boys were at a greater risk for anaemia than girls.

Malaria parasitaemia and anaemia were not associated to any great extent in this study. Sumbele *et al.*, (2016) however reported that children who had malaria had a higher risk (χ 2 = 3.96, P = 0.047) of being anaemic than those without malaria.

5.3 Vitamin A deficiency and its determinants

Analysis of the cross-sectional baseline data revealed that the participants across the three groups were vitamin A deficient. This result agrees with other related studies (Singh and West 2004; Ribeiro-Silva *et al.*, 2014; De Lima *et al.*, 2017). The mean serum retinol concentration at baseline ranged from 22.3 ± 5.6 µg/dl to 23.1 ± 7.3 µg/dl (Table 4.3). A lower range of mean serum retinol concentration (14.39 ± 5.4 to 16.6 ± 5.2 µg/dl) was reported by a similar study on Ghanaian pupils (Egbi *et al.*, 2018). The level of deficiency of vitamin A observed in the current study at baseline (Intervention group A - 31.5 %; intervention group B - 29.6 % and control group C - 35.2 %) could be considered as of severe public health importance per WHO cut-off values. The reason being that at least 20 % of the study participants in each group were vitamin A deficient (WHO, 2009).

Prevalence of vitamin A deficiency (VAD) reported in this study at baseline was higher compared to earlier cross-sectional studies conducted among children under five years in Nigeria and Kenya (NCPD/ICF *et al.*, 2015; Abolurin *et al.*, 2018). However, the level of VAD was lower than recorded in other studies (42 %, 41 %, 49.6 %, 70 %) done among

Ghanaian school children in general and in Adansi South district and Wa West district (Sarpong *et al.,* 2015; GMSR, 2017). The rare consumption of animal products by children in the present study, may have led to the rate of low vitamin A level for participants. Besides, the irregular intake of yellow and orange-fleshed fruits and green leafy vegetables known to be rich sources of carotenoids, may have led to the high prevalence of vitamin A deficiency among the study groups at base line.

Over the years, home or industrially fortified foods have served as a tool for addressing population-wide nutrient deficiencies such as VAD. The enforcement of food fortification strategies improved vitamins and mineral content of fortified foods appreciably for public health benefits. Staples like cereals, oils and condiments, and refined sugar were successfully fortified with vitamin A. Studies were carried out to demonstrate that consumption of vitamin A fortified foods enhanced vitamin A status (Saeterdal *et al.,* 2012). Vitamin A fortified milk and breakfast cereals in the United States of America have been included in dietary guidelines as good sources of vitamin A (NIH, 2016). In the current study, however, the dietary consumption pattern of participants did not include fortified breakfast cereals or milk to help improve their vitamin A status.

There was no association established in the current study between the presence of malaria and vitamin A deficiency. However, the high prevalence of vitamin A deficiency recorded in this study at baseline could be partly attributed to malaria infection. As reported by an earlier study, school pupils aged 10 years and below with malaria presented a two-fold greater chance of having moderate to severely low levels of vitamin A and a greater chance of having borderline vitamin A deficiency suggesting greater vulnerability of young school children (Wirth *et al.,* 2017). A review of malaria infection in African children showed that a low concentration of serum retinol was common in children

infected with malaria (Sanjoaquin and Molyneux, 2009). This corroborates the high malaria and high vitamin A deficiency prevalence recorded at baseline in the current study (Table 4.3). The immune system functions well with a sound vitamin A status, which protects the body against diseases. Literature has it that both innate and specifically acquired mechanisms are involved in the immune response against malaria suggesting that, low serum retinol level impair antibody response to malarial antigens (Omer *et al.*, 2000). The high prevalence of vitamin A deficiency at baseline in this present study could have led to the high prevalence of malaria seen in the various groups: A, B, C at baseline, 46 %, 26 % and 40 % respectively. A study among pre-school children that investigated the relationship between vitamin A deficiency and malaria found that 40.8 % of the children had the abnormal status of vitamin A during the time of malaria infection. This may have occurred because the vector also utilizes vitamin A for growth and development in its life cycle (Sanjoaquin and Molyneux, 2009). A negative association was however observed between the level of vitamin A and malaria in an observational cross-sectional study carried out in adults and in children (Sanjoaquin and Molyneux, 2009; Essuman *et al.*, 2010).

The results at the end of the study showed that the level of malaria parasitaemia and the prevalence of vitamin A deficiency reduced to 14 %, 10 % and 10 %; 10 %, 20 % and 29 % respectively across the three groups. The beta-carotene content of *Amaranthus cruentus* and *Solanum macrocarpon* in intervention diets A and B probably led to the increase in serum retinol concentration with a subsequent reduction in the prevalence of vitamin A deficiency within group A and group B as recorded by the end of the study. The observed rise in retinol concentration also improved antibody response to malarial antigens thereby contributing to the reduced level of malaria infection at the end of the study.

It was observed that some households in the community started having access to palm fruits and mangoes which were in season getting to the end of the intervention period. Palm fruits and mangoes are known to contain provitamin A and their consumption at the household level getting to the end of the intervention period might have contributed to the rise in the mean serum retinol concentrations within all study groups which might have registered some decrease in the prevalence of vitamin A deficiency across the study groups.

A pair wise comparison analysis revealed that, children in group A (fed with school lunch plus fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder - FACSMP) recorded significantly higher mean serum retinol concentration (+ 4.5 µg/dl; p = 0.021) compared to their counterparts in group C who consumed only the regular school lunch. The presence of FACSMP and ACSMP in the intervention diets may have contributed to the significant change in mean serum concentration recorded for these two groups.

According to Fernández-García *et al*., (2012), various factors may influence carotenoid bioavailability. These may include the type and nature of the carotenoid consumed and the food structure as well as the extent of processing. Other researchers have corroborated this by confirming that dietary factors such as the chemical nature of the nutrients, food matrix, nutrients interactions and other organic components within foods affect bioavailability and absorption of nutrients such as pro-vitamin A carotenoids from plant-based foods (Gibson, 2007; Haskell *et al.,* 2012). Beta-carotene being a precursor of vitamin A is easily converted to retinol when blood levels of retinol are depleted (Tanumihardjo, 2002). In the current study, the powdered nature of the green leafy vegetables and the fact that the powder was given one-minute heat treatment, could have helped improve beta-carotene bioavailability in the green leafy vegetables powder. According to Van Het Hof *et al.,* (2000), heating plant tissues substantially improve

carotenoids bioavailability, possibly by alterations in plant cell walls or other barriers to the release and absorption of carotenoids.

Dietary fat has also been shown to improve beta-carotene absorption (Brown *et al.,* 2004; Kopec *et al.,* 2014). The oil used as an ingredient in the preparation of the meals also contributed to improving the bioavailability of the carotenoids in the intervention diets. Carotenoids are fat-soluble precursors of vitamin A and require a fatty medium for efficient absorption. The above effect could not be realized in the control diet even though the control diet was also prepared with the same type and quantity of fat. That could be due to the absence of the vegetables powder in the control diet. The fish present in the FACSMP contributed to the provision of amino acids needed for the formation of retinol-binding protein (RBP). In the blood, retinol-binding protein is considered a specific carrier of retinol. It carries retinol from the liver to periphery tissues for action. Supplementation of culture medium of hepatocytes with amino acids maintained retinol-binding protein production and secretion for several days in an animal study (Dixon and Goodman, 1987). The findings of this research work showed that the difference in mean serum retinol concentrations between group B and C were not significantly different. This may have been because of the absence of fish powder in the diets they received.

It was established through binary logistic regression analysis conducted in the current study that the absence of anaemia and maternal marital status associated significantly with vitamin A deficiency (Table 4.12). Participants whose parents were married had about 94 % less risk of becoming deficient in vitamin A (OR = 0.056; 95 % CI, 0.004 – 0.024; p = 0.036) compared to children of single mothers. This finding is corroborated by results from Mboho and Bassey, 2013, who reported a significant association between maternal marital status and nutrition status of children. Adequate nutrition, food and nutrition security can be threatened by an adverse change in marital status. This may be because the

adverse effects of being a single mother on a child's nutritional status and over all well-being are hinged on economic resources, which include time available to the mother. Single mothers are usually less economically resourced, with little time for monitoring the eating habits of their children (Dearden *et al*., 2013). In another related study, malnutrition was higher in both rural and urban unmarried women compared to married women and their children (Teller and Yimar, 2000). Also, children with a single surviving parent were found to be one and half times more likely to develop anaemia and vitamin A deficiency (95% CI = 0.6, 3.9) than children having both parents alive (Al-Zain, 2013; Senthamaria, 2015; Legason *et al.*, 2017). Being an unmarried or a single mother and poor could have adverse socioeconomic outcomes, which could adversely affect the well-being of children (Manning and Brown, 2006; Hannan and Halpin, 2014). Higher odds were recorded for child food insecurity among household heads that were single mothers as compared to households that had children with mothers and the presence of stepfathers and stepfamilies (Balistreri, 2018), thus demonstrating a protective effect of marriage, beyond economic resources. Additionally, there is evidence to show that unmarried mothers experience a high rate of major depressive illness and distress (Avison and Davies, 2005), and they spend less time with their children than married mothers (Kendig and Bianchi, 2008). Low socioeconomic status and depressive illnesses in unmarried mothers, therefore, could influence the quality of childcare provided to children and this may lead to the development of vitamin A deficiency or low serum retinol level in children. A study investigating the determinants of low serum retinol level in children, however, did not establish an association between marital status and vitamin A deficiency (Sachdeva *et al.*, 2011).

Results from the current study also indicated that, children who had no anaemia had about 84% (OR = 0.164; 95 % CI, 0.045 – 0.598; p = 0.006) reduced risk of becoming vitamin A deficient (Table 4.12). Several studies have reported a possible association between vitamin A deficiency and anaemia. (Yamamura *et al.,* 2004; Aini *et al.,* 2007; Khan *et al.,* 2008; Kapil and Kapil 2018). This is as a result of vitamin A's involvement in red blood cells formation, improvement in haemoglobin concentration and reducing susceptibility to infections (Aini *et al.,* 2007; Khan *et al.,* 2008; Kapil and Kapil, 2018).

5.4 Interaction between dietary intake, anaemia and vitamin A status

Results obtained after analysis of the food frequency questionnaire data showed that cereals and grains were the staples mostly consumed by the study participants. Roots and tubers, as well as legumes, were also relied on as staples (Table 4.6). Frequent consumption of these staples could predispose the children to iron deficiency, anaemia and vitamin A deficiency. As demonstrated by Zimmerman (2005), the low availability of iron from grains and leguminous foods could be the cause of iron deficiency among African children. Other studies have shown that the poor bioavailability of iron from grains and leguminous diets could be the dominant cause of the high prevalence of anaemia in most regions of the world specifically Sub Saharan Africa: 42 % in Swaziland; 91 % in Burkina Faso and 63 % in Ghana (Magalhães and Clement, 2011; Abdul-Razak *et al.,* 2012). Cereal and legume-based diets are sources of anti-nutritional factors including iron inhibitors like polyphenols, tannins and phytates (Madode *et al.,* 2011; Woyengo and Nyachoti, 2013). These inhibitors affect the bioavailability and absorption of iron when ingested during and shortly before or after a meal (Woyengo and Nyachoti, 2013).

The study showed that all participants in intervention group A satisfied their minimum iron requirement at the end line, such that the prevalence of inadequacy (Barr, 2014) of iron intake was absent among children in that group. All three groups recorded an increase

in the number of participants that met the nutrient adequacy ratio (NAR > 1) from baseline to end line for protein and iron intake. Recall bias, which usually results in over or under estimation of food intake, may have contributed to the increases in NAR for protein and iron across all the three groups (Castell *et al.*, 2015).

For pro-vitamin A and ascorbic acid, the number of children who met NAR > 1 from the start of the study to the end of the study declined for the control group C and intervention group B. Only a small number of participants satisfied the estimated average requirement for ascorbic acid which is known to enhance non-haem iron bioavailability (Tetens *et al*., 2007). The food consumption data based on the food frequency questionnaire and 24hour dietary recall revealed that many study participants across the three groups scarcely consumed any fruits. This might be the reason why their dietary vitamin C intake was low at both baseline and end line. Children in group A and group B who received the green leafy vegetables powder could have obtained an appreciable level of vitamin C by the end of the study since green leafy vegetables are good sources of ascorbic acid (Gupta *et al.,* 2013; Lawal *et al.,* 2015), however, the drying and milling process the vegetables were taking through during powder preparation may have led to the loss of some of the vitamin C they contained. Studies have indicated that processing methods such as drying and cooking result in the reduction of vitamin C content in vegetable (Agbemafle *et al*., 2012; Gupta *et al.,* 2013; Lawal *et al.,* 2015).

Across the three groups, significant increases were recorded with regards to dietary intake for protein, iron and beta-carotene (Table 4.6). The mean change in protein intake was significant between intervention groups A and B. Between intervention groups A and C (fed with school lunch), significant changes in mean iron and beta-carotene intakes were recorded. The rich nutrient content of the powders added to the school lunch could have supported the increase in the amount of iron and beta-carotene consumed between group

A and B. This is because, aside the powders, all the children in the three groups received the same treatment from the study.

In the context of the current study, protein and iron content of the FACSMP (Appendix C) (fed to intervention group A), compares with previous studies (Torheim *et al.*, 2003, Rathnayake *et al.,* 2012; Egbi *et al.,* 2015), that the quality of the net nutrient of a diet improves with improved dietary diversity. In the current study, beside the protein in the FACSMP, were iron, folate, zinc and beta-carotene from the *Amaranthus cruentus* and the *Solanum macrocarpon* powder. These could have helped improve the overall nutrient content of the diets for group A and B compared to the diets of the control (Appendix C). The additional impact of dietary diversity concerning the FACSMP was demonstrated as the overall increase in mean haemoglobin and mean serum retinol levels of study participants in intervention group A compared to children in group B and group C.

The heam iron as well as the MFP factor enhanced the bioavailability and absorption of non-haem iron in the stews and soups with fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* composite powder. The proteins in the fish provided amino acids that aided the production of retinol-binding proteins, which binds and transports vitamin A from the liver to action sites.

Results from the food frequency questionnaire also indicated that all the participating children consumed very little or no meat and meat products including poultry. From observation during data collection, the quantity of fish consumed was not adequate to compensate for the non-consumption of other animal and animal products. Fish, meat and poultry are very good sources of haem iron, which are highly bioavailable with better absorption rate (20 to 30 %) compared to (5 % to 15 %) non-haem iron (Young *et al.*, 2018). The low monthly income of parents in the current study, (less than 500 Ghana

Cedis) might have made meat and meat products, which are expensive, less affordable to them. According to Yakwezi (2012), in many developing countries, meat is considered a very expensive commodity in rural areas restricting the poorest people access to the commodity.

5.5 Anthropometric measurements nutritional status

Mean weight and mean height increased in the three groups of the study with p = 0.001 and p = 0.002 respectively (Table 4.5). The findings also showed significant differences in mean weight and height within group A, B and C. The study revealed some level of stunting, underweight and wasting among the study participants. All three groups recorded slight differences in the prevalence of stunting, underweight and wasting from baseline to end line, but these increases were not statistically significant (Table 4.9). Prevalence of anthropometric indices recorded in the current study according to WHO cut off values for public health significance, was low for underweight and stunting but acceptable for wasting (WHO, 1995). The prevalence of stunting and underweight in the current study was lower compared reports by 2014 Ghana demographic and health survey. Over the years, the Ghana demographic and health survey (2003, 2008, 2014), has reported steady decline in the level of stunting (35 %, 28 %, 19 %), underweight (18 %, 14 %, 11 %) and wasting (8 %, 9 %, 5 %) in children below 5 years (GSS, 2014). The minimal changes recorded within and between groups in anthropometric nutrition status (stunting, underweight and wasting) could be due to the short time (four months) used for executing the intervention study. Contrary to a nutrition intervention study conducted in Iran, a significant decrease in the prevalence of wasting was recorded among the 3 to 6-year-old experimental group compared to their counterparts in the control group (Zavoshy *et al.*, 2012).

5.6 Limitations of the study

A samples size of 265 was initially estimated however, 55 of the children could not be included due to hospitalization, relocation and refusals. The refusals were based on religious and cultural reasons including taboo to donate biological samples. Thirty-nine children out of the 201 left were found to have severe malaria and or severe anaemia (children with haemoglobin < 7.5 g/dl) after baseline screening so they were not included in the study.

The participants were four to eight years old pupils taking part in the Ghana School Feeding Program (GSFP). The study findings can therefore not be generalized to cover pupils not benefiting from the GSFP and children who are not in school.

