Science Journal of Public Health
2014; 2(5): 454-460
Published online September 20, 2014 (http://www.sciencepublishinggroup.com/j/sjph)
doi: 10.11648/j.sjph.20140205.22
ISSN: 2328-7942 (Print); ISSN: 2328-7950 (Online)

Evaluation of beta-carotene, iron and other micronutrient status of rats fed sun or shade-dried fluted pumpkin (*Telfariaoccidentalis*) leaves and its product, the leaf curd

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To cite this article:
Onoja, I. U., Nnam N. N.. Evaluation of Beta-Carotene, Iron and other Micronutrient Status of Rats Fed Sun or Shade-Dried Fluted Pumpkin (*Telfariaoccidentalis*) Leaves and its Product, the Leaf Curd. *Science Journal of Public Health.*
Vol. 2, No. 5, 2014, pp. 454-460. doi: 10.11648/j.sjph.20140205.22

Abstract: The effects of differently processed leaves and the curd of fluted pumpkin (*Telfariaoccidentalis*)on beta carotene, iron, ascorbate, zinc, copper and calcium status of rats were evaluated. Fluted pumpkin leaf was divided into four (4) portions. One was shade-dried, another was sun-dried and the other was used to produce leaf curd. The last portion was not processed and served as the control. All the processed samples were milled to fine flour andeach of the pumpkin leaf flour was incorporated into rat chow for a 28-day study period. Twenty (20) male weanling rats were divided into four (4) groups of five (5) rats each. They were housed in individual metabolism cages and fed diets and water *ad libitum*. Blood samples were drawn before and after the experiment by ocular puncture and were used for biochemical analysis. Blood plasma was used to determine haemoglobin while serum was used to determine pro- vitamin A, ascorbate, ferritin, iron, copper, zinc and calcium. The liver was individually removed and analysed for liver ferritin, ascorbate and other micronutrients. The results showed that the rats fed rat chow supplemented with dried leaf curd had higher serum beta carotene, ascorbate, and ferritin than those of the other groups. The rats fed rat chow supplemented with shade dried-fluted pumpkin leaf had higher ($p<0.05$) haemoglobin level, liver beta-carotene and ascorbate than the other groups. The result showed that processing fluted pumpkin leaf into leaf curd improved serum beta-carotene, serum and liver ascorbate, serum ferritin and liver iron of rats more than other processing methods.

Keywords: Beta-Carotene, Iron, Micronutrient, Rats, Fluted Pumpkin, Leaf Curd

1. Introduction

Green leafy vegetables like other foodstuffs used in Nigeria are subjected to quite a variety of processing procedures in the preparation for consumption. These procedures include rinsing, cutting, chopping or lacerating, washing, drying, blanching, boiling and combinations of some of these. The production of *Telfariaoccidentalis* leaves has been on the increase in Nigeria due to increased awareness of its nutritional value. The young succulent vigorous shoots and leaves are used as cooked vegetables [(1)] and frequently eaten as pot herbs [(2)]. *Telfariaoccidentalis* is of commercial importance and the crop is grown mainly for the leaves, which constitute an important component of the diet in many West African countries [(1)].

The nutritional importance of the plants cannot be over emphasised. The importance is well kwon from year to year but little is known about its toxicology and mechanism of action. *Telfariaoccidentalis* is widely consumed in the tropics and contributes to the dietary requirements of people in developing countries, particularly, among the low income groups. Moreover, it has some myths and speculations about the medicinal value of the plant leaves especially with respect to treating anaemia in pregnant women, children and in malaria induced anaemia

The seeds are intolerant to desiccation and stock for the planting season is maintained locally by strong fully matured fruits [(3)]. A diet preparation of *Telfariaoccidentalis* is said to cause a dose dependent increase in the measure of haematological indices (PVC, haemoglobin concentration, RBC count, WBC count, etc), [(4)]. According to Tindal [(5)], the chemical composition of Telfariaoccidentalis leaves includes protein (4.3%), fats (0.8%), fibre (2.3%), carbohydrate (CHO) (2.5%), Ca (1.71mg/100g), and iron (Fe) (0.03mg/100g), thiamine (0.08%), riboflavin (2.07%). Most of these components stimulate the production of blood cells from the bone marrow. Also, methanol extract from the leaves of Telfariaoccidentalis is reported to reduce the plasma glucose, cholesterol, creatinine and triglyceride levels of diabetic rats but had no effect on the plasma protein level [(6)].

