Original Research Article

Effect of Soybean, Groundnut, Crayfish inclusion and Vitamin C-Fortified Formulated Food Blends on Biochemical, Hematological, Protein Quality and Performance Indices in Weanling Albino Rats

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ABSTRACT

In this study, food staples comprising of Zea mays (maize), Sorghum bicolor (guinea corn), Glycine max (soybean), Arachis hypogea (groundnut) and Procambarus clarkii (crayfish) were traditionally processed and formulated into maize-guinea corn based weaning diets at 16% protein level. A replica of each diet was formulated with inclusion of vitamin C (50 mg/100 g diet). Fifty four 3-week old weanling albino rats (Rattus norvegicus) weighing between 36-39 g were placed into 13 groups of 4 rats per group and administered different diet groups ranging from maize- guinea corn base diet alone, through staples-inclusive diet (singly and in combination) and their vitamin c fortified replica diets, and standard commercial infant formula (Cerelac®) diet. Animals were sacrificed on the 28th day from onset of feeding and evaluated for biochemical, hematological and biological parameters; and sensory evaluation also conducted on the diets. Results showed significant increase in serum concentrations of protein, urea and creatinine in the staples-inclusive diets compared to base diet and also in the vitamin C- fortified diets compared to non-fortified diets. The formulated diets caused significant increase in the levels of red blood cell (RBC), hemoglobin (Hb) and packed cell volume (PCV) and insignificant changes in white blood cell (WBC) and platelet counts compared to base diet. These parameters were insignificantly affected by diet containing both soybean and groundnut compared to that of soybean alone. Fortification with vitamin C also increased the levels of these parameters significantly. The presence of soybean and other food staples, singly or in combination in the diet caused significant increase ($p < 0.05$) in total body weight gain and organ weights compared to control. Incorporation of food staples enhances the protein quality of the diets while fortification with vitamin C increased only the biological value. On the acceptability rating, only the base gruel-crayfish mix showed significantly higher taste and overall acceptability. Therefore, the inclusion of the food blends comprising of soybean, groundnut and crayfish improved the nutritive content, hematopoietic potential, protein quality, taste and overall acceptability of the maize-guinea corn based weaning diet. These were further improved by fortification of the diets with vitamin C, while crayfish played a prominent role in taste and overall acceptance of the formulated diets. Fortification also significantly bridges the gap between the formulated weaning diets and Cerelac®, a standard commercial weaning formula in Nigeria.

Keywords: soybean, groundnut, crayfish, vitamin C, weanling, biochemical, hematological, protein quality, sensory evaluations.

1. INTRODUCTION

The growth of infants is largely determined by the prevailing feeding practices. Nutritional requirements in infants are usually age dependent as the need for micronutrients (such as iron and zinc), for example, increases rapidly between ages 4-6 months of life [1][2]. Exclusive breastfeeding for 6 months and inclusion of complementary diets afterwards as recommended by the World Health Organization (WHO), supplies nutritional needs of infants at early stage and satisfies increased mineral demand after 6 months of life [1].

Poor nutrition contributes to stunted growth and micronutrient deficiencies as well as poor immune system development [3][4]. Adequate protein intake is important for antibody production, and inadequate intake of micronutrients such as vitamins A, C, E and zinc predisposes the body to infection [5]. Also iron deficiency has been reported to impact negatively on neurodevelopment and behaviour of 1-2 years old children [6].

Poor diets are major contributors to the problems associated with weaning. The scientifically prepared, commercially available weaning foods may not have been widely accepted due to unaffordability and reports of sensitivity and adverse reactions of some of its components [7]. This has led to dependence on locally produced weaning diet which most at times is nutritionally, nonstandard.

Locally (in Nigeria), weaning foods are usually formulated from gruels of fermented maize, millet and/or guinea corn. Crayfish, soybean and groundnut may be added. For economic reasons, animal proteins are usually not incorporated in the diets. Feeding is usually to provide volume that will keep the infant from being hungry without recourse to nutritional quality, and the result is obstructed growth and nutritional deficiencies [8][9]. There have been reports of positive effects of inclusion of cereals, legumes, vegetable and fruits in infants' diets and also supplementation with mineral and vitamin sources [10][11][12].

Vitamins are micronutrients that are needed by the body for metabolic activities, cell growth and development. They are involved in the production of nervous system chemicals; formation of genetic materials, hormones and blood vessels and also serve as catalysts for generation of active metabolic enzymes [13][14]. Two vitamin groups are known: the fat soluble vitamins (Vit. A, D, E, K) and the water soluble vitamins; B-complex and C [15].

Vitamin C is required for normal physiological activities of the human body. Some involvements of vitamin C include; the generation and metabolism of folic acid tyrosine and tryptophan; in the hydroxylation of catecholamine, proline, glycine and lysine carnitine; lowering of blood cholesterol by production of bile acids from cholesterol and improvement iron absorption in the guts through ferric to ferrous reduction. Vitamin C also is a good antioxidant that is capable of protecting the body from toxins, pollutants and free radicals [16].