Consumption of mangoes and palm fruits which were in season getting to end of study could be a confounding factor for beta-carotene and total carotenoids intake.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

- The study demonstrated that the consumption of stews and soups with fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder (FACSMP): -
    - increased the mean haemoglobin concentration and reduced the prevalence of anaemia among the study participants
    - increased mean serum retinol concentration but did not reduce the prevalence of vitamin A deficiency of the study participants

- There were no significant changes in the anthropometric indicators of nutritional status (underweight, stunting and wasting).
- Malaria parasitaemia was prevalent among the participants at baseline.
- Low serum retinol level was significantly associated with anaemia hence is an important factor to consider when controlling anaemia among the study participants.
- Marital status of mothers as well as anaemia status significantly associated with low serum retinol level as such are critical factors to consider when managing vitamin A deficiency among the study participants.

Clearly then, feeding of fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder (FACSMP) significantly reduced the anaemia status of study participants. The intervention therefore had the potential to improve haemoglobin concentration and to control anaemia among the study participants.

The study thus rejects the hypothesis that consumption of fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder will not significantly improve heamoglobin concentration and reduce prevalence of anaemia among study participants.

6.2 Recommendations

It is recommended that:

- The consumption of fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder be further investigated among a larger population of school children involved in the Ghana School Feeding Programme to ascertain whether it will be established as a dietary strategy for minimizing anaemia and vitamin A deficiency.
- Further study should be conducted among school children of similar age who are mildly or moderately anaemic to determine the effectiveness of the fish fortified *Amarantus cruentus* and *Solanum macrocarpon* powder in improving their anaemia status
- Further research ought to be carried out among children who are moderately vitamin A deficient to determine the effectiveness of the fish fortified green leafy vegetables powder in controlling vitamin A deficiency among them.

REFERENCES

Abdul-Razak, A., Moretti, D., Zimmermann, M. B., Armar-Klemesu, M., & Brouwer, I. D. (2012). Whole cowpea meal fortified with NaFeEDTA reduces iron deficiency among Ghanaian school children in malaria endemic area. *Journal of Nutrition, 142*, 1836 – 1842.

Abeshu, M. A., Lelisa, A., & Geleta, B. (2016). Complementary Feeding: Review of Recommendations, Feeding Practices, and Adequacy of Homemade Complementary Food Preparations in Developing Countries - Lessons from Ethiopia. *Frontiers in Nutrition, 3*, 41.

Abiodun, O. & Akinoso, R. (2014). Textural and sensory properties of trifoliate yam (Dioscorea dumetorum) flour and stiff dough 'amala'. *Journal of Food Science and Technology, 52*, 2894 – 2901.

Abioye, V. & Aka, M. (2015). Proximate composition and sensory properties of moringa fortified maize-ogi. *Nutrition Food Science, 10*, 4172/2155 – 9600.

Abolurin, O. O., Adegbola, A. J., Oyelami, O, A., Adegoke, S. A., Bolaji, O. O. (2018). Prevalence of Vitamin A deficiency among under-five children in South-Western Nigeria. *Nigerian Post Graduate Medical Journal*, 25 (1), 13 – 16.

Abu-Ouf, N. M., & Jan, M. M. (2015). The impact of maternal iron deficiency and iron deficiency anaemia on child's health. *Saudi Medical Journal*, 36(2), 146–149.

Abuya, B. A., Ciera, J., & Kimani-Murage, E. (2012). Effect of mother's education on child's nutritional status in the slums of Nairobi. *BMC Pediatrics, 12*, 80.

Acho, C. F., Zoue, L.T., Akpa, E. E., Yapo, V. G., & Niamke, S. I. (2014). Leafy vegetable consumed in Southern Côte d`Ivoire: a source of high value nutrients. *Journal of Animal and Plant Science, 20*(3), 3159 – 3170.

Achouri, I., Aboussaleh, Y., Sbaibi, R., El Hioui, M., & Ahami, A. (2015). Prevalence of iron deficiency anaemia and associated factors among urban school children in Kenitra, North West of Morocco. *The American Journal of Innovative Research and Applied Sciences, 1*, 332 – 337.

Addis, G., Asfaw, Z., & Woldu, Z. (2013). Ethnobotany of wild and semi-wild edible plants of Konso ethnic community, South Ethiopia. *Ethnobotany Research Application, 11*(20), 121 – 142.

Adenipenkun, C. O., & Oyetunji, O. J. (2010). Nutritional Values of some tropical vegetables. *Journal of Applied Bioscience, 35*, 2294 – 2300.

Adewumi, T. Oyeyinkaa, S. & Oyeyinkab, A. (2018). Moringa oleifera as a food fortificant: Recent trends and prospects. *Journal of the Saudi Society of Agricultural Sciences, 2,* 127-136.

Adhikari, P. M., Chowta, M. N., Ramapuram, J. T., Rao, S., Udupa, K., & Acharya, S. D. (2016). Prevalence of Vitamin B12 and folic acid deficiency in HIV-positive patients and its association with neuropsychiatric symptoms and immunological response. *Indian Journal of Sexually Transmitted Diseases and AIDS, 37*(2), 178 – 184.

Agbemafle, R., Obodai, E. A., Adukpo, G. E., & Amprako, D. N. (2010). Effects of boiling time on the concentrations of vitamin c and beta-carotene in five selected green vegetables consumed in Ghana. *Pelagia Research Library*, 3 (5), 2815 – 2820.

Age-Related Eye Disease Study Research Group (2006). A randomized, placebo controlled, clinical trial of high-dose supplementation with vitamins C and E and beta-carotene for age cataract and vision loss: AREDS report no. 9. *Archive of Ophthalmology, 119*, 1439 – 1452.

Agrawal, S., & Agrawal, P. (2013). Vitamin A supplementation among children in India: Does their socioeconomic status and the economic and social development status of their State of residence make a difference? *International Journal of Medicine and Public Health, 3*(1), 48-54.

Aguayo, V. M. (2017). Complementary feeding practices for infants and young children in South Asia. A review of evidence for action post 2015. *Maternal and Child Nutrition, 13*(S2), e12439.

Ahmed, F., Prendiville, N., & Narayan, A. (2017). Micronutrient deficiencies among children and women in Bangladesh: progress and challenges. *Journal of Nutritional Science*, 5, 46.

Aini, N. U., Al-Mekhlafi, H. M., Azlin, M., Shaik, A., Sa'iah, A., Fatmah, M. S., Ismail, M.G., Firdaus, A. M. S., Aisah M. Y., Rozlida A. R. (2007). Serum iron status in Orang Asli children living in endemic areas of soil-transmitted helminthes. *Asia Pacific Journal of Clinical Nutrition*, 16, 724–730.

Ajao, O. K., Ojofeitimi, E., Adebayo, A., Fatusi, A. & Afolabi, O. (2010). Influence of Family Size, Household Food Security Status, and Child Care Practices on the Nutritional Status of Under-five Children in Ile-Ife, Nigeria. *African Journal of Reproductive Health, 14*, 117-26.

Akhtar, S., Ahmed, A., Randhawa, M. A., Atukorala, S., Arlappa, N., Ismail, T., & Ali, Z. (2013). Prevalence of vitamin A deficiency in South Asia: causes, outcomes, and possible remedies. *Journal of Health, Population, and Nutrition, 31*(4), 413 - 23.

Akhwale, W. S. 1., Lum, J. K., Kaneko, A., Eto, H., Obonyo, C., Björkman, A., Kobayakawa, T. (2004). Anaemia and malaria at different altitudes in the western highlands of Kenya. *Acta Tropical*, 91(2), 167 - 75.

Al Qahtani S. A. (2018). Drug-induced megaloblastic, aplastic, and haemolytic anaemias: current concepts of pathophysiology and treatment. *International Journal of Clinical Experimental Medicine, 11*(6), 5501 – 5512.

Alatiah, G. A. (2013). Serum vitamin A and Nutritional Status of Children fed Gari and Beans Stew. Department of Nutrition and Food Science, University of Ghana, Legon Accra. Mphil Thesis.

Alegbejo, J. O. (2013). Nutritional value and utilization of Amaranthus. *Journal of Pure and Applied Sciences*, 6(1), 136 – 143.

Alexandrescu, D. T., Dasanu, C. A., & Kauffman, C. L. (2009). Acute scurvy during treatment with interleukin - 2. *Clinical and Experimental Dermatology, 34*, 811–814.

Allen, L. (2006). Guidelines on food fortification with micronutrients, World Health Organization, Geneva, 112 – 117.

Al-Mekhlafi, H. M., Al-Zabedi, E. M., Al-Maktari, M. T., Atroosh, W. M., Al-Delaimy, A. K., Moktar, N., Sallam, A. A., Abdullah, W. A., Jani, R., & Surin, J. (2013). Effects of vitamin A supplementation on iron status indices and iron deficiency anaemia: a randomized controlled trial. *Nutrients*, 6(1), 190 - 206.

Al-Zain, B. F. (2013). Anaemia and nutritional status of pre-school children in North Gaza, Palestine. *International Journal of Scientific and Technology Research,* 1, 86 – 91.

American Societ of Haematology (2017). Annual meeting report, Atlanta Georgia, USA.

Ami, N., Bernstein, M., Boucher, F., Rieder, M., & Parker, L. (2016). Canadian Paediatric Society, Drug Therapy and Hazardous Substances Committee Folate and neural tube defects: The role of supplements and food fortification. *Paediatrics and Child Health, 21*(3), 145 - 54.

Amo-Adjei, J., & Kumi-Kyereme, A. (2014). Fruit and vegetable consumption by ecological zone and socioeconomic status in Ghana. *Journal of Biosocial Science*, 471–- 19.

AOAC, Official methods of analysis (AOAC) of AOAC international 18th edition, 2010.

Appleton, K. M., Hemingway, A., Saulais, L., Dinnella, C., Monteleone, E., Depezay, L., Morizet, D., Armando Perez-Cueto, F. J., Bevan, A., & Hartwell, H. (2016). Increasing vegetable intakes: rationale and systematic review of published interventions. *European Journal of Nutrition*, 55(3), 869 - 96.

Arise, A. K., Arise, R. O., Sanusi, M. O., Esan, O. T., & Oyey, S. A. (2014). Effect of Moringa oleifera flower fortification on the nutritional quality and sensory properties of weaning food. *Journal of Food Science and Technology, 6*(2), 65 - 71.

Arlappa, N., Laxmaiah, A., Balakrishna, N., Harikumar, R., & Brahmam, G. N. V. (2016). Clinical and sub-clinical vitamin A deficiency among rural pre-school children of Maharashtra, India. *Annals of Human Biology, 35*(6), 606 – 614.

Asare-Marfo D, Birol E, Gonzalez C. Mourad M., Salomon, P., Jana S., & Manfred Z. (2014). Prioritizing countries for biofortification interventions using country- level data. HarvestPus working paper. Available at: https://pdfs.semanticscholar.org/15e0/66d09812bbe76eacc5c1d6db98efbcceb20a.pdf

Atimati, A. O., Abiodun, P. O., & Ofovwe, G. E. (2013). Relationship between vitamin A status and anaemia among school age children in Benin. *Niger Journal of Pediatrics*, (4), 379 –383.

Augusto, R. A., Cobayashi, F., & Cardoso M. A. (2015). Associations between low consumption of fruits and vegetables and nutritional deficiencies in Brazilian schoolchildren, 18(5), 927 – 935.

Avison, W. R., & Davies, L. (2005). Family structure, gender, and health in the context of the life course. *Journal Gerontology, Series B Psychological Sciences and Social Sciences*, 60, S113 – S1S6.

Awasthi, S., Peto R., Read, S., Clark, S., Pande, V., Bundy, D. & The DEVTA (Deworming and Enhanced Vitamin A) team. (2013). Vitamin A supplementation every 6 months with retinol in 1 million pre-school children in north India: DEVTA, a cluster-randomized trial. *Lancet*, 381, 1469 – 77.

Awoyale, W., Maziya-Dixon, B., Sanni, L. & Shittu, T. A. (2010). Nutritional and sensory properties of amala supplemented with distiller's spent grain (DSG*). Journal of Food, Agriculture and Environment*, 8, 66 – 70.

Ayogu, R. N. B., Okafor, A. M., & Ene-Obong, H. N. (2015) Iron status of schoolchildren (6–15 years) and associated factors in rural Nigeria, *Food & Nutrition* Research, 6, 59 - 26223.

Ayogu, R. N., Nnam, N. M., Ibemesi, O., & Okechukwu, F. (2016). Prevalence and factors associated with anthropometric failure, vitamin A and iron deficiency among adolescents in a Nigerian urban community. *African Health Sciences*, 16(2), 389-98.

Baech, S. B., Hansen, M., Bukhave, K., Jensen, M., Sørensen, S. S., Kristensen, L., Purslow, P. P., Skibsted, L. H., & Sandström, B. (2003). Non-haeme-iron absorption from a phytate-rich meal is increased by the addition of small amounts of pork meat. *American Journal of Clinical Nutrition*, 77(1), 173 - 9.

Baily, R. L., West, K. P., & Black, R. E. (2015). The epidemiology of global micronutrient deficiencies. *Annals of Nutrition and Metabolism*, 66(2), 22 – 33.

Bain, L. E., Awah, P. K., Geraldine, N., Kindong, N. P., Sigal, Y., Bernard, N., & Tanjeko, A. T. (2013). Malnutrition in Sub-Saharan Africa: burden, causes and prospects. *The Pan African medical Journal*, *15*(120).

Baker, R. D., Greer, F. R. & Committee on Nutrition, (2010). Diagnosis and prevention of iron deficiency and iron-deficiency anaemia in infants and young children (0 – 3 years of age). *American Academy of Pediatrics*, 126, 1040 – 1050.

Balarajan, Y., Ramakrishnan, U., Ozaltin, E., Shankar, A. H. S. & Ubramanian, S. V. (2011). Anaemia in low-income and middle-income countries. *The Lancet, 378*(9809), 2123 – 35.

Balistreri, K. S. (2018). Family Structure and Child Food Insecurity: Evidence from the Current Population Survey. *Social Indicators Research, 138*(3), 1171 – 1185.

Baltussen, R., Knai, C., & Sharan, M. (2004). Iron fortification and iron supplementation are cost-effective interventions to reduce iron deficiency in four sub regions of the world. *Journal of Nutrition, 134*(10), 2678 - 84.

Barr, S. I., DiFrancesco, L., & Fulgoni, V. L. (2014). Breakfast consumption is positively associated with nutrient adequacy in Canadian children and adolescents. *The British Journal of Nutrition,* 112(8), 1373 - 83.

Benn, C. S., Fisker, A. B., Jørgensen, M. J., & Aaby, P. (2008). Conflicting evidence for neonatal vitamin A supplementation. *Vaccine,* 26(33), 4111 - 2.

Berti, C., Faber, M., & Smuts, C. M. (2014). Prevention and control of micronutrient deficiencies in developing countries: current perspectives. *Nutrition and Dietary Supplements Journal*, 6, 41 – 57.

Bertolaso, C., Groleau, V., & Stallings, A. V. (2014). Fat-soluble vitamins in cystic fibrosis and pancreatic insufficiency: efficacy of a nutrition intervention. *Journal of Pediatric, Gastroenterology and Nutrition,* 54(4), 443 – 448.

Bhutta, Z. A., Haider, B. A., Cousens, S., & Black, R. E. (2008). Response to "Conflicting evidence for neonatal vitamin A supplementation (letter). *Vaccine*, 26(34), 4304 - 5.

Birhanu, M., Gedefaw, L., & Asres, Y. (2018). Anaemia among School-Age Children: Magnitude, Severity and Associated Factors in Pawe Town. *Ethiopian Journal of Health Science*, 28(3), 259-266.

Boeing, H., Bechthold, A., Bub, A., Ellinger, S., Haller, D., & Kroke, A. (2012). Critical review: vegetables and fruit in the prevention of chronic diseases. *European Journal of Nutrition*, 51(6), 637 – 663.

Bonifant, C. M., Shevill, E., Chang, A. B. (2014). Vitamin A supplementation for cystic fibrosis. *Cochrane Database of Systematic Reviews*, 5, 06751.

Bonsu, K. O., Fontem, D. A., Nkansah, G. O., Iroume, R. N., Owusu E. O., Schippers R. R., 2002. Diversity within the Gboma eggplant (Solanum macrocarpon), an indigenous vegetable from West Africa. *Ghana Journal of Horticulture*, 1, 50 – 58.

Bora, P. (2014). Anti-nutritional factors in foods and their effects. *Journal of Academic and Industrial Research*, 3, 285 - 290.

Bothwell, T. H., Baynes, R. D., MacFarlane, B. J., MacPhail, A. P. (1989). Nutritional iron requirements and food iron absorption. *Journal of Internal Medicine*, 226(5), 357 - 65.

