

PRODUCTION, ASSESSMENT AND SAFETY ASPECT OF WEANING FOOD FROM SORGHUM, CRAYFISH AND GARDEN EGG

ABSTRACT

This study examined the production, assessment and safety aspect of weaning food from sorghum, crayfish and garden egg. Sorghum was fermented, garden egg and crayfish were cleaned and processed into flours and formulated into blends. Laboratory analyses were done such as proximate composition, mineral composition, lipid profile, haematological studies of rat fed commercial and formulated diets. Also, growth rate, feed intake and body weight were determined. Data generated were analyzed. The results showed that the moisture content observed in the diets was very low. The lowest moisture content was observed in diet O (10.41%), the highest protein was from diet L (26.35%). The highest ash content was observed in diet L (5.35%). Diet L (8.82%) had the highest fat content. The highest calcium content was found in diet L (26591.10mg/kg), next was A (26158.86mg/kg), M (23405.84mg/kg), I (16291.12mg/kg), O (13298.34mg/kg), and F (6011.12mg/kg). Diet A (103.40mg/kg) had the highest iron content, next was M (91.69mg/kg), L (89.74mg/kg), O (77.66mg/kg), I (77.07mg/kg), and X (28.19mg/kg). The lowest LDL was observed in rats fed diet M (14.0mg/dl), next was rats fed diet L (16.0mg/dl), S (17.50mg/dl) and O (24.0mg/dl). The body weight of the rat at day 0 ranges between 32.10-33.85g, at day 4 (39.60-47.90g), at day 8 (38.85 – 52.75g), at day 12 (38.70 -54.35g), at day 16 (41.80-74.25g), at day 20 (46.30 -84.85g) at day 24 (47.85 -86.35g), and at day 28 (50.15 -90.35g). The highest WBC counts were diets M and O with the same values ($10.0 \times 10^9/L$), next was A and S with the same values ($9.0 \times 10^8/L$), L ($8.0 \times 10^8/L$), I ($7.0 \times 10^8/L$) and X ($4.0 \times 10^8/L$) in that decreasing order. The highest PCV count was diet I (43%) next was A (42%), O (41%), M (40%), L (39%), X (38%) and S (37%). This study showed that the use of sorghum, crayfish and garden egg (vegetable) at combination of 60: 30: and 10 respectively could improve optimal health and growth.

Key words: Weaning, Proximate, lipid, Sorghum, Safety

INTRODUCTION

Scientifically, it has been proved that breast milk is the perfect food for the infant during the first six months of life. It has all the nutrients needed by an infant to maintain optimal health and growth. In addition, breast milk protects infants against the two leading causes of infant mortality, upper respiratory infections and diarrhea [1]. At the age of six months and above, the child's birth weight is expected to have doubled, and then breast milk is no longer enough to meet the nutritional needs of the growing infant. Nutritious complementary foods are then introduced, it is also known as weaning foods, which typically starts from six to twenty-four months of age in developing countries [2].

Guinea corn also known as sorghum is a cereal grain that originated in Africa and is consumed in the world because of its resistance to drought. Guinea corn contains important nutrient that is often milled into flour to make bread, porridge, pancakes and Kunun drink. This product gives numerous nutritional and therapeutic benefits. Guinea corn contains the same protein than

Comment [W1]: May consider restructuring the sentence (use of words such as further, whilst, whereas, subsequently might help) **corrected**

Comment [W2]: Add little about the sample information like number of samples prepared, their composition etc so that readers can relate/understand your results mentioned in abstract **ok**

Comment [W3]: Check spelling **ok**

Comment [W4]: Consider restructuring and combining these sentences. **ok**

Comment [W5]: Using what method? **corrected**

Comment [W6]: Spell out full when mentioning first time in manuscript **noted**

Comment [W7]: Must mention a little like sample size and breed of rats earlier in the abstract (methodology flow) **corrected**

Comment [W8]: Spell out full when mentioning first time in manuscript **corrected**

Comment [W9]: Improvement among whom? Is it children of specific age or adults or elderly? Please specify **corrected**

Comment [W10]: Upper case **Corrected**

Comment [W11]: A good abstract should be written in a flow of background, objective(s), methodology, result(s), conclusion(s) in a way that readers from different backgrounds can understand easily. Please consider restructuring. **corrected**

Comment [W12]: Avoid starting a paragraph on this note especially without a supporting reference. **OK**

Comment [W13]: i.e. **ok**

Comment [W14]: Consider restructuring this sentence **ok**

Comment [W15]: Which nutrient? **noted**

Comment [W16]: Like? **ok**

other grains. It is used as food, fodder for animals, and for the production of alcoholic beverages. It can resist drought and heat, and is important in arid regions. It is an important food crop in Africa, Central America, and South Asia, and is the fifth most important cereal crop grown in the world. Guinea corn is one of the nutritional high light foods, its mineral content in ¼ cup serving contains 13mg calcium 2.1mg iron, 13.8mg phosphorus, and these are essential minerals needed for bone health and strength.

Garden egg (*Solanum*), a widespread plant belonging to the family Solanaceae, has over 1000 species worldwide with at least 100 indigenous species in Africa and adjacent islands; these include a number of valuable crop plants and some toxic ones. Represented in Nigeria by 25 species including those domesticated with their leaves, fruits or both eaten as vegetables or used in traditional medicine. Among them are two African eggplants, *S. aethiopicum* L. and *S. macrocarpon* L., which are widely cultivated in Nigeria and across the African continent [3][4].

