NUTRITION-SENSITIVE INTERVENTION WITH SELECTED AFRICAN INDIGENOUS LEAFY VEGETABLES AMONG SCHOOL-GOING CHILDREN IN MACHAKOS COUNTY, KENYA

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DECLARATION

This is my original work and has not been submitted for the award of degree in any other university

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DEDICATION

This work is dedicated to:

My beloved Wife Abigael Nandra,

Our children Grace, Jerryjoel and Billypaul, and

My mother Lucy Ndombi and My father Patrick Ndombi

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ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectrometry
AILVs	African Indigenous leafy vegetables
ANOVA	Analysis of Variance
BC	Beta carotene
Baseline	Pre-intervention (At the beginning of the study period)
BHT	Butylated Hydroxyl Toluene
CL	Confidence limit
DCM	Dichloromethane
DDI	Deuterium dilution isotope
ENA	Emergency Nutrition Assessment
Endline	Post-intervention (At the end of the study period)
FANTA	Food and Nutrition Technical Assistance
FAO	Food Agriculture Organization
FFM	Fat Free Mass
FFQ	Food Frequency Questionnaire
FM	Fat Mass
FTIR	Fourier Transform Infrared Spectrometry
GOK	Government of Kenya
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
IAEA	International Atomic Energy Agency
IDA	Iron Deficiency Anemia
KDHS	Kenya Demographic Health Survey
KNBS	Kenya National Bureau of Statistics
NACOSTI	National Commission for Science, Technology and Innovation
PLWA	People living with aids
PPM	Parts Per Million
RDA	Recommended Daily Allowance
SD	Standard Deviation

SMART	Standardized Monitoring and Assessment of Relief and Transition
SPSS	Statistical Package for Social Sciences
TBW	Total Body Water
UNICEF	United Nations International Children Educational Fund
USAID	United States Agency for International Development
UV	Ultra Violet
VAD	Vitamin A Deficiency
VIS	Visible
W.H.O	World Health Organization

ABSTRACT

The 2014 Kenya Demographic Health Survey (KDHS) and Micronutrient Survey report showed malnutrition among children aged 5-11 years in Kenya. In particular, malnutrition in Machakos County manifested as stunting (26.3 %), wasting (6.3 %), underweight (12.7 %), marginal vitamin A deficiency (VAD) (33.9 %), anemia (16.5 %), zinc deficiency (82.5 %) and iron deficiency (9.4 %). African Indigenous Leafy Vegetables (AILVs) such as *Vigna unguiculata* and *Amaranthus cruentus* can be used to fight malnutrition in school-going children, based on their high micronutrient levels, but they need to be made more available to these children. The cultivation of AILVs as well as their consumption can be optimized through school garden establishments and the effect of the AILVs' consumption on the children's nutrition status accurately determined. This is to generate data to support the recommendation by 2014 KDHS that food based intervention through garden establishment can fight malnutrition. However, empirical data to support this recommendation employs determining body mass index, a limited technique as compared to deuterium dilution isotope (DDI) method that measures fat free mass (FFM) and fat mass (FM). The objective of the study was to determine the effect of consuming school garden-sourced vegetables on nutritional status of school children in Machakos County, Kenya. This was an experimental cross over design study with 4 weeks in between phases, phase I (13 weeks) and II (12 weeks). In phase I study subjects (children aged 6-10 years) who met the inclusion criteria were grouped as experimental (Kangundo, N=66) and control (Kilalani, N=46). The experimental group fed on a recipe of *Vigna unguiculata* and *Amaranthus cruentus* grown in school gardens of Kangundo and Kilalani primary, Machakos, with an accompaniment of a mixture of maize grains and beans once a day, 5 days a week per phase while the control group had only the accompaniment. The baseline information on dietary practices, morbidity, socio-demographic, economic factors and anthropometry of children were collected using a structured questionnaire. Hemoglobin (HB) was measured by a hemoglobinometer, while levels of Fe and Zn in raw and cooked recipe and in blood samples were determined using AAS procedure. The body FFM and FM was determined by DDI method and saliva analyzed by Fourier Transform Infrared (FTIR) spectroscopy, while serum retinol and BC were analyzed by HPLC. Baseline results showed poor consumption of indigenous vegetable (< 20 %), high morbidity (> 40 %), low socio-economic status of the parents/guardians (> 95.6 % who earned <Ksh.10, 000 month), the anthropometry and low HB results indicated malnutrition. The garden sourced vegetable recipe contained sufficient levels (mg/100g) of Fe 55.465 ± 0.419, Zn 3.430 ± 0.054, and BC 4.299 ± 0.010 to meet the RDA for children. At end line, the study subjects' body composition as indicated by the FFM and FM as well as the Fe, Zn and BC levels significantly improved ($p < 0.001$) during both intervention phases. Further, there was a positive micronutrient impact, as shown by significantly higher levels of Fe, Zn, BC, Retinol and HB in the experimental group as compared to the control group at end line ($p < 0.001$) during both phase I and II. Since consumption of AILVs improves nutritional status of school going children the study recommends promotion of school gardens and consumption of school-garden sourced AILVs to improve nutrition of school going children.

CHAPTER ONE

INTRODUCTION

1.1 Background

Micronutrient malnutrition is chronic, debilitating and kills young children and pregnant women especially among the economically challenged population in developing countries (KNBS, 2010; WHO, 2014). Malnutrition includes both under nutrition and over nutrition (UNICEF, 2009). The former is caused by less food supply, poor diets, low bioavailability, poverty, high food prices, lack of nutrition information that would lead to diversification of local foods, and presence of infections. The latter is due to the intake of foods that are poor in micronutrients and have high calories from fat and sugar, as well as very little physical exercises leading to cases of type II diabetes, cancer, and heart diseases in young and middle aged people (Hawkes, 2006). A study on the relationship between overweight and micronutrient consumption reported that insufficient intake of vitamin A favours overweight by altering thyroid metabolism (Leao and dos Santos, 2012). The study highlighted the importance of nutrition education offered during nutrition intervention and the promotion of healthy eating habits to control obesity and its complications.

Malnutrition is common in a number of developing countries including Kenya (KDHS, 2014). Its manifestation among children is characterized by underweight, stunted growth, protein energy malnutrition (PEM), iodine deficiency, vitamin A deficiency (VAD), iron deficiency anemia (IDA) and zinc deficiency (WHO, 2014). Fifty percent of all countries in Africa and South East Asia are faced with VAD and its prevalence in

under-fives is estimated to be 84 % in Kenya. It causes illness and death in infants and the effect on young children and pregnant women is debilitating, resulting in a compromised immune system (WHO, 2014; KDHS, 2014). Globally nearly 250 million preschool children are vitamin A deficient and over 250,000 turn blind every year with over 125,000 deaths reported within a year of losing their sight (WHO, 2014). Iron deficiency is the cause of anemia in about 15% of the worlds' population and its prevalence is 50 % and 10 % in developing and developed countries, respectively, with approximately 60 % in Africa (KDHS, 2014). Apart from being common in infants and pre-school age children, anemia is ranked the eighth leading cause of disease in adolescent girls and women of reproductive age in developing countries (World Bank, 2009).

Some of the most important micronutrients deficient among low income people in developing countries include β-carotene, zinc, iron and iodine. Beta carotene is the source of serum retinol (vitamin A) whereby the consumption of 2-4 mg/day of β-carotene is sufficient to maintain a retinol serum level above 0.70 µmol/L needed to prevent VAD (WHO, 2014). The recommended daily allowances (RDA) of beta carotene in children and adults are 0.6-1.1 and 0.8-1 mg/day, respectively (WHO, 2014). The consumed beta carotene is metabolized into vitamin A (retinol) hence serum retinol levels are a measure of the vitamin A status of a person. Concentrations greater than 1.05 µmol/L indicate normal serum retinol levels while levels of 0.70-1.05 µmol/L indicates moderate VAD and levels less than 0.70 µmol/L indicate severe VAD (WHO, 2000).

The RDA for iron is 8-13 mg/ day for 10-18 years old (UNICEF, 2009) and 9-13 years is 8 mg/day for both girls and boys while for 4-8 years is 10 mg/day (Otten *et al*., 2006). Hemoglobin levels less than 12 g/dl for girls aged 11 and above and less than 13 g/dl for boys is indicative of anemia, 12-16 and 13-16 g/dl for girls and boys, respectively of ages 11-18 years is normal (Hess *et al*., 2007). The human body has no mechanism of storing zinc and the levels of zinc in the body may be depleted according to the body metabolism in response to the body needs. Therefore, zinc serum concentration depends on dietary zinc intake and a concentration less than 7µmol/L is interpreted as zinc deficiency but a range of 11.0-23.0µmol/L is normal (Hess *et al*., 2007). The RDA for zinc is 7 mg/day for children aged 9-13 years and 5 mg/day for 4-8 years while for adult men it is 11mg/day and for adult women it is 8 mg/day (Miller *et al*., 2007).

Since 2008, Kenya has had severe food insecurity problems, attributed to droughts, climate change, and high proportion of arid and semi-arid land, floods, high cost of domestic food production, high global food prices, displacement of farmers, and armyworm and locust invasion of farms (KDHS, 2014). Almost half of the Kenyan population is poor and there is evidence that Kenya did not manage to achieve the millennium development goals (MDGs) regarding eradication of extreme hunger and poverty (KDHS, 2014). Despite this, the country's Gross Domestic Product growth rose from 4.6 % to 5 % in 2013, the contributing factors being the agricultural sector. The government of Kenya has made nutrition a priority in the 47 counties and has given a national plan to be realized by the year 2030. This plan is to reduce severe and moderate

stunting by one-third, if possible eliminate iodine deficiency, and drastically reduce anemia by about 30 %, under-five mortality to 20 deaths per 1000 live births by 2035. These can only be realized by reducing common preventable causes of child mortality, including under nutrition (KDHS, 2014). This comes in the wake of the 2014 Kenya Demographic Health Survey (KDHS) and Micronutrient Survey's report that revealed malnutrition among children aged 5-11 years in Kenya (KDHS, 2014).

A number of approaches such as micronutrient supplementation, food fortification and agricultural practices have been implemented to address malnutrition. However, agricultural practices if well harnessed are a more effective nutrition-sensitive approach and in addition they generate income (FAO, 2012; KDHS, 2014). Agri-foods and especially those of indigenous origin such as the African indigenous leafy vegetables (AILVs), contain high levels of β-carotene, vitamin C, iron, zinc and chromium (Chege, 2012; Nawiri *et al*., 2013; Wakhanu *et al*., 2015; Egbi *et al*., 2018). Many intervention studies include the use of recipes prepared from indigenous vegetables. A recipe is a set of special instructions that include a list and amount of the ingredients required to make a particular dish (Habwe *et al*., 2009). Usually a combination of at least two indigenous vegetables increases the taste and may be more appealing hence increases indigenous vegetables' acceptability and consumption (Habwe *et al*., 2009).

In an effort to address malnutrition, different recipes have been used in intervention studies with parameters including hemoglobin, beta carotene, zinc and iron being monitored among children (Winichagoon *et al*., 2006; Chege, 2012; Nawiri *et al.*, 2013;

Egbi *et al*., 2018). The studies are generally in agreement that vegetable recipes increase the stated parameters. For example, cowpeas and amaranth are dark green vegetables which have been shown to be effective in improving children's serum beta carotene and can thus be employed to combat VAD and iron deficiency anemia. According to Nawiri and co-workers (2013), a cooked recipe consisting of sundried amaranth and cowpea leaves improved the levels of beta carotene, retinol, and hemoglobin in preschool children from Machakos District, a semi-arid region in Kenya (Nawiri *et al*., 2013). Chege (2012) did a study in which a recipe was prepared by incorporating dried amaranth leaves in maize flour. Children in Kajiado County, Kenya fed on porridge made from this recipe. The researcher reported a significant increase in the serum retinol, zinc, iron and vitamin A at endline in the experimental study children.

Machakos County is a semi-arid region in Kenya that experiences food insecurity. An extract of the statistics for Machakos County from the 2014 KDHS and Micronutrient Survey report indicate malnutrition manifested as stunting (26.3%), wasting (6.3%), underweight (12.7 %), marginal VAD (33.9 %), anemia (16.5 %), zinc deficiency (82.5 %) and iron (9.4 %)(KDHS, 2014).

There are over 210 AILVs in Kenya including spider plant (*Cleome gynandra*), African nightshade (*Solanum nigrum*), pumpkin (*Curcubita moschata*), cowpea (*Vigna unguiculata)*, Amaranthus (*Amaranthus cruentus)*, jute mallow (*Corchorus olitorius*) and slender leaf (*Crotalaria brevidens*) (Abukutsa-Onyango, 2007; Muhanji *et al*., 2011; Gido *et al.*, 2016). These vegetables could be used to fight malnutrition in

Machakos County if their availability is enhanced. However, their use in the fight against malnutrition is limited by lack of certified seeds and reliable production regimes, seasonal production (rain-fed), high postharvest losses and wastages, poor transportation, low value addition and negative attitude towards their consumption (Berinyuy and Fontem, 2011).

The scarcity of vegetables in Machakos County is attributed to among other factors, their seasonality. Nevertheless, it was worthwhile to explore the establishment of school gardens that grow AILVs in this region as a nutritive sensitive intervention. The benefits of school gardens include, but are not limited to, their influence on policy developers to integrate agri-food systems into solutions to malnutrition and optimize the production of AILVs to increase their accessibility and availability to the vulnerable groups. School gardens will also enhance the participation of the school children together with their parents and provide an opportunity for them to access nutrition information on diet diversification, and inculcate in the school going children the value of consuming indigenous vegetables. School gardens will increase consumption of fresh vegetables directly sourced from gardens, hence reduce post-harvest loss of nutrients as is the case with market-sourced vegetables, and still alleviate the fears of consuming vegetables suspected to be grown in sewages. This would be in line with the KDHS (2014) and Micronutrient Survey reports' recommendation that diversified food-based intervention through vegetable garden establishments is the best approach to fight malnutrition.

Many studies report nutrition intervention outcomes based on body mass index (BMI) and this may underestimate or overestimate nutrition outcomes (Paula *et al*., 2011; Thompson and Subar, 2013; Javed *et al*., 2015). Further, this misleads prediction of health and nutritional outcomes because an increase in BMI could be due to an increase in FM and a severe decrease in FFM, an indication of a poor health outcome (Ejlerskov *et al*., 2014). The deuterium dilution isotope (DDI) method was introduced by international atomic energy agency (IAEA) to fill this gap (IAEA, 2010; Silva *et al*., 2013). Ndung'u (2017) used DDI method to validate BMI among school children aged 8-11 years in Nairobi, Kenya while Diouf (2018) determined the FFM and FM of Senegalese children aged 8-11 years to validate bioelectric impedance analysis. In these studies the DDI method was found to be more sensitive than BMI in determining nutrition outcomes. DDI method was therefore employed in a nutrition-sensitive intervention study among school going children in Machakos County, Kenya towards addressing malnutrition.

1.2 Statement of the problem

The KDHS and Micronutrient Survey's report of 2014 presents high prevalence of malnutrition in developing countries such as Kenya (KDHS, 2014). Malnutrition manifests through underweight, stunted growth, protein energy malnutrition (PEM), iodine deficiency, vitamin A deficiency (VAD), iron deficiency anemia (IDA) and zinc deficiency (WHO, 2014). Some global interventions such as food supplementation and food fortification are costly and unsustainable. On the contrary, consumption of plant foods like African indigenous leafy vegetables (AILVs) provide variety of

micronutrients and are more affordable for populations of low living standards such as those in developing countries. In this regard, their promotion for cultivation as well as consumption is called for. Kenya has over 210 locally available AILV varieties although their availability is challenged by seasonality, poor accessibility, bioavailability of micronutrients, and negative perception that lead to their low consumption (Biodiversity International, 2007; Kimiywe, *et al*., 2007; Muhanji *et al*., 2011). This continues to present malnutrition cases among school-going children and other vulnerable groups. A solution is envisaged in a recommendation made by the KDHS (2014) and Micronutrient survey report that calls for the establishment of school garden to grow AILVs. The benefits of establishing and promoting school gardens to grow AILV cannot be underscored for they will optimize production of AILVs, increase accessibility of AILVs, and provide nutritive information. Further, gardens are sustainable and easier to manage, are sources of fresh vegetables hence minimize post-harvest losses and alleviate fear on source of vegetable as consumers will harvest them directly for use.

The promotion of school gardens to fight malnutrition can only be a positive intervention outcome if the consumption of garden-sourced selected vegetables leads to significant increase in serum levels of micronutrients in the targeted individuals and positive changes in the FFM and FM of these individuals as well. The accurate assessment of the serum micronutrient levels and the FFM and FM changes in the targeted individuals is a critical step in evaluating the intervention outcome. This calls for more accurate and reliable methods of body composition measurement. Previous intervention studies to address malnutrition have employed Body Mass Index (BMI)

which faces a challenge in underestimating or overestimating nutrition outcomes and in addition fails to distinguish between FFM and FM (Thompson and Subar, 2013).

In this study, deuterium dilution isotope (DDI) method was used to determine FFM and FM changes during a nutrition intervention among school children. The method has been identified by the International Atomic Energy Agency (IAEA) as an accurate, reliable, safe and convenient chemical method for such use in field studies. With the benefits of establishing and promoting school gardens on one hand, and the advancement in chemical methods of assessment for a nutrition intervention on the other, scientific data is presented from the findings of this study. In view of the advantages of school gardens, primary schools were targeted for vegetable garden establishment because they are best placed to achieve homogeneity and control of the intervention studies.

1.3 Null hypothesis

A nutritive-sensitive intervention with school garden-sourced *Vigna unguiculata* and *Amaranthus cruentus* recipe does not improve the nutritional status of school going children in Machakos County, Kenya.

1.4 Objectives

1.4.1 General objective

To evaluate the nutritional status of school going children in Machakos County, Kenya after a nutritive-sensitive intervention with school garden-sourced *Vigna unguiculata* and *Amaranthus cruentus* (AILVs).

1.4.2 Specific objectives

i. To evaluate baseline information on dietary practices, morbidity, socio-demographic, economic factors and anthropometry of children study subjects in Kangundo and Kilalani primary schools in Machakos County, Kenya.
ii. To determine the levels of iron, zinc and beta-carotene in raw and cooked recipe of school garden-sourced *Vigna unguiculata* and *Amaranthus cruentus*.
iii. To determine at endline the effect of school garden-sourced *Vigna unguiculata* and *Amaranthus cruentus* vegetable recipe on body FFM and FM of children study subjects in Kangundo and Kilalani primary schools in Machakos County, Kenya.
iv. To determine at endline the effect of school garden-sourced *Vigna unguiculata* and *Amaranthus cruentus* vegetable recipe on hemoglobin and serum retinol, β-carotene, zinc and iron of children study subjects in Kangundo and Kilalani primary schools in Machakos County, Kenya.

1.5 Justification of the study

This study aims to promote the establishment of school gardens that grow AILVs and recommend the incorporation of these garden-sourced vegetables in school lunch programs to address malnutrition among school-going children in Machakos County and beyond.

The benefits of establishing and promoting school gardens to grow AILV cannot be underscored. Among them is the influence on policy development from stakeholders to integrate agri-food systems into solutions to malnutrition, optimize the production of AILVs and increase their accessibility and availability to the vulnerable groups. Further, school gardens will enhance the participation of the school children together with their parents and provide an opportunity for them to access nutrition information on diet diversification. This will also inculcate in the school going children the value of consuming indigenous vegetables and influence parents to establish home gardens.

