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Toxicity of Soursop Leaf Powder and Its Relevance in Determining The Micronutrient Status in Formulated Complementary Food

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Abstract

Complementary food was produced from blends of hungry rice (A), pigeon pea (P) and soursop leaves (S). The raw materials were washed with portable water, dried at room temperature, milled with hammer mill, fermented for 24 hours at 28 ± 2 °C (28 ± 2 °C), oven-dried at 50 °C for 12 hours, remilled, sieved to 1 mm pore size and packaged in polyethylene bags for further analysis. The samples were in the ratio of 70:30:0 (sample APS), 65:30:5 (sample APS1), 60:30:10 (sample APS2) and 55:30:15 (sample APS3). Toxicity test for lethal dose (LD50) was carried out on the soursop leaves. Bioassay was carried out with male albino rats for 28 days including acclimatization period of 7 days. Feed intake and weight gain of experimental rats were recorded daily and weekly. Blood serum was collected before and after feeding trials for analyzing bioavailability of the selected micronutrients. The data were subjected to one-way analysis of variance. Means were separated using the Duncan's multiple range test and significance was accepted at probability level of 0.05 %. The toxicity test (LD50) indicated safety of soursop leaf as an infusion (oral administration) at lower doses of 10-1000 mg/kg body weight of rats. The bioassay revealed that food intake was significantly ($p < 0.05$) different among the samples in the first, second and third week. Rats that ate normal rat chow had the highest food intake while the rats that ate APS3 had the lowest food intake. Weight gain was highest in rats that ate rat chow while it was lowest in the rat that ate APS3. Bioavailability of selected micronutrient revealed that calcium content had the highest bioavailability in rats fed with rat chow and lowest in AP. Sample of APS1 had the highest iron bioavailability (47.83 %) among the fortified samples and the rat chow. Zinc had the highest bioavailability (52.86 %) in APS1. The work revealed that selected vitamins were most available in APS2 and the selected minerals were most available in sample APS1.

Keywords: *Pigeon pea, Acha, Soursop leaf powder, Complementary food, Micronutrient, Bioassay.*

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I. INTRODUCTION

Complementary foods are solid or semisolid foods introduced to the diets of infants after six months of exclusive breast feeding. Although exclusive breast feeding provides all the necessary nutrients needed for the infant development, after six months, the child will need more nutrients (vitamins, minerals, proteins and carbohydrates) which cannot be gotten from breast milk alone. During this period, any solid or semisolid food given to young children are known as complementary foods. Usually, such foods are also used to rehabilitate undernourished infants between 4-6 months especially those recovering from illness (Fernandez *et al.*, 2002).

Insufficient intake of nutrients by infants has been the major cause of malnutrition especially in most developing countries, this has resulted to deficiency disorders among developing children. In order to tackle this problem, appropriate feeding practices has to be put into place; this is to ensure that the infants are given the correct adequate food intake and also at the right time. Such practices should not only be seen as a means of introduction to the family diet, but also as a way of enhancing the nutritional well-being of infants and providing nutrient which is needed for their ideal growth and development (Fernandez *et al.*, 2002).

According to Solomon (2005), complementary foods used for the growth and development of infants may vary depending on geographical location and availability of raw materials. For most rural and poor urban mothers, they prepare complementary foods for their infants using staple crops (cereals, tubers, legumes and condiments) (Solomon, 2005; Guptill *et al.*, 1993). However, due to insufficient preparation and improper processing methods applied during production of traditional/home-made complementary foods, the end product often results in affordable

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food with poor texture and high volume, filling but lack adequate nutrients which are needed for growth and development (Fernandez *et al.*, 2002). However, despite these challenges, home-based diets remain an easy adaptable alternative for complementary feeding practice when adequate measures are taken during preparation and processing. This may be developed in line with a country dietary infant feeding guideline, taking into consideration the accessibility and budget-friendly of commercial instant infant cereal formula in that area.

Commercial infant instant cereal formula is known to be highly nutritive, palatable and safe to consume for the infant, but due to their high-priced, it usually cannot be reachable for low-income earners. As a result, these families have no other alternative than to rely on traditional developed meals to feed their young ones. The traditional developed foods such as pap and porridges have a lot of disadvantages. They include low protein, because they are made from single cereals, loss of micronutrients due to poor processing and cooking methods. This gives way to nutrient malnutrition.

