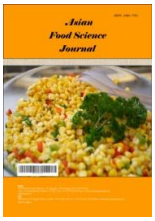

Asian Food Science Journal

20(6): 77-90, 2021; Article no.AFSJ.68675
ISSN: 2581-7752

Development, Evaluation and Safety Aspect of Enriched Weaning Food from Cereal, Legume and Vegetable

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/AFSJ/2021/v20i630311
Editor(s):
(1) Dr. Ho Lee Hoon, Universiti Sultan Zainal Abidin (UniSZA), Malaysia.
Reviewers:
(1) Reginold Jebitta, Kalasalingam University, India.
(2) Timur Chis Ovidius, University Constanta, Romania.
Complete Peer review History: http://www.sdiarticle4.com/review-history/68675

Original Research Article

Received 15 March 2021
Accepted 21 May 2021
Published 26 May 2021

ABSTRACT

This study examined the development, evaluation and safety aspect of enriched weaning food from cereal, legume and vegetable. Maize was fermented, soybean was defatted and carrot was sliced, and all were processed to flour. The flours were formulated to blends. Laboratory analysis were done such as proximate, minerals, haematology and lipid studies of rat fed both formulated and commercial diets as well as the growth and feed intake of the rats. The data obtained were analyzed. The results showed that the moisture content observed was low ranging from 5.07% - 12.91%. The lowest moisture (5.07%) was defatted soybean flour; this was followed by fermented maize flour (9.71%) while the highest moisture (12.91%) was seen in carrot powder. Carrot had the highest Ca (5850.0ppm), followed by defatted soybean (4700ppm), the least was maize (2900ppm). The highest count for white blood cell (WBC) was observed in rat fed rat pellet ie diet G ($11.8x10^9$). The high density lipoprotein (HDL) of rats fed different diets ranged from 31 to79mg/dl with group F (31.0 mg/dl) and C (79 mg/dl) which had the lowest and highest HDL respectively. At day 4, the highest (62.20g) feed intake was observed for rats that fed on diet C, next B (60.70 g), diet D (54.20 g), diet G (53.20 g), diet E (45.10 g), diet F (43.80 g). The least feed intake was diet A (39.6 g) in that decreasing order. This study has showed that diet C was outstanding among the diets and should be used to improve the body weight of infant, increased the HDL, reduced LDL, improves haematological parameters and improves nutritional qualities.

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Keywords: Weaning; food; cereal; legume; vegetable.

1. INTRODUCTION

The problem of infant malnutrition in the developing world has stirred efforts in research, development and extension by local and international organizations. Consequently, the formulation and development of weaning foods from local and readily available materials have received a lot of attention in many countries.

Malnutrition a major health problem in developing countries and contributes to infant death, low physical and intellectual development of infants, as well as low resistance to diseases and consequently stifles development. Protein-energy malnutrition occurs during transitional phase when children are weaned from liquid to semi-solid or fully adult foods. During this period, children need nutritionally balanced, calorie-dense supplementary foods in addition to mother's milk because of the increasing nutritional demands of the growing body [1,2].

Complementary foods are foods other than breast milk or infant formula (liquids, semisolids, and solids) given to infant to provide nutrients [3]. Complementary foods are mostly produced from food such as, cereals (wheat, maize and rice,) legumes (such as soybeans, cowpeas) etc. Formulation of complementary foods can be made by one or combination of more than one plant product (cereal such as maize, millet and sorghum combined with legume such as cowpea, groundnut, soybeans etc).

Maize (*Zea mays*) is prepared and consumed in a variety of ways and its economic value is increased through the development of technologies which process it into value added products and thus promoting its production and consumption. Fermentation of maize has some advantages. It helps in the introduction of probiotic bacteria so by consuming fermented foods, beneficial bacteria and enzymes are being added to overall intestinal flora for important health benefits [4]. The breakdown of some of the sugars and starches in food during fermentation makes for easy digestibility of fermented foods [5,6]. Other advantages of fermentation include the increase in availability of vitamins and minerals and the removal of some natural compounds that interfere with the absorption of nutrients [7].

One major ways of using maize is by fermenting into pap (cereal gruel often used as complementary food). Maize starch can be used for products such as custard (vanilla flavoured corn starch) often given as complementary food to infants. The disadvantage of ogi is that the traditional production process has been implicated in nutrient losses [8]. On the other hand, germination (malting or sprouting) is a simple and traditional method that can be used at home. It involves hydrating and holding the grains at room temperature to germinate. During germination, both endogenous and newly synthesized enzymes begin to modify seed constituents, starch; protein and fat are hydrolysied by amylolytic, proteolytic and lipolytic enzymes, respectively [9]. This non-thermal process has the advantages of reduction of anti-nutritional factors, including phenolic compounds, phytic acid, trypsin inhibitors and oligosaccharides [10], enhancement of organoleptic qualities due to a softening of texture and an increase in the flavour of various cereals [11],

Soybean (*Glycine max*) a legume species common in East Asia, widely grown for its edible bean with numerous uses [12]. Soy beans have low content of carbohydrates and high content of proteins, and furthermore, contain a number of health promoting compounds. Together, soybean oil and protein content add up to 60% of the dry beans by weight (protein at 40% and oil at 20%). The remainder makes up 35% carbohydrate and about 5% ash. Soybean is made up of 8% seed coat or hull, 90% cotyledons and 2% hypocotyl axis or germ [12].

