Int. J. Food, Nutrition and Public Health, Vol, 9, No. 1, 2017

MORINGA OLEIFERA LEAF CONSUMPTION ON THE VITAMIN A AND HAEMATOLOGICAL STATUS OF SCHOOL CHILDREN IN ADA-EAST DISTRICT, GHANA

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ABSTRACT

Background: Vitamin A deficiency (VAD) could be prevented by the consumption of pro-vitamin A (β-carotene)-rich *Moringa oleifera* (*M. oleifera*) leaves. *M. oleifera* grows well in many developing countries, and dried leaves also retain a high percentage of β-carotene on storage; they could thus serve as an excellent, convenient source of this pro-vitamin A carotenoid. This study assessed the efficacy of the supplementation of dried *M. oleifera* leaves on the vitamin A and haematological status of children in Ada-East district, Ghana.

Methodology: Children aged 5–12 years were supplemented three times a week at 0.2 g/kg bodyweight for nine weeks. Background data were collected on the socio-demographic status of the children. Data were collected on vitamin A and haematological status of the children at baseline and at the end of the study. Malaria and hookworm morbidity were also assessed at baseline and at the end of the study.

Results: There was a significant increase in mean serum vitamin A levels after supplementation (end of study) in the intervention group ($p<0.05$), but not in the control group. Haematological parameters Haemoglobin (HGB), Red Blood Count (RBC), Haematocrit (HCT), Mean Corpuscular Volume (MCV) did not show any significant changes in either group ($p<0.05$). A bivariate analysis, however, showed a significant association between vitamin A and haematological indices in the intervention group, but not in the control group.

Conclusion: *M. oleifera* leaf consumption significantly increased the vitamin A status of children in Ada-East district, Ghana, and has the potential of increasing the haematological status of children. It could thus play a major role as a food-based strategy in vitamin A deficiency control.

Keywords: *Moringa oleifera* leaves; Vitamin A deficiency; Intervention; β-carotene. Ghana, Vitamin A status

INTRODUCTION

Vitamin A deficiency (VAD) is a major public health problem worldwide, particularly in developing countries (Aguayo and Baker, 2005). An estimated 195 million pre-school children have VAD globally (WHO, 2009). The consequences of VAD include sub-optimal child growth, as well as effects on metabolism, reproduction, which embraces embryonic development and spermatogenesis in males (Clagett-Dame and Knutson, 2011), and resistance to infection, with the most severe effect being xerophthalmia, which could eventually lead to blindness (WHO, 1995). Vitamin A deficiency also affects the mobilisation of iron from storage organs for haematopoiesis. Vitamin A supplementation has been demonstrated to produce significant elevations in blood Haemoglobin (HGB), Haematocrit (HCT), Erythrocytes (RBCs), serum iron and the percentage of transferrin saturation (%TS), but had no effect on Total Iron Binding Capacity (TIBC) or serum ferritin (Mejia and Chew, 1988; Hodges et al., 1978; Bloem et al., 1989). In many developing countries, where rates of VAD and anaemia are high (Semba and Bloem, 2002), interventions that will improve vitamin A status and eliminate VAD could thus also improve haematological parameters and prevent anaemia. Interventions such as dietary diversification and promoting the consumption of carotene-rich (α-carotene, β-carotene and β-cryptoxanthin) foods such as orange-fleshed sweet potatoes, mangoes, carrots, yellow maize, yellow yam and dark green leafy vegetables such as amaranth and drumstick (*M. oleifera*) leaves, could be an important VAD prevention strategy in low income groups. This would be in place of the periodic administration of large doses of vitamin A supplements to infants (0–59 months) currently practised (Kandlakunta et al., 2008).

M. oleifera is a dark green leafy vegetable with a high β-carotene content. Most studies (Fuglie, 1999; Glover-Amengor and Mensah, 2012; Nambiar and Seshadri, 2001; Seshadri et al., 1997) reported high levels of β-carotene in *M. oleifera* per 100 g leaves; these values are comparable to levels found in amaranth and spinach. Furthermore, studies indicated that up to 90% of the β-carotene content of dried leaves was retained on storage (Glover-Amengor et al., 2012; Seshadri et al., 1997). Therefore, *M. oleifera* leaves could help in alleviating the seasonal scarcity of vitamin A-rich foods, ensure adequate supply of β-carotene, and be available for convenient use.

