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Protein Quality Evaluation and Haematological Parameters of Rats Fed Compmementary Diets Prepared from Pearl Millets (*Penniselum glaucum*), Groundnut (*Arachis hypogeae*) and *Moringa oleifera*

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Abstract
The aim of the study was to determine protein quality and hematological parameters of complementary diets prepared from Millet, Groundnut and Moringa oleifera by feeding albino rats. A basal diet (Nitrogen-free) was formulated along with four different 10% protein diets and cerelac as control. The 30 weanling rats used in the experiment were divided into 6 groups of 5 rats each, and each group was allotted to the different diets. The Biological value (70.74 - 78.29%), true protein digestibility (65.03-66.03%), protein efficiency ratios (2.79 -3.94) and and Protein retention efficiency (70.88-79.52) of formulated complementary diets were found to be significantly (P<0.05) higher compared to Ogi (BV=17.64%, TPD=14.78%, PER=1.52, PRE =22.88), but, these values were found to be lower than Cerelac values (BV= 81.71%, TPD=70.6%, PER=4.50, PRE= 63.68). The result showed that the hematological parameters(packed cell volume, red blood cells and hemoglobin) of rats fed the formulated diets were within the normal range. The result for erythrocytes sedimentation rate (ESR), monocytes, basophils, mean cell heamoglobin concentration (MCHC), mean cell heamoglobin (MCH) and mean cell volume (MCV), showed there were no significant difference observed between the values of animals fed with the formulated diets and control food samples (ogi or Cerlac) (p<0.05). The weight gained by the organs (heart, liver, spleen and kidney) of animals fed with the experimental complementary food samples were observed to be significantly higher than those animals placed on ogi, but found to be lower than those rats fed with Cerelac (p<0.05). In the overall, the rats fed with formulated diets showed good growth and development, with no adverse biochemical or hematological effect and therefore maybe used as an ideal weaning food to improve the nutritional status of children.
Key words: Moringa Oleifera, Protein quality, hematological, Biological Value

Introduction

Access to adequate diet, care, water and sanitation are the basic conditions required for growth and healthy development of children but, absence of any one of these, would result in protein-energy malnutrition (de Onis *et. al*., 2000). The feeding of an infant in early years of life influences an individual's whole life. Complementary feeding period is the period in life when breast milk is no longer sufficient or enough to meet the nutritional needs of the infant at the age of 6 months (Caulfield, 1999). Therefore, the World Health Organization (WHO) recommended commencement of complementary feeding at this juncture (WHO, 2000). This practice leads to the gradual transition of an infant to the consumption of family foods. During this period, if infant or children fails to receive sufficient quantities of breastfeeding and appropriate complementary foods they can become malnourished (Osundahunsi and Aworh, 2003; Issaka *et al*., 2015).

Poor infant feeding practices have been implicated as factors responsible for nutritional problem among children in Nigeria (Anigo *et al*., 2009). According to Dang *et al* (2005), the main limiting factor for providing children with nutritious complementary foods is the limited opportunities to access such foods. In order to reduce the high rate of morbidity and mortality,

researchers have focused on the exploitation and utilization of under used plant resources. A number of readily available cereals and legumes in Nigeria, have been found to have nutrients that could complement one another if properly processed and blended (Osundahunsi and Awohr, 2002; Gernah *et al*., 2012; Akinwande *et al*., 2014; Ajibola *et al*., 2016).

The traditional complementary foods in Nigerian and other part of developing countries are cereal based and are characterized by low protein (e.g., lysine and tryptophan) (Oyarekua, 2011), low energy density and bulky (Ukegbu and Anyika, 2012).

Food processing methods such as germination, fermentation, roasting, drying and milling have been reported to enhance the nutrient density, palatability, nutrient bioavailability and food safety of complementary foods suitable for infant mixtures. In Nigeria, processing technology such as germination, fermentation, milling and roasting of cereals are widely practiced (Kumari *et al*., 2017). Many nutrients in which are in bound forms in the food are unlocks by germination, thereby increasing nutrient bio-availability, energy density and acceptability of the food (Ajibola *et. al*., 2016).

Cereal grains belong to the family *graminaeae* and are one of the most widely cultivated and consumed crops worldwide. The major cereals cultivated in Nigeria, especially in Northern part where cereals are the main sources of energy and protein in their diets are sorghum, rice, millet, rice and maize.(Muhammad *et al*., 2013). The main nutrient component of cereals grains which make them important in food preparations are their starch and proteins contents. In cereal carbohydrate makes up 79–83% (the dry matter), 7–14% protein (depending on the grain). Cereals are low in the amino acids tryptophan and methionine, and although potential breeding may produce cereals higher in the amino acid lysine, it remains the limiting amino acid in cereals.

Legumes are unique foods because of their rich nutrient content, including starch, vegetable protein, dietary fibre, oligosaccharides, phytochemicals (especially the isoflavones in soy) and minerals. The carbohydrate and dietary fibre contents of legumes contribute to their low glycemic indices. Groundnut, *Arachis hypogaea* L. also known as peanut or earthnut is principally cultivated for its edible oil and protein rich kernels seeds,. The protein content in the groundnut kernel ranges from 16.2 to 36%. The major seed proteins globulins (salt soluble) of the groundnut seed contains about 18.3% nitrogen, albumins (water soluble) and glutamines (acid or alkaline soluble). These proteins are used in several food products for their functional properties, such as emulsifying and foaming capacity, or for their nutritional properties. Groundnut seeds provide a wide range of mineral elements such as calcium, magnesium, potassium, sodium, iron, and certain trace elements such as manganese, copper, zinc, and boron in appreciable amounts. Groundnut seeds are also known to be an excellent source of certain vitamins (especially vitamins E, K and the B group), as well is flavones and phytostrogen, and other antioxidant compounds.