Carrot (*Dacus carota*) is very important crop of *Apiaceae* family. It is a root vegetable with distribution. Carrot were first used for medical purposes and gradually turned food. Written records in Europe indicated that they were cultivated prior to the tenth century. The colours of carrot flesh may be white, yellow, orange, red, purple, or very dark purple [13]. Carrot is an excellent source of antioxidant compounds with the richest vegetable source of the pro-vitamin A carotenes. Carrots' antioxidant compounds are useful in the protection against cardiovascular disease and cancer and also enhance good vision, especially night vision. They have a unique combination of three flavonoids: kaempferol, quercetin and luteolin [14]. They are also rich in other phenols [15]. This study aimed at the production, evaluation, and safety aspect of enriched weaning food produced from cereal, legume and vegetable.

2. MATERIALS AND METHODES

Source of materials: Yellow maize (*Zea mays*), soybean (*Glycine max)* and carrot ((*Dacus carota*) were purchased in Benin and Auchi market, Edo State, Nigeria.

2.1 Preparation of Fermented maize Flour

Two and half (2.5) kilograms of maize was prepared by the traditional wet milling process. In this process, the maize were sorted, washed and steeped in sufficient water at room temperature for 72 hours. The water for steeping was changed daily and on the 3rd day, it was drained and wet milled with a disc attrition mill. The wet milled slurry/ gruel was sieved using a muslin cloth. The slurry was allowed to settle overnight and the supernatant decanted. The wet cake was recovered by squeezing excess water with cheese cloth and sun-dried for three days. It was later dried in a cabinet drier at 50°C for 8 hours. The dried meal was dried-milled with a hammer mill and sieved. The fermented maize flour were packed in cellophane and stored in a cool dry place until needed for product formulation.

2.2 Preparation of Carrot Powder

One (1) kilogram of carrot was washed with water to remove soil. The cleaned carrots were diced and sun- dried. The sun-dried carrot was further dried using cabinet drier at 50°C for eight hours, and thereafter were dry-milled with hammer mill and then sieved. The dried carrot meal was packed in cellophane for product formulation.

2.3 *In vitro* Starch Digestibility (IVSD)

The IVSD was determined according to the method described by [16]. 50mg each of the samples was mixed with 1ml of 0.2M phosphate buffer (pH 6.9). 0.5 ml of pancreatic alpha amylase (100 unit /mg) was added to the sample and incubated at 37°C for 2hrs. After incubation, 2ml of 3.5-DNS reagent was added immediately. The mixture was heated for 5-15mins in a boiling water bath. After heating, 1.0 ml of 40% potassium- sodium tartarate solution was added to the rest test tubes and allowed to cool at room temperature (35°C). The solution was filtered through 0.45 nm filter and made up to 25 ml with distilled water. Absorbance was measured at a wavelength of 550nm. A blank was run simultaneously. A standard curve was prepared using maltose and the values were expressed as mg maltose per 100 mg of sample

2.4 Proximate Analysis

Proximate analyses were carried out on the samples using standard [17] methods. Moisture content was calculated after drying at 105°C to constant weight in an air oven (Thermo Scientific-UT 6200, Germany). Lipids were estimated by exhaustive extraction of known weight of samples with petroleum ether using rapid Soxhlet extraction apparatus (Gerhardt Soxtherm SE- 416, Germany). Determination of protein was by Kjeldahl method. The efficiency of the nitrogen values were corrected with acetanilide values and multiplied by the factor of 6.25 to obtain the protein value. Ash was determined gravimetrically after incineration in a muffle furnace (Carbolite AAF-11/18, UK) for 24 h at 550°C. Crude fibre was obtained by difference after the incineration of the ash-less filter paper containing the insoluble materials from the hydrolysis and washing of moisture free defatted sample (0.5 g). Carbohydrate content was determined by difference: 100% - (% MC + % Ash + % Crude protein + % Fat + % Crude fibre).

Table 1. Formulation of fermented maize, defatted soybean and carrot complementary food

	Levels of substitution		
Sample Id	**Fermented maize flour**	**Defatted soy flour**	**Carrot powder**
A	100.00	0.00	0.00
B	85.00	10.00	5.00
C	60.00	30.00	10.00
D	65.00	20.00	15.00
E	50.00	50.00	0.00

2.5 Determination of Mineral Composition

The method of [17] was used to determine the mineral composition of the composite flours. The minerals analyzed for were Potassium, Calcium, Sodium, and Iron

2.6 Determination of Vitamin

Vitamin A was analyzed using the high performance liquid chromatography (HPLC) method described by [18]. Ascorbic acid was extracted with metaphosphoric acid and acetic acid and quantified by fluorometric analysis according to the method of AOAC (1995). All samples were analyzed in triplicate.

