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Improving Blood Retinol Concentrations with Complementary Foods Fortified with *Moringa oleifera* Leaf Powder – A Pilot Study

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Vitamin A deficiency (VAD†) remains a major public health issue and is reported to be the cause of about 6 percent of child deaths under the age of 5 years in Africa. Inadequate dietary intake of vitamin A-rich foods is a major cause of VAD. *Moringa oleifera* leaf powder (MLP) is rich in nutrients particularly vitamin A and its use in infant feeding has been explored. This pilot study was designed to test the efficacy of MLP in improving blood retinol concentrations among infants in a rural district in Ghana. A subset of infants participating in a randomized controlled trial (ISRCTN14377902) were randomly assigned to receive one of the three study foods (*MCL-35g* and *MS-5g* both of which were fortified with MLP, and a third food, *CF-35g*, a cereal legume blend which served as the control food) in a feeding intervention that lasted for 6 weeks. Primary outcome of the pilot study was retinol levels measured in 5 ml of whole blood at baseline and endline using the iCheck™ Fluoro device. A total of 103 infant-mother pairs were recruited at baseline, of which 65 completed the study. All the infants in the study were vitamin A deficient at both baseline and endline when compared to the World Health Organization (WHO) threshold of 0.70 μmol/l. There was however a marginal non-significant increase in blood vitamin A concentrations for all three groups at endline, with higher numerical increases seen in the two *Moringa* supplemented groups. VAD is a significant public health problem and MLP could be an affordable and sustainable means of combatting the issue. The efficacy of MLP in improving vitamin A status of infants however needs to be ascertained in well-designed trials involving larger numbers of infants and which will last for longer periods. Such studies will also be beneficial in helping to establish the long-term acceptability of complementary foods that incorporate MLP in the target population.

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†Abbreviations: CWC, Child welfare clinic; MLP, *Moringa oleifera* leaf powder; RCT, Randomized Controlled Trial; UMKD, Upper Manya Krobo District; UNICEF, United nations international children's emergency fund; VAD, Vitamin A Deficiency; WHO, World Health Organization; HH, Household head.

Keywords: Vitamin A deficiency, *Moringa oleifera* leaf powder, infants and young children

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INTRODUCTION

Worldwide, undernutrition consisting of wasting, stunting, fetal growth restriction, and deficiencies of vitamin A and zinc, coupled with sub optimal breastfeeding, underlies approximately 3.1 million deaths among children younger than 5 years, annually [1]. Globally, 5.2 million preschool age children are affected by night blindness and 190 million preschool age children have low serum retinol concentrations according to the WHO. In Africa, vitamin A deficiency is responsible for about 6 percent of child deaths under the age of 5 years [2].

The typical diet for complementary feeding in most developing countries with a high prevalence of malnutrition, consists mostly of starchy staples, such as a cereal (maize, rice) or tuber (cassava, yams), with limited amounts of fruits, vegetables, legumes, and few foods of animal origin. Consequently, micronutrient deficiencies, including vitamin A deficiency and iron-deficiency anemia, are common among infants and children living in developing countries [3]. Improving the quality of diets of infants and young children during the complementary feeding period is one of the most cost-effective strategies for reducing morbidity and mortality and improve health [4].

The leaves of *Moringa oleifera*, a fast-growing and drought-resistant tree found in nearly all tropical countries, is currently emerging as an important food source in West Africa [5]. A review research paper pooled together the findings of several studies that investigated the nutritional value of *M. oleifera* leaves and determined its contribution to diets of children aged 1 to 3 years (Table 1) [6]. The study found a wide variability in the nutrient content of both the fresh and dried leaves and recommended that nutrition intervention studies that intended to include *M. oleifera* as part of supplemental feeding programs, needed to analyze samples periodically throughout the program to ensure that planned nutrient targets are being met continuously [6]. This notwithstanding, the nutritional profile of *M. oleifera* leaves suggest that it might be beneficial in improving nutritional quality, particularly vitamin A, of complementary foods in low-resource settings. Glover-Amengor *et al.*, [7] have also reported that the leaves of *M. oleifera* could be harvested and cheaply dried with solar dryers and milled to form a nutrient-rich powder that could be stored for use in rural households. A study that investigated the use of solar and mechanical dryers for *Moringa oleifera* leaf powder (MLP) production found that while both methods of drying offer convenient storage conditions in terms of moisture, water activity, and acceptable nutrient levels, solar dryers would have an advantage in terms of minimized cost of production since solar energy is readily available in the tropics [8]. There has been a recent interest in the promotion of MLP

as a nutritionally valuable ingredient in complementary foods blends. In a study conducted in Tanzania, children aged 6 to 24 months who were patients of a nutrition rehabilitation unit, were randomly allocated to receive either 250 g of maize porridge to which 25 g of MLP was added or 250 g of plain maize porridge daily for 3 months (2 months at the rehabilitation unit and 1 month at home) [9]. The study reported a significant difference in hemoglobin levels of infants at endline ($p < 0.05$) with infants who received MLP having higher hemoglobin concentrations [9]. Further, weight-for-age z scores (WAZ), weight-for-length z scores (WLZ) and mid-upper arm circumference (MUAC) improved significantly for infants in the MLP supplemented group when compared to infants in the control group [9]. There was however no statistically significant difference in the length-for-age z scores (LAZ).