In this work, maize-guinea corn based diets containing blends of indigenous (Nigerian) food staples: soybean, groundnut, crayfish; and vitamin c were evaluated for effects, on biochemical and hematological parameters; nutrient and mineral absorption capacity; iron and protein status of weanling rats.

2. MATERIAL AND METHODS.

2.1 Materials

2.1.1 Chemicals and Equipment

Some equipment used in the experiment include spectrophotometer (Surgispec SM -23D, Surgified medical,England) and Hemtocrit centrifuge (Great Medicals, England). Chemicals used were of analytical grade and product of BDH Ltd, Poole England. Regent kits (Randox, UK) were used for biochemical assays.

2.1.2 Samples

Food staples including maize (Zea mays), guinea corn (Sorghum bicolor), crayfish (Procambarus clarkii), groundnut (Arachis hypogea) and soybean (Glycine max) were procured from Aba, Abia State Nigeria. Also a commercially produced infant formula, Cerelac® and vitamin c tablets were purchased from a Pharmacy shop in the same town. Samples were properly stored prior to use.

2.1.3 Experimental Animals

Three weeks old weanling albino rats (Rattus norvegicus) weighing between 36-39g were obtained from and kept in the animal house of the Department of Biochemistry, University of Port Harcourt, Nigeria. The animals were placed in 13 groups of 4 animals per group. They were acclimatized for 10 days in a well ventilated metallic cage at normal room temperature (25+50C). They were given free access to water and commercially prepared animal feed throughout the period. Animals were weighed at the end of acclimatization period and at the beginning of administration of formulated diets.

2.2 Methods

2.2.1 Samples Preparation

The maize-guinea corn gruel was prepared by traditional method as described by Akingbala et al. [17]. Soybean and crayfish were processed according to the method reported by Nwachinemerem et al. [18]. The groundnut was processed based on the method described by Nkama et al. [19]. The formulation of weaning diet was done at 16% protein level using the Pear Square Method as described by Nwachinemerem et al. [18]. Similarly, formulation of vitamin C- fortified weaning diet was done using Pearson Square method at 16% protein level as shown in Table 1 below.

Table 1. Diet formulation with vitamin C at 16% protein level (Pearson Square method)

Diet Group	Maize-guinea corn (MG) (g)	Soybean (S) (g)	Groundnut (GR) (g)	Crayfish (CR) (g)	Vitamin C (Vit C) (mg)	Cerelac® (g)	Sum (g)
1	100.00g	-	-	-	-	-	100.00g
2	100.00g	-	-	-	50mg	-	100.00g
3	78.40g	21.60g	-	-	-	-	100.00g
4	78.40g	21.60g	-	-	50mg	-	100.00g
5	62.80g	-	37.20g	-	-	-	100.00g
6	62.80g	-	37.20g	-	50mg	-	100.00g
7	82.60g	-	-	17.40g	-	-	100.00g
8	82.60g	-	-	17.40g	50mg	-	100.00g
9	70.60g	10.80g	18.60g	-	-	-	100.00g
10	70.60g	10.80g	18.60g	-	50mg	-	100.00g
11	71.65g	5.40g	18.60g	4.35g	-	-	100.00g
12	71.65g	5.40g	18.60g	4.35g	50mg	-	100.00g
13	-	-	-	-	-	100.00g	100.00g

2.2.2 Experimental Design

Diet administered to each group of experimental rats was 100 g in weight with 50mg of vitamin C added to replicate diets. The rats were weighed before feed administration and subsequently, reweighed at intervals of one week over a period of four weeks. Then feed, equivalent to 0.28g/g weight of rat were administered to the rats on daily basis with adjustments made as rat weight increased.

Animals in Group 1 were fed maize-guinea corn (MG) base diet alone. Group 3 received base gruels in combination with soybean (MGS). Group 5 received base gruels mixed with groundnut (MGGR). Group 7 rats were fed base gruel/crayfish (MGCR) diet. Group 9 received diet containing base gruel and combination of groundnut and soybean (MGGRS). Group 11 received base gruel with groundnut, soybean and crayfish blend (MGGRSCR). Groups 2, 4, 6, 8, 10 and 12 received vitamin c-fortified (50 mg) diet replicates of groups 1,3,5,7,9 and 11 respectively. Group 13 rats were fed with equivalent amount of diet standard, Cerelac® alone.

The experimental animals were fed for 4 weeks, with the daily feed intake and faecal weight promptly recorded. The animal body weights were recorded weekly, with the final weight taken on the 28th day, prior to sacrifice. The animals were sacrificed under chloroform anaesthesia and blood collected through cardiac puncture and placed in appropriate sample bottles for biochemical and hematological analysis.