2.7 Animal Husbandry and Experimental Design

Thirty five healthy Wistar rats with mean weight of 38.1-50.9 g 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 3 days in well ventilated cages. The rats were divided into seven groups of 5 animals each. Each of the rats was given an identification mark in form of an indelible mark on tail, head and back. The rats in each group were fed with either formulated or commercial diet (Cerelac). The five formulated diets include Sample A (100% fermented maize flour), Sample B (Maize 85%, soybean 10% and carrot 5%), Sample C (maize 60%, soybean 30% and carrot 10%), Sample D (maize 65%, soybean 20% and carrot 15%), Sample E (maize 50% and soy bean 50%) (Table1). Samples F and G were used as control (Cerelac and growers mash respectively). The rats were acclimatized on a commercial diet (rat pellet) and water *ad libitum* for 3 days prior to commencement of the experiment which lasted for 28 days.

Growth performance study: The growth Performance was studied with the method described by [19].

Feed Intake (FI)

This is the sum of the quantity of feed consumed by the rats for 28 days.

2.8 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 [20].

2.9 The Red Blood Cells Count was determined by Haemocytometry Method

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

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

2.10 White Blood Cells (WBC) or Total Leucocyte 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 11 mark. The fluid and blood were mixed well and the first few drops of blood were discarded by holding the pipette vertically. The counting chamber was charged by holding the pipette vertically. The counting chamber 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.

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

2.11 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. Therea fter, the centrifuge was spuned and the tubes were removed and the percentage packed volume was read using micro-haematocrit reader according to the method [20].

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 Neubaucer counting chamber. Their characteristic Mauve-pink colour was used in their identification.

Determination of haemoglobin estimation: The conventional method (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 left for 5 minutes (for the haemoglobin to be changed into acid haematin). More acid was thereafter added and the mixture was stirred until the colour of the test solution matched that of the coloured glass standard. The level of the fluid in the tube was read and the haemoglobin content was expressed in percentage.

Determination of Leucocytes (differential white blood cell count): The differential white blood cell count (Neutrophils, Lymphocytes, monocytes, Eosinophils and Basophils) was done by making a thin film of blood on a smooth edged slide. It was allowed to dry on a bench protected from dust, ants, flies, and other insects. The blood film was fixed 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 thoroughly and the smear was covered with the dilute May Grunwald stain for 3 minutes. The stain was tipped off and replaced with diluted Giemsa's stain (5%) for 9 minutes. The stain was washed off with buffered water (pH 6.8) and clean water was dropped on the slide which was allowed to stay for 30 seconds. The water was tipped off and the slide was allowed to dry. It was then examined microscopically (McArthur microscope) for the identification of Neutrophils (cytoplasm stained pink with small mauve granules), Eosinophils (cytoplasm stained pink with large red granules), Basophils (cytoplasm contained dark mauve-blue granules) Monocytes (cytoplasm stained dull grey-blue) while lymphocytes (cytoplasm stained blue).

2.12 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, 8^{TH} edition (Genstat Procedure Library Release PL16). Also, data were represented by simple descriptive bar chart.

3. RESULTS AND DISCUSSION

There was significant difference ($p<0.05$) in the proximate composition of plant raw materials used in this study (Table 2). The moisture content observed in this study was low ranging from 5.07% -12.91%. The lowest moisture (5.07%) was defatted soybean flour; this was followed by fermented maize flour (9.71%) while the highest moisture (12.91%) was seen in carrot powder. The results for yellow fermented maize flour (9.71%) was lower compared to 13.67% reported by [21] also lower than 12% moisture reported by [22]. The low moisture content in this study suggests moderate moisture level needed for long storage period. Raw material with high moisture content is prone to deterioration and microbial infestation. For protein, soybean had the highest amount of protein and was significantly different ($p<0.05$) from that of carrot powder (10.63%) and the least was fermented maize (6.14%). The ash content showed that soybean had the highest value (8.87%), next was Carrot powder (7.07%) and the least was fermented maize (1.13%) in decreasing order. Carrot powder had the highest fiber (6.50%), followed by soybean flour (2.46%), the least fiber was seen in fermented maize (1.09%) although relatively low, but the presence of fiber in foods is known to be beneficial. Fiber has some physiological effects in the gastrointestinal, tract. These effects include variation in faecal water, faecal bulk, elimination of bile acids and neutral steroids which lower the body cholesterol pool. Fat content was higher in soybean flour (14.11%) compare to others; this suggests that soya bean

may be a viable source of oil, going by their crude fat contents.

The carbohydrate content (70.54%) in maize significantly increased ($p<0.05$) compared to soybean flour (28.295) and carrot powder (57.70%). The high carbohydrate contents70.54% observed in maize suggests that the flour could be used in managing protein-energy malnutrition since there is enough quantity of carbohydrate to derive energy from, in order to spare protein so that protein can be used for its primary function of building the body and repairing worn out tissues rather than as a source of energy. Besides, maize is mostly a carbohydrate based food hence, it was not out of place for the non substituted (100% whole maize) to have high carbohydrate content compared to others.

There was significant difference ($p<0.05$) in the mineral composition of the raw plant materials (Maize, soybean and carrot) used in this study. Carrot had the highest Ca (5850.0ppm), followed by defatted soybean (4700mmm), the least was maize (2900ppm). This trend was also seen in Potassium, sodium and vitamin A (Table 3). The highest Fe content (126) was observed in defatted soybean, and was significantly different ($p<0.05$) from fermented maize and carrot which had 0.0 and 20.0 respectively. This trend was observed in Phosphorus with defatted soybean having 2400ppm, carrot (1700.46ppm) and fermented maize the least (20.0ppm). There was significant difference ($p<0.05$) in the energy values of the raw materials used in this study. The highest energy value was recorded in fermented maize (407.7Kcal/100g), this was followed by soybean (344.9 Kcal/100g) and the least energy (313.6 Kcal/100g) was observed in carrot. The highest energy value of fermented maize in this study was expected because maize is a carbohydrate based food.