This approach will also increase consumption of fresh vegetables directly sourced from gardens, hence reducing post-harvest loss of nutrients in market-sourced vegetables, and alleviate the fear of consuming vegetables grown in sewages. These will lead to improved micronutrients among school children by promoting more diversified diets and will reduce the occurrence and severity of infectious diseases among school children. Consequently absenteeism will be reduced and children's performance at school will improve.

Additionally this study will come up with a report that can be used by national policy makers to increase accessibility of nutrient-rich foods at school levels through encouraging the development of school gardens.

This study will promote the use of an accurate method to determine the impact of the nutrition intervention because the true relationship between food intake and food intake impact on body composition of nutrition intervention target subjects shows a gap due to unreliable assessment methods. The use of BMI has been the most common method of measuring nutrition intervention impact on the bodies of individuals. However, recent research has reported BMI's failure to distinguish FM and FFM in different races of populations and children. Additionally BMI also fails to detect the influence of puberty, gender, age and ethnicity on body composition of individuals. This has led to inaccurate prediction of health and nutritional outcomes. The deuterium dilution isotope (DDI) method is an advanced chemical method recommended by International Atomic Energy Agency (IAEA) to fill the aforementioned gap. This method determines FFM and FM changes during intervention, and is accurate, simple to carry out, more reliable and suitable for children. The use of this method in the present study will add value by providing empirical data to support the use of school gardens that grow ALVs to resolve the malnutrition menace.

1.6 Scope and limitations

There are over 210 indigenous vegetables in Kenya but two of these, *Vigna unguiculata* and *Amaranthus cruentus,* which are commonly consumed by children, were used to prepare the recipe without regard to the different species. The nutrients beta carotene, zinc and iron that interact to impact on nutrition status were assessed assuming minimal synergetic effects of other phytochemicals that might have been present in the vegetable recipe.

There was generally inability to control other food intakes away from school although a 24 hour dietary recall was used to monitor micronutrient and energy intakes. The duration of the intervention for each phase of the study was limited by the school calendar dates.

The effect of intervention employed the Deuterium Dilution Isotope chemical method to determine the FFM and FM changes in the children study subjects while AAS and HPLC was used to determine the levels of some nutrients in raw, cooked recipes and serum of the children study subjects. Prior to the study, the subjects were de-wormed to account for one of the factors that affect the bioavailability of nutrients. Gender was not considered in statistical treatment of data. The duration of the intervention for each phase of the study was limited by the school calendar dates.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

A review of malnutrition, the role of school vegetable gardens in addressing malnutrition, the levels of beta carotene, zinc and iron in indigenous vegetables as well as their chemistry and roles is presented. The DDI method in the assessment of nutrition outcomes is described.

2.2 Malnutrition

Incidences of malnutrition are common in a number of developing countries including Kenya (KDHS, 2014). About 50 % of all countries in Africa and South East Asia are faced with vitamin A deficiency (VAD). About 250 million preschool children in Asia and Africa are vitamin A deficient with over 250,000 turning blind every year and over 125,000 deaths being reported within a year of losing their sight (WHO, 2014). Iron deficiency is the cause of anemia in about 15% of the worlds' population and its prevalence is 50 % and 10 % in developing and developed countries respectively, with approximately 60 % in Africa (KDHS, 2014). Apart from being common in infants and pre-school age children, anemia is ranked the eighth leading cause of disease in adolescent girls and women of reproductive age in developing countries (UNICEF, 2019). A number of approaches such as micronutrient supplementation, food fortification and agricultural practices are implemented to address malnutrition (WHO, 2014). Micronutrient supplementation and food fortification are short term, costly hence unsustainable among the low income groups (Bhutta, 2013). However, Foods of plant

origin like African indigenous leafy vegetables (AILVs) provide variety of micronutrients and are more affordable for populations of low living standards such as those in developing countries, where in addition they generate income (FAO, 2012; KDHS, 2014).

Malnutrition among children in Kenya is common and is characterized by underweight, stunted growth, wasting, iodine deficiency, vitamin A deficiency (VAD) , iron deficiency anemia (IDA) and zinc deficiency (WHO, 2014). Vitamin A deficiency leads to xerophthalmia manifested as night blindness, eye infections, slow growth rate and dry eyes, and causes morbidity and mortality in infants. Its prevalence in under-fives is estimated to be 84 % in Kenya, leading to a compromised immune system, and is the leading cause of preventable blindness in children and increases the risk of disease and death from severe infections (KDHS, 2014). In pregnant women VAD causes night blindness and may increase the risk of maternal mortality. Vitamin A deficiency is a health problem in more than half of all countries, especially in Africa and south East Asia, hitting hardest young children and pregnant women in low income countries (WHO, 2014).

The 2014 KDHS and Micronutrient Survey finding indicates malnutrition in children aged 5-11 years manifested as stunting (26.3 %), wasting (6.3 %), underweight (12.7 %), marginal VAD (33.9 %), anemia (16.5 %), zinc deficiency (82.5 %) and iron (9.4 %)(KDHS, 2014). For Machakos County the statistics indicated malnutrition as stunting (26.5 %), wasting (6.5 %) and underweight (8.1%) (KDHS, 2014). Anthropometric

measurements are used to assess the nutritional status of individuals in a target population. This is done through weight and height measurement of the individuals and conversion to Z scores and BMI to classify the nutritional status of the target population. There are three types of Z scores from anthropometric measurements: weight for age (WFA) Z score, Height for age (HFA) Z score, and weight for height (WFH) Z score, which if less than -1 standard deviation (SD), express underweight, stunting and wasting respectively. An extract of the statistics for Machakos County from the 2014 KDHS and Micronutrient Survey report indicate malnutrition in children aged 5-11 years manifested as HFA < -3SD (7.1 %), HFA < -2SD (26.5 %), WFH< -3SD (2.5 %), WFH< -2SD (6.5 %). These figures reveal stunting and chronic malnutrition in the county. Hospital reports in Machakos County indicate cases of malnutrition among children in public primary schools in the area among them Kangundo and Kilalani primary schools.

The government of Kenya has made nutrition a priority in all the counties and aims to reduce severe and moderate stunting by one-third, if possible eliminate iodine deficiency, and drastically reduce anemia by about 30 %, by reducing common preventable causes of child mortality, including under nutrition (WHO, 2014). This comes in the wake of the 2014 Kenya Demographic Health Survey (KDHS) and Micronutrient Survey's report that revealed malnutrition among children aged 5-11 years in Kenya (KDHS, 2014). Ultimately, diversified food-based intervention through vegetable garden establishments to fight malnutrition was recommended by the Kenya Demographic Health Survey and Micronutrient Survey's report (KDHS, 2014).

2.3 School gardens

School gardens have been incorporated into primary school education system since the 19th century and are continuously gaining popularity (Doerfler, 2011) due to the realization that eating locally grown foods improves nutrition. In the United States of America, for example, school gardening is becoming a national movement (Blair, 2009). School gardens have been successful in providing nutrition knowledge on the importance of fruit and vegetable consumption (Doerfler, 2011) and have transformed children's food attitudes and habits since children consume 20 % of their dietary intake at school (WHO, 2004; Evans *et al*., 2012) with research showing that dietary habits developed in childhood persist through life (Mukherjee and Chaturvedi, 2017). Ultimately, school gardens have contributed towards reducing the risks of chronic heart disease, over nutrition and under nutrition (Heim *et al*., 2009; Blair, 2009).

There has also been an attempt to launch school gardens in Kenya to contribute to efforts to fight malnutrition. For example, the Kenya School Garden Initiative Organization was launched in 2005 to inculcate in the school children positive values on sustainable agriculture and nutrition as a basis for livelihood and as a source of nutrition information (Foeken *et al*., 2010). The Organization has established 11 school gardens in Nakuru County, Kenya and has engaged about 410 students from highlands and semi-arid parts of Kenya in garden establishments that act as demonstration and seed multiplication centers for communities, contributing towards food security and biodiversity conservation (Foeken *et al*., 2010). These gardens grow cabbages, kales, potatoes, maize, beans and spinach but hardly indigenous vegetables. The Ministry of

Education and Agriculture has also partnered with another organization called Sustainable Organic Farming to establish school gardens in Kakamega and Vihiga Counties, Kenya.

In Kenya, the Ministry of Planning and National Development estimates that 50 % of children in pre-primary school, 10 % in primary and 5 % in secondary schools rely solely on school lunches (Foeken *et al*., 2010). Nutrition intervention would achieve a positive transformation among school-going children if coordinated within school feeding programs and nutrition education. Ultimately, healthier food choices at home would be consumed since children are more likely to consume similar fruits and vegetables as those grown in school gardens (Morris and Zidenberg-Cherr, 2002). Homogeneity in the management and use of school gardens provide an excellent opportunity to reduce inequalities in health to the school going children (WHO, 2004; Van Cauwenberghe *et al*., 2010). The impact of school garden for nutritive intervention is however challenged by poor intervention evaluations, perception on vegetable consumption, inadequate funding, poor study designs, lack of adequate follow-up time, little community and parent participation (Somerset and Markwell, 2009; Wang *et al*., 2009).

2.3.1 Soil sampling and analysis

Recent research has shown the link between soils and human health (Brevik and Sauer, 2015) to the extent that growing vegetables in soils deficient in micronutrients will drastically reduce these micronutrients in the vegetables (Cakmuk *et al*., 2010; Cakmuk

et al., 2017; Shukla *et al*., 2014). Globally, about one-third of arable soils are deficient in micronutrients, especially zinc (Cakmuk *et al*., 2017). Vegetables grown in soils deficient in zinc and iron also end up with low zinc and iron in their leaves and seeds. Lack of adequate micronutrients in the soil may cause consumption of food crops deficient in micronutrients leading to micronutrient deficiency in humans (Manzeke *et al*., 2019).

There is a strong correlation between iron levels in soils and prevalence of iron deficiency anemia which can be explained by poor accumulation of iron in vegetables grown in iron deficient soils (Shukla, 2014). Zinc and iron deficiencies have been noticed to increase with the nature and extent of the deficiencies varying from types of soil, plant genotypes and crop management. Iron deficiency is common especially in alkaline soils and also those soils with high levels of phosphorus, zinc, manganese, copper and nickel. Additionally poor drained soils can induce iron deficiency. Zinc and iron deficiencies in soils could be overcome by augmenting soils by addition of chemical fertilizers.

Nutrients in vegetables vary depending on their availability in the soil at different collection sites and plant vegetable uptake (Makokha *et al*., 2019). The nutritional content of vegetables differs due to type of vegetable and the geographical area of production (Makokha *et al*., 2019). In one of the studies on soil micronutrients, zinc and iron levels in *Cleome gynandra,* L and *Amaranthus hybridus,* L varied in Kongwa, Muheza and Arumeru districts in Tanzania (Makokha *et al*., 2019). Iron and Zinc levels

in *Corchorus olitorius*,L and *Corchorus trilocularis*, L in Muheza district were lower than those in Morogoro district in Tanzania (Makokha *et al*., 2019). Heavy metal content significantly varied in ALVs planted in different locations in urban and periurban Nairobi, Kenya (Mutune *et al*., 2014).

In order to know the soil requirements before planting, one needs to do soil sampling and testing. Ten sampling spots are randomly chosen from each sampling unit and its surface litter removed. Soil to a depth of about 15 cm is cut at the sampling spot using a spade. Thick slices of soil from top to bottom of exposed face of the soil cut are removed and put in a clean container. The samples are thoroughly mixed and foreign particles like stones, roots and pebbles removed. The mixed sample is reduced to about 500 g by quartering technique (Simmons *et al.*, 2014). The final soil sample is sent to a soil laboratory in a clearly labeled polythene bag for soil fertility analysis. In the laboratory parameters like pH, amounts of nitrogen, phosphorus and minerals present (potassium, calcium, magnesium, manganese, copper, iron, zinc and Sodium) are tested.

Trace elements (Fe, Zn and Cu) in the soil samples are commonly extracted according to Mehlich Double Acid method (Mehlich *et al.*, 1962) and the concentrations of the elements Fe, Zn and Cu determined using Atomic Absorption Spectrophotometer. Phosphorus is extracted according to Olsen method (Watanabe and Olsen, 1965) and its concentration determined in UV-VIS spectrophotometer set at 880 nm. Total organic carbon is extracted according to the calorimetric method (Anderson and Ingram, 1993) and the carbon concentration read on the UV-VIS spectrophotometer at 600 nm. Total

nitrogen in the soil sample is extracted according to Kjeldahl method (Page *et al*., 1982) and the total nitrogen concentration determined by distillation followed by titration with diluted standardized H_2SO_4. Soil pH and electrical conductivity were determined in a 1:1 (w/v) soil – water suspension with pH – meter and conductivity meter respectively (Mehlich *et al.*, 1962). The results obtained help to establish the types and amounts of fertilizers required for application. Inorganic compounds $ZnSO_4$ and ZnO are the most commonly used Zn fertilizers because they are cost effective while for iron application of $FeS0_4.7H_20$ alleviates soil iron deficiency.

2.4 African indigenous leafy vegetables

There are over 45,000 species of African indigenous leafy vegetables (AILVs) in sub-Saharan Africa and Kenya is home for about 210 species (Abukutsa-Onyango, 2007; Muhanji *et al*., 2011; Gido *et al.*, 2016). Common AILVs include spider plant (*Cleome gynandra*), African nightshade (*Solanum nigrum*), pumpkin (*Curcubita moschata*), cowpea (*Vigna unguiculata)*, Amaranthus (*Amaranthus cruentus)*, jute mallow (*Corchorus olitorius*) and slender leaf (*Crotalaria brevidens*) (Muhanji *et al*., 2011; Gido *et al.*, 2016). These vegetables are known to contain high levels of beta carotene, zinc, non-hem iron, chromium, calcium, fluorine, magnesium, phosphorus, potassium, sodium, zinc, dietary fiber and proteins (Orech *et al*., 2009; Wakhanu *et al.*, 2015). The challenges in AILVs availability include seasonality (rain dependent), high cost of production, lack of certified seeds, while their consumption is limited due to negative perception and lack of nutritional knowledge on diet diversification (Biodiversity International, 2007; Kimiywe *et al*., 2007; Onyango *et al*., 2008). However, their consumption has been shown to address

malnutrition among children in Kenya and beyond (Chege, 2012; Nawiri *et al*., 2013; Egbi *et al*., 2018).

2.4.1 Amaranth

Amaranth (*Amaranthus cruentus*, L) is the most commonly consumed indigenous vegetable in Kenya as it is cheap, tasty and locally available (Moraa *et al*., 2008). The seeds germinate in 3-15 days and leaf harvesting can be done from 3 weeks onwards as prolonged harvesting delays onset of flowering (Fasuyi and Akindahunsi, 2009). Amaranth has many species that easily adapt to different climates and well drained sandy, loamy and clay soils of pH 4-7 (Wambugu and Muthamia, 2009)

Amaranthus leaves are nutritious as they contain high levels of protein, iron, zinc, carotenoids, calcium, anti-oxidants and vitamin C to provide the required daily allowances (RDAs) of children (GoK, 2008). Further, they have low anti-nutrient (phytic acid and oxalate) content compared to other AILVs and its potential in fighting malnutrition in children from semi-arid and arid areas is reported (Chege, 2012; Nawiri *et al*., 2013). Some nutrients in amaranth increase and others decrease with age of the plant as shown for beta carotene and zinc in table 2.1 (Makobo *et al*., 2010; Cherioyot, 2011; Duya *et al*., 2018). Beta carotene levels increase with age unlike the decrease in zinc which is attributable to its distribution to the reproductive parts of the plant (Makobo *et al*., 2010; Cherioyot, 2011).

Table 2.1: Levels of beta carotene and Zinc at different ages of amaranths plant

Age (days)	Beta carotene content (mg/100g DW)	Weeks after germination	Mean zinc content (mg/100g DW)
25	38.288	3	7000
50	40.592	4	1000
75	45.831	5	800
		6	1500
		7	1000
		8	1000

Source**:** Cherioyot (2011). DW means dry weight.

2.4.2 Cowpea

The cowpea (*Vigna unguiculata,* L) is among the most important food legume crop in the semi-arid tropics and well-adapted where other food legumes do not perform well. It fixes atmospheric nitrogen through its root nodules, and grows well in poor soils with more than 85% sand and with less than 0.2 % organic matter and low levels of phosphorus (Singh *et al*., 2003). Cowpea has high levels of iron, zinc and beta carotene and this makes it a potential vegetable in the provision of micronutrients to school children (Abukutsa-Onyango, 2007; Chikwendu *et al*., 2014). The mineral content of cowpea has been found to be higher compared to other conventional vegetables like spinach, cabbage and lettuce (Flyman and Afolayan, 2007). Table 2.2 shows mean levels of micronutrients in cooked cowpea leafy vegetables (Wakhanu *et al*., 2015) and table 3 shows the mineral content of cowpea harvested at different ages of the plant (Flyman and Afolayan, 2008).

Table 1.2: Mean levels of micronutrients in cooked cowpea leafy vegetable

Micronutrient	Mean levels
Beta carotene	4.30-6.24mg/100g FW
Vitamin E	3.80-7.78 mg/100g FW
Vitamin C	0.48-1.55 mg/100g FW
Zinc	5.72-7.38 mg/100g DW
Iron	97.5-179.83 mg/100g DW
chromium	3.89-7.63 mg/100g DW

Source: Wakhanu *et al.* (2015). FW and DW mean fresh weight and dry weight, respectively.

Table 2.3: Levels of Zn and Fe in raw cowpea leafy vegetable harvested at different ages

Days after planting	Stage	Zinc (mg/100g)	Iron (mg/100g)
21	1	382.62	161.33
28	2	184.73	238.00
35	3	70.57	299.73
44	4	67.18	278.33
50	5	44.43	282.18
57	6	29.21	338.10
64	7	22.36	283.93

Source: Flyman and Afolayan (2008)

A study undertaken by Flyman and Afolayan (2008**)** showed higher zinc content in cowpea at initial stages but a decrease towards the final stage just like with amaranth. The iron content increased from stage 1 to 6 before decreasing and this means that in general iron content increases with leaf age (Table 3). Similarly beta carotene levels in cowpea also increase with vegetable age (Bergquist, 2006). The bioavailability of either zinc or iron at any stage of vegetable maturity is influenced by the interactions between the two (Flyman and Afolayan, 2007). Iron inhibits zinc absorption when the levels of iron are twice those of zinc.

2.5 Intervention studies employing AILVs

In an effort to address malnutrition, different recipes have been used in intervention studies where parameters such as hemoglobin, βeta carotene, Zn and Fe are monitored among children (Winichagoon *et al.*, 2006; Chege, 2012; Nawiri *et al.*, 2013). The studies are generally in agreement that vegetable recipes increase the stated parameters.

In North East Thailand, Winichagoon and co-workers (2006) undertook a study on the efficacy of a multi-nutrient-fortified seasoning powder on the hemoglobin, zinc and iron status of primary school children, aged 5-14 years. In the 31 week study, subjects (n=569) were categorized into fortified group and unfortified group (Table 4). The seasoned powder was fortified with vitamin A, zinc and iron and the effect of the intervention on the hemoglobin and selected micronutrient serum levels were found to be significant ($P < 0.001$) for hemoglobin and serum zinc but not for iron. There was some evidence of a significantly higher hemoglobin concentration in the fortified groups but no significant changes in serum iron and retinol were observed. The study concluded that consumption of a micronutrient seasoning powder improved zinc and hemoglobin count of the study children.