Moringa oleifera belongs to the family moringaceae, genus moringa which has 14 species. It is widely cultivated in northern parts of Nigeria and many countries in Africa for human food, livestock forage, medicine, dye, and water purification (Fagwalawa *et al.,* 2015). The leaves, fruits, flowers and matured pods of this tree are edible and they form a part of traditional diet in many countries of the tropics and sub-trophic. Moringa seed has also known to combat malnutrition in infant and nursing mothers, has other impressive range of medicinal uses with high nutritional value (Siddhuraju and Becker, 2003).

The present *study* seeks to evaluate the protein quality and haematological parameters of rats fed the formulated diets from millet, groundnut and M*oringa oleifera*

Materials and Methods

Food Materials

The preparation of the three raw food materials used in the formulation of the complementary foods was as described by Anigo *et al*., (2010) and Zakari *et al*., (2007) (Figure 1)

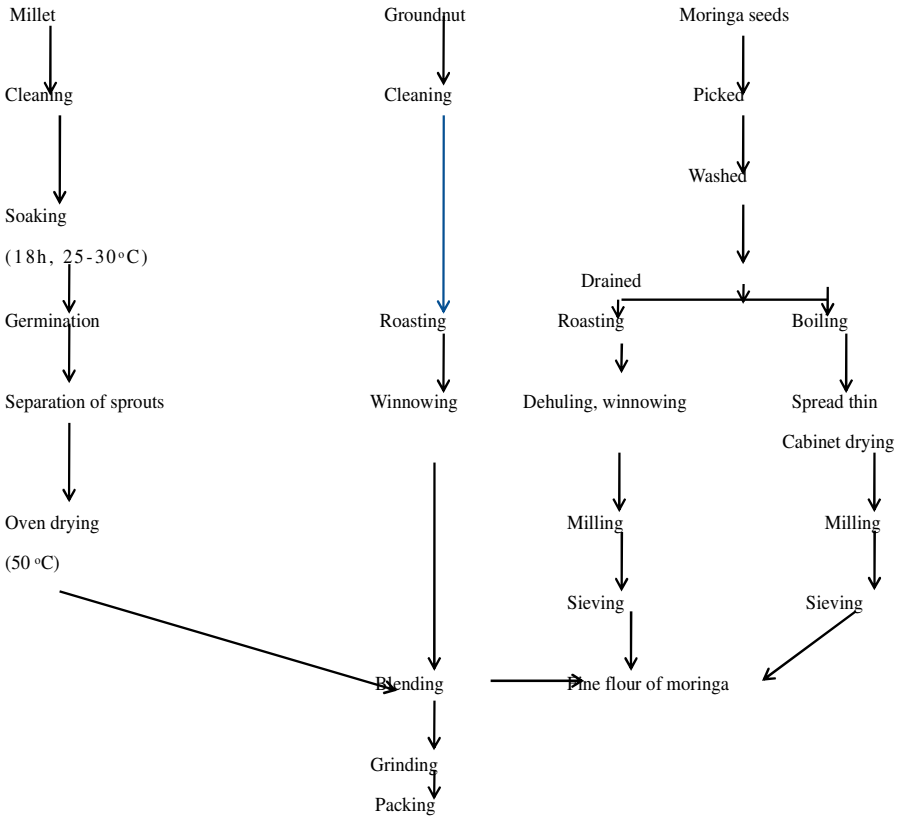

Fig. 1: The flow chart for preparation of Formulated food blend

Sources :Anigo *et al*, (2010) and Zakari *et al*, (2007).

Experimental design and composition of the diets

The experimental diets which consists of formulated diets (millet, groundnut and *moringa oleifera diets*) and commercial weaning food (*cerelac*) were prepared at 10% protein level (isonitrogenous diets). A Basal diet (ordinary ogi) was also prepared. The diets
were prepared by mixing the complementary foods to a calculated protein level of 10% and supplemented with other basic ingredients like glucose (5%), sucrose (10%), non-nutritive cellulose (5%), vegetable oil (5%), vitamin premix (3%), mineral premix (3%), salt (0.2%) and cornstarch added to make up to 100%. Corn starch was used to dilute the protein content of the diets. Composition of experimental diet is shown in Table 1.

Animal Studies

Twenty-one (21) day old weaned rat (Wistar strain) weighing between 30 g and 60g at the beginning of experiment were obtained from Department of Biochemistry, Federal University of Technology, Akure. Thirty (30) healthy albino rats (males and females) of 4 weeks old maturity randomly distributed into six (6) groups containing five animals per group. The rats were housed in individual metabolic cages. They were kept in metabolic cages made of Perspex sheets. The rats

were allowed to stabilize on a commercial diet (Concentrate) and water *ad libitum* for 7 days. After the acclimatization period, the animals were then re-weighed and grouped into six groups of five rats each per group such that the differences in their mean weights were ±2 g. Four groups of animals were administered with the formulated diets (FMGM3, FMGM5, GMGM3 and GMGM6) while the remaining two groups of animals were administered with cerelac (a commercial weaning food) and ordinary ogi. The weighed diets in feeding cups were moistened with warm water before feeding. Food and water were provided *ad libitum* to the rats for 28 day Weighed diets and water were given ad libitum for 28 days and unconsumed diets were collected and weighed daily.