2.2.3 Analysis

2.2.3.1 Biochemical Analysis

Blood collected in the plain bottles were allowed to clot and then centrifuged for 5 min at 5000 rpm to obtain the serum. The serum obtained was used for biochemical assay using Randox (UK) commercial test kits.

The serum total protein was determined by biuret method as described by Lubran [20]. Serum alkaline phosphatase was determined by colorimetric method as reported by Wright et al. [21]. The serum urea was assayed by Berthelot method as described by Weatherburn [22] and reported by Osigwe et al. [23]. The Jaffe method was used to assay for the serum creatinine concentration, as reported by Toora and Rajagopal [24]. Serum lactate dehydrogenase (LDH) was assayed by spectrophotometric method as reported by Vassault et al. [25].

2.2.3.2 Haematological Analysis

Blood sample collected in EDTA bottles were used to assay for some haematological parameters. The microscopic method was used to ascertain the platelet count as reported by Briggs et al. [26]. The haematocrit method was used to estimate the percentage packed cell volume (PCV) as reported by Green and Ezeilo [27]. Hemoglobin concentration was determined by haemometric method as reported by [28]. The haemocytometric method was adopted in both the red blood cell and leucocyte counts, as reported by Tavares-Diasi et al. [29].

2.2.3.3 Biological Evaluations

Faeces of the experimental rats were collected on a daily basis. They were dried in oven at 1050C for 24hours and then powdered and stored for faecal nitrogen analysis. Feed consumption was recorded as the difference between weighed given feed and leftover /spillage feed after feeding.

2.2.3.4 Faecal and Carcass Nitrogen Evaluation

The kjedahl method of protein determination [30] was used to determine the faecal and carcass nitrogen of the weanling rats.

2.2.3.5 Protein Efficiency Ratio (PER)

Protein Efficiency Ratio is a growth method for expressing the biological value of proteins and it is presented as the weight gained or lost per gram of protein consumed over a period of time [31].

$$\text{PER} = \frac{\text{Weight gain}}{\text{Protein consumed}}$$

2.2.3.6 Net Protein Ratio (NPR)

This is a measure of the protein required for growth and maintenance. It is expressed as the ratio of the difference in weight gain of test animal and weight loss of non-protein group to protein intake of test animal [31].

$$\text{NPR} = \frac{\text{weight gain of test animal} - \text{weight loss of non protein group}}{\text{Protein intake of test animal}}$$

2.2.3.7 Net Protein Utilization (NPU)

This is the quantity of nitrogen from the eaten diet that is retained and used by the cells [31]. In calculation, it is given as

$$\text{NPU} = \frac{B - (BK - IK)}{I}$$

B = Total body nitrogen of animals on test diet
BK = Total body nitrogen of animals on non protein diet.

I = Nitrogen intake of animals on protein diet

IK = Nitrogen intake by animals on non protein diet.

2.2.3.8 Biological Value (BV)

This was originally defined by [32] as the fraction of the absorbed nitrogen that is retained in the body. Biological value is determined by analysis of the food and faeces of the test animal [31].

$$\text{BV} = \frac{\text{Retained Nitrogen x 100\%}}{\text{Absorbed Nitrogen}}$$

2.2.3.9 Sensory Evaluation

This was carried out using 5-point hedonic scale sensory evaluation method as reported by Graham et al. [33]. Gruels were made from the formulated diets by mixing uniformly with water to a desired consistency and steaming for some times with continuous stirring. The prepared gruels were scored by panellist who evaluated the food on the basis of appearance, smell, consistency and taste. The scores were as follows; 5=like very much, 4=like lightly, 3=like, 2=dislike, 1=dislike very much.

2.2.3.10 Statistical Analysis

Data analyses were performed by analysis of variance (ANOVA) using IBM, SPSS version 21. Comparisons were made using post hoc multiple comparisons at 0.05 level of significance. Values represent mean + standard error mean (SEM).

3. RESULTS AND DISCUSSION

3.1 Effect of formulated feed blends and Vitamin C Fortification on the Biochemical Parameter in Weanling Albino Rats

The effect of formulated feed blends and vitamin C fortification on the biochemical parameter in weanling albino rats is shown in Table 2. The inclusion of the food staples, soybean, groundnut and crayfish, singly or in combination caused significant increase in the concentrations of serum total protein, urea and creatinine compared to the maize-guinea corn gruel diet base. These food staples have been reported as protein sources which could readily provide amino acids for endogenous protein synthesis [34][35]. This is unlike the maize and guinea corn which contain, predominately, carbohydrate [36]. It is also known that urea and creatinine directly or indirectly result from protein metabolism [37].

The results also showed significant increase ($p<0.05$) and decrease ($p<0.05$) in the concentrations of serum total protein and urea respectively for groups that received vitamin C fortified diets, compared to the non-fortified diet fed groups. Also the total protein and urea concentrations of the animal group fed vitamin C fortified diet showed insignificant changes ($p>0.05$) compared to the Cerelac® control.

