# Nutrition Intervention with African Indigenous Leafy Vegetables Among School-Going Children in Machakos County, Kenya

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## ABSTRACT

**The 2014 Kenya Demographic Health Survey (KDHS) and Micronutrient Survey's report revealed malnutrition among children aged 5-11 years in Kenya and recommended diversified food-based intervention through vegetable garden establishments to fight malnutrition. School gardens growing African Indigenous Leafy Vegetables (AILVs) (*Amaranthus cruentus* and *Vigna unguiculata*) were established at Kangundo and Kilalani primary schools in Machakos County 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 interchange of the role 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 grains and beans once a day, 5 days a week per phase. The control group fed only on the accompaniment. Baseline and endline study children's serum Zn and Fe levels were analyzed by Atomic Absorption Spectroscopy while retinol and β-carotene by High Performance Liquid Chromatography. Endline analysis in both phases I and II showed the mean serum Fe, Zn, retinol and β-carotene were significantly higher ($p < 0.001$) for respective experimental groups. Findings support the use of vegetable garden-sourced AILVs in schools to fight malnutrition among school going children.**

## INTRODUCTION

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 undernutrition and overnutrition (UNICEF, 2008). 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). 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 Southeast 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 preschool 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 deficiency among low income people in developing countries include β-carotene, zinc, iron and iodine.

Some of the global interventions to address malnutrition include food supplementation and food fortification but they are costly and unsustainable in comparison to the use of local food systems (Bhutta *et al*., 2013). Local foods of plant origin like African indigenous leafy vegetables (AILVs) will provide much of the micronutrients in addition to being more affordable for a population of low economic status such as in developing countries. In order to achieve this, their promotion for cultivation as well as consumption is required.

The benefits of establishing and promoting school gardens that grow AILV cannot be underscored ranging from the influence on policy development from stakeholders to integrate Agri-food systems into solutions for malnutrition, optimize the production of AILVs and increase their accessibility and availability to the vulnerable groups. Furthermore, 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 hence influences 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 fears of the source of the vegetables.

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; FAO, 2005; Evans *et al*., 2012). Ultimately, they have contributed towards reducing the risks of chronic heart disease, overnutrition and undernutrition (Heim *et al*., 2009 and Blair, 2009). Researchers have shown that dietary habits developed in childhood persist through life (Mukherjee and Chaturvedi, 2017).

Amaranth (*Amaranthus cruentus* and *Amaranthus hybridus*) is the most commonly used vegetable in Kenya because it is affordable, tasty and locally available (Moraa, 2008). Amaranth has many species that easily adapt to different climates and soil types (Wambugu and Muthamia, 2009) and can grow in both wet and dry seasons to a height of about 2 meters in well drained sandy, loamy and clay soils of pH 4-7. Its seeds and leaves contain high levels of protein, iron, zinc, carotenoids, calcium, antioxidants and vitamin C (Ng'ang'a *et al*., 2008 and Platkin, 2008). The cowpea (*Vigna unguiculata*) is among the most important food legume crops 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 β-carotene and this makes it a potential vegetable in the provision of micronutrients to school children (Abukutsa *et al*., 2007 and Chikwendu *et al*., 2014).

A number of nutrition intervention studies using AILVs have been conducted to show the potential of AILVs in combating malnutrition among school-going children. Chege (2012)

reported a significant increase in the serum retinol, β-carotene, zinc and iron of children in Kajiado County, Kenya after feeding on porridge prepared from fermented maize flour incorporated with dried amaranth leaves (*A. cruentus*), for a period of 6 months. Nawiri and co-workers (2013) reported a significant increase in the mean serum β-carotene, retinol and hemoglobin in pre-school children from Machakos County, a semi-arid region in Kenya, after feeding on a cooked recipe consisting of sun dried amaranth and cowpea leaves. The study concluded that one of the most effective and sustainable ways to mitigate VAD was through diversified diets as a food-based strategy (Nawiri *et al*., 2013). In a similar study carried out in Ghana, the effect of green leafy vegetables powder consisting of a mixture of eggplant (*Solanum macrocarpon*) and amaranth (*A. cruentus*) on anemia and vitamin A status of Ghanaian school children aged 4-9 years was reported (Egbi *et al*., 2018). The consumption of the vegetable powder increased the mean serum hemoglobin and retinol levels of the study subjects in the experimental group. The study concluded that composite green leafy vegetable powder if consumed has the potential to reduce anemia among children. Similarly, Black and co-workers (2013) fed malnourished Aboriginal children on a diet of fruits and vegetables for a period of 12 months and reported an improvement in hemoglobin and iron concentrations in children's sera.