Table 1: Composition of diet g/100g(for complementary diets)

INGREDIENTS	Ogi	Cerelac	Diet 1 FMGM3	Diet 2 FMGM5	Diet 3 GMGM3	Diet 4 GMGM6
Corn Starch	71.8	11.81	18.44	18.69	26.61	26.41
Cerelac	-	59.99				
Diet1			53.56			
Diet2				53.11		
Diet3					45.19	
Diet4						45.39
Glucose	5.00	5.00	5.00	5.00	5.00	5.00
Sucrose	10.00	10.00	10.00	10.00	10.00	10.00
Cellulose	5.00	5.00	5.00	5.00	5.00	5.00
Vegetable oil	5.00	5.00	5.00	5.00	5.00	5.00
Min\Vit Premix	3.00	3.00	3.00	3.00	3.00	3.00
salt	0.2	0.20	0.20	0.20	0.20	0.20
Total	100	100	100	100	100	100

FMGM3 : 55% fermented Millet +25%Groundnut + 20%Moringa 0leifera, GMGM5 70% Fermented Millet +10%Groundnut + 20%Moringa 0leifera, GMGM3 : 55%Germinated Millet +25%Groundnut + 20%Moringa 0leifera FGMGM6 55% Germinated Millet +20%Groundnut + 25%Moringa 0leifera,CERELAC; Ogi

The weekly intake and changes in body weight were recorded for the four weeks and the Protein efficiency ratio (PER) was calculated. Feed efficiency ratio (FER weight gained/g intake) and mean weight gain were calculated. The physical appearances of the animals were recorded. They were then killed, and blood for hematological analysis was quickly drawn by jugular incision and put in ethylenediaminetetraacetate (EDTA) solution. Blood for serum protein and serum albumin was drawn into tubes and centrifuged for 30 minutes. The serum was stored in a refrigerator for subsequent analysis. The liver, heart, kidney and spleen were immediately removed from the carcass, washed in a cold buffer and weighed. The values were subsequently expressed in g/kg of body weight (Agbede and Aletor, 2003). The nitrogen contents of fecal matter and urine were determined using AOAC (1995) methods. The biological value (BV), true digestibility (TD), net protein utilization (NPU), protein efficiency ratio (PER), and net protein ratio (NPR) were calculated according to the method describe by Onabanjo *et al.*, (2009).

Haematological Evaluations

Blood Collection

On the 28 day of the experiment period, all the rats were starved for 3 hours and weighed and thereafter each rat was anesthetized with chloroform inside a dessicator and sacrificed. Blood samples from each rat were collected into sample bottles containing a few milligram of dried ethylenediamine acid powder (EDTA) before haematological analysis was done as described by Lamb (1981).

Haematological Analysis

The packed cell volume (PCV) was determined by centrifuging about 75 µl of each blood sample in heparinized capillary tubes in a haematocrit microcentrifuge for 5 minute. Using normal saline as the diluting fluid, the total red blood cell (RBC) and white blood cell (WBC) counts were determined. The haemoglobin concentration (Hb) was estimated using the cyano-methaemoglobin concentration method, while the lymphocyte, neutrophil, monocyte, basophill and eosinophil were determined according to the method described by Lamb (1981).

Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 16.0 for Windows were used to analyse the results. The mean and standard error of the mean (SEM) of the triplicate determination were calculated. The Significant difference between parameters was determined by one-way analysis of variance (ANOVA) and the means were separate using Duncan's Multiple Range Test (DMRT) at $p<0.05$.

Results and Discussion

Protein quality values of the formulated diets and control food samples are shown in Table2. The food intake of rats fed with formulated diets, *Cerelac* and *Ogi* ranged from 297.54g (FMGM3) to 409.69g (GMGM6). Food intake of animals fed with *Cerelac* diet was significantly ($P<0.05$) higher than that of animals fed with formulated diets except for GMGM which was comparable to that of *Cerelac*. The weight gain of the experimental animals ranged between 0.8g (Ogi) and 22.0g (*Cerelac*). It was observed that the weight gains of animals fed with formulated diets were lower than those of animals placed on *Cerelac* but were higher than those of animals fed with *Ogi*. This observation agree with the observation of Ajibola *et al.,* (2016) who reported that animals fed with ogi did gain less weight than those placed on formulated diets fed with oven-dried crayfish-ogi and toasted crayfish-ogi. A significant difference ($P<0.05$) was observed between the animals fed germinated formulated diets and those fed with fermented formulated diets. The feed Efficiency ratio (FER) of the experimental diets varied from 0.01 (Ogi) to 0.06g (*Cerelac*). The FER of rats fed with the formulated diets were observed to be significantly lower ($P<0.05$) when compared with those fed with Cerelac but higher than those fed with Ogi. The Net Protein Ratio (NPR) of the diets varied from 1.43 (ogi) to 4.97 (GMGM6). The NPR of the formulated diets were higher than than those of *Cerelac* and Ogi diets. Similar report was obtained by Gernah *et al.,* (2012).