Leaf curd is a paste-like substance formed from boiled leaf filtrate. Leaf curd is a concentration of the filtrate. Curd could be gotten from both plants and animals. In animals (milk) it is a thick substance that forms when it becomes sour. In the processing of soy milk, the topmost layer which is removed during boiling because of its toxicity forms the curd. Leaf concentrate is an extremely nutritious food made by mechanically separating indigestible fibre and soluble anti nutrients from much of the proteins, vitamins and minerals in certain fresh green plant leaves. This is because it is so rich in beta carotene, iron and high quality protein. Leaf concentrate is very effective in combating malnutrition, especially anaemia and vitamin A deficiency. These are prevalent among children and pregnant women in most developing countries [(7)]. Other nutrients, especially minerals in leaf curds are concentrated; it could form an ingredient for the formulation of infant and children's foods. Minerals are not easily lost when cooking except the water is thrown away; the nutrients which could be lost as a result of processing the leaf curd are those that are heat labile. In producing leaf curd, heating is usually done at a very low heat (65-70^{O}C), so some of the heat labile nutrients would be retained.

The World Health Organisation (WHO) has classified Nigeria among the 34 countries in the world with serious problems of nutritional blindness and xerophtalmia. Data from Participatory Information Collection (PIC) survey done in Nigeria showed that the prevalence of vitamin A deficiency (VAD) in 1993 was 9.2% in children and 7.2% in mothers [(8)]. Iron deficiency anaemia affects more than 3.5 billion people in the developing world [(9)].It has been noted that the prevalence of these micronutrients deficiencies are more in developing countries than in developed countries. WHO/OMS [(10)] reported that VAD is a public health problem in 118 countries, especially in Africa and South-East Asia. Young children and pregnant women are vulnerable. The most affected groups in developing countries are pregnant women (56%), school-age children (53%), non-pregnant women (44.6%) and preschool children (42%) [(11)].

The problems associated with these micronutrient deficiencies are much and irreversible proceeding death. In children, it greatly increases the chances of morbidity and disability. Maternal night blindness was associated with almost four fold increase in the risk of mortality [(12)].Based on the diverse effects of iron and vitamin A deficiencies, it is important that preventive measures capable of combating these deficiencies be adopted, especially diversification of diets at the reach of the low income groups.

Dietary diversification using locally available foods within the communities appears to be a more feasible approach in rural communities than other approaches to combat the problem of hidden hunger. However, the problem with this approach is dietary bulk and bioavailability of nutrients, particularly in the complementary infant food. This is because the stomach capacity of infants is too small (200-250ml). They will consume little quantity of food at a time, which may not meet the recommended requirement for all the nutrients [(13)]. It is necessary to reduce the bulk of infant foods so that the little quantity consumed would be concentrated to meet the nutrient requirements of the infant. This study would address the problem of dietary bulk in infants feeding by developing leaf curd from fluted pumpkin (*Telfariaoccidentalis*) leaf. This effort is directed towards improving micronutrients intake of the infants particularly the vitamin A and iron status since fluted pumpkin leaf is a good source of the nutrients. The leaf curd would be used as supplement to infant food.

2. Materials and Methods

Fluted pumpkin (*Telfariaoccidentalis*) used for the study were purchased from Nsukka main market in Enugu state.

2.1. Preparation of Sample

Fluted pumpkin leaves were picked to remove stems, flowers, unwanted particles, washed in clean deionized water and divided into four equal portions. One portion was analysed fresh which served as control. The other two portions were dried, one under shade and the other under sunlight. The fourth portion was used to produce the leaf curd.

2.2. Preparation of Fluted Pumpkin Leaf Curd

One portion of fresh fluted pumpkin leaves was homogenized in a laboratory homogenizer (HOBART MIXER) to obtain vegetable puree. The puree were mixed with deionized water in a ratio of 1:3 (puree to water) to get a homoginized mixture. The mixture was filtered through a clean cloth to obtain a clean filtrate. The filtrates were simmered at 75^{O}C until the leaf curd was formed. The production of the fluted pumpkin leaf curd is shown in Fig 1.