Bourne, R. R. A., Flaxman, S. R., Braithwaite, T., Cicinelli, M. V., Das, A., Jonas, J. B. (2017). Vision Loss Expert Group. Magnitude, temporal trends, and projections of the global prevalence of blindness and distance and near vision impairment: a systematic review and meta-analysis. *Lancet Global Health*, 5(9), 888 – 97.

Brown, M. J., Ferruzzi, M. G., Nguyen, M. L., Cooper, D. A., Eldridge, A. L., Schwartz, S. J., & White, W. S. (2004). Carotenoid bioavailability is higher from salads ingested with full fat than with fat-reduced salad dressings as measured with electrochemical detection. *American Journal of Clinical Nutrition*, 80, 396 – 403.

Bruinsma J. (2003). World agriculture: towards 2015/2030. An FAO perspective. Rome, Food and Agriculture Organization of the United Nations/London, Earthscan.

Burke, R. M., Whitehead, R. D., Figueroa, J., Whelan, D., Aceituno, A. M., Rebolledo, P. A., Revollo, R., Leon, J. S., & Suchdev, P. S. (2018). Effects of Inflammation on Biomarkers of Vitamin A Status among a Cohort of Bolivian Infants. *Nutrients*, 10(9), 1240.

Burri, B. J. (2011). Evaluating sweet potato as an intervention food to prevent vitamin A deficiency. *Comprehensive Reviews in Food Science and Food Safety*, 10(2), 118 - 130.

Camaschella, C., & Nai, A. (2015). Ineffective erythropoiesis and regulation of iron status in iron loading anaemias. *British Journal of Haematology*, 72(4), 512 – 523.

Conrad, M. E., Umbreit, J. N., & Moore, E. G. (1999). Iron absorption and transport. *American Journal of Medical Science*, 318, 213 – 29.

Cao, C., & Fleming, M. D. (2016). The placenta: the forgotten essential organ of iron transport. Nutrition reviews, 74(7), 421– 431.

Carbone, E., T. & Zoellner J. M. (2012). Nutrition and Health Literacy: A Systematic Review to Inform Nutrition Research and Practice. *Journal of the American Academy of Nutrition and Dietetics*, 112(2), 254 – 65.

Castell, G. S., Serra-Majem, L., & Ribas-Barba, L. (2015). What and how much do we eat? 24-hour dietary recall method. *Nutrition Hospitalaria*, 31(3), 46 – 48.

Chakravarty, I. (2000). Food-based strategies to control vitamin A deficiency. *Food and Nutrition Bulletin*, 21:2.

Chan, Q., Stamler, J., Brown I. J., Daviglus, M. L., Van Horn, L. & Dyer, A. R. (2014). Relation of raw and cooked vegetable consumption to blood pressure: the INTERMAP Study. *Journal of Human Hypertension*, 28(6), 353 – 359.

Chander, A., Chopra, R., & Batra, N. (2013). Vitamin A deficiency: An eye sore. *Journal of Medical Nutrition and Nutraceuticals*, 2(4), 1 – 5.

Chebaya, P., Karakochuk, C. D., March, K. M., Chen, N. N., Stamm, R. A., & Kroeun, H. (2017). Correlations between maternal, breast milk and infant vitamin B12 concentrations among Mother–Infant Dyads in Vancouver, Canada and Prey Veng, Cambodia: An Exploratory Analysis. *Nutrients* MPDI. 9, 270.

Cheesbrough, M. District Laboratory Practice in Tropical Countries. Cambridge University Press, Cambridge 1998.

Chhabra, S., & Mishra, S. (2018). "Persisting Problem of Deficiency of Vitamin A in Rural Preschool, Primary School Children". *Pediatrics*, 7.5, 445 - 450.

Christen, W. G., Liu, S., Schaumber, D. A., & Buring, J. E. (2005). Fruit and vegetable intake and the risk of cataract in women. *American Journal of Clinical Nutrition*, 81(5), 1417 - 1422.

Clasen, T. F., Alexander, K. T., Sinclair, D., Boisson, S., Peletz, R., Chang, H. H., Majorin, F., & Cairncross, S. (2015). Interventions to improve water quality for preventing diarrhoea. The *Cochrane Database of Systematic Reviews*, (10), CD004794.

Coles, C., Levy, A., Gorodischer, R., Dagan, R., Deckelbaum, R., Blaner, W. & Fraser, D. (2004). Subclinical vitamin A deficiency in Israeli-Bedouin toddlers. *European Journal of Clinical Nutrition*, 58,796 – 802.

Collings, R., Harvey, L. J., Hooper, L., Hurst, R., Brown, T. J., Ansett, J., King, M., & Fairweather-Tait, S. J. (2013). The absorption of iron from whole diets: a systematic review. *The American Journal of Clinical Nutrition*, 98(1), 65 – 81.

Costa, M. H., de Souza, A. I., Braga, B. M., & Filho, M. C. (2013). Coexistence of Anaemia and vitamin A deficiency in women of childbearing age in the Northeast region of Brazil. *Reviews of Nutrition*, 26(5), 1678 – 9865.

D'alessandro, A., & Liumbruno, G. M. (2018). Red blood cell storage and clinical outcomes: new insights. Blood transfusion = *Trasfusione del sangue*, 15(2), 101 – 103.

D'Ambrosio, D. N., Clugston, D., & Blaner, W. S. (2011). Vitamin A Metabolism: An Update. *Nutrients*, 3(1), 63 – 103.

Da Cunha, M. S. B., Campos H. N. A., and Arruda, S. F. (2018). Effect of vitamin A supplementation on iron status in humans: A systematic review and meta-analysis. *Critical Review of Food Science and Nutrition*, 6, 1 – 15.

Dalby-Payne, J. R., & Elliott, E. J. (2011). Gastroenteritis in children. *BMJ Clinical Evidence*, 0314.

Darlow, B. A., & Graham, P. J. (2016). Vitamin A supplementation to prevent mortality and short and long-term morbidity in very low birthweight infants. *Cochrane Database Systems Review*, 22(8).

Darnton-Hill, I., & Mkparu, C. U., (2015). Micronutrients in Pregnancy in Low- and Middle-Income Countries. *Nutrients*, 7(3), 1744 – 1768.

Daucher, L., Amouye, P., Hercberg, S., & Dallongeville, J. (2006). Fruit and vegetable consumption and risk of coronary heart disease: a meta-analysis of cohort studies. *Journal of Nutrition*, 136, 2588 - 2592.

De Benoist B., McLean E., Cogswell M., Egli I. & Wojdyla D. (2008). Worldwide prevalence of anaemia 1993–2005. World Health Organization Global Database on Anaemia. Geneva: World Health Organization. 1 – 55.

De Lima, B. M., & Garcez, S. L., Oliveira, I. K. F., Marize, S., DA Paz, M. R. S., Paiva, A. A. (2017). Vitamin A deficiency and factors associated with retinol levels in public school students. *Revista de Nutrição*, 30, 605 - 614.

De Mast, Q., Syafruddin, D., Keijmel, S, Riekerink, T. O., Deky, O., & Asih, P. B. (2010). Increased serum hepcidin and alterations in blood iron parameters associated with asymptomatic P. Falciparum and P. vivax malaria. *Haematologica*, 95(7), 1068 – 1074.

De Saveur, S. A., & Broin, M. (2010). MORINGA growing and processing moringa leaves. Moringa news/Moringa Association of Ghana. *Biodiversity International*, 15–29.

Dearden, K., Crookston, B., Madanat, H., West, J., Penny, M., & Cueto S. (2013). What difference can fathers make? Early paternal absence compromises Peruvian children's growth. *Journal of Maternal and Child Nutrition*, 9, 143-154.

Demissie, T., Ali, A., Mekonnen, Y., Haider, J., & Umeta, M. (2009). Demographic and health-related risk factors of subclinical Vitamin A deficiency in Ethiopia. *Journal of Health Population and Nutrition*, 27666.

Diedhiou, B. M. & Jalal, C. (2018). Current Situation of Micronutrient Deficiencies in West Africa. Rice fortification in Africa. *Nutrition International*.

Dixon, J. L., & Goodman, D. S. (1987). Effects of nutritional and hormonal factors on the metabolism of retinol- binding protein by primary cultures of rat hepatocytes. *Journal of Cellular Physiology*, 130 (1), 6 – 13.

Djuikwo, V. N., Ejoh, R. A., Gouado, I., Mbofung, C. M., & Tanumihardjo, S. A. (2011). Determination of major carotenoids in processed tropical leafy vegetables indigenous to Africa. *Journal of Biomedical and Life Sciences*, 2, 793 – 802.

Dougnon, V. T., Bankolé, H. S., Christian, J. R., Klotoé, J. R., Gbaguidi, F. A., Dougnon, G., Gbénou, J., Assogba, M.F., Atègbo, J-C., Sahidou, S., Rhin, B. H., Loko, F., Boko, M., & Edorh, A. P. (2012). Phytochemical screening, nutritional and toxicological analyses of leaves and fruits of *Solanum macrocarpon* (Solanaceae) in Cotonou (Benin). *Food and Nutrition Sciences*. 3(11).

Egbi, G. (2012). Prevalence of vitamin A, zinc, iodine deficiency and anaemia among 2-10 year- old Ghanaian children. *AJFAND*, 12(2), 5946 – 5958.

Egbi, G., Ayi, I., & Saalia, F. K. (2015). Impact of cowpea-based food containing fish meal served with vitamin C–rich drink on iron stores and haemoglobin concentrations in Ghanaian schoolchildren in a malaria endemic area. *Food and Nutrition Bulletin*, 36(3), 264 - 275.

Egbi, G., Gbogbo, S., Mensah, G. E., Glover-Amengor, M., & Steiner-Asiedu, M. (2018). Effect of green leafy vegetables powder on anaemia and vitamin-A status of Ghanaian school children. *BMC Nutrition*, 4, 27.

Egbi, G., Steiner-Asiedu, M., Kwesi, F. S., Ayi, I., Ofosu, W., Setorglo, J., Klobodu, S. S., & Armar-Klemesu, M. (2014). Anaemia among school children older than five years in the Volta Region of Ghana. *The Pan African Medical Journal*, 17(1), 10.

Eilander, A., Gera, T., Sachdev, H. S., Transler, C., van der Knaap, H. C. M., Kok, F. J. & Osendarp, S. J. M. (2010). Multiple micronutrient supplementation for improving cognitive performance in children: systematic review of randomized controlled trials. *The American Journal of Clinical Nutrition*, 19(1), 115 – 130.

Eltayeb, M. S., Elsaeed, A. E., Mohamedani, A. A., & Assayed, A. A. (2016). Prevalence of anaemia among Quranic school (Khalawi) students (Heiran)in Wad El Magboul village, rural Rufaa, Gezira State, Central Sudan: a cross sectional study. *The Pan African Medical Journal*, 24, 244.

Engle-Stone, R. Perkins., A., Clermont, A., Walker, N., Haskell, M. J., Vosti, S. A., & Brown, K. H. (2017). Estimating Lives Saved by Achieving Dietary Micronutrient Adequacy, with a Focus on Vitamin A Intervention Programs in Cameroon, *The Journal of Nutrition*, 147 (11), 2194S – 2203S.

Ewusie, J. E., Ahiadeke, C., Beyene, J., & Hamid, J. S. (2014). Prevalence of anaemia among under-5 children in the Ghanaian population: estimates from the Ghana demographic and health survey. *Public Health Journal*, 14, 626.

Faber, M, & Laubscher, R. (2008). Seasonal availability and dietary intake of bcarotene-rich vegetables and fruit of 2-year-old to 5-year-old children in a rural South African setting growing these crops at household level. *International Journal of Food Science Nutrition*, 59, 46–60.

Faber, M., & Laurie, S. (2010). A home-gardening approach developed in South Africa to address vitamin A deficiency. Food based approaches (FBAs) for combating micronutrient deficiencies. *CABI*, 163 – 182.

Faber, M., Van Jaarsveld, P., & Laubscher, R. (2007). The contribution of dark-green leafy vegetables to total micronutrient intake of two-to five-year-old children in a rural setting. *Water SA*, 33, 407 – 412.

Fairweather-Tait, S. J., de Sesmaisons, A. (2018). Approaches used to estimate bioavailability when deriving dietary reference values for iron and zinc in adults. *Proceedings of the nutrition Society*, 27, 1 - 7.

Fairweather-Tait, S. J., Wawer, A. A., Gillings, R., Jennings, A., & Myint, P. K. (2014). Iron status in the elderly. *Mechanisms of Ageing and Development*, 136 - 137(100), 22 - 8.

Food and Agriculture Organization & World Health Organization. 2014. Second International Conference on Nutrition. Report of the joint FAO/WHO secretariat on the Conference. Rome, FAO, and Geneva, WHO.

Food and Agriculture Organization, IFAD, and World food progemme. 2014. The state of food insecurity in the world. Strengthening the enabling environment for food security and nutrition. Rome, FAO.

Food and Agriculture Organization, IFAD, UNICEF, WFP and WHO. 2017. The state of food security and nutrition in the world 2017. Building resilience for peace and food security. Rome, FAO.

Fernández-García, E., Carvajal-Lérida, I., Jaren, M., Garrido-Fernández, J., & Pérez-Gálvez, A., & Hornero-Méndez, D. (2012). Carotenoids bioavailability from foods: From plant pigments to efficient biological activities. *Food Research International*, 46, 432 - 450.

Ferreira, M. S. L., Santos, M, C. P., Moro, T. M. A., Basto, G. T., Andrade, R. M. S., & Goncalves, E. C. B. A. (2016). Fromulation and characterization of functional foods based on fruit and vegetable residue flours. *Journal of Food Science and Technology*. 52(2), 822 - 830.

Finlay, J. E., Özaltin, E., & Canning, D. (2011). The association of maternal age with infant mortality, child anthropometric failure, diarrhoea and anaemia for first births: evidence from 55 low- and middle-income countries. *BMJ Open*, 1, e000226.

Food and Agriculture Organization, 2012. The State of World Fisheries and Aquaculture (2012). FAO Fisheries and Aquaculture Department. Rome, FAO. 209.

Food and Agriculture Organization (2018). The State of World Fisheries and Aquaculture - Meeting the sustainable development goals. Rome. CC BY-NC-SA 3.0 IGO.

Garg, M., Sharma, N., Sharma, S., Kapoor, P., Kumar, A., Chunduri, V., & Arora, P. (2018). Bio-fortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. *Frontiers in Nutrition*, 5, 12.

Gebremedhin, S. (2014). Effect of a single high dose vitamin A supplementation on the haemoglobin status of children aged 6-59 months: propensity score matched retrospective cohort study based on the data of Ethiopian Demographic and Health Survey 2011. *BMC Pediatrics*, 21(14), 79.

Gebreselassie, S. G., Gase, F. E., & Deressa M. U. (2013). Prevalence and correlates of prenatal vitamin A deficiency in rural Sidama, Southern Ethiopia. *Journal Health Population and Nutrition*, 31(2), 185 – 94.

Gereklioglu, C., Asma, S., Korur, A., Erdogan, F., & Kut, A. (2016). Medication adherence to oral iron therapy in patients with iron deficiency anaemia. *Pakistan Journal of Medical Sciences*, 32(3), 604-7.

Gesquiere, I., Lannoo, M., Augustijns, P., Matthys, C., Van der Schueren, B., & Foulon, V. (2014). Iron deficiency after Roux-en-Y gastric bypass: insufficient iron absorption from oral iron supplements. *Obesity Surgery*, 24(1), 56 – 61.

Getachew, A., Guadu, T., Tadie, T., Gizaw, T., Gebrehiwot, M., Cherkos, D. H., Menberu, M. A., & Gebrecherkos, T. (2018). "Diarrhea prevalence and sociodemographic factors among under-five children in rural areas of north Gondar zone, Northwest Ethiopia," *International Journal of Pediatrics*, 8.

Getaneh, Z., Enawgaw, B., Engidaye, G., Seyoum, M., Berhane, M., Abebe, Z., Asrie, F., & Melku, M. (2017). Prevalence of anaemia and associated factors among school children in Gondar town public primary schools, northwest Ethiopia: A school-based cross-sectional study. *PLOS ONE*, 12(12), e0190151.

Ghana Micronutrient Survey report (2017). University of Ghana, groundwork, University of Wisconsin-Madison, KEMRI-Wellcome Trust, UNICEF, Accra, Ghana.

Ghana Statistical Service (GSS), Noguchi Memorial Institute for Medical Research (NMIMR), and ORC Macro. 2004. Ghana Demographic and Health Survey 2003. Calverton, Maryland: GSS, NMIMR, and ORC Macro.

Ghana Statistical Service (GSS), Ghana Health Service (GHS), and ICF Macro. 2009. Ghana Demographic and Health Survey 2008. Accra, Ghana: GSS, GHS, and ICF Macro.

Ghana Statistical Service (GSS), Ghana Health Service (GHS), and ICF International. 2015. Ghana Demographic and Health Survey 2014. Rockville, Maryland, USA: GSS, GHS, and ICF International.