Table 2.2: Hemoglobin and serum levels of Zn, Ferritin and retinol in North East Thailand study subjects

Serum parameter	Unfortified	Fortified	P value
Hemoglobin (g/L)	120.6 ± 9.63 (N= 257)	121.3 ± 9.54 (N=261)	0.008
Serum ferritin (µg/L)	36.0 (N=235)	35.1 (N=225)	0.606
Zinc (µmol/L)	10.9 ± 1.67 (N=261)	11.2 ± 1.63 (N=247)	0.011
Retinol (µmol/L)	1.4 ± 0.36 (N=256)	1.3 ± 0.31 (N=241)	0.274

Source: Winichagoon *et al.* (2006)

Chege (2012) conducted a study on the efficacy of dried amaranth leaves (*Amaranthus cruentus*), incorporated in fermented maize flour, on vitamin A, iron and zinc status of children in Kajiado County, Kenya. In this study the sample size was calculated using a formula by Hseih (2006) which provided a minimum sample size of 37 children per group. However, to cater for 10 % attrition 55 children were recruited for experimental and 45 children for control. The experimental children fed on the intervention recipe for a period of 6 months. This recipe contained 6.81 mg/100 g beta carotene, 12.05 mg/100 g iron and 5.55 mg/100 g zinc. The serum levels of beta carotene and retinol were analyzed by HPLC, zinc levels by AAS while iron levels were analyzed as ferritin using enzyme-linked immunosorbent assay (ELISA).

The study reported a significant increase ($p = 0.047$) in the serum retinol (0.679 ± 0.227 to 0.853 ± 0.233 µmol /L), beta carotene ($p = 0.046$) from 0.162 ± 0.106 to 0.539 ± 0.209 µmol /L). There was also a significant increase ($p = 0.044$) of zinc from 9.94±1.2 to 12.78±1.4 µmol/L and iron ($p=0.043$) from 10.18±1.6 to 13.11±1.7 µmol/L at endline

for the experimental group. The experimental group's serum retinol mean content at baseline and endline were 0.679 ± 0.227 and 0.853±0.233 µmol/L respectively. The experimental group's serum beta carotene mean content at baseline and endline were 0.162 ± 0.106 and 0.539 ± 0.209 µmol/L respectively. The experimental group's serum zinc mean content at baseline and endline were 9.94±1.2 and 12.78±1.4 µmol/L respectively. The experimental group's serum iron mean content at baseline and endline were 10.18±1.6 and 13.11±1.7µmol/L. The study concluded that incorporation of the dried amaranth leaves into fermented maize flour significantly raised the content of beta carotene, iron and zinc of maize flour and ultimately consumption of this recipe for six months significantly increased the serum beta carotene, iron and zinc of the experimental children. This study highlighted the potential of amaranth in fighting malnutrition in children from semi-arid and arid areas.

Cowpeas and amaranth are dark green vegetables which are effective in improving children's serum beta carotene and can thus be employed to combat VAD and iron deficiency anemia. According to the study by Nawiri and co-workers (2013), a cooked recipe consisting of sundried amaranth and cowpea leaves improved the levels of beta carotene, retinol, and hemoglobin in preschool children from Machakos District, a semi-arid region in Kenya. In this study, the experimental group (n=51) was fed on the vegetable recipe accompanied by a paste made from white flour for 13 weeks while the control group (n = 25) was fed on white cabbage and the paste. The serum levels of beta carotene and retinol were analyzed by HPLC while hemoglobin count was done using a portable battery operated hemocue analyzer (Nawiri *et al*., 2013). The baseline means

serum beta carotene for the experimental and control groups were 0.1 µmol/L while at endline it was 0.5 µmol/L for the experimental group and 0.2 µmol/L for the control group ($p = 0.001$). The serum mean retinol levels for both the experimental and control groups were 0.6 µmol/L at baseline, while at end line they were 0.8 and 0.7 µmol/L for the experimental and control groups respectively.

The study by Nawiri *et al.* (2013) further looked at the effect of this recipe on the hemoglobin count of the study children. There was an increase in hemoglobin count at endline but not statistically significant. However, the number of children who attained hemoglobin count above 12.0 mg/L as recommended by WHO increased at endline. This was possibly due to the increase in serum retinol which led to the mobilization of iron from liver stores and was incorporated in red blood cells of the subjects. The study demonstrated improved retinol levels in the experimental group after an intervention with the recipe for 13 weeks. The findings further supported the hypothesis that one of the most effective and sustainable way to mitigate VAD was through diversified diets as a food based strategy. Amaranth and cowpea as indigenous leafy vegetables have therefore a great potential for use in combating malnutrition among school children in Kenya.

In a similar study carried out in Ghana, the effect of green leafy vegetables powder on anemia and vitamin A status of Ghanaian school children aged 4-9 years was investigated (Egbi *et al*., 2018). This was an intervention study that lasted 12 weeks where the experimental group received the vegetable powder against those in the

control group who did not. The powder consisted of a mixture of eggplant (*Solanum macrocarpon*) and amaranth (*Amaranthus cruentus*). Baseline data on anthropometry and morbidity patterns of the study children were collected. The socio-demographic and economic data of the parents /caregivers and were collected as well. The baseline dietary data revealed low dietary diversity among the children. Vitamin A concentration was assessed by HPLC while hemoglobin count was done by means of a hemoglobinometer. The consumption of the vegetable powder increased the mean serum hemoglobin and retinol levels of the study subjects in the experimental group. The mean hemoglobin concentration for the experimental group was 121.9±13.5 g/l and that of the control was 113.4±8.5 g/l at endline ($p = 0.001$). At baseline the mean serum retinol concentration for the experimental group was 16.97± 7.74 and 26.96 ± 6.86 µg/dl at endline ($p = 0.001$) while at baseline the mean serum retinol concentration for the control group was 16.79 ± 8.74 µg/dl and was 24.35±5.50 µg/dl at endline ($p < 0.05$). The study highlighted the potential of composite green leafy vegetable powder in reducing anemia among children.

2.6 Effect of nutrients on body mass

The effects of consumption of AILVs, food supplements and fortified foods on body composition have been highlighted in a number of studies (Winichagoon *et al.*, 2006; Chege, 2012; Egbi *et al*., 2018). The changes of free fat mass (FFM) of an individual is due to growth of lean tissue as a result of micronutrients like zinc, iron, chromium and pro-vitamins like beta carotene obtained from diet. Since vegetables have been demonstrated to contain high levels of these micronutrients, their consumption is likely to

have an effect on the FFM of an individual. The exact mechanism of how zinc influences growth is unknown, however, zinc stimulates appetite and energy intake to enhance FFM (Arsenault *et al*., 2008). In one study, Zinc given to 6-8 months old Peruvian children, who suffered from mild to moderate stunting, increased their FFM by 0.41 kg than those who did not get the zinc supplement, leading to the conclusion that stunted children could be zinc deficient (Arsenault *et al*., 2008). Zinc plays a critical role in growth and development; it is cofactor for enzymes that control cell division and proliferation. Zinc deficiency impairs the synthesis of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and protein which has implications on the FFM (MacDonald, 2000). Iron is also another important cofactor in the synthesis of amino acids and its deficiency has an effect on metabolism of glucose, lipid biosynthesis and amino acid biosynthesis. Iron is required for energy generation and also for DNA and RNA synthesis which in turn affects FFM (Zhang, 2014).

A study was carried out on the effect of zinc supplementation on growth and body composition in children with sickle cell disease (Babette *et al*., 2002). The study reported that the mean FFM for girls who received zinc supplementation significantly increased by 0.87 kg. Study subjects in the experimental group had increased linear growth and weight, increased appetite and food consumption more than in the control group who received placebo. The study concluded that zinc intake increased linear growth, weight, appetite and food consumption in children. Further, zinc deficiency is manifested as poor growth and delayed skeletal and sexual maturation common in children (Babette *et al*., 2002).

Similarly a group of 20 children who received oral zinc supplementation, 20 mg /day mixed with multivitamin, showed significant increase in appetite and significant weight gain in comparison to those who only received oral multivitamin. Increase in weight was associated with FFM increase (Shakur *et al*., 2009). The children showed 80 % weight increase associated with FFM (lean body tissue) without increase in body fat. The study reported that weight gain of zinc supplemented children was associated with formulation of lean tissue instead of adipose tissue and that zinc improves test equity, hence increased appetite (Shakur *et al*., 2009).

Vegetable soup containing peanut butter and skimmed milk powder fortified with vitamin-mineral complex was given to people living with HIV-Aids (PLWA) in a study in Senegal (Adama *et al*., 2016). This recipe contained vitamin A (rich in beta carotene), zinc, iron, calcium and magnesium among others. The control group was not fed on the recipe. Consumption of the recipe for 3 months by experimental groups showed a significant increase in body weight, FFM, FM, and haemoglobin than in the control groups. The experimental group's FFM and FM increased by 11.8 % and 10.7 % respectively (Adama *et al*., 2016). The increase in FFM in PLWA was attributable to vitamins and minerals in the recipe.

Marcia *et al*. (2015) did an intervention study in which pre-pubertal, non-zinc deficient (8-9 years old) children were divided into two groups: experimental (31) and control (31). Of these 62 children examined, 32 were boys and 30 were girls. The experimental group received zinc supplementation while the control was given placebo in a three months

intervention. The experimental group showed significant increase in soft tissue, mainly FFM than the control group (Marcia *et al.,* 2015). The percentage FFM increase ranged from 80.03 ± 4.64 to 81.13 ± 4.35 and percentage FM ranged from 18.87 ± 4.35 to 19.97 ± 4.80.

Studies on the effect of the combination of iron, zinc and vitamins on FFM have also been done. For example, Farhmida *et al.* (2007), carried out a study on the effect of a combination of zinc, iron and vitamin A on 800 infants aged 3 months in rural East Lumbok, West Nusa Tenggora for a period of 6 months. Weight, length and micronutrient status were determined. Zinc alone disadvantaged the haemoglobin and iron status of the subjects. Both zinc and iron combination improved both the zinc and the iron status while zinc, iron and vitamin A realized the highest increase in vitamin A and Haemoglobin. The height of the study subjects increased by 1.1- 1.5 cm more than those with placebo. The study concluded that zinc intake can only have a positive effect on FFM if low haemoglobin, Iron status and Vitamin A are also addressed and corrected (Farhmida *et al*., 2007).

The above study agreed with earlier findings by Berger *et al.* (2006) who investigated the efficacy of combined iron and zinc supplementation on micronutrient status and growth in Vietnamese infants. In the investigation 915 infants were divided into groups: Group 1 received only 10 mg of iron daily for six months, group 2 received 10 mg of zinc, group 3 received 10 mg iron plus 10 mg zinc and group 4 received a placebo. Weight gain was higher in the zinc group while serum ferritin and haemoglobin was higher in the iron and

iron plus zinc groups (22.6 and 20.6 g/l for haemoglobin; 36.0 and 24.8 mg/l for serum ferritin) and lower in placebo groups (haemoglobin: 6.4 and 9.8 g/l serum ferritin -18.2 and -16.9 mg/l) (Berger *et al*., 2006).

Fabiansen *et al.* (2017) investigated the effectiveness of food supplements in increasing fat-free-tissue accretion in children with moderate acute malnutrition in Burkina Faso. To assess FFM, a dose of 6 g of Deuterium oxide (D_2O) (99.8 %) diluted in 5 g of mineral water was weighed and given orally after collection of pre-dose saliva samples. Post-dose saliva samples were collected after 3 hours equilibration period. For each assessment, D_2O abundance was measured in duplicate using FTIR. Deuterium dilution space was calculated and converted to TBW using a 1.044 factor to adjust for proton exchange. FFM was calculated as TBW/ hydration, using ages and sex specific hydration coefficients. FM was calculated as weight minus FFM. Baseline mean weight was 6.91kg and baseline mean percentage FFM was 83.5 %. At endline the investigation revealed a weight increase of 0.90 kg and FFM increase of 93.5 %. The study concluded that children with malnutrition when nutritionally rehabilitated with zinc, iron, and vitamin A put on predominately Fat Free Tissue (Fabiansen *et al*., 2017). In another study Lora *et al.* (2015) demonstrated that ready- to-use supplementary food increased Fat Free Mass and BMI in Haitian school-aged children. The FM of experimental children increased by 0.73 $\pm$ 0.34kg compared to the control who never received the supplement (Lora *et al*., 2015).

There is a correlation of the beta carotene to vitamin A conversion factor with BMI in individuals such that those with more body fat have a lower capability to convert beta

carotene to vitamin A (Tang *et al*., 2003). In one study a rich vegetable diet was intervened on 9-13 year old Filipino school children for 9 weeks to determine if there was any relationship between BMI with serum carotenoid during the intervention period (Ribaya-Mercado *et al*., 2008). There was an inverse correlation of serum beta carotene levels with BMI (Wise *et al*., 2009). These findings also agreed with those of Etyang *et al*. (2003) who investigated on the serum retinol, iron status and body composition of lactating mothers in Nandi District of Kenya and reported that serum retinol, ferritin and FFM negatively correlated with breast milk fat.

2.7 Effect of preparation and cooking methods on nutrients in AILVs

Mixing different AILVs in recipe preparation increases their taste, is more appealing, increases their acceptability, and consumption (Habwe *et al*., 2009). Preparation and cooking methods of vegetables can enhance the nutrient content of the recipe through ingredients added or reduce the nutrient content through heat degradation and loss through leaching (Msuya *et al*., 2008; Wakhanu *et al*., 2015).

The loss of vitamins like beta carotene, for example, is attributed to oxidative degradation and *trans*/*cis* isomerization as a result of thermal processing during the preparation and cooking (Mulokozi *et al*., 2004). Boiling vegetables causes greater losses of beta carotene than other cooking methods (Mazzeo *et al*., 2011; Seongeung *et al*., 2018). In one study by Mulokozi and co-workers (2004) methods of vegetable preparations like chopping, washing and fermenting caused zinc and iron losses ranging from 14-51 % while losses due to cooking ranged from 6-34 %. The effect of different cooking methods and

microbial fermentation on the levels of phytic acid and oxalate in selected African indigenous vegetable recipes from Lake Victoria Basin, Kenya was reported by Wakhanu *et al.* (2015). The study reported that cooking methods and microbial fermentation significantly reduced phytic acid and oxalates in vegetables, and thus cooking methods may increase the bioavailability of micronutrients. Other studies reported that washing vegetable leaves in water, chopping and cooking by boiling may reduce their iron, zinc, magnesium and calcium because more vegetable surface area gets into contact with water thereby increasing leaching. Further, heat destroys some micronutrients like vitamins (Hettie and Beulah, 2011; Tiwari and Cummins, 2013; Bongoni *et al*., 2014).

The most common methods used for cooking vegetables are steaming, roasting, boiling, frying, sautéing, microwave and pressure-cooking. In order to retain as much nutrients as possible cooking time should not exceed 30 minutes (Wakhanu *et al*., 2015). However, use of oil, tomato and lemon during cooking helps retain certain carotenoids and minerals and frying vegetables in oil greatly enhances the absorption of minerals they contain since thermal processing in oil releases the micronutrients (Ngegba, 2007).

2.8 Role of selected nutrients in human nutrition

The combination of iron, zinc and vitamin A influences micronutrient levels in the human body due to their synergistic effects (Sonja *et al*., 2005). For example, beta carotene when assimilated as vitamin A (retinol) increases iron status implying vitamin A deficiency makes iron incorporation into hemoglobin less effective (Sonja *et al*., 2005). In this regard, a strong positive correlation between serum retinol levels and hemoglobin concentrations

are reported with lower vitamin A status at baseline (Hinderaker *et al*., 2002; Nawiri *et al*., 2013) while intervention studies with foods fortified with vitamin A improved hemoglobin concentrations in the blood of children and expectant mothers. However, with very high serum levels of vitamin A, there is no additional effect on hemoglobin concentrations (Mulokozi, 2003). On the contrary, iron deficiency causes the accumulation of vitamin A in the liver and the reduction of vitamin A in the plasma hence vitamin A is not metabolized since the utilization and absorption of vitamin A is reduced in such cases (Jang *et al*., 2000). Nutritional surveys indicate a high prevalence of vitamin A deficiency and a high prevalence of anemia occurrence in the same population (WHO, 2014). Further, anemia often occurs in individuals with night blindness, leading to the conclusion that anemia is among the underlying causes of night blindness. Vitamin A increases body immunity which in turn decreases anemia due to infection (Wieringa *et al*., 2004).

Serum levels of retinol are affected by bioavailability and bioconversion of the beta carotene in the consumed foods. The factors that affect the bioavailability and bioconversion of beta carotene ingested are food matrix in the intestine, genetics, type of beta carotene, molecular level linkages, amounts of beta carotene ingested, amount of lipids ingested, intestinal infections, age, gender and nutritional status of the individual and interactions of other carotenoids with beta carotene (Solomons and Orozco, 2003). Munoz *et al.* (2000) reported that zinc and iron supplementation improved indicators of vitamin A status in school children in Mexico. Although the effect of vitamin A on zinc metabolism is unknown, a combination of zinc and vitamin A supplementation increases the serum retinol and serum zinc concentrations (Rahman *et al*., 2000).

2.8.1 Beta carotene and retinol

Beta carotene is a pro-vitamin A carotenoid, compounds responsible for the orange, red and yellow colors seen in fruits, vegetables, plant leaves, flowers, crustaceans, some fish (like salmon), insects and some birds. Beta carotene molecule is unstable due to its conjugated system of double bonds hence is destroyed by light, high temperatures and acids, and undergoes oxidative degradation through a free radical process if stored for long. It absorbs UV radiation at maxima of 450 nm (Nyambaka and Ryley, 2001).

A molecule of beta carotene has two β-ionine rings and can be cleaved enzymatically (Figure 2.1), to give two molecules of vitamin A (retinol) (Michele *et al*., 2001; Nyambaka and Ryley, 2001). Beta-carotene is converted to retinal by symmetrical cleavage of the central chain by an enzyme called β-carotene 15, 15'-monooxygenase (Michele *et al*., 2001; Ronda *et al*, 2014). In this reaction, β-carotene is cleaved to form two retinal molecules; with the addition of a water molecule at the noncyclical end (Figure 2.1). Vitamin A exists in humans in several forms that include retinol, retinol esters, retinal and retinoic acid (Ronda *et al.*, 2014). The alcohol form, retinol, predominates in the circulation but it is too toxic for storage. Instead, the liver stores retinol as retinyl esters or palmitate (Ronda *et al.*, 2014). The active form of vitamin A is the aldehyde form, retinal. Retinoic acid is the form in tissues responsible for the biological actions of vitamin A in cellular division and differentiation. Retinol has a β-ionone ring attached at one end of an all-*trans*-unsaturated nonene chain with two methyl groups and a hydroxyl group at the opposite end. The heavy degree of unsaturation and the cis-trans isomerism dictate the properties of retinol (Michele *et al*.,

2001; Ronda *et al.*, 2014).