2.3. Diet Formulation

Three experimental diets were formulated to provide 2.1 mg beta-carotene per day to weanling rats. A weight of 792.4 g dried fluted pumpkin leaf and 354.2 g curd was blended, respectively with 15 g rat chow to provide daily food intake of the rats. The rat chow served as the control diet. Altogether, four diets were used for the study.

Table 1. *Experimental diets*

Diet I	Diet II	Diet III	Diet IV
2100g rat chow (Control)	2100g rat chow 393.4g Shade-dried fluted pumpkin leaf.	2100g rat chow 399g Sun-dried fluted pumpkin leaf	2100g rat chow 354.2g dried leaf curd

Table 1 show the formula used to prepare the experimental diets and the control

2.4. Animal Housing and Feeding

Twenty male weanling rats weighing 45-55 g were purchased from the Department of Veterinary Pathology, University of Nigeria, Nsukka.

The animals were divided into four groups of 5 rats each on the basis of body weight. They were housed in individual stainless steel metabolic cages and fed the diet and deionized water *ad libitum* for 28 days. The last 7 days were used for balance study. The animals were weighed prior to access to test diets. Blood samples were drawn before and after the experiment by ocular puncture. At the end of the experiment, the rats were sacrificed by decapitation. The liver were individually removed and analysed for liver ferritin, beta-carotene and ascorbate, and some micronutrients (Fe, Cu, Zn and Ca).

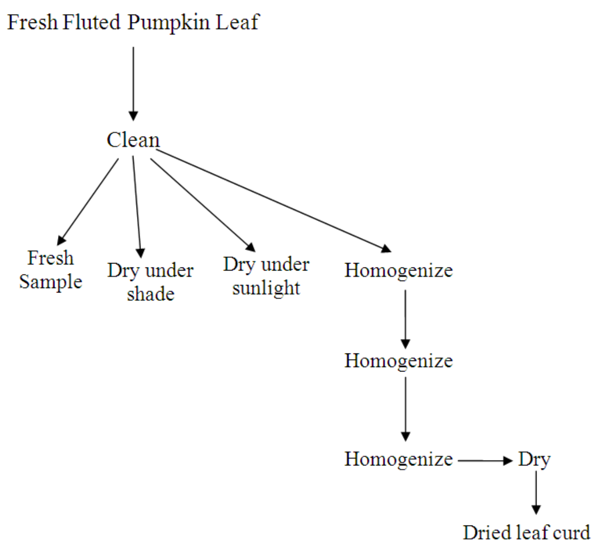

Fig. 1. *Flow diagram for leaf curd preparation*

2.5. Analysis of Blood and Liver Samples

2.5.1. Haemoglobin Determination

Twenty microliters (20 µl) of blood were measured, preferably by using a standardized automatic pipette and 4ml of diluent (providing a 1 in 201 dilution) was added. This was mixed and 5 minutes allowed for completion of the reaction (if it is not capillary blood). The absorbance of the sample was measured against the diluent as a blank in matching curvette. The haemoglobin concentration of the sample was then determined by reference to the calibrated curve showing photometer readings versus haemoglobin concentration [14].

2.5.2. Serum Beta-Carotene

A given volume (100µl) of serum was diluted with ethanol and methanol, which denatured plasma proteins and the vitamin A was extracted with a suitable organic solvent. After centrifugation, an aliquot of the organic phase was injected onto a normal or reversed phase High Pressure Liquid Chromatography (HPLC) column, followed by an eluting solvent of suitable polarity. Carotene which is eluted as a sharp peak within 1-6 minutes, was detected by a sensitive UV detector set at 325-328 nm. β-carotene was quantitated by use of peak height ratios or peak area ratio relative to an internal standard (retinyl acetate).

2.5.3. Apparatus

Analytical microbalance, ultraviolet visible spectrophotometer centrifuge, desk top, vortex mixer, rotary evaporator, High performance liquid chromatography system, equipped with a variable wavelength detector or suitable filters, Nitrogen or other inert gas, water bath, 40-50^{O}C, constant temperature, micropipette 10 ml, 15µl, 100 µl, glass test tubes, 10-75 mm or conical centrifuge tubes with polythene screw caps.