Eggplants/garden eggs (Hausa: Dauta; Igbo: afufa or añara; Yoruba: igbagba), are highly valued constituents of the Nigerian foods and indigenous medicines; they are commonly consumed almost on daily basis by both rural and urban families. This research examined the proximate and phytochemical of plant food materials used as weaning food produced from sorghum, crayfish and garden egg.

Seafood contains nutrient including vitamins; fat soluble (A, D, E, and K) and water soluble example C and the B complex [5]. Vitamins A and vitamin D are found in fish liver oils and in small amounts in the fatty tissues of fish. Some fish oils, example cod liver oil, are high in vitamin D, providing more than 200% of the recommended. Crayfish is among the cheapest animal protein sources in Nigeria. The composition of Fish flesh are mainly water, protein and fat with traces of carbohydrates, amino acids and other non-protein nitrogenous extracts, various minerals and vitamins [6]. The production of composite flours from Guinea corn, crayfish and garden egg could help to improve the nutritional status of the infants. Fermentation of plant foods (sorghum) provide means through which their nutritional worth can be improved upon.

The objectives of this study was to develop, evaluate and determine the safety aspect of some quality attributes of sorghum, crayfish and garden egg flour blends as a complementary food in Nigeria.

MATERIALS AND METHODS

Source of Materials:

Guinea corn (*Sorghum bicolor*), crayfish (*Cambarus* sp) and garden egg (*Solanum melongena*) used in this experiment were purchased from Auchi, Edo State, Nigeria.

Preparation of materials:

Four kilograms of sorted sorghum grains were cleaned and soaked in clean tap water in a covered container. The soaked grains were allowed to ferment at room temperature (37°C) for 24 hours. After fermentation, the water was drained and dried at 80°C for 3hrs. Four kilograms of sorted and cleaned crayfish was sun dried. Three kilogram of garden eggs were sorted, cleaned, sliced thinly and dried. All the dried samples ie guinea corn, garden egg and crayfish were separately milled.

Formulation of composite flours:

The composites were formulated in the ratio shown below using Random sampling method (RSM). The controlled experiment was 100% sorghum

Comment [W17]: Reference?

Comment [W18]: Used **ok**

Comment [W19]: reference? **ok**

Comment [W20]: Please rephrase/restructure the sentence and provide reference. **ok**

Comment [W21]: Please rephrase **Done**

Comment [W22]: Is this your primary/secondary objective? If yes, then it should not be included anywhere in between **ok**

Comment [W23]: Please rephrase **Done**

Comment [W24]: Either vitamins A and D or vitamin A and vitamin D **ok**

Comment [W25]: Reference? **ok**

Comment [W26]: It doesn't match to the objective in abstract (what about weaning food?) **corrected**
Is it for infants or general population? **infant**

Comment [W27]: What dried? Residue or water? **effected**

Comment [W28]: Random sampling of what? Formulations were not made using materials mentioned in previous paragraph? **Corrected**

Table 1: Formulation of fermented sorghum, crayfish and garden egg

Samples	Level of subscription		
ID	Fermented sorghum	Crayfish	Garden egg
A	100.00	20.00	5.00
I	113.64	25.00	7.50
L	60.00	30.00	10.00
M	80.00	25.00	11.70
O	80.00	25.00	7.50

Comment [W29]: How did you came up with these ID's? Did you use any software or online randomizer? **I used software (Design expert)**

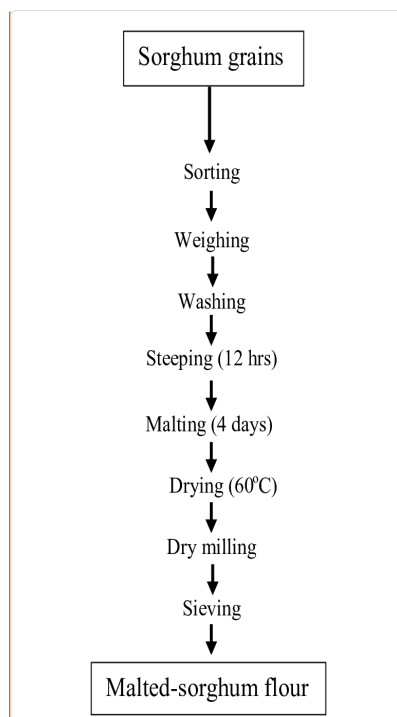


Fig 1: Flow chart for the processing of sorghum flour

Comment [W30]: Steps mentioned in this figure doesn't match with text under "Preparation of materials". Please cross-check and rectify. **corrected**

Also, please avoid repeating the information in text and figures. You may either provide a figure or in-text information.

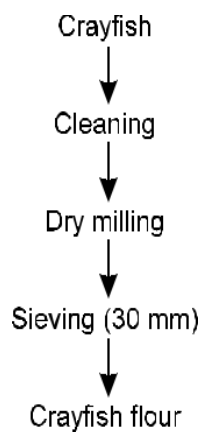


Fig. 2: Flow chart for the production of crayfish flour

Comment [W31]: Steps mentioned in this figure doesn't match with text under "Preparation of materials". Please cross-check and rectify. **corrected**
Also, please avoid repeating the information in text and figures. You may either provide a figure or in-text information.

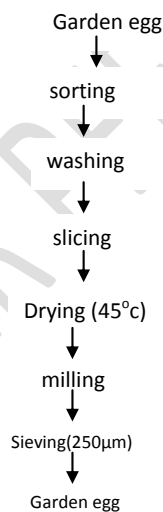


Fig. 3: Flow chart for the production of garden egg flour

Proximate analysis:

Composition of the flour blends was calculated according to standard assay [7]. Crude protein was calculated by Microkjeldah method. Fat and ash content were evaluated by Soxhlet extraction and ashing method respectively. Carbohydrate was determined by difference while gross energy was also calculated [7]. All assays were performed in triplicate.