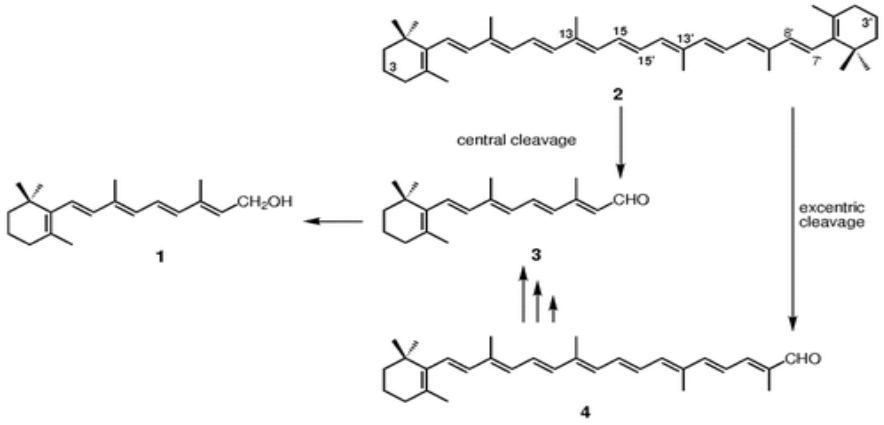

Figure 2.2 Central cleavage pathways of beta carotene by intestinal enzymes

Source: Michele *et al*. (2001). 1. retinol, 2. β-carotene, 3. β-retinal and 4. apo-carotenals.

The Figure 2.1 shows enzymatic cleavage of β-carotene (2) by two routes to form retinol (1). Central cleavage (the first route) leads to retinol (1). Excentric cleavage (second route) yields apo-carotenals (4), which are subsequently degraded to β-retinal (3).

Beta carotene is epidemiologically linked with the prevention of several chronic, degenerative diseases in humans. Its conjugated double bond system makes it effective singlet oxygen quencher and anti-oxidant (Tilman *et al.*, 2010). It therefore protects the body from effects of oxidation and neutralizes free radicals, making it anti-tumor, anti-cancer and anti-aging. It is also effective in the treatment of skin disorders as well as enhancing immune function in the body by protecting phagocytes from self-destruction. It is essential for growth and development, and for prevention of xerophthalmia, measles and diarrhea in children on conversion to vitamin A (retinol) (WHO, 2014).

That is why VAD is responsible for blindness in many children especially in Asia and sub-Saharan Africa (KDHS, 2014; WHO, 2014).

Consumption of 2-4 mg/day of beta carotene is sufficient to maintain a retinol serum level above 0.70 µmol/L needed to prevent VAD (WHO, 2014). The RDA in children and adults are 0.6-1.1 and 0.8-1 mg/day respectively (WHO, 2014). The Consumed beta carotene is metabolized into vitamin A (retinol) hence serum retinol levels are a measure of the vitamin A status of a person. Concentrations greater than 1.05 µmol/L indicate normal serum retinol levels while a figure of 0.70-1.05µmol/L indicates moderate VAD and a figure less than 0.70 µmol/L indicates severe VAD (WHO, 2000).

2.8.2 Iron

Iron is obtained in the form of non-hem iron from vegetables and as hem iron from meat and is stored in the reticulo-endothelial system as ferritin and hemosiderin, and is necessary for the manufacture of hemoglobin, which transports oxygen to tissues. Iron deficiency, therefore, leads to anemia making it the most common micronutrient deficiency in the world responsible for anemia (De-Regil *et al*., 2013; Haider *et al*., 2013) and has a devastating effect on brain functions (Lozoff, 2007; Prado and Dewey, 2014). Iron deficiency reduces oxygen-carrying capacity and interferes with aerobic functions during infancy which may have irreversible adverse effects on cognitive development.

One of the priorities of World Health Organization (WHO) is to reduce iron deficiency anemia that is prevalent in low-income countries through increased iron supplementations and food fortification (Bates-Earner *et al*., 2016). However, iron supplementation is a direct source of iron into the body without following the route of absorption via foods and this is dangerous as excess iron in the body may stimulate pathogens in the body to cause diseases (Veenemans *et al*., 2011; Zlotkin *et al*., 2013).

The absorption and regulation of iron in the body is mediated by a hormone called hepcidin that is secreted by the liver and soluble non-hem iron is reduced from Fe^{3+} to Fe^{2+} by duodenal cytochrome B (Andrew *et al*., 2017). The Fe^{2+} enters the enterocyte through the divalent metal transporter I (Powell *et al*., 2013). The Fe^{2+} as intracellular iron is stored temporarily or transported from the enterocyte into the blood circulation through facilitation by a single transporter called ferroportin. The Fe^{2+} is then oxidized to ceruloplasmin by ferroxidase hephaestin and distributed to other organs and tissues by chaperone transporter transferrin (Andrew *et al*., 2017).

Ferroportin determines the movement of Fe^{2+} in and out of the enterocyte through the action of hepcidin. When hepcidin binds to ferroportin, the latter is internalized and degraded. Consequently the movement of Fe^{2+} from enterocyte is curtailed or blocked (Andrew *et al*., 2017). This blocked iron in the enterocyte stimulates a regulation mechanism that reduces entrance of iron into the enterocyte through divalent metal transporter I. The curtailed iron will eventually be excreted in stool at the death of the enterocyte. Iron absorption is therefore a highly regulated process (Andrew *et al*.,

2017). Hepcidin is the main inhibitor of iron transport from cells into the blood circulation. Suppression of hepcidin therefore raises iron absorption. Hepcidin is usually suppressed by iron deficiency and is elevated by iron overload (Cercamondi and Egli, 2010; Drakesmith and Prentice, 2012; Prentice *et al*., 2012; Glinz *et al*., 2015). Transferrin binds to Fe^{2+} in blood circulation to ensure nonuse by pathogens in the blood to cause disease. However, if there is an iron overload through supplementation, for example, transferrin will be overwhelmed and some unbound iron will be available for microbial use leading to disease (Lounis *et al*., 2001; Portugal *et al*., 2011; Clark *et al*., 2014). For this reason iron supplements are not the best for iron intervention, the best sources being non hem foods.

Iron deficiency could also be due to inadequate folic acid, riboflavin, copper, vitamin A, vitamin B_{12} and zinc intake (WHO, 2005). The RDA for iron is 8-13 mg /day for 10-18 years old and 10 mg /day for 4-8 years old (UNICEF, 2009; Otten *et al*., 2006). Hemoglobin levels less than 12 g /dl for girls aged 11 and above and less than 13 g /dl for boys is indicative of anemia (WHO, 2008; WHO, 2014). A study on the health outcomes of a subsidized fruit and vegetable program for disadvantaged Aboriginal children reported an improvement in hemoglobin and iron concentrations in children's sera. At baseline the iron levels were 12.7 µmol /L and at endline were 13.2 µmol /L. The hemoglobin count was 12.68 g /dL at baseline and 12.82 g /dL at endline (Black *et al*., 2013).

2.8.3 Zinc

Zinc is an essential trace element that influences the absorption, transport and utilization of vitamin A (King, 2011) through the synthesis of retinol binding protein hence its deficiency impairs this important function. Zinc is also a co-factor in the conversion of retinol to retinal, a necessary step in prevention of xerophthalmia. The enzyme that plays a major role in the oxidative conversion of retinol to retinal is zinc-dependent. Studies have shown that plasma retinol levels increase in individuals who receive zinc supplementation (Babette *et al*., 2002; Berger *et al*., 2006; Farhmida *et al*., 2007).

Zinc is also useful in blood clotting and proper insulin and thyroid function (Saper and Rash, 2008). The conversion of beta carotene to retinol is accelerated by thyroxin and hyperthyroidism. Zinc has antioxidant properties and helps protect the body cells from damage caused by free radicals, which contribute to the aging process, heart diseases and cancer (Saper and Rash, 2008).

Zinc may also help speed the healing of gastric ulcers (Saper and Rash, 2008). Healthy prostate gland in men is linked to zinc. Zinc is also required in the manufacture of testosterone and deficiency may induce low sperm count and loss in libido. Zinc is lost in ejaculation since the sperms need it to swim towards the egg (Hambidge, 2000) and should be taken daily to maintain a steady state because the body has no specialized zinc storage system. Zinc serum concentration depends on dietary zinc intake and a concentration less than 7 µmol/L is interpreted as zinc deficiency but a range of 11.0-23.0µmol/L is normal (Hess *et al*., 2007). The RDA for zinc is 7 mg/day for children

aged 9-13 years and 5 mg/day for 4-8 years while for adult men it is 11mg/day and for adult women it is 8 mg/day (Miller *et al*., 2007).

2.9 Impact measurement of nutrition interventions

Nutrition status measurement is done to identify individuals at risk of malnutrition to develop health policies and for effective nutrition intervention assessment. A nutrition intervention ideally results in changes in body composition of individuals. The two compartment model of body composition divides the body into Fat Mass (FM) and Fat Free Mass (FFM). FFM includes the mass of bone, muscle, connective tissue, water and organs such as liver, kidney and adrenal glands (Wells and Fewtrill, 2006; IAEA, 2010). FM is also called adipose tissue where fats are stored. The quantities and distribution of body fat and the composition of FFM (lean mass) are parameters used to measure nutrition intervention outcomes (Wells and Fewtrill, 2006) thus making body composition an indicator of both undernutrition and overnutrition (Ploeg *et al*., 2014; Bowen *et al*., 2015).

The true relationship between food intake and body composition shows a gap due to unreliable assessment methods. There are several ways of measuring nutrition intervention impact on the bodies of individuals. These include BMI measurement, Skinfold Thickness measurement, Waist circumference measurement, Bioelectric Impedance Analysis, Dual Energy X-ray Absorptiometry, Magnetic Resonance Imaging, Densitometry and Total Body Electrical Conductivity (Wells and Fewtrell, 2006).

2.9.1 Body mass index

This is the most commonly employed method of nutrition status measurement and involves the measurements of height and weight (anthropometric measurements) and is calculated as weight/ height^2 (Mei *et al*., 2002). Therefore, BMI is related to nutrition status by anthropometric measurements. These measurements are converted to Z scores and percent of median in order to identify and classify malnutrition into stunting, wasting and underweight (WHO, 2000). Z-scores of less than – 1.99 Standard deviation (SD) indicate underweight, stunting and wasting, those between – 2.99 to – 2 SD indicate moderate malnutrition while scores of less than – 3 SD indicate very severe malnutrition (WHO, 2000).

Many studies report nutrition outcomes based on BMI and this underestimate or overestimate nutrition outcomes (Thompson and Subar, 2013). Though easier to determine, BMI fails to distinguish FM and FFM in different races of populations and children (Paula *et al*., 2011; Javed *et al*., 2015). For example, an increase in BMI could be due to increase in FM and severe decrease in FFM and thus mislead prediction of health and nutritional outcomes, since a decrease in FFM is indicative of poor health outcome (Hoffman *et al*., 2000; Ejlerskov *et al*., 2014). Puberty, gender, age and ethnicity influence body composition of individuals but BMI does not detect these factors (Salamone *et al*., 2014). However, BMI is a useful parameter in predicting health outcomes in adults such as obesity, type II diabetes and eating disorders.

2.9.2 Deuterium dilution isotope method

The deuterium dilution isotope (DDI) method was introduced by international atomic energy agency (IAEA) for accurate determination of FFM and FM. The method determines FFM and FM changes during intervention, is accurate, simple to carry out, more reliable and suitable for children (Silva *et al*., 2013). Further, deuterium is non-radioactive isotope hence safe (IAEA, 2010). Ndung'u (2017) used DDI method to validate BMI, physical activity and dietary practices as methods for FM assessment among school children aged 8-11 years in Nairobi, Kenya. However, DDI method has not been widely employed in Kenya to assess nutrition intervention unlike the BMI one. Further, the impact of indigenous vegetables on the FFM of children has not been assessed by DDI method in Kenya. A number of studies outside Kenya have employed this method to assess the impact of food supplements on FFM of study subjects cutting across all ages.

The DDI method measures body composition by determining Total Body Water (TBW) because water in the body is exclusively found within the FFM compartment hence its estimation enables the determination of the FFM (IAEA, 2010). The body water pool naturally contains a small amount of deuterium (^2H) which represents the natural abundance of ^2H in body water and is usually close to 0.015 % of hydrogen (IAEA, 2010). After collection of a baseline saliva, urine or milk sample, a known quantity of deuterium oxide (^2H_2O) also known as D_2O (99.8 or 99.9 % ^2H) is ingested. The dosing is done as per the weights of the study subjects. Children aged 6-10 years with a weight less than 30 Kg are given 6g-10g of deuterium (Fabiansen *et al*., 2016). The ^2H_2O equilibrates with the body water within a few hours (IAEA, 2010). The amount of

deuterium in body water above that naturally present is known as the enrichment and reaches a ‘plateau’ after 3-4 hours in the body water after ingestion by which time deuterium is uniformly distributed in saliva, urine, plasma and milk (for lactating mothers). Consequently body fluid samples can be collected 3 or 4 hours after dosing. Participants shouldn’t drink water during equilibration period. After equilibration saliva, urine, plasma and milk contain the same concentration of deuterium and any can be used for the determination of the deuterium enrichment in the body.

Saliva is easier to use since its equilibration is faster than urine, plasma and milk. Working with saliva has lower risk of contamination and study subjects can collect saliva on their own (Bonne and Wong, 2012). Deuterium in saliva can be analyzed by Fourier Transform Infrared (FTIR) spectrometry (Khasekheli *et al*., 2013) and measured enrichment reported in mg $^{2}H_2O$ per kg H_2O (ppm) (IAEA, 2010).

In a study by Ndung’u (2017) using DDI method to validate BMI, 85.4 % of the children found normal by BMI measurement were found obese by DDI method. Diouf (2018) determined the FFM and FM of Senegalese children aged 8-11 years (n=151) using DDI method to validate bioelectric impedance analysis in predicting total body water (TBW) and adiposity among the children. In this study deuterium enrichment in saliva samples of the children was measured using FTIR spectroscopy. The mean (± SD) body weight, height, BMI and Height for- age-Z score (HAZ) were 28.2 ± 6.5, 137.2 ± 7.8, -1.34 ± 1.20 and -0.19 ± 1.07, respectively, with 3 children suffering from stunting (HAZ < -2 z-score). The mean (± SD) TBW (kg), FFM (kg) and FM (kg) was

17.2 ± 2.7, 22.8 ± 5.7 and 4.4. Only 1.9 % of the children were obese by BMI but 11% were obese by DDI method. This finding highlighted the limitation of BMI in the determination of body composition in children.

2.10 Study area

The study was conducted in Kangundo and Kilalani mixed primary schools in Machakos County, which is a semi-arid region that experiences food insecurity most of the times. It lies within the southern region of Kenya with a poverty rate of 24.1 %. It has a population of 1,098,584 with an area of approximately 5,952.90 sq.km (Kenya Census, 2009). Human settlement and agricultural activities in the area have resulted in deforestation. These attributes contribute to environmental degradation and slowed development, giving rise to high poverty levels (Boitt and Odima, 2017).

Soil fertility in the study area is low and soils are generally shallow, and are of red and brown clay with poor drainage and soil erosion common. Land is degraded and subsistence farming fails due to poor rains and yet the inhabitants mostly depend on local markets for supply of vegetables. Although a few house holds plant vegetables like kales, white cabbage, spinach and cowpea, there is little diet diversification predosposing the children to severe malnutrition. Reports from one of the hosiptals near the study schools showed that diarrhoea, skin diseases, respiratory diseases, clinical malaria and anemia were common among children, a pointer to malnutrition. Few inhabitants are in formal employment and quite a number get income from manual labour (Boitt and Odima, 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research design

This was a food intervention study conducted in Kangundo and Kilalani primary schools in Machakos County, Kenya in the year 2018. The study adopted an experimental cross- over design that involved two phases, I (13 weeks) and II (12 weeks), with 4 weeks between the phases to enable interchange of the role of study subjects as experimental and control groups during phase II. *Amaranthus cruentus* and *Vigna unguiculata* as AILVs were grown in school gardens of Kangundo and Kilalani primary, Machakos and used for intervention and effect measured using levels of nutrients and FM and FFM.

The experimental group fed on a recipe of *Vigna unguiculata* and *Amaranthus cruentus* with an accompaniment of a mixture of maize and beans once a day, 5 days a week per phase while the control group had only the accompaniment. The baseline information on dietary practices, morbidity, socio-demographic, economic factors and anthropometry of children was collected using a structured questionnaire. Hemoglobin (HB) was measured by a hemoglobinometer. Levels of Fe and Zn in raw and cooked recipe and in blood samples were performed using AAS while beta carotene (BC) by HPLC. The end line effect of the vegetable recipe on body FFM and FM was determined by DDI method and saliva analyzed by FTIR while serum retinol and BC were analyzed by HPLC.

Parents/caregivers were allowed in school during all procedures including sample collection, deworming and feeding. Prior to any field procedures, meetings were conducted in each school for parents, agricultural officers, health officials, headteachers and class teachers to explain the study purpose, procedures (including blood and saliva sampling by qualified laboratory medical staff). A structured questionnaire was administered to parents/caregivers to collect socio-demographic, economic data. Parents also signed consent forms to allow their children to participate in the study.

3.2 Inclusion criteria

Children aged 6-10 years and whose parents or guardians gave consent were selected for inclusion (appendix I) and the children who were selected must have not been ill or hospitalized two weeks prior to the study and had been dewormed .

3.3 Selection of study groups

Kangundo and Kilalani primary schools were purposively selected from 67 public primary schools within the study area based on their accessibility, number of children, accessibility to water for irrigation, land for school garden and an ongoing lunch programme.

In phase I, 76 children aged 6-10 years, from Kangundo primary school were assigned to the experimental group while 49 in Kilalani primary were assigned to the control group. In phase II, the roles of the schools were inter changed. This gave a sample size

of n=125 (including 10 % of the calculated sample) as determined using equation [3.1] (Cochran, 1963).

$$n = \frac{Z^2 pq}{e^2} \quad \text{……..} \quad \text{……...[3.1]}$$

Where

Z = confidence limits of the survey results. For 95% confidence level, Z=1.9.

P = proportion of the population with the attribute of interest (the prevalence of malnutrition among children aged 5-11 years in Machakos County is 8.1 % weight for age) =0.081.

q = (1-p) the proportion of population without the attribute of interest =0.919.

e = desired precision of the estimate (5 %) =0.05.

The sample number was 125 from the two schools but following drop outs, there were 66 and 46 study subjects in the experimental and control groups respectively at the end of phase I and 46 and 66 study subjects in the control and experimental groups respectively at the end of phase II.

3.4 Establishment of school gardens

3.4.1 Soil sampling and analysis

The school gardens in the experimental school in both phases I and II was divided into three sampling units. Ten (10) sampling spots were randomly chosen from each sampling unit and its surface litter removed. Soil to a depth of about 15 cm was cut at the sampling spot using a spade. Thick slices of soil from top to bottom of exposed face

of the soil cut were removed and put in a clean container. The samples were thoroughly mixed and foreign particles like stones, roots and pebbles were removed. The mixed sample was reduced to about 0.5 Kg by quartering technique (Simmons *et al.*, 2014). The final soil sample was sent to Kenya Agricultural Research Institute (KARI), Nairobi in a clearly labeled polythene bag for soil fertility analysis where parameters, pH, amounts of nitrogen, phosphorus and minerals present (potassium, calcium, magnesium, manganese, copper, iron, zinc and Sodium) were tested. The results obtained helped to establish the types and amounts of fertilizers required for application.