The results of Biological values (BV) True Digestibility (TD), Protein Efficiency Ratio (PER) and Protein Retention Efficiency (PRE) are presented in Table2. The biological values (BV) of f0rmulated complementary food samples ranged from 70.74 % for FMGM3 to 78.27% for GMGM6 sample. For Protein efficiency ratio (PER), value ranged between 2.78 (FMGM3) and 3.94 (GMGM6) True protein digestibility of germinated food samples ranged between 65.03% for GMGM3 and 66.03% for GMGM6 while that of fermented food samples ranged from 60.40% for FMGM3 to 65.27% for FMGM5 sample. Comparatively, the biological values (BV), true protein digestibility (TPD), protein efficiency ratios (PER) and and Protein retention efficiency (PRE) of formulated complementary diets were found to be significantly ($P<0.05$) higher compared to Ogi (BV=17.64%, TPD=14.78%, PER=1.52, PRE =22.88), but, these values were found to be lower than *Cerelac* values (BV= 81.71%, TPD=70.6%, PER=4.50, PRE= 63.68). The Protein Advisory Group (PAG) guidelines (1971) recommends a PER of not less than 2.1 preferably not less 2.3 for complementary foods (PAG, 1971) while FAO/WHO (1989)

recommended values of 70% and 2.7 for BV and PER respectively. All the formulated diets were higher than the PAG and FAO/WHO recommended values for BV and PER and therefore met the required standards of BV and PER. According to Oser (1959), a protein material is said to be of good nutritional quality when its BV is 70% and above. Since the BV and PER obtained in this study is within the ranged of the set standard, these indicated that the protein contents in the formulated complementary foods would adequately support growth and development in infant. Generally, germination and fermentation significantly improved PER and Net Protein Ratio (NPR) of foods. PER and NPR are indices of protein quality. The PER indicates the relationship between weight gain in the test animals and corresponding protein intake, while NPR relates the weight changes in the animals placed on the test diets to those red the control diets. NPR therefore is a more accurate measure of protein quality than PER as it allows for the evaluation of maintenance requirement and results are independent of feed intake. The high values of TPD, PER and NPR of both the fermented and germinated complementary diets could be attributed to the enzymic degradation of macromolecules of protein and carbohydrate into smaller units, thereby increasing in the surface area of substances for a facilitated digestion and subsequent absorption by the experimental animals.

Table 2: Evaluation of Protein quality formulated food samples and control

Parameters	FMGM3	FMGM5	GMGM3	GMGM6	CERELAC	OGI
Weight gained (g)	13.33^{b}	9.50^{d}	13.00^{c}	13.00^{c}	22.00^{a}	0.80^{e}
Food intake (g)	297.54^{e}	326.60^{d}	397.65^{b}	409.69^{a}	400.71^{a}	369.42^{c}
Feed Efficiency Ratio(FER) (g)	0.05^{b}	0.03^{c}	0.03^{c}	0.03^{c}	0.06^{a}	0.01^{d}
Nitrogen Retention	0.51^{e}	0.86^{c}	0.78^{d}	0.98^{b}	1.32^{a}	0.00^{f}
Net Protein Utilization (NPU)(%)	60.43^{e}	66.17^{c}	65.13^{d}	68.18^{b}	71.56^{a}	16.58^{f}
Biological value(BV)(%)	70.74^{e}	76.32^{c}	75.23^{d}	78.27^{b}	81.71^{a}	17.64^{f}
Protein Efficiency Ratio(PER)	2.78^{e}	3.61^{c}	2.88^{d}	3.94^{b}	4.50^{a}	1.52^{f}
Net Protein Ratio(NPR)	4.43^{d}	4.92^{b}	4.89^{c}	4.97^{a}	3.98^{e}	1.43^{f}
Protein Retention Efficiency	70.88^{d}	78.72^{b}	78.24^{c}	79.52^{a}	63.68^{e}	22.88^{f}
True Protein Digestibility (TPD)(%)	60.40^{e}	65.27^{c}	65.03^{d}	66.20^{b}	70.65^{a}	14.78^{f}

Means (±SEM) with different superscripts in a row are significantly different at P<0.05
FMGM3 : 55% fermented Millet +25%Groundnut + 20%Moringa 0leifera, GMGM5 : 70% Fermented Millet +10%Groundnut + 20%Moringa 0leiferaGMGM3 : 55%Germinated Millet +25%Groundnut + 20%Moringa 0leifera FGMGM6 55% Germinated Millet +20%Groundnut + 25%Moringa 0leifera, CERELAC; Ogi

Table3: Relative organ weights of experimental rats (g/100g live weight)

Parameters	FMGM3	FMGM5	GMGM3	GMGM6	CERELAC	Ogi
Liver	1.34^{c}	1.80^{b}	1.87^{b}	1.89^{b}	2.36^{a}	1.24^{d}
Kidney	0.21^{d}	0.22^{c}	0.22^{c}	0.23^{b}	0.24^{a}	0.20^{e}
Heart	0.22^{d}	0.23^{c}	0.22^{d}	0.24^{b}	0.27^{a}	0.21^{e}
Spleen	0.30^{b}	0.29^{c}	0.29^{c}	0.30^{b}	0.32^{a}	0.15^{d}

Means (±SEM) with different superscripts in a row are significantly different at P<0.05
Key: FMGM3 : 55% fermented Millet +25%Groundnut + 20%Moringa 0leifera, GMGM5:70% Fermented Millet +10%Groundnut + 20%Moringa 0leifera, GMGM3 : 55%Germinated Millet +25%Groundnut + 20%Moringa 0leifera,FGMGM6 55% Germinated Millet +20%Groundnut + 25%Moringa 0leifera, CERELAC; Ogi

These observations is agreement other studies that reported on significant increases in PER in rats as a result of fermentation and germination of cereals and legumes (Ikujenola and Fashakin, 2005; Gernah *et al.,* 2012)

The influence of the experimental diets on the organs of Albino rats fed with the formulated complementary food samples and control samples are presented in Table 3. The liver, kidney, heart and spleen values ranged from 1.34 to 1.80g, 0.21 to 0.22g, 0.22 to 0.23g and 0.29 to 0.30 g for fermented products respectively whereas for germinated products the values varied from 1.87 to 1.89g, 0.22 to 0.23g, 0.22 to 0.24g and 0.29 to 0.30 g respectively. The weight gained by the liver of the rats fed with FMGM3 food sample was lower than those fed with other formulated complementary food samples.