M. oleifera leaves have been reported to improve retinol and haemoglobin levels in laboratory

animals (Nambiar and Seshadri, 2001). Its safety to laboratory animals has also been demonstrated (Asiedu-Gyekye et al., 2014), and its *β*-carotene has also been reported to be highly bioavailable *in vitro* (Yang et al., 2006). However, whether the leaves can improve retinol levels in humans is not yet clear (Thurber and Fahey, 2009). In addition, existing evidence on *M. oleifera*'s potential for improving retinol and haemoglobin levels in humans is anecdotal (Fuglie, 1999 and Fahey, 2005). If found to be efficacious in improving the vitamin A status in humans, *M. oleifera* would be a less expensive source of vitamin A for tropical countries; this is because it easily adapts in those countries with favourable climatic conditions for its production (Manh et al., 2005). If efficacy is demonstrated, it could contribute to the improvement of the diets of rural households that mostly derive their nutrition from plant food sources; these include cereals, legumes and vegetables (Babu, 2000). This study therefore assessed the efficacy of *M. oleifera* leaf supplementation on the vitamin A and haematological status of children in the Ada-East district, Ghana. Specifically, the study assessed the vitamin A and haematological status of children, before and after *M. oleifera* leaf consumption.

SUBJECTS AND METHODS

Study Area

The study was conducted in the Ada-East district in the Greater Accra region of Ghana, from May to July 2012. This was as a follow-up to the *M. oleifera*-leaf-fortified dishes acceptability study that was