Kenya has over 210 locally available AILV varieties and yet there is evidence from the Kenya Demographic Health Survey (KDHS) and Micronutrient Survey's report of 2014 of high prevalence of malnutrition in arid and semi-arid areas in Kenya (KNBS, 2010 and WHO, 2014). An extract of the statistics from the report for Machakos County, a semi-arid area in Kenya, indicate malnutrition 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 %) (KDHS, 2014). Over years, scientific findings continue to be disseminated to resolve the malnutrition menace that manifests through underweight, stunted growth, protein energy malnutrition (PEM), iodine deficiency, vitamin A deficiency (VAD), iron deficiency anemia (IDA) and zinc deficiency (WHO, 2014). These local AILV varieties can be used to mitigate malnutrition in semi-arid and arid areas like Machakos but their availability is challenged by seasonality, poor accessibility, bioavailability of micronutrients, and negative perception that lead to their low consumption (Kimiywe, *et al*., 2007; Onyango *et al*., 2008; Muhanji *et al*., 2011. A solution is envisaged in a recommendation made by the 2014 KDHS and Micronutrient survey report that calls for the establishment of school gardens that grow AILVs and promote their consumption as well. The purpose of this study was to establish school gardens in Kangundo and Kilalani primary schools in Machakos County, Kenya that grow *A. cruentus* and *V. unguiculata* vegetables, and assess at endline the effect of the consumption of a recipe prepared from these school garden-sourced vegetables on hemoglobin and serum retinol, β-carotene, zinc and iron of children study subjects in these selected schools.

## MATERIALS AND METHODS

This was a 13-week food intervention study conducted in Kangundo and Kilalani primary schools in Machakos County, Kenya in the year 2018. The study involved children aged 6-10 years who were fed on a recipe of *A. cruentus* and *V. unguiculata.* Kangundo and Kilalani primary schools were purposely 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 the school had an ongoing lunch program. A non-randomized pretest-posttest control group design was adopted where groups were intact and did not interrupt existing research settings, allowing high degree of external validity by reducing the reactive effects of the experimental procedure. Therefore, the treatment effect reported can be generalized across populations, settings, treatment variables, and measurement instruments. The study was conducted from beginning of January to end of

November 2018 in 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.

*Sampling and sample size.*

In Phase I, children aged 6-10 years, from Kangundo primary school were assigned to the experimental group while those in Kilalani primary were assigned to the control group. In Phase II, the roles of the schools were interchanged. In Phase I, there were 76 study subjects in the experimental group and 49 for the control group for a sample size of n=125 (including 10% of the calculated sample) that was determined using equation [1] (Cochran, 1963).

$$n = \frac{Z^2 pq}{e^2} \ldots [1]$$

Where

Z = confidence limits of the survey results. For 95% confidence level, Z=1.96

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.

Following dropouts, there were 66 and 46 study subjects in the experimental and control groups respectively in phase I. The study was reviewed and approved by the National Ethical Review Committee at Kenyatta University and permit NACOSTI/P/15/3659/5730 obtained from the National Commission for Science, Technology and Innovation (NACOSTI).

*Intervention procedure.*

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 grains and beans once a day, 5 days a week per phase. The control group fed only on the accompaniment. Parents/caregivers were allowed in school during all procedures including sample collection, deworming and feeding. Prior, meetings were held between the researcher, parents and pupils to explain the study purpose, procedures and roles during the study. For inclusion, consent was sought from parents or guardians. Children who had been ill or hospitalized two weeks prior to the study were excluded. School vegetable gardens growing *A. cruentus* and *V. unguiculata* were established in the experimental school during the respective phase and the vegetables harvested at 21 days of age and thereafter as consumption need arose.