During elemental analysis the soil samples were extracted according to Mehlich Double Acid method (Mehlich *et al.*, 1962) and the concentration determined using flame spectrophotometric procedures. Phosphorus was extracted according to Olsen method (Watanabe and Olsen, 1965) and its concentration determined in UV-VIS spectrophotometer set at 880 nm. Total organic carbon was extracted according to the calorimetric method (Anderson and Ingram, 1993) and the carbon concentration was read on the UV-VIS spectrophotometer at 600 nm. Total nitrogen in the soil sample was extracted according to Kjeldahl method (Page *et al*., 1982) and the total nitrogen concentration was determined by distillation followed by titration with diluted standardized H_2SO_4. Soil pH and electrical conductivity were determined in a 1:1 (w/v) soil – water suspension with pH – meter and conductivity meter respectively (Mehlich *et al.*, 1962). Trace elements (Fe, Zn and Cu) in the soil samples were extracted according to Mehlich Double Acid method (Mehlich *et al.*, 1962) and the

concentrations of the elements Fe, Zn and Cu determined using Atomic Absorption Spectrophotometer.

3.4.2 Cultivation of vegetables

Green amaranth was planted on a quarter of an acre while cowpea was planted on a half an acre at the experimental school. Couch grass and other weeds were eliminated by spraying with Roundup herbicide. Furrows were made and 4.0 kg of Diammonium Phosphate (DAP) fertilizer was applied in the furrows (Martens and Westermann, 2016) prior to planting cowpea Seeds (type M66) sourced from Kenya Agricultural Research Institute in Katumani, Kangundo sub-county. Green amaranth Seeds were broadcasted on top of the prepared soil and covered with maize stovers (mulching) to provide humus and retain moisture required for germination. Green amaranth was top dressed with Calcium Ammonium Nitrate (CAN) two weeks after germination and a dose of superglo was applied to make them leafier and develop more biomass. The vegetables were ready for harvest and use as recipe for the intervention 21 days after germination (appendix VIII) and were subsequently harvested at ages 35, 50 and 75 days, respectively.

3.5 Baseline procedures

3.5.1 Questionnaire administration

A structured questionnaire (Appendix II) was pretested before it was administered to parents/caregivers of the children. Pretesting allowed the researcher to adjust the questionnaire by removing some ambiguous parts and adding some parts relevant to the objectives of the study. Each of the respondents was given the appropriate questionnaire in the sampled school. The researcher with the assistance of the research team explained

to the respondents the purpose of the study and how to fill the questionnaire. Any parts of the questionnaire not well understood by the respondents were clarified by the researcher. The questionnaire gathered data on demographic and socio-economic characteristics, baseline data on the methods of preparation and consumption patterns of vegetables and morbidity patterns (respondents recalled the incidences of child illness for the previous two weeks, types of illness and their frequency).

3.5.2 Anthropometric measurements and deworming

The weight and height of the study subjects was taken by a qualified nutritionist using a healthcare weighing machine (Salter scale model 2006) and UNICEF scale respectively (Appendix VIII). Weight measurement, taken to 0.1 g accuracy was done in triplicates and mean weight calculated. Anthropometric data sheet was used to record collected data on weight, height, and of the study children (appendix IV).The weight and height indices were converted to Z-scores and BMI to classify the nutrition status of the target population. Age was obtained from the class teachers records that had been verified using the child's health card, birth certificate or baptismal card. The study subject was then dewormed using an anthelminthic drug (albendazole syrup, 10 mls of 400 mg/child).

3.5.3 Dietary intake assessment

Dietary intake was assessed by use of a 24-hour dietary recall which helped to determine the number of meals taken per day and the adequacy of the nutrients. Food frequency table and 24 hour dietary recall sheet (Appendix III) were used to record

collected data on dietary practices and nutrition status of the target population. Amounts of food were estimated using the commonly used household equipment. Calibration table was developed to help in establishing the amounts of foods. The respondents were asked to state the type and amount of ingredients cooked for the entire household, amount of food cooked, amount served to the target child and what remained to determine the amount of food consumed by the child. The amount of ingredients consumed in grams was then calculated and used to analyze the amount of nutrients consumed. To establish the food frequency, food items were listed and the respondents asked to state how often the foods were consumed. The types of vegetables eaten, their source and preparation methods were also established.

3.5.4 Saliva sampling

The sampling procedure was performed according to International Atomic Energy Agency (IAEA) standard operating procedures (IAEA, 2010). Deuterium oxide (D_2O) liquid (99.8%) was diluted with tap water by adding 800g (800ml) of tap water to 200g (180ml, density of D_2O is 1.105g/ml) of D_2O. The weight of both the D_2O and the added tap water was recorded to 0.01g. The study subjects consumed 30 ml. of the diluted D_2O liquid and were starved and with minimal motions for three hours to allow the D_2O to equilibrate with body water, and minimize water loss through sweating. A pre-dose and post dose (after consumption of deuterium oxide (D_2O) liquid/water) saliva collection was done by qualified medical staff by placing two cotton balls in the mouth of the study subjects for 2 minutes. Post-dose saliva was sampled 3 hours after administration of the D_2O dose in a similar manner. The cotton balls were sodden with

saliva and transferred to a clean 20 ml syringe. The saliva was squeezed out of the cotton balls into another clean sterile vial using a syringe plunger and the vial tightly sealed and labelled. Collected samples were kept in cool boxes and transported to Kenyatta University laboratory and kept in the freezer at -80°C.

3.5.5 Blood sampling

Blood sampling for both the experimental and control subjects was performed at baseline during both phases I and II and involved trained personnel from the Ministry of Health (Kangundo Hospital). Approximately 5 ml of the subjects' blood was collected through venipuncture of an antecubital vein (peripheral vein), using sterile non-toxic, non-pyrogenic, and Revital Healthcare syringes. For Hemoglobin count, approximately 1 ml of the sampled blood was sucked into a cuvette and placed in a portable Diaspect hemoglobin counter (Diaspect Medical G mbH Von-cancrin-Strol 63877 Sailaaf, Germany). The remaining blood sample was dispensed into trace-element free tubes, immediately wrapped in aluminum foil to shield them from light and transported within one hour on ice packs in a cool box to Kangundo hospital laboratory for centrifugation to obtain serum. The centrifuge (Hettich Zentrifugen EBA 20, D-78532 Tuttingen-Germany) was set at 2500 rpm at room temperature for 10 minutes. The separated serum was transferred into clearly labeled cryo tubes and transported on ice packs in a cool box to Kenyatta University laboratory for refrigeration at -80 ^{o}C. The entire sample in the cryo tube was used for the analysis of beta carotene, retinol, iron and zinc.

3.6 Endline saliva and blood sampling

The procedures of saliva sampling (section 3.5.4) and blood sampling (section 3.5.5) were repeated after the intervention period of 13 weeks for subjects in both the experimental and control groups during both phases I and II. Biochemical data sheet was used to record collected data on serum levels of selected micronutrients of the study children (appendix IV) and body composition of the study children (Appendix V).

3.7 Vegetable recipe preparation and intervention

In each of the schools, two assistants were recruited and trained on vegetable recipe preparation and serving, working in collaboration with the school cooks. During both phases, the experimental school was supplied with vegetable preparation and cooking accessories which included fuel, tomatoes, onions, cooking oil and salt. The harvested *Amaranthus cruentus* and *Vigna unguiculata* vegetables were cleaned under clean running water and whole leaves were chopped into small pieces and mixed in the ratio of 1:1 (wt./wt.). A portion of the raw chopped mixture *Amaranthus cruentus* and *Vigna unguiculata* vegetables harvested at 35 days was reserved for laboratory quantification of iron, zinc and beta-carotene. The other portion of the mixture was cooked as per the local community procedures of boiling the vegetables before frying with oil and adding tomatoes and onions. A portion of the cooked vegetables was reserved for laboratory micronutrient analysis while the remaining was used for feeding intervention. Each study subject in the experimental group consumed on average 80 g (wet weight) of the recipe of *Amaranthus cruentus* and *Vigna unguiculata* expected to meet RDA for children. The vegetable recipe was served together with a mixture of cooked beans and maize (being part of the school feeding programme) once a day for 5 days a week, for

13 weeks (Appendix VIII).

3.8 Chemicals and reagents

Chemicals and reagents used were: all-trans-beta carotene standard and all-trans-retinol standard (Types I, Sigma chemicals), de-ionized water, ethanol, Butylated Hydroxyl Toluene (BHT), hexane, dichloromethane (DCM), methanol, acetonitrile, 0.5 % ascorbic acid (Aldrich chemicals), acetone, methanolic potassium hydroxide, 10 % sodium chloride solution, anhydrous sodium sulphate, concentrated sulphuric acid, hydrogen peroxide, ferric nitrate, zinc nitrate and deuterium oxide liquid. The solvents used in HPLC analysis were HPLC grade.

3.9 Cleaning of glassware

All glassware was thoroughly cleaned with soap and tap water, rinsed 3 times with acetone and air blown to dry before use for the preparation of solutions.

3.10 Analytical instruments

The equipment used in this study were a high performance liquid chromatography (HPLC) instrument (Shimadzu CTO-AS VP 230V), Fourier Transform Infra-red Spectrometer (IR Tracer-100 FTIR SHIMADZU), and Atomic Absorption Spectrophotometer (Buck Scientific, model 210 VGP).

HPLC column type was Luna 5U C18(2), column length of 250 mm , internal diameter of 4.6 mm at a column temperature of 30°C and pump pressure of 16.4 Mpa. The

mobile phase for the reversed phase isocratic elution of serum extracts for beta carotene analysis consisted of a mixture of acetonitrile: DCM: methanol in the ratios of 70:20:10 (v: v: v) containing 0.1% BHT. The mobile phase flow rate was 2.0 ml/min with a retention time of 8 minutes. Beta-carotene was monitored using an SPD-20A prominence UV-Vis detector at 452 nm at a sensitivity of 0.0100 absorbance units' full scale (aufs). In analysis for retinol, the mobile phase was an aqueous binary mixture of acetonitrile and water in ratios of 85:15 (v:v) containing 0.1% BHT as an antioxidant with a flow rate of 1.5 ml/min and a 15 minute run time. Retinol was monitored using a UV-Vis detector (Deuterium lamp) and detected at a wavelength of 325 nm.

The FTIR instrument set with absorbance as the measurement mode, apodization was square triangle, number of scans was 32, resolution was 2.0 and range was minimum 2300 cm^{-1} and maximum 2900 cm^{-1}.

The electronic balance (Shimadzu Corporation Japan AT x 224, max.220 g, min. 10 mg with a readability of 0.1mg), accurate to 0.0001g, was used for weight measurements of chemicals and reagents.

3.11 Laboratory procedures

3.11.1 Extraction of beta carotene from serum

Beta-Carotene was extracted from the serum samples obtained in section 3.5.5 according to the procedures by Hosotani and Kitagawa (2003). Frozen serum samples were left to thaw for 20 minutes and 200µl aliquots pipetted into serum vials using a

micropipette and diluted with 200 µl double distilled de-ionized water. The mixture was de-proteinised by vortex mixing for 30 seconds with 400 µl ethanol, containing Butylated Hydroxyl Toluene (BHT) (0.0599g/ml), to prevent the oxidation of beta carotene(Howe and Sherry, 2006). To extract beta carotene, 3 ml hexane was added, vortex mixed and centrifuged at 800 rpm at 5°C for 15 minutes. This was repeated twice and the resultant supernatant from the three extractions combined and evaporated under a stream of nitrogen at 30 °C. The residue after evaporation was re-dissolved in 150µl dichloromethane (DCM): methanol (4:1), vortex mixed and ultrasonically sonicated for 10 seconds and passed through a single use membrane filter (0.45µm pore size) before injection into the HPLC column.

3.11.2 Extraction of beta carotene from raw vegetables and vegetable recipe

Extraction and analysis was done in replicates following the procedure according to Hosotani and Kitagawa (2003). Exactly 25g of the fresh vegetable recipe was homogenized by blending with 50 ml de-ionized water containing 0.5 % ascorbic acid for 5 minutes. Five grams of the resultant mixture was extracted with 50 ml of acetone-hexane mixture (3:2 v/v) containing 0.1% BHT in a 250 ml conical flask. The resulting mixture was shaken with a mechanical shaker for 10 minutes at moderate speed and then centrifuged for 10 minutes to separate the layers. The organic layer was transferred to a separating funnel and 25 ml of 0.5 M methanolic potassium hydroxide added to saponify the potentially interfering oils. The saponified extract was shaken and allowed to settle for 30 minutes then washed with 100 ml of 10 % sodium chloride solution followed by three 100 ml portions of distilled water to remove acetone while discarding

the aqueous layer continuously. The extract was then dried by filtration over anhydrous sodium sulphate. The filtrate was concentrated in a rotary evaporator at 45 ^{o} C and reconstituted in methanol to 50 ml in a 100 ml volumetric flask. All the containers of the extract were covered with aluminum foil to minimize the destruction of beta carotene.

3.11.3 Extraction of retinol from serum

The procedure according to Hosotani and Kitagawa (2003) was adopted for serum retinol extraction. Frozen samples (section 3.5.5) were left to thaw for 20 minutes then 300 µl aliquots of serum was pipetted into serum vials using a micropipette and diluted with 300 µl double de-ionized water. The resulting mixture was de-proteinised by vortex mixing for 30 seconds with 600 µl ethanol containing BHT (0.0599 g/ml) as an antioxidant (Howe and Sherry, 2006). Extraction was repeated twice with 2 ml hexane and the combined supernatant evaporated under a stream of nitrogen at 30 °C. The residue was dissolved in 70 µl ethyl acetate and vortex- mixed for 10 seconds. The sample was diluted with 200 µl of the freshly prepared mobile phase (an aqueous binary mixture of acetonitrile and water in ratios of 85:15 (v: v) containing 0.1% BHT as an antioxidant) which had been filtered and ultrasonically degassed for one hour before use.

3.11.4 Extraction of iron and zinc from serum

Serum samples were left out to thaw for ten minutes and 1000 µL of the serum was taken from the sample tubes and transferred to 100 ml beakers. Exactly 20 ml of

distilled water was added to the samples followed by 8 ml of concentrated sulphuric acid and 2ml of hydrogen peroxide. The samples were then placed on a hot plate at 150 ºC for 10 minutes and thereafter filtered through whatman filter paper grade one. The filtrate was transferred into 100 ml volumetric flask then topped up to the mark with distilled water and transferred into 60 ml storage containers for AAS analysis for iron and zinc.

3.12 Method validation

The accuracy of methods used was verified by determining the percentage recovery of the analyte in the serum and saliva samples using equation [3.2].

$$\text{Recovery} = \frac{C_s - C_x}{C_{add}} \; X \; 100 \quad \text{[3.2]}$$

C_s: Concentration determined in the spiked sample

C_x: Concentration determined in unspiked sample

C_{add}: Expected additional concentration due to spiking

Precision was determined by repeatability and by calculating the relative standard deviation (RSD) of repeated measurements (Lu Ning-wei *et al.*, 2016) of the test sample according to equation [3.3].

$$\text{RSD} = \frac{S}{X} \; x \; 100 \quad \text{[3.3]}$$

Where S is the standard deviation of the replicate measurement of the test sample and X is the mean concentration of the repeated measurement of the test sample.

3.12.1 HPLC calibration of beta carotene

A stock solution containing 100 µg/mL of all-trans-beta carotene was prepared by

dissolving 0.0100g of the all-trans-beta carotene standard in hexane containing 0.1% BHT (w/v) in a 100ml volumetric flask and made it to the mark (Howe and Sherry, 2006). The stock solution was degassed ultrasonically for 20 seconds to homogenize the solution before preparation of working standards. Working standard solutions (20, 40, 60, 80 and 100 µg/mL) were prepared from the stock solution by pipetting 20, 40mL, 60 and 80mL into 100 mL volumetric flasks and each of the solution was diluted to 100 mL mark with a mixture of methanol and dichloromethane (DCM) in the ratio 9:1 (v: v). The blank and the working solutions were run in an HPLC column and a calibration curve was generated by plotting peak areas against concentration and its regression equation obtained (appendix VI).

3.12.2 HPLC calibration of retinol

The stock solution containing all-trans-retinol was prepared by dissolving 25 mg of all-trans-retinol standard in hexane and volume made to 250 ml in a volumetric flask. The working standards (2, 4 and 6 µg/mL) were prepared from the stock solution by pipetting 2, 4 and 6 mL of the stock solution into 250 mL volumetric flasks and diluted to the mark with an aqueous binary mixture of acetonitrile: water in ratios of 85:15 (v: v) containing 0.1 % BHT as an antioxidant (Howe and Sherry, 2006). The working standards were run in the HPLC instrument connected to a UV-Vis detector set at a wavelength of 325 nm. The generated peak areas for each of the run working standards were plotted against their respective concentration to obtain the standard calibration curve and its regression equation (appendix VI).

3.12.3 Calibration of iron and zinc in serum

A standard iron and zinc stock solutions of 1000 ppm were separately prepared by weighing 1.083 g of ferric nitrate and 0.724 g of zinc nitrate, respectively, in a small beaker and dissolving it in 50 ml of distilled water. The resulting solution was quantitatively transferred into a 250 ml volumetric flask and topped to the mark using distilled water. A working standard of 100 ppm was prepared from the stock solution by serial dilution. Working standards of 0, 4, 6 and 8 ppm were then prepared by pipetting 0, 4, 6 and 8 mL of the 100 ppm standard solution respectively into 100 mL volumetric flask and topped up to 100 mL using distilled water. The standards were then aspirated into the AAS instrument to obtain their absorbance. A standard calibration curve was obtained by plotting absorbance versus the respective concentration of the standards to give the regression equation (appendix VI).

3.12.4 Calibration of deuterium oxide

Calibration and quantification of deuterium oxide was done according to the IAEA (2010) procedures. To prepare 1000 ppm of the calibration standard, 2 g of deuterium oxide was diluted to 1L with water and transferred to a clean, dry glass bottle with a polytetrafluoroethylene (PTFE) lined screw cap and stored in a cool place. A similar volume of water was kept for use as a blank to measure the background spectrum. Weighing of the water and the deuterium water was done using an electronic balance. Working standards of 0, 100, 200, 400, 600, 800, 1000, 1500 and 2000 ppm were prepared by pipetting 5, 10, 20, 30, 40, 50 and 100 mL of 2000 ppm of D_2O stock solution and then diluting with water in 100mL volumetric flask.

The FTIR instrument was switched on and initialized to obtain background spectrum. Exactly 1mL of each of the working standards was introduced to the FTIR cell using a 1mL syringe and scanned. The peak area of the working standards was plotted against the concentration of the respective working standard to obtain a standard calibration curve and its regression equation (appendix VI).

Linearity test of concentration and limit of detection for each method were also done. The linearity domain was checked from the standard calibration curve. The linearity of the calibration curve is given by y = mx-c equation, where the calculated blank sample absorbance is given by the intercept c and the method sensitivity is given by the slope and the degree of linear relation between the signal and concentration is shown by the correlation coefficient R^2. Limit of detection (LOD) were calculated using equation [3.4] (EURACHEM guide, 2017) using the determined absorbance values for 10 replicates of the blank solution, then transformed into concentration values in order to be compared with the data obtained from the calibration curve.

$$LOD = \bar{X}_{blank} + 3S_{blank} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots [3.4]$$

$\bar{X}$ is the mean concentration of the replicates and S is the standard deviation.