Vitamin C has been reported to help protect oxidative damage to proteins in patients with low basal antioxidant [38][39]. Also the administration of vitamin C significantly increased the concentration of serum proteins and protein fractions, especially gamma globulins in healthy mice and rams [40][41]. Vitamin C has also been shown to promote protein synthesis through increased anabolic and myogenic gene expression [42]. The epigenetic regulation of vitamin C over protein expression has also been reported [43][44] and through its role in hydroxylation, proteins associated with glycolysis, glucose uptake, cell proliferation, iron hemostasis, erythropoiesis and angiogenesis are synthesized [45].

The significant reduction of urea level associated with the various vitamin C fortified diets may indicate the role of vitamin C in urinary elimination of urea or its facilitation of protein synthesis rather than catabolism. It has been associated with synthesis and metabolism of tryptophan, folic acid and tyrosine. Being a cofactor in mixed function oxidase, it also has a role to play in the hydroxylation of lysine, proline, glycine, catecholamine and carnitine [16][46]. The urinary urea is produced by hydrolytic cleavage of L-arginine by the enzyme arginase. Hepatic arginase also regulates metabolism of L-arginine to ornithine and formation of L-proline which are necessary for collagen synthesis [47]. Vitamin C was reported to enhance collagen synthesis and may also influence the arginase activity leading to low serum urea level [14]. There was no effect of vitamin C fortification on the other parameters: creatinine, alkaline phosphatase (ALP) and lactate dehydrogenase (LDH).

The consecutive significant increases in serum creatinine due to separate inclusions of soybean, groundnut and crayfish respectively in the diet may result from high protein content of the food staples [18]. Though soybean contain more protein than groundnut [35], the presence of high antinutritional factors may limit dietary protein metabolism and effect serum creatinine concentration [48]. Inclusion of both groundnut and soybean in the diet (Group 9) significantly ($p < 0.05$) reduced the serum protein level without significant impact on creatinine concentration compared to separate inclusions of the gruels (groups 3 and 5). Here creatinine concentration was

significantly increased compared to soybean diet (Group 3). Inclusion of all 3 staples (Group 11) caused no significant changes in the concentration of all the parameters.

Table 2. Effect of formulated feed blends and Vitamin C Fortification on the biochemical

parameter in weanling albino rats

Group	Diet	Total Protein (g/dL)	Urea (mg/dL)	ALP (µ/L)	Creatinine (mg/dL)	LDH (µ /L)
1	MG	4.00±0.31^{a}	10.66±0.00^{a}	191.25±6.25^{a}	0.51±0.06^{a}	277.50±8.54^{a}
2	MGVITC	5.35±0.18^{b}	8.35±0.41^{b}	189.25±3.28^{a}	0.50±0.04^{a}	277.00±9.17^{a}
3	MGS	7.75±0.25^{c}	19.25±1.03^{c}	180.00±9.13^{a}	0.68±0.05^{b}	266.50±6.91^{a}
4	MGSVITC	9.61±0.39^{d}	16.00±0.00^{d}	179.75±6.46^{a}	0.65±0.07^{b}	267.50±5.91^{a}
5	MGGR	7.00±0.41^{c}	18.50±3.70^{c}	190.50±6.09^{a}	0.78±0.05^{c}	261.75±4.71^{a}
6	MGGRVITC	8.50±0.65^{e}	16.25±1.75^{d}	181.75±5.94^{a}	0.85±0.07^{c}	275.50±6.74^{a}
7	MGCR	7.82±0.52^{c}	17.25±1.49^{e}	178.50±5.39^{a}	0.80±0.08^{c}	278.25±6.52^{a}
8	MGCRVITC	9.73±1.10^{d}	16.75±2.21^{d}	197.00±23.36^{a}	0.85±0.18^{c}	275.75±24.2^{a}
9	MGGRS	6.98±0.50^{f}	18.25±1.38^{c}	190.50±11.23^{a}	0.93±0.05^{c}	273.50±2.90^{a}
10	MGGRSVITC	8.80±0.72^{e}	16.75±2.39^{d}	183.50±9.46^{a}	0.85±0.06^{c}	265.00±65.60^{a}
11	MGGRSCR	6.00±0.19^{f}	18.50±0.96^{c}	183.50±2.72^{a}	0.73±0.05^{c}	271.50±20.60^{a}
12	MGGRSCRVITC	8.50±0.64^{e}	16.00±2.38^{d}	180.25±4.54^{a}	0.75±0.07^{c}	268.75±4.50^{a}
Control	Cerelac®	8.70±0.65^{e}	16.70±2.12^{d}	182.50±2.96^{a}	0.88±0.05^{c}	270.00±2.97^{a}

Values represent Means ± Standard Error Mean (SEM) of triplicate analysis. Values bearing different superscripts down the column are significantly different (p<0.05) from each other. MG= maize/guinea corn; MGS= maize/guinea corn/soybean; MGGR= maize/guinea corn/groundnut; MGCR= maize/guinea corn/crayfish; MGGRS= maize/guinea corn/ groundnut/soybean; MGGRSCR= maize/guinea corn/groundnut/soybean/crayfish; VitC= + Vitamin C.