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 *A. cruentus* and *V. 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). The mixture was cooked as per the local community procedures of boiling the vegetables before frying with oil and adding tomatoes and onions. Each study subject in the experimental group consumed on average 80g (wet weight) of the recipe of *A.s cruentus* and *V. unguiculata* expected to meet RDA for children. The consumption of other foods by the study children was monitored by 24-hour dietary recall.

The baseline and endline data on levels of serum retinol and β-carotene as well as blood hemoglobin, zinc and iron were obtained. Zinc and iron levels were analyzed by Atomic Absorption Spectroscopy (AAS) (Buck Scientific, model 210 VGP) while retinol and β-carotene by High Performance Liquid Chromatography (HPLC) (Shimadzu CTO-AS VP 230V).

*Blood sampling and laboratory procedures.*

Blood sampling for both the experimental and control subjects was performed at baseline and endline during both phases I and II by trained personnel from the Ministry of Health from nearby Kangundo Hospital. About 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. 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) for Hemoglobin count. 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 the same day on ice packs in a cool box to Kenyatta University chemistry laboratory for refrigeration at -80 $^{o}$C in readiness for analysis.

Procedures according to Hosotani *et al.* (2003) were adopted in the extraction of β-carotene from serum samples. 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 deionized water. The mixture was deproteinized by vortex mixing for 30 seconds with 400 µl ethanol, containing Butylated Hydroxytoluene (BHT) (0.0599g/ml), to prevent the oxidation of β-carotene (Howe and Sherry, 2006). To extract β-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. 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 β-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.

A stock solution containing 100 µg/mL of all-trans-β-carotene was prepared by dissolving 0.0100g of the all-trans-β-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 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL and 100 µg/mL) were prepared from the stock solution by pipetting 20mL, 40mL, 60mL 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. The serum samples were then run in the HPLC column to obtain their peak areas and their concentration extrapolated from the calibration curve using the regression equation.

To extract retinol the procedure according to Hosotani *et al.* **(**2003) was adopted. Frozen serum samples 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 distilled deionized water. The resulting mixture was deproteinized by vortex mixing for 30 seconds with 600 µl ethanol containing BHT (0.0599g/ml) as an antioxidant (Howe and Sherry, 2006). Extraction was repeated twice with 2ml 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 which had been filtered and ultrasonically degassed for one hour before use.

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 µg/mL, 4 µg/mL and 6 µg/mL) were prepared from the stock solution by pipetting 2mL, 4mL and 6mL 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 then run in the HPLC instrument. 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 generated peak areas for each of the run working standards were plotted against their respective concentration to obtain a standard calibration curve and its regression equation. The samples were then run in the HPLC instrument to obtain their peak areas from which the concentration of the samples was obtained.

To extract iron and zinc, serum samples were left out to thaw for ten minutes in natural light and 1000µL of the serum was taken from the sample tubes and transferred to 50 ml beakers. Exactly 20 ml of distilled water was added to the samples followed by 8ml of concentrated sulphuric acid and 2ml of hydrogen peroxide. The samples were then placed on a hot plate at 150ºC for 10 minutes and thereafter filtered through Whatman filter paper grade one. The filtrate was transferred into a 50 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.

To analyze iron in the serum samples a standard iron stock solution of 1000 ppm was prepared by weighing 1.083 g of ferric nitrate 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 100ppm was prepared from the stock solution by serial dilution. Working standards of 0 ppm, 4 ppm, 6 ppm and 8 ppm were then prepared by pipetting 0 mL 4 mL, 6 mL and 8 mL of the 100ppm 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 and used to work out the concentrations of the samples. To analyze zinc in the serum samples, a standard zinc stock solution of 1000 ppm was prepared by accurately weighing 0.724 g of zinc nitrate 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 100ppm was prepared from the stock solution by serial dilution. Working standards of 0 ppm, 4 ppm, 6 ppm and 8 ppm were then prepared by pipetting 0 mL 4 mL, 6 mL 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 and used to work out the concentrations of the samples.