3.12.5 Quantification of beta carotene

The samples (serum and vegetable) were then run in the HPLC column to obtain their peak areas and their concentration extrapolated from the calibration curve using the

regression equation Y = 54140.7X – 216928. In the regression equation Y is the peak area and X is the concentration of the sample.

3.12.6 Quantification of retinol

The samples were then run in the HPLC instrument to obtain their peak areas. To obtain the concentration of the samples, peak areas were inserted into the regression equation (Y= 996266X-98853.8) of the Retinol calibration curve. In the regression equation Y is the peak area and X is the concentration of the sample.

3.12.7 Quantification of iron and zinc

The extracted samples were then aspirated into the AAS instrument to obtain their absorbance. The concentration of the samples were obtained by inserting the absorbance into the regression equation (Y = 0.138X-0.0100 for iron and Y = 0.270X-0.003 for zinc), where Y is the absorbance and X is the concentration of iron and zinc in the sample (Appendix VI).

3.12.8 Quantification of deuterium in saliva samples

The vials with saliva samples were allowed to thaw for one hour before centrifugation for 15 minutes at 1000 rpm to remove bubbles. The samples were then introduced into the FTIR instrument and the peak areas determined from which the concentration of deuterium oxide was obtained by regression analysis. To obtain the body free fat mass (FFM), the total body water of study subjects was determined by dividing the gravimetrically determined mass of the dose of deuterium water consumed in mg by the

respective reading of the FTIR instrument in ppm (mg /kg). The TBW of the study subject was then used to work out the subject's FFM (IAEA, 2010).

3.13 Permit and ethics

The study was reviewed and approved by the National Ethical Review Committee at Kenyatta University (Appendix IX), while permit NACOSTI/P/15/3659/5730 obtained from the National commission for science, technology and innovation (NACOSTI). To protect participant confidentiality, unique identifiers were assigned to each study participant and a password protected linking file was stored on a separate secure server, only accessible by the study investigator. Questionnaire data was collected by trained data collectors, uploaded and securely stored in a database on a secure password-protected server. All handwritten field notes from the data collection, data-collection forms, and notes were secured in a locked file cabinet in a secured location.

3.14 Data analysis

Statistical data analysis was performed using Statistical Package for Social Sciences (SPSS) version 21 software. Data on dietary practices, morbidity patterns, demographic, socio-economic characteristics and nutritional status were described by use of means and percentages and presented in form of figures and tables. Independent t-test was used to compare the mean FFM, hemoglobin levels, serum levels of Zn, Fe, beta carotene and retinol between the experimental groups and control groups at baseline and endline in both phases I and II while paired t-test was used to compare the percentage means. ANOVA was used to compare the nutrient content of the fresh and cooked vegetables at

three different ages of growth. Emergency nutritional assessment (ENA) for Standardized monitoring and assessment of relief and transition (SMART) software was used to convert anthropometric data to Z- scores. Nutri-Survey software was used to analyze 24-hour recall data to establish the nutrient intakes (vitamin A, zinc, iron, protein and energy) of the study subjects. All significance levels were determined at 95 % confidence level and $p = 0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Baseline information

4.1.1 Demographic and socio-economic characteristics

Table 4.1 shows demographic data on gender, age, occupation, income bracket and marital status of respondents in Kangundo (experimental) and Kilalani (control) per group assignment during phase I of the study.

Table 4.1: Demographic data of respondents

	Experimental group (%)	**Control group (%)**
Gender		
Male	16.3	29.2
Female	83.7	70.8
Age bracket		
21-30	25	36
31-50	65.9	48
51-70	9.1	16
Occupation		
Self employed	47.7	32
Employed	18.2	36
Unemployed	34.1	32
Income bracket		
<2,000	37.8	48
2,001-4,000	33.3	32
4,001-6,000	6.7	16
6,001-8,000	4.4	4
8,001-10,000	13.3	0
>10,000	4.4	0
Marital status		
Married	88.6	80
Single	4.5	4
Widowed	6.8	8
Separated	0	8

In terms of gender, there were more female than male in both the experimental and control groups thus with over 70.8 % female respondents in each case. The majority (over 50 %) of the respondents were aged between 31-50 years.

Findings indicated that most respondents fell in the bracket of being self-employed or were unemployed for the experimental group while in the control group the distribution across the three categories that described their occupation was fairly uniform. However, irrespective of their category of occupation, more than 60 % of the respondent had earning of less than ksh 4000 per month, this pointing well the extent of poverty levels. It was noted that despite the school feeding programme, where every parent/guardian contributed 4 kg of maize and 1 kg of beans per month per pupil, some parents could not afford and their children only started having lunch on recruitment to participate in the study.

4.1.2 Food sources and consumption of indigenous vegetables

Data obtained from the questionnaire analysis on food sources, consumption and preparation of indigenous vegetable is given in Table 4.2.

Table 4.2: Food sources and consumption of vegetables

	% Experimental group (n=66)	% Control group (n=46)
(i)Common vegetable consumed	18.5	20.2
Kale (*B.carinata*)		
White cabbage	18.1	20.2
Spinach	16.8	18.5
Cowpea	15.9	17.7
Jute mallow	2.2	8.1
Amaranth	15.9	15.3
Night shade (*S. nigrum*)	8.6	0
Spider plant(*C. gynandra*)	3.8	0
(ii)Methods of vegetable preparation		
Boiling	4.7	29.4
Sautéing	93.0	47.1
Steaming	2.3	23.5
(iii) Sources of food		
Purchased from the market	30.4	69.6
Home garden	67.4	26.1
Donated	2.2	4.3

A list of common vegetables consumed in Kenya was presented to gather information on their preferences. These included cabbages, spinach and kale which are exotic among the indigenous ones cowpea, amaranth, jute mallow, nightshade and spider plant. Although the exotic ones were more preferred the study was inclined to AILVs. This was not unique since AILV have been reported to be underutilized amidst the challenges of their availability (Egbi *et al*., 2018).

Cowpea and amaranth were found to have higher preference among the indigenous vegetables (range 15-20%) and were therefore used to prepare the vegetable recipe in this study. The study investigated on the most common method of preparation of the

vegetables among three common ones boiling, steaming and Sautéing. Sautéing of vegetables in oil was the most preferred method of cooking. Notably, over 90 % of respondents in the experimental group preferred this method against about 50 % in the control group. The study scope did not establish the immediate reason to this though factors such as income, education level, and socio-economic factors are attributed to such trends (Egbi *et al*., 2018). The preference on sautéing gave basis of using the same method in the recipe preparation in this study. The method holds an advantage that it releases nutrients and makes them more bioavailable.

Findings indicated that among the possible sources of vegetables that include market sources, donated and grown in home gardens most of the respondents in the experimental group sourced their vegetables form home gardens (67.4 %) against most of the respondents in the control group who purchased vegetables (69.6 %). Vicinity to the local market among other unestablished factors such as the socio-economic factors would be attributed to these findings (Egbi *et al*., 2018).

4.1.3 Morbidity patterns

The morbidity was investigated for sickness one month prior to the intervention and on even health conditions. The results are as presented in table 4.3.

Table 4.3: Morbidity patterns of the respondents

	Experimental group (%)	**Control group (%)**
(i) Sickness record 1 month prior to intervention		
Yes	40	56
No	60	44
(ii) Medical condition		
Anemia	63.2	25
Heart related condition	57.1	45
Any physical deformity	18.8	19
Unmentioned medical condition	40.5	24

A majority of the study subjects in the experimental group were reported to have anemia (63.2 %) unlike those in the control group (25 %). Anemia is common among pre-school children and is closely related to chronic micronutrient deficiencies. The prevalence of anemia at a lower percentage in the control group as compared to the experimental can be attributed to the socio-economic status of the parents of the control group. However, prevalence at 25 % was still alarming. These findings indicated high morbidity in the study subjects.

4.2 Twenty-four hour dietary recall

Table 4.4 presents the results for 24-hour dietary recall to show the mean intakes of nutrients at baseline and endline among the two study groups.

Table 4.4: Comparison of mean intakes of nutrients between baseline and endline among the two study groups

Nutrient	Mean dietary intakes					
	Control group (n=46)			Experimental group (n=66)		
	Baseline	Endline	p-value	Baseline	Endline	p-value
Energy/ kcal	761.67+98.4	793.86+120.9	0.001	717.08 +102.6	728.69 + 99.0	<0.001
Protein/ g	25.03 + 7.5	27.18 +7.0	<0.001	21.08 + 6.7	22.75 + 6.9	<0.001
Iron/mg	6.88 + 1.3	6.80 +1.0	0.346	7.37 + 1.4	8.32 + 1.3	<0.001
Zinc/mg	5.16 + 1.4	5.16 + 1.4	0.966	4.49 + 1.2	4.88 + 1.3	<0.001
Vit. A/µg	431.66+101.6	429.28+101.9	0.427	386.04 + 106.7	409.66 + 105.98	<0.001

N/B: Paired t-test, 95 % CL, p = 0.05.

The results showed that although the control group children had significantly higher intakes of mean energy and protein at endline than at baseline ($p < 0.05$), the mean intakes of zinc, iron and beta carotene among the control group children were not significantly different between the two stages of dietary analysis. Notably, the experimental group children had significantly higher intakes of all the nutritional components analyzed at endline than at baseline ($p < 0.001$).

The higher mean intakes of energy and protein at endline than at baseline in both groups could be attributed to the mixture of maize and beans consumed by both groups at lunch time plus other sources at home. Maize has high calories and beans have high protein content. However, the significant higher intake of the three micronutrients in the experimental group than control could be attributed to the vegetable recipes that contained high levels of iron, zinc and beta carotene. The mean energy intake for the children both in the control and experimental group was lower than the RDA (Table 4.4). The mean energy intake for the control group increased from 761.67 + 98.4 at baseline to 793.86 + 120.9 Kcal/day at the endline but was still below the RDA of 1400 Kcal/day. The mean energy intake for the control group increased from 717.08 + 102 at baseline to 728.69 + 99.0 Kcal/day at the endline but still was equally below the RDA of 1400 Kcal/ day. The experimental group children had significantly higher intakes of all the nutritional components analyzed at endline than at baseline ($p < 0.001$). The baseline mean iron intake increased from 7.37 + 1.4 to 8.32 + 1.3 mg/day at endline to meet the RDA of 8mg/day (UNICEF, 2009). The mean iron intake for the control group was below the RDA of 8mg/day.

The mean zinc intake for the control group at baseline and endline was not significantly different though it met the RDA of 5 mg /day (Miller *et al*., 2007). However, the endline mean zinc intake in the experimental group was significantly higher than at baseline though slightly below the RDA of 5 mg /day due to losses in vegetable preparation, cooking and less bioavailability. This significant increase could be due to the supply of zinc from the vegetable recipe that the experimental group consumed. The

mean vitamin A for the experimental group at baseline increased from 386.04 ± 106.7 to 409.66 ± 105.98 µg at endline to meet the RDA for vitamin A for children, which is 400 µg (WHO, 2000).

Table 4.5 presents the percentage contribution to RDA of the control group and experimental group at baseline and endline (independent t-test) and table 4.6 shows a comparison of the mean dietary intakes (percentage RDA) at baseline and endline for both the control group and experimental group (paired t-test).

Table 4.5: Study children's dietary intakes (% RDA)

Nutrient	**Dietary intakes (% RDA)**					
	Baseline			**Endline**		
	Control (n=46)	**Expt. (n=66)**	**p-value**	**Control (n=46)**	**Expt. (n=66)**	**p-value**
Energy	108.49 ± 8.3	107.62	0.590	113.06 ± 12.4	109.38	0.077
Protein	91.03 ± 20.2	±8.4	0.818	99.25 ± 16.7	±7.4	0.512
Iron	77.98 ± 10.8	90.29 ± 9.8	0.977	77.12 ± 8.8	97.55 ± 6.0	<0.001
Zinc	75.92 ± 10.9	78.05	0.768	76.13 ± 9.7	88.11 ± 9.7	<0.001
Vit. A	83.63 ± 12.6	±11.4	0.922	83.20 ± 12.7	83.31 ± 9.8	0.013
		76.54			89.16	
		±10.9			±12.0	
		83.86				
		±12.9				

N/B: Independent t test, 95 % CL, p = 0.05

Table 3: Study children's dietary intakes (% RDA)

Nutrient	Dietary intakes (% RDA)					
	Control (n=46)			Experimental (n=66)		
	Baseline	Endline	p-value	Baseline	Endline	p-value
Energy	108.49 ± 8.3	113.06 ± 12.4	0.002 <0.001	107.62 ± 8.4	109.38 ± 7.4	<0.001 <0.001
Protein	91.03 ± 20.2	99.03 ± 16.7	0.389	90.29 ± 9.8	97.55 ± 6.0	<0.001
Iron	77.98 ± 10.8	77.12 ± 8.8	0.797	78.05 ± 11.4	88.11 ± 9.7	<0.001
Zinc	75.92 ± 10.9	76.13 ± 9.7	0.453	76.54 ± 10.9	83.31 ± 9.8	<0.001
Vit. A	83.63 ± 12.6	83.20 ± 12.7		83.87 ± 12.9	89.16 ± 12.0	

N/B: Paired t- test, 95 % CL, p = 0.05

From the results there was a significant difference in the dietary adequacy (percentage fulfillment of RDAs) between the diets of the control and experimental groups at baseline, with regard to all the nutritional components analyzed ($p < 0.05$) for all the nutrients (table 4.5). After 13 weeks of intervention the experimental group achieved significantly higher dietary adequacy (higher percentage fulfillment of RDAs) with regard to the micronutrients (Fe, Zn and vitamin A) than the control Group ($p <0.05$). However, after 13 weeks the percentage fulfillment of RDAs for energy and proteins were not significantly different between the two groups ($p > 0.05$). This could be attributed to the mixture of maize and beans consumed by both groups at lunch time plus other sources at home. Maize has high calories and beans have high protein content.

The results (table 4.6) showed that even though the percentage RDAs for the three micronutrients (Fe, Zn and vitamin A) did not vary significantly among the control group children at both baseline and endline stages ($p > 0.05$), percentage fulfillment of

energy and protein RDAs amongst children in the control group significantly differed at baseline and endline, with the children achieving greater percentage RDAs of both energy and proteins after the intervention ($p < 0.05$). Experimental group children achieved greater percentage RDAs for all the nutrients analyzed at endline as compared to baseline, (p values < 0.05).

4.3 Soil fertility analysis for school gardens

The soil fertility analysis report for gardens in the two schools, Kangundo and Kilalani Primary are presented in Table 4.7.

Table 4: Soil test report for Kangundo and Kilalani primary schools

	Kangundo primary school		**Kilalani primary school**	
Field lab. No/2015	2133	2134	2135	2136
Soil depth	top	Sub-top	top	sub-top
Soil pH	8.08	7.53	7.41	7.58
Total nitrogen (%)	0.06	0.06	0.06	0.04
Total org. carbon (%)	0.51	0.57	0.52	0.32
Phosphorus ppm	14.00	5.00	4.00	3.00
Potassium (%)	1.27	1.04	0.42	0.32
Calcium (%)	3.50	3.90	2.60	2.20
Magnesium (%)	2.89	1.36	1.41	1.15
Manganese (%)	0.60	0.40	0.53	0.69
Copper ppm	6.19	9.63	2.81	3.10
Iron ppm	65.4	52.9	124	116
Zinc ppm	28.4	46.1	16.2	15.5
Sodium (%)	0.40	0.42	0.20	0.24
Electrical conductivity. (mS/cm)	0.18	0.11	0.11	0.21

The pH of the soils in both schools was alkaline and phosphorus was deficient, thus necessitating application of diammonium phosphate and calcium ammonium nitrate. All

other soil nutrients (nitrogen, organic carbon, phosphorus, potassium, calcium, magnesium, manganese, copper, iron, zinc and sodium) were adequate for vegetable growth (Martens and Westermann, 2016).

4.4 Method validation

4.4.1 Recovery

The percentage recovery of the analyte in the serum and saliva samples was determined to verify the accuracy of each method and the results are shown in Table 4.8.

Table 4.8: Percentage recovery of the analyte

Method	Analyte	$C_{x\ (ppm)}$	$C_{add\ (ppm)}$	$C_{s\ (ppm)}$	% Recovery
HPLC	Beta carotene	0.49	5	5.46	99.4
HPLC	Retinol (vit. A)	0.76	5	5.78	100.4
AAS	Iron	14.32	5	19.3	99.6
AAS	Zinc	0.72	5	5.75	100.6
FTIR	FFM	406.36	100	507.4	101.0

The percentage recovery ranged from 99.4 to 101.0 %, indicating that the methods of analyses used were accurate (Eurachem Guide, 2017).

4.4.2 Repeatability

Precision was determined by calculating the relative standard deviation (RSD) of repeated measurements of the test sample and the results presented in Table 4.9.

Table 4.9: Relative standard deviation (RSD) of repeated measurements of the parameter

Parameter	Mean n=3)	SD	%RSD
beta carotene	0.49	0.01	2.66
Retinol (vit. A)	0.76	0.02	2.64
Fe	14.32	0.17	1.19
Zn	0.72	0.02	2.09
FFM	18.84	0.24	1.27

The relative standard deviation ranged from 1.19 % to 2.66 % which is in agreement with work done by Lu Ning-wei *et al.* (2016) that showed that the relative standard deviation of less than 3 % is sufficiently precise.

4.4.3 Linearity and limit of detection

Linearity test of concentration and limit of detection (LOD) for the various methods are shown in Table 4.10.

Table 4.10: Linearity test of concentration and limit of detection

Analyte	LOD	Regression equation	R^2
beta carotene	4.01	Y = 54140.7X - 216928	0.9986
Retinol (vit. A)	0.0992	Y = 996266X -98853.8	0.9962
Iron	3.673	Y = 0.138X -0.01	0.999
Zinc	2.579	Y = 0.27X -0.003	0.999
Deuterium oxide	4776.89	Y = 0.001X + 0.016	0.993

The R^2 values ranged from 0.993 to 0.999, meaning that the instrumental response to concentration was above 99.3 %. The R^2 values being closer to 100 % indicate that the established calibration curves are linear over the respective range of the concentration

of the working standards that were used. The calibration curves for iron, zinc, beta carotene and deuterium are shown in Appendix VI.

4.5 Levels of nutrients in raw and cooked vegetables

Table 4.11 shows the mean levels of beta-carotene, zinc and iron in raw school garden-sourced *Vigna unguiculata* and *Amaranthus cruentus* leaves harvested after 21, 35 and 50 days of planting.

Table 4.11: Levels of micronutrients in raw vegetable leaves at different days of maturity

Days of maturity	Micronutrient content of vegetables in mg/100g DW					
	Cowpea leaves (n=3)			Amaranth n=3)		
	Fe	Zn	beta carotene	Fe	Zn	beta carotene
21 days	30.497±0.031[a]	3.320±0.040[b]	4.110±0.017[a]	73.260±0.066[c]	6.253±0.055[b]	5.747±0.040[a]
35 days	41.910±0.036[b]	2.907±0.032[a]	4.430±0.062[a]	84.707±0.045[b]	5.820±0.010[b]	8.823±0.012[b]
50 days	55.890±0.092[c]	2.433±0.038[a]	5.123±0.025[b]	99.567±0.372[a]	4.023±0.078[a]	9.867±0.059[c]
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Mean ± SD followed by the same small letter within the same column do not differ significantly from one another (one-way ANOVA, SNK-test, $\alpha = 0.05$).