The weight gained by the organs of animals fed with the experimental complementary food samples were observed to be significantly higher than those animals placed on ogi, but found to be lower than those rats fed with *Cerelac* ($p<0.05$). The relative organ weight of liver, heart, kidney and spleen were not affected by the various diets samples meaning that the complementary mixes are toxicological free and therefore safe for human consumption.

Table 4. shows the effect of the formulated diets on hematological properties of albino rats. The value of blood components is an indication of availability of nutrients for synthesis of blood cells. Pack cell volume (PCV) of albino rats fed with the fermented food samples ranged from 44% for FMGM3 to 48% for FMGM5, while germinated complementary foods ranged from 45% for GMGM3 to 48% for GMGM6. The values obtained for PCV for rats fed with FMGM3 (44) and GMGM3 (45) were lower than those rats fed with *Cerelac*. Red blood cells (RBC) of rats fed with FMGM3 (7.65×10^3 mm^3) sample had the least value, while those fed with GMGM6 (8.15×10^3 mm^3) sample had highest value. Similarly, it was also observed that the red blood cells of rats fed with either germinated or fermented food samples were higher than those fed with 'Ogi', and also comparable with those rats fed with *Cerelac*. The hemoglobin (Hb) concentration of animals placed on germinated foods ranged from 13.30 g/dl to 14.60 g/dl with lowest value obtained for FMGM3. The heamoglobin concentration of rats fed with the formulated complementary foods were significantly higher than those in 'Ogi' group ($p<0.05$), while those fed with FMGM5 and GMGM6 were found to be comparable with rats in *Cerelac* group. The hemoglobin and pack cell volume values of rats on the formulated diets were within the acceptable range indicating adequate iron status. This could be associated with the iron content of the Moringa Oleifera seed used in the formulation of the diets can be said to be a good sources of non-heme iron (Shiriki *et al*., 2015). Studies have shown that non-heme iron from plant sources are not readily absorbed in the body as heme iron from animal sources which are well absorbed and therefore, there is need to consume foods such as fruits that will enhance iron absorption (Brown *et al., 1*998; Onabanjo *et al.,* 2009). The findings in this study shows that if plant proteins are adequately combined could substitute for animal sources which are usually too expensive for the poor. The result for erythrocytes sedimentation rate (ESR), monocytes, basophils, mean cell heamoglobin concentration (MCHC), mean cell heamoglobin (MCH) and mean cell volume (MCV), showed there were no significant difference observed between the values of animals fed with the formulated diets and control food samples (ogi or *Cerlac*) ($p<0.05$); however for other parameters, such as lymphocytes, neutrophils and Eosinophils significant difference ($p<0.05$) were observed between the rats fed with the formulated diets and those placed on ogi and C*erelac* (Table 4).

Table 4: Hematological properties of albino rats fed with formulated diets, Ogi and Cerelac

Parameters	FMGM3	FMGM5	GMGM3	GMGM6	*Ogi*	CERELAC
ESR(mm^3)	1.00±0.00^a	1.00±0.00^a	1.00±0.00^a	1.00±0.00^a	1.00±0.00^a	1.00±0.00^a
PVC (%)	44.00±0.09^d	48.00±0.13^a	45.00±0.06^c	48.00±0.31^a	42.00±0.09^e	47.00±0.03^b
Hb (g/dl)	13.30±0.03^b	14.60±0.04^a	13.60±0.04^b	14.60±0.15^a	12.30±0.10^c	14.30.±0.02^a
WBC (x 10^3 mm^3)	12.90±0.04^b	10.60±0.03^d	11.50±0.02^c	12.20±0.03^b	13.90±0.21^a	11.05±0.03^c
RBC (x 10^6 mm^3)	7.65±0.02^b	8.10±0.01^a	7.80±0.01^b	8.15±0.01^a	7.45±0.03^b	8.00±0.01^a
MCHC (g/dl)	33.30±0.31^a	33.20±0.31^a	33.20±0.37^a	33.20±0.37^a	33.10±0.12^a	33.70±0.20^a
MCH (pg)	30.20±0.26^a	29.50±0.24^b	29.60±0.04^b	30.00±0.21^a	29.70±0.04^b	30.00±0.05^a
MCV (fl)	90.50±0.61^a	88.80±0.42^c	89.20±0.47^b	90.30±0.59^a	89.70±0.17^b	90.00±0.04^a
Neutrophils (%)	45.00±0.32^a	31.00±0.20^d	44.00±0.51^b	30.00±0.71^e	42.00±0.05^c	24.00±0.03^f
Lymphocytes	50.00±0.47^e	69.00±0.51^f	49.00±0.42^c	68.00±1.32^c	52.00±0.26^d	72.00±0.20^a
Monocytes (%)	0.00±0.00^c	0.00±0.00^c	1.00±0.00^b	2.00±0.00^a	2.00±0.00^a	0.00±0.00^c
Eosinophils (%)	5.00±0.00^b	0.00±0.00^e	6.00±0.00^a	0.00±0.00^e	3.00±0.00^d	4.00±0.00^c
Basophils (%)	1.00±0.00^a	1.00±0.00^a	1.00±0.00^a	1.00±0.00^a	1.00±0.00^a	1.00±0.00^a