Another study conducted in Burkina Faso compared two groups of malnourished children (aged 6 to 59 months); one group received a 10-g daily dose of MLP for 6 months, while the control group did not receive any MLP. The average daily weight gain (8.9 ± 4.30 g/kg/day) observed in children receiving the MLP was significantly higher than in children (5.7 ± 2.72 g/kg/day) who did not receive any MLP, but there was no significant improvement in hemoglobin levels in either group [10]. Kouevi *et al.*, [11] investigated the nutritional properties of infant weaning foods developed in West Africa and found that although most complementary food blends were low in energy, vitamin A, vitamin C, iron, and calcium, nutrient content could be improved with the addition of MLP. The above-mentioned findings indicate that, including MLP in diets of infants could be an affordable and sustainable means of combatting micronutrient deficiencies. Findings from such studies may be contributing to the increasing interest in the use of MLP to fortify complementary foods in developing countries. We are, however, not aware of any studies that have looked at using MLP to improve vitamin A levels of infants aged 6 to 12 months.

Serum retinol < 0.70 $\mu\text{mol/l}$ and the prevalence of night blindness above various population-specific thresholds are used in classifying vitamin A deficiency in countries as a public health issue [12] and currently preschool age children in Africa are at high risk [13]. An important step in combatting vitamin A deficiency is to be able to determine the extent of the problem by measuring blood vitamin A levels with a technique that is simpler and more convenient than traditional vitamin A determination methods. A device manufactured by Bionalyt Inc. and named iCheck™ Fluoro, is a state-of-the-art portable fluorometer that can be used to determine vitamin A quantitatively in food and biological fluids. The device operates by the use of innovative technology to measure the fluorescence of vitamin A in a sample [14]. A study

Table 1. Contribution of fresh and dried *Moringa oleifera* leaves to the nutrient needs of children aged 1-3 years. Source: Adapted from Witt [6]. Used with permission.

Nutrient	Nutrient content of <i>Moringa oleifera</i>		Recommended nutrient intake	Percentage of recommendation provided	
	5g (1 Tbsp) <i>Moringa</i> leaf powder	20g (1 cup) Fresh <i>Moringa</i> leaves		5g (1 Tbsp) <i>Moringa</i> leaf powder	20g (1 cup) Fresh <i>Moringa</i> leaves
Energy (kcal, MJ)	15.2kcal, 0.064MJ	17.3kcal, 0.072MJ	1098kcal, 4.6MJ	1	2
Protein (g)	1.2	1.76	13	9	14
Fiber, Total Dietary (g)	2.0	1.3	19	11	7
Ca (mg)	95	106	700	14	15
Mg (mg)	23.65	5.2-30.2	80	29	6.5-38
Fe (mg)	1.625	2.16	7(14)	23(12)	31(15)
Zn (mg)	0.12	0.06-0.26	3(6)	4(2)	2(1)
Thiamin (mg)	0.13	0.05	0.5	26	9
Riboflavin (mg)	0.06-1.0	0.15	0.5	12-200	29
Niacin (mg)	0.41	0.74	6	7	12
Vitamin B-6 (mg)	0.12	0.24	0.5	24	48
Folate (µg)	27	41	150	18	27
Vitamin A (µg, RAE)	182	258	300	61	86
Vitamin c (mg)	8.6	32.4	15	57	216
Vitamin E (mg)	2.8-5.6	5	6	46-93	83

by Aglago *et al.*, compared the iCheck™ Fluoro kit to High-Performance Liquid Chromatography (HPLC) in assessing serum retinol and indicated that iCheck measurement provided good correlations with HPLC for serum concentrations and that it may offer dependable means for testing serum retinol measurements done with no significant time delay [15].

Our aim was to conduct a pilot study to determine the efficacy of *Moringa* leaf powder in improving vitamin A levels of infants in the complementary feeding age, by measuring and comparing blood retinol concentrations of infants (with iCheck™ Fluoro) at baseline, and after 6 weeks of feeding with MLP. The pilot study was intended to fine-tune study procedures for a larger trial.

METHODS

Study Design

The study was a randomized controlled trial with three study arms comprising two experimental arms and one control arm. Study infants were recruited from Child Welfare Clinics (CWCs) in the Upper Manya Krobo

(UMKD) district. The first arm received MLP as part of a cereal-legume blended flour (*MCL-35g*). The second arm (*MS-5g*) received MLP as a food supplement to be added to infants' usual complementary foods, while the third arm (*CF-35g*) which served as the control arm received a cereal-legume blend complementary food (*Weanimx*) without *Moringa* leaf powder. Energy and nutrient content of the study foods were determined by proximate analysis and are reported in Table 2. An acceptability trial of study foods was conducted before being used for the study. The trial found the study foods acceptable to infants and care givers in the study population [16]. Infants were randomly assigned to receive one of the three study foods using simple randomization procedures and depending on the CWC they were drawn from. The trial could not be blinded because the study foods were very dissimilar in appearance.