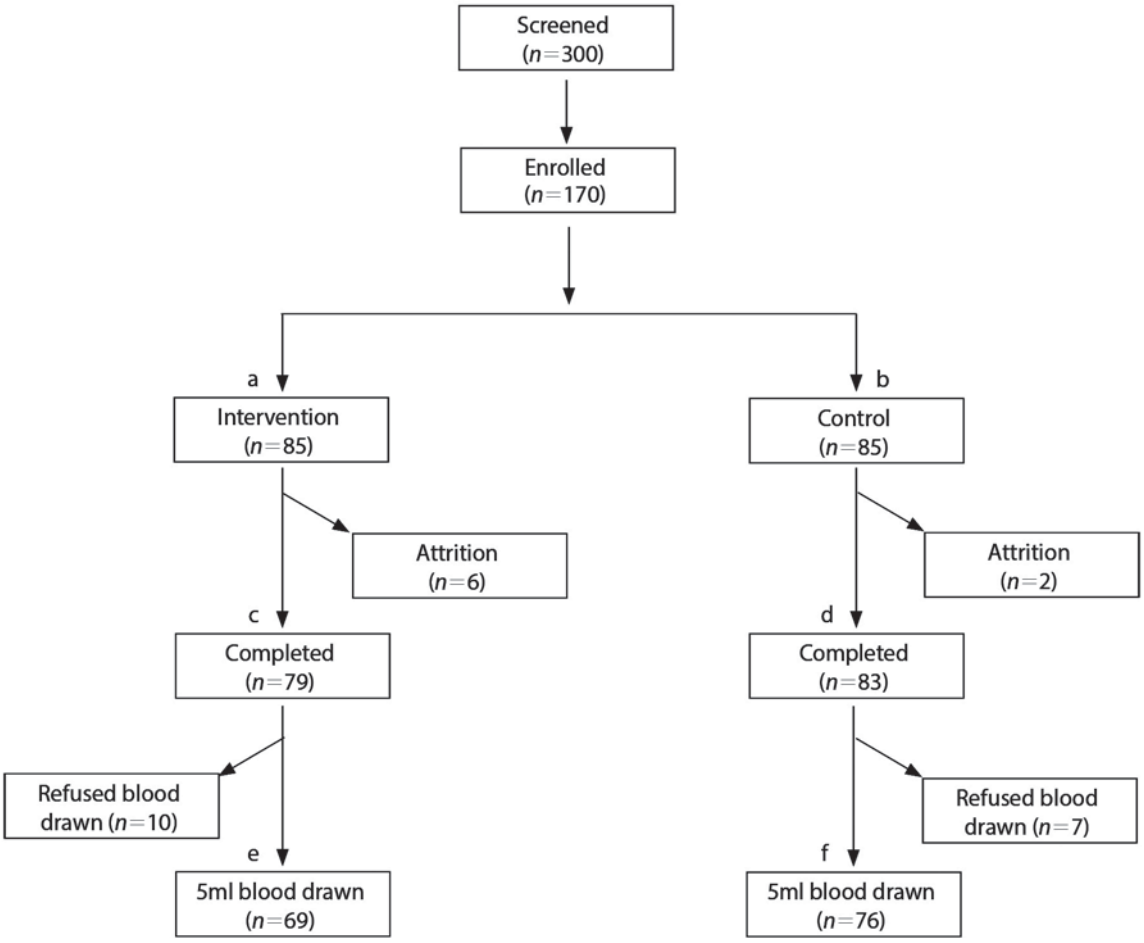

Figure 1 Flow Chart for participant recruitment Into Study

Source: Devised by authors

conducted in the same district (Glover-Amengor et al., 2016). The district has a total land area of 463 square kilometres and a population of 71,671: of this total 37,659 (52.6%) are females, and 34,012 (47.4%) are males (GSS, 2014). Predominantly, the indigenous people in the Ada-East district are farmers and fishermen, mostly cultivating onions, pepper, tomatoes and cassava (Ada-East District Assembly, 2013). The district is a malaria endemic area.

Study Population

Children eligible for the study were those aged 4–12 years, of both sexes, residing in the study area, attending schools selected for the intervention, and not weighing more than 26 kg ($n=300$). The schools, which were purposively selected, were in coastal communities of low socioeconomic status, had kindergartens, and were not participating in the Government School Feeding Programme (GSFP). The selection of non-GSFP schools was to eliminate the possible introduction of foods containing vitamin A from the school menu.

Study Design

The study was a randomised, un-blinded controlled feeding trial. To determine a difference of 0.25g/dl in Haemoglobin (Hb) levels at alpha (significance level)=0.05, 90% power and 80% follow-up, a sample size of 82 was calculated for each of the two arms of the study. Eighty-five children were recruited into each arm to make 170. Children were randomly assigned to two groups – Intervention and Control groups. Some children dropped out of the study (Intervention, $n=6$; Control, $n=2$). These were not included in the final sample. Others also completed the study, but refused the final blood draw (Intervention, $n=10$; Control, $n=7$); these were also excluded from the final sample. At end of study, therefore, the numbers were Intervention, $n=69$ and Control, $n=76$.

Intervention

Leaf Processing and Nutrient Determination

M. oleifera leaves were randomly sampled three times at two-week intervals from a field in Accra in the months of March and April 2012. Leaves were transported to the Council for Scientific and Industrial Research-Food Research Institute (CSIR-FRI) where they were solar-dried. The detailed processing procedure was described in a previous article (Glover-Amengor et al., 2016). Duplicate samples of dried leaves were analysed for various parameters; these included moisture (AOAC 925.10, 1990), pH (Radiometer, Copenhagen), minerals (AOAC, 2005) and β-carotene (Karnjanawipagul et al., 2010). Nutrient levels were determined separately in each replicate. The replicates were thoroughly mixed together and then milled using a locally fabricated stainless steel hammer mill (0.8 mm particle size); the milled product was packaged in clean polythene bags and kept at room temperature (27C to 28C).

Feeding

The Intervention (I) group received *M. oleifera*-leaf-fortified dishes at 0.2 g/kg body weight. The Control (C) group received same quantity of non-leaf fortified dish. All ingredients for food preparation were provided by the research team, and the same research team prepared and served the food throughout the study. Three dishes, based on the outcome of a consumer acceptability study (Glover-Amengor et al., 2016), were selected for the intervention. These were fortified porridge (composite white maize, groundnut and white cowpea meal) (a sweetened dish), *waakye* (boiled red cowpea and rice), and groundnut soup (salted dishes). The children were supplemented three times a week for nine weeks, this being the full span of a school term. Porridge was served with doughnut or biscuits; twice a week this was alternated with either *waakye* or groundnut soup served with rice balls or *gari*. There was no feeding at the weekends. The groundnut soup was prepared from whole peanut butter to provide oil that enhances the absorption of β-carotene. Similarly, the composite flour used in the porridge preparation contained peanuts to provide both protein and oil; the gravy used in serving *waakye* was made with coconut oil and smoked herring.