Ghana Statistical Service (2012). 2010 Population and Housing Census: Summary Report of Final Results Accra: Ghana Statistical Service.

Ghose, B., & Yaya, S. (2018). Fruit and vegetable consumption and anaemia among adult non-pregnant women: Ghana Demographic and Health Survey. *Peer Journal*, *6*, e4414.

Gibson, R. S. (2005). Principles of nutritional assessment. 2nd edition. Oxford University Press.

Gibson, R. S. (2007). The Role of Diet- and Host-Related Factors in Nutrient Bioavailability and Thus in Nutrient-Based Dietary Requirement Estimates. *Food and Nutrition Bulletin*, 1(28), 1.

Gibson, R. S. (2011). Strategies for preventing multi-micronutrient deficiencies: a review of experiences with food-based approaches in developing countries. Combating micronutrient deficiencies: food-based approaches. Rome, Italy. CAB International and FAO, 7 – 27.

Gibson, R. S. (2014). Enhancing the performance of food-based strategies to improve micronutrient status and associated health outcomes in young children from poor resource households in low-income countries: Challenges and solutions." In improving diets and nutrition: Food-based approaches. Wallingford, Oxfordshire: Rome, Italy. CAB International and FAO.

Gibson, R. S. Perlas, L., & Hotz, C. (2006). Improving the bioavailability of nutrients in plant foods at the household level. *Proceedings of the Nutrition Society*, 65, 160 – 168.

Gibson, R. S., & Hotz, C. (2001). Dietary diversification/modification strategies to enhance micronutrient content and bioavailability of diets in developing countries. *British Journal of Nutrition*, 85(2), S159 - S166.

Gibson, R. S., Anderson, V. P. (2009). A review of interventions based on dietary diversification or modification strategies with the potential to enhance intakes of total and absorbable zinc. *Food Nutrition Bulletin*, 30(1), S108 - 43.

Gillooly, M. T. H. Bothwell, T. H., Torrance, J. D., & MacPhail, A. P. (2007). The effects of organic acids, phytates and polyphenols on the absorption of iron from vegetables. *British Journal of Nutrition*, 49(3), 331 – 342.

Girerd-Barclay, E., & Tiwari, K. (2002). Prevention and control of nutritional anaemia, a South Asia priority United Nations Children's Fund. Regional Office for Asia.

Glinz, D., Hurrell R. F., Ouattara, M., Zimmermann, M. B., Brittenham, G. M., Adiossan, L. G., Righetti, A. A., Seifert, B., Diakité, V. G., & Wegmüller, T. (2015). The effect of iron-fortified complementary food and intermittent preventive treatment of malaria on anaemia in 12- to 36-month-old children: A cluster-randomized controlled trial. *Malaria Journal*, 14, 347.

Grantham-McGregor, S., & Baker-Henningham, H, (2010). Iron Ddeficiency in childhood: Causes and consequences for child development. *Annales Nestlé*, 68, 105–119.

Gregory J. F., (2012). Accounting for differences in the bioactivity and bioavailability of vitamers. *Food & Nutrition Research*, 56, 10.

Greiner, T. (2017). Combatting vitamin A deficiency: overcoming obstacles to optimize the food-based approach. *World Nutrition*, 8(2).

Gretchen, A. S., Finucane, M. M., De-Regil, L. M., Paciorek, C. J., Flaxman, S. R., Branca, F., Peña-Rosas, J. P., Bhutta, Z. A., & Ezzati, M. (2013). Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995-2011: a systematic analysis of population-representative data. *Lancet Global Health*, 1, 16 – 25.

Grote, V., Verduci, E., Scaglioni, S., Vecchi, F., Contarini, G., & Giovannini, M., Koletzko, B., & Agostoni, C. (2015). Breast milk composition and infant nutrient intakes during the first 12 months of life. *European Journal of Clinical Nutrition*, 70(2).

Grubbens, G. J. H., & Denton, O. A. (2004). Plant Resources of Tropical Africa 2. Vegetables. PROTA Foundation, Wageningen. Netherlands/Backhuys Publishers, Leiden, Netherlands/CTA, Wageningen, Netherlands. 668.

Gupta, S., Gowri, B. S., Lakshmi, A. J., & Prakash, J. (2013). Retention of nutrients in green leafy vegetables on dehydration. *Journal of Food Science and Technology*, 50(5), 918-25.

Habte, D., Asrat, K., Magafu, M. G. M. D., Ali, I. M., Benti, T., Abtew, W., Tegegne, G., Abera, D., & Shiferaw, S. (2013). Maternal risk factors for childhood anaemia in Ethiopia. *African Journal of Reproductive Health / La Revue Africaine de la Santé Reproductive*, 17(3), 110 – 118.

Hallberg, L., Hoppe, M., Anderson, M., & Hulthen, L. (2003). "The role of meat to improve the critical iron balance during weaning." *Pediatrics*, 111(4), 1 864 - 70.

Hannan, C., & Halpin, B. (2014). The Influence of Family Structure on Child Outcomes: Evidence for Ireland. *The Economic and Social Review*, 45. 1 - 24.

Harrison, E. H. (2012). Mechanisms involved in the intestinal absorption of dietary vitamin A and pro vitamin A carotenoid. *Molecular and Cell Biology of Lipids*, 1821(1), 70 – 77.

Haskell, M. J. (2012). The challenge to reach nutritional adequacy for vitamin A: β-carotene bioavailability and conversion-evidence in humans. *American Journal of Clinical Nutrition*, 96, 1193 –1203.

He, F. J., Nowson, C. A., & Macgregor, G. A., (2006). Fruit and vegetable consumption and stroke: meta-analysis of cohort studies. *Lancet*, 367320 – 326.

Hoffman, R. (2017). Iron; Deficiency and Toxicity. Intelligent Medicine Lifestyle, 1, 1 – 5.

Horimoto, Y., & Lim, L. T. (2017). Effects of different proteases on iron absorption property of egg white hydrolysates. *Food Research International*, 95, 108 - 116.

Hu, F. B., Rimm, E. B., Stampter, M. J., Ascherio, A., Spiegelman, D., & Willett, W. C. (2000). Prospective study of major dietary patterns and risk of coronary heart disease in men. *American Journal of Clinical Nutrition*, 72, 912 – 921.

Hudak, L., Jaraisy, A., Haj, S., & Muhsen, K. (2017). An updated systematic review and meta-analysis on the association between Helicobacter pylori infection and iron deficiency anaemia. *Annals of Gastroenterological Surgery - Helicobacter*, 22(1).

Huh, E.C. A., Hotchkiss, J., Brouillette, & Glahn, R. P. (2004). Carbohydrate fractions from cooked fish promote iron uptake by Caco-2 cells. *Journal of Nutrition*, 134, 1681-1689.

Hurrell, R. F. (1997). Bioavailability of iron. *European Journal of Clinical Nutrition*, 51, S4.

Iannotti, L. L., Delnatus, J. R., Odom, A. R., Eaton, J. C., Griggs, J. J., Brown, S., & Wolff, P. B. (2015). Determinants of anaemia and haemoglobin concentration in Haitian school-aged children. *The American Journal of Tropical Medicine and Hygiene*, 93(5), 1092-8.

Iglesias Vázquez, L., Valera, E., Villalobos, M., Tous, M., & Arija, V. (2019). Prevalence of anaemia in children from Latin America and the Caribbean and effectiveness of nutritional interventions: Systematic review and meta¯ analysis. *Nutrients,* 11(1), 183.

Imdad, A., Herzer, K., Mayo-Wilson, E., Yakoob, M. Y., & Bhutta, Z. A. (2010). Vitamin A supplementation for preventing morbidity and mortality in children from 6 months to 5 years of age. *Cochrane Database Systems and Revision*, 12, 8524.

Imdad, A., Yakoob, M. Y., Sudfeld, C., Haider, B. A., Black, R. E., & Bhutta, Z. A. (2011). Impact of vitamin A supplementation on infant and childhood mortality. *BMC Public Health,* 11(3), 20.

Indonesia Ministry of Health. 2017. Indonesia minimum service standards for nutrition: Technical brief for district health administrators. Washington, DC: FHI 360.

Jamil, K. M., Brown, K. H., Jamil, M. (2012). Daily consumption of orange- fleshed sweet potato for 60 days increased plasma β- carotene concentration but did not increase total body vitamin a pool size in Bangladeshi Women. *The Journal of Nutrition*, 142, 1896 – 902.

Jáuregui-Lobera I. (2014). Iron deficiency and cognitive functions. *Neuropsychiatric disease and treatment*, *10*, 2087 – 2095.

Jemberu, Y., Zegeye, M., Singh, P., & Abebe, H. (2017). Consumption of vitamin A rich foods and associated factors among children aged 6 - 23 months in Kachabira District, Southern Ethiopia, *International Journal of Food Science and Nutrition Engineering*, 7(1), 1 - 10.

Ji, X., Cui, N., & Liu, J. (2017). Neurocognitive function is associated with serum iron status in early adolescents. *Biological Research for Nursing*, 19(3), 269-277.

Jimenez, C., Leets I., Puche, R., & Anzola, E. (2010). A single dose of vitamin A improves haemoglobin concentration, retinol status and phagocytic function of neutrophils in preschool children. *British Journal of Nutrition*, 103(6), 798 – 802.

Jimoh, K., & Olatidoye, O. (2009). Evaluation of physicochemical and rheological characteristics of soybean fortified yam flour. *Journal of Applied Bioscience*, 13, 703 – 706.

Johnson, E. J., & Russell R. M. (2011). Beta-carotene. In: Coates P. M., Betz J. M., & Blackman, M. R. Encyclopedia of Dietary Supplements. 2nd edition, London and New York: *Information Healthcare*, 115 – 20.

Johnson-Wimbley, T. D., & Graham, D. Y. (2011). Diagnosis and management of iron deficiency anaemia in the 21st century. *Therapeutic advances in gastroenterology*, 4(3), 177 - 84.

Kapil, R., & Kapil, U. (2018). Determinants of anaemia in South East Asian countries. *Blood Research and Transfusion Journal*, 2575 - 8934.

Karim, O. R., Kayode, R. M. O., Oyeyinka, S. A., & Oyeyinka A. T. (2013). Proximate, mineral and sensory qualities of 'amala' prepared from yam flour fortified with moringa leaf powder. *Food Science and Quality Management*, 12, 10 – 22.

Karim, O., Kayode, R., Oyeyinka, S., & Oyeyinka, A. (2015). Physicochemical properties of stiff dough 'amala' prepared from plantain (Musa Paradisca) flour and Moringa (Moringa oleifera) leaf powder. *Food Health and Disease*, 4, 48 – 58.

Kawarazuka, N., & Béné, C. (2011). The potential role of small fish species in improving micronutrient deficiencies in developing countries: Building evidence. *Public Health Nutrition.* 14, 1927 - 38.

Kelebek, H., Selli, S., Kadiroğlu, P., Kola, O. Kesen, S., & Uçar, B. (2017). Bioactive compounds and antioxidant potential in tomato pastes as affected by hot and cold break process. *Food Chemistry*, 220, 31 – 4.

Kendig, S. M., & Bianchi, S. M. (2008). Single, cohabiting, and married mothers' time with children. *Journal of Marriage and Family*, 70(5), 1228 – 1240.

Khan, M. R., Ahmed, F., Banu, C. P., Qazi, M. R., Akhtaruzzaman, M. (2008). The coexistence of other micronutrient deficiencies in anaemic adolescent school girls in rural Bangladesh. *European Journal of Clinical Nutrition*, 62(3), 365 – 72.

Kishwar, F., Tahira, A., Hanif, I., Asif, H., & Kalsoom, S. (2015). Iron deficiency anaemia; prevalence in children of Lahore. The Professional Medical Journal, 20. 1122 - 1125.

Kokore, B. A., Yopo, B. C. Kouakou, L. K., & Bleyere., M. A. (2013). Haematological status and anaemia prevalence among children aged 5 to 11 years in school canteens in Abidjan (Côte d'Ivoire). *International Journal of Current Pharmaceutical Review and Research*, 5(13), 37– 50

Kopec, R. E., Cooperstone, J. L., Schweiggert, R. M., Young, G. S., Harrison, E. H, Francis, D., M., Clinton, S. K., & Schwartz, S. J. (2014). Avocado Consumption Enhances Human Postprandial Pro-vitamin A absorption and conversion from a novel high β-carotene tomato sauce and from carrots. *Journal of Nutrition,* 14(144), 1158 – 66.

Korenromp, E. L., Armstrong- Schellenberg, J. R. M., Williams, B. G., Nahlen, B. L., & Snow, R., W. (2004). Impact of malaria control on childhood anaemia in Africa – a quantitative review. *Tropical Medicine and International Health,* 9(10), 1050 – 1065.

Kreemer, K., & Zimmermann, M. (2007). Nutritional anaemia: Sight and Life Press Basel.

Krinsky, N. I., Landrum, J. T., & Bone, R. A. (2003). Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. *Annual Review of Nutrition,* 23, 171 – 201.

Kwenin, W. K. J., Wolli, M., & Dzomeku, B. M. (2011). Assessing the nutritional value of some. African Indigenous green leafy vegetables in Ghana. *Journal of Animal and Plant Science,* 10(2), 1300 – 1305.

Lanier, J. B., Park, J. J., Callahan, R. C. (2018). Anaemia in older adults. *American Family Physician,* 98(7), 437 -442.

Lawal, O. O., Essien, N. C., Essien, N. M. & Ochalla, F. (2015). Vitamin C content of some processed green leafy vegetables. *European Journal of Experimental Biology*, 5(2), 110 – 112.

Laxmaiah, A., Nair M. K., Arlappa, N., Raghu, P., Balakrishna, N., & Rao, K. M. (2010). Prevalence of ocular signs, subclinical vitamin A deficiency, and its determinants among rural preschool children in India. *Public Health Nutrition,* 15(4), 568 – 77.

Lazarte, S. S., Mónaco, M. E., Jimenez, C. L., Achem, M. S. C. L., Terán, M. M., & Issé, B. A. (2015). Erythrocyte catalase activity in more frequent microcytic hypochromic anaemia: Beta-thalassemia trait and iron deficiency anaemia. *Advances in Hematology*, 7.

Legason, I. D, Atiku, A. I., Ssenyonga, R., Olupot-Olupot, P., & Barugahare, J. B. (2017). Prevalence of anaemia and associated risk factors among children in North-western Uganda: A Cross Sectional Study. *BMC Hematology*, 17, 10.

Li, Y., Jiang, H., & Huang, G. (2017). Protein hydrolysates as promoters of non-naem iron absorption. *Nutrients,* 9(6), 609.

Liu, R. H. (2013). Health-Promoting Components of fruits and vegetables in the diet. *Articles of Advances in Nutrition,* 4(3), 384 – 392.

Lock, K., Pomerleau, J., Causer, L., Altmann, D. R., & McKee, M. (2005). The global burden of disease attributable to low consumption of fruit and vegetables: implications for the global strategy on diet. World Health Organization, 83, 100 – 108.

Lockyer, s. A. White, J. L. & Buttriss, B. (2018). Biofortified crops for tackling micronutrient deficiencies – what impact are these having in developing countries and could they be of relevance within Europe. *Nutrition Bulletin,* 43, 319 – 357.

Lopez, A., Cacoub, P., Macdougall, I. C., Peyrin-Biroulet, L. (2016). Iron deficiency anaemia. *The Lancet Seminar*, 387, 10021, P907 - 916.

Low, J. W., Arimond, M., & Osman, N. (2007). A food- based approach introducing orange- fleshed sweet potatoes increased vitamin A intake and serum retinol concentrations in young children in rural Mozambique. *The Journal of Nutrition.* 137, 1320 – 7.

Lund, N., Biering-Sørensen, S., Andersen, A., Monteiro, I., Camala, L., Jørgensen, M. J., Aaby, P., Benn, C. S. (2014). Neonatal vitamin A supplementation associated with a cluster of deaths and poor early growth in a randomized trial among low-birth-weight boys of vitamin A versus oral polio vaccine at birth. *BMC Pediatrics,* 14, 214.

Mactier, H., & Weaver, L. T. (2005). Vitamin A and preterm infants: what we know, what we do not know, and what we need to know. *Archive of Disease and Child Fetal Neonatology,* 90, 103 – 8.

Madodé, Y. E., Houssou, P. A., Linnemann, A. R., Hounhouigan, D. J., Nout, M. J. R., & Van Boekel, M. A. J. S. (2011). "Preparation, consumption, and nutritional composition of West African cowpea dishes". *Ecology of Food and Nutrition,* 50, 115 - 136.

Magalhães, R. J. S., & Clements, A. C. A. (2011). Spatial heterogeneity of haemoglobin concentration in preschool-age children in sub-Saharan Africa. *Bulletin of the World Health Organization,* 89, 459 - 68.