Recruitment

Beginning December 2014, infants aged 4 to 11 months were recruited after the study had been explained to their mothers or caregivers and consent was sought and obtained. When infants attained age 8 to 12 months, they

Table 2. Nutrient content of study foods.

	<i>MCL-35g</i>	<i>MS-5g</i>	<i>CF-35g</i>
Daily ration g/day	35	5	35
Energy (kcal)	144.64	10.25	142.15
Protein (g/100g)	6.40	1.38	5.88
Iron (mg/100g)	3.78	1.13	2.53
Vitamin A ($\mu\text{g RE}/100\text{g}$)	51.95	24.82	0.27

CF-35g - control, *MCL-35g* – Moringa with Weanimix, *MS-5g* – Moringa as Sprinkles

were assigned to receive one of the three study diets based on the CWC from where the infant was recruited. On account of ethical concerns about providing no treatment to infants who could have tested low on certain assessments (e.g. hemoglobin) at baseline, there was no placebo group.

Study Site

The study was carried out in the Upper Manya Krobo district (UMKD) of the Eastern Region of Ghana. where agriculture is the main economic activity of the people of the district, employing about 80 percent of the population, most of whom are subsistence farmers with very few commercial ones [17].

Study Population and Eligibility

The study population included infants under two years of age attending weight monitoring sessions at the Child Welfare Clinics in the UMKD.

Inclusion Criteria

Infants who were aged between 8 to 12 months at the time of recruiting, who were still being breastfed, had no congenital abnormalities, had been assigned maternal and child health cards, and whose mothers or caregivers planned to stay at the study site for the duration of the study were eligible for enrollment in the study. Infants who did not satisfy the above criteria or who had known intolerances to any of the ingredients of the study foods were excluded from the study.

Sample Size

A sample size of 35 infants per study arm was conveniently determined. Infants were recruited for the study after screening for eligibility. Informed consent was obtained from parents and caregivers, after which infants were randomly assigned to receive one of the three study foods (*CF-35g*, *MCL-35g*, or *MS-5g*).

Study Procedures

Mothers and caregivers of recruited infants were visited at home by trained field assistants to verify

eligibility, to explain the study protocol in detail, and to obtain written informed consent. Study foods were delivered to the infants on a bi-weekly basis. Cooking demonstrations were carried out to teach mothers how to prepare study foods. Mothers were instructed to feed foods 2 to 3 times daily, 7 days a week. The feeding intervention lasted for 6 weeks. During the period, each infant received a total of four follow-up home visits.

Data Collection

A structured pre-tested questionnaire was administered at home to mothers/care givers of infants. It was written in English and then translated into *Krobo* (the local dialect of the people of the UMKD) and pre-tested on a group of non-participating mothers. The questionnaire was back-translated into *Krobo* to ascertain its accuracy before the pre-testing. The questions were adjusted and incorporated accordingly. Field workers were drawn from the local community and taken through rigorous training in the translation and administration of the questionnaires as well as the use of study equipment for anthropometry and blood retinol measurements before the field work commenced.

Baseline Data

Following the recruitment of study participants into the study, the following baseline data were collected:

Household and maternal characteristics – Household head's (HH) gender, age, and marital status; total number of members in household, total number of children under 5 years in the household, level of education of respondent and HH; main occupation of HH and respondent; household vegetable garden, types of vegetables grown in the garden, main use of vegetables; current household main source of food; household food security. Data on maternal (weight and height) and family demographics were also collected at baseline.

Anthropometry – Measurements of infant weight and length and maternal weight and height were taken. Infant weight and length measurements were taken in accordance with WHO guidelines. Infants were weighed naked on a digital infant scale (model 1583, Tanita, Quick