Means (±SEM) with different superscripts in a row are significantly different at P<0.05*:*
FMGM3 : 55% fermented Millet +25%Groundnut + 20%Moringa 0leifera, GMGM5 70% Fermented Millet +10%Groundnut + 20%Moringa 0leifera, GMGM3 : 55%Germinated Millet +25%Groundnut + 20%Moringa 0leifera FGMGM6 55% Germinated Millet +20%Groundnut + 25%Moringa 0leifera, CERELAC; Ogi

The biochemical properties of rat fed on formulated complementary diets are presented in Table 5. The evaluation of creatinine and urea in serum helps to assess Glomerular Filtration Rate (GFR) by renal function. The rise in these two important chemical substances in the blood marked the progression of kidney damage. However, neither creatinine nor urea is directly toxic and they are only a measure of kidney function (Renuga *et al.,* 2009). The creatinine value of rat fed on the formulated diet varied from 27.46 mg/dl in GMGM3 to 41.40 mg/dl in FMGM5, while that OF Ogi and *cerelac* (control samples) were 26.63 mg/dL and 25.70 mg/dL respectively. Studies have shown that the quantity of creatinine in serum depends on their generation, glomerular filtration and tubular secretion of serum creatinine, which may indicates the degree of kidney function (William *et al.,* 1998; Mohamad *et al.,* 2008). In this study all the creatinine level of the rat fed on the formulated diets and control samples were observed to be within the normal ranged when compared with the report of Kunitoshi *et al.* (1997), this implies that the experimental diets had no negative effect on the kidney glomeration filtration rate or functionality. The serum creatinine was also observed to be higher in rats fed the formulated diets (27.46 -41.40mg/dl) than in ogi (26.63mg/dl) and cerelac (25.79mg/dl). This suggests that there were greater changes in the daily turnover of the total muscles mass in rats fed the formulated diets which was not the case in rats fed with *ogi* and *cerelac.* The Urea values of rats fed on experimental formulated diets ranged from 1.54 mg/dl in FMGM5 to 3.01 mg/dl in FMGM3 while the values for rats fed on cereals (1.94 mg/dl) and Ogi (1.86 mg/dl) were comparable to CNT were comparable to the formulated diets. Plasma urea values for rats fed with the formulated diets were observed to be comparable to those rats fed with Ogi and cerelac. The results obtained in this study were found to be within the pediatric reference range of 1.5-4.0 mmol/L when extrapolated to humans (Solomon, 2005). Thus affirming that the formulated diets are adequate in protein and amino acid content and would support normal liver and metabolic functions. Total blood protein of the rat fed on formulated diet ranged between

4.84g/dL in FMGM3 and 5.66 g/dL in GMGM6, while that of control samples *Cerelac* (4.06 g/dL) and Ogi (3.87g/dL).

Table 5: Effects of Formulated diets on Biochemical Parameters in Albino Wister Rat

Parameters	FMGM3	FMGM5	GMGM3	GMGM6	Ogi	CERELAC	*Standard
Creatinine(mg/dl)	36.61±0.18^{b}	41.40±0.39^{a}	27.46±0.31^{c}	28.29±0.61^{c}	26.63±0.16^{c}	25.79±0.94^{c}	0.2 -0.8
Urea(mg/dl)	3.01±0.33^{a}	1.54±0.83^{d}	1.56±0.69^{d}	2.05±0.83^{b}	1.86±0.36^{c}	1.94±0.70^{c}	7 -20
Total protein(g/dL)	4.84±0.84^{b}	4.84±0.88^{b}	5.63±0.63^{a}	5.66±0.66^{a}	4.06±0.60^{b}	3.87±0.87^{c}	6.00-8.00
Albumin(g/dL)	3.20±0.71^{b}	3.20±0.75^{b}	4.12±0.38^{a}	4.67±0.44^{a}	3.22±0.7^{b}	3.58±0.80^{b}	3.50-5.00
Globulin(g/dL)	1.64±0.03^{a}	1.64±0.07^{a}	1.51±0.09^{b}	0.99±0.03^{c}	0.84±0.05 ^{d}	0.29±0.02^{e}	<3.00
AST(µ/L)	59.32±0.83^{a}	52.76±0.84^{c}	53.64±0.22^{c}	52.76±0.51^{c}	55.52±0.43^{b}	46.28±0.21^{d}	45.7-80.8
ALT(µ/L)	124.28±0.57^{a}	103.21±0.42^{d}	104.82±0.14^{d}	112.14±0.86^{c}	120.35±0.71^{b}	112.67±0.85^{c}	`17.5-30.2
ALP(µ/L)	120.43±0.47^{a}	110.43±0.4^{b}	121.30±0.41^{a}	113.04±3.48^{b}	124.34±0.73^{a}	129.13±0.43^{a}	56.8-128