Mahajan, S. K., & Aundhakar, S. C. (2015). A Study of the prevalence of serum vitamin B12 and folic acid deficiency in Western Maharashtra. *Journal of Family Medicine and Primary Care,* 4(1), 64 – 68.

Mainasara, A. I., Uko, K., Jiya, E., Erhabor, N., Osaro U., Muhammad, N., Sanusi, S., Garba, M. A., Muhammad, L. S. H., Lukman, S., Onuigwe, L., Danyaro, F. M & Bello, Z. (2017). Prevalence of anaemia among children attending paediatrics department of UDUTH, Sokoto, North-Western Nigeria. *International Blood Research and Reviews*, 7, 1 - 10. 10.9734.

Malin, A. S., Qi, D., Shu, X. O., Gao, Y. T., Friedmann, J. M., Jin, F., & Zheng, W. (2003). Intake of fruits, vegetables and selected micronutrients in relation to the risk of breast cancer. *International Journal of Cancer*, *105*(3), 413–418.

Mandaliya, K., Bwayo, J. J., & McClelland, R. S. (2004). Use of serum retinol-binding protein for prediction of vitamin A deficiency: effects of HIV-1 infection, protein malnutrition, and the acute phase response. *American Journal of Clinical Nutrition*, 79, 218–25.

Manning W. D., & Brown, S. (2006). Children's economic well-being in marriage and cohabiting parent families. *Journal of Marriage and Family*, 68(2), 345 – 62.

Marcelline, U., Umulisa, N., Munyaneza, T., Karema, C., Maniga, J., & Barugahare, J. B. (2016). The impact of malaria and gastointestinal helminthiasis co-infection on aneamia and severe malaria among children in Bugesera District, Rwanda. *International Journal of Tropical Disease and Health*, 13(4), 1–7.

Martinez-Torres, C., & Layrisse, M. (1971). Iron absorption from veal muscle. *American Journal of Clinical Nutrition*, 24, 531 - 540.

Mboho, M., & Bassey, R. (2013). Mothers' variables as determinants of nutritional status of children 0-5 Years in Nsit Ibom Local Government Area of Akwa Ibom State, Nigeria. *Academic Research International*, www.journals.savap.org.pk.

McCauley, M. E., van den Broek, N., Dou, L., & Othman, M. (2015). Vitamin A supplementation during pregnancy for maternal and newborn outcomes. *Cochrane Database of Systematic*, 10.

McDevitt, M. A., Xie, J., Gordeuk, V., & Bucala, R. (2004). The anaemia of malaria infection: The role of inflammatory cytokines. *Current Hematology Report*, 3, 97 – 106.

McKay, D. M., Shute, A., & Lopes, F. (2017). Helminths and intestinal barrier function. *Tissue Barriers*, 5(1), e1283385.

McNeil, C. J., Beattie, J. H., Gordon, M. J., & Pirie, L. P. (2011). Differential effects of nutritional folic acid deficiency and moderate hyperhomocysteinemia on aortic plaque formation and genome-wide DNA methylation in vascular tissue from ApoE-/-mice. *Clinical Epigenetic*, 2, 361 – 368.

McNamara, K., & Wood, E. (2019). Food taboos, health beliefs, and gender: understanding household food choice and nutrition in rural Tajikistan. *Journal of Health Population and Nutrition*, 38, 17.

Mesfin, F., Berhane, Y., & Worku, A. (2015). Anaemia among primary school children in Eastern Ethiopia. *PLoS One*, 10(4), 0123615.

Milman, N. (2011). Anaemia still a major health problem in many parts of the world. *Annals of Hematology*, 90, 369 – 377.

Mondal, R. K., Monoj, M., & Rayhan, S. (2014). The impact of maternal education on child health; Evidence from Bangladesh. *Asian Journal of Social Sciences and Humanities*, 3, 19 - 27.

Morris, M. C., Evans, D. A, Tangney, C. C., Bienias, J. L., & Wilson, R. S. (2006). Associations of vegetable and fruit consumption with age-related cognitive change. *Neurology.* 2467(8), 1370 - 6.

Muhanji, G., Roothaert, R., Webo, C., & Stanley, M. (2011) African indigenous vegetable enterprises and market access for small-scale farmers in East Africa. *International Journal of Agricultural Sustainability*, 9(1), 194 – 202.

Munayco, C. V., Ulloa-Rea, M. E., Medina-Osis J., Castro-Salazar, C., & Munarriz-Villafuerte, J. (2013). Evaluation of the impact of multiple micronutrient powders on children anaemia in three regiones in Peru. *Revista Peruana de Medicina. Experimental Salud Públication*, 30, 229 – 234.

Muthayya, S., Eilander, A., Transler, C., Thomas T., van der Knaap, H. C., & Srinivasan, (2009). Effect of fortification with multiple micronutrients and n´3 fatty acids on growth and cognitive performance in Indian schoolchildren: The CHAMPION (Children's Health and Mental Performance Influenced by Optimal Nutrition) Study. *American Journal of Clinical Nutrition*, 89, 1766 – 1775.

Mwanri, L., Worsley, A., Ryan, P., & Masika, J. (2000). Supplemental vitamin A improves anaemia and growth in anemic school children in Tanzania. *The Journal of Nutrition*, 130, 2691 - 6.

Nair, M. K., Augustine, L. F., & Konapur, A. (2016). Food-based interventions to modify diet quality and diversity to address multiple micronutrient deficiency. *Frontiers in Public Health*, 3, 10 – 3389.

Najati, N., & Gojazadeh, I. M. (2010). Maternal and neonatal complications in mothers aged under 18 years. Patient Prefer Adherence, 4, 219 – 22.

Natesh, N. H., Abbey, L. & Asiedu, S. K. (2017). An overview of nutritional and anti-nutritional factors in green leafy vegetables. *Horticulture International Journal*, 1(2).

National Council for Population and Development and ICF International. 2015. Kenya Demographic and Health Survey 2014. Nairobi, Kenya and Rockville, MD: KNBS and the DHS Program/ICF International.

National Health and Nutrition Examination Survey (NHANES) III (2010). Center for disease control and prevention.

National Institute of Health - NIH, (2016). Strengthening knowledge and understanding of dietary supplement. Pages 1 – 8.

Ngesa, O., & Mwambi, H. (2014). Prevalence and risk factors of anaemia among children aged between 6 months and 14 years in Kenya. *Public Library Science one*, 9(11).

Ngnie-Teta, I., Receveur, O., & Kuate-Defo, B. (2007). Risk factors for moderate to severe anaemia among children in Benin and Mali: insights from a multi-level analysis. *Food and Nutrition Bulletin*, 28(1), 76 – 89.

Njunda, A. L., Fon, S. G., Assob, J. C. N., Nsagha, D. S., Kwenti, T. D. B., & Kwenti, T. E. (2015). Coinfection with malaria and intestinal parasites, and its association with anaemia in children in Cameroon. *Infectious Disease and Poverty*, 4, 43.

Nnamani, C. V., Oselebe, H. O., & Agbatutu, A. (2009). Assessment of nutritional values of three underutilized indigenous leafy vegetables of Ebonyi State, Nigeria. *African Journal of Biotechnology*, 8 (9), 2321 – 2321.

Noguchi Memorial Institute for Medical Research (NMIMR). Procedure for analyzing serum and breast-milk retinol. Department of Nutrition. NMIMR, Legon Ghana 1997.

Nordin, S. M., Boyle, M., & Kemmer, T. M. (2013). Academy of nutrition and dietetics. position of the academy of nutrition and dietetics: nutrition security in developing nations: sustainable food, water, and health. *Journal of the Academy of Nutrition and Dietetics*, 113(4), 581 – 595.

Oboh, G., Ekperigin, & Mutiu, K. (2005). Nutritional and haemolytic property of eggplant (Solanum macrocarpon) leaves. *Journal of Food Composition and Analysis,* 18. 153 – 160.

Obse, N., Mossie, A., Gobena, T. (2013). Magnitude of Anaemia and associated risk factors among pregnant women attending atenatal care in Shella Woreda, West Arsi Zone, Oromia Region, Ethiopia. *Ethiopia Journal of Health and Science*, 23(2), 165 – 73.

O'Byrne, S. M., & Blaner, W. S. (2013). Retinol and retinyl esters: biochemistry and physiology. *Journal of Lipid Research*, 54(7), 1731 - 43.

Odunlade, T. V., Famuwagun, A. A., Taiwo, K. A., Gbadamosi, S. O. Oyedele, D. J., & Adebooye, O. C. (2017). Chemical composition and quality characteristics of wheat bread supplemented with leafy vegetable powders. *Hindawi Journal of Food Quality*, 9536716, 7.

Oelofse, A., Faber, M., Benadé, J. G., Benadé, A. J. S., & Kenoyer, D. G. (1999). The nutritional status of a rural community in KwaZulu-Natal, South Africa: the Ndunakazi project. *Central African Journal of Medicine*, 45, 14 – 19.

Ogunka-Nnoka, C. U., Ekrika, D. (2018). Nutritional profiling of garden egg stalk Solanum macrocarpum L. (Solanales: Solanaceae). *Brazilian Journal of Biological Sciences*, 5(10), 265 - 275.

Oguntibeju, O. (2015). Parasitic Infestation and Anaemia: The Prevalence in a rural hospital setting. *West Indian Medical Journal*. 4.

Oguntibeju, O. O., Truter, E. J., & Esterhuyse A. J. (2013). The role of fruit and vegetable consumption in human health and disease prevention. INTECH. *Diabetes mellitus - insights and perspectives,* (10), 5772 – 50109.

Okaka, J. C., Okaka, A. N. C., (2009). Role of ascorbic acid in the prevention of iron deficiency anaemia. *Biomedical Research*, 16 (1), 40 – 44.

Oladeinde, B. H., Omoregie, R., Olley, M., Anunibe, J. A., Onifade, A. (2012) Malaria and anaemia among children in low resource statesin Nigeria. *Iranian Journal of Parasitology*, 7, 31 - 37.

Oliveira-Menegozzo, J. M., Bergamaschi, D. P., Middleton, P. & East, C. E. (2010). Vitamin A supplementation for postpartum women. *Cochrane Database Systems Review*, 6(10).

Olmedilla-Alonso, B., Cuadrado-Vives, C., Jiménez, S., & Beltrán de Miguel, B. (2011). Vitamin A status in young adults: serum and diet markers and their correlation with visual function. *Annals of Nutrition and Metabolism*, 8(3).

Olorode, O. O., Idowu, M. A., & Ilori, O. A. (2013). Effect of benoil (*Moringa oleifera*) leaf powder on the quality characteristics of 'Ogi'. *American Journal of Food and Nutrition*, 3(2), 83 – 89.

Omelonye, K. E., Okolo, C, A., Nwibo, D. D., Chukwuka, C. O., Onyeka & Nwibo, M. I., & Ubachukwu, P. O. (2015). Prevalence and management of diarrhea among children in Nsukka, Nigeria. *Animal Research International*, 12, 2107 - 2113.

Omer, F. M., Kurtzhals, J. A., Riley, E. M. (2000). Maintaining the immunological balance in parasitic infections: a role for TGF-beta? *Parasitol Today*, 16, 18 – 23.

Oruch, R., & Pryme, I. (2012). The biological significance of vitamin A in humans: A review of nutritional aspects and clinical considerations. Simplex Academic Publishers, 1. 19.

Osei-Boadi, K., Lartey, A., Marquis, G. S., & Colecraft, E. K. (2012). Dietary intakes and iron status of vegetarian and not vegetarian children in selected communities in Accra and Cape Coast, Ghana. *African Journal Food Agriculture Nutrition and Development*, 12(1), 1684 – 5374.

Özdemir, N. (2015). Iron deficiency anaemia from diagnosis to treatment in children. *Turk pediatri arsivi*, 50(1), 11-9.

Ozsoylu, S., & Aytekin, M. N. (2011). Vitamin D deficiency and anaemia. *Annals of Hematology*, 90 (6), 737 – 737.

Paintal, K., & Aguayo, V. M. (2016). Feeding practices for infants and young children during and after common illness. Evidence from South Asia. *Maternal and Child Nutrition*, 12(1), 39-71.

Pearson, D. (1976). Chemical Analysis of Foods. 7th Ed., Church Hill Livingstone, London, UK, 72 - 73, 138 - 143, 488 - 496.

Pem, D., & Jeewon, R. (2015). Fruit and Vegetable Intake: Benefits and progress of nutrition education interventions. *Iran Journal of Public Health*, 44(10), 13, 09 – 21.

Platel, K., & Srinivasan, K. (2016). Bioavailability of micronutrients from plant foods: an update. *Critical Review of Food Sciences and Nutrition*, 56(10), 1608 – 19.

Pomeranz Y., Meloan C.E. (1994) Determination of Moisture. In: Food Analysis. *Springer*, Boston, MA.

PROFAV (2011). Promotion of fruit and vegetables for health. African Regional Workshop Arusha, Tanzania: 26– 30 September.

Pullan, R. L., Gitonga, C., Mwandawiro, C., Snow, R. W., & Brooker, S. J. (2013). Estimating the relative contribution of parasitic infections and nutrition for anaemia among school-aged children in Kenya: a subnational geostatistical analysis. *British Medical Journal*, 3(2).

Ramakrishnan, U. (2002). Prevalence of micronutrient malnutrition worldwide. *Nutrition. Reviews*, 60(2), 46 – 52.

Rameshwar, S. K. V., Udaykumar, P., Balakrishna, N., Vijayaraghavan, K., & Sivakumar, B. (2006). Effect of micronutrient supplementation on health and nutritional status of schoolchildren: growth and morbidity. *Nutrition*, S8 –S14.

Rana, M., Wong-See, D., Katz, T., Gaskin, S., & Lochhead, A. (2014). Fat-soluble vitamins deficiency in children and adolescents with cystic fibrosis. *Journal of Clinical Pathology*, 67(7), 775 – 783.

Rathnayake, K. M., Madushani, P., & Silva, K. (2012). Use of dietary diversity score as a proxy indicator of nutrient adequacy of rural elderly people in Sri Lanka. *BMC Research Notes*, 5, 469.

Reboul E. (2013). Absorption of vitamin A and carotenoids by the enterocyte: focus on transport proteins. *Nutrients*, 5(9), 3563–3581.

Reddy, M. B., Hurrell, R. F., & Cook, J. D. (2006). Meat consumption in a varied diet marginally influences non-haeme iron absorption in normal individuals. *Journal of Nutrition*, 36(3), 576 – 581.

Ribeiro-Silva, R. C, Nunes, I. L, & Assis, A. M. O. (2014). Prevalence and factors associated with vitamin A deficiency in children and adolescents. *Journal of Pediatrics*, 90(5), 486 - 92.

Ricachenevsky, F., K., Vasconceloes, M. W., Johnson, A. A. T., Sperotto, R. A., & Shou, H. (2018). Improving the nutrition content and quality of crops, promises, achievements and future challenges - Current status of biofortified crops. *Frontiers in Nutrition*, 5(12).

Righetti, A. A., Adiossan, L. G., Ouattara, M., Glinz, D., Hurrell, R. F., N'Goran, E. K., Wegmüller, R., Utzinger, J. (2013). Dynamics of anaemia in relation to parasitic infections, micronutrient status, and increasing age in South-Central Côte d'Ivoire. *The Journal of Infectious Diseases*, 207, 1604–1615.

Riso, P., Brusamolino, A., Scalfi L., Porrini, M., & Matrics, P. X. (2004). Bioavailability of carotenoids from spinach and tomatoes. *Journal of Nutrition Metabolism and Cardiovascular Disease*, 14 (3), 150 – 156.

Rivera, J. A., Hotz, C., González-Cossío T., Neufeld, L., & García-Guerra, A. (2003). The effect of micronutrient deficiencies on child growth: A review of results from community-based supplementation trials. *The Journal of Nutrition*, 133(11), 1 4010S – 4020S,

Rodriguez-Amaya, D. B., & Kimura, M. (2004). HarvestPlus handbook for carotenoid analysis. HarvestPlus Technical Monograph Series 2.

Rogers, L. M., Boy, E., Miller, J. W., Green, R., Sabel, J. C., & Allen, L. H. (2003). High prevalence of cobalamin deficiency in Guatemalan schoolchildren: Associations with low plasma holotranscobalamin II and elevated serum methylmalonic acid and plasma homocysteine concentrations. *American Journal of Clinical Nutrition*, 7, 433 – 440.

Roos, N., Wahab, M. A., Chamnan, C., & Thilsted, S. H. (2007). The role of fish in food-based strategies to combat vitamin A and mineral deficiencies in developing countries. *Journal of Nutrition*, 137, 1106 – 1109.