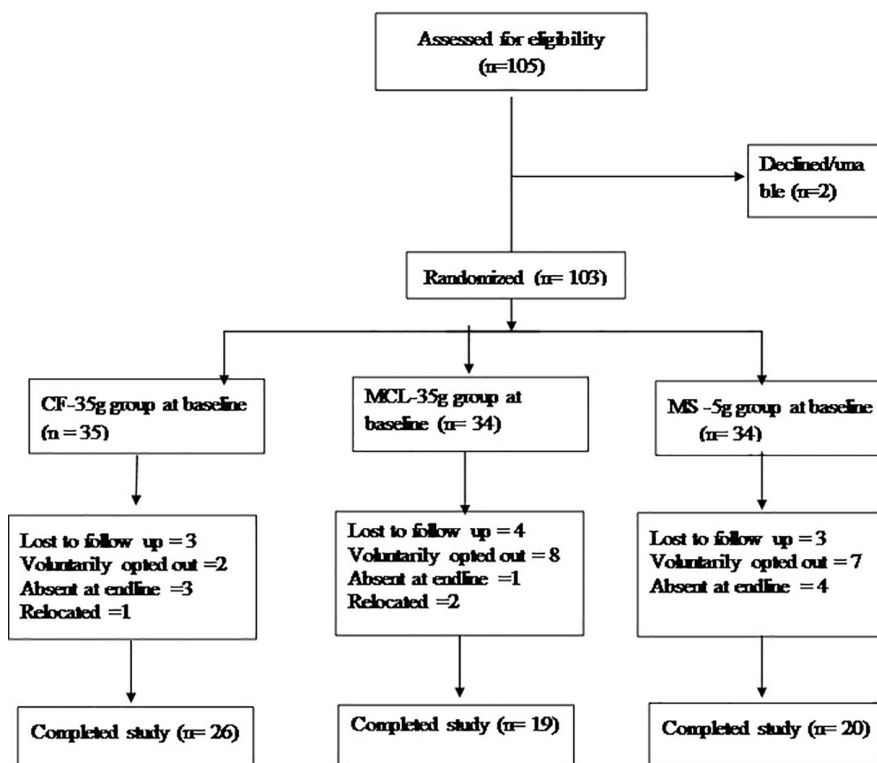


Figure 1. Flow of infants through the study.

Medical, Snoqualmie, WA) to the nearest 100 g. Recumbent length was measured with an infantometer (model 447, Infantronic Digital Infantometer; Quick Medical) to the nearest 0.1 cm. Two trained field assistants were responsible for taking measurements – while one ensured that the top of the infant’s head gently touched the head board, the other ensured that the child’s legs were fully extended before taking the reading to ensure accurate results. Maternal weight (to the nearest 0.1kg) was measured using an adult digital scale (model BF-508, Omron Healthcare, USA); participants were asked to step onto the center of the scale platform with their feet slightly apart in order to obtain measurement. Maternal height was measured (to the nearest 0.1 cm) using a stadiometer (model 213 Seca, Germany); Participants were asked to stand with their backs against a height board and their eyes in the Frankfort plane, for the correct reading to be taken. Prior to taking measurements, mothers were asked to remove shoes, head gear and other bulky outer clothing that could affect accuracy of the weight and height measurements. Each parameter was measured twice to ensure reliability. Procedures were clearly explained to participants in their local dialects before measurements were taken.

Child Morbidity Occurrence – Information on di-

arrhea (defined as three or more watery stools per day), symptoms of respiratory infections cough, (tachypnea/dyspnea, lower chest wall in-drawing and fever) in the previous 2 weeks were collected by caregiver interviews at baseline and during the bi-weekly visits. Further questions regarding symptoms (fever, chills, loss of appetite) suggestive of malaria were also asked.

Biochemical Data – To measure blood retinol concentration, 0.8 to 1 ml of blood was drawn from each infant by a qualified phlebotomist. The blood samples were stored in 5 ml EDTA bottles (to prevent coagulation of blood samples), after which the bottles were wrapped with aluminum foil and stored in an ice chest with ice packs to preserve them. The purpose of the aluminum foil was to prevent sunlight (UV light) from coming into direct contact with the blood samples. The blood samples were then transported to the Asesewa Government Hospital laboratory where the iCheck™ Fluoro (Bioanalyt Inc, Germany) device was set up. In the laboratory, 0.5 ml of blood was drawn from the EDTA bottle and injected into a reagent vial. The reagent vial was then shaken vigorously for 10 seconds and allowed to stand for 5 minutes after which the reading was taken with the iCheck™ Fluoro device. The laboratory analysis was done the same day as the blood samples were taken by trained study team

Table 3. Background characteristics of study participants at baseline.

	<i>CF-35g</i> (n = 35)	<i>MCL-35g</i> (n = 34)	<i>MS-5g</i> (n = 34)	<i>p-value</i>
Infant sex, males (%)	62.90	58.80	73.50	0.42
Infant age (Mean \pm SD)	9.0 \pm 1.30	9.1 \pm 1.40	9.3 \pm 1.40	0.48
Infant height (cm)	68.11 \pm 2.84	68.51 \pm 2.78	68.00 \pm 11.45	0.59
Infant weight (kg)	7.98 \pm 1.07	7.87 \pm 0.97	8.89 \pm 6.31	0.38
Length for age z-scores (LAZ)	-0.89 \pm 1.17	-0.85 \pm 1.04	-1.2 \pm 1 .10	0.21
Weight for Age z-scores (WAZ)	-0.57 \pm 1.18	-0.6 \pm 1.22	-0.9 \pm 1.10	0.88
Weight for Length z-scores (WLZ)	-0.1 \pm 1.13	-0.3 \pm 1.17	-0.3 \pm 1.00	0.71
Hemoglobin concentration of infants (g/dL)	10.40 \pm 1.13	10.36 \pm 1.36	10.17 \pm 1.29	0.73
Birth order (Mean \pm SD)	2.71 \pm 2.15	3.09 \pm 2.07	2.88 \pm 1.90	0.34
Mother's age (years) (Mean \pm SD)	26.77 \pm 9.34	27.68 \pm 7.66	25.82 \pm 6.45	0.63
Mother's education (%) Primary - JHS	82.90	91.20	73.50	0.73
Number of people in household (Mean \pm SD)	6.80 \pm 2.41	6.32 \pm 3.23	6.18 \pm 2.36	0.60
Use public water (%) (Public tap/borehole/well)	74.30	44.10	35.30	0.04
Infant morbidity (%)				
Vomiting	5.70	20.60	8.80	0.13
Diarrhea	28.60	11.8.0	26.50	0.19
Cough	28.60	29.40	32.40	0.94
Nasal discharge	45.70	26.50	50.00	0.11
Fever	40.00	38.20	50.00	0.33