Means (±SEM) with different superscripts in a row are significantly different at P<0.05*:*
FMGM3 : 55% fermented Millet +25%Groundnut + 20%Moringa 0leifera, GMGM5 70% Fermented Millet +10%Groundnut + 20%Moringa 0leifera, GMGM3 : 55%Germinated Millet +25%Groundnut + 20%Moringa 0leifera FGMGM6 55% Germinated Millet +20%Groundnut + 25%Moringa 0leifera, CERELAC; Ogi

The total blood protein concentration of rat fed on formulated diets were significantly (P<0.05) higher than rats fed on *cerelac*, buts were comparable to those rats fed on Ogi. The albumin values of rats fed on the formulated diets ranged between 3.20 g/dl in FMGM3 and 4.67 g/dl in GMGM6, while those fed on the control samples (ogi and *cerelac*) were 3.22 g/dL and 3.58 g/dL respectively. The albumin values of germinated formulated diets were found to be significantly higher (p<0.05) than those rats fed on formulated on fermented formulated diets as well as those rats on Ogi and *cerelac*. in this study it was observed that the total blood proteins and serum albumin concentrations of rats fed on the formulated dough meals were comparatively lower than normal range values for serum protein (6 to 8 g/dL) and abumin (3.5 to 5.0 g/dL), this maybe attributed to the nature of protein source, that is, from plant. It has been shown that the bioavailability of plant proteins is usually lower compare to animal-based proteins, and that the concentration of plasma proteins, especially albumin, depends on the amount of protein intakes and its quality (Fujita *et al.,* 1978). For the globulin values, the results indicated that the values ranged from 0.09 mg/dL in GMGM6 to 1.64 mg/dL in FMGM5. The globulin values of rats fed on the formulated diets were significantly (p<0.05) higher globulin values of *cerelac* and Ogi. The Aspartate aminotransferase (AST) values of rats placed on the formulated diets ranged from 52.76 µ/L to 77.83 µ/L.

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline Phosphate (ALP) are all enzyme marker use to determine the functionality of the liver. The AST values for experimental food samples were within the normal range value (45.70 – 80.50 µ/L). For Alanine aminotransferase (ALT), the values of rat fed with formulated diets ranged from 103.21 µ/L in FMGM5 to 124.28 µ/L in FMGM3, while that of rats fed on ogi was 120.35 µ/L and *cerelac* was 112.67 µ/L. Statistically, the ALT values of rats fed on FMGM3 diet was significantly (p<0.05) higher than other formulated diets, ogi and *cerelac*. The ALP values of rats fed on formulated diets ranged from 110.43 µ/L in FMGM5 to 121.30 µ/L in GMGM3, while that of Ogi and *cerelac* were 124.34 µ/L and 129.13 µ/L, respectively. Statistically, there was no significance (P>0.05) between FMGM5, FMGM5, Ogi and *cerelac*.

The AST/ALT ratios of experimental diets ranged between 0.46 and 0.51 for GMGM6 and GMGM3, respectively. The results obtained in this study showed that there was no significant

difference (P>0.05) between FMGM3, FMGM5, Ogi and *cerelac*. The AST/ALT ratio were observed to be higher in the formulated diets than in *cerelac* but the values were within the normal values (<1.0). This observation further indicates that the formulated diets are suitable for consumption, and the consumption of the food samples would not cause any toxic effect to the liver cells.

Conclusion

The results obtained in this study indicated a better growth promoting quality of the proteins of the formulated diets than ordinary ogi. The organ weight measurement and hematological indices of rats fed with formulated diets were better than that of ordinary ogi and compared favorably with that of rats fed on cerelac. The study further revealed that both the fermented and germinated diets would support growth better than the traditional ogi commonly use as weaning foods in Nigeria and therefore may be used as a substitute for ogi.

References

Ajibola C. F., Fagbemi T.N. and Osundahunsi O. F. (2016) Nutritional quality of weaning Foods formulated from Maize gruel 'Ogi' and Crayfish using combined traditional processing technology. Advances in Research. 6(4): 1-11.

Brown, K.H., Dewey, K.G. and Allen, L.H. 1998. Complementary Feeding of Young Children in Developing Countries: A Review of Current Scientific Knowledge. WHO/NUT/98, World Health Organization, Geneva, Switzerland.

Agbede J.O, Aletor V.A. Comparative evaluation of weaning foods from *Glyricidia* and *Leucaena* leaf protein concentrates and some commercial brands in Nigeria. Journal of the Science of Food and Agriculture. 2003;84:21-30.

Anigo K. M., Ameh D. A., Ibrahim S. and Danbauchi S. S. (2009). Infant feeding practices and nutritional status of children in North Western Nigeria. In Asian Journal Clinical Nutrition. 1(1):12-22.

Anigo K. M, Ameh D. A., Ibrahim S. and Danbauchi S. S. (2010). Nutrient composition of complementary food gruels formulated from malted cereals, soybeans and groundnut for use in North-western Nigeria. African Journal of Food Science. 4(3):65-72.

AOAC. Association of official analytical Chemists Offical Methods of Analysis 16th ed. Washington D.C; 1995.

Caulfield L.E., Huffman S.L., Piwoz E.G. (1999). Interventions to improve intake of complementary foods by infants 6 to 12 months of age in developing countries: impact on growth and on the prevalence of malnutrition and potential contribution to child survival. Food Nutr Bull., 20:183-200.

de Onis M., Frongillo E. A. and Blossner M. (2000). Is malnutrition declining? An analysis of changes in levels of child malnutrition since 1980. Bulletin of the World Health Organization,78(10):1222-1232.