The weight of the pupils ranged from 12 kg to 25.4 kg with a mean weight of 21.1 kg. The package was designed to give 0.2 g leaf powder/kg body weight. A 90 ml ladle and a 15 ml spoon were used to give the children portions of food in proportion to their body weight: these measures were weighed to determine the weight of food. Feeding bowls and spoons were provided for the children; these were cleaned thoroughly after each session

by the research team and stored away. The meal was used as a vehicle for the delivery of *M. oleifera* leaf powder. The study was not blinded due to the intense green colour of *M. oleifera* leaves that made blinding difficult. The serving of food started at 11:30a.m. that is just before the lunch break. The Control group received an equal amount of food, but without *M. oleifera* leaf powder. Each child was given a code. Sheets of paper were used to record compliance. Separate sheets were given to the Intervention and Control groups. Each child's name was called out during the feeding sessions and ticked to indicate presence or absence.

Compliance was defined as the number of days that a child received and ate the whole portion of food, expressed as a percentage of the total number of days of feeding. The food was prepared in the community by the research team and served to the children on the same day. Hot food was transported in food warmers to the schools. The research team did the serving and supervised the consumption with the assistance of the class teachers. Children were encouraged, but not forced to eat the food. Before commencement of the feeding trial, 5 ml of venous blood was collected from each child by venepuncture, and parts of this was used for the determination of vitamin A, Haemoglobin (HGB), Haematocrit (HCT), Red Blood Cells (RBC) and Mean Corpuscular Volume (MCV) and malaria parasitaemia. At the end of the 9-week trial, 5 ml of venous blood was again collected from each child (a day after the last feeding) by venipuncture (van Jaarsveld et al., 2005; de Pee and West, 1995) and all tests carried out at baseline were repeated.

Data Collection

Socio-Demographic Data

Before commencement of the intervention, background data were collected on the socioeconomic status of the children and their parents or caregivers.

Assessment of Vitamin A and Haematological Status

Five ml of blood, drawn from each child by venipuncture by phlebotomists was used for analysis using standard methods. Blood samples on separator gels, after coagulation, were spun in a centrifuge at 5,000 rpm for 5 minutes in a dark room. The serum was collected into Eppendorf tubes. Serum retinol was determined by High-Performance Liquid Chrom (HPLC) (Shimadzu SPD – 6A detector; Shimadzu LC – 6A pump and Shimadzu CR-5A printer) and Hitachi Spectrophotometer (U–1100). A full blood count was performed on Ethylenediaminetetraacetic Acid (EDTA), Whole Blood (WB) samples in the laboratory using Sysmex KX – 21N (Kobe, Japan), an automated haematology analyser. Haemoglobin (HGB), Red Blood Cells (RBCs), Haematocrit (HCT), and Mean Corpuscular Volume (MCV) were determined. All biochemical and haematological parameters were determined at baseline and at the end of the study. The children were also screened for malaria parasites and hookworm at baseline and post-test.

Statistical Analysis

Data were analysed using SPSS version 21.0. Means, standard deviations and frequencies were generated. Statistical analysis included baseline data comparisons between groups for differences in haematological and biochemical variables by using independent-sample t-tests. For children in the Control and Intervention groups who completed the study, Fishers' and chi-square test (for the percentages) and t-tests (for the continuous variables) were used to determine whether changes from baseline to end of study were statistically significant. Baseline and post-test comparisons within groups were conducted using paired *t*-tests.

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

RESULTS

Vitamin A and Haematological Parameters

Mean vitamin A levels in the Intervention and Control groups at baseline were 0.69±0.33 and 0.77±0.32, respectively. At the end of the study, mean vitamin A levels in the Intervention and Control groups were 1.03±0.32 and 0.71±0.28, respectively. The mean baseline haemoglobin levels were 10.64±1.00 and 10.57±1.21 in the Intervention and Control groups respectively, while at the end of the study, the levels were 10.65±1.02 and 10.82±1.10 in the Intervention and Control groups respectively (Table 2).