CF-35g- control, *MCL-35g* – Moringa with Weanimix, *MS-5g* – Moringa as Sprinkles

members.

Dietary Data Collection – Two-day 24-hour dietary recall of all foods and drinks consumed by infants were collected from mothers/primary caregivers by trained research assistants at baseline. Common containers used for feeding infants such as bowls, cups, and spoons were used to estimate amounts of food that had been consumed. Additional questions to prompt mothers/primary caregivers about foods such as snacks and drinks that could easily be forgotten in the recall and special dietary issues (e.g. foods that the child does not eat or purportedly reacts to) were also asked.

Intervention

For infants receiving *MCL-35g* and *CF-35g*, each mother/caregiver was given two 250 g sachets (placed in a clean transparent plastic container with a firmly closing lid) for use within the 2-week period. Approximately 35g of the flours, equivalent to 2 heaped tablespoons was to

be used to prepare meals for infants each day. An extra 10 g package of food was added to the plastic container for each child just in case other individuals within or outside the home wanted to taste the food. For the third study arm, fourteen 5g sachets of *MS-5g* (MLP) was supplied to infants in their homes every two weeks. One sachet (5g) was to be sprinkled on the child's usual complementary food everyday while the food was very hot or freshly taken off the fire. Mothers were given plastic bowls with spoons to feed their infants and a cake of soap to wash their hands before preparation of the foods. Mothers were taught how to prepare porridge from the *MCL-35g* flour as well as how to use the flour to prepare other local meals such as "banku/akple," "aprapransa," and "kakra." Trained field workers demonstrated the preparation of the recipes. Mothers were instructed to feed the study foods 2 to 3 times/day to infants. Each mother was instructed to continue breastfeeding as well as feed their infants of other complementary foods aside the study

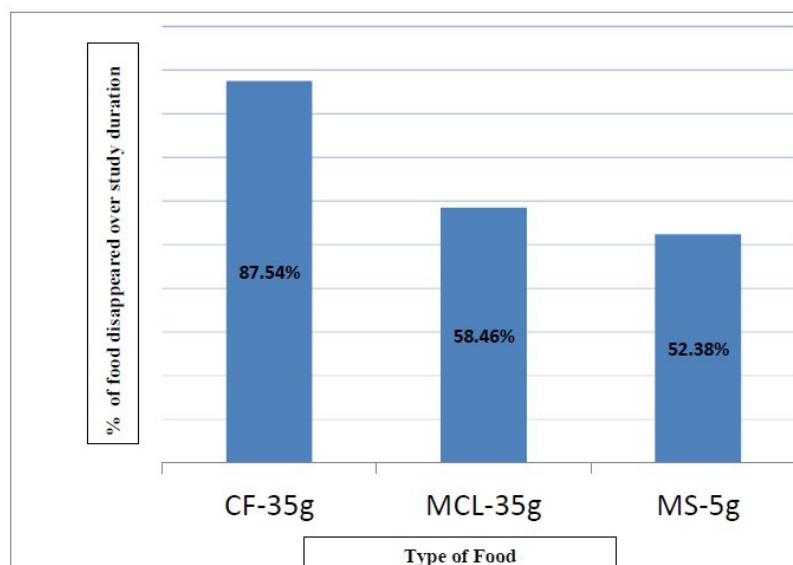


Figure 2. Adherence of infants to study foods over the study period.

Table 4. Baseline and end line energy and nutrient intakes of study infants by group.