2.5.4. Reagents

Retinyl acetate, all-trans (highest parity), Retinal, all-trans (highest parity).

2.5.5. Procedure

Quantities of 100 µl of serum (or plasma), 15 µl of internal standard solution {~4µg/ml of retinyl acetate or propionate or of all trans 9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-tetrareanol} and 100 µl of methanol were transferred to a conical centrifuge tube (706 × 0.9 cm). The contents of the tube were mixed with a vortex-type mixer. A quantity of 200 µl of extraction solvent (petroleum ether dichloromethane:isopropanol, 80:19.3:0.7, respectively by volume) was added and the tube (polyethelyene cap) capped. Extraction was done by interrupted mixing on the vortex-type mixer for 60 seconds. After centrifugation (3000 rpm for 2 minutes), 100 µl of the supernate was injected on top of the column by the use of a Hamilton 710 syringe, 100 µl capacity. Elution was carried out with the same solvent as used for extraction.

2.5.6. Measurement of Haemoglobin Concentration

The cyanomethmoglobin method as specified by the International Committee for Standardization in Haematology [14] were used. The concentration of HiCN was measured in a suitably calibrated spectrophotometer or colorimeter.

2.5.7. Serum and Liver Ferritin Concentration

These were measured by a two-site immunoradiometric assay and a radioimmunoassay as given in Bothwell*et al.*[15].

2.5.8. Measurement of Serum and Liver Iron Concentration

The method recommend by the International Committee for Standardization in Haematology[16] for the estimation of serum or plasma and liver depend on liberating the iron from its binding with transferring and estimating it by complexing with a suitable chromogen.

2.5.9. Serum and Liver β-Carotene

A given volume (~100 ml) of serum or plasma and liver tissue were diluted with ethanol or methanol, which denature the proteins and the β-carotene were extracted with a suitable organic solvent, centrifuged and an aliquot of the organic phase was injected into a normal or reversed phase HPLC column.

2.6. Statistical Analysis

Data collected were subjected to one way analysis of variance (ANOVA). The Least Significance Difference (LSD) tests were used to separate means [17 -18].

3. Result

Table 2. *Mean serum beta-carotene, ascorbate and ferritin of the rats before and after feeding*

	*GROUP I	GROUP II	GROUP III	GROUP IV	LSD
Serum β-carotene (µg/ml)					
After	0.9472^{c}	1.8730^{b}	0.6424^{d}	2.9448^{a}	0.213
Before	0.6916^{c}	1.0442^{b}	0.3968^{d}	1.5242^{a}	0.2606
% Increase	0.2556 (37%)	0.8288 (79.4%)	0.2456 (61.9%)	1.4206 (93.2%)	
Serum ascorbate (mg/100ml)					
After	14.524^{c}	20.000^{b}	20.758^{b}	23.810^{a}	2.5832
Before	12.143^{a}	14.048^{a}	12.619^{a}	13.571^{a}	3.1055
% Increase	2.381 (19.6%)	5.952 (42.4%)	8.139 (64.5%)	10.239 (75.5%)	
Serum Ferritin (ng)					
After	24.296^{a}	25.044^{a}	27.699^{a}	28.516^{a}	5.2142
Before	22.724^{a}	21.182^{a}	22.338^{a}	20.800^{a}	3.6445
% Increase	1.572 (6.9%)	3.862 (18.2%)	5.361 (24.0%)	7.716 (37.1%)	

n=3
*a-d: Values with the same letters are statistically similar (p>0.05) and those with different letters are different (p<0.05)
* GROUP I - Control group (rats fed rat chow only)
GROUP II - Rats fed rat chow and shade-dried fluted pumpkin leaf
GROUP III - Rats fed rat chow and sun-dried fluted pumpkin leaf
GROUP IV - Rats fed rat chow and dried leaf curd

Table 2 show that there was 37% increase in the Serum β-carotene of the control group (group 1) however, all othere groups fed the test diet (group II – IV) significantly had higher Serum β-carotene after feeding on the test diet. The percentage increase was highest in the group IV that were fed rat chow and dried leaf curd. The trend was also the same for Serum ascorbate and Serum Ferritin. Hence here the vitamins were more concentrated the dried leaf curd.

