Original Research Article

PROTEIN QUALITY EVALUATION OF WEANING FOODS FORMULATED FROM MAIZE GRUEL 'OGI' AND CRAYFISH USING COMBINED TRADITIONAL PROCESSING TECHNOLOGY.

ABSTRACT

Aims: To investigate the protein quality of high nutrient dense weaning foods produced from maize gruel 'ogi' and crayfish using combined traditional processing techniques (germination, fermentation and toasting).
Study design: Two-way ANOVA
Place and Duration of Study: Department of Food Science and Technology, Federal University of Technology, Akure, Ondo State, Nigeria, between January 2013 and November 2014.
Methodology: Germinated-fermented ogi slurry was produced from maize. This slurry was processed into flours using oven drying and toasting method in order to obtain two different blends (oven-dried and toasted maize gruel crayfish enriched 'ogi'). The developed blends were evaluated for microbial quality and tested biologically by feeding them to experimental rats.
Results: The total mesophilic bacteria count of the *ogi* blends ranged from 1.2 to 2.5x 10^3 cfu/g. Mold (1.0 x 10^3) were found in both oven-dried and toasted enriched *ogi* blends. Yeasts were found only in oven-dried enriched *ogi* blend (1x 10^3cfu/g). Coliform, staphylococcus and salmonella were not detected in all the formulated weaning diets. The weight gain value recorded over a 28day experimental period for the test animal on crayfish-enriched *ogi* blends showed that they supported growth than ordinary *ogi* but their performance was not quite optima as that of Cerelac and Casein reference diets. The protein efficiency ratio of rats fed on Casein diet was similar (p= 0.05) to those of rats fed on crayfish-enriched *ogi* and Cerelac diet. The results of Net protein ratio, True digestibility, Biological value and Net protein utilization of rats fed on Casein and Cerelac diet were significantly higher (p=0.05) than those of rats fed on crayfish-enriched *ogi* diets. The weight of the heart, liver, spleen and kidney of rats fed on crayfish-enriched *ogi* blends were significantly higher (p=0.05) than that of ordinary *ogi* but similar to that of rats fed on Casein and Cerelac diets. The packed cell volume, haemoglobin concentration and red blood cell of the experimental rats were not significantly (p=0.05) influenced by the dietary treatment while white blood cell and erythrocyte sedimentation rate were influenced by dietary treatment.
Conclusion: Crayfish enriched ogi has potential as a functional weaning food with adaptable production technology especially among rural dwellers.

Keywords: Quality evaluation, crayfish, enrichment, Ogi, germination, fermentation, toasting

1. INTRODUCTION

When breast milk is no longer enough to meet the nutritional needs of the infant at the age of four or six months and above, complementary foods (i.e., traditional or commercial weaning foods) should be added to the diet of the child. Several commercial weaning foods are marketed in Nigeria, but they are too expensive for people of low socio-economic status, especially those in the rural areas [1]. The most popular traditional weaning food in Nigeria which is fermented maize gruel known as 'ogi' has been implicated in the etiology of protein – energy malnutrition (PEM) in children during weaning period due to the low nutritive value characterized by low protein, low energy density and high bulk [2]. There is therefore a need to develop weaning foods with adequate protein that will support growth in children from low cost local food sources using methods suitable at village level or at home.

Crayfish which is classified as an animal polypeptide is a freshwater crustacean resembling small lobster and it is commonly found in Nigerian coastal water. Crayfish is relatively cheap, affordable and readily available throughout the year. A review of nutritional value of crayfish showed that it is a good source of protein (36 -45%) with a superior biological value, true digestibility, net protein utilization, high content of essential amino acid, and protein efficiency is favourable compared to casein [3, 4]. It very low in carbohydrate but rich in vitamin D, A and mineral elements such as calcium, potassium copper, zinc and iodine, [5, 6].

Germination, fermentation and toasting of cereals are affordable and widely practiced processing techniques in Africa which enhance the nutritional quality of foods [7]. Fermentation enhances the nutrient of foods through biosynthesis and bioavailability of vitamins, essential amino acids, reducing the antinutrients and improving the protein quality and fibre digestibility [8, 9]. Germination unlocks many nutrients which are in bound forms in the food and thereby increases nutrient bio-availability, energy density and acceptability of the food [10, 11]. Dahiya and Kapoor [12] also reported that toasting reduces anti-nutrients, improves the taste and nutrient quality of the food product and lowers the moisture content of such food product thereby increasing its shelf life.