The availability of nutrients in complementary foods for use by the body is important to note. This is due to the presence of chemical compounds that bind nutrients and makes them unavailable. They are often known as anti-nutrients. They reduce the bioavailability of some micronutrients and fiber content. As a result, it is recommended to use dehulled legumes and cereals (e.g., corn meal). Meeting micronutrient needs from complementary foods appears to be a great challenge. In order to provide adequate amount of important nutrients (iodine, vitamin A, iron and zinc) which are necessities for child development and growth in complementary diets, micronutrient fortification and supplementation needs to be adopted. According to FAO/WHO (1998) and Kennedy *et al*. (2003), the inclusion of food-based approaches like diversification of diets, fortification and biofortification of foods could serve as a solution to the above-mentioned case. Kennedy *et al*. (2003) further made a suggestion as regards food fortification and biofortification. The two could serve as a less costly alternative means of addressing and intervening on all public health concerns and also provide food for low-income earners.

The food-based dietary guidelines were published by the Federal Ministry of Health (FMOH) in Nigeria (FMOH, 1999). According to the regulation, the use of staple commodities such as starchy roots and tubers, cereals together with pulses, vegetables and fruits and possibly proteins gotten from animal sources were recommended for the production of complementary foods to promote and encourage dietary diversification. With the recommendations put into place, different factors negatively affecting absorption of micronutrient and means to curb micronutrient malnutrition have been put into review in several nutrition forays (NNN, 2000; NACMD, 2003). These can be seen by the promising results reported by many researchers on the potentials of grains, pulses, vegetables and fruits in the developing of complementary foods (Badamosi *et al.*, 1995; Owolabi *et al.*, 1996; FAO, 1997; Okoh, 1998). Hungry rice (*Digitaria exilis*) is a cereal which is rich in certain amino acids such as methionine and cysteine. Both amino acids are essential to the health of humans, and are lacking in nowadays major cereals; wheat, rice, sorghum and others. Pigeon pea (*Cajanus cajan)* on the other hand, is a legume known for its relatively high amount of protein. They are good sources of lysine but deficient in sulphur amino acids viz-a-viz methionine and cysteine. Pigeon pea when combined with cereals, makes a well-balanced meal. Soursop (*Annona muricata)* leaf is a glossy dark green vegetable which contains minerals and vitamins healthy for human health. The soursop leaf is not usually consumed due to paucity of information on its nutritional and toxicological content. Therefore, with all said, it is important to blend and develop such varieties of foods found in different localities and studies on the biochemical properties of the developed product carried out to ascertain its possible use as complementary diet. It is also important to take into consideration during formulation; processing methods that will help prolong the shelf-life reduce the frequency of preparation. This study, production, quality and toxicological evaluation of hungry rice and pigeon pea complementary food fortified with soursop leaves, aims at ways of boosting the nutritional status of traditional complementary meals, and also the promotion of the use of staple underutilized foodstuffs that are indigenous to some states in Nigeria in formulating composite blends that can be highly nutritive, readily available and budget-friendly to both rural and poor urban mothers.

II. Materials and Methods

A. Procurement of raw material

Acha grains (*Digitaria exilis*) and pigeon pea (*Cajanus cajan)* were purchased from Ogbete main market, Enugu state Nigeria. Soursop leaves *(Annona muricata*) were handpicked from a botany garden in University of Nigeria Nsukka and authenticated by a botanist in Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

B. Preparation of samples

1. Processing of acha flour

Acha grains (*Digitaria exilis*) were processed according to procedure used by Echendu *et al.* (2009). Fifteen kilogram of acha grains were screened to remove unwanted substances, washed severally with tap water to remove extraneous materials, dried at 50 ºC in a laboratory oven (model no DHG 9101-LSA) for 24 hours to constant weight, milled with a hammer mill and sieved through 1 mm pore sieve.