*Method validation.*

The accuracy of methods used in the study was verified by determining the percentage recovery of the analyte in the serum samples using equation [2].

$$\text{Recovery} = \frac{C_s - C_x}{C_{add}} X\ 100 \ldots\ldots. [2]$$

$C_s$: 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 calculating the relative standard deviation (RSD) of repeated measurements of the test sample according to equation

$$\text{RSD} = \frac{S}{\underline{X}}\ x\ 100 \ldots\ldots [3]$$

Where s is the standard deviation of the replicate measurement of the test sample and $\underline{X}$ is the mean concentration of the repeated measurements of the serum sample. Linearity test of concentration and limit of detection 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 [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.

$$LOD = \bar{x}_{blank} + 3s_{blank} \quad [4]$$

*Data analysis.*

Data was analyzed using Statistical Package for Social Sciences (SPSS) software version 21. Independent t-test was used to compare the mean hemoglobin levels, mean serum levels of Zn, Fe, β-carotene and retinol between the experimental groups and control groups at baseline and endline in both phases I and II. Paired t-test was used to compare the mean hemoglobin levels, mean serum levels of Zn, Fe, β-carotene and retinol at baseline and endline for the experimental school and the control school in both phases I and II. All significance levels were determined at 95% confidence level and p=0.05.

## RESULTS

The percentage recovery of the analyte in the serum samples was determined to verify the accuracy of each method and the results are shown (Tables 1, 2, and 3). The percentage recovery ranged from 99.4 to 100.6. The results (Table 1) confirm that the methods of analyses used were accurate and fit for analysis of each parameter.

Precision was determined by calculating the relative standard deviation (RSD) of repeated measurements of the test sample and the results presented (Table 2). 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 relative standard deviation of less than 3% is sufficiently precise. Thus, the obtained results show a good precision for each parameter. Linearity test of concentration and limit of detection were also done, and results shown (Table 3). The $R^2$ values ranged from 0.996 to 0.999. The $R^2$ values indicate that the established calibration curves are linear over the respective range of the concentration of the standards. Therefore, the method response is linearly related to the analyte concentration and thus fit for the analysis.

Table 4 presents the baseline and endline serum zinc, iron, β-carotene, retinol levels and hemoglobin (Hb) count for the phase I and II control and experimental groups. From the results the endline analysis in both phases I and II showed the mean serum Fe, Zn, retinol, β-carotene and hemoglobin count were significantly higher ($p < 0.001$) for the respective experimental groups.

## DISCUSSION

In phase I the end line mean hemoglobin, mean serum levels of Fe, Zn, β-carotene and retinol for the experimental group (Kangundo school) were significantly higher than baseline means ($p<0.05$) but not significantly different for the control (Kilalani school) ($p>0.05$). This observation could be attributed to the consumption of the AILV recipe by the experimental group and non-consumption by the control group. This was confirmed in phase II when the roles of the experimental and control schools in phase I were interchanged The endline hemoglobin count and the end line mean serum levels of Fe, Zn, β-carotene and retinol for the experimental group (Kilalani school) were significantly higher than at baseline ($p<0.05$) while for the control (Kangundo school) there was no significant difference between baseline and endline means ($p>0.05$). The vegetable recipe is known to contain iron, beta carotene, zinc and other micronutrients and thus availed these micronutrients to the experimental group. Iron is obtained in the form of non-heme iron from vegetables and is necessary for the manufacture of hemoglobin. Consequently, the provision of iron, zinc and vitamin A micronutrients in a combination controls the individual micronutrient deficiencies and has synergistic effects due to the interactions between

the micronutrients (Sonja *et al*., 2005). Zinc is an essential trace element (King, 2011) that influences the absorption, transport and utilization of vitamin A. Zinc deficiency limits the body's ability to mobilize vitamin A stores from the liver and transport vitamin A to body tissues. The enzyme that plays a major role in the oxidative conversion of retinol to retinal is zinc dependent.