Ross, A. (2006). Vitamin A and Carotenoids. In: Shils M, Shike M, Ross A, Caballero B, Cousins R. Modern nutrition in health and disease. 10th edition Baltimore, MD: Lippincott Williams and Wilkins, 351 – 75.

Ruel, M. T. (2001). Can food-based strategies help reduce vitamin A and iron deficiencies? A review of recent evidence. *International Food Policy Research Institute*, Washington, DC.

Ruel, M. T., Minot, M., & Smith, L. (2005). Patterns and determinants of food consumption in Sub-Saharan Africa. A multicountry comparison. International Food Policy Institute, Washington DC, USA.

Sachdeva, S., Alam, S., Beig, F. K., Khan, Z., & Khalique, N. (2011). Determinants of vitamin A deficiency amongst children in Aligarh District, Uttar Pradesh. *Indian Pediatric Journal*, 48 (17), 853 – 4.

Saeterdal, I., Mora, J. O., & De- Regil, L. M. (2012). Fortification of staple foods with vitamin A for preventing vitamin A deficiency. *Cochrane Database of Systematic Reviews*, 9, 10068.

Sanjoaquin, M. A., & Molyneux, M. E. (2009). Malaria and vitamin A deficiency in African children: a vicious circle? *Malaria Journal*, 8, 134.

Sankar, T.V., Anandan, R., Suseela, M., Asha, K. K., Laxmanan, P. T., Varkey, J., Aneesh, P. A., & Mohanty, B. P. (2013). Chemical composition and nutritional value of Anchovy (Stolephorus commersonii) caught from Kerala coast, India. *European Journal of Experimental Biology*, 3, 85 - 89.

Santos, E. N., Velarde, L. G. C., & Ferreira, V. A. (2010). Associação entre deficiência de vitamin A variáveis socioeconômicas, nutricionais e obstétricas em gestantes. *Ciênc Saúde Colet*, 15(1), 1021 – 30.

Sarpong, N., Owusu-Dabo, E. Kreuels, B. Fobil, J. N., Segbaya, S., Amoyaw, F. Hahn, A., Kruppa, T., & Jürgen M. (2015). Prevalence of malaria parasitaemia in school children from two districts of Ghana earmarked for indoor residual spraying: a cross-sectional study. *Malaria Journal*, 14, 260.

Schippers, R. R. (2002). African indigenous vegetables: An overview of the cultivated species. Chatham, UK: Natural Resources Institute /ACP-EU Technical Centre for Agricultural and Rural Cooperation.

Siva, P. M., Sobha, A., & Manjula, V. D. (2016). Prevalence of anaemia and its associated risk factors among adolescent girls of Central Kerala. *Journal of Clinical and Diagnostic Research*, 10(11), LC19 – LC23.

Schreinemachers, P., Patalagsa, M. A., & Uddin, M. N. (2016). Impact and cost-effectiveness of women is training in home gardening and nutrition in Bangladesh, *Journal of Development and Effectiveness*, 8(4), 473 – 488.

Scrimshaw, N. S., & Wallerstein, M. B. (2012). Nutrition policy implementation: Issues and xeperience. Plenum press. New York and London.

Semba, R. D., & Bloem, M. W. (2002). The anaemia of vitamin A deficiency: epidemiology and pathogenesis. *European Journal of Clinical Nutrition*, 56, 271 – 281.

Semba, R. D., & Bloem, M. W. (2008). Nutrition and health in developing countries. Totowa, USA: Humana Press, 2008.

Semedo, R. M. L., Santos, M. M. A. S., Baião, M. R., Luiz, R. R., & da Veiga, G. V. (2014). Prevalence of anaemia and associated factors among children below five years of age in Cape Verde, West African. *Journal of Health, Population and Nutrition*, 32(4), 646 – 657.

Senthamarai, M., Shankar, J. A. R. P., & Majeed, N. A. (2015). Prevalence of anaemia and malnutrition in children and adolescent in selected orphanages in rural areas of Salem district, rural medicine. *Indian Journal of Rural Health Care*, 91.

Singh, V., & West, K. P. Jr. (2004). Vitamin A deficiency and xerophthalmia among school-aged children in Southeastern Asia. *European Journal Clinical Nutrition*, 58(10), 1342 - 9.

Sinha, A., Jonas, J. B., Kulkarni, M., & Nangia, V., (2011). Vitamin A deficiency in school children in urban central India: The central India children eye study. *Archive of Ophthalmology*, 129(8), 1095 – 1096.

Sivakumar, B., & Reddy, V. (1998). Absorption of labelled vitamin A in children during infection. *British Journal of Nutrition*, 27, 299 – 304.

Slavin, J. L., & Lloyd, B. (2012). Health benefits of fruits and vegetables. *Advances in nutrition (Bethesda, Md.)*, 3(4), 506–516.

Solomons, N. W. (2006). Vitamin A. In: Bowman B, Russell R, eds. Present knowledge in nutrition. 9th edition Washington, DC: *International life Sciences Institute*, 157 – 83.

Sommer, A. (2008). Vitamin A deficiency and clinical disease: A historical overview. *Journal of Nutrition*, 138, 1835 – 1839.

Sommer, A., & Davidson, F. R. (2002). Assessment and control of vitamin A deficiency: The Annecy accords. *Journal of Nutrition*, 132, 2845 – 2850.

Stevens, G. A., Finucane, M. M., De-Regil, L. M., & Paciorek, C. J. (2017). Prevalence of anaemia and severe anaemia in young children. *Pakistan Journal of Medical Sciences*, 1(1), 16 – 25.

Stevens, G. A., Finucane, M. M., De-Regil, L. M., Paciorek, C. J., Flaxman, S. R., & Branca, F. (2013). Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995 – 2011: a systematic analysis of population-representative data. *Lancet Global Health*, 1, 16 – 25.

Sumbele, I. U., Sama, S. O., Kimbi, H. K., & Taiwe, G. S. (2016). Malaria, moderate to severe anaemia, and malarial anaemia in children at presentation to hospital in the Mount Cameroon area: A Cross-Sectional Study. *Anaemia*, 5725634.

Syed, S., Addo, O. Y., De la Cruz-Góngora, V., Ashour, F. A., Ziegler, T. R., & Suchdev, P. S. (2016). Determinants of Anaemia among school - aged children in Mexico, the United States and Colombia. *Nutrients*, 8(7), 387.

Szajewska, H., Ruszczynski, M., & Chmielewska, A. (2010). Effects of iron supplementation in non-anemic pregnant women, infants, and young children on the mental performance and psychomotor development of children: a systematic review of randomized controlled trials1–3. *The American Journal of Clinical Nutrition*, 91, 1684 – 90.

Tacon, A. G. J., & Metian, M. 2009. Fishing for aquaculture: non-food use of small pelagic forage fish–a global perspective. *Reviews in Fisheries Sciences*, 17 (3), 305 – 317.

Tanumihardjo, S. A., Permaesih, D., Dahro, A. M., Rustan, E., Muhilal, Karyadi, D., & Olson, J. A. (2002). Comparison of vitamin A assessment techniques in children from two Indonesian villages. *American Journal of Clinical Nutrition*, 60, 136 – 141.

Tata, C. Y., Ickowitz, A., Powell, B., & Colecraft, E. K. (2019). Dietary intake, forest foods, and anaemia in Southwest Cameroon. *PLoS ONE*, 14(4), e0215281.

Teller, H., & Yimar, G. (2000). Levels and determinants of malnutrition in adolescent and adult women in southern Ethiopia. *Ethiopian Journal of Health Development*, 14(1), 57 – 66.

ter Borg, S., Verlaan, S., Mijnarends, D. M., Schols, J. M. G. A., de Groot L. C. P. G. M., Luiking, Y. C. (2015). Macronutrient Intake and Inadequacies of Community-Dwelling Older Adults, a Systematic Review. *Annals of Nutritional and Metabolism*, 66, 242 - 255.

Tetens, I., Bendtsen, K. M., Henriksen, M., Ersboll, A.K., Milman, N. (2007). The impact of a meat- versus a vegetable-based diet on iron status in women of childbearing age with small iron stores. *European Journal of Nutrition*, 46, 439 – 445.

The Administrative Committee on Coordination and Sub-committee on Nutrition (ACC/SCN) 2000. Fourth report on the world's nutrition situation. In collaboration with IFPRI, Geneva.

Thiam, S., Diène, A. N., Fuhrimann, S., Winkler, M. S., Sy, I., Ndione, J. A., Schindler, C., Vounatsou, P., Utzinger, J., Faye, O., & Cissé, G. (2017). Prevalence of diarrhoea and risk factors among children under five years old in Mbour, Senegal: a cross-sectional study. *Infectious diseases of poverty*, 6(1), 109.

Thilsted, S. H., James, D., Toppe, J., Subasinghe, R., & Karunasagar, I. (2014). Maximizing the contribution of fish to human nutrition. Second International Conference on Nutrition ICN2. FAO, WHO.

Thompson, B., & Amoroso, L. (2011). Combating micronutrient deficiencies: Food-based approaches. CAB and Food and Agriculture Organization of the United Nations, Rome, Italy.

Thuret, I. (2017). Biological diagnosis of iron deficiency in children. *Archives of Paediatrics*, 24(5S), 5S5 – 5S13.

Tolentino, K., & Friedman, J. F. (2007). An update on anaemia in less developed countries. *American Journal of Tropical Medicine and Hygiene*, 77(1), 44 – 51.

Torheim, L., Barikmo, I. Parr, L. C., & Hatløy, A. (2003). Validation of food variety as an indicator of diet quality assessed with a food frequency questionnaire for Western Mali. *European Journal of Clinical Nutrition*, 57, 1283 – 91.

Tucker, K. L., Hallfrisch, J., Qiao, N., Muller, D., Andres, R., & Fleg, J. L. (2005). The combination of high fruit and vegetable and low saturated fat intakes is more protective against mortality in aging men. *Journal of Nutrition*, 135, 556 – 5561.

Turgeon, O, H., Blanchet, R., Gagné, D., Lauzière, J., & Vézina, C. (2016). Using soluble transferrin receptor and taking inflammation into account when defining serum ferritin cutoffs improved the diagnosis of iron deficiency in a group of Canadian preschool inuit children from Nunavik. *Anaemia*, 643, 02 – 14.

Udoidung, N. I., Opara, D. C., Okon, O. K., & Opara K. N. (2015). The Impact of intestinal parasitic infections on the nutritional status of rural and urban school-aged children in Nigeria. *International Journal of MCH and AIDS*, 1, 73 – 82.

Underwood, B. A. (2004). Vitamin A deficiency disorders: international efforts to control a preventable pox. *Journal of Nutrition*, 134, 231 - 6.

United Nation Standing Committee of Nutrition. (2004). Nutrition for improved development outcomes. Proceeding of the 5th report on the world nutrition situation. Chapter 1. Geneva: SCN.

United Nations Children's Fund (2012). The state of the world's children 2012: Children in an urban world. United Nations Plaza, New York, NY 10017, USA.

United Nations Children's Fund Data (2013). Vitamin A deficiency. https://data.unicef.org Statistics by Topic Nutrition.

United Nations Children's Fund, United Nations University, World Health Organization (2001). Iron deficiency anaemia assessment, prevention, and control: a guide for programme managers. Geneva: World Health Organization, (WHO/NHD/01.3).

United Nation Standing Committee of Nutrition. (2004). Nutrition for improved development outcomes. Proceeding of the 5th Report on the World Nutrition Situation. Chapter 1. Geneva: SCN.

Usunobun, U., & Igwe, V. C. (2016). Phytochemical analysis, mineral composition and in vitro antioxidant activities of Solanum macrocarpon leaves. *International Journal of Health*, 4, 62 - 65.

Válka, J., & Čermák J. (2018). Differential diagnosis of anaemia. *Vnitr Lekarstvi Summer*, 64(5), 468 - 475.

Van Het Hof, K. H., de Boer, B. C. J., Tijburg, L. B. M., Lucius, B. R. H. M., Zijp, I., & West, C. E. (2000). Carotenoid bioavailability in humans from tomatoes processed in different ways determined from the carotenoid response in the triglyceride-rich lipoprotein fraction of plasma after a single consumption and in plasma after four days of consumption. *Journal of Nutrition*, 130, 1189 – 1196.

Vanbuskirk, M. K., Ofosu, A. Amy, K & Donna, M. D. (2014). Pediatric anaemia in rural Ghana: A cross-sectional study of prevalence and risk factors. *Journal of Tropical Pediatrics*, 60(10), 10.1093.

VAST (1993). Vitamin A supplementation in Northern Ghana: effects on clinic attendances, hospital admissions, and child mortality. Ghana VAST study team. *Lancet*, 342(8865), 2503, 7 - 12.

West, K. P., & Mehra, S. (2009). Vitamin A intake and status in populations facing economic stress of nutrition sales. *The Journal of Nutrition*, 140, 201.

West, K. P., (2002). Extent of vitamin a deficiency among preschool children and women of reproductive age. *Journal of Nutrition*, 132(9), 2857S – 66S.

West, K. P., Christian, P., Labrique, A. B., Rashid, M., Shamim, A. A., Klemm, R. D., Massie, A. B., Mehra, S., Schulze, K. J., Ali, H., Ullah, B., Wu, L. S., Katz, J., Banu, H., Akhter, H. H. & Sommer, A. (2011). Effect of vitamin A or beta carotene supplementation on pregnancy-related mortality and infant mortality in rural Bangladesh: a cluster randomized trial. *JAMA*, 305(19), 1986 - 95.

Wirth, J. P., Petry, N., Tanumihardjo, S. A., Rogers, L. M., McLean, E., Greig, A., Garrett, G. S., Klemm, R. D. W., & Rohner, F. (2017). Vitamin A supplementation programs and country-level evidence of Vitamin A deficiency. *Nutrients*, 9(3), 190.

Wirth, J. P., Woodruff, B. A., Engle-Stone, R., Namaste, S. M., Temple, V. J., Petry, N., Macdonald, B., Suchdev, P. S., Rohner, F., Aaron, G. J. (2017). Predictors of anaemia in women of reproductive age: Biomarkers reflecting inflammation and nutritional determinants of anaemia (BRINDA) project. *The American Journal of Clinical Nutrition*, 106(1), 416S-427S.

Wiwanitkit, (2007). Tropical Anaemia. Nova Science Publishers, Inc. New York.

Wodaye, A. Y., Beyene, A. S., & Roba, H. S. (2016). Prevalence of xerophthalmia and associated factors among school age children of Fadis, Oromia regional state, Eastern Ethiopia: School based cross-sectional study. *Journal of Public Health and Epidemiology*, 8(9), 175 - 183.

World Bank. (2017). Country literacy rate, adult female (% of females ages 15 and above) UNESCO Institute for Statistics. World. East Asia & Pacific. Europe & Central Asia. https://data.worldbank.org/indicator/

World Health Organization, (2001). Iron deficiency anaemia: assessment, prevention, and control. A guide for programme managers. Geneva: World Health Organization, 114. (Technical Report WHO/NHD/01.3).

World Health Organization, (2006). WHO Child growth standards: Training course on child growth assessment, interpreting growth Indicators. Geneva, World Health Organization. 1 – 46.

World Health Organization, (2008). Worldwide prevalence of anaemia 1993-2005: WHO global database on anaemia. Bruno de Benoist, Erin McLean, Ines Egli and Mary Cogswell. Geneva: World Health Organization.

World Health Organization, (2009). Global prevalence of vitamin A deficiency in populations at Risk 1995 – 2005: WHO global database on vitamin A deficiency. Geneva.

World Health Organization, (2011). Serum retinol concentrations for determining the prevalence of vitamin A deficiency in populations. Vitamin and mineral nutrition information system. Geneva.

World Health Organization (2011). The global prevalence of anaemia. WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland.

World Health Organization, (2017). Vitamin A supplementation in infants and children 0-59 months of age.

World Health Organization (2018). Healthy diet. https://www.who.int/news-room/factsheets/detail/healthy-diet).

World Health Organization, UNICEF (2004). Focusing on anaemia: towards an integrated approach for effective anaemia control: joint statement by the World Health Organization and the United Nations Children's Fund. 1(2), 1.

World Health Organization, UNICEF/UNU (2015): Iron deficiency anaemia: assessment, prevention and control, a guide for programme managers. Geneva: World Health Organization; 2001. WHO/NHD/01.3.

Woyengo, T., & Nyachoti, C. (2013). "Review: Anti-nutritional effects of phytic acid in diets for pigs and poultry–current knowledge and directions for future research." *Canadian Journal of Animal Science*, 93(1), 9 – 21.

Yakwezi, S. N. (2012). European commission, research directorate confirming the international role of community research. The contribution of simple agro-processing industries. 7 (1), 2 – 7.