There was significant difference ($p<0.05$) in the mineral composition of the raw plant materials (Maize, soybean and carrot) used in this study. Carrot had the highest Ca (5850.0ppm), followed by defatted soybean (4700mmm), the least was maize (2900ppm). This trend was also seen in Potassium, sodium and vitamin A (Table 3). The highest Fe content (126 ppm) was observed in defatted soybean, and was significantly different ($p<0.05$) from fermented maize and carrot which had 0.0 and 20.0 respectively. This trend was observed in Phosphorus with defatted soybean having 2400ppm, carrot (1700.46ppm) and fermented maize the least (20.0ppm).

Table 2. Proximate composition of raw plant materials used

	Treatment			
Proximate parameter	**Fermented maize**	**Defatted Soybean**	**Carrot**	**SED**
Moisture (%)	9.71^{b}	5.07^{c}	12.91^{a}	0.03
Protein (%)	6.14^{c}	41.2^{a}	10.63^{b}	0.02
Ash (%)	1.13^{c}	8.87^{a}	7.07^{b}	0.02
Fiber (%)	1.09c	2.46^{b}	6.50^{a}	0.01
Fat (%)	11.39^{b}	14.11^{a}	5.19^{c}	0.03
Carbohydrate (%)	70.54^{a}	28.29^{c}	57.70^{b}	0.05
Energy (Kcal/100g)	407.7^{a}	344.9^{b}	313.6^{c}	0.47

Means with the same superscript along the rows are not significantly different (p>0.05); SED=Standard error of difference of means

Table 3. Mineral composition of raw plant materials used

	Treatment			
Mineral composition (ppm)	**Fermented maize**	**Defatted soybean**	**Carrot**	**SED**
Calcium (Ca)	2900^{c}	4700^{b}	5850^{a}	47.1
Potassium (K)	1100^{c}	21700^{b}	24800^{a}	47.1
Sodium (Na)	177.0^{c}	240.0^{b}	2425.0^{a}	14.52
Iron (Fe)	0.0^{c}	126.0^{a}	20.0^{b}	2.62
Zinc (Zn)	4.00^{a}	23.0^{a}	13.0^{b}	1.89
Phosphorus (P)	20.0^{c}	2400.25^{a}	1700.46^{b}	0.94
Vitamin A (uit/g)	163.5^{c}	262.09^{b}	846.56^{a}	2.13

Means with the same superscript along the rows are not significantly different (p>0.05); SED=Standard error difference of means

There was significant increase ($p<0.05$) in ash content of the samples with increasing substitution of soy and carrot. Sample E had 4.93%, C (2.66%), D (1.79%) B (1.69%), the least ash content was A (0.27%) which had no soy and carrot in the formulation (Table 1). The ash values ranged between 0.27 - 2.66%. It was higher than 0.56-2.00% of complementary food formulated from fermented maize, soybean and carrot flours [23]

Fibre content of these samples was low ranging from 0.31% - 2.19%. This range is within the fibre content [24,25]. Fibre plays a role in the increased and utilization of nitrogen and absorption of some other micronutrients. The low fibre content is in agreement that food used for complementary feeding should contain low fibre as high fibre can lead to high water absorption and displacement of nutrient and energy needed for the growth of children less than two years [26,27].

There was significant difference ($p<0.05$) in the fat content of the flour blends. The highest was observed in sample E (11.74%), next was sample D (11.26%), A (11.10%), B (10.19%) and sample C (10.14%) in that decreasing order. Fat is important in the diets of infants and young children as it provides high energy density and facilitates the absorption of fat soluble vitamins. It also provides essential fatty acids such as omega-3 and omega-6 polyunsaturated fatty acids (PUFA) needed for proper neural development in infants and young children [28]. Low fat is beneficial as it ensures long product shelf stability by reducing susceptibility to oxidative rancidity. There was significant difference ($p<0.05$) among the blends based on energy value. The highest energy value was observed in Sample A (413.7Kcal/100g), it was followed by Sample B (391.8 Kcal/100g), Sample D (388.4 Kcal/100g), Sample C (373.6Kcal/100g) and E (369.4Kcal/100g) in that decreasing order. The highest energy observed in Sample A was expected because it contained 100% whole maize (Table 1) which is carbohydrate based food and it agreed with the highest energy value also reported in Table 2.

The analysis of variance showed significant difference ($p<0.05$) in the mineral content of the blends (Table 5). The results showed that sample A consisting of 100% maize had the highest content of Ca (4000.0 ppm), this was followed by Sample D (1850 ppm), Sample C (850ppm), Sample E (333 ppm) and Sample B (300 ppm) in that decreasing order. Calcium plays a vital role in structural and physiological functions. It is present more abundantly in the body. It helps for the development of strong bones and teeth.