There was a significant increase in vitamin A levels in children in the Intervention group ($p<0.05$), whilst those in the Control group experienced a slight but non-significant decrease in serum vitamin A levels (Table 2). In addition, 37 (53.6%) of children were vitamin A deficient at baseline, whilst 9 (13%) were deficient at the end of the study in the Intervention group (serum retinol <0.70 µmol/l). However, in the Control group, 35 (46.7%) and 40 (52.6%) children were vitamin A deficient at baseline and the end of the study respectively (Table 3).

At baseline, 53 (76.8%) of children in the Intervention group were anaemic as against 62 (81.6%)

Table 1 Univariate Relationships of Child and Caregiver Characteristics and, Vitamin A and Haematological Indicators

Characteristic	*n (%)*	*VitaminA µmol/L (SD)*	*HGB g/dL (SD)*	*RBC×104/µl (SD)*	*HCT % (SD)*	*MCV fL (SD)*
Sex of child						
Male	90 (52.9)	0.70 (0.27)	10.54 (1.05)	4.17 (0.39)	32.52 (2.58)	78.23 (5.80)
Female	80 (47.1)	0.76 (0.32)	10.66 (1.14)	4.20 (0.48)	32.99 (3.07)	79.34 (5.51)
Child's age (years)						
5–7	66 (38.8)	0.67 (0.27)*	10.40 (1.06)	4.17 (0.48)	32.53 (2.98)	78.64 (5.67)
8–10	85 (50.0)	0.78 (0.31)	10.73 (1.12)	4.22 (0.42)	32.90 (2.84)	78.29 (5.71)
11–12	19 (11.2)	0.69 (0.24)	10.69 (0.99)	4.05 (0.30)	32.64 (2.20)	80.87 (5.28)
Formal education						
No education	153 (90.0)	0.72 (0.29)	10.58 (1.09)	4.19 (0.42)	32.70 (2.83)	78.55 (5.66)
Middle school/JHS	17 (10.0)	0.79 (0.33)	10.72 (1.11)	4.17 (0.58)	33.12 (2.77)	80.08 (5.80)
Occupation						
Unemployed	9 (5.3)	0.72 (0.17)	10.74 (0.77)	4.15 (0.32)	32.94 (2.19)	79.63 (4.79)
Trader	26 (15.3)	0.74 (0.35)	10.67 (1.38)	4.16 (0.48)	32.63 (2.79)	79.21 (5.43)
Farmer	130 (76.5)	0.72 (0.27)	10.58 (1.07)	4.20 (0.43)	32.74 (2.88)	78.35 (5.78)
Student	5 (2.9)	0.96 (0.54)	10.22 (0.53)	3.84 (0.28)	32.84 (3.33)	83.64 (3.54)
Refuse disposal						
Refuse dump	124 (72.9)	0.70 (0.26)	10.63 (1.10)	4.20 (0.46)	32.80 (2.86)	78.53 (5.81)
Burned	41 (24.1)	0.80 (0.34)	10.42 (1.06)	4.09 (0.35)	32.28 (2.69)	79.17 (5.56)
Buried	5 (2.9)	0.77 (0.52)	11.16 (0.95)	4.43 (0.26)	35.04 (2.19)	79.12 (2.83)
Toilet facility						
Pit latrine	5 (2.9)	1.01 (0.60)	11.10 (0.62)	4.20 (0.38)	33.68 (2.42)	80.50 (5.36)
Public toilet	4 (2.4)	0.87 (0.07)	10.13 (0.62)	3.80 (0.15)	30.80 (1.31)	81.20 (3.16)
Bush	161 (94.7)	0.72 (0.28)	10.60 (1.10)	4.19 (0.44)	32.76 (2.85)	78.58 (5.73)
Sleep under a bed-net						
Yes	166 (97.6)	0.72 (0.29)	10.59 (1.10)	4.18 (0.43)	32.71 (2.85)	78.66 (5.68)
No	4 (2.4)	0.89 (0.19)	10.90 (0.54)	4.49 (0.60)	33.90 (1.20)	80.25 (6.21)

RBC=*Red* blood cells; HGB=Haemoglobin; HCT=Haematocrit; MCV=Mean corpuscular volume; *significant at $p<0.05$

Source: Devised by authors

in the Control group. Those with normal haemoglobin levels were 16 (23.2%) in the Intervention group and 14 (18.4%) in the Control group. The mean haemoglobin was 10.63 g/dl in the Intervention group, and 10.56 g/dl in the Control group, with an overall mean HGB of 10.59 g/dl (Table 2).