Comment [W32]: Do not simply mention method's name. Either explain or provide a suitable reference. **ok**

Mineral analysis:

The mineral contents of the products were determined as described by [8]. 2g oven-dried sample were ashed at 600°C using a muffle furnace. The ashed form was transferred into 250ml glass beaker and 120ml conc. HNO₃ and 10ml H₂O₂ added. The component was heated at 90°C for duration of 1 hr, allowed to cool and filtered and the filtrate transferred into 250ml volumetric flask which was later made up to the mark with deionised water. After gentle shaking to mix, 2ml was transferred into 250ml using a pipette and diluted to the mark with deionised water. Stock solution of 1000mg/kg of elements was prepared using deionised water. Dilution comprising of each element was made with deionised water and together with the test sample were analyzed using an atomic absorption spectrophotometer.

Comment [W33]: By whom?

Comment [W34]: ash

Comment [W35]: Restructure/ consider fragmenting into shorter sentences. done

Animal Husbandry and Experimental Design

Thirty five healthy Wistar rats with mean weight of 32.10-33.85g were obtained from an Animal farm attached to Federal University of Technology Akure, Nigeria. The rats were allowed to acclimatize with the laboratory condition for 2 days in well ventilated cages. The rats were divided into seven groups of 5 animals each. Each rat was given an identification mark on the tail, head and back. The rats in each group were fed with either formulated or commercial diet (Cerelac). The formulated diets are seen in Table 1. Samples X and S were used as control (Sorghum and Cerelac). The rats were acclimatized on a commercial diet (rat pellet) and water *ad libitum* for 2 days prior to commencement of the experiment which lasted for 28 days.

Comment [W36]: Choose correct heading. Experimental design should be fine. ok

Comment [W37]: How did you end up with this figure? What about sample size calculation?

Comment [W38]: Animal ok

Comment [W39]: Rat ok

Comment [W40]: Is this your study design? Crossover study? ok

Comment [W41]: Table 1 has no mention of sample X and S please refer to table 2 both are 100%

Growth performance study: The growth performance was studied by the method described [9].

Comment [W42]: By whom? Please see reference section

Feed Intake (FI)

This was calculated using the sum of the quantity of feed consumed by the rats during the period of the experiment which lasted for 28 days.

Collection and Analysis of Blood Sample

The rats were anaesthetized with chloroform vapour twelve hours (12 h) after last day of feed administration, and blood samples were collected by cardiac puncture into a set of plain and fluoride oxalate sample bottles for haematological and lipid studies [10]

The Red Blood Cells Count was determined by Haemocytometry Method

Procedure: Blood was drawn up to 0.5 mark of RBC pipette and RBC diluting fluid was added to make up 101 marks. The fluid was mixed with the blood and the first few drops of blood were discarded by holding the pipette vertically. The counting chamber was charged with drop of blood that mixed with diluting fluid and the chamber was left undisturbed for few minutes while the four corners of the chamber were visualized under a low power (10x) objective and cells were counted in all the four marked squares.

Comment [W43]: This is not a heading

Total RBC/L = $\frac{\text{Number of cells counted} \times \text{diluting factor}}{\text{Area counted} \times \text{depth of fluid}}$

White Blood Cells (WBC) or Total Leucocytes Count (TLC)

Total Leucocyte count was determined by haemocytometer method. Blood was drawn up to 0.5 mark of the WBC pipette and WBC diluting fluid was added up to 1 mark. The fluid and blood were thoroughly mixed while the first few drops of blood were thrown away by holding the pipette vertically. The counting chamber was charged by holding the pipette vertically and was charged with a drop of blood that has mixed with diluting fluid and the chamber was left undisturbed for few minutes and the four corners of the chamber and the middle were visualized under a low power (10x) objective and cells were counted in all the four marked squares [10]

$$\text{Total WBC/L} = \frac{\text{Number of cells} \times \text{diluting factor}}{\text{Area counted} \times \text{depth of fluid}}$$

Packed Cell Volume (PCV)

Blood sample was filled to 75% of capillary tube through capillary action, one end of tube was sealed with plasticine and placed in micro-haematocrit centrifuge and the centrifuge was set at 12 rpm (revolution per minute) for 5 minutes. Thereafter, the centrifuge was spun and the tubes were removed and the percentage packed volume was read using micro-haematocrit reader according to the method [10]

Determination of platelets: The platelets were determined by diluting the blood in one percent (1%) ammonium oxalate which haemolysed the red blood cells. The platelets were then counted in a definite area using the rulings of an improved Neubauer counting chamber. Their characteristic Mauve-pink colour was used in their identification.

Determination of haemoglobin: Sahli's haemoglobinometer was employed for the estimation of haemoglobin (Hb) content of the blood. Using the Sahli haemoglobinometer, The colour of the test solution was filled to 20ml mark with 10N hydrochloric acid, 0.02ml of blood was added and the content of the test tube was mixed using glass rod. It was allowed for 5 minutes (haemoglobin changed into acid haematin). More acid was added and mixture stirred until colour of the test solution matched that of the colored glass standard. The level of the fluid in the tube was read and the haemoglobin content was expressed in percentage.