The results in the present study compare with findings reported by Chege (2012) in a study on the efficacy of dried amaranth leaves (*A. cruentus*), incorporated in fermented maize flour, consumption on vitamin A, iron and zinc of children in Kajiado county, Kenya. The study found a significant increase in the serum iron, zinc, retinol, and β-carotene at endline for the experimental group. The iron mean levels increased from 10.18±1.6µmol/L to 13.11±1.7µmol/L which represented 28.78 % increase. In the present study the phase I mean levels of iron significantly increased from baseline to endline by 22.34 %. The experimental group's serum zinc mean content reported by Chege (Chege, 2012) increased from 9.94±1.2µmol/L to 12.78±1.4µmol/L (28.57 % increment). In the present study the mean zinc levels significantly increased from baseline to endline by 24.57%. The mean serum retinol and β-carotene results of the present study are consistent with those reported by Chege (2012). In that study the experimental group's serum retinol mean content significantly increased from 0.679±0.227µmol/L to 0.853±0.233µmol/L representing 25.62 increment while in the present study the mean retinol levels increased from 0.523±0.001 to 0.7760±0.20, representing 48.37% increment. The experimental group's serum β-carotene mean content reported by Chege (2012). at baseline and endline were 0.162±0.106µmol/L and 0.539±0.209µmol/L respectively. In the present study the mean beta carotene levels increased from 0.168±0.00 to 0.491± 0.13µmol/L.

Also, comparable with the present study were the findings of Nawiri *et al.* (2013) and Egbi *et al.* (2018) in similar intervention studies. Nawiri *et al.* (2013) reported that a cooked recipe consisting of sun dried amaranth and cowpea leaves improved the levels of β-carotene, retinol, and hemoglobin in preschool children from Machakos District, a semi-arid region in Kenya. The experimental group fed on a recipe of sun-dried amaranth and cowpea for 13 weeks while the control group fed on white cabbage leaves for the same period of time. The student t-test showed that the endline mean values of β-carotene and retinol in the sera of the experimental group were significantly higher than for the control group. The baseline means serum β-carotene for the experimental group significantly increased from 0.1µmol/L to 0.5µmol/L at endline with no significant change for the control group. At the same time the baseline means serum retinol for the experimental groups significantly increased from 0.6µmol/L to 0.8µmol/L. Egbi *et al.* (2018) reported that consumption of green leafy vegetable powder increased serum mean hemoglobin and retinol levels of the study subjects in a 13 weeks' intervention study on school children in Ghana aged 4-9 years. At baseline the mean serum retinol concentration for the experimental group was 16.97±7.74µg/dl (0.819651µmol/L) and it significantly increased to 26.96±6.86 µg/dl (1.30217µmol/L) at endline. At baseline the mean serum retinol concentration for the control group was 16.79±8.74µg/dl (0.810957µmol/L) and significantly increased to 24.35±5.50 µg/dl (1.1761µmol/L) at endline.

The mean hemoglobin concentration reported by Egbi *et al.* (2018) for the experimental group was 121.9±13.5g/l and that of the control was 113.4±8.5g/l at endline. These findings are again in agreement with the findings of the present study. In the present study the baseline mean hemoglobin count for the phase I experimental group was 112.918±16.58g/L (11.291g/dL) but at endline was 118.541±17.39g/L (11.8541g/dL). For the control group at baseline and endline the mean hemoglobin counts were 112.424±12.01g/L (11.2424g/dL) and 112.445±9.93 g/L (11.2445g/dL) respectively. In Phase II baseline mean hemoglobin count in the experimental group significantly increased from 112.437 ± 8.63 g/L to 116.516 ± 16.61 g/L at endline. The increase in the hemoglobin count over

a period of 13 weeks suggests that feeding on the vegetable recipe has the potential of combating iron deficiency anemia in children.

The results of the present study further collaborate with findings in a study on the health outcomes of a subsidized fruit and vegetable program for disadvantaged Aboriginal children (Black *et al*., 2013). The study reported an improvement in hemoglobin and iron concentrations in children's sera. The hemoglobin count was 12.68g/dL at baseline and 12.82g/dL at endline which compares well with mean values reported in the present study. At baseline the mean iron levels in the Aboriginal children were 12.7µmol/L and at endline were 13.2µmol/L. Just as there were no significant differences in the mean serum retinol, β-carotene, zinc and iron levels in the control group at baseline and endline, there were also no significant differences in hemoglobin count in the control group at baseline and endline.