Yamaguchi, M. (2012). Role of carotenoid β-cryptoxanthin in bone homeostasis. *Journal of Biomedical Science*, 19, 36 – 51.

Yamamura, C. M., Sullivan, K. M., Frits van der Haar, Auerbach, S. B., & Iohpd, K. K. (2004). Risk Factors for vitamin A deficiency among preschool aged children in Pohnpei, Federated States of Micronesia. *Journal of Tropical Pediatrics*, 50(1).

Young, I., Parker, H. M., Rangan, A., Prvan, T., Cook, R. L., Donges, C. E., Steinbeck, K. S., O'Dwyer, N. J., Cheng, H. L., Franklin, J. L., & O'Connor, H. T. (2018). Association between Haem and non-haem iron intake and serum ferritin in healthy young women. *Nutrients*, 10(1), 81.

Yudhistira, B., Affandi, D. R., & Nusantari, P. N. (2017). Effect of green spinach (*Amaranthus tricolor L.*) and tomato (*Solanum lycopersicum*) addition in physical, chemical, and sensory properties of marshmallow as an alternative prevention of iron deficiency anaemia. IOP Conf. Series: *Earth and Environmental Science*, 102 (2018) 012007.

Zavaleta, N., Loza, D., Egoavil, P., Sanchez J., Mosqueira, R., & Neufeld, L. (2013). Efficacy of different schemes of supplementation with micronutrient powders on anaemia and micronutrient status in infants. *FASEB Journal*, 27, 36 – 38.

Zavoshy, R., Noroozi, M., Jahanihashemi, H., & Kiamiri, D. (2012). Nutritional intervention on malnutrition in 3 - 6 years old rural children in Qazvin Province, Iran. *Pakistan Journal of Biological Sciences*, 15, 347 - 352.

Zeitlin M. F., Megawangi R., Mara Kramer E. and Armstrong H. C. (1992). Mothers and children's intakes of vitamin A in rural Bangladesh. *American Journal of Clinical Nutrition*, 56, 136 – 147.

Zijp, I. M., Korver, O., & Tijburg, L. B. (2000). Effect of tea and other dietary factors on iron absorption. *Critical Reviews in Food Science and Nutrition*, 40(5), 371 – 398.

Zimmermann, M. B., Chaouki, N., & Hurrell, R. F. (2005). Iron deficiency due to consumption of a habitual diet low in bioavailable iron: A longitudinal cohort study in Moroccan children. *AJCN*, 81, 115 – 121.

Zuma, M. K., Kolanisi, U., & Modi, A. T. (2018). The potential of integrating pro-vitamin A-bio-fortified maize in smallholder farming systems to reduce malnourishment in South Africa. *International Journal of Environmental Research and Public Health*, 15(4), 805.

APPENDICES

Appendix A: Map of Adaklu District with study area

Appendix B: Field Questionnaires

Effect of fish fortified Amaranthus cruentus and Solanum macrocarpon powder on anaemia and vitamin A status of 4 to 8 - year old school children in Kodzobi, Adaklu District, Volta Region.

Semi-structured field questionnaire for socio-economic health and dietary data gathering

Participant ID No: …… Date of Interview:…….

Questionnaire number:…….. Community name:…….

A. Socio- demographic information

Child data

1. Child name:…… 2. Child sex: a. Male [ ] b. Female [ ]

3. Child age: …… 4. Date of birth (DOB):…….

5. Source of verification of DOB (e.g. birth certificate, growth monitoring booklet)

……..

6. Name of school:…….....

7. Class:……

8. Number of siblings: …….....

Father Data

9. Age: ……

10. Marital status:
    a. Single [ ]
    b. Married [ ]
    c. Separated [ ]
    d. Divorced [ ]
    e. Widowed [ ]
    f. Other (specify) ……..

11. Number of wives or partners: ……

12. Educational status:
    a. Primary [ ]
    b. JHS/Middle school [ ]
    c. SHS [ ]
    d. Training college [ ]
    e. Vocational/Technical [ ]
    f. University [ ]
    g. No formal education [ ]

13. Primary occupation:
    a. Fisherman [ ]
    b. Trader [ ]
    c. Teacher [ ]
    d. Farmer [ ]
    e. Other (specify)…….

14. Secondary occupation:……..

15. Religion:……

16. Ethnicity: a. Ewe [ ] b. Akan [ ] c. Ga [ ] d. Fante [ ] e. other (specify)……

17. Monthly income: ……..

18. Telephone number……..

Mother Data

19. Age: ……

20. Marital status:
    a. Single [ ]
    b. Married [ ]

c.Separated [ ]

d. Divorced [ ]

e. Widower [ ]

f. Other (specify) ……..

21. Educational status:

a. Primary [ ]

b. JHS/Middle school [ ]

c. SHS [ ]

d. Training college [ ]

e. Vocational/Technical [ ]

f. University [ ]

g. No formal education [ ]

22. Primary occupation:

a. Fisherman [ ]

b. Trader [ ]

c. Teacher [ ]

d. Farmer [ ]

e. Other (specify)…….

23. Secondary occupation:……

24. Religion: …….

25. Ethnicity: a. Ewe [ ] b. Akan [ ] c. Ga [ ] d. Fante [ ] e. other (specify)……

26. Monthly income:…….

27. Telephone number……..

B. Child Health/ Nutrition

29. When your child is sick, where do you usually take him? a. Hospital/ Health Center [ ]

b. Native doctor [ ] c. Self Medicate at home [ ] d. Prayer camp [ ]

e. Other (specify)……..

30. Has your child been ill in the past two weeks or past one month? a.Yes [ ] b. No [ ]

*(If **Yes**, continue, if **No**, skip to question 33)*

31. If yes, where was your first point of call? A. Hospital/ Health Center [ ] b. Native doctor [ ] c. Self Medicate at home [ ] d. Prayer camp [ ] e. Other (specify)……..

32. If yes, what was the disease? a Malaria [ ] b. Diarrhea [ ] c. Dysentery [ ] d. Catarrh [ ] e. Other (Specify) …….

33 Has your child received anti-malaria medicine in the past 4 months? a.Yes []

b.No [ ]

34. Has your child received Vitamin A capsules before? a. Yes [ ] b. No [ ]

*(If **Yes**, continue, if **No**, skip to question 37)*

35. What was the colour of capsule he/she received? a. Blue [ ] b. Red [ ] c. other, specify.

36. When did he / she receive the last one?

a. One month ago [ ]

b. Three months ago [ ]

c. Six months ago [ ]

d. More than six months ago [ ]

37. Is your child receiving iron supplement? a. Yes [ ] b. No [ ]

*(If **Yes**, continue, if **No**, skip to question 39)*

38. If 'yes', when was the last one?

a. In less than one month [ ]

b. Between one and two months ago [ ]

c. more than two months ago [ ]

39. Has your child received de-wormer in the last 3 months? a. Yes [ ] b. No [ ]

40. For meal preparation, what kind of salt do you use at home? a. Rock salt [ ] b. Annapurna [ ] c. Other (specify)……..

41. Does your child sleep inside an insecticide treated mosquito net? a. Yes [ ] b. No [ ]

42. What is the sleeping time for your child in the evening? ……

43. Does your child have Health Insurance Card? a. Yes [ ] b. No [ ]

C. Water and Sanitation

44. What is your drinking water source?

a. Bore hole []

b. Well [ ]

c. Dam [ ]

d. Tap [ ]

e. Others (specify)……..

45. How do you dispose off your refuse? a. Refuse damp [ ] b. Dust bin [ ] c. Dugout pit [ ] d. other (specify)……

46. Where do you ease yourself? a. KVIP [ ] b. Water closet [ ] c. Free range pit [ ] d. Pit latrine [ ] e. other (specify)……

47. Observe the house for the presence of the following:

a. Trapdoor……. a. Yes [ ] b. No [ ]

b. Net on windows……. a. Yes [ ] b. No [ ]

c. Covered stored water…… a. Yes [ ] b. No [ ]

d. Mosquito larvae in stored water…… a. Yes [ ] b. No [ ]

D. Cultural practices

48. Do you have any food taboos in your community (especially children)?

a. Yes [ ] b. No [ ]

If Yes, what are these foods?

1…… 2……

3…… 4…….

49. Why are children forbidden from eating these foods? (Write reason for each food listed).

1……

2……..

3…….

4……

50. What are the social amenities available in this community?

a. Electricity: …… a. Yes [ ] b. No [ ]
b. Pipe – borne water …… a. Yes [ ] b. No [ ]
c. Toilet…….. ….. a. Yes [ ] b. No [ ]
d. Market …….. a. Yes [ ] b. No [ ]
e. Health facility…….. a. Yes [ ] b. No [ ]

E Food Security

51. How many meals do you have in a day? a. Once [ ] b. Twice [ ] c. Thrice [ ]
d. More than thrice [ ]

52. How often do you buy foods stuff from the market? a. Daily [ ] b. weekly [ ]
    c. monthly [ ] d. others, specify …….
53. Do you have a backyard garden? a. Yes [ ] b. No [ ].
54. If yes. what vegetables do you grow in your garden?
    a. Okro [ ] b. Gboma [ ] c. Aleefi/fotete [ ] d. Nkontomire [ ] e. Ayoyo/Sigri [ ] f. Tomato [ ] f. Pepper [ ] h. Garden eggs [ ] i. Other (specify)…….

F: Food frequency questionnaire for School Child

Food group	**Never**	**Daily**	**No. of times week**	**Monthly (…… /31)**	**Seldom (…. /per …..)**	**Yearly (/12)**
Animal Products						
• Fish						
• Milk						
• Egg						
• Poultry						
• Meat						
• Wole/ayikoko						
• Crab						
• mushroom						
• snail						
Dark Green leafy Vegetables						
• Ayoyo						
• Nkotomire						
• Allefu						
• Gboma						
• Garden eggs						
• Tomatoes						
• Bean leaf						
• Carrot						
• Cabbage						
• Okro						
• Cassava leaves						
Fruits						
• Mango						
• Pawpaw						
• Orange						
• Banana						
• Guava						
• Watermelon						
• Pineapple						
• Coconut						

• Pear						
• Tangerine						
Cereals /Grain						
• Maize						
• Rice						
• Millet						
• Guinea corn						
Legumes						
• Cowpea						
• Groundnuts						
• Soybeans						
• Bambara beans						
Roots and Tubers						
• Cassave						
• Cocoyam						
• Yam						
• Plantain						
• Sweet potato						
Fat and Oil						
• Palm oil						
• Palm kernel oil						
• Groundnut						
• Shea butter						
• Coconut oil						
• Margarine						
• Soya oil						
• Vegetable oil						
Others						
• Toffees						
• Ice cream						
• Biscuit						
• Meatpie						
• Koose/agawu						
• Roasted plantain						
• Fried plantain						
• Roasted cassava						
• Vitamins, herbs and other supplements						
Drinks						
• Asana						
• Soobolo						
• Fruit juice						
• Coke, fanta etc						

G: 24-hour dietary recall

Please tell me all the foods **your child** ate at home and at school in the past 24 hours. I will ask you to estimate the quantities of each meal your child ate.

Meal	**Time (am/pm)**	**Description of food or drink**	**Quantity**		
			Cost (Ghȼ)	**Household measure**	**Amount (g / ml)**
Breakfast					
Snack					
Lunch					
Snack					
Supper					
Snack					

Effect of fish fortified Amaranthus cruentus and Solanum macrocarpon powder on anaemia and vitamin A status of 4 to 8 - year old school children in Kodzobi, Adaklu District, Votal Region.

Anthropometric data sheet

Child ID number:……. Date of measurement:……..

Name of child:……. Interviewer's code:…….

Weight (kg): i: ……... ii: …….. iii: …… Average: ……

Height (cm): i: ……... ii: …….. iii: …… Average: ……

Name/Signature of interviewer: ……..

Checked by: ……..

Date:…….

Appendix C. Nutrient composition of powders

Powder	Nutrient composition of intervention powders per 100g						
	Moisture %	Ash %	Protein (g)	Fat (g)	Iron (mg)	Zinc (mg)	Carotenoids (µg)
FACSMP	7.7 ± 0 .4	23.7 ± 0.3	33.4 ± 1.3	4.5 ± 0.8	82.6 ± 1.8	5. 9 ± 0.2	6299.1± 0.4
ACSMP	8.0 ± 0.2	20.7 ± 0.4	29.9 ± 0.9	4.2 ± 0.4	78.0 ± 2.9	5.7 ± 0.1	6331.0 ± 0.2

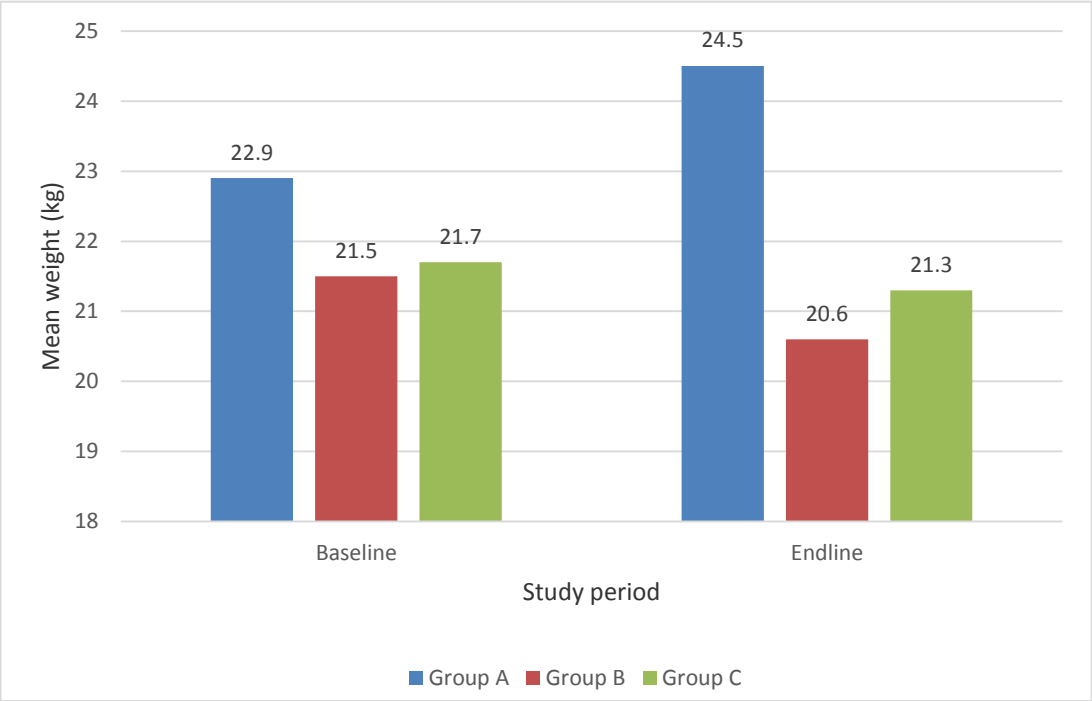
FACSMP: Fish fortified *Amaranthus cruentu* and *Solanum macrocarpon* powder; ACSMP: *Amaranthus cruentus* and *Solanum macrocarpon* Powder

Appendix D: Biochemical and Anthropometric variables of study participants without Anaemia at baseline