The differences in mean changes in vitamin A and haematological indices at the end of the study

Table 2 Vitamin A and Haematological Indices of Study Groups at Baseline

Haematological Indices	*Intervention Group Mean±SD*	*Control Group Mean±SD*	*Total Mean±SD*	*p-value*
Vitamin A (µmol/l)	0.69±0.27	0.77±0.31	0.73±0.29	0.053
RBC×104/µl	4.21±0.43	4.15±0.44	4.18±0.44	0.370
HGB (g/dL)	10.63±0.97	10.56±1.20	10.59±1.08	0.664
HCT (%)	32.89±2.76	32.59±2.89	32.74±2.82	0.485
MCV (fL)	78.38±5.52	79.02±5.83	78.70±5.67	0.460

RBC=Red blood cells; HGB=Haemoglobin; HCT=Haematocrit; MCV=Mean corpuscular volume
Source: Devised by authors.

Table 3 Vitamin A and Haematological Status of School-Aged Children by Study Group

Indices	*Intervention Group*		*Control Group*	
	Baseline n (%)	*Posttest n (%)*	*Baseline n (%)*	*Posttest n (%)*
Vitamin A (µmol/L)				
<0.7[a]	37 (53.6)	9 (13.0)	35 (46.7)	40 (52.6)
<1.05[b]	63 (91.3)	39 (43.5)	64 (85.3)	74 (97.4)
RBC×104/µl				
Low	24 (34.8)	20 (29.0)	28 (36.8)	17 (22.4)
Normal	42 (60.9)	46 (66.7)	46 (60.5)	58 (76.3)
High	3 (4.3)	3 (4.3)	2 (2.6)	1 (1.3)
HGB (g/dL)				
Anaemia	53 (76.8)	56 (81.2)	62 (81.6)	59 (77.6)
Normal	16 (23.2)	13 (18.8)	14 (18.4)	17 (22.4)
HCT (%)				
Low	63 (91.3)	67 (97.1)	72 (94.7)	74 (97.4)
Normal	6 (8.7)	2 (2.9)	4 (5.3)	2 (2.6)
MCV (fL)				
Low	37 (53.6)	48 (69.6)	40 (52.6)	48 (63.2)
Normal	32 (46.4)	21 (30.4)	36 (47.4)	28 (36.8)
Malaria parasite				
No MPS	55 (79.7)	47 (68.1)	64 (84.2)	54 (71.1)
Symptomatic	13 (18.8)	20 (29.0)	11 (14.5)	22 (28.9)
Max. parasitimia	1 (1.4)	2 (2.9)	1 (1.3)	0 (0.0)

RBC=*Red* blood cells; HGB=Haemoglobin, HCT=Haematocrit; MCV=Mean corpuscular volume
[a] <0.7µmol/l;
[b] <1.05µmol/l=cut-offs to define vitamin A deficiency.
Source: Devised by authors.

Table 4 Vitamin A and Haematological Indices of Children According to Study Group with Time

Indices	*Intervention Group*				*Control Group*				*Differences in mean changes at end of study*
	Baseline Mean±SD	*Posttest Mean±SD*	*Change*	*p-value*	*Baseline Mean±SD*	*Posttest Mean±SD*	*Change*	*p-value*	
Vitamin A (µmol/L)	0.69±0.33	1.03±0.32	0.33±0.25	<0.001**	0.77±0.32	0.71±0.28	−0.06±0.22	0.094	0.39*
RBC×104/µl	4.18±0.44	4.27±0.44	0.08±0.59	0.250	4.16±0.42	4.28±0.36	0.12±0.54	0.052	−0.04#
HGB (g/dL)	10.64±1.00	10.65±1.02	0.01±1.29	0.963	10.57±1.21	10.82±1.10	0.26±1.46	0.131	−0.25#
HCT (%)	32.73±2.86	32.73±2.51	0.00±3.34	0.994	32.57±2.81	33.01±2.90	0.44±3.83	0.324	−0.43#
MCV (fL)	78.59±5.77	77.09±5.21	−1.50±7.65	0.109	78.93±5.57	77.33±5.63	−1.59±7.25	0.060	0.10#
MALPARA	419.83±199.23	711.94±274.87	292.12±350.12	0.407	459.09±190.60	418.41±135.31	−40.68±208.54	0.846	332.80