Table 3. *Mean serum iron, zinc and copper of the rats before and after feeding*

	*GROUP I	GROUP II	GROUP III	GROUP IV	LSD
Serum Iron (mg/dl)					
After	0.1348^{b}	0.1098^{bc}	0.1018^{c}	0.1694^{a}	0.0275
Before	0.1252^{a}	0.0712^{b}	0.0616^{b}	0.1192^{a}	0.0153
% Increase	0.0096 (7.7%)	0.0386 (54.2%)	0.0402 (65.3%)	0.0502 (42.1%)	
Serum Zinc (mg/dl)					
After	0.1376^{d}	0.1700^{c}	0.2654^{a}	0.2188^{b}	0.0221
Before	0.1300^{a}	0.1326^{a}	0.1322^{a}	0.1324^{a}	0.0098
% Increase	0.0076 (5.9%)	0.0374 (28.2%)	0.1332 (100.7%)	0.0864 (65.3%)	
Serum Copper (mg/dl)					
After	0.1832^{c}	0.2593^{b}	0.3232^{a}	0.3323^{a}	0.0234
Before	0.1486^{b}	0.1828^{a}	0.1956^{a}	0.2052^{a}	0.0255
% Increase	0.0346 (23.3%)	0.0765 (41.9%)	0.1276 (65.2%)	0.1271 (62.2%)	

n =3
*a-d: Values with the same letters are statistically similar (p>0.05) and those with different letters are different (p<0.05)
* GROUP I - Control group (rats fed rat chow only)
GROUP II - Rats fed rat chow and shade-dried fluted pumpkin leaf
GROUP III - Rats fed rat chow and sun-dried fluted pumpkin leaf
GROUP IV - Rats fed rat chow and dried leaf curd

Table 3 show that there was 7.7% increase in the serum iron of the control group (group 1) however, all othere groups fed the test diet (group II – IV) significantly had higher Serum iron after feeding on the test diet. The percentage increase was highest in the group III that were fed rat chow and sun-dried fluted pumpkin leaf. Serum zinc and Serum Copper followed the same trend as well. Hence here the minerals were more concentrated the sun-dried fluted pumpkin leaf.

Table 4. *Mean serum calcium and haemoglobin of the rats before and after feeding*

	*GROUP I	GROUP II	GROUP III	GROUP IV	LSD
Serum Calcium (g/dl)					
After	0.0146^{b}	0.0276^{a}	0.0298^{a}	0.0304^{a}	0.0058
Before	0.0112^{c}	0.0204^{ab}	0.0196^{a}	0.0228^{a}	0.0032
% Increase	0.0034 (30.4%)	0.0072 (35.2%)	0.0102 (52.0%)	0.0076 (33.3%)	
Haemoglobin (g/dl)					
After	14.9672^{a}	15.0562^{a}	14.8522^{a}	14.2944^{b}	0.5065
Before	10.2784^{a}	9.9672^{ab}	9.9508^{ab}	9.5084^{b}	0.5387
% Increase	4.6888 (45.6%)	5.089 (51.1%)	4.9014 (49.3%)	4.786 (50.3%)	

n=3

*a-d: Values with the same letters are statistically similar (p>0.05) and those with different letters are different (p<0.05)

* GROUP I - Control group (rats fed rat chow only)

GROUP II - Rats fed rat chow and shade-dried fluted pumpkin leaf

GROUP III - Rats fed rat chow and sun-dried fluted pumpkin leaf

GROUP IV - Rats fed rat chow and dried leaf curd

Table 4 show that there was 30.4% increase in the serum calcium of the control group (group 1) however, all othere groups fed the test diet (group II – IV) significantly had higher serum calcium after feeding on the test diet. The percentage increase was highest in the group III that were fed rat chow and sun-dried fluted pumpkin leaf. Haemoglobin however, increased highest in the group II rats fed rat chow and shade-dried fluted pumpkin leaf.