An integrated approach that combines a variety of the traditional food processing techniques in the preparation of weaning food, including the addition of small amount of animal-source foods has been reported to be the best strategy to improve the nutrient content and bioavailability of micro- nutrients in plant-based diets in resource-poor settings [13] . The combination of two or more food processing techniques is more effective in removing antinutritional factors in cereal and thereby produces high nutrient dense weaning food [14]

The present study is therefore developed to produce high nutrient dense weaning foods from ogi flour and crayfish flour mixes using a combination of traditional processing techniques and evaluates the microbiological and in vivo protein quality of the formulated diets.

2. MATERIAL AND METHODS

2.1 Materials

White maize *(Zea mays*) and white crayfish (*euastacus spp)* were purchased from Oba market in Akure Ondo state Nigeria.

2.2 Preparation of germinated-fermented crayfish-enriched ogi flour

The maize grains were soaked overnight after which they were malted for 3 days. The malted grains were dried for 24 hours and the radicules were removed. The malted grains were steeped in water for 24 hours for fermentation to take place. The fermented and malted grains were wet-milled, wet-sieved and the slurry obtained was allowed to settle, packed in muslin cloth and dewatered. *Ogi* cake obtained was pulverized, sieved and divided into two portions. One portion was oven dried at 50 ^{o}C for 24 hours. The second portion was toasted using open cast iron at 70^{o}C to 80^{o}C. The crayfish were cleaned, beheaded and milled to obtain the flour. Blends were made by mixing adequate proportion of *ogi* flour and crayfish flour to obtain blends of 18% level of protein. The mixing ratios were determined by using Quarto pro 8 software programme.

2.3 Microbiological analysis

The formulated weaning diets were examined microbiologically using the procedure of Olutiola *et al*., [15] after serial dilution. The total microbial load was determined using nutrient agar in a plate count while molds and yeasts were examined using potato dextro agar. Staphylococcus aureus, coliform and salmonella were determined using manitol salt agar, macconkey agar and deoxycholate citrate agar respectively.

2.4 Animal Experiments

The freshly formulated weaning foods, Cerelac and Casein were fed to rats for biological evaluation of protein

2.5 Experimental Diets

The test diets contained formulated diets and cerelac so as to provide 10% protein. Glucose (5%), 12% sucrose, 5% cellulose, 10% vegetable oil, 2% mineral mixture, 1% vitamin mixture and 0.2% NaCl to make 100%. A control diet containing 10% casein was formulated. Basal diet (ordinary *ogi*) was formulated such that there was no nitrogen furnished by any of the ingredient. Composition of experimental diets is as shown in Table 1. Diet 1 is the basal diet (ordinary *ogi*), diet 2 is the control (casein diet) while diets 3, 4 and 5 are Cerelac, oven dried crayfish enriched *ogi* and toasted crayfish enriched *ogi* respectively. All the experimental diets were hand milled starting with the minute components to ensure uniform and proper blending of all the ingredients. They were thereafter put into a well-sealed plastic container, labeled and stored at 4^{o}C prior to use.

Table 1: Composition of experimental diet (g/100g)

Ingredients	N-free diet	Casein diet	Cerelac diet	Oven-dried enriched ogi diet	Toasted enriched ogi diet
Ordinary ogi	71.80	60.30	5.63	16.24	16.24
Casein	-	11.50	-	-	-
Cerelac	-	-	66.67	-	-
Oven-dried enriched ogi	-	-	-	55.56	-
Toasted enriched ogi	-	-	-	-	55.56
Glucose	5.00	5.00	5.00	5.00	5.00
Sucrose	10.00	10.00	10.00	10.00	10.00
Non-nutritive cellulose	5.00	5.00	5.00	5.00	5.00
Vegetable oil	5.00	5.00	5.00	5.00	5.00
Mineral mixture	2.00	2.00	2.00	2.00	2.00
Vitamin mixture	1.00	1.00	1.00	1.00	1.00
NaCl	0.2	0.2	0.2	0.2	0.2
Total	100.00	100.00	100.00	100.00	100.00

