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Protein Quality, Haematological and Histopathological Studies of Rats Fed with Maize-based Complementary Diet Enriched with Fermented and Germinated Moringa Oleifera Seed Flour

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Abstract

This study is about the production of Maize-based complementary diet enriched with fermented and germinated moringa oleifera seed holds great promise in alleviating malnutrition so prominent among Nigerian children less than five years. However, there is scanty scientific information on its protein quality and safety which is necessary prelude to trials on human subjects. This study hence reports a controlled feeding trial involving 30 weanling wister rats housed in individual standard metabolic cages under room temperature condition. Following a subsequent daily feeding of the rats for 28 days, the protein quality of the diet with the haematological and histological studies was conducted. Results showed that the diets had a statistically significant effect on the growth rate on the test rats when compared with the control. The protein quality evaluation of the diets showed that the protein efficiency ratio ranged from 2.10 to 2.38 while the biological value ranged from 62.01 to 89.01 %. The true protein digestibility and protein rating were 55.79 to 79.25 % and 35.42 to 48.61 respectively. The relative weight of organs of the rats fed with the complementary diets showed that the weights of the kidney and liver ranged from 0.57 to 0.76g and 2.87 to 3.60g respectively. The growth performance of the rats fed with the formulated complementary diets showed that the formulated diets contributed to the growth status of the animals indicating that protein quality of the diets could support the growth and development of the infants. The haematological indices showed that the packed cell volume of the sample ranged from 36.67 to 40.67% and the red blood cell counts was 5.00 to 5.93 ($\times 10^6$ mm^3). The white blood cell counts ranged from 3.47 to 3.98 (×103mm3) while the mean corpuscular haemoglobin and mean corspucularhaemoglobin concentration ranged from 10.04 to 11.71Pg and 33.10 to 33.45% respectively. Moreover, rat's biopsy (histopathology) revealed no necrosis in the observed livers of the rats fed with the diets. Evidently, ogi-fermented moringa oleiefera seed complementary diet has no established detrimental effect and may therefore be safe for humans.

Keyword: Biological value; Complementary diet; Haematological; Histological; Protein quality

Abbreviations: PTD: Protein True Digestibility; PR: Protein Rating; NPU: Net Protein Utilization NPR: Net Protein Retention; PCV: Packed Cell Volume

Introduction

Background

The end of the 20th century was marked by drastic increase in the incidence of protein energy malnutrition, chemical hazards as well as other safety issues[1]. In a bid to significantly establish the protein quality and safety of raw or processed food materials for human consumption, it is customary to test same on animal models which could provide relevant information. New complementary food meant toprovide nutritional support for improving growth and continued reduction in child mortality/morbidity are by no means an exceptional. Food processing techniques such as fermentation and germination have the potential to enhance the nutrient bioavailability, nutrient density, food safety, storage stability, palatability, and convenience of complementary foods suitable for infant mixtures[2]. Fermentation and Germination of cereals are affordable and widely practiced processing techniques in Africa[3]. Fermentation enhances the nutrient of foods through biosynthesis and bioavailability of vitamins and essential amino acids, reduction of antinutrients, improving the protein quality and fibre digestibility [4,5]. Germination unlocks many nutrients which are in bound forms in the food, thereby increasing nutrient bio-availability, energy density and acceptability of the food [6].

As far as safety of complementary food is concerned, enlargement of the pancreas and inhibited growth of rats fed sub-optimally processed soybeans foods and theobroma cacao induced fetal malformation[7], hence preclinical experimentation using animal model is therefore in order. Ogazi et al.[8] in reporting the effects of 'soya musa' (an extruded soybean-plantain baby food) on sixteen wistar rats observed that they had normal growth, packed cell volume and white blood cell counts. In evaluating the biological effect of a cassava-soy weaning food on rat organs (small intestine, pancreas, liver and heart), Babajide et al.[9] observed no significant difference in the organ weight of the rats fed the test diets as compared with cerelac- a commercial weaning diet. Rats fed on extruded weaning foods based on peanut, maize and soybean were observed by Plahar et al.[10] to have between 60-100-fold increases in mean weight gain over the control.It was also reported that haemotological data of test animals showed normal values for white blood cell count, red blood cell count, haemoglobin level and packed cell volume for all