2. Processing of pigeon pea flour

Pigeon peas were processed into flour by the method described by Fasoyiro *et al.* (2010). 10 kg of the pigeon pea seeds were sorted and moistened with 50 ml of water for easy dehulling of the seed coat. The moistened seed was left to equilibrate for 15 minutes. The seeds were then roasted on electric fryer (Essentials Model CTW-250) for 10 minutes at 150 °C. Mortar and pestle were used to dehull the roasted seeds, the seed coat was removed through winnowing. Hammer mill was used to

mill the roasted seeds and 1 mm sieve size was used to sieve out the flour. The flour was packaged inside a polyethylene bag till further use.

3. Processing of soursop leaves

The soursop leaves were processed using the method described by Eka *et al.* (2012). 10kg of soursop leaves were washed, with tap water 3 times, dried at room temperature 25ºC for 3 days and milled into powder and sieved with 1mm sieve size.

C. Formulation of composite flours

The composite flours were formulated with the following ratio, 70 percent cereal: 30 percent legume as described by FAO/WHO/UNU (1985). The blends were fermented naturally at room temperature for 0, 24, 48 and 72 hours. The best blend was selected based on the nutritional composition of the fermented composite. Table 1 presents the proportion of acha, pigeon pea and soursop leaf powder used for complementary food formulation (%).

Table 1. Proportion of acha, pigeon pea and soursop leaf powder used for complementary food formulation (%).

Acha flour	Pigeon flour	Soursop flour
70	30	0
65	30	5
60	30	10
55	30	15

D. Analysis of complementary food blends

Animal feeding experiment

Forty (40) healthy male albino rats with average initial weight of 45 - 50 g obtained from the University of Nigeria, Nsukka, Department of Veterinary Pathology, were divided into five (5) groups of eight (8) rats each based on weight of the body. Individual metabolic wire-mesh bottomed cages were used to house the rats at a temperature of 28 ± 2 ºC. The animals were fed *ad libitum* with drinking water using provided nipple drinkers and feed (test diets) supplied in troughs. A different experimental diet was fed to each group of 8 replicates for 28 days including the period of acclimatization. The body weight was measured weekly for 28 days whereas the feed intake was measured individually twice weekly. Selected micronutrient status was determined through the collection of blood serum. Table 2 shows the test diet fed to the rats.

E. Acute toxicity test of soursop leaf powder (LD 50)

The method of Lorke (1983) was used for the acute toxicity test of the leaves of soursop. Thirty-six (36) albino rats were utilized in this study. The test involved two stages. In the first stage, the animals were divided into three (3) groups of six rats each and were fed orally on leaf powder at a rate of 10, 100 and 1000 mg/kg of rat body respectively. The rats were watched for 24 hours for signs of weakness, drowsiness and death after none died and in the second stage, they were fed at a rate of 1600, 2900 and 5000 mg/kg body weight, administered to the rat for 24 hours.

Table 2. Composition of test diet (%)

Raw material		Ratios			
	AP 70:30:0	APS1 65:30:5	APS2 60:30:10	APS3 55:30:15	RFED ---
Acha	70	65	60	55	-
Pigeon pea	30	30	30	30	-
Soursop leaf	-	5	10	15	-
Rat chow	-	-	-	-	Rat chow

Key: AP = Acha - pigeon pea blend 70:30: APS1 = Acha, pigeon pea and soursop leaf powder blend 65:30:5 %; APS2 = Acha, pigeon pea and soursop leaf powder blend 60:30:10 %; APS3 = Acha, pigeon pea and soursop leaf powder blend 55:30:15 %.

F. Determination of serum vitamin C

The serum vitamin C was determined according to the method of Oyaizu, 1986.

Reagents:

1. DTC Reagent : 0.4 g of thio urea, 0.05 g of copper sulphate and 4 g of 2, 4-dinitro phenyl hydrazine weighed and dissolved in 100 ml of 9 N H_2SO_4.
2. 85 % Sulphuric acid
3. Stock ascorbic acid: 10 mg of ascorbic acid was weighed and dissolved in 10 ml of 5 % TCA (1mg/ml).
4. Working standard: 1 ml of the stock was diluted to 10 ml with 5 % TCA (100 µg/ ml).
5. Preparation of the extract: 10 ml of the extract was dissolved in 10 ml of respective solvents and used for estimation.