Comment [W44]: Incomplete sentence corrected

Determination of Leucocytes: The differential white blood cell count (Neutrophils, Lymphocytes, monocytes, Eosinophils and Basophils) was carried out by making a thin film of blood on a smooth edged slide. Dry was allowed on a bench free from dust, ants, flies, and other insects. Blood film was fixed by a covered staining jar of methyl alcohol for 3 minutes. Ten (10) ml of May Grunwald Stain (mixture of 5g of May Grunwald powder and 1 liter of methanol) and 10ml of buffered water (pH 6.8) was mixed and smear was covered with the dilute May Grunwald stain for 3 minutes. The stain was off and replaced with diluted Giemsa's stain (5%) for 9 minutes. The stain was washed using buffered water (pH 6.8) and pure water was allowed on the slide which lasted for 30 seconds. The water was wiped off and the slide was allowed to dry

and examined microscopically (McArthur microscope) for Neutrophils, Eosinophils, Basophils, Monocytes and lymphocytes.

Statistical Analysis

Data generated were subjected to one-way analysis of Variance (ANOVA) in randomized block to test significant variations ($P < 0.05$) among mean values obtained. The values used for each treatment were in triplicate. Where significant differences existed Duncan's multiple range test was applied to indicate where the differences occurred using Genstat statistical package 2005, 8TH edition (Genstat Procedure Library Release PL16). Also, data were represented by simple bar chart.

Comment [W45]: P ok

Comment [W46]: Please restructure done

RESULTS AND DISCUSSION

Table 2: Proximate composition of raw materials (flour)

Samples Proximate (%)	X	Y	Z	SEM
Moisture	9.55 _b	12.30 ^a	9.31 ^b	0.06
Ash	2.48 ^c	3.19 ^b	5.13 ^a	0.06
Protein	7.49 ^c	17.40 ^a	12.69 ^b	0.04
Fat	1.59 ^b	6.72 ^a	6.80 ^a	0.15
Fiber	0.49 ^b	1.05 ^b	3.14 ^a	0.21
Carbohydrate	78.41 ^a	59.34 ^c	62.94 ^b	0.28

Comment [W47]: What is it? Standard error mean? yes

Means with the same superscript along the rows are significantly not different ($p > 0.05$)

Sample X = sorghum

Sample Y = Crayfish

Sample Z = Garden egg

Comment [W48]: Not the correct way of reporting? ok

The analysis of variance showed significant differences ($p < 0.05$) in the proximate composition of the raw plant materials (Table 2).

Comment [W49]: On what attributes? Proximate analysis generally

The moisture content of all the raw samples were significantly ($p < 0.05$) low. However, sample Y had the highest (12.30%) moisture content and it was significantly higher ($p < 0.05$) than other samples. The high moisture content of sample Y could be attributed to the fact that it is an animal product; also the relative increase in the moisture content in local weaning food may be attributed to a variation in the treatment during the drying process of the diets and the storage condition. This was followed by moisture content of sample X (9.55%) which was not significantly different ($p > 0.05$) from sample Z (9.31%). The low moisture content of the various samples reflects their shelf stability. [11] reported that low moisture content within the range of 9.31-12.30% suggest moderate moisture level needed for long storage of processed flour and plant raw material. High moisture content makes food prone to deterioration and microbial infestation. The high moisture content of the local diets may affect the storage quality of the foods. High moisture content in food has been shown to encourage microbial growth [12].

Comment [W50]: Which local weaning food? Sorry it is a sample

Comment [W51]: Who reported? Pls refer to reference section

Sample Z had the highest (5.13%) ash content and was significantly different ($p < 0.05$) from sample Y (3.19%) and Sample X (2.48%) in decreasing order. According to [13] the ash content of weaning food should not exceed $5\text{g.}100\text{g}^{-1}$.

Comment [W52]: According to whom? Pls see reference section

The highest protein content was observed in sample Y (17.40%), this was followed by Sample Z (12.69%) and the least was Sample X (7.49%). The highest protein content observed in sample Y suggest its source (animal source). Animal foods are the highest quality protein sources, animal protein are complete protein and they contain all essential amino acids in correct proportion. Proteins are important both in quantity and quality, for rapid growth and development of a child. The poor protein levels of traditional complementary foods have been a major concern in infant feeding. The use of this formulation could serve as a practical means of upgrading the protein levels of the traditional sorghum/maize based complementary foods.

Comment [W53]: Please restructure/rephrase done

Comment [W54]: Reference mine yet to be published

The crude fiber in all the samples was very low, the lowest was Sample X (0.49%), followed by Sample Y (1.05%) and the highest was Sample Z (3.14%) in that increasing order. The low crude fiber found in these raw materials is of importance because feeds that are high in fiber are less digestible than those low in fiber.

The fat content in these diet was low and the lowest was Sample X (1.59%) which was significant different ($p < 0.05$) from sample Y (6.72%) and Z (6.80%). However, there was no significant difference ($p > 0.05$) between Sample Y and Z. Fat in food increases the energy density, fat can also provide essential fatty acids like that of n-3 and n-6 Polyunsaturated Fatty Acids (PUFA's) needed to ensure proper neural development. Even though the fatty acid composition of these local diets were not determined, research carried out by [14] and his colleagues on the fatty acid composition of some Nigerian weaning foods, revealed that weaning foods are devoid of arachidonic and decosahexanoic acids, but high in linoleic and linolenic acids.

Comment [W55]: Please restructure this sentence and provide reference

Comment [W56]: By whom?

The low crude fiber content in all these samples is nutritionally appreciated except Sample Z (3.14%) which had the highest, next was sample Y (1.05%) while the least was X (0.49%). The crude fiber content of infant foods is expected to be below [13] as food with high fiber content tends to cause indigestion in infants. Hence, samples with low fiber content were rated good as potential complementary foods.