2.6 Animal Management

A total of 30 weaning albino rats of the wistar strain purchased from the department of biochemistry, University of Ilorin, Kwara state, Nigeria. The rats were at about 21- 25 days old weighing 30-65g. The rats were housed individually in stainless steel metabolic cages. The animals were acclimatized to the laboratory environment by feeding them on normal diets for seven days. The animals were then reweighed and grouped into five of six rats each per group such that the differences in their mean weights were ±2g. Food and water were provided *ad libitum* to the rats for 28days, during which daily feed consumption and weight were recorded. Variations in weight of the experimental animals were taken every three days. A five day (day 24 - 28) feacal and urine collection was done individually on daily basis for rat in each metabolic cage. The urine from each cubicle was collected into small urine container. About 1ml of concentrated sulphuric acid was added to each urine container as a preservative measure against fungal and other microbial growth. The daily feacal collection (day 24 - 28) were dried at 70 ^{o}C, weighed and powdered prior to laboratory analyses [16]. Duplicate samples of urine and faeces were taken for nitrogen determination [17].

2.7 Blood collection

At the end of the experiment, all the rats were starved for 3hours and weighed. Before sacrifice, each rat was anaesthetized with chloroform inside a dessicator. Blood was

collected into Bijour bottles containing a speck of dried ethylenediamine tetracetic acid (EDTA) powder and used for haematological studies [18].

2.8 Organ measurements

The heart, lungs, spleen, kidneys and liver were removed, blotted free of blood and weighed [18].

2.9 Haematological studies

The packed cell volume (PCV) was estimated by spinning about 75µl of each blood sample in heparinised capillary tubes in a haematocrit microcentrifuge for 5min, and the total red blood cell (RBC) and white blood cell (WBC) counts were determined [18, 19]. The haemoglobin concentration (Hb) was estimated using the cyano-methaemoglobin concentration method, while the lymphocyte, neutrophil, monocyte, basophill and eosinophil were determined [18, 19].

2.10 Statistical analysis

All analyses were carried out in triplicates. Means were tested for differences using Analysis of Variance (ANOVA) using Statistical Analysis System Software (SAS version 9.2, SAS institute, Cary, NC). Significant differences between mean values were determined by Duncan's Multiple Range Test and accepted at $P \leq 0.05$. Data are reported as mean ± standard deviation from the mean.

3. RESULTS AND DISCUSSION

3.1 Microbial analysis

The result of *coliform, staphylococcus, salmonella*, mould, yeast and total viable count of the formulated diets are shown in Table 2. *Coliform, Staphylococcus and Salmonella* spp were absent in the formulated diets. This shows that the food will be fit for human consumption. The total viable count in all the formulated diets are below the maximum level of 1.0 x105 recommend by PAG [20]. However, all the formulated weaning diets would require cooking before feeding to children during which most of these microorganisms would be destroyed. The reduction in the total viable count of toasted enriched ogi diets may be due to toasting which was done at high temperature. (70-80^{o}C) and might have destroyed all the pathogenic microorganisms.

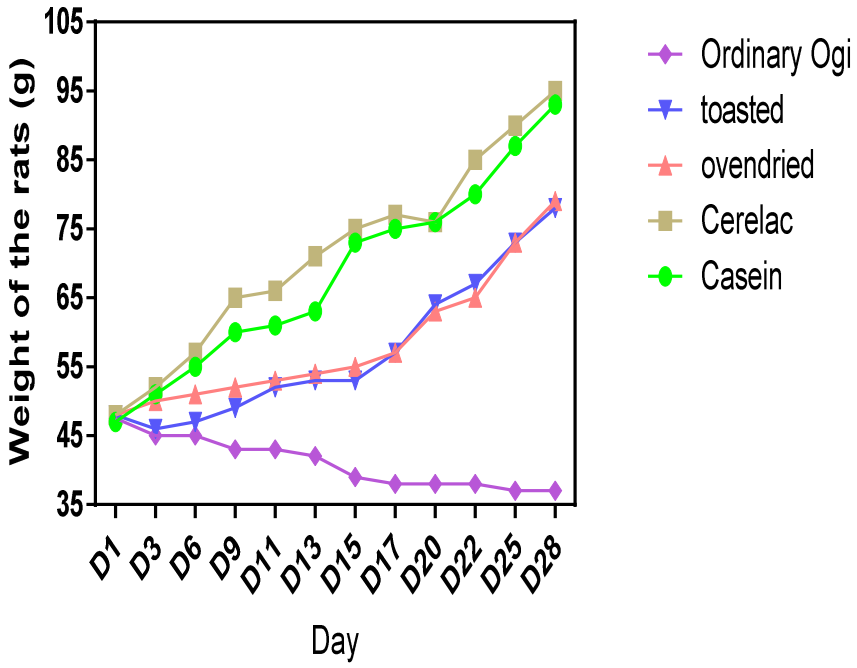
Table 2: Microbiological quality of enriched *ogi* (cfu/g)