The significant increase of hemoglobin in the experimental group at endline can be accounted for by the metabolic interactions of these selected micronutrients (Sonja *et al*., 2005). The increase in the serum retinol may have led to the mobilization of iron (Nawiri *et al*., 2013). Retinol (vitamin A) is involved in the release of iron from the liver and the spleen stores and the incorporation of iron directly into the hemoglobin (Van Stuijvenberg *et al*., 1997) therefore retinol positively affects iron metabolism and also makes iron incorporation into hemoglobin more effective (Sonja *et al*., 2005). Previous studies have reported a strong positive correlation between serum retinol levels and hemoglobin concentrations with findings that the correlation is stronger with lower vitamin A status at baseline (Fishman *et al*., 2000; Dijkhuizen *et al*., 2001 and Hinderaker *et al*., 2002). Additionally intervention studies have demonstrated that foods fortified with vitamin A improve hemoglobin concentrations in the blood of children and expectant mothers (Mwanri *et al*., 2000; Semba and Bloem, 2002) and yet it has also been demonstrated in previous studies that if the serum levels of vitamin A are very high there will be no additional effect of vitamin A on hemoglobin concentrations (Villamor *et al*., 2000; Mulokozi, 2003). It is apparent that both iron and vitamin A are required for erythropoiesis (red blood cell production). Several mechanisms linking retinol to iron metabolism have been proposed (Roodenberg *et al*., 2000) with the suggestion that retinol influences iron metabolism through its effect on erythropoiesis because less erythropoiesis occur with vitamin A deficiency since less iron is incorporated into red blood cells.

The findings in the present study are also collaborated with findings in another different recent study by Egbi *et al* (2018) on the effect of green leafy vegetables powder, which consisted of a mixture of eggplant (*Solanum macrocarpon*) and amaranth (*A. cruentus*), on anemia and vitamin A status of Ghanaian school children. It was found that consumption of green leafy vegetables powder significantly increased serum mean, hemoglobin, and retinol levels of the study subjects in a three months intervention study on school children aged 4-9 years. The present study notes that it took about 13 weeks of intervention with the AILVs to bring about a significant change in the nutrition status of the school going children. This was in agreement with previous intervention studies. For example, the intervention period for Chege and co-workers was six months (24 weeks). Both studies of Nawiri *et al.* (2013) and Egbi *et al.* (2018) took each 13 weeks of intervention.

Though the present study was limited by the general inability to control other food intakes away from school, a 24-hour dietary recall was used to monitor micronutrient and energy intakes. These food intakes by the study children at home contributed to the micronutrient levels assessed. However, it was not a coincidence that results obtained in phase II took the trend of those in phase I despite this limitation, leading to the conclusion that the positive nutrition impact most likely was due to the consumption of the vegetable recipe. It was also difficult to explain why the control group's mean hemoglobin and mean serum levels of the selected micronutrients did not reduce significantly at endline in phase II, since they had stopped feeding on the vegetable recipe at school. Perhaps the 4 weeks in between phase I and II was not long

enough to allow wash out of the micronutrients. Additionally, foods eaten away from school may have played a role in this observation. Another limitation was that the micronutrients β-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.

## CONCLUSION

Despite the limitations highlighted in the present study, the results still point to positive nutritional intervention with school garden-sourced *A. cruentus* and *V. unguiculata* among the school going children in Machakos County, Kenya. Further, food-based intervention through vegetable garden establishments as recommended by KDHS and Micronutrient Survey's report (2014) has the potential to fight undernutrition among school-going children in Kenya and beyond. Since there was a positive micronutrient impact, as shown by significantly higher levels of Fe, Zn, β –carotene, retinol and hemoglobin in the experimental group, the study recommends establishment of AILV school gardens and promotion of consumption of AILVs to improve nutrition status of school-going children. The selected AILVs should be incorporated in the school lunch programs especially in semi-arid and arid areas and their daily consumption for a minimum period of 13 weeks is needed to realize a positive micronutrient impact in the target group. For further studies, the synergetic effects of other minerals and phytochemicals that might have been present in the vegetable recipe other than iron, zinc, and β-carotene on the nutrition status of target subjects need further investigation, together with the effect of anti-nutrients like phytic acid, oxalates, and phenols on bioavailability of the selected micronutrients.