Energy and micronutrients	<i>CF-35g</i> Baseline n=35 Endline n=26	<i>MCL-35g</i> Baseline n=34 Endline n=19	<i>MS-5g</i> Baseline n=34 Endline n=20
Energy (kcal)			
Baseline	356.945±244.11	426.92±249.29	448.875±253.18
Endline	570.3±324.33	554.945±221.70	598.28±399.51
Protein (g)			
Baseline	11.965±16.99	11.27±7.29	11.625±7.27
Endline	15.38±8.91	18.88±8.96	17.28±10.75
Carbohydrate (g)			
Baseline	73.705±111.32	73.81±36.71	70.74±42.52
Endline	94.375±56.67	82.76±32.45	95.48±71.99
Fat (g)			
Baseline	10.575±10.93	14.71±14.75	9.827±10.83
Endline	16.345±14.42	17.54±10.68	32.865±59.51
Iron (mg)			
Baseline	5.075±4.47*	8.78±4.56*	6.135±3.87*
Endline	8.43±5.82	11.456±6.58	9.145±7.36
Vitamin A (µg/RE)			
Baseline	280.87±635.73	703.035±809.99	632.335±715.66
Endline	642.38±944.29	634.025±619.59	896.98±770.69

CF-35g- control, *MCL-35g* – Moringa with Weanmix, *MS-5g* – Moringa as Sprinkles. Values are reported as means and standard deviations. *Significant at p = 0.026.

Table 5. Serum vitamin A concentrations at baseline and end line for infants who completed the study.

Study Group	Serum Retinol Concentrations ($\mu\text{mol/l}$)		
	Baseline	Endline	p-value
CF-35g	0.45 \pm 0.16	0.55 \pm 0.16	0.09
MCL-35g	0.52 \pm 0.18	0.63 \pm 0.38	0.14
MS-5g	0.57 \pm 0.20	0.65 \pm 0.20	0.82

CF-35g- control, MCL-35g – Moringa with Weanimix, MS-5g – Moringa as Sprinkles

foods. All study foods were supplied to infants without charge. Each mother/infant pair was paid a total of four visits (bi-weekly) by a field worker over the 4-month feeding duration. The purpose of the visit was to monitor how much food the infants had eaten, to obtain mothers' feedback on their experiences with the use of the food as well as to monitor infant morbidity using a questionnaire.

Outcome Measure

The primary outcome of this pilot study was blood retinol concentration and was measured at baseline and after 6 weeks of feeding with complementary foods that incorporate MLP. Anthropometric and hemoglobin measurements were also taken at baseline and after 6 weeks. A record of infant's dietary intake (both study and non-study foods) were collected by 24-hour recalls for 2 days (1 weekday and 1 weekend day), at baseline and at 6 weeks. Morbidity data (diarrhea, symptoms of respiratory infections, and fever) was collected at baseline and thereafter, every 2 weeks, during the time of the home visits when project foods were delivered to the mothers/caregivers.

Data Management

Completed data forms were collected and reviewed daily by the researchers. Data forms were stored in secured cabinets which were kept under lock and key. Data was entered using Microsoft Excel (2010) by trained research staff and stored in a secure password protected computer.

Data Analyses

Data analyses were performed using SPSS Version 20, (IBM, USA). Analysis of covariance was used to determine any significant differences between the three study groups in blood retinol concentrations at end line. Paired t-test was used to examine within group differences in blood retinol concentrations from baseline to endline. Dietary intakes collected from the two day 24-hour recall were converted to energy and nutrients using a food database (RIING food composition database, Nutrition Department, University of Ghana) and ESHA-F-pro software.

Ethical Approval

The study protocol was approved by the Ethical Review Committee of the Ghana Health Service (GHS-ERC: 07/09/14), and the Institutional Review Board of the Noguchi Memorial Institute for Medical Research, University of Ghana (NMIMR –IRB CPN -106/14-14). Permission to use the study foods was obtained from the Food and Drugs Authority. Written permission to carry out the study in the Upper Manya Krobo District was obtained from the District Health Administration and written informed consent was obtained from the parents of each infant. The trial was registered with the ISRCTN registry with trial number – ISRCTN14377902. This study was financially supported by the International Development Research Centre (IDRC), Canada, Research grant No 104519-009 (IDRC RC) to the University of Ghana; the UG-Carnegie PhD research grant No B8739 (R01), and the Association of African Universities (AAU).

RESULTS

Background Characteristics of Study Participants

Sixty-five out of a total of 103 mother and infant pairs completed the study. The flow of infants through the study is reported in Figure 1. Background characteristics of study participants is shown in Table 3. Mean infant age was 9 months for infants in each study group. At baseline, there were more males than females in each of the study arms.

The mean weight of the infants in the CF-35g and MCL-35g groups was 7.98 kg and 7.87kg respectively, while the mean weight of infants in the MS -5g group was highest at 8.89 kg. Mean length averaged 68 cm for infants in all the three study arms. Infant birth order for each group averaged three.

The mean hemoglobin concentration of the infants in the three groups was comparable at 10 g/dL for each group at baseline. Mean maternal age ranged between 25 to 27 years for the three study groups, while majority of the mothers in all three groups were educated up to the Junior High School level; 83 percent, for the control group, CF -35g and 91 percent and 74 percent for the

Table 6. Comparison of serum vitamin A at endline for infants who completed the study.