G. Procedure

To 1 ml of the extract DTC was added and incubated at 37° C for 3 hours. After incubation 1.25 ml of 85 % $H_2SO_4$was added under ice-cold condition. The mixture was kept at room temperature for 30 minutes. The absorbance was measured at 540 nm against blank using UV/V is spectrometer. The experiments were repeated in triplicate. The results were expressed as mg of ascorbic acid /g of extract.

H. Determination of serum vitamin E

Determination of vitamin E was performed according to the method of Prieto *et al*. (1999).

Reagents:

1. Alpha tocopherol reagent: 28 mM sodium phosphate and 4 mN ammonium molybdate was weighed and dissolved in 100 ml of 0.6 M sulphuric acid.
2. Standard α-tocopherol: 10 mg of tocopherol was weighed and dissolved in 100 ml ethanol (0.1mg/ml).
3. Preparation of extract: 10 ml of the extract was weighed and dissolved in 10 ml of respective solvents and used for estimation.

Procedure:

An x Aliquots of the prepared extract was mixed with α-Tocopherol reagent α-tocopherol. The respective solvents were used as blank. All the tubes were capped and incubated in a boiling water bath at 95° C for 60 - 90 minutes. Samples were cooled to room temperature, the absorbance of each samples were measured calorimetrically at 695 nm against blank in Perkin Elmer UV spectrophotometer. The experiments were repeated in triplicates and calculated using a standard graph and the values were expressed as α- tocopherol / g of extract.

I. Determination of serum vitamin A

Determination of vitamin A was performed according to the method of Prieto *et al*. (1999). Blood samples were taken from the rats centrifuged at 5000 rpm for 10 minutes and 2 ml of serum fraction was collected. A 500 ml serum aliquot from each sample in the presence of standard (250 ml) was treated with 500 ml methanol and vortexed for 30 minutes. Vitamins were extracted with 4.0 ml hexane and the original layer was removed. The organic solvent was evaporated with a stream of nitrogen and the residue was redissolved in 0.5 ml of methanol. The samples were appropriately diluted with carrier solution and analyzed for retinol. The absorbance was taken at 562 nm using a spectrophotometer (Genway model 6000). Retinol was used as standard.

J. Determination of serum zinc

Determination of zinc was performed according to the method described by Johnsen *et al*. (1987). Fifty microliter (50 µl) of distilled water was poured into a blank tube, Fifty (50 µl) of the standard was pipetted to its tube, pipette 50 µl of serum to the sample tube, 1000 µl of reagent was added to all incubate and allow to stand for 8 minutes at 25 ^{o}C. The absorbance was measured at 560 nm using a spectrophotometer (Genway model 6000).

K. Determination of serum iron

Determination of iron was performed according to the method described by Ramsey (1952). A 0.5 ml volume of serum was diluted to 5 ml using distilled water. Then 1.5 ml of acetate buffer pH 4.5 was added. 1 ml of 2.5 % hydroquinone and 1 ml of 0.1 % of α, α di-pyridine were added and pH adjusted to 4.5 with 20 % sodium hydroxide. The solution was made up to 10 ml using distilled water absorbance taken at 520 nm against reagent blank, using a spectrophotometer (Genway model 6000).

L. Determination of serum calcuim

Determination of calcuim was performed according to the method described by Sarkar and Chauhan (1967). Fifty microliter of distilled water was pipette into cuvette and 12.5 µl of standard was pipette into its tube, 12.5 µl of serum was added to the sample tube and 500 µl of reagent was added to all incubate and mix for 5 minutes at 25 ^{o}C. The absorbance was measured at 560 nm using a spectrophotometer (Genway model 6000).

M. Data analysis and experimental design

The experiment was based on completely randomized design (CRD). Data analysis were carried out using one-way analysis of variance (ANOVA) and Duncan's multiple range test with SPSS version 20. Significance was accepted at $p \leq 0.05$.

III. RESULTS

A. Acute toxicity test (LD50) of rat fed with soursop leaf powder

Table 3 shows the acute toxicity test of rats fed with soursop leaf powder**.** In the first phase of the test, the rats showed no change in behavior or death in animals when observed for 24 hours. In the second phase, rats showed weakness and drowsiness but no death was recorded within 24 hours of administration. There was no change in the nature of their stool, urine and eye colour. No mortality was observed at all dose levels for 24 hours. Orally, 5000 mg/kg of soursop leaf powder was well tolerated in rats after 24 hours. Hence, the LD50 was estimated to be <5000 mg/kg (orally).