The mean level ranges of the micronutrients (mg /100g) in the two vegetables were Fe (30.4967 ± 0.031-99.5667 ± 0.372), Zn (2.4333 ± 0.038-6.2533 ± 0.055) and beta carotene (4.41100 ± 0.017-9.8667 ± 0.059). These levels were sufficient to meet the RDA for the study subjects according to UNICEF (2009). One way ANOVA showed that the raw amaranth leaves at all the three different stages of growth had significantly higher mean levels of all the three micronutrients than the cowpea leaves ($p < 0.001$; 95% CL, $p = 0.05$).

As the plant aged, the levels of iron and beta carotene generally increased significantly ($p < 0.001$) supporting positive correlations between plant age and nutrient content (Bergquist, 2006).

The decrease in zinc however is due to the distribution of the products of photosynthesis to the reproductive parts of a plant (Makobo *et al*., 2010; Cherioyot, 2011). Further, iron inhibits zinc absorption when the iron levels are more than twice those of zinc and that the bioavailability of either zinc or iron at any age of vegetable is influenced by the interactions between the two (Flyman and Afolayan, 2007). Table 4.12 presents a comparison of levels of iron, zinc and beta carotene content in both raw and cooked mixtures of the two vegetables harvested at 35 days.

Table 4.12: Levels micronutrients in raw and cooked vegetable recipe harvested at 35 days

Nutrient	Raw(n=3)	Cooked(n=3)	P value	loss	% loss
Iron	63.302	55.465 ±	< 0.001	7.837	12.38
Zinc	±0.001	0.419	< 0.001	0.946	21.62
Beta	4.376 ± 0.033	3.430 ± 0.054	< 0.001	2.327	35.12
carotene	6.626 ± 0.007	4.299 ± 0.010			

N/B: Independent t test, 95 % CL, p = 0.05

The mean zinc content in the recipe was 3.430 mg /100g ± 0.054 while the mean beta carotene content in the recipe was 4.299 mg /100g ± 0.010 and these were able to meet the RDA of the study subjects (Miller *et al*., 2007).

The levels of the three micronutrients in the cooked vegetables were significantly lower than in the raw vegetables ($p < 0.001$). Methods of preparation and cooking significantly reduced the mean iron, zinc and beta carotene content in the vegetables recipe ($p<0.001$). The highest content reduction was of beta carotene (35.12 %), followed by zinc (21.62 %), and iron (12.38 %). The loss of beta carotene is attributed to oxidative degradation and *trans*/*cis* isomerization as a result of thermal processing during the preparation and cooking procedure (Mulokozi *et al*., 2004).

The percentage mean losses reported in the present study are comparable to those reported by other studies (Mulokozi *et al*., 2004). Methods of preparation caused losses ranging from 14-51 % were reported while losses due to cooking ranged from 6-34 % (Mulokozi *et al*., 2004). The loss of iron and zinc during preparation and cooking could

be attributable to leaching into the water rather than their destruction. Chopping vegetables into small pieces may have led to significant iron and zinc losses because more surface area got into contact with water and increased leaching.

4.6 Effect of intervention on FFM and FM

Table 4.13 presents the mean free fat mass (FFM), free mass (FM) and body mass index (BMI) of the phase I and II control and experimental groups.

Table 5: Levels and percentage of body FFM, FM and BMI of the experimental and control groups in phase I and II

Phase I								
	Experimental (n=66)			**Control (n=46)**			**Baseline P- value**	**Endline P- value**
	Baseline	**Endline**	**P-value**	**Baseline**	**Endline**	**P-value**	**Expt. Vs Control**	**Expt. Vs Control**
BMI(Kg/m^2)	15.112 ± 1.40	15.126 ± 1.45	0.861	14.747 ± 1.22	14.968 ± 1.16	0.177	0.156	0.540
FFM (Kg)	18.866 ± 2.64	20.097 ± 2.80	<0.001	18.340 ± 2.27	19.001 ± 2.29	0.074	0.275	0.003
FFM (%)	77.508 ± 4.95	80.420 ± 4.90	<0.001	77.701 ± 3.89	77.533 ± 3.82	0.64	0.826	0.001
FM (kg)	5.488 ± 1.41	4.927 ± 1.47	<0.001	5.291 ± 1.21	5.553 ± 1.36	0.076	0.443	0.041
FM (%)	22.492± 4.95	19.712 ± 5.12	<0.001	22.299 ± 3.89	22.467 ± 3.82	0.64	0.826	0.001
Phase II								
	Experimental (n=46)			**Control (n= 66)**			**Baseline P- value**	**Endline P- value**
	Baseline	**Endline**	**P-value**	**Baseline**	**Endline**	**P-value**	**Expt. Vs Control**	**Expt. Vs Control**
BMI(Kg/m^2)	14.868 ± 1.09	14.887 ± 1.91	0.802	15.138 ± 1.25	15.136 ± 1.42	0.863	0.530	0.431
FFM (Kg)	18.597 ± 2.19	19.916 ± 2.95	0.002	20.107 ± 2.75	20.097 ± 2.79	0.977	0.004	0.743
FFM (%)	77.224 ± 2.34	81.058 ± 4.64	<0.001	80.400 ± 4.05	80.320 ± 5.10	0.692	0.001	0.437
FM (kg)	5.460 ± 1.26	4.636 ± 1.23	<0.001	4.916 ± 1.47	4.909 ± 1.44	0.809	0.042	0.267
FM (%)	22.776 ± 2.34	18.942 ± 4.64	<0.001	19.683 ± 5.02	19.580 ± 4.90	0.603	0.001	0.418

Independent and paired t tests, 95 % CL, p = 0.05

All the experimental groups showed significant improvements in the mean FFM and not for BMI in both phases I and II. In both phases, subjects in both control and experimental groups had similar indices of BMI ($p = 0.156$ and 0.540). On the other hand, subjects in both control and experimental groups had different indices of FFM (0.275 and 0.003) and FM (0.443 and 0.041) at baseline entities. The effect of intervention is projected from changes in the indices at endline. At endline, FFM increased as FM decreased as would be attributed to a positive impact of the intervention for the experimental group which consumed the vegetable recipe. The body's FFM is inversely proportional to FM, the constant of proportionality being total body mass. Increase in FFM implies growth of soft tissue as a result of increased cell division due to the nutrients supplied by the AILVs consumed (MacDonald, 2000). Hence the percentage FFM for the control group which was 77.70074 ± 3.89 at baseline would be explained to decrease to 77.53346 ± 3.82 at endline.

Studies including those of Shakur *et al.* (2009), Marcia *et al.* (2015); Lora *et al.* (2015) and Fabiansen *et al.* (2017) support nutritive intervention to address malnutrition. In these studies, all the experimental groups showed significant improvements in body compositions. The interventions involved experimental group subjects receiving zinc supplementation (Marcia *et al*., 2015), iron and vitamin A supplements (Fabiansen *et al*., 2017). A nutrition intervention ideally results in changes in body composition of individuals. Notably, the study findings indicate that changes were significant for FFM ($p = 0.002$) and FM ($p < 0.001$) unlike for BMI ($p = 0.802$). While the latter therefore showed that the intervention had no significant effect on the nutritional status of both

the experimental and control group, the converse was true using the DDI method. While the most commonly employed method of nutrition status measurement is BMI, nutrition outcomes based on BMI have been underestimated or overestimated and fail to distinguish FM and FFM (Paula *et al*., 2011; Thompson and Subar, 2013; Javed *et al*., 2015). This finding highlighted the limitation of BMI in the determination of body composition in children.This is consistent with findings by Ndung'u (2017) and Diouf *et al*. (2018). Ndung'u (2017) used DDI method to validate obesity measurement by BMI and demonstrated that a high percentage of the children found to be normal by BMI measurement turned to be obese by DDI method measurement. In fact, the excessive fatness by DDI method was three times higher than that by BMI (Diouf *et al*., 2018).

4.7 Levels of serum micronutrients and hemoglobin count

Table 4.14 presents the baseline and endline serum zinc, iron, beta carotene, retinol levels and hemoglobin (Hb) count for the phase I and II control and experimental groups.

Table 4.14: Levels of serum micronutrients and hemoglobin at baseline and endline for experimental and control groups in phase I and phase II

Phase 1								
Serum constituents	**Experimental n=66**			**Control n=46**			**Baseline p-values**	**Endline p-values**
	Baseline	**Endline**	**p-value**	**Baseline**	**Endline**	**p-value**	**Expt. vs control**	**Expt. vs Control**
Fe (µg µ/L)	11.644±1.81	14.246±1.51	<0.001	11.634±0.23	11.874±0.21	0.097	0.978	<0.001
Zn (mg/L)	0.582 ±0.15	0.725±0.17	<0.001	0.577± 0.10	0.584±0.11	0.706	0.843	<0.001
BC(µmol/L)	0.168±0.00	0.491± 0.13	<0.001	0.167±0.00	0.172±0.03	0.392	0.076	<0.001
Retinol (µmol/L)	0.523±0.001	0.760±0.20	<0.001	0.523±0.01	0.530±0.11	0.686	0.585	<0.001
Hemoglobin (g/L)	112.918±16.58	118.541±17.39	<0.001	112.424±12.01	112.445±9.93	0.985	0.855	<0.021
Phase 2								
Serum constituents	**Experimental n= 46**			**Control n=66**			**Baseline p-values**	**Endline p-values**
	Baseline	**Endline**	**p-value**	**Baseline**	**Endline**	**p-value**	**Expt. vs control**	**Expt. vs Control**
Fe (µg/L)	11.852±1.13	14.320±1.71	<0.001	14.239±1.80	14.235±1.80	0.090	<0.001	0.802
Zn (mg/L)	0.589±0.12	0.719±0.15	<0.001	0.712± 0.15	0.708±0.14	0.064	<0.001	0.698
BC (µmol/L)	0.169±0.05	0.492±0.13	<0.001	0.488± 0.09	0.485± 0.13	0.075	<0.001	0.752
Retinol (µmol/L)	0.528±0.09	0.765±0.20	<0.001	0.759± 0.20	0.756± 0.19	0.058	<0.001	0.796
Hemoglobin (g/L)	112.437±8.63	116.516±16.61	<0.001	118.524±17.61	118.522±17.31	0.15	0.15	0.542

Independent and paired t- tests, 95 % CL, p=0.05.

Studies including those of Black *et al.* (2013), Winichagoon *et al.* (2006), Chege (2012), Nawiri *et al.* (2013), Marcia *et al.* (2015) and Egbi *et al.* (2018) support nutritive intervention to address malnutrition. In these studies, all the experimental groups showed significant improvements in serum micronutrients levels. The interventions involved experimental group subjects receiving zinc supplementation (Marcia *et al*., 2015), iron and vitamin A supplements (Fabiansen *et al*., 2017). A nutrition intervention is expected to result in changes in serum levels of micronutrients of individuals. Notably, the current study findings indicate significant improvements ($p < 0.001$) in the levels of iron, zinc, beta-carotene, retinol and Hb count among the experimental group children during both phases of study which was attributable to the consumption of the vegetable recipe. These findings corroborate with those of Black *et al.* (2013), Winichagoon *et al.* (2006), Chege (2012), Nawiri *et al.* (2013), Marcia *et al.* (2015) and Egbi *et al.* (2018).

The mean serum zinc values in the findings by Marcia *et al.* (2015) are slightly higher than those reported in the present study. This could be due to the difference in the sources of zinc and the amounts consumed by the study subjects. The source in the study by Marcia *et al.* (2015) was in form of supplements while in the present study was mainly from the consumed vegetable recipe. The micronutrient bioavailability from the vegetables is affected by anti- nutrients present in the vegetable, among other factors like intestinal matrix of the study subjects. The body also has no mechanism of storing zinc and the levels of zinc in the body may be depleted according to the body metabolism in response to the body needs and functions. The study by Nawiri *et al.*

(2013) supports the findings in the current study that dark green vegetables are effective in improving children's Hb count and serum beta carotene and retinol and could be used with success to combat VAD and iron deficiency anemia. On the other hand no significant changes were observed in serum micronutrient levels in control group children in both phases of study ($p > 0.05$). This is attributable to non-consumption of the vegetable recipe by the control groups.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

(i) Baseline results showed poor consumption of indigenous vegetable (< 20 %). Cowpeas and amaranth were acceptable AILVs for study subjects when cooked by sautéing method. Morbidity was high at baseline (> 40 %), anthropometry and low HB results indicated malnutrition. About 50 % of the study subjects had been in good health condition prior to the study although anemia (a form of malnutrition) was a prevalent illness. The socio-economic status of the parents/guardians was low (> 95.6 % earned <Ksh.10, 000 month) hence the respondents (parents/guardians) were generally of low income standards.

(ii) The garden sourced vegetable recipe contained sufficient levels of Fe, Zn, and BC that is able to meet the RDA for children. A consumption of an average of 80 g (wet weight) of the recipe of *Amaranthus cruentus* and *Vigna unguiculata* met RDA of Fe and BC for children. However, the mean zinc content in the recipe was 3.430 mg /100g. An average of 160 g (wet weight) of the recipe would have to be consumed at every serving to meet the RDA for Zn.

(iii) At end line, the study subjects' body composition as indicated by the FFM and FM as well as the Fe, Zn and BC levels significantly improved ($p < 0.001$) during both intervention phases.

(iv) There was a positive micronutrient impact, as shown by significantly higher levels of Fe, Zn, BC, Retinol and HB in the experimental group as compared to the control group at end line ($p < 0.001$) during both phase I and II.

(v) Further**,** Deuterium Dilution Isotope method is more sensitive than BMI in determining nutrition intervention outcomes in children. The null hypothesis in this study is therefore rejected.

5.2 Recommendations

Despite the limitations highlighted in the present study, there was a positive micronutrient impact, as shown by significantly higher levels of Fe, Zn, BC, retinol and hemoglobin in the experimental group that consumed the garden sourced selected AILVs as compared to the control group at end line during both phase I and II. Since consumption of AILVs improves nutritional status of school going children the study makes the following recommendations.

(i) Promotion of school gardens and consumption of school-garden sourced AILVs should be done to improve nutrition of school going children.

(ii) Deuterium Dilution Isotope method, which gave results that were able to capture significant difference than BMI from the intervention, is recommended for use in the determination of nutrition intervention outcomes among children in field studies.

(iii) Each study subject in the experimental group consumed on average 80 g (wet weight) of the recipe of *Amaranthus cruentus* and *Vigna unguiculata* to meet RDA of the selected micronutrients for children. However, to meet the RDA for zinc one would have to consume an average of 160 g (wet weight) of the recipe at every serving.

Further, a different method of recipe preparation like steaming should be used and compared with sautéing to determine which of the two leads to more zinc being available.

(iv) Since planted vegetables draw their micronutrients from the soil, soil sampling and analysis should be done to determine levels of iron, zinc and other important elements in soils before establishment of vegetable gardens. Any deficiency in the soils should be corrected through appropriate fertilizer application.

(v) Further study needs to be done to compare the impact of the nutrition intervention in the present study between children from rural and urban schools. Additionally, another study is recommended to compare the impact of the nutrition intervention in the present study between male and female children in the same schools.

(vi) Synergetic effects of other minerals and phytochemicals that might be present in the recipe other than Fe, Zn, and BC on the nutrition status of children need further investigation.

(vii) The effect of anti-nutrients like phytic acid, oxalates, and phenols on bioavailability of the selected micronutrients in the present study also needs further investigation.

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APPENDICES

Appendix I: Consent form

Dear respondent,
My name is John Wakhanu Akello (P.o Box 43844-00100 Nairobi, Tel 0722347648), a PhD student from Kenyatta University. I am undertaking a research study on **NUTRITION-SENSITIVE INTERVENTION WITH AFRICAN INDIGENOUS LEAFY VEGETABLES AMONG SCHOOL-GOING CHILDREN IN KENYA**

Instruction:-Please read and understand (interpretation will be made where necessary) the information provided before signing or impressing using a thumb print to ascertain that both yourself (as parent/caretaker) and the child will participate in this study when called upon.

1. This study will maintain confidentiality and information from this study will be used for the purpose of this study only.
2. Participation in this study is voluntary and there will be no payment to you or from you that is associated with this study.
3. The child to be recruited in the study is my own (or I am the caretaker) and is in standard two and aged between 6-10 years.
4. The child will be either in the experimental group (_____) or control group (_____).
5. The child(_____) in the **experimental group** will receive a recipe of mixed fresh cowpea and amaranth leaves(50:50 wt./wt.) accompanied by the regular school meal which comprises of a mixture of maize and beans daily for five days week during the period May 2015-April 2016.(parents with children in the control group should not tick this section

[ ]

6. Any assessment planned to be undertaken on the child in both the experimental and control group during the study period will be communicated to me in advance. I accept the following measurements (**tick where applicable**)

a. Monitoring dietary practices of the child
[ ]

b. Assessment of morbidity patterns information on monthly basis

c. The child's weight and height to be taken at least 3 times during the study period
[ ]

d. The child's stool will be assessed foe worm infestation at least twice
[ ]

e. The child's serum (from blood) will be extracted for measurement of vitamin A, zinc and iron at the beginning and at the end of the study
[ ]

f. Deuterium oxide will be administered to the child and its composition in saliva will be measured at the beginning and at the end of the study

[ ]

7. At the end of the study there may be/not a change in the measurements made during the study

8. Any risks that may be suspected to be associated with the intervention (such as allergy, diarrhea, vomiting and intolerance due to consumption of the vegetable recipe) will lead to withdrawal of the child from the study and followed by appropriate information and counseling for both parent and child.

9. The direct/indirect benefits associated with this study have been clearly explained to me during a meeting at my child's school.

Your participation will be highly appreciated.

Thank you.

_____

John Wakhanu Akello

Respondent's consent

Please indicate your willingness to participate in the study

I_____ hereby undersigned accept that both my child and I will participate in the study and that I clearly understand the information on this consent form.

Yes----------No--------- Signature/Thumbprint----------

Appendix II: Structured questionnaire for parent/caregiver

Research Topic: NUTRITION-SENSITIVE INTERVENTION WITH AFRICAN INDIGENOUS LEAFY VEGETABLES AMONG SCHOOL-GOING CHILDREN IN KENYA

School_____Questionnaire No._____Date_____

Name of child_____

Dear respondent,

My name is John Wakhanu Akello (P.O Box 43844-00100 Nairobi, Tel 0722347648), a PhD student at Kenyatta University. I am undertaking above research study.

Instruction:-Please read and understand (interpretation will be made where necessary) the information provided before responding to the questions herein. The information will be confidential and will only be used for the purpose of this study. Please tick/fill to answer the questions below.

Your relationship with the child: Parent [ ] guardian [ ] Caretaker [ ]

Section 1: Demographic and socio- socio-economic characteristics of the respondent

Gender: Male [ ] Female [ ]

Age (years): below 20 [ ] 21-30 [ ] 31-50 [ ] 51-70 [ ] above 70 [ ]

Education levels: none [ ] Primary [ ] secondary [ ] vocational [ ] tertiary college [ ]

Occupation: Self-employed [ ] Employed [ ] Unemployed [ ]

Marital status: Married [ ] single [ ] Widowed [ ] separated [ ]

Monthly income (Ksh): less than 2000 [ ] 2001-4000 [ ] 4001-6000 [ ] 6001-8000 [ ] 8001-10000 [ ] above 10000 [ ].