Table 1: Percentage recovery of the analyte.

| Method | Analyte | $C_{x\ (ppm)}$ | $C_{add}$ (ppm) | $C_s$ (ppm) | Recovery % |
|---|---|---|---|---|---|
| **HPLC** | B-carotene | 0.49 | 5 | 5.46 | 99.4 |
| **HPLC** | Retinol | 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 |
| | | n=5 | | | |

$C_s$: Concentration determined in the spiked sample
$C_x$: Concentration determined in unspiked sample
$C_{add}$: Expected additional concentration due to spiking

Table 2: Relative standard deviation (RSD) of repeated measurements of the test sample.

| Parameter | Mean | SD | %RSD |
|---|---|---|---|
| **β-carotene** | 0.49 | 0.01 | 2.66 |
| **Retinol** | 0.76 | 0.02 | 2.64 |
| **Fe** | 14.32 | 0.17 | 1.19 |
| **Zn** | 0.72 | 0.02 | 2.09 |
| | | | |

RSD (relative standard deviation) and SD (standard deviation) of 5 samples

Table 3: Linearity test of concentration and limit of detection (LOD).

| Analyte | LOD | Equation | $R^2$ |
|---|---|---|---|
| **β-carotene** | 4.01 | Y = 54140.7X - 216928 | 0.9986 |
| **Retinol** | 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 |

Table 4a: Levels of serum micronutrients and hemoglobin at baseline and endline for experimental and control groups in Phases I and II.

**PHASE I**

| Serum constituents | EXPERIMENTAL (KANGUNDO) N=66 | | CONTROL (KILALANI) N=46 | |
|---|---|---|---|---|
| | Baseline | Endline | Baseline | Endline |
| Fe (µg/L) | 11.644±1.81[a] | 14.246±1.51[b] | 11.634±0.23[a] | 11.874±0.21[a] |
| Zn (mg/L) | 0.582 ±0.15[a] | 0.725±0.17[b] | 0.577± 0.10[a] | 0.584±0.11[a] |
| β-carotene (µmol/L) | 0.168±0.00[a] | 0.491± 0.13[b] | 0.167±0.00[a] | 0.172±0.03[a] |
| Retinol (µmol/L) | 0.523±0.001[a] | 0.7760±0.20[b] | 0.523±0.01[a] | 0.530±0.11[a] |
| Hemoglobin (g/L) | 112.918±16.58[a] | 118.541±17.39[b] | 112.424±12.01[a] | 112.445±9.93[a] |

**PHASE II**

| Serum constituents | EXPERIMENTAL (KILALANI) N=46 | | CONTROL (KANGUNDO) N=66 | |
|---|---|---|---|---|
| | Baseline | Endline | Baseline | Endline |
| Fe (µg/L) | 11.852±1.13[a] | 14.320±1.71[b] | 14.239±1.80[a] | 14.235±1.80[a] |
| Zn (mg/L) | 0.589±0.12[a] | 0.719±0.15[b] | 0.712± 0.15[a] | 0.708±0.14[a] |
| β-carotene (µmol/L) | 0.169±0.05[a] | 0.492±0.13[b] | 0.488± 0.09[a] | 0.485± 0.13[a] |
| Retinol (µmol/L) | 0.528±0.09[a] | 0.765±0.20[b] | 0.759± 0.20[a] | 0.756± 0.19[a] |
| Hemoglobin (g/L) | 112.437±8.63[a] | 116.516±16.61[b] | 118.524±17.61[a] | 118.522±17.31[a] |

There is no significant difference between mean values with the same superscript for each serum constituent at baseline and endline, but a significant difference exists between those with different superscripts at 95% confidence level, p=0.05, paired t- test (detailed Table 4b).

Table 4b: Levels of serum micronutrients and hemoglobin at baseline and endline for experimental and control groups in Phases I and II

| **Phase I** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Serum constituents | Experimental n=66 | | | Control n=46 | | | independent t t-test Baseline p-values | independent t t-test Endline p values |
| | Baseline | Endline | Paired t-test p-value | Baseline | Endline | Paired t-test 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 |
| β-carotene (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 II** | | | | | | | | |
| 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 |
| β-carotene (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 | 0.150 | <0.001 | 0.542 |

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

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