	counts	
Micro-organism	**Ovendried**	**Toasted**

Coliform	0	0
Staphylococcus	0	0
Salmonella	0	0
Molds (sfu/g)	$1x10^3$	$1x10^3$
Yeast	$1x10^3$	0
Total viable count	$1.8x10^3$	$1.2x10^3$

3.2 Performance and nutrient utilization of rats fed with enriched ogi, casein and Cerelac

The weight changes of the experimental animals are presented in Fig 1. The basal diet (ordinary *ogi* with low protein) failed to support growth. The casein diet gave the highest change in growth closely followed by Cerelac. The performance change in growth of oven-dried enriched *ogi* and toasted enriched *ogi* diet was not quite as good as those of Casein and Cerelac. However, it may be observed that the oven-dried enriched *ogi* and toasted enriched ogi diet ranked very closely. Ibironke, [21] showed that commercially available diets supported growth more than the diet formulated from vegetable proteins and cereals. In contrast, Ibironke et al. [6] reported that the diet formulated from fermented maize flour and crayfish (10% and 15%) supported growth more than the milk based commercial diet. Ordinary ogi diet did not show any appreciable growth. This may be due to the fact that the diet lacked adequate nutrient such as protein. This agrees with the previous results that cereals are deficient in essential amino acid, such as lysine and tryptophan, hence, was not nutritionally adequate to promote growth [3,11,12].



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165 D…….1042010 = Days of feeding

166 Fig. 1: Growth rate of rats fed with different weaning diets and casein

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168 Food intake, gain in body weight and feed efficiency ratio of the experimental animals

169 The results of food intake, protein intake and gain in body weight of the experimental rats are presented in
170 Table 3. The food intake of rats fed on the formulated diets, Cerelac and Casein ranged from 148.5g (oven-
171 dried enriched ogi) to 182.2g (cerelac diet). Food intake was significantly (p=0.05) higher in the group fed
172 with casein and cerelac than rats fed on the oven-dried and toasted enriched ogi. The food intake of the
173 animals fed with commercial diet agreed with their growth rate. Similar observation was reported by Ibironke,
174 [21]. Food intake of rats fed on toasted enriched ogi diet was the same with rats fed on oven dried enriched
175 ogi diet. A similar trend was observed in the protein intake of the different groups being highest (18.5g) in the
176 cerelac and lowest (14.9g) in oven dried crayfish enriched ogi diet. The gain in body weight of growing rats
177 ranged from 33.0 g to 46.8 g. The gain in body weight of rats fed on oven dried CF enriched *ogi* and toasted
178 CF enriched *ogi* diets were the same and significantly (p=0.05) higher than those fed on ordinary ogi but
179 were lower than those fed on Casein and Cerelac. The rats fed on ordinary *ogi* lost weight. Although the gain
180 in body weight of the rats fed with cerelac and casein group were almost the same, the food intake of the
181 Cerelac group was (though not-significant) less. This shows that the casein protein was slightly better
182 assimilated than that of cerelac. Similar result was reported by Dahiya and Kapoor [12]. It can be deduced
183 that casein and cerelac protein were better assimilated than those of oven-dried and toasted CF enriched *ogi*
184 diets.