Section 2: Morbidity patterns of the child

1. Has the child been sick during the last two weeks? Yes [ ] No [ ]

2. If yes, what were the signs of this sickness: Diarrhea [ ] Vomiting [ ] Fever [ ] Loss of appetite [ ]

3. Does your child visit the hospital for any of the following? Anemia: Yes [ ] No [ ] immunization: Yes [ ] No [ ] heart disease: Yes [ ] No [ ] Vitamin A supplementation: Yes [ ] No [ ] Deworming: Yes [ ] No [ ] Any Physical deformity: Yes [ ] No [ ]

4 (a) Does your child suffer from any known medical condition not mentioned above? Yes [ ] No [ ]

(b) If yes, state the condition_____

Section 3: Vegetables consumed by child and preparation:

1. Do you have a home vegetable garden? Yes [ ] no [ ]. If yes, which vegetables do you grow_____?

2. Which vegetables are consumed by your child at home: *Sukuma* [ ] white Cabbage [ ] Spinach [ ] *Kunde* [ ] *Murenda* [ ] Amaranth [ ] *Managu* [ ] *Sagaa* [ ] others [ ] specify_____

3. Which method is used to prepare them?

Boiling [ ] Frying [ ] Steaming [ ] Juicing [ ] Eaten uncooked [ ]. Others [ ] specify_____

4. Which vegetable does your child prefer most at home? _____

Section 4: Other information on feeding practices

1. What is the main source of food consumed by the child?
Purchased from the market [ ] home garden [ ] Donated [ ]
2. Number of meals normally taken by the child per day? _____
3. Is your child allergic to the following foods? (Control group don't respond).
Green amaranth yes [ ] no [ ] cowpea yes [ ] no [ ] bean stew yes [ ] no [ ]
4. If yes, how is the allergy manifested? Rashes [ ] Vomiting [ ] Diarrhea [ ] Pain [ ] Pimples [ ] Swellings [ ] others [ ] if any other specify_____
5. Is your child taking any food supplements? Yes [ ] no [ ]. If yes, what kind? _____
How often does the child take the supplements? Daily [ ] weekly [ ] monthly [ ]
6. How many meals does your child eat at home per day? 1 [ ] 2 [ ] 3 [ ] more than 3 [ ].
7. Do you feel the amount of food at home satisfies your child? Yes [ ] no [ ] not always [ ].
8. Does your child eat breakfast before going to school? Yes [ ] no [ ]. If yes, what is served for breakfast?
Brown porridge [ ] white porridge [ ] tea with escort [ ] tea without escort [ ]. Juice [ ]
Others [ ]. Specify_____
9. Does your child carry a snack to school to eat at break time before lunch? Yes [ ] no [ ]. If yes specify the snack_____
10. Does your child eat lunch at school? Yes [ ] no [ ]. If yes, which meals are served during lunch? _____
11. Do you think the food served to your child during lunch is enough? Yes [ ] no [ ]
12. Do these meals include leafy vegetables? Yes [ ] no [ ]. If yes, specify the type of vegetables_____
13. Do you think your child enjoys eating amaranth and cowpea leafy vegetables? Yes [ ] no [ ] If no, what is the reason? _____
Don't like taste [ ] in our home we don't eat [ ].other [ ]
Specify_____
14. Has your child been absent from school in the last one term? Yes [ ] no [ ]. If yes, what was the reason?
Illness [ ] unable to pay school levies [ ] funeral [ ] hunger [ ] others [ ]. Specify_____

Appendix III: Dietary practices

Food frequency table and 24-hour dietary recall sheet

This is to establish the meals taken per day, ingredients and the amount in the meals, the weight of the food cooked the weight of the food given to the child and to calculate the amount of ingredients consumed in grams by the child.

Part 1: Food frequency table

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

Food Item	Frequency per Week	Food Item	Frequency per Week
Maize		Eggs	
Maize flour		Liver	
Rice		Milk	
Sorghum		Fish	
Cassava		‘’Omena’’	
Millet		Meat	
Raw bananas		Groundnuts	
kales		Avocado	
Cabbage		Mangoes	
Spinach		Papaws	
Carrots		Passion fruits	
Tomatoes		Ripe bananas	
Pumpkins		Oranges	
sweet potatoes		Guava	
Beans		Watermelon	
Peas		Herbs	
Green grams		Supplements	
Irish Potatoes		Seasoning	

Part II: 24 Hour dietary recall sheet

				Cooked Dishes			**Uncooked food**		**Official**
Meal	**Time**	**Type of dish/food name**	**Ingredients**	**Weight of empty plate (g)**	**Weight of plate + food (g)**	**Amount taken(g)**	**Weight of empty plate (g)**	**Weight of plate + food taken(g)**	**Amount taken (g)**
Breakfast									
Snack 1									
Lunch									
Snack 2									
Supper									
Snack 3									

Appendix IV: Anthropometric and biochemical measurement data sheet.

1. The age of the child (in years) _____
2. Sex of the child [ ] Male [ ] Female
3. Baseline weight of the child. 1st reading _____ kg
   2nd reading _____kg
   Average weight _____ kg
4. Baseline height of the child 1st reading_____cm.
   2nd reading _____ cm
   Average height _____cm.
5. Fat Free Mass (FFM) _____kg Fat Mass (FM) _____kg
6. Baseline Serum average levels.

Zinc serum levels_____ Beta carotene levels _____
Serum retinol levels_____ Serum Iron levels _____
Hemoglobin count_____

7. Post intervention Serum average levels.

Zinc serum levels_____ Beta carotene levels _____
Serum retinol levels_____ Serum Iron levels _____
Hemoglobin count_____

8. Endline weight of the child. 1st reading_____ kg
   2nd reading _____kg
   Average weight _____ kg
9. Endline height of the child 1st reading_____cm.
   2nd reading _____ cm,
   Average height _____cm.
10. Endline Fat Free Mass (FFM) _____kg
11. Endline Fat Mass (FM) _____kg

Appendix V: Data sheet for TBW estimation by DDI METHOD

Please tick/fill to answer the questions below.
Person performing the test: _____ Date: _____/_____/_____

1. Participant

Name of child _____ Code/ID: _____
Weight: _____kg Height/length: _____cm BMI_____ kg/m^2
Date of birth: _____/_____/_____ Age: _____ years
Gender: Male [ ] Female [ ]
Healthy: Yes [ ] No [ ]
Notes (health):_____
_____

2. Dose

Dose bottle number: _____
Dose weight: _____g
Did the participant fast overnight? Yes [ ] No [ ]
If not, how long was the fast before the dose? _____
Was the container opened just before the dosage? Yes [ ] No [ ]
Was the dose consumed correctly? Yes [ ] No [ ]
If not, what was the weight of the dose not consumed? _____
The container was rinsed with 2 × 50 mL water. Yes [ ] No [ ]
The same straw was used. Yes [ ] No [ ]
Notes: _____

3. Specimen times

Time of baseline saliva sample: _____
Time dose was taken: _____
Post-dose saliva samples taken after:
2 hours [ ] 3 hours [ ] 4 hours [ ]

Appendix VI: Calibration curves

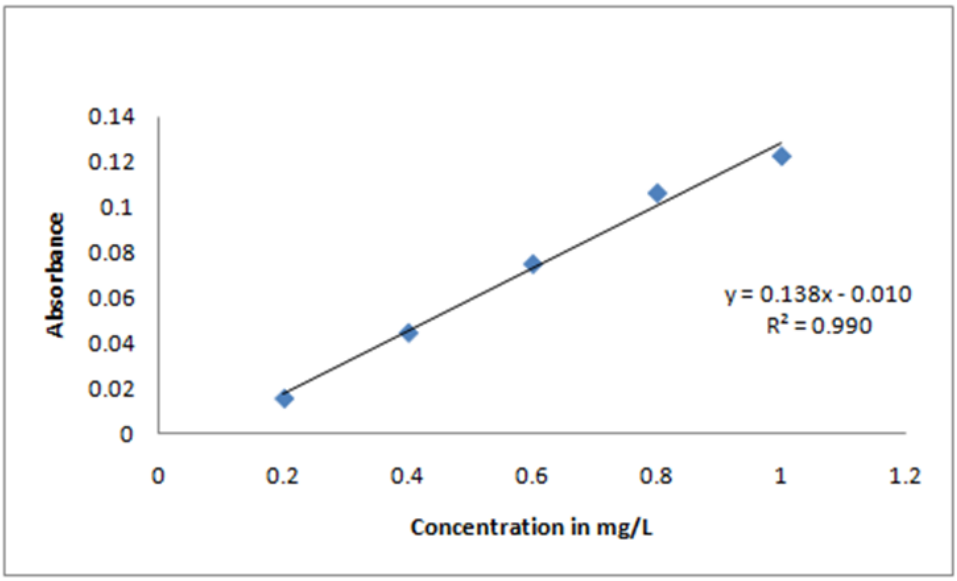

Figure 4.3: Iron standard calibration curve

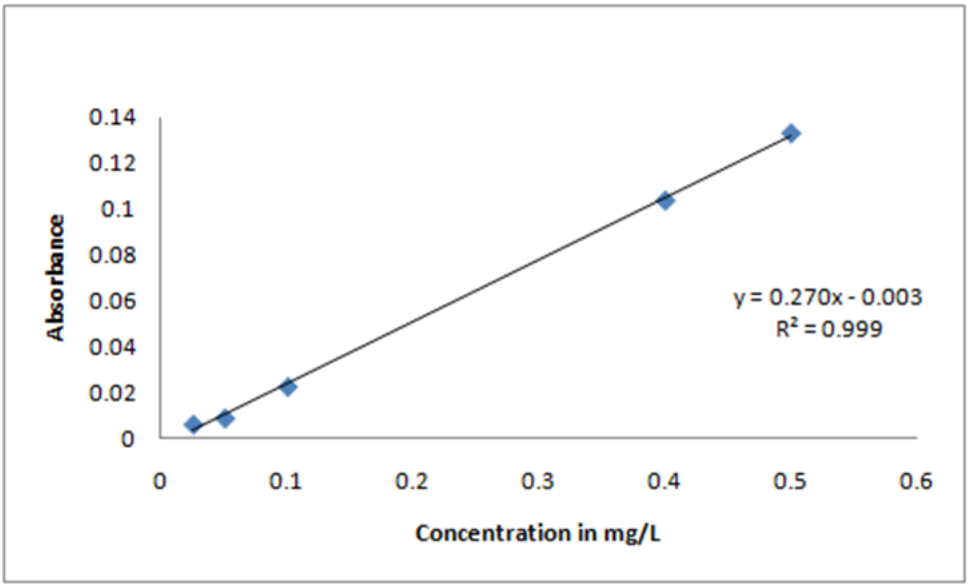

Figure 4.4: Zinc standard calibration curve

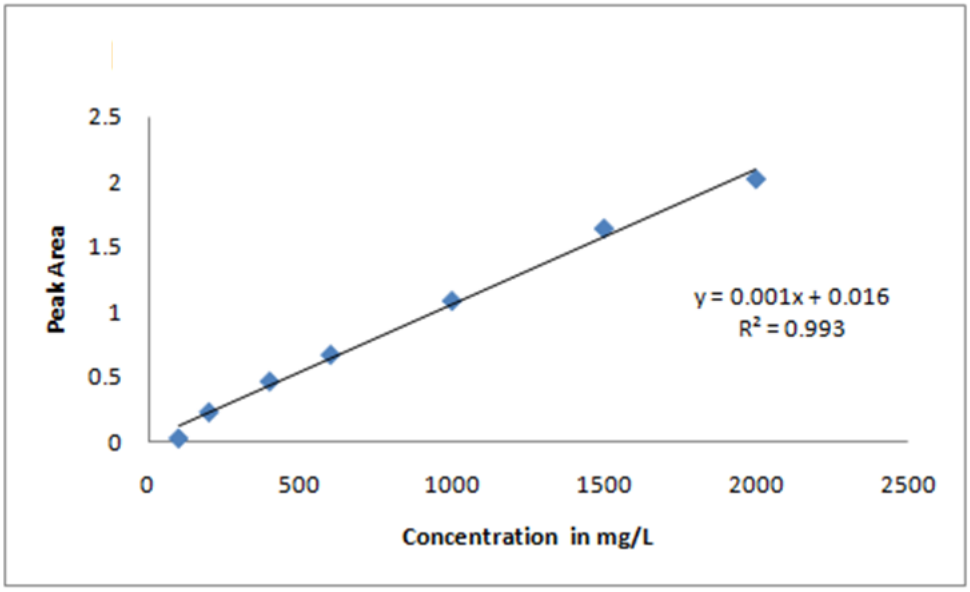

Figure 4.5: Deuterium oxide standard calibration curve

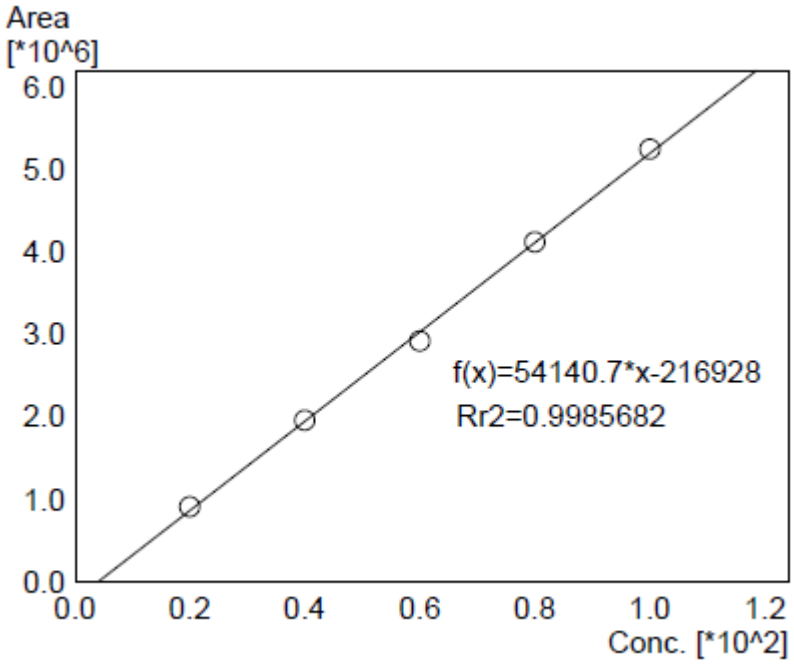

Figure 4.6: beta carotene standard calibration curve

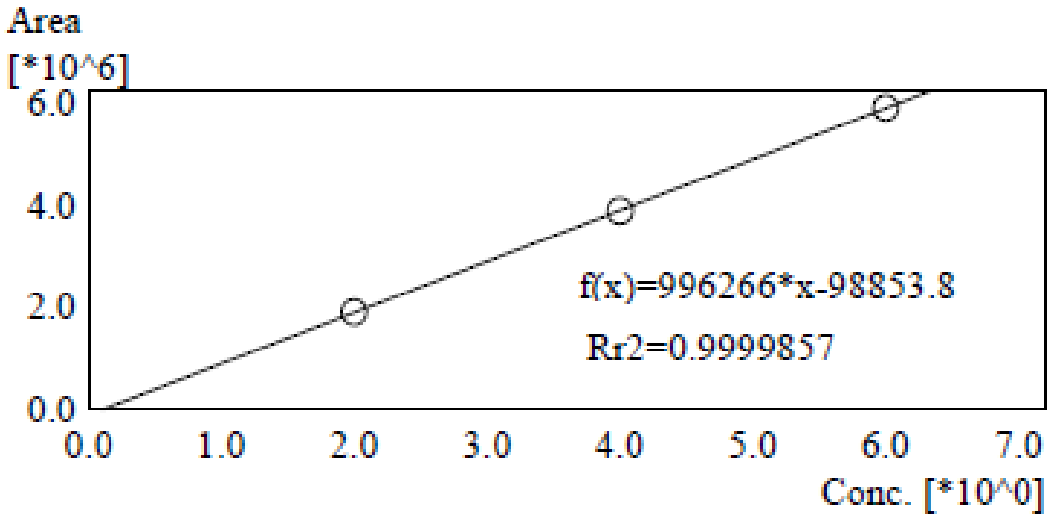

Figure 4.7: Retinol calibration curve

Appendix VII: Publication

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Consumption of African Indigenous Vegetables Improves Children's body Fat Free Mass in Machakos County, Kenya

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Abstract: School gardens growing African Indigenous Leafy Vegetables (AILVs) (*Amaranthus cruentus* and *Vigna unguiculata*) were established in Kangundo and Kilalani primary schools in Machakos County, Kenya and children aged 6-10 years (Kangundo, $N = 66$; Kilalani, $N = 46$) that met the inclusion criteria participated as study subjects. There were two phases, I (13 weeks) and II (12 weeks) with 4 weeks in between to enable crossover of the school as either experimental or control. AILVs were grown in gardens of the experimental school. Study subjects in the experimental group were fed on the AILVs recipe with an accompaniment of a mixture of cooked maize and beans once a day, 5 days a week per phase. The control group fed only on the accompaniment. Body Mass Index (BMI) was determined and a prescribed dose of deuterium oxide was administered and deuterium enrichment determined by Fourier Transform Infrared Spectrometry for % Fat Free Mass (FFM) in children's saliva at baseline and endline. Serum Zn and Fe levels were analyzed by Atomic Absorption Spectroscopy at baseline and endline. Endline analysis in both phase I and II showed the % FFM, mean serum Fe and Zn were significantly higher ($p < 0.001$) only for the experimental group. Food-based intervention through vegetable garden establishments has potential to eradicate malnutrition among school-going children in Kenya. Further, finding by previous studies that DDIM is more accurate in determining nutrition intervention outcomes in children than BMI is supported.

Appendix VIII: Sample pictorials of the study activities

Figure 4.8: Cowpea vegetables at 3 weeks at Kangundo primary school garden

Figure 4.9: A study subject taking weight measurement

Figure 4.10: study subjects feeding on a recipe of cowpeas and amaranth with a mixture of maize and beans

Appendix IX - Study permit and authorization

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

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Our Ref: KU/R/COMM/51/413

Date: 26th February, 2015

Prof. Hudson Nyambaka
Kenyatta University
P.O. Box 43844 -00100, Nairobi.

Dear Prof. Hudson,

APPLICATION NUMBER PKU/301/1286 – "NUTRITION-SENSITIVE INTERVENTION WITH AFRICAN LEAFY VEGETABLES TO IMPROVE NUTRITION STATUS OF SCHOOL GOING CHILDREN IN KENYA."

1. IDENTIFICATION OF PROTOCOL
The application before the committee is with a research topic, **"Nutrition-Sensitive Intervention with African Leafy Vegetables to Improve Nutrition Status of School Going Children in Kenya"**. Received on 4th February, 2015, discussed on 17th February, 2015.

2. APPLICANT
Prof. Hudson Nyambaka

3. SITE
Kangundo Sub-County Machakos County, Kenya

4. DECISION
The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines **AND APPROVED that the research may proceed for a period of ONE year from 26th February, 2015.**

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

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

KENYATTA UNIVERSITY OFFICE OF THE CHAIRMAN 26 FEB 2015 ETHICS REVIEW COMMITTEE P.O. BOX 43844 - 00100 NAIROBI

PROF. NICHOLAS K. GIKONYO
CHAIRMAN ETHICS REVIEW COMMITTEE

I Prof. Hudson Nyambaka .....accept the advice given and will fulfill the conditions therein.

Signature..... Dated this day of 8th April ..... 2015.

cc. Vice-Chancellor