RBC= Red blood cells; HGB= haemoglobin; MCV= Mean corpuscular volume; HCT= haematocrit

**Significant* differences were observed in difference in changes between the Intervention and Control groups at $p<0.05$

***Differences* significant within-group change comparison at $p<0.05$

#*No* significant differences were observed in difference in changes between the Intervention and Control groups at $p<0.05$. MALPARA= Malaria parasite density; Parasite density was determined on the basis of the number of parasites per 200 white blood cells (WBCs) on a thick blood film assuming a total WBC count of 8000/µl.

Source: Devised by authors.

are shown in Table 4. The change in vitamin A was statistically significant, but there were no significant changes in HGB, HCT, RBC and MCV. The effect of *M. oleifera* leaf consumption on the vitamin A status of the children is shown in Table 5. At a vitamin A level of <0.7µmol/l, there was a significant improvement in vitamin A status; however, above 0.7µmol/l, the increase in the vitamin A level was not significant. Bivariate corrections between vitamin A and haematological indices in the Intervention and Control groups are shown in Tables 6A and 6B respectively. Vitamin A correlated positively and significantly with HGB and HCT in the Intervention group, but not in the Control group.

Table 5 Effect of *M. Oleifera* Leaf Consumption on Vitamin A Status of Children 512 Years

Vitamin A (µmol/L)	*Intervention Group*			
	Baseline Mean±SD	*Endline Mean±SD*	*Change*	*p-value*
<0.7	0.45±0.16	0.94±0.29	0.49±0.29	<0.001*
>0.7	0.91±0.20	1.13±0.34	0.22±0.30	

Source: Devised by authors.

Table 6A Bivariate Correlation between Vitamin A and Haematological Indices among Children in the Intervention Group

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

VITA=*Vitamin* A; RBC=Red blood cells ($\times 10^4$/µl); HGB-=Haemoglobin; HCT=Haematocrit; MCV=Mean corpuscular volume (fL).

**Correlation* is significant at the 0.05 level (2-tailed)

#*Correlation* is significant at the 0.01 level (2-tailed)

Source: Devised by authors.

Table 6B Bivariate Correlation between Vitamin A and Haematological Indices among Children in the Control Group

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

VITA=Vitamin A; RBC=Red blood cells ($\times 10^4$/µl); HGB=Haemoglobin; HCT=Haematocrit; MCV=Mean corpuscular volume (fL).

#Correlation is significant at the 0.01 level (2-tailed); No significance at $p<0.05$.

Source: Devised by authors..

DISCUSSION

Subclinical vitamin A deficiency existed in the study groups at baseline, as 37 (53.6%) children were vitamin A deficient in the Intervention group, while 35 (46.7%) were vitamin A deficient in the Control group (serum retinol <0.70µmol/l). At the end of the study, however, 9 (13%) and 40 (52.6%) children were vitamin A deficient in the Intervention and Control groups respectively (Table 3). This implies that *M. oleifera* leaf consumption improved serum retinol levels in the Intervention group. Furthermore, in the Intervention group, those children who were vitamin A deficient at baseline (serum retinol <0.70 µmol/l), significantly improved in vitamin A status ($p<0.001$). At the same time, there was no significant change in the serum retinol levels of those children who had retinol levels above 0.7 0 µmol/l (Table 5). Those with serum vitamin A <0.70µmol/l at baseline had an average value of 0.45µmol/l, but at end of study, this value rose to 0.94µmol/l, resulting in a change of 0.49 µmol/l ($p<0.001$). In those with vitamin A level >0.70µmol/l at baseline, the change was from 0.91µmol/l to 1.13µmol/l, resulting in a non-significant change of 0.22µmol/l ($p<0.001$). These

findings were similar to those obtained by Ullah et al. (2011), Lala and Reddy (1970) and Zagre et al. (2003) who supplemented green leafy vegetables and red palm oil respectively. They found increases in serum retinol after supplementation, and observed that there was higher absorption in those with lower levels of serum retinol at baseline.