185 **Table 3: Performance and nutrient utilization of rats fed with enriched ogi, casein and Cerelac**

parameters	casein	cerelac	Oven-dried enriched ogi	Toasted enriched ogi
Food intake (g)	164.6[a]	182.2[a]	148.5[b]	149.6[b]
Protein intake (g)	17.8[a]	18.5[a]	14.5[b]	15.0[b]
Gain in body weight (g)	46.5[a]	45.8[a]	33.8[b]	33.5[b]
Nitrogen consumed	0.61	0.62	0.47	0.56
Feacal nitrogen	0.15	0.20	0.20	0.23
Urinary nitrogen	0.09	0.13	0.14	0.17
Nitrogen retained	0.37	0.28	0.13	0.16

186 Values followed by different superscript on the same row are significantly different (p=0.5)

187 The results of Feed efficiency ratio (FER), protein efficiency ratio (PER) and net protein ratio (NPR) of the
188 rats fed on Casein, Cerelac, oven-dried CF enriched *ogi* and toasted CF enriched *ogi* are shown in Fig 2.
189 The feed efficiency ratio (FER) of different diets varied from 0.22g (toasted enriched ogi diet) to 0.26g
190 (casein diet). The FER of rats fed on casein and cerelac were not significantly different (P=0.05) but were
191 higher than those of rats fed on toasted CF enriched ogi and oven dried CF enriched ogi diets. The corrected
192 PERs of the different diets varied from 2.3 (toasted CF enriched *ogi*) to 2.5 (Casein diet). There was no
193 significant difference (p=0.05) in the PER of the different diets obtained in the present study and this is
194 similar to those reported by Osundahunsi and Aworh, [22] in the weaning foods prepared from maize-tempe
195 based diet with PER ranging from 2.3 to 2.5. The net protein ratio (NPR) of the diets ranged from 2.6
196 (toasted enriched ogi) to 3.25 (Casein diets). NPR is a more accurate measure of protein quality than PER
197 as it allows for the evaluation of maintenance requirement and results are independent of feed intake. The
198 NPR performance of the formulated diet was not quite optimal when compared to that of the standard
199 (casein). Similar report was obtained by Fashakin, [23].

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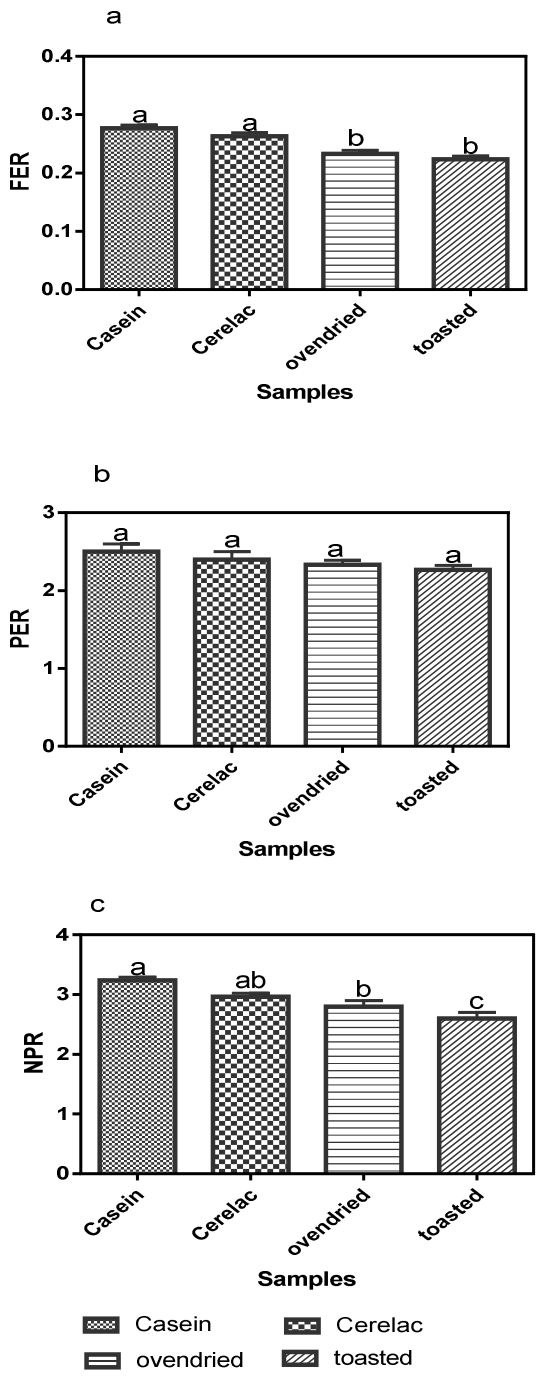