Outcome variable	CF-35g (n=)	MCL-35g (n=80)	MS-5g (n=74)	P
Serum vitamin A at endline ($\mu\text{mol/l}$)	0.55 \pm 0.16	0.63 \pm 0.38	0.65 \pm 0.32	0.83

CF-35g- control, MCL-35g – Moringa with Weanimix, MS-5g – Moringa as Sprinkles; ¹Data are mean \pm standard deviation; ²P values compare all 3 groups using analysis of covariance (ANCOVA), with adjustment for infant age, sex and baseline hemoglobin and serum vitamin A levels. Significance was set at $p \leq 0.05$

Table 7. Morbidity occurrence among infants during the study.

Morbidity	CF-35g	MCL-35g	MS-5g
Vomiting (%)	8.6	8.8	8.8
Diarrhea (%)	22.9	8.8	26.5
Cough (%)	22.9	38.2	11.8
Nasal Discharge (%)	28.6	50	8.8
Fever (%)	28.6	44.1	32.4

CF-35g- control, MCL-35g – Moringa with Weanimix, MS-5g – Moringa as Sprinkles

MCL -35g and MS -5g groups respectively. With respect to sources of drinking water, majority, (74 percent) of study participants in the CF-35g group had tap water/boreholes/wells (improved sources of drinking water), as their main water source, compared 44 percent to and 35 percent of study participants in the MCL-35g and MS-5g groups respectively. Morbidity of infants at the time of the baseline interview is reported in Table 3. A high prevalence of fever (50 percent) and nasal discharge (50 percent) were reported for infants in the MS -5g group at baseline Table 4.

Blood Retinol Concentrations

Mean blood retinol concentrations of infants at baseline and at endline are reported in Table 5. There was a marginal increase in retinol levels from baseline to endline in all three study groups. The differences however were not statistically significant at endline (Table 6) for any of the study groups. However, at endline the mean retinol concentration in the intervention groups MCL-35g and MS-5g were slightly higher numerically, (0.63 $\mu\text{mol/l}$ and 0.65 $\mu\text{mol/l}$ respectively) when compared with that of the control group (0.55 $\mu\text{mol/l}$). There were also no significant differences in blood retinol levels from baseline to endline within any of the three study groups.

Energy and Nutrient Intakes

Table 4 presents the baseline and end line energy and nutrient intakes in all three groups. Average energy

and nutrient intakes increased at endline for all the study groups. With the exception of iron intake at baseline, there were no significant differences among the three groups for energy and the nutrients analyzed. A significant difference ($p = 0.026$) was observed among the three groups for iron intake at endline.

Adherence to Study Foods

Adherence to study foods was measured as the disappearance rate (percentage of food disappeared over the duration of the study) and is presented in Figure 2. A significant difference in adherence was observed among the three study groups with infants in the CF-35g (control) arm, having significantly higher ($p \leq 0.05$) adherence (87.54 percent) than infants in the two intervention arms (58.56 percent and 52.38 percent for MCL-35g and MS-5g respectively). The difference observed in adherence among infants between the two intervention groups was however not significant.

Morbidity

The occurrence of diarrhea, vomiting, cough, nasal discharge, and fever among study infants (as reported by mothers or caregivers) over the duration of the study are presented in Table 7. Generally, there were few reports of morbidity over the study period among infants in the three groups. The highest morbidity occurrence was observed in the MCL-35g group where 50 percent of the infants were reported to have

had nasal discharge over the duration of the study.

DISCUSSION

Background Characteristics

The majority of infants in this study were males (Table 3). Darton-Hill et al [18] have reported that there is evidence to suggest that vitamin A deficiency is commonly reported to occur up to about ten times more frequently in males than in females. The relationship between sex and vitamin A concentrations will have to be ascertained in the larger trial.

Study infant's weights and heights were within the normal limits for infants in the complementary feeding age according to WHO growth charts [19].

The hemoglobin concentration of study infants in each group averaged 10g/dl. This level falls within the normal range of hemoglobin levels for infants in that age group, which is 9.5g/dl to 13g/dl [20].

Findings of our study showed that study infants were averagely third in birth order for each group. A study by Sarvar et al [21] that investigated micronutrient deficiencies in children aged 1 to 5 years showed that children with higher birth order showed higher prevalence of micronutrient deficiencies. The relationship between birth order and vitamin A status will need to be investigated in the larger trial as this information will be useful in formulating policy for vitamin A supplementation.

In our study, the educational level of majority of the mothers were educated up to Junior High School level for each study arm (Table 3). The educational status of parents or caregivers is believed to play a major role on the nutritional status of children. A study by Netto et al showed that father's educational level was significantly associated with serum retinol levels with each extra year the father had spent in education being associated with an increase of 0.052µg/dL ($p = 0.013$) in serum retinol concentration [22]. Another study conducted in Ethiopia to determine the demographic and health-related risk factors of subclinical VAD, however showed that, level of education of mother had no association with subclinical vitamin A deficiency among the children [23]. It will be beneficial to investigate and ascertain any associations between educational level of parents/caregivers and serum retinol levels in the larger trial.