Sample E had the highest amount of K (13700 ppm), next was Sample B (11850ppm), and the least was Sample C (542.0 ppm), Potassium keeps muscles and nervous system working properly. Sodium content ranges from 219.0 to 550.0 ppm. Sodium is both an electrolyte and mineral. It helps to keep the water (the amount of fluid inside and outside the body's cells) and electrolyte balance of the body. Sodium is also important in how nerves and muscles work. Fe ranges from 4.0 to 71.0 ppm. Iron is essential for the formation of blood cells and prevention of anaemia in infants and children. The body needs iron to transport oxygen from lungs to the rest of the body. Zn ranges from 5.0 to 17.0 ppm. Zinc helps the body immune system to fight off illnesses and infections, helps with cell growth and helps heal wounds, such as cuts. Phosphorus ranges from 1795.07 to 378.80 ppm. Phosphorus (P) is the second most abundant mineral in the body and approximately 80% is found in the teeth and bones [29]. Vitamin A

Table 4. Proximate composition of flour blends

	Sample					
Proximate Composition	**A**	**B**	**C**	**D**	**E**	**SED**
Moisture (%)	9.68^{c}	11.05^{a}	9.45^{d}	10.21^{b}	7.91^{e}	0.02
Protein(%)	5.69^{e}	10.03^{d}	18.11^{b}	14.44^{b}	26.78^{a}	0.03
Ash(%)	0.27^{e}	1.69^{d}	2.66^{b}	1.79^{c}	4.93^{a}	0.02
Fiber(%)	0.44^{d}	0.31^{e}	2.19^{a}	1.28^{b}	0.89^{c}	0.02
Fat(%)	11.10^{c}	10.19^{d}	10.14^{d}	11.26^{b}	11.74^{a}	0.04
Carbohydrate (%)	72.82^{a}	66.73^{b}	57.44^{d}	61.03^{d}	47.84^{e}	0.06
Energy (Kcal/100g)	413.7^{a}	391.8^{b}	373.6^{d}	388.4^{c}	369.4^{e}	0.73

Means with the same superscript along the rows are not significantly different (p>0.05); SED=Standard error difference of means

Table 5. Mineral and pH composition of flour blends

	Minerals (ppm)							
Samples	**Ca**	**K**	**Na**	**Fe**	**Zn**	**P**	**Vit A (uit/g)**	**pH**
A	4000.0^{a}	2117.0^{d}	432.7^{c}	15.0^{d}	7.0^{c}	500.73^{d}	388^{e}	3.81^{e}
B	300.0^{d}	11850.0^{a}	491.0^{b}	46.0^{b}	13.0^{ab}	1432.02^{b}	976^{d}	4.38^{d}
C	850.0^{c}	542.0^{d}	219.0^{e}	4.0^{e}	5.0^{c}	378.80^{e}	1155^{c}	5.67^{b}
D	1850.0^{b}	9250.0^{c}	550.0^{a}	35.0^{c}	9.0^{bc}	900.01^{c}	1369^{b}	5.44^{c}
E	333.0^{d}	13700.0^{a}	357.0^{d}	71.0^{a}	17.0^{a}	1795.07^{a}	1858^{a}	6.09^{a}
SED	65.2	1054.2	4.90	2.11	2.03	2.83	0.36	0.01

Means with the same superscript down the column are not significantly different (p>0.05); SED=Standard error difference of means

ranges from 388.0 to 1858.0ppm. Vitamins A increased with increasing amount of carrot and soybeans inclusion. The pH ranges from 3.81 to 6.09, this reflect that these flours are shelf stable and could be stored for a longer time without deterioration and insect infestation.

Hematological parameters are important indices of the physiological and pathological status for both animals and humans [30]. It can also be used to determine the extent of deleterious effect of foreign compounds, including plant extracts, on the blood of the albino rats [31]. Heamatological parameters can be used to explain blood relating functions of plant extract or its products [31,32 and 20].

Table 6 below showed the haematological results of Wistar rats fed different diets. There were significant differences ($p<0.05$) in the haematological parameters evaluated.

The highest count for white blood cell (WBC) was observed in rat fed rat pellet ie diet G ($11.8x10^{9}$). However, this was not significantly different ($p>0.05$) from WBC of rat fed diet D ($11.5x10^{9}$), next was diet F ($8.9x10^{9}$) which was commercial diet (control) , then diet C ($7.34x10^{9}$), diet E ($7.14x10^{9/}$L), while the least count was rat fed diet B ($1.1x10^{9/}$L) but was not significantly different from WBC of rat fed diet A ($4.3x10^{9}$L). This experiment revealed that the WBC of rat fed diet D (65% fermented maize, 20% soybean and 15% carrot) was not significantly different ($p>0.05$) and it compete favourably with the commercial diet (cerelac). This could be attributed to its highest amount of carrot (15%) in the formulation (Table 1) reflecting higher vitamin A (848.56uit/g) (Table 3) influencing the WBC. WBC is important in defending the body against infection [33]. 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 (Neutrophiles, eosinophiles, Monocyte, lymphocytes and Basophiles) narrows down to give specific information [33]. The function of lymphocytes is primarily its involvement in a variety of immunological functions, such as immunoglobulin production and modulation of immune defense [34].