Table 3. Acute toxicity test (LD50)

Phase 1	Dosage (mg/kg body weight)	Mortality
Group 1	10	0/6
Group 2	100	0/6
Group 3	1000	0/6
Phase 2		
Group1	100	0/6
Group 2	2900	0/6
Group 3	5000	0/6

Values are means of ± standard deviation of triplicate determinations. Each group (0/3) = no death among the 3 rats.

B. Weekly food intake of the rat fed with the complementary food and normal rat chow for 28 days

Food intake was significantly ($p \leq 0.05$) different among the samples in the first, second and third week. It was observed that from Table 4 rats that fed on normal rat chow (RFED) had the highest food intake while rats that fed on sample APS3 had the lowest food intake. Samples APS2 and APS3 had the lowest feed intake of 0.91 and 4.27, respectively. Samples AP and APS1 had food intake of 20.87 and 8.17, respectively. From week 1 to week 3, RFED had the highest food intake of 9.26 - 9.71 g followed by sample AP which had 6.11 - 7.74 g from week 1 to week 3. Among the fortified blends, sample APS1 had the highest food intake from week 1 to week 3 (2.52 - 3.10 g). Samples APS2 and APS3 had the lowest food intake as shown in Table 4.

Table 4. Weekly feed intake of the rats fed with the blends in grams (g).

Sample	Week 1	Week 2	Week 3	Total
AP	$6.11^{d} \pm 3.43$	$7.02^{d} \pm 4.35$	$7.74^{d} \pm 3.88$	20.87
APS1	$2.52^{c} \pm 3.04$	$2.55^{c} \pm 3.04$	$3.10^{c} \pm 3.81$	8.17
APS2	$1.93^{b} \pm 2.94$	$1.24^{b} \pm 2.58$	$1.10^{b} \pm 1.74$	4.27
APS3	$0.91^{a} \pm 1.14$	N R	N R	0.91
RFED	$9.71^{e} \pm 6.43$	$9.26^{e} \pm 6.77$	$9.29^{e} \pm 7.26$	28.26

Values are means ± standard deviation of 7days determinations. Values with different superscripts in the same column are significantly different at $p \leq 0.05$. Key: AP = Acha - pigeon pea blend 70: 30 %; APS1 = Acha, pigeon pea and soursop leaf powder blend 65:30:5 %; APS2 = Acha, pigeon pea and soursop leaf powder blend 60: 30: 10 %; APS3 = Acha, pigeon pea and soursop leaf powder blend 55: 30: 15 %; RFED = normal rat chow, NR = No result.

C. Average weekly body weight of rats fed complementary food

Table 5 shows the average weekly body weight gain of rats fed complimentary food and normal rat chow for 28 days. Samples AP, APS1, APS2 and APS3 had total body weight gain of 321.30, 293.17, 266.45 and 204.75 respectively from week 1 to week 4. The low values in body weight gain in rats fed with sample APS2 and APS3 was as a result the mortality of two rats that was fed with sample APS2 and all of the rats that was fed with sample APS3. Rats fed with sample RFED had the highest body weight gain of 549.42 from week 1 to week 4.

D. Selected Serum micronutrient status of the rats fed with the complementary food blends and normal rat chow.

The rats fed with normal rat chow (RFED) had the highest calcium content in their blood serum (7.63 mg/100g) while the rats fed with sample AP had the least calcium content (7.54 mg/100g). There was no significant ($p \leq 0.05$) difference in the calcium content of the blood serum of the rat that ate the complementary food and those fed normal rat chow. Rats fed with sample APS1 had the highest iron content while rats fed with sample APS2 had the lowest iron content. Iron content of the rats showed a significant ($p \leq 0.05$) difference among certain samples. The rats that were fed with sample APS1 had the highest zinc content (58.85 mg/100g) as shown in Table 6. Rats fed with sample AP and APS3 had comparable zinc values. There was no significant ($p \leq 0.05$) difference in the zinc content of the blood serum of the rat that ate the complementary food and normal rat chow. Vitamin A content showed no significant ($p > 0.05$) difference between the control (AP), fortified blends (APS1 and APS2) and the normal rat chow (RFED). Rats fed with sample APS2 had the highest vitamin A content (0.76 µg/ml) while the rats that eat the normal rat chow (RFED) had the lowest vitamin A content (0.30 µg/ml).