The main source of drinking water for majority of study participants (74 percent) in the control arm (CF-35g) was public tap/boreholes/wells (Table 3), with smaller percentages of study participants in the two intervention groups (MCL-35g – 44 percent and MS-5g – 35 percent) also depending on public tap/boreholes/wells as major sources of drinking water. All other study participants depended on river/pond/stream water, which are poorer sources of drinking water. de

Queiroz et al [24] identified poor water sources as an associated factor for children in urban areas being susceptible to VAD. Intestinal parasites from poor water sources can reduce the absorption of vitamin A and in some instances, be associated with clinical VAD [25].

Blood Retinol Concentrations

According to the WHO threshold for classifying VAD biochemically ($< 0.70 \mu\text{mol/l}$), all the children in the study were vitamin A deficient both at baseline and at the end of the study (Table 5). However, at endline, the mean blood retinol concentrations in the two experimental groups (MCL-35g – $0.63 \mu\text{mol/l}$ and MS-5g – $0.65 \mu\text{mol/l}$) approached the WHO threshold of $0.7 \mu\text{mol/l}$ for VAD classification, when compared to infants in the control group (CF-35g – $0.55 \mu\text{mol/l}$). The differences however were not significant at endline for any of the study groups. There were also no significant differences in blood retinol levels from baseline to endline within any of the three study groups. A larger trial that lasts for longer might be able to better ascertain the effects of MLP supplementation on blood retinol levels. The increase in blood retinol levels in the two *Moringa* supplemented groups at endline however, are supported by some studies. Glover-Amengo et al [7], showed that supplementing diets with MLP could improve the vitamin A status of school age children. Another study by Ullah et al [26] in their study which investigated serum retinol levels in a small population after feeding with extracts from a dark green leafy vegetable for seven days showed that, serum retinol increased in majority of subjects after they were fed. Results from another study by Persson et al [27], showed substantial increase in serum vitamin A after schoolchildren who had been previously dewormed, were fed for six weeks with dark green leafy vegetables. We reiterate however that, dark green leafy vegetables other than leaves of *M. oleifera* were used in the studies by Ullah et al and Person et al, mentioned above.

Babu [28] has also reported that *Moringa* is superior in providing vitamin A compared to other vegetables in a study conducted in Malawi and advocates the usefulness of indigenous knowledge on local foods in planning rural nutrition interventions.

However, de Pee and West [29] have reported that plant sources of beta-carotene were not effective in improving the serum vitamin A concentrations in lactating women who were fed with a special wafer prepared from plant-based beta-carotene rich sources.

Assessment of Dietary Intake

Our findings from the dietary intake assessment showed that, there were increases in energy and nutrient intakes in all the three study groups from baseline to

endline in the treatment groups as well as the control group. The differences however were not statistically significant for energy and nutrient intakes at endline. Regarding adherence to the study foods, findings of this study, clearly showed that, the control food (*CF-35g*), was more acceptable to the study population than the two intervention foods that incorporated MLP (*MCL-35g* and *MS-5g*) (Figure 2). MLP has unique sensory (color, taste, odor) characteristics and Oyeyinka and Oyeyinka [30] have reported a reduction in the acceptability and overall sensory characteristics of foods with increasing levels of MLP supplementation. The findings of this study thus raises the issue of acceptability of complementary foods that are fortified with MLP and calls for further studies to investigate levels of MLP supplementation in complementary foods that will be acceptable to infants and young children.

The study had some limitations. The sample size at endline was small as some of the participants of the study dropped out for a variety of reasons. Specifically, some mothers/caregivers who voluntarily opted out of the study in the two *Moringa* groups indicated that infants got tired of eating the *Moringa*-fortified foods while other mothers were unwilling to have another sample of blood drawn from their infants for the serum vitamin A determination at endline. Another limitation was the fact that, the measurement of morbidity occurrence and adherence over study period was not objective. While measurement of morbidity occurrence was based on reports from mothers/caregivers, the measurement of adherence was based on the disappearance rate of study foods given to study participants. In addition to the use of the 24-hour recall method for dietary assessment, the aforementioned methods were sources of bias that could influence study findings. These limitations notwithstanding, findings of this pilot study will go a long way to help in fine-tuning procedures and refining the hypotheses for the larger trial.

CONCLUSION

In the planning of food-based interventions that employ food diversification to combat VAD, MLP may be considered for use as a fortificant in complementary foods to improve the vitamin A levels of infants. In this study, the observation of a marginal increase in serum vitamin A concentrations for infants in all three groups notwithstanding, vitamin A levels of all infants were low (both at baseline and at endline) when compared to the WHO threshold of $0.70\mu\text{mol/l}$. This implies that, VAD remains a major public health issue in the study population and in Ghana for that matter. The efficacy of MLP in improving vitamin status of infants in the complementary feeding age however needs to be ascertained in a well-designed larger trial that will last for a longer period. Such

a study will also be beneficial in helping to establish the long-term acceptability of complementary foods that incorporate MLP in the target population.

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