The highest carbohydrate was reported in Sample X (78.41%), this was followed by Sample Y (62.94%) and the least was Sample Y (59.34%) in a decreasing order. Foods high in carbohydrates are an important part of a healthy diet. Carbohydrate provides the body with glucose, which is converted to energy used to support bodily functions and physical activity.

Table 3: Proximate composition of the diets

Proximate Composition (%)	A	I	L	M	O	SEM
Moisture	10.71 ^b	11.73 ^a	10.42 ^c	10.72 ^b	10.41 ^c	0.02
Protein	18.10 ^d	19.35 ^c	26.35 ^a	23.09 ^b	23.06 ^b	0.02
Ash	3.30 ^d	3.01 ^e	5.35 ^a	4.26 ^b	3.60 ^c	0.04
Fat	7.89 ^c	5.67 ^e	8.82 ^a	7.05 ^d	8.10 ^b	0.03
Fiber	1.40 ^c	1.34 ^{cd}	2.32 ^a	1.64 ^b	1.29 ^d	0.02
Carbohydrate	58.61 ^b	58.92 ^a	46.76 ^e	53.26 ^d	53.56 ^c	0.04

Means with the same superscript along the rows are not significantly different ($p > 0.05$)

Sample A=100.0 sorghum, 20.0 crayfish, 5.0 garden egg

Sample I=113.64 sorghum, 25.0 crayfish, 7.50 garden egg

Sample L= 60.0 sorghum, 30.0 crayfish, 10.0 garden egg

Sample M= 80.0 sorghum, 25.0 crayfish, 11.70 garden egg

Sample O= 80.0 sorghum, 25.0 crayfish, 7.50 garden egg

Comment [W57]: Is it standard error mean or p value? **Standard error mean**

Comment [W58]: Not the correct way of reporting

The analysis of variance has showed significant difference ($p < 0.05$) in the proximate composition of the different diets.

The moisture content observed in the diets was very low. The lowest moisture content was observed in diet O (10.41%), it was not significantly different ($p > 0.05$) from diet L (10.42%), next was diet A (10.71%) which was not significantly different from diet M (10.72%) while the highest was diet I (11.73%).

The highest protein was from diet L (26.35%) and was significantly different from other diets, this was followed by diet M (23.09%) and was not significantly different ($p > 0.05$) from diet O (23.06%), next was diet I (19.35%) while the least protein was diet A (18.10%). Protein is essential for normal growth and development of children since they help the body to synthesize new tissues and repair worn out tissues. They are also components of hormones, enzymes and other vital processes in the body. The protein obtained in these formulated diets was high. Diets composed of cereals/legumes mixed with some animal protein source (10 – 20 %), have been reported to be sufficiently high in amino acids to meet recommended nutrient intakes (Fernandez *et al.*, 2002). There was significant difference ($p < 0.05$) in the ash content of the various diet.

Comment [W59]: Reference? **mine**

The highest ash content was observed in diet L (5.35%) and was significantly different ($p < 0.05$) from other diets, this was followed by diet M (4.26%), diet O (3.60%), diet A (3.30%) and diet I (3.01%). The high level of ash in the blends may indicate high amount of mineral in these blends.

There was significant difference ($p < 0.05$) in the fat content of the diets. Diet L (8.82%) had the highest fat content and was significantly different ($p < 0.05$) from other diets, next was diet O (8.10%), diet A (7.89%), M (7.05%) and diet I (5.67%) in that decreasing order. Fat provides essential fatty acids like that of n-3 and n-6 Polyunsaturated Fatty Acids (PUFA's) needed to ensure proper neural development. Even though the fatty acid composition of the formulated diets were not determined, research carried out by [14] on the fatty acid composition of some Nigerian weaning foods, revealed that the foods were devoid of arachidonic and decosahexanoic acids, but high in linoleic and linolenic acids.

Comment [W60]: By whom?

Diet L (2.32%) had the highest fiber content, next was diet M (1.64%), A (1.40%), I (1.34%) and the least diet O (1.29%).Crude fiber adds bulk to food to facilitate bowel movements (peristalsis) and prevent many gastrointestinal diseases

There was significant difference ($p < 0.05$) in the carbohydrate content of the diets. The highest was found in diet I (58.92%) and was significantly different ($p < 0.05$) from other diets, next was diet A (58.61%), O (53.56%), M (53.26%) and least L (46.76%) in that decreasing order.

Table 4: Mineral composition of the different diets

Minerals (mg/kg)	A	I	L	M	O	X	SEM
Potassium	6304.35 ^e	5827.06 ^f	9068.31 ^b	7915.66 ^c	7606.39 ^d	10159.49 ^a	0.404
Calcium	26158.86 ^b	16291.12 ^d	26591.10 ^a	23405.84 ^c	13298.34 ^e	6011.12 ^f	0.403
Sodium	6957.02 ^b	6704.73 ^c	8024.70 ^a	6321.70 ^e	6543.06 ^d	957.94 ^f	0.201
Iron	103.40 ^a	77.07 ^d	89.74 ^c	91.69 ^b	77.66 ^d	28.19 ^e	0.204

Means with the same superscript along the rows are not significantly different ($p > 0.05$)

Sample A=100.0 sorghum, 20.0 crayfish, 5.0 garden egg
Sample I=113.64 sorghum, 25.0 crayfish, 7.50 garden egg
Sample L= 60.0 sorghum, 30.0 crayfish, 10.0 garden egg
Sample M= 80.0 sorghum, 25.0 crayfish, 11.70 garden egg
Sample O= 80.0 sorghum, 25.0 crayfish, 7.50 garden egg

There was significant difference ($p < 0.05$) in the mineral composition of the diets. Minerals are vital to the functioning of many body processes and are critical players in nervous system functioning, other cellular processes, water balance, and structural (e.g. skeletal) systems [15].