The serum β-carotene level of the rats fed chow and dried leaf curd increased from 1.524µg/ml to 2.9448 µg/ml after the feeding trial. The group had the highest (p<0.05) gain in β-carotene and was followed by the group of rats fed rat chow and shade-dried fluted pumpkin leaf. The serum ascorbate, serum ferritin and serum iron followed the same trend as the serum β-carotene. The group fed rat chow and dried leaf curd had the highest (p<0.05) gain. The group of rats fed rat chow and sun-dried fluted pumpkin leaf had the highest (p<0.05) increase in serum zinc, serum copper and serum calcium, respectively. The group of rats fed rat chow and shade-dried fluted pumpkin leaf had the highest haemoglobin level (15.0562 g/dl) after the feeding trial.

Table 5. *Liver beta-carotene, ascorbate, ferritin, iron, copper, zinc and calcium levels of the rats.*

Nutrients	GP I	GP II	GP III	GP IV	LSD
β-carotene (µg/g)	3.2000^{c}	10.148^{a}	5.4410^{b}	6.4700^{b}	2.195
Ascorbate (mg/100ml)	114.2900^{b}	147.868^{a}	120.2390^{b}	145.4330^{a}	15.736
Ferritin(ng)	54.0130^{b}	55.635^{b}	69.835^{a}	45.880^{c}	6.721
Fe (mg/g)	0.2110^{c}	0.2308^{b}	0.1704^{d}	0.2488^{a}	0.015
Cu (mg/g)	0.0411^{b}	0.0424^{b}	0.0443^{b}	0.0773^{a}	0.013
Zn (mg/g)	0.3400^{d}	0.3892^{a}	0.3892^{a}	0.3622^{c}	0.017
Ca (g)	0.1878^{c}	0.3114^{a}	0.2618^{b}	0.2278^{b}	0.036

n=3

*a-d Values with the same letters are statistically similar (p>0.05) and those with different letters are different (p<0.05)

* GROUP I - Control group (rats fed rat chow only)

GROUP II - Rats fed rat chow + shade-dried fluted pumpkin leaf

GROUP III - Rats fed rat chow and sun-dried fluted pumpkin leaf

GROUP IV - Rats fed rat chow and dried leaf curd

Table 5 shows the liver β-carotene, ascorbate, ferritin, iron, copper, zinc and calcium of the rats. The liver β-carotene level of the rats fed rat chow and shade-dried leaf sample were higher (p<0.05) than that of the other groups. The liver ascorbate levels of rats fed rat chow and shade-dried sample and the group fed rat chow and dried leaf curd were similar (p>0.05) but significantly different (p<0.05) from those fed rat chow and sun-dried pumpkin leaf.

The liver ferritin level of the group fed sun-dried sample with rat chow was higher (p<0.05) than all the others. The liver ferritin of rats fed rat chow and shade-dried leaf was comparable (p>0.05) to those of the control group. The iron level of the group of rats fed rat chow and dried leaf curd was higher (p<0.05) {0.2488 mg/g} than that of the other groups. The liver copper followed the same trend as that of the iron. The group of rats fed rat chow and processed fluted pumpkin leaf had higher calcium than the control. The zinc (Zn) and calcium (Ca) levels of rats fed rat chow and shade-dried fluted pumpkin was higher than the other groups.

4. Discussion

Beta-carotene: The increase observed in the serum beta-carotene level of the four groups of rats could be attributed to the controlled and quantity of feed fed to the animals and continuous daily intake of the processed leaves which is a source of the nutrient. This is in line with Haskell *et al.*, [19] who observed a positive impact on vitamin A pool size in population at risk of VAD after daily consumption of cooked pureed leafy vegetable. All the groups had serum beta carotene level above the normal range (0.3-2.5 µg/ml) after the feeding trial. The higher increase (93.2 %) in serum beta-carotene of the group fed rat chow with dried leaf curd than others could be due to removal of antinutrients during the processing to make the nutrients readily available in the body. The higher liver beta-carotene of the group fed rat chow and shade dried sample could be due to higher beta-carotene level of the sample. The rats must have stored much of the nutrients in the liver. Drying under the shade conserved the nutrient.

Ascorbate: The higher increase in serum ascorbate (75.5 %) of the group fed rat chow and dried leaf curd than the other groups suggests that the rat readily absorbed the nutrient more than the others. The serum ascorbate levels of the rats were within the normal range of 0.06-0.2 mg/dl. The higher liver ascorbate of the groups fed rat chow and dried leaf curd or shade dried sample (145.4 vs 147.9 mg/100g) than the other groups (114.3 vs 120.3 mg/100g) could be due to better absorption of ascorbate in the leaves fed to the groups.