There was no significant difference ($p>0.05$) in the neutrophils of rat fed diet A (34%), B (30%) and E (35%). For lymphocytes, the highest count was in rats fed diet B but was not significantly different ($p>0.05$) from lymphocyte of rats fed diet C (75%), E (76%) F (70%) and G(76%). Neutrophiles is mainly responsible for phagocytosis of pathogenic micro organism during the first few hours after their entry into tissues [33].

The highest count for monocytes was seen in rats fed diet E (9%) and was significantly different ($p<0.05$) from monocytes of other groups. This was however, followed by monocytes of rats fed diet A (8%), next was diet B (6%), diet C (5%) in that decreasing order. Rats fed diet D (4%) was not significantly different ($p>0.05$) from those of group F (4%). Monocytes are responsible for defense of tissues against microbial agents; It increases with bacterial infection and decreases with stress [35].

Basophiles counts increase upon sensitization to an antigen (or allergen). There was no significant difference ($p>0.05$) in the basophils of rats fed diet B(0%) and rats fed diet F (0%). Also, there was no significant difference ($p>0.05$) in the basophils of rats fed diet A (1%),

C (1%), D (1%) and E (1%). Diet G had the highest basophils count (4%) which was significantly different ($p<0.05$) from other groups. The low basophiles could be as a result of some residual anti-nutrients present in the diet which must have affected this parameters. Basophils are chiefly responsible for allergic and antigen response by releasing the chemical histamine causing vasodilation [35].

The highest eosinophils was recorded in rats fed diet A (3%) and was not significantly different ($p>0.05$) from rats fed diet C (3%) and diet E (3%). Also no significant difference ($P>0.05$) was recorded for eosinophils of rats fed diet B (2%) from Diet D (2%) and diet G (2%). Eosinophiles are responsible for allergic reactions and disorders, It increases with allergic conditions and decreases with stress and/or infection [35,33 and 31].

The highest haemoglobin (Hb) was recorded in rats fed diet E (14g/dl) and was not significantly different ($p>0.05$) from rats fed diet B (13.60 g/dl), C (13.10g/dl) and diet F (12.70g/dl). The least was recorded in rats fed diet A (11.70g/dl).The highest haemoglobin in rats fed diet E (14g/dl) could be attributed to the high amount (50%) of soybean in the diet (Table 1) and the increase in the protein 41.2% of defatted soybean (Table2)which could have influenced the Fe content 126ppm (Table 3). Heme consists of iron and a pigment called porphyrin, which gives blood its red colour. Hemoglobin plays the important role of carrying oxygen and carbon dioxide through the blood.

Higher packed cell volume (PCV) which is important in the diagnosis of anemic condition increased significantly ($p < 0.05$). It was observed that rats fed diet B had the highest PCV (43%), next was E (42%), D and G (41%) no significant difference ($p>0.05$), F and C same value (38.0%) no significant difference ($p>0.05$), the least was rat fed diet A (35%) in that decreasing order.

Platelets count showed no significant difference ($p>0.05$) between rats fed diet C ($5.72x10^9$/L) and rats fed diet F ($4.78x10^9$/L), next B ($3.95x10^9$/L), diet E ($3.88x10^9$/l) and D ($3.21x10^9$/L). The least platelet was in rats fed diet A ($2.0x10^9$/L) in that decreasing order. Platelets are tiny blood cells that help the body form clots to stop bleeding.

Red blood cells (RBC) which are important in the diagnosis of anaemic condition increased significantly ($p < 0.05$). The highest red blood cell (RBC) count was observed in rats fed diet D ($7.13x10^{12}$), this was not significantly different ($p>0.05$) from RBC of rat in other groups. However, the least RBC count was in rat fed diet A ($4.3x10^{12}$). The red blood cell count in this study was higher compared to the RBC obtained by [35].

From this study, it was obvious that PCV, Hb and RBC increased with increasing amount of soybean and carrot, this may possibly have been influenced by the amount of minerals present including iron (Fe) and Copper (Cu) (Table 5) which is important in haemoglobin synthesis. Besides, it is also an indication that these rats never suffer from anaemic condition.

The analysis of variance showed significant difference ($p<0.05$) in the lipid profile (Fig. 1) of the rats fed different diets. The total cholesterol (TC) showed that rats with the lowest TC was observed in rats fed diet F (76.0mg/dl) and was significantly different ($p<0.05$) from rats fed other diets. The next in increasing order were diet G (81mg/dl), A (86.0mg/dl), E (86mg/dl), B (111.0mg/dl), C (124mg/dl) and D (126.0mg/dl). The TC of rat fed diet A (86mg/dl) was not significantly different ($p>0.05$) from rats fed diet E (86mg/dl). Total cholesterol is the total amount of cholesterol in your blood.

The high density lipoprotein (HDL) of rats fed different diets ranged from 31 to79mg/dl with group F (31.0 mg/dl) and C (79 mg/dl) which had the lowest and highest HDL respectively, and this was significantly different ($p<0.05$) from others. HDL of group B (69mg/dl) was not significantly different ($p>0.05$) from HDL of group D (65.33mg/dl)

The Low density lipoprotein (LDL) of rats were significantly low ($p<0.05$) with rats fed diet E the lowest, this was followed in increasing order by diet G (24mg/dl), diet A(25mg/dl), diet F (26mg/dl), diet B (28mg/dl), diet C (31mg/dl) and diet D(39mg/dl). The LDL observed in these rats was very low and it ranged from 18.0 mg/dl to 39.0 mg/dl.