Table 5. Weekly body weight and total weight of rats before and after being fed with the complementary food blends and normal rat chow in grams (g).

Sample	Initial Weight	Week 1	Week 2	Week 3	Week 4	Total
AP	57.38^{a} ± 11.10	65.00^{a}± 11.10	70.48^{a} ± 10.03	84.48 ± 10.61	101.34^{a} ± 11.49	321.30
APS1	65.35^{a} ± 4.28	79.93^{b} ± 3.28	64.08^{a} ± 5.02	66.33± 13.50	82.83^{b} ± 13.60	293.17
APS2	63.37^{a} ± 5.37	85.00^{bc} ± 2.41	62.95^{a} ± 3.46	59.10± 4.67	59.40^{bc} ± 4.95	266.45
APS3	63.15^{a} ±8.02	89.15^{c} ± 1.96	61.60^{a} ± 5.62	54.00 ±0.00	N R	204.75
RFED	74.87^{b} ± 8.45	99.77^{d}± 1.84	125.64^{b} ± 9.43	153.96 ± 24.06	170.05^{d} ±33.89	549.42

Values are means ± standard deviation of 7days determinations. Values with different superscript in the same column are significantly different at $p \leq 0.05$. Key: AP = Acha - pigeon pea blend 70: 30 %; APS1 = Acha, pigeon pea and soursop leaf powder blend 65:30:5 %; APS2 = Acha, pigeon pea and soursop leaf powder blend 60: 30: 10 %; APS3 = Acha, pigeon pea and soursop leaf powder blend 55: 30: 15 %; RFED = normal rat chow NR= No result.

There was no significant ($p \leq 0.05$) difference in the vitamin C content of the rats that fed on the control (AP), fortified blends (APS1 and APS2) and the normal rat chow (RFED). Rats fed with sample APS2 had the highest vitamin C content (0.79 mg/100g) while rats fed with APS1 had the lowest vitamin C content (0.5 mg/100g). Vitamin E content showed a significant ($p > 0.05$) difference among the samples. Sample APS2 had the highest vitamin E content (0.76 mg/100g) while normal rat chow (RFED) had the lowest (0.64 mg/100g).

Table 6. Selected serum micronutrient status of the rats fed with the complementary food blends and normal rat chow.

Samples	Calcium (mg/dl)	Iron (µmol/l)	Zinc (µg/dl)	Vitamin A (µg/ml)	Vitamin C (mg/dl)	Vitamin E (mg/dl)
AP	7.54^{a} ± 0.24	14.52^{a} ± 2.44	44.48^{a} ± 3.31	0.47^{a} ±0.31	0.74^{a}± 0.15	0.73^{ab} ± 0.18
APS1	7.61^{a} ± 0.16	38.14^{b}± 0.79	58.85^{a} ± 4.15	0.50^{a} ± 0.31	0.54^{a} ± 0.20	0.68^{a} ± 0.18
APS2	7.60^{a} ± 0.14	12.72^{a} ± 0.89	46.16^{a} ± 0.14	0.76^{a} ±0.36	0.79^{a} ± 0.23	0.76^{b} ± 0.39
RFED	7.63^{a} ± 0.08	27.45^{ab} ±12.47	44.39^{a} ± 15.89	0.30^{a} ±0.22	0.53^{a} ± 0.22	0.64^{a} ± 0.08

Values are means ± standard deviation of duplicate determinations. Values with different superscript in the same column are significantly different at $p \leq 0.05$. Key: AP = Acha - pigeon pea blend 70: 30 %; APS1 = Acha, pigeon pea and soursop leaf powder blend 65:30:5 %; APS2 = Acha, pigeon pea and soursop leaf powder blend 60: 30: 10 %; APS3 = Acha, pigeon pea and soursop leaf powder blend 55: 30: 15 %; RFED = normal rat chow.