The highest potassium was recorded in diet X (10159.49 mg/kg), next was diet L (9068.31 mg/kg), M (7915.66 mg/kg), O (7606.39 mg/kg), A (6304.35 mg/kg) and I (5827.06 mg/kg) in that decreasing order. Potassium is needed for regulating the water balance of cells, the use of carbohydrates and the building of proteins. It acts against disturbances of the cardiac rhythm and intervenes in the regulation of the osmotic pressure of the cell.

The highest calcium content was found in diet L (26591.10 mg/kg), next was A (26158.86 mg/kg), M (23405.84 mg/kg), I (16291.12 mg/kg), O (13298.34 mg/kg), and F (6011.12 mg/kg). Calcium plays a major role in the constitution of the skeleton, and also in various metabolic functions such as muscle activity, nerve stimuli, enzymatic and hormonal activities and oxygen transport [16].

Diet L (8024.70 mg/kg) had the highest sodium content, next was B (6957.02 mg/kg), I (6704.73 mg/kg), O (6543.06 mg/kg), M (6321.70 mg/kg) and X (957.94 mg/kg).

Comment [W61]: Is it standard error mean or p value? **Standard error of mean**

Comment [W62]: Not the correct way of reporting the results

Sodium is involved in the acid-base balance and the water balance of the body. It promotes nerve function and muscle contraction.

Diet A (103.40mg/kg) had the highest iron content, next was M (91.69mg/kg), L (89.74mg/kg) and O (77.66mg/kg), I (77.07mg/kg), and X (28.19mg/kg). Iron (Fe) is an essential nutrient in diets especially in neonate diets because it is essential for the development of baby's brain; it helps in neurological and cognitive development. It is very important that babies consume a steady supply of adequate iron at every stage of growth. Iron deficiency may hinder the development of the central nervous system. Iron is also involved in the formation of hemoglobin, myoglobin and enzymes play a key role in many metabolic reactions [17].

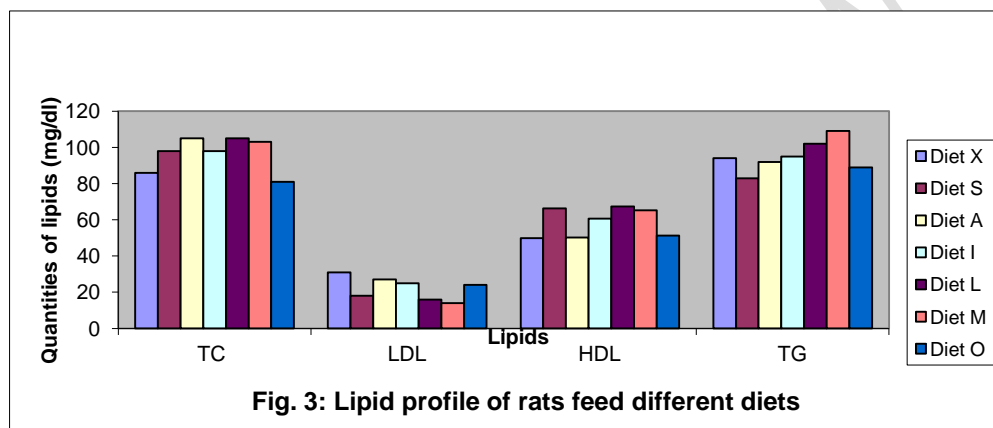
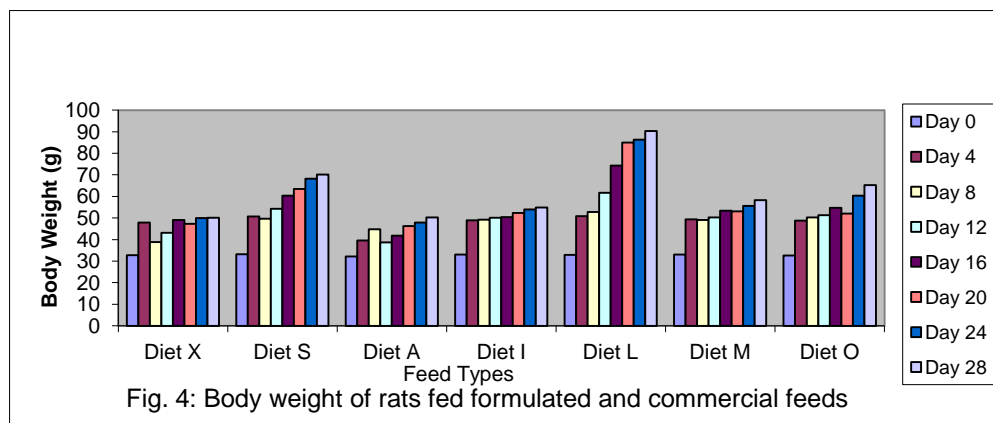