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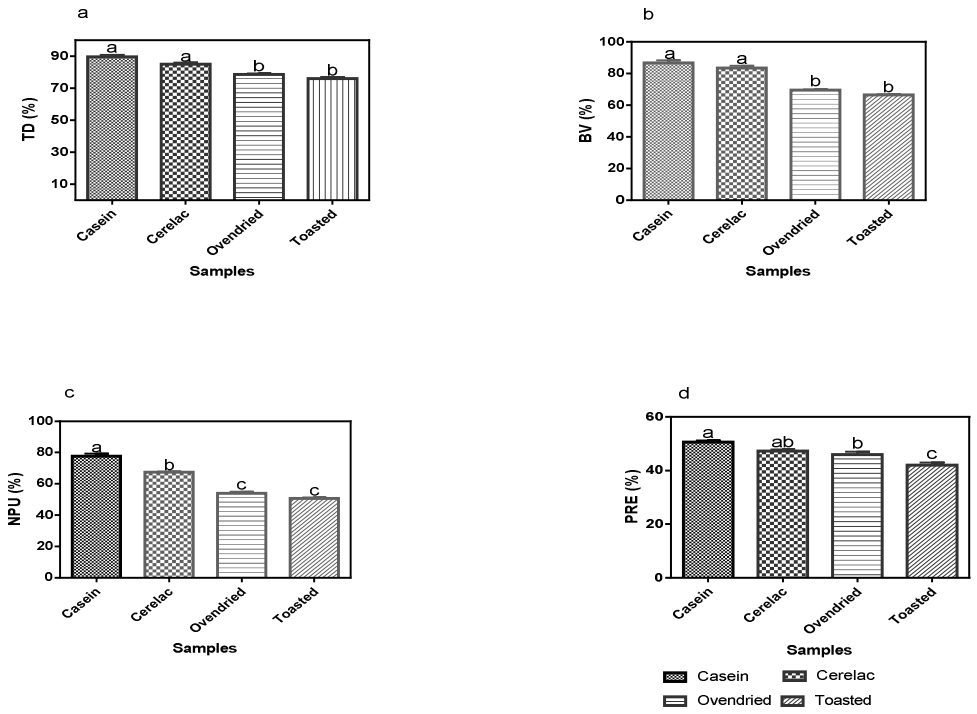
202 Fig. 2: (a) The Feed Efficiency Ratio (FER), (b) Protein Efficiency Ratio (PER) and (c) Net Protein Ratio
203 (NPR) of rats fed on different weaning diets and casein

204 The results of true digestibility (TD), biological value (BV), net protein utilization (NPU) and protein retention
205 efficiency (PRE) are illustrated in (Fig 3). TD, BV, NPU and PRE ranged from76.0 to 89.8%, 66.5 to 87.6%,

206 50.5 to77.4% and 42 to 51 respectively. The TD, BV and NPU of casein and cerelac were higher (p≤ 0.05)
207 than those of oven-dried *and* toasted CF enriched *ogi* . The result obtained in the present study is similar to

208 those observed by Obizoba [24], who reported BV values of 67.6 to 75.9% and NPU values of 51.8 to 62.3%
209 in the weaning food prepared from malted wet-milled corn plus crayfish. The lower values of TD, BV and
210 NPU in the toasted diet may be due to roasting as it adversely affects the availability of some amino acids.
211 Similar report was obtained by Dahiya and Kapoor, [12] who reported that PER, TD, BV and NPU decreased
212 on roasting. The effect of roasting on availability of amino acid can be minimized by roasting at a reduced
213 temperature. Since protein retention efficiency (PRE) was obtained by multiplying NPR by 16, the trend of
214 the result obtained for PRE is similar to that of NPR (Fig. 2.).



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216 Fig. 3: (a) The True Digestibility (TD), (b) Biological Value (BV), (c) Net protein Utilization (NPU) and (d)
217 Protein Retention Efficiency of rats fed with different weaning diets and casein

218 **3.3 Organ weights**

219 The weight of some vital organs of rats fed cerelac, casein, oven dried and toasted CF enriched ogi and
220 ordinary ogi are shown in Table 4. The heart weight, the liver weight, the spleen weight and kidney weight
221 ranged from 0.20 to 0.37g, 2.19 to 4.4g, 0.13 to 0.42 g and 0.35 to 0.78g respectively. The weights of the
222 heart, the kidney, the spleen and liver of rats fed on oven dried and toasted CF enriched ogi compared
223 favorably with those of standard (Casein and Cerelac). This indicates that the formulated diets may not result
224 in abnormal development of the vital organs.