E. Percentage serum status of selected minerals and baseline of rats after ingestion with complimentary food and rat chow

Table 7 shows percentage bioavailability of selected minerals before and after feeding with the complementary food blends and normal rat chow. The calcium content was not significantly ($p \leq 0.05$) different for all the samples. The bioavailability of calcium for rats fed with sample AP was 7.54 mg/dl after ingestion, compared to 5.04 mg/dl baseline which had an increase of 49.60 %. Rats fed with sample APS1 had the highest calcium content among the fortified samples with an increase of 50.99 %. Sample RFED had the highest available calcium content of 7.63 mg/dl after ingestion as compared to 5.04 mg/dl baseline and as such had the highest percentage increase (51.39 %) compared to sample AP and the fortified samples (APS1 and APS2). Iron content was not significantly ($p \leq 0.05$) different for all the samples and the normal rat chow. Samples AP and APS2 decreased by 43.72 and 50.70 %, respectively compared to baseline iron content 25.8 µmo/l.

Sample APS1 had the highest percentage increase of iron content (47.83 %) among the complementary food samples and the rat chow.

Samples AP and RFED had the lowest and comparable zinc content (44.48 and 44.39 µg/dl) respectively. Samples AP and

RFED had percentage increase 15.53 % and 15.30 %, respectively.

Table 7. Percentage difference in serum mineral status of rats before and after feeding with the complementary food and normal rat chow.

Minerals	Calcium (mg/dl) AP, APS1, APS2, RFED	Iron (µmol/l) AP, APS1, APS2, RFED	Zinc (µg/dl) AP, APS1,APS2, RFED
BASELINE	5.04 5.04 5.04 5.04	25.8 25.8 25.8 25.8	38.5 38.5 38.5 38.5
AFTER INGESTION	7.54 7.61 7.60 7.63	14.52 38.14 12.72 27.45	44.48 58.85 46.16 44.39
PERCENTAGE DIFFERENCE (%)	49.60 50.99 50.79 51.39	-43.72 47.83 -50.70 6.40	15.53 52.86 19.90 15.30

Key: AP = Acha - pigeon pea blend 70: 30 %; APS1 = Acha, pigeon pea and soursop leaf powder blend 65:30:5 %; APS2 = Acha, pigeon pea and soursop leaf powder blend 60: 30: 10 %; RFED = normal rat chow.

F. Percentage serum status of selected vitamins and baseline of the rats after ingestion with complimentary food and rat chow

The vitamin A content was not significantly ($p \leq 0.05$) different for all the samples. The bioavailability of vitamin A for sample AP had 88 % increase where APS1 had 100 % increase. Sample APS2 had 204 % increase where sample RFED had the lowest vitamin A (20 %) increase after ingestion of the complementary food. The more the quantity of soursop leaf in the blend the more the vitamin A content of the food and the vitamin A available in blood. Vitamin C content of sample APS2 had the highest increase of 68.09 %. Sample RFED had lowest vitamin C content of 12.77 % increase. Vitamin E content showed a non-significant ($p \leq 0.05$) difference among the samples. Sample APS2 had the highest percentage increase of vitamin E (28.81 %). Sample RFED had the lowest vitamin C content (0.64 mg/dl) and expectedly the lowest percentage increase (8.47 %).

Table 8. Percentage difference in serum micronutrient status of vitamins before and after feeding with complementary food and normal rat chow.

Vitamins	Vitamin A (µg/dl) AP, APS1, APS2, RFED	Vitamin C (mg/dl) AP, APS1, APS2, RFED	Vitamin E (mg/dl) AP, APS1, APS2, RFED
BASELINE	0.25 0.25 0.25 0.25	0.47 0.47 0.47 0.47	0.59 0.59 0.59 0.59
AFTER INGESTION	0.47 0.50 0.76 0.30	0.74 0.54 0.79 0.53	0.73 0.68 0.76 0.64
PERCENTAGE DIFFERENCE (%)	88.00 100 204 20.0	57.45 14.89 68.09 12.77	23.73 15.25 28.81 8.47

Key: AP = Acha - pigeon pea blend 70: 30 %; APS1 = Acha, pigeon pea and soursop leaf powder blend 65:30:5 %; APS2 = Acha, pigeon pea and soursop leaf powder blend 60: 30: 10 %; RFED = normal rat chow.