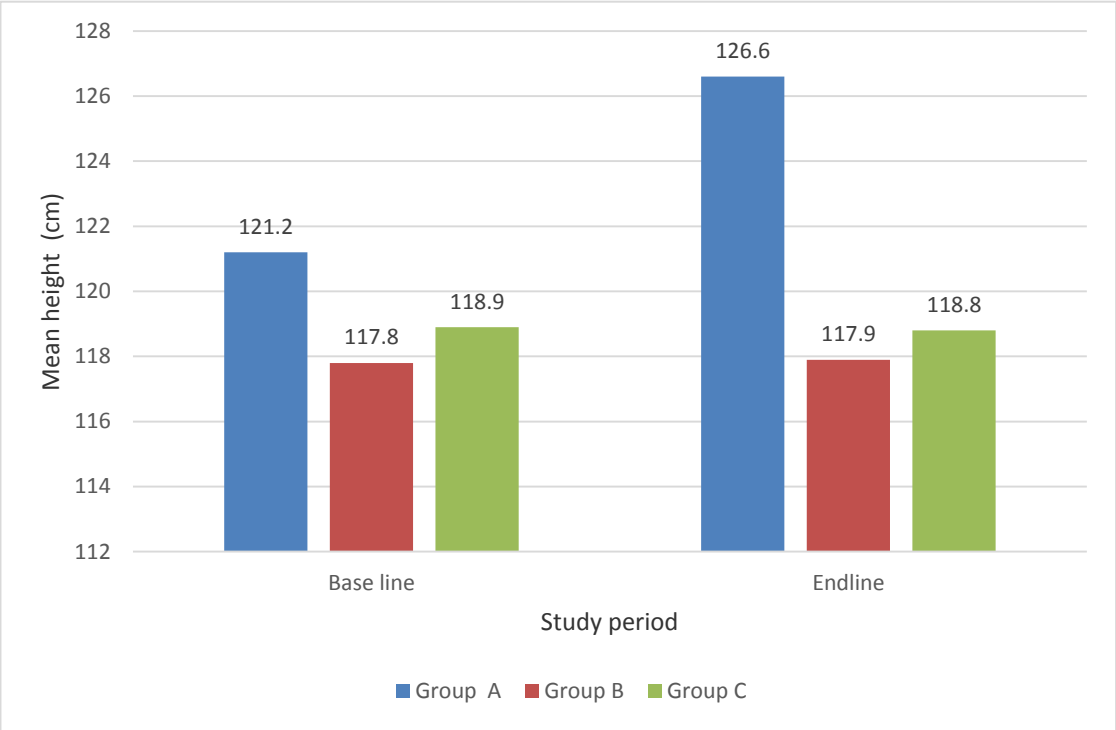
Variable	Group A	Group B	Group C	p-value*
Mean haemoglobin concentration g/dl (mean ± SD)				
Baseline	12.45 ± 0.59	12.30 ± 0.96	12.23 ± 0.66	0.662
End line	12.56 ± 1.45	12.25 ± 1.33	12.00 ± 1.18	0.412
Difference	0.11 ± 0.86	-0.05 ± 0.37	-0.23 ± 0.52	
Prevalence of anaemia (Haemoglobin conc. <11.5g/dl) n (%))				
Baseline	40 (0.00)	25 (0.00)	31 (0.00)	0.662
End line	7 (16.67)	12 (48.00)	9 (29.03)	0.412
Difference	-33 (-82.50)	-13 (-52.00)	-22 (-70.97)	
Mean serum retinol concentration (µg/dl)				
Baseline	22.76 ± 6.78	23.79 ± 5.19	22.65 ± 7.90	0.877
End line	27.67 ± 7.52	25.77 ± 5.01	24.19 ± 6.06	0.240
Difference	4.91 ± 0.74	1.98 ± 0.18	-1.54 ± 1.84	
Prevalence of VAD (serum retinol concentration <20 µg/dl) n (%)				
Baseline	10 (100.00)	5 (100.00)	9 (100.000)	0.916
End line	2 (20.00)	2 (40.00)	7 (77.78)	0.166
Difference	-8 (-80.00)	-3 (-60.00)	-2 (-22.22)	
Effectiveness (%)	-80.00	-60.00	-22.22	
Prevalence of malaria n (%)				
Baseline	17 (100.00)	5 (100.00)	9 (100.00)	0.330
End line	3 (17.65)	3 (60.00)	2 (22.22)	0.693
Difference	-14 (-82.35.0)	-2 (-40.00)	-7 (-77.78)	
Mean weight (kg) Mean ± SD				
Baseline	23.42 ± 3.88	23.04 ± 5.02	21.94 ± 4.41	0.598
End line	24.96 ± 4.12	22.61 ± 4.58	21.61 ± 5.10	0.065
Difference	-1.54 ± 0.24	-0.43 ± 0.44	-0.33 ± 0.69	
Height (cm) Mean ± SD				
Baseline	122.50 ± 7.77	119.59 ± 12.10	117.26 ± 12.98	0.310
End line	126.90 ± 9.81	120.49 ± 10.82	118.09 ± 12.91	0.040
Difference	4.40 ± 2.04	0.90 ± 1.28	0.83 ± 0.07	
Z-scores				
WAZ				
Baseline	-0.07 ± 0.76	-0.17 ± 0.93	-0.62 ± 0.86	0.109
End line	-0.19 ± 0.83	-0.45 ± 0.90	-0.77 ± 0.87	0.112
Difference	-0.12 ± 0.07	-0.28 ± 0.03	-0.15 ± 0.01	

HAZ				
Baseline	-0.03 ± 1.14	-0.52 ± 1.18	0.82 ± 1.21	0.104
End line	-0.03 ± 1.12	-0.51 ± 1.05	-0.93 ± 1.17	0.045
Difference	0.00 ± 0.02	0.01 ± 0.13	-1.75 ± 0.04	
BMIZ				
Baseline	0.01 ± 0.74	0.16 ± 0.84	-0.21 ± 0.82	0.415
End line	-0.14 ± 0.67	-0.22 ± 0.93	-0.28 ± 0.88	0.859
Difference	-0.15 ± 0.07	-0.38 ± 0.09	-0.07 ± 0.06	
Underweight (WAZ < -2SD) n (%)				
Baseline	0 (0.00)	2 (100.00)	3 (100.00)	0.283
End line	0 (0.00)	2 (100.00)	3 (100.00)	0.283
Difference	0 (0.00)	0 (0.0)	0 (0.0)	
Stunting (HAZ < -2SD) n (%)				
Baseline	0 (0.00)	2 (100.00)	9 (100.00)	0.016
End line	0 (0.00)	2 (100.00)	6 (66.67)	0.047
Difference	0 (0.00)	0 (0.00)	3 (33.33)	
Wasting (BAZ < -2SD) n (%)				
Baseline	0 (0.00)	0 (0.00)	2 (100)	0.351
End line	0 (0.00)	0 (0.00)	2 (100)	0.351
Difference	0 (0.00)	0 (0.00)	0 (0.00)	

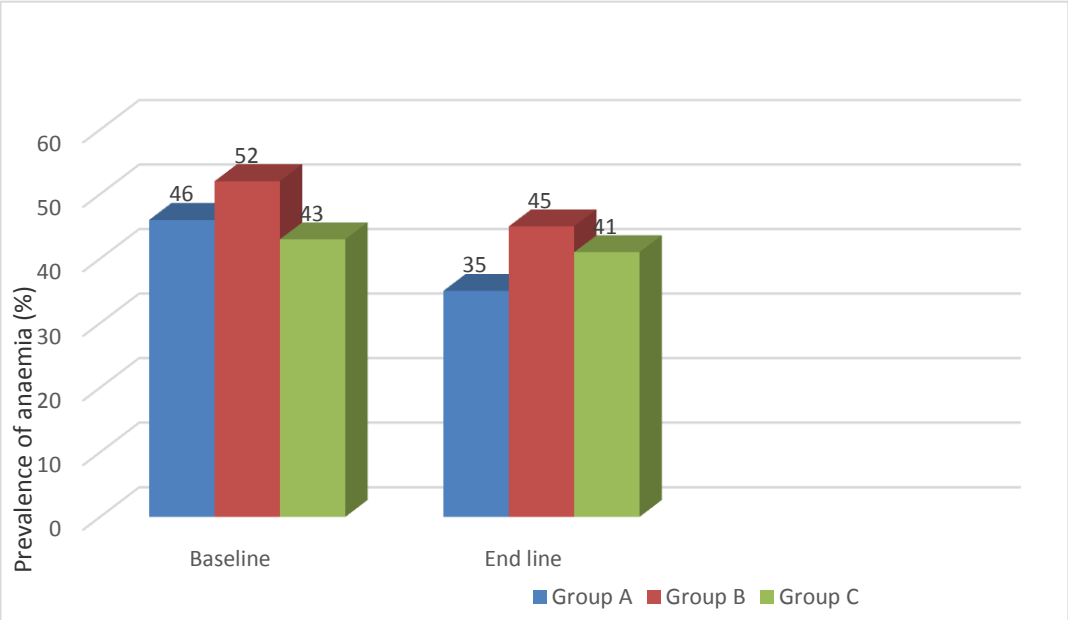
Appendix E: Mean weight of participants per group at baseline and end line



Appendix F: Mean height of participants at baseline and end line per study group



Appendix G: Prevalence of anaemia per study group at baseline and end line



Appendix H: Recommended dietary allowance (RDA) and Estimated average requirements (EAR) for 4 to 8 - year olds

Nutrients	RDA	EAR
Protein (g/d)	19 g/day	0.76g/kg/body weight
Iron (mg/d)	10 mg/day	4.1mg/day
Vitamin A	400 µg/day	275 µg/day
Vitamin C (mg/d)	25 mg/day	

Appendix I: Haemoglobin level to diagnose anaemia at sea level (g/dl)

	Status of anaemia			
Age group	**No Anaemia**	**Mild**	**Moderate**	**Severe**
Children 6 – 59 months	≥ 11.0	10.0 to 10.9	7.0 to 9.9	< 7.0
Children 5 – 11 years	≥ 11.5	11.0 to 11.4	8.0 to 10.9	< 8.0

Source: WHO 2011

Appendix J: Parental consent form

CONSENT FORMS

Title: Effect of fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder on anaemia and vitamin A status of 4 to 8 - year old Ghanaian school children.

Principal investigator: Margaret Mary Tohouenou

Address: Department of Nutrition and Food Science

School of Biological Sciences

College of Health and Applied Science

University of Ghana, Legon

P. O. Box LG, 25

Tel. No. + 233 246 383 475

awonana@yahoo.co.uk

Research Purpose/General Information about the Research

The purpose of this research is to investigate how the consumption of food powder prepared from dried green leafy vegetables (*Amaranthus cruentus* and *Solanum macrocarpon* local known as Aleefu/Fotete and Gboma), and anchovies' powder, added to school lunch will change the haemoglobin and vitamin A levels in the blood of children who will participate in this study. We will thus require information about your children's dietary intake at home and at school. We will collect 4 ml of blood sample (2 ml at the beginning of the study and another 2 ml at the end of the study) from your child to determine their haemoglobin as well as their serum retinol levels. There will be a nutrition intervention that will last for 4 months. Your child will be fed with the regular school lunch plus 5 g + 0.6 g (about a tablespoon full) of fish fortified green leafy vegetables powder, school lunch plus 5 g (about one tablespoon full) of green leafy vegetables powder or school lunch only. It is our hope that the information and the blood samples taken will help us find out the effect of fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder on anaemia and vitamin A status of the participating school-aged children.

Possible Risks and Discomforts

This research may pose a little discomfort to your child as a result of the blood samples that will be collected. We will ensure that all necessary precautions are taken to ensure minimum discomfort to your child as only qualified Phlebotomist and experienced staff will attend to your child.

Costs

There is no cost to participants in this study. Participation is totally free.

Possible Benefits

Though you may not directly benefit from this study, by the conclusion of the study, your participating child may have improved levels of vitamin A and or haemoglobin. In addition, findings from the study will benefit society and especially the Ghana school feeding programme

Confidentiality

All data collected will be kept in strict confidence and used for purely study purposes. No access will be given to any unqualified person to you and your child's information. We will protect your information as best as we can. Please note that neither you or your child will be named in any report or publication that comes out of this study.

Compensation

There will be no form of monitory compensation for you or your child for participating in this study.

Leaving the study

Feel free to allow or not to allow your child to take part in this study. You are free to change your mind anytime if you do not wish your child to continue or participate in this study even after agreeing to do so.

If you have a problem or have other questions about the study, kindly call Margaret Mary Tohouenou on **0246 383 475.**

Your rights as a participant

Ethical clearance for this study was requested for and granted by the Institutional Review Board of the Noguchi Memorial Institute for Medical Research at University of Ghana, Legon. Direct all questions and concerns you may have on your rights as a study participant to Rev. Dr. Ayete-Nyampong, Chairperson, NMIMR-IRB, on telephone number 0208152360.

AGREEMENT WITH VOLUNTEER

The document above detailing the proceedures, benefits and risks for the study entitled "Effects of fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder on anaemia and vitamin A status of Ghanaian school children" has been read and explained to me. I have had the opportunity to have any concerns and questions of mine about the study answered to my satisfaction. I agree and confirm my participation as a volunteer.

……

Volunteer's signature or thumb print

……

Date

A witness shall sign here, in case the volunteer cannot read by themselves.

The procedures, benefits and risks of this study were read and explained to the volunteer in my presence. All questions raised by the volunteer have been answered and the volunteer has agreed to participate in the study.

……

Signature of witness

……

Date

Appendix K: Permission letter to Ghana Education Service (GES)

PERMISSION TO CONDUCT A STUDY ON EFFECT OF FISH FORTIFIED AMARANTHUS CRUENTUS AND SOLANUM MACROCARPON POWDER ON ANAEMIA AND VITAMIN A STATUS OF GHANAIAN SCHOOL CHILDREN IN KODZOBI ADAKLU DISTRICT, VOLTA REGION.

We wish to seek permission from the Ghana Education Service to conduct this study entitled: **Effect of fish fortified *Amaranthus cruentus* and *Solanum macrocarpon* powder on anaemia and vitamin A status of Ghanaian school children.** The study is being carried out by the Nutrition and Food Science Department and the Department of Nutrition of the Noguchi Memorial Institute of Medical Research. Both departments are from the University of Ghana, Legon.

The study will examine the influence of green leafy vegetables powder and its fish fortified variant on the anaemia and vitamin A status of 4 to 8 - year old school children and associated factors.

The study will involve basic school children who are four to eight years old in a public school that is participating in the Ghana school feeding programme. The study will comprise about two hundred and sixty children, which we hope to obtain from a randomly selected public school.

The study will involve weight and height measurements and taking of blood and stool samples of participants. The children and their parents will be interviewed to ascertain their dietary practices. The children will be fed five times in a week with a lunch at school.

The study will initially be explained to parents and participants, after which their consent will be sought before the study begins.

At the end of the study, we hope to establish the influence of fish fortified green leafy vegetables powder on anaemia and vitamin A status and ascertain the factors that may be associated with the risk of developing anaemia and vitamin A deficiency in the participating school children. Appropriate interventions will then be profered to help prevent anaemia and vitamin A deficiency in these children.

We hope our request will be granted.

Yours sincerely

Dr. Godfred Egbi

(Head, Department of Nutrition, Noguchi Memorial Institute of Medical Research)

Appendix L: Permission to head of selected school

PERMISSIONS TO HEADS OF SELECTED SCHOOL

PERMISSIONS TO UNDERTAKE A STUDY ON EFFECT OF FISH FORTIFIED AMARANTHUS CRUENTUS AND SOLANUM MACROCARPON POWDER ON ANAEMIA AND VITAMIN A STATUS OF SCHOOL CHILDREN IN KODZOBI ADAKLU DISTRICT, VOLTA REGION

We wish to seek permission from you to carry out a study on the topic: **Effect of fish fortified Amaranthus cruentus and Solanum macrocarpon powder on anaemia and vitamin A status of school children in Kodzobi, Adaklu District, Volta Region.** Two departments namely the Department of Nutrition and Food Science and the Department of Nutrition of the Noguchi Memorial Institute of Medical Research, both of the University of Ghana are collaborating to conduct this study.

One public school has been randomly selected from a list from the Ghana Education Service (GES) and your school happened to be the one selected. The study has been approved by the GES and School Health and Education Programme Office. Ethical clearance has also been obtained from the Institutional Review Board of the Noguchi Memorial Institue of Medical Research.

About two hundred and sixty children who are between 4 to 8 years old will be involved in the study. Weight and height measurement as well as bood and stool samples will be taken from participating children. Dietary practices information will also be taken through interviews. Before any child is enrolled, consent will be sought from both parents and children after explaining the study to them.

We will be grateful if this permission is granted

Yours sincerely.
Dr. Godfred Egbi
(Head, Department of Nutrition, Noguchi Memorial Institute of Medical Research)

Appendix M: Ethical clearance

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH
Established 1979
A Constituent of the College of Health Sciences
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Ghana
My Ref. No: IR 22
Your Ref. No:

3rd September, 2014

ETHICAL CLEARANCE

FEDERALWIDE ASSURANCE FWA 00001824 **IRB 00001276**

NMIMR-IRB CPN 001/14-15 **IORG 0000908**

On 3rd September 2014, the Noguchi Memorial Institute for Medical Research (NMIMR) Institutional Review Board (IRB) at a full board meeting reviewed and approved your protocol titled:

TITLE OF PROTOCOL : **The effect of mixed green leafy vegetables powder on vitamin A and anaemia status of Ghanaian school children**

PRINCIPAL INVESTIGATOR : **Godfred Egbi, PhD**

CO – INVESTIGATORS : **Eric. Harrison MHSc, Dr. Irene Ayi, Dr. Faribu K. Saalia & Prof. Matilda Steiner-Asiedu**

Please note that a final review report must be submitted to the Board at the completion of the study. Your research records may be audited at any time during or after the implementation.

Any modification of this research project must be submitted to the IRB for review and approval prior to implementation.

Please report all serious adverse events related to this study to NMIMR-IRB within seven days verbally and fourteen days in writing.

This certificate is valid till 2nd September, 2015. You are to submit annual reports for continuing review.

Signature of Chair: .....

Mrs. Chris Dadzie
(NMIMR – IRB, Chair)

cc: Professor Kwadwo Koram
Director, Noguchi Memorial Institute
for Medical Research, University of Ghana, Legon