3.2 Effect of formulated feed blends and vitamin C fortification on Hematological Parameter in Weanling Albino Rats

The results of effect of vitamin C fortified formulated feed blends on the hematological parameter in weanling albino rats are presented in Table 3.

The results showed that inclusions of the staples, soybean, groundnut and crayfish in feed formulation either singly or in combinations caused significant increase ($p < 0.05$) in the levels of red blood cell (RBC), hemoglobin (Hb) and packed cell volume (PCV) and insignificant changes in white blood cell (WBC) and platelet counts compared to control. As protein sources [35][49], theses staples can provide the needed amino acids for generation of the protein component of the red blood cell. Also the iron and copper content of crayfish and soybean have been reported to be of significance [18]. Both minerals play vital roles in the synthesis of red blood cells [50][51]. This may explain the insignificant changes in Hb and PCV values with separate inclusions of soybean and crayfish in the diet.

Combination of soybean and groundnut in the diet resulted in insignificant changes in the RBC, Hb and PCV values compared to soybean diet alone and significant increase in same parameters compared to groundnut alone. This could indicate soybean as a better contributor to haematopoiesis than groundnut. A high concentration of calcium, as reported for groundnut [18] was observed to limit haematopoiesis [52]. The combination of the trio of soybean, groundnut and crayfish only resulted in significant increase in RBC and not Hb or PCV compared to soybean-groundnut combination group. This could also be due to iron deficiency anemia as a result of high contributions of calcium to the diet by soybean and crayfish leading to competition for absorption between dietary calcium and iron, and eventually low incorporation of iron into hemoglobin. [52].

There was significant increase ($p<0.05$) in RBC, Hb and PCV for all groups that received vitamin C fortified diets, compared to the non-fortified diet groups. This is insignificant ($p>0.05$) compared to the Cerelac® control. Vitamin C is involved in synthesis of proteins associated with cell proliferation, iron homeostasis and erythropoiesis among others [45]. It enhances the absorption of iron in the gut by reducing ferric to ferrous state [53].

Table 3. Effect of formulated feed blends and vitamin C fortification on the hematological parameter in weanling albino rats

Group	Diet	RBC (4.8-7.8 x 10^{6} /μl)	WBC (4.5-11 x 10^{3}/ μl)	HB (11.00-17.50mg/dL)	PCV (30-41%)	Platelet count (150-450 x 10^{3}/ μl)
1	MG	3.00±0.00^{a}	5.25±1.08^{a}	8.70±1.14^{a}	24.00±2.68^{a}	240.00±0.41^{a}
2	MGVITC	4.70±0.41^{b}	5.00±1.31^{a}	9.00±0.71^{b}	28.75±1.25^{b}	250.00±1.79^{a}
3	MGS	5.80±0.41^{c}	5.50±0.65^{a}	14.48±0.37^{c}	31.50±1.19^{c}	220.00±1.71^{a}
4	MGSVITC	7.70±0.41^{d}	5.00±1.73^{a}	16.57±0.15^{d}	39.00±2.41^{d}	220.00±0.41^{a}
5	MGGR	4.60±0.25^{b}	5.23±2.26^{a}	10.22±1.28^{b}	29.25±0.75^{b}	245.00±0.62^{a}
6	MGGRVITC	7.50±0.75^{d}	5.21±0.63^{a}	16.25±0.25^{d}	37.75±0.48^{d}	255.00±0.41^{a}
7	MGCR	6.00±0.48^{e}	5.20±0.41^{a}	14.30±0.31^{c}	31.75±0.85^{c}	220.00±0.85^{a}
8	MGCRVITC	7.75±0.25^{d}	5.00±1.19^{a}	16.60±0.00^{d}	38.70±2.27^{d}	245.00±0.91^{a}
9	MGGRS	5.00±0.71^{c}	5.75±0.85^{a}	14.13±0.28^{c}	31.50±1.04^{c}	248.00±0.48^{a}
10	MGGRSVITC	6.75±1.32^{e}	5.00±0.41^{a}	16.88±0.08^{d}	36.75±1.49^{d}	245.00±0.48^{a}
11	MGGRSCR	5.59±0.71^{b}	5.00±0.65^{a}	14.18±0.23^{c}	30.75±0.48^{c}	235.00±0.51^{a}
12	MGGRSCRVITC	7.50±0.64^{d}	5.00±0.41^{a}	16.00±0.32^{d}	37.25±1.79^{d}	230.00±0.65^{a}
Control	Cerelac	7.95±0.48^{d}	5.50±0.41^{a}	16.28±0.42^{d}	38.75±2.29^{d}	255.00±0.38^{a}

Values represent Means ± Standard Error Mean (SEM) of triplicate analysis. Values bearing different superscripts down the column are significantly different ($p<0.05$) from each other. MG= maize/guinea corn; MGS= maize/guinea corn/soybean; MGGR= maize/guinea corn/groundnut; MGCR= maize/guinea corn/crayfish; MGGRS= maize/guinea corn/ groundnut/soybean; MGGRSCR= maize/guinea corn/groundnut/soybean/crayfish; VitC= + Vitamin C.

3.3 Effect of formulated feed blends and vitamin C fortification on the Performance Indices in Weanling Albino Rats

The results of effect of vitamin C fortified formulated feed blends on the performance indices; average feed intake; average faecal weight; weight gain and percentage liver, heart and kidney weights in weanling albino rats are presented in Table 4. The result showed that vitamin C fortification caused no significant changes in all the parameters compared to the non- fortified equivalent diets. This is an indication that vitamin C did not affect overall growth performance of experimental animals. There were no significant changes in average feed intake and faecal weight between the test groups and the control, a pointer to uniform consumption and utilization of available feed by experimental animals. The presence of soybean and other food staples, singly or in combination in the diet caused a significant increase ($p < 0.05$) in total body weight gain and organ weights compared to control. This also supports the assertion of higher nutritive potential of the food staples over the base diet. Essential amino acids, for example, are needed for normal growth and development and in low concentration, may not be nutritionally adequate to provide normal growth in infants [54].

Table 4. Effect of formulated feed blends and vitamin C fortification on the performance indices in weanling albino rats

Group	Diet	Av. Feed Intake (g/28 days)	Av. Fecal Weight ((g/28 days)	Weight Gain (g)	% liver Weight	%heart Weight	%kidney weight
1	MG	79.15±7.69^a	17.65±3.25^a	15.05±5.8^a	1.30±0.93^a	0.50±7.21^a	0.27±1.31^a
2	MGVITC	79.18±8.34^a	17.25±3.22^a	17.11±1.21^a	1.41±5.25^a	0.51±7.51^a	0.27±8.40^a
3	MGS	80.70±4.80^a	15.95±3.09^a	30.00±1.98^b	2.01±3.55^b	0.80±1.81^b	0.42±1.51^b
4	MGSVITC	79.09±6.62^a	15.13±2.98^a	32.01±0.11^b	2.00±4.51^b	0.80±2.50^b	0.42±1.51^b
5	MGGR	80.00±9.46^a	15.00±3.03^a	29.95±4.10^b	1.85±1.55^b	0.79±1.95^b	0.50±8.31^b
6	MGGRVITC	81.34±11.53^a	14.49±2.81^a	30.91±0.81^b	1.99±2.01^b	0.82±0.81^b	0.41±8.21^b
7	MGCR	79.25±9.50^a	15.91±2.82^a	29.00±3.41^b	2.05±4.51^b	0.80±5.01^b	0.42±4.51^b
8	MGCRVITC	80.00±10.78^a	15.15±2.99^a	30.55±2.88^b	1.99±1.22^b	0.83±0.31^b	0.52±3.81^b
9	MGGRS	79.50±9.43^a	16.04±3.00^a	28.25±3.41^b	1.85±2.51^b	0.88±0.51^b	0.42±2.51^b
10	MGGRSVITC	81.75±8.79^a	15.20±2.91^a	31.85±2.85^b	2.00±0.25^b	0.81±0.51^b	0.42±3.81^b
11	MGGRSCR	79.63±8.51^a	16.08±3.04^a	28.41±3.51^b	1.88±0.31^b	0.80±1.11^b	0.50±3.65^b
12	MGGRSCR VITC	82.75±10.93^a	15.84±2.75^a	30.95±1.99^b	1.95±4.21^b	0.82±1.91^b	0.54±3.60^b
Control	Cerelac	83.25±9.58^a	15.00±0.74^a	31.95±0.85^b	1.99±2.77^b	0.88±1.81^b	0.54±1.85^b

Values represent Means ± Standard Error Mean (SEM) of triplicate analysis. Values bearing different superscripts down the column are significantly different ($p<0.05$) from each other. MG= maize/guinea corn; MGS= maize/guinea corn/soybean; MGGR= maize/guinea corn/groundnut; MGCR= maize/guinea corn/crayfish; MGGRS= maize/guinea corn/ groundnut/soybean; MGGRSCR= maize/guinea corn/groundnut/soybean/crayfish; VitC= + Vitamin C.

3.4 Effect of formulated feed blends and vitamin C fortification on The Protein Quality Characteristics in Weanling Albino Rats.

The results of effect of vitamin C fortified formulated feed blends on the protein quality characteristics of weanling albino rats are presented in Table 5. Parameters analyzed include nitrogen intake, protein intake, carcass nitrogen, faecal nitrogen, feed conversion ratio (FCR), net protein ratio (NPR), protein efficiency ratio (PER), net protein utilization (NPU), and biological value (BV). The result showed a significant increase ($p<0.05$) in the values of all the parameters for the test groups (groups 3 to12) compared to control (groups 1 and 2). This low protein quality exhibited by the base cereals (maize and guinea corn) could also be attributed to lack of essential amino acids [55][56]. Except for biological value (BV), there was insignificant changes in the values of all the parameters for the groups fed with vitamin C fortified feed blend compared to the groups fed non-fortified feed equivalents. Biological value which is a proportion of digested dietary protein converted to body protein may have been significantly increased due to the ability of vitamins to aid the absorption of some amino acids into the cells [57]. The FCR, NPR and PER of groups1 and 2 were negligible. Also vitamin C has been shown to promote protein synthesis via increased anabolic and myogenic gene expression [42].

Table 5. Effect of formulated feed blends and vitamin C fortification on the protein quality characteristics in weanling albino rats

Group	Nitrogen Intake (g/day)	Protein Intake (g)	Carcass Nitrogen (g)	Faecal Nitrogen (g/day)	FCR	NPR	PER	NPU	BV
1	0.56±0.85^{a}	8.25±0.05^{a}	1.98±0.05^{a}	0.02±1.67^{a}	-	-	-	49.33±1.43^{a}	43.88±0.77^{a}
2	0.65±0.11^{a}	8.24±0.11^{a}	2.01±0.35^{a}	0.01±0.18^{a}	-	-	-	50.33±1.21^{a}	44.22±1.99^{a}
3	5.22±0.23^{b}	35.01±1.55^{b}	4.55±0.81^{b}	0.68±0.95^{b}	0.33±0.54^{a}	2.28±0.05^{a}	2.14±1.87^{a}	88.21±0.51^{b}	89.55±0.28^{b}
4	5.35±0.41^{b}	35.01±0.25^{b}	5.10±0.41^{b}	0.59±2.25^{b}	0.32±1.28^{a}	2.28±1.55^{a}	2.11±2.31^{a}	88.31±0.61^{b}	94.61±0.41^{c}
5	5.41±0.25^{b}	36.55±1.45^{b}	4.68±1.25^{b}	0.71±1.85^{b}	0.35±1.47^{a}	2.33±2.10^{a}	2.12±1.57^{a}	89.25±0.11^{b}	83.22±0.55^{b}
6	5.51±0.75^{b}	36.55±1.45^{b}	5.25±2.10^{b}	0.65±1.22^{b}	0.36±0.89^{a}	2.35±1.59^{a}	2.11±2.34^{a}	89.25±0.28^{b}	93.41±1.28^{c}
7	5.45±0.92^{b}	36.18±1.22^{b}	4.82±1.91^{b}	0.70±1.85^{b}	0.35±1.82^{a}	2.24±0.71^{a}	2.13±1.21^{a}	88.41±5.41^{b}	83.45±1.05^{b}
8	5.51±0.51^{b}	36.25±0.95^{b}	5.35±0.01^{b}	0.67±1.25^{b}	0.35±0.09^{a}	2.31±1.97^{a}	2.15±0.28^{a}	88.71±1.91^{b}	94.45±0.28^{c}
9	5.08±1.15^{b}	35.54±2.21^{b}	4.61±0.53^{b}	0.69±1.11^{b}	0.34±0.05^{a}	2.25±1.48^{a}	2.98±4.11^{a}	88.11±0.66^{b}	83.95±0.11^{b}
10	5.12±1.51^{b}	35.55±2.51^{b}	5.11±0.77^{b}	0.59±1.28^{b}	0.31±0.11^{a}	2.11±0.58^{a}	2.98±2.81^{a}	88.21±1.91^{b}	93.96±1.21^{c}
11	5.35±0.88^{b}	34.95±1.25^{b}	4.65±2.12^{b}	0.71±2.57^{b}	0.33±1.12^{a}	2.24±0.95^{a}	2.89±2.11^{a}	87.11±1.21^{b}	83.71±0.89^{b}
12	5.41±0.85^{b}	35.88±2.31^{b}	5.51±1.44^{b}	0.62±2.57^{b}	0.33±1.49^{a}	2.45±1.78^{a}	2.20±1.50^{a}	87.22±1.48^{b}	94.01±1.88^{c}
13	5.58±1.80^{b}	39.33±1.88^{b}	5.99±0.01^{b}	0.65±1.85^{b}	0.35±1.21^{a}	2.39±0.05^{a}	2.95±1.50^{a}	89.89±1.53^{b}	96.18±0.28^{c}

Values represent Means ± Standard Error Mean (SEM) of triplicate analysis. Values bearing different superscripts down the column are significantly different ($p<0.05$) from each other. MG= maize/guinea corn; MGS= maize/guinea corn/soybean; MGGR= maize/guinea corn/groundnut; MGCR= maize/guinea corn/crayfish; MGGRS= maize/guinea corn/ groundnut/soybean; MGGRSCR= maize/guinea corn/groundnut/soybean/crayfish; VitC= + Vitamin C; 1= rats fed with maize/guinea corn; 2= rats fed with maize/guinea corn + vitamin C; 3= rats fed with maize/guinea corn/soybean; 4= rats fed with maize/guinea corn/soybean + vitamin c; 5= rats fed with maize/guinea *corn/groundnut; 6= rats fed with maize/guinea corn/groundnut + vitamin c; 7= rats fed with maize/guinea corn/crayfish; 8= rats fed with maize/guinea corn/crayfish + vitamin c; 9= rats fed with maize/guinea corn/groundnut/soybean; 10= rats fed with maize/guinea corn/groundnut/soybean + vitamin c; 11=rats fed with maize/guinea corn/groundnut/soybean/crayfish; 12= rats fed with maize/guinea corn/groundnut/soybean/crayfish + vitamin c; 13= rats fed with cerelac (control); - = negligible; P.E.R = Protein efficiency ratio; B.V= Biological Value; NPU= Net protein utilization; FCR = Feed conversion ratio; NPR= Net protein ratio.*

3.5 Acceptability Rating of the Sensory Attributes of the Formulated Diets

The results of the acceptability rating of the sensory attributes of the formulated diets are presented in Table 6.

The result showed that for appearance, smell, taste and overall acceptability, the base cereals (maize-guinea corn) diet had the lowest rating, while the commercial standard diet had the highest rating. Addition of the food staples (soybeans, groundnut and crayfish), singly or in combinations caused insignificant changes in ratings of all the sensory attributes compared to one another, except for base gruel-crayfish mix which showed significantly higher taste and overall acceptability ratings. This conforms to the studies of [58] and [59] that reported crayfish as popular, nutritious and among the most frequently used food ingredients in Nigeria. These may contribute to its high overall acceptability rating.

Table 6. Acceptability rating of the sensory attributes of the formulated diets

Group	Diets	Appearance	Smell	Taste	Overall acceptability
1.	MG	3.55 ± 0.11^{a}	3.55 ± 0.11^{c}	3.52 ± 0.88^{b}	3.99 ± 1.25^{a}
2.	MGS	4.95 ± 0.28^{b}	4.95 ± 0.51^{d}	4.85 ± 0.11^{a}	4.55 ± 2.57^{b}
3.	MGGR	4.85 ± 1.25^{b}	4.95 ± 0.51^{d}	4.45 ± 1.15^{a}	4.65 ± 1.02^{b}
4.	MGCR	4.77 ± 2.55^{b}	5.00 ± 1.88^{d}	4.85 ± 0.11^{ac}	4.85 ± 1.02^{d}
5.	MGGRS	4.85 ± 1.88^{b}	4.99 ± 0.22^{d}	4.45 ± 1.15^{a}	4.66 ± 2.58^{b}
6.	MGGRCRS	4.95 ± 1.11^{b}	4.95 ± 0.51^{d}	4.33 ± 2.58^{a}	4.55 ± 2.57^{b}
7.	Cerelac®	5.00 ± 1.85^{c}	5.00 ± 1.11^{e}	4.85 ± 1.25^{c}	5.00 ± 1.11^{c}

Values represent Means ± Standard Error Mean (SEM) of triplicate analysis. Values bearing different superscripts down the column are significantly different ($p<0.05$) from each other. MG= maize/guinea corn; MGS= maize/guinea corn/soybean; MGGR= maize/guinea corn/groundnut; MGCR= maize/guinea corn/crayfish; MGGRS= maize/guinea corn/ groundnut/soybean; MGGRSCR= maize/guinea corn/groundnut/soybean/crayfish; VitC= + Vitamin C.

4. CONCLUSION

The inclusion of the food blends comprising of soybean, groundnut and crayfish improved the nutritive content, hematopoietic potential, protein quality, taste and overall acceptability of the maize-guinea corn based weaning diet. Except for performance indices and some aspects of protein quality indicators, these were further improved by fortification of the diet with vitamin C, with crayfish playing a prominent role in taste and overall acceptance of the formulated diet. Fortification also significantly bridges the gap between the formulated weaning diet and Cerelac®, a standard commercial weaning formula in Nigeria.

ETHICAL APPROVAL

Author hereby declare that this research, involving animal studies, was carried out after due approval (of research relevance and design, including research ethics in animal handling and patients consent) by the departmental postgraduate committee, Department of Biochemistry, Faculty of Science, University of Port Harcourt, Nigeria.

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