There was significant difference ($p<0.05$) in the triglycerides (TG) of rats based on the diets. The TG ranges from 72 mg/dl to 102 mg/dl. There was no significant difference ($p>0.05$) in the TG of rats fed diet A (75mg/dl), diet B (72mg/dl) and diet C (75mg/dl).

Table 6. Haematological studies of wistar rat fed different diets

	Formulated and commercial diets fed to rats							
Haematology	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**SED**
WBC (10^9/L)	$4.3x10^{9cd}$	$1.1x10^{9d}$	$7.34x10^{9bc}$	$11.5\ x10^{9a}$	$7.14x10^{9bc}$	$8.9x10^{9ab}$	$11.8\ x10^{9a}$	$1.78x10^9$
Neutrophils (%)	34.00^a	30.00^{ab}	21.00^{cd}	28.00^b	35.00^a	25.00^{bc}	18.00^d	2.30
Lmyphocyts (%)	54.0^c	80.0^a	75.0^{ab}	67.0^b	76.0^{ab}	70.0^{ab}	76.0^{ab}	5.09
Monocytes (%)	8.00^b	6.00^c	5.00^d	4.00^e	9.00^a	4.00^e	3.00^f	$0.3X10^4$
Basophils (%)	1.00^{ab}	0.00^b	1.00^{ab}	1.00^{ab}	1.00^{ab}	0.00^b	4.00^a	$1.6x10^3$
Eosinophils (%)	3.00^a	2.00^{ab}	3.00^a	2.00^{ab}	3.00^a	1.00^c	2.00^{ab}	$0.7x10^3$
Haemoglobin (g/dl)	11.70^c	13.60^{ab}	13.10^{ab}	13.20^{ab}	14.00^a	12.70^{abc}	12.30^{bc}	$0.5x10^3$
PCV (%)	35.00^c	43.00^a	38.00^{bc}	41.00^{ab}	42.00^a	38.00^{bc}	41.00^{ab}	$1.4X10^3$
Platelet (10^9/L)	$2.0x10^{9d}$	$3.95x10^{9bc}$	$5.72x10^{9a}$	$3.21x10^{9cd}$	$3.88x10^{9bc}$	$4.78x10^{9ab}$	$2.66x10^{9cd}$	$6.13x10^9$
Rbc (10^{12}L)	$4.35x10^{12a}$	$5.82x10^{12a}$	$6.48x10^{12a}$	$7.13x10^{12a}$	$6.11x10^{12a}$	$6.64x10^{12a}$	$4.79x10^{12a}$	$1.2x10^{12}$

Means with the same superscript along the rows are not significantly different (p>0.05); Group A rat fed whole maize 100%; Group B rats fed 85% maize, 10%soybean and 5%carrot; Group C rat fed 60% maize, 30%soybean and 10%carrot; Group D rats fed 65%maize, 20%soybean and 15% carrot; Group E rats fed 50%maize and 50%soybean, Group F rats fed Cerelac (control); Group G rats fed rat pellet (control); SED=Standard error difference of means

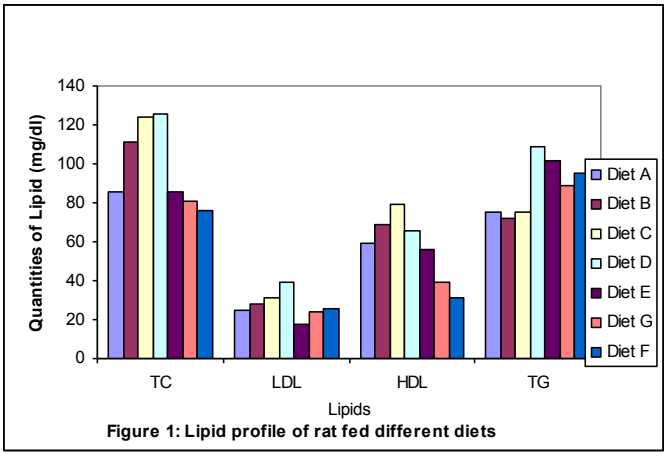

Fig. 1. Lipid profile of rat fed different diets

From the bar chart above (Fig. 2), there was significant increase ($p<0.05$) in the body weight of the rats fed different diets. The mean range of the rat's body weight ranges from 37.90 to 50.80 g.

At day 0, group A rats had the lowest body weight (37.90g), this was significantly different ($p<0.05$) from rats in group D (50.80g). It was also observed that rats in group A had significant reduction ($p<0.05$) in body weight from day 4 to day 8 (36.16-34.68g), this reduction could be attributed to diet (whole maize at 100%) having low nutritive composition (Table 4) Rats in group C had significant increased ($p<0.05$) from day 4-8 (55-70g). Throughout the period, rat in other groups except group A experienced increased body weight. At day 24 and 28, rats fed diet C had increased body weight which was significantly different ($p<0.05$) from weight of rats from other diets at day 28 (133g). However, it was not significantly different ($p>0.05$) at day 24 (113g). At day 24, body weight of rats fed diet D (104g) was not significantly different ($p>0.05$) from body weight of rats fed diet C. This could be attributed to the high feed intake of the rat (Fig. 3). From this study, it was observed that diet C (60%maize, 30%soybean and 10%carrot) fed to rats in group C could have enhanced their body weight (Fig. 3).