Ferritin: The higher increase (37.1 %) in the serum ferritin level of the group fed rat chow and dried leaf curd suggests an association between intake of leaf curd and level of serum ferritin. The higher levels of ferritin in the groups supplemented with fluted pumpkin leaves than the control is in line with Alada(4) who observed a dose-dependent increase in the haematological indices of rats fed *Telfariaoccidentalis* leaves. Prior to the feeding trial, all the groups of rats were below the normal value of 25-200 ng/ml but after the feeding, the group fed the differently processed leaves had values above 25ng/ml. The lower liver ferritin (storage iron) for the group fed rat chow and dried leaf curd than the other groups might be due to mobilization of the iron from the liver to the serum.

Iron: The higher increase in serum iron level observed with the group supplemented with dried leaf curd, sun and shade dried samples than the control was due to the supplementation effect of the leaf. However, the group fed rat chow and sun-dried samples had a higher percent (63.3 %) increase than all the other groups which could be attributed to higher absorption of iron from the sun-dried leaves. All the groups had serum iron level within the range of 0.05-0.017 mg/dl. This supports Alada(4) who reported increased haematological factors in rats fed *T. occidentalis* leaves. Iron is a well-established haematopoetic factor and its deficiency produced anaemia. Naiho *et al*. (20) also reported increased haematological parameters in wistar rats fed leaves of *Rauwfoliavomitoria* (family, *Apocynaceae*). The higher liver iron in the group fed rat chow and dried leaf curd than the other groups supports the work of Kennedy (7) who reported that leaf curd is a rich source of iron. Nnam(21) recorded an improved vitamin A and iron status on children fed baobab leaf pulp (a traditional food plant rich in micronutrients). The study showed that the baobab fruit pulp is a good source of vitamin C, which improved bioavailability of iron in diets of Nigerian school children.

Zinc: The higher increase in serum zinc (Zn) of the groups fed the processed leaves could be due to the effect of the leaf supplement. However, the higher increase of over 100.7% observed in the group fed rat chow with sun-dried samples shows that sun-drying concentrated the nutrient more and this was also true for the liver zinc.

Copper: The increase in serum copper level of the rat fed rat chow and processed leaves could be attributed to drying which concentrated the nutrient. All the groups had copper level above the normal range (0.09-0.15 mg/dl). The higher increase of 65.2 % observed in the group fed rat chow with sun-dried samples than in the other groups showed that sun-drying improved copper absorption.

Calcium: The higher calcium (Ca) increase of the group fed processed fluted pumpkin leaves than the control was due to the supplementation effect of the leaf. All the groups were within the normal range (0.008-0.013 g/dl). The higher liver Ca level of the groups fed rat chow and the different processed leaves is equally an evidence of Ca in the leaves.

Haemoglobin: The higher haemoglobin level of the group fed rat chow and the leaves could be attributed to the haematopoietic factors which were obtained from the leaf and its concentrate (4, 7, 20). The rats in all the groups prior to feeding were all anaemic (below the normal range of 11-19.2 g/dl). Ingestion of the feed (rat chow) together with supplementation with the prepared *Telfariaoccidentalis* leaves markedly improved their haemoglobin level and reduced anaemia. This is in line with Alada(4) whose work supports the claim of the local people that *T. occidentalis* leaf is a blood tonic and could be used to cure anaemia.

5. Conclusion

The results of this study showed that feeding rats with rat chow supplemented with the processed leaves improved both serum and liver beta-carotene, ascorbate, ferritin, iron, copper, zinc and calcium as well as the haemoglobin concentration. The processing of fluted pumpkin (*T. occidentalis*) leaves into leaf curd reduced bulk and increased micronutrient density of the vegetable thereby improving its nutritional quality. The rich nutrient potentials of the curd could be employed in infant food formulation. Based on the results of the study, the following recommendations were made:Fluted pumpkin leaf curd should be used as an ingredient in the formulation of complementary food to increase nutrient density, also shade-dried and leaf curd samples should be produced from popular and lesser known seasonal green leafy vegetables.

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