225

Table 4: Organ weights (g) of rats fed with enriched-ogi, casein, cerelac and ordinary 'ogi'

Dietary group	Heart (g)	liver (g)	Spleen (g)	Kidney (g)
Cerelac	0.28[b]	4.02[ab]	0.31[ab]	0.72[ab]
Casein	0.37[a]	4.49[a]	o.42[a]	0.78[a]
Oven-dried Enriched 'ogi'	0.29[b]	3.79[b]	0.29[b]	0.55[ab]
Toasted Enriched 'ogi'	0.31[ab]	3.80[b]	0.29[b]	0.53[ab]
Ordinary'Ogi' diet	0.20[c]	2.19[c]	0.13[c]	0.35[b]

Values with different superscript on the same column are significantly different (p=0.5)

3.3 Haematological parameters of rats fed with cerelac, casein, oven-dried, and toasted enriched ogi and ordinary ogi

The results of haematological parameters of rats fed with cerelac, casein, oven-dried, and toasted enriched ogi and ordinary ogi are shown in Table 5. The blood indices varied: packed cell volume (PCV) 30.25 to 33.0 %, haemoglobin concentration (Hb) 10.03 to 11.28%, red blood cell (RBC) 64.45 to 73.08 $x10^{5}$, white blood cell (WBC) 52.0 to 101.5 x $10^{5,}$ erythrocyte sedimentation rate 1.23 to 1.65, lymphocyte 50.18 to 53.15%, basophil 2.0 to 11.25%, neutrophil 30.5 to 37.35% and monocytes 7.75 to 10.0%. Low PCV, HBc and serum protein have been associated with protein deficiency [25]. The PCV, Hb and RBC of rats fed on basal diet were lower than those fed on Casein, Cerelac and experimental diets. Similar results were reported by Osundahunsi and Aworh [22]. The values obtained for PCV, RBC, WBC and Hb for the rats fed on formulated diets were similar to those fed Casein and Cerelac. The results obtained for haematological indices in this study are similar to the values reported for infant foods formulated from *Telfairia occidantalis* leaf protein concentrate by Agbede et al. [26]. This suggests that the feeding of formulated diets will support haematopoietic activities of the body.

Table 5: Haematological parameters of rats fed with various diets

Parameters	Toasted enriched ogi	Oven-dried enriched ogi	Casein	Cerelac	Ogi
Packed cell volume (%)	30.50	30.75	32.00	33.00	30.25
Haemaglobin (g/100ml)	10.38	10.25	10.73	11.28	10.03
Red blood cell (x 10^5)	67.55	67.30	70.98	73.08	64.45
White blood cell (x10^2)	76.63^{ab}	52.01^{b}	66.51^{ab}	68.01^{ab}	101.5^{a}
Erythrocyte sedimentation rate	1.23^{b}	1.35^{ab}	1.38^{ab}	1.35^{ab}	1.65^{a}
Lymphocytes (%)	53.15	51.00	50.18	51.00	54.25
Monocytes (%)	9.00	8.00	7.75	9.00	10.00
Eosinophil (%)	2.50	2.75	2.00	2.00	3.50
Basophil (%)	1.25^{ab}	1.75^{a}	1.25^{ab}	1.50^{a}	2.01^{a}
Neutrophil (%)	36.75^{a}	30.51^{b}	37.25^{a}	36.01^{a}	31.25^{b}

Conclusion

The performance of rats fed the formulated diets was not quite optimal when compared with the rats fed Cerelac and Casein. The performance of the formulated diets were significantly (p=0.05) better than that of ordinary *ogi*. The haematological indices and organ weight measurement of the rats fed the formulated diets were better than that of ordinary *ogi* and compared favourably with that of rats fed with standard casein and Cerelac. The study indicated that oven dried enriched *ogi* and toasted enriched *ogi* will support growth in children than ordinary *ogi* which is currently in use as traditional weaning foods in Nigeria. The implications of these findings are far reaching since all the components used in the formulation are obtained from local market and toasting is a processing method that can easily be practiced at home. Adoption of toasted enriched ogi may make the product a potentially more functional and more accessible weaning food.

COMPETING INTERESTS

We declared no competing interests exist

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