The Fig. 3 is the feed intake of rats on different diets. There was significant difference ($p<0.05$) in the feed intake of the rats fed on different diets.

At day 4, the highest (62.20g) feed intake was observed for rats that fed on diet C(60% maize, 30% soybean and 10% carrot), next B (60.70g = 85% maize, 10 soybeans and 5%carrot), diet D (54.20g= 65% maize, 20%soybean and 15% carrot), diet G (53.20g=rat pellet), diet E (45.10g= 50%maize and 50% soybean), diet F (43.80g =Cerelac). The least feed intake was diet A (39.6g= 100% maize) in that decreasing order.

At day 8, the feed intake increased for all the groups. Probably the animals became used to the diets. The highest was diet C (67.30g) and was not significantly different ($p>0.05$) from diet B (65.9g), next was diet D (62.20g). The high acceptance of the diets (B,C and D) could be attributed to the inclusion of carrot in the diets which probably made it attractive and added taste when consumed by the rats.

At day 12, the highest feed intake in rat was observed in diet D (89.1g) next was DC(75.9g), , F (67.3g), G (63.8g), B (61.0g), E (57.2g) and the least A (51.0g) in that decreasing order.

At day 12 to 16 there was significant increase ($p<0.05$) in feed intake in diet D (89.1 to 91.8), C (75.9 to 89.2g) and E (57.2 to61.0g) respectively. Throughout the period feed intake for diet C and D increased significantly $p<0.05$ and consistently. At 20 days feed intake for rat that fed on diet C=95.60g, D=86g, 24days feed intake of rat that fed on diet D= 99.60g, C=98.40g and at 28 days feed intake of rat that fed on diet D= 104g and C= 103g, there was no significant difference ($p>0.05$) in feed intake of rat fed Diets C and D.

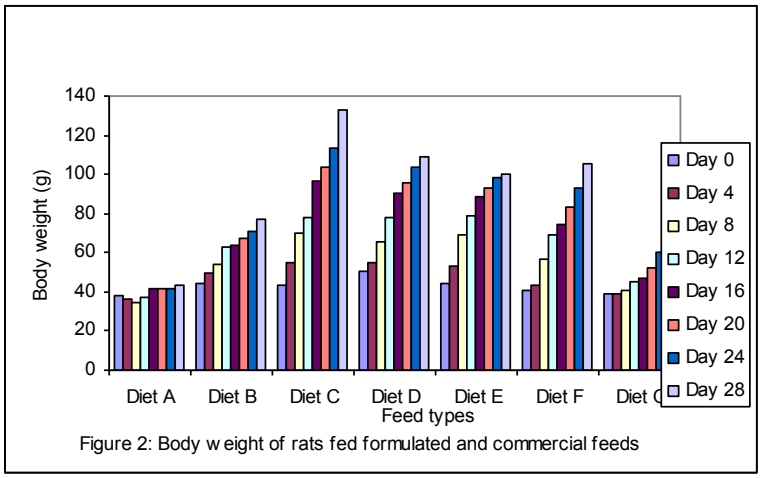

Fig. 2. Body weight of rats fed formulated and commercial feeds

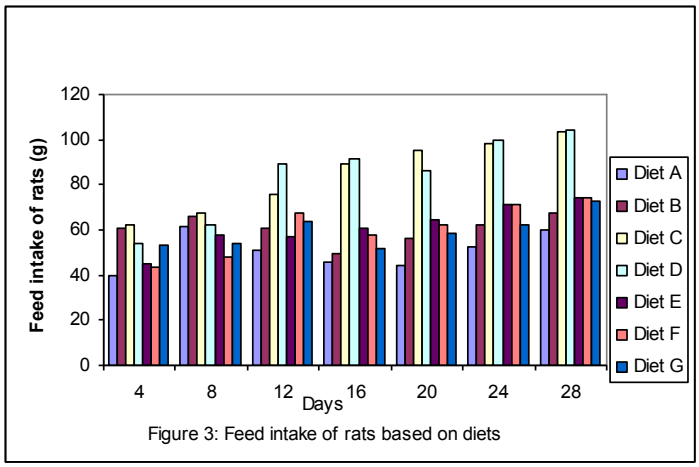

Fig. 3. Feed intake of rats based on diets

Throughout the period of this study the commercial feeds (F and G ie control) significantly increased ($p<0.05$) consistently from day 16 to 28.

5. CONCLUSION

Maize, soybean and carrot are locally available and affordable raw materials that can be used by mothers as home-based complementary foods. This study has showed that diet C which had 60% maize, 30%soybean and 10% carrot was outstanding among the diets and should be used to improve the body weight of infant, increased the HDL, reduced LDL, improves haematological parameters (such as white blood cells, PCV, platelet etc), improve the diet intake with high nutritional qualities.

ACKNOWLEDGEMENT

Author wishes to thank Tetfund for funding this research and for the opportunity to contribute to scientific development.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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