Agte et al. (2006), Persson et al. (2001) and Tyssandier et al. (2002) also reported that β-carotene levels increased when children were supplemented with dark green leafy vegetables and other carotenoid-rich foods respectively. In a well-controlled study, Jalal (1991) found an increase in serum retinol when he supplemented red sweet potato and dark green leafy vegetables. Similarly, Vuong et al. (2002) reported increases in plasma retinol level in children when they supplemented the fruit *Momordica cochinchinensis* (*GAC*), while van Jaarsveld et al. (2005) reported an increase in vitamin A status of primary school children supplemented with orange-fleshed sweet potato using a Modified-Relative-Dose-Response (MRDR) test.

The implication of these findings is that in low socio-economic countries where vitamin A status is mostly marginal (Singh et al., 2001), plant-based β-carotene-rich foods, including green leafy vegetables such as *M. oleifera* leaves, could serve as alternative sources of vitamin A to children in place of high-dose capsule supplementation, which is expensive in terms of health infrastructure and personnel, and animal source foods, which are also unaffordable to low income groups.

Although de Pee and West (1995) did not find any significant improvement in serum retinol when they supplemented dark green vegetables in breastfeeding women, but only had significant levels in those women supplemented with β-carotene enriched wafer, they found a significant increase in serum β-carotene levels. This implies that β-carotene in the leaves was absorbed by study participants, similar to the findings of Persson et al. (2001) and Tyssandier et al. (2002). In addition, all the women supplemented had serum retinol levels above 0.70µmol/l, (0.89µmol/l in the vegetable group, 0.84µmol/l in the enriched wafer group, and 0.81µmol/l in the control wafer group) at baseline. This meant that, although marginal, the women were not vitamin A deficient at baseline (cut-off $<$70µmol/l), so bioconversion of β-carotene to vitamin A in these women could be low as these two processes have an inverse relationship with each other (Solomons, 2001 and Wardlaw, 1999). It is more enhanced in people with very low vitamin A status (Lala and Reddy, 1970; Zagre et al., 2003).

According to de Saint Saveur and Broin (2010), 10 g of *M. oleifera* leaf powder a day will meet 50–100% of the vitamin A needs of all age ranges (depending on the recommended daily intake of each age range). For children in the age range 4–6 years whose daily requirement is 200RE/day, 10 g of *M. oleifera* leaf powder (containing 4,000–8,000µg retinol eq/100 g) will meet 100% of their vitamin A needs. If well promoted, therefore, *M. oleifera* leaves could serve as a less expensive source of vitamin A for tropical countries where it grows, and whose nutrition is mostly plant based. Daily consumption of the leaves will not pose any problem of vitamin A toxicity as opposed to the ingestion of high doses of preformed vitamin A, because β-carotene absorbed from the leaves is stored, and is only converted to vitamin A in response to the body's needs. Moreover, all other nutrients, such as the B-vitamins and minerals present in *M. oleifera* leaves, will be available to the consumer.

Haematological Indices

There was no significant change in the haematological indices (RBC, HGB, HCT and MCV), both in the Intervention and Control groups at the end of the study (Table 4). However, vitamin A correlated positively and significantly with haemoglobin and haematocrit ($p<0.05$) in the Intervention group (Table 6A). RBC also correlated positively with HGB and HCT $p<0.01$). This is expected because one major role of vitamin A in the body is the mobilisation of iron from storage organs for haematopoiesis. The correlation of vitamin A with the haematological indices in the Intervention group confirms this important role of vitamin A in the body (Hashizume et al., 2004), as the increase in vitamin A levels also caused the haemoglobin levels to increase. There was no significant correlation between vitamin A and haemoglobin in the Control group (Table 6B) because vitamin A levels did not increase in the Control group. There were no hookworm infections among the children studied. Malaria parasitaemia, although present in both the Intervention and Control groups at baseline and the end of the study, did not cause any significant changes in the haematological indices.

CONCLUSION

M. oleifera leaf consumption significantly increased the vitamin A status of children in the Ada-East district, Ghana, and has the potential of increasing the haematological status of the children. It could thus play a major role as a food based strategy in vitamin A deficiency control.

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