IV. DISCUSSION

Results showed that there was no death or change in the rats behavior after 24 hours of administration. However, in the second phase, a weakness and drowsiness were observed. Additionally, the LD50 was estimated to be <5000 mg/kg (orally). The toxicity test (LD50) obtained was a clear indication of safety of soursop leaf powder for internal and external use at lower doses. This agreed with Arthur *et al.* (2011) on acute and sub-chronic toxicity of *Annona muricata* leaves extract.

Food intake was different among the samples. Nwala *et al.*, 2013, reported that in rats, food intake may be influenced by a variety of factors such as the taste, smell, texture of the test diets among other factors. The low food intake of APS2 and APS3 could probably be attributed to the colour, taste and smell of the blends. This could be as a result of the strong smell and colour of soursop leaf powder, which was more in APS2 and APS3 samples. Low food intake could also be attributed to poor palatability, aroma and nitrogen source. The higher food intake of the experimental diet samples AP and RFED (20.87 and 28.26) might be due to a combination of these factors (Ene-Obong and Obizoba, 1995). Animals are known to eat more food when it has good organoleptic appeal according to Ihekoronye and Ngoddy (1985) and Nnam (2001).

Rats fed with sample RFED had the highest body weight gain. Weight loses in APS2 and APS3 might probably be attributed to the presence of anti-nutrient factors contained in the food which may interfere with protein metabolism and food acceptance (Nwala *et al.,* 2013). Weight gain is an indication that the diet supported growth (Arueya and Osundahunsi, 2014). This probably suggests that among the fortified samples APS1, APS2 and APS3, APS1 (acha, pigeon pea and soursop leaf powder blend 65:30:5 % was superior to the others.

No significant difference was observed in the calcium and zinc content of the blood serum of the rat that ate the complementary food and those fed normal rat chow. However, iron content showed a significant difference among samples. Rats fed with sample APS2 had the highest vitamin A content while the rats that eat the normal rat chow had the lowest vitamin A content. This suggests the higher the quantity of soursop leaf powder in the fortified blends the higher the vitamin A content of the rat serum. The values in this study were lower than the plasma vitamin A reported by Mohammed and El-Nahal (2013), of rats fed on carrot blends (88.3 µg/ml), rats fed on parsley blends (84.3 µg/ml) and rats fed with spinach blends had (84.9 µg/ml). Magda and Dalia (2013) also reported vitamin A of rats fed cowpea and carrot, wheat, faba beans and carrots, wheat, white beans and carrots, wheat, cowpea and parsley, wheat, cowpea and spinach and wheat, faba beans and spinach as 87.37, 85.63, 91.97, 80.23 and 85.37 µg/ml. This might be as a result of mineral retention being a function of food intake, fermentation period, fecal and urinary mineral outputs among other factors as reported by Odumodu, 2013.

Vitamin E content showed a significant difference among the samples. Umar *et al.* (2010) reported calcium, iron and zinc levels in serum of weanly albino rats fed millet and maize blend based complementary food as follows: millet 60 %, soybeansn16 %, groundnut 16 %, crayfish 5 % and palm oil 5 % and maize 60 %, soybeansn16 %, groundnut 16 %, crayfish

5 % and palm oil 5 % 11.81, 6.41, 0.36 and 8.35, 8.38 and 0.93 mg/dl for calcium, iron and zinc respectively. The values obtained in this study for iron and zinc were higher than the above values reported by Umar *et al.* (2010) for zinc and Iron while calcium values were higher than those recorded by Umar *et al.* (2010). The decrease in iron content may probably be due to the presence of anti-nutrient or loss of nutrient during processing of the food samples.

V. Conclusion

Acha-pigeon pea was fortified with varying level of soursop leaf powder. the presence of soursop leaf powder increased the protein and ash content but decreased carbohydrate content. sample rfed had the highest feed intake and weight gain in the rats fed but had the lowest serum micronutrient status in vitamins and minerals except for calcium, where it had comparable values with the fortified samples. control sample ap had low serum micronutrient status except in vitamin a and c. hence, it can be concluded that soursop leaf has the potential of been utilized, if this potential is harnessed it could help our country solve food security issues.

Conflict Of Interest

The author declares no conflict of interest.

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