Fig. 3: Lipid profile of rats fed different diets

The analysis of variance showed significant differences ($p < 0.05$) in the lipid status of the various rats fed different diets (Figure 3). There was significant difference ($p < 0.05$) in the total cholesterol (TC) of rats fed different diets. Rat fed diet A had the highest (105.50mg/dl) TC but was not significantly different ($p > 0.05$) from TC of rats fed diet L (104.50mg/dl), TC of rat fed diet M (103.50mg/dl), TC of rat fed diet S (infant formula) 98.0mg/dl while TC of rat fed diet X (infant formula) had 86.50mg/dl in decreasing order. All the rats fed different diets had low values of low density lipoprotein (LDL). However, the lowest LDL was observed in rats fed diet M (14.0mg/dl), next was rats fed diet L (16.0mg/dl), S (17.50mg/dl), O (24.0mg/dl) while the highest LDL was seen in the control diet X (31.0mg/dl) in increasing order. Rats fed diet L had a significant ($p < 0.05$) increase in high density lipoprotein (HDL) (67.25mg/dl), followed by rats fed diet S (66.30mg/dl), M (65.30mg/dl), and the least HDL was observed in rats fed diet X (49.80mg/dl) (control). There was also significant difference ($p < 0.05$) in the Triglyceride (TG) of the various rats fed different diets. The rats fed diet L had the highest TG (67.25mg/dl), followed by rats fed diet S (66.30mg/dl), M (65.30mg/dl), I (60.70mg/dl), O (51.30mg/dl), A (50.20mg/dl), and X (49.80mg/dl) in decreasing order.



The analysis of variance has showed significant differences ($p < 0.05$) in the body weight of the rats fed formulated and commercial diets. The body weight of the rat at day 0 ranges between 32.10-33.85g, at day 4 (39.60-47.90g), at day 8 (38.85 – 52.75g), at day 12 (38.70 - 54.35g), at day 16 (41.80-74.25g), at day 20 (46.30 -84.85g) at day 24(47.85 -86.35g), and at day 28 (50.15 -90.35). At day 0, there was no significant difference ($p > 0.05$) in the body weight of the rat fed different diets; diet A (32.10g), I (33.10g), L(32.85g), M(33.15g), O (32.65g), S (33.20G), and X(32.75g).However, there was significant ($p < 0.05$) progressive increased in the body weight of the rats from 4 to 28 days. At 4 days, rats fed diet L (50.85g) had the highest bodyweight, this was followed by diet S (50.75g), M (49.30g), I (48.90g), O (48.75g), and the least was seen in diet A (39.60g). This trend was observed at 8 days but with diet X (38.85g) as the least. Throughout the period, rat fed diet L compete favorably with rats fed diet S(infant formula) and had significant ($p < 0.05$) body weight at day 8(52.75g while S= 49.60g), day 12 (61.70 while S=54.35g) day 16 (74.25g while S= 60.35g), day 20 (84.85g while S= 63.49g), day 24 (86.35 while S= 68.25g) and day 28 (90.35g while S= 70.10).

There was significant difference ($p < 0.05$) in the feed intake based on diets fed to the rats. At 4 days, rats fed diet I had the highest feed intake (53.50g), however, it was not significantly different from diets L (45.10g) and M (45.63g), next was diet S (43.20) and this was not significantly different from diet A (43,20g), the least feed intake was observed in diet X (35.60g).At 8 -28 days, the feed intake increased for diet L (62.83g - 72.90g), S (63.10-70.20g), M (62.56 -69.70g), A (56.20-60.40) and O (46.60-60.30g). Although, the feed intake increased for diet X from 8-24 days but with a decreased in the feed intake at 28 days (51.40g). It was obvious that the formulated diet had increased in the feed intake throughout the feeding period while the control, diet X (sorghum) had reduction in the feed intake at 28 days. The reduction in the feed intake at 28 days is an obvious indication of gradual dislike of the feed (sorghum).