Fagwalawa L.D., Yahaya S.M. and Umar D.I. (2015). A comparative Proximate Analysis of Bark, Leaves and Seed of Moringa Oleifera. International Journal of Basic and Applied Chimical Sciences. 5(4): 56-62.

FAO/WHO. (1989). Protein quality evaluation, Report of the joint FAO/WHO Expert consultation Bethesda, MD., USA

Gernah D.I., Ariahu C.C. and Ingbiam E.K (2012). Nutritional and Sensory Evaluation of Food formulations from malted and fermented maize(*Zea mays L.*) fortified with

Deffatted Sesame (*sesamun indictum* L) Flour. African Journal of food, Agriculture, Nutrition and Development. 12: 6614-6633.

Ikujenlola, V. A. and Fashakin, J. B. (2005). The physico-chemical properties of a complementary diet prepared from vegetable diets. Journal of Food Agriculture Environment and Environment. 3 (3&4): 23-26.

Issaka A. I., Agho K. E., Page A. N., Burns P. L., Stevens G.j. and Dibley M. J. (2015a). Determinants of suboptimal complementary feeding practices among children aged 6-23 months in four anglophone West African countries. Maternal and Child Nutrition. 11(suppl. 1).14 -30.

Kumari, V., Sangeeta, C. S and Jyoti, S. (2017). Nutritional Evaluation of Indigenously Developed Weaning Food from Malted Sorghum Incorporated with Soyabean and Raw Banana Flour. International Journal of Current Microbiology and Applied Sciences. 6 (6). 1264-1271

Kunitoshi, I., Yoshiharu, I and Koshiro, F. (1997). Risk factors of end stage renal disease and serum creatinine in a community based mass screening. Kidney International, 51:850-854.

Lamb G. N. (1981). Manual of veterinary laboratory technique. CIBA - GEIGY, Kenya: 96-107.

Mila´n -Carrillo J., Valde´z-Alarco´n C., Gutie´rrez-Dorado R., Ca´rdenas-Valenzuela O.G., Mora-Escobedo R., Garzo´nN-Tiznado J.A., Reyes-Moreno C. (2007). Nutritional properties of quality protein Maize and chickpea extruded based weaning food. Plant Foods for Human Nutrition. 2007;62:31-37.

Mohammed, S. M. A., Muna, A. B., Leela, B. M., Ali, F. A and Abdulmajeed, M. H. (2008). Hematological changes post- hemo and peritoneal dialysis among renal failure patients in Sudan. Saudi Journal of Kidney Disease and Transplantation, 19(2): 274-279.

Muhammad H. S., Muhammad F. S., Muhammad S., Niaz A. Q. and Safia M. (2013). The importance of cereals (Poaceae: Gramineae) nutrition in human health: A review. J. Cereals Oilseeds Vol. 4(3):32-35.

Onabanjo O.O., Maziya-Dixon B., Oguntona C. R. B. and Dixon A. G.O. (2009). Protein quality evaluation and hematological parameters of rats fed complementary diets. J. Food, agriculture and Environment. 7(2): 139-142.

Oser B. L. (1959). An integrated essential amino acid index for predicting the biological value of proteins. In A. A. Albanese (Ed). Protein and amino acid nutrition. New York; Academic Press 295-311.

Osundahunsi O. F and Aworh, A. C. (2002). A Preliminary Study on the use of Tempe-Based Formula-Plant. Foods and Human Nutrition, 57 (3-4):365-376.

Osundahunsi O. F and Aworh, A. C. (2003). Nutritional evaluation with emphasis on protein quality of maize-based complementary food enriched with soyabean and cowpea tempe. International Journal of Food Science and Technology, 38: 809-813

Oyarekua M.A. (2011).Evaluation of the nutritional and microbiological status of co-fermented cereals/cowpea 'OGI'. Agric. Biol. J. N. Am., 2011, 2(1): 61-73.

PAG (Protein Advisory Groupof the United Nations). (1971). Protein rich mixtures for use as weaning foods. PAG guideline no.8. FAD/WHO/UNICEF, New York. 1-7

Renuga, D. T. S., Gunasekaran, S., Wesley, H. J and Joybell, I. S. A. (2009). Analysis on renal failure patient's blood samples: Characterization and efficacy study. Indian Journal of Science and Technology, 2(2): 46-50.

Shiriki D., Igyor M.A. and Gernah D. I. (2015). Nutritional evaluation of complementary food formulation from Maize, Soybean and Peanut fortified with *Moringa oleifera* leaf powder. Food and Nutrition Sciences. 6:494-500

Siddhuraju P. and Becker K. (2003). Effect of various domestic processing methods on anti-nutrients and in vitro protein and starch digestibility of two indigeneous varieties of Indian Tribal Pulse, Mucuna pruriens var. utilis. Journal of Agricultural and Food Chemistry, 49:3058-3068.

Solomon M. (2005). Nutritive Value of three potential complementary foods based on cereals and legumes. In African Journal of Food and Nutritional Sciences. 5(2):1-14.

Ukegbu, Patricia.O. and Anyika, Julie .U. (2012). Chemical analysis and nutrient adequacy of maize gruel (pap) supplemented with other food sources in ngor-okpala LGA, Imo State, Nigeria. Journal of Biology, Agriculture and Healthcare, 2(6):13-21.

WHO. (2000). Child and Adolescent Health and Development: Nutrition and Infant Feeding. Pan American Health Organization/WHO. Guiding principles for complementary feeding of the breastfed child (2000).