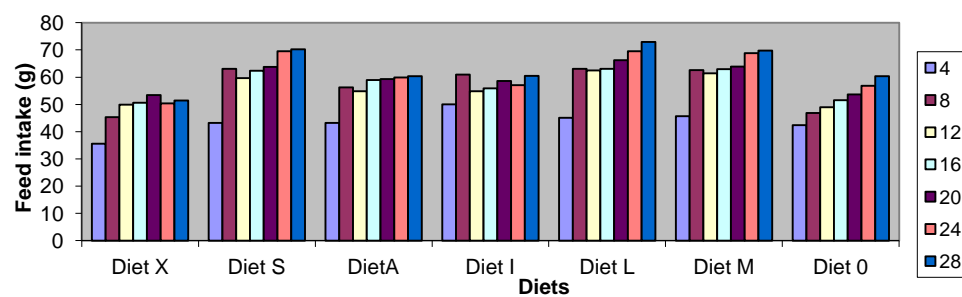


Figure 5: Feed intake of rats based on diets

Table 5: Hematological studies of rats fed different diets

Haematology	Formulated and commercial diets fed to rats							
A	I	L	M	O	S	X	SEM	
WBC(10^8 /L)		9.0x10 ^{8a}	7.0x10 ^{8a}	8.0x10 ^{8a}	10.0x10 ^{9a}	10.0x10 ^{9a}	9.0x10 ^{8a}	4.0x10 ^{8a} 3.2x10 ⁸
Neutrophils(%)		28.00 ^b	32.50 ^a	19.50 ^d	27.00 ^b	21.50 ^{cd}	23.50 ^c	32.50 ^a 0.945
Lymphocytes(%)		72.50 ^{ab}	77.50 ^a	72.50 ^{ab}	61.00 ^d	76.00 ^a	66.50 ^{bc}	51.00 ^e 2.041
Monocytes(%)		6.00 ^{ab}	8.00 ^a	5.50 ^{abc}	3.00 ^{cd}	2.50 ^d	4.50 ^{bcd}	5.00 ^{bcd} 0.756
Basophils(%)		0.50 ^a	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a	0.50 ^a	1.00 ^a 0.24
Eosinophils(%)		2.50 ^a	3.50 ^a	2.50 ^a	2.50 ^a	2.50 ^a	1.50 ^a	2.50 ^a 0.756
Haemoglobin(g/dl)		14.21 ^{ab}	13.34 ^{ab}	13.56 ^{ab}	13.27 ^{ab}	14.77 ^a	12.92 ^{ab}	12.81 ^b 0.527
PCV		42.00 ^a	43.00 ^a	38.00 ^a	40.00 ^a	41.00 ^a	37.00 ^a	38.00 ^a 1.704
Platelet(10^9 /L)		3.0x10 ^{9ab}	2.0x10 ^{9ab}	5.0x10 ^{9a}	4.0x10 ^{9ab}	3.0x10 ^{9ab}	4.0x10 ^{9a}	5.1x10 ^{8b} 8.8x10 ⁸
RBC(10^{12} /L)		4.0x10 ^{12bc}	6.0x10 ^{12ab}	5.0x10 ^{12ab}	6.0x10 ^{12a}	6.0x10 ^{12ab}	5.0x10 ^{12abc}	4.0x10 ^{12c} 3.1x10 ¹¹

Means with the same superscript along the rows are not significantly different ($p>0.05$)

Comment [W63]: Please use either haematology (US) or hematology (UK) throughout the manuscript and should avoid using both. **ok**

Comment [W64]: Is it standard error mean or p value?

Comment [W65]: Not the correct way of reporting the results

The analysis of variance has showed that there are significant differences ($p < 0.05$) in the haematological status of rat fed different diets. Hematological parameters are important indices of the physiological and pathological status for both animals and humans

There was no significant difference in ($p > 0.05$) in the WBC of rats fed different diets. The white blood cell count however cannot give a definite or specific information about infections, toxicity, allergy, immuno-suppression and poisoning but the result of a differential white blood cell count (Neutrophils, eosinophils, Monocyte, lymphocytes and Basophils) narrows down to give specific information [18].

However, the highest WBC counts were diets M and O with the same values ($10.0 \times 10^9/L$), next was A and S with the same values ($9.0 \times 10^8/L$), L ($8.0 \times 10^8/L$), I ($7.0 \times 10^8/L$) and X ($4.0 \times 10^8/L$) in that decreasing order.

Significant difference was observed ($p < 0.05$) in the neutrophils of the rat fed different diets. The highest neutrophils were in diets I and X with the same count (32.50%), next was A (28.0%), M (27.0%), S (23.50%), O (21.50%) and L (19.50%) in decreasing order. Neutrophils are mainly responsible for phagocytosis of pathogenic microorganism during the first few hours after their entry into tissues

For Lymphocytes, Diet I had the highest count (77.50%) and was not significantly different ($p > 0.05$) from diet O (76%), L (72.50%) and A (72.50%). Lymphocytes are involved in immunological functions, such as immunoglobulin production and modulation of immune defense [19]. They are type of white blood cell. Lymphocytes at excess levels suggest an infection or other inflammatory condition.

Significant difference ($p > 0.05$) was not observed in the monocytes of diet A (6%), I (8%), L (5.50%) and the least monocytes was sample O (2.60%). Monocytes are responsible for defense of tissues against microbial agents; It increases with bacterial infection and decreases with stress.

The basophils and Eosinophils counts in the different diets fed to rats were not significantly different ($p > 0.05$). The low basophiles could be as a result of some residual anti-nutrients present in the diet which must have affected these parameters. Basophils are chiefly responsible for allergic and antigen response by releasing the chemical histamine causing vasodilation (Lewis *et al.*, 2006).

Comment [W66]: Different citation style corrected

The Hemoglobin count was not significantly different ($p > 0.05$) in the rats fed with different diets except for diet X (12.81 g/dl). Hemoglobin plays the important role of carrying oxygen and carbon dioxide through the blood.

Significant difference ($p > 0.05$) was not recorded in the PCV results of the rats fed different diets. Packed cell volume (PCV) is important in the diagnosis of anemic condition. The highest PCV count was diet I (43%) next was A (42%), O (41%), M (40%), L (39%), X (38%) and S (37%).

Significant difference ($p > 0.05$) existed in the platelets count of the diets fed to rats except for diet X ($5.1 \times 10^8/L$). Platelets are blood cells responsible for clots to stop bleeding.

Significant difference ($p>0.05$) was not recorded in the RBC count of the diets fed to the rats except for diet X ($4.0 \times 10^{12}/L$). Red blood cells (RBC) are useful to diagnose anemic condition. The red blood cell count in this study was higher compared to the RBC obtained by (Lewis, 2006).

Comment [W67]: Different citation style corrected

Conclusion

This study has shown that affordable, available and acceptable complementary food could be produced from Sorghum, crayfish and garden egg. The formulated diets were able to compete favourably with the commercial diets. The results from this study showed that the formulated diets had low LDL, high HDL, high nutritional content which enhanced the growth rate, body weight, feed intake and better haematological properties all these would influence the growth of children and aimed at combating the problem of malnutrition among infants and children in Nigeria and other developing countries.

Comment [W68]: Have you done the price analysis also?

Comment [W69]: Have you done sensory evaluation test? Yes, it is contained in another paper

Ethical Approval

As per international standard and university standard, ethical approval has been collected and preserved by the authors

Comment [W70]: Please restructure the sentence and provide the ethical approval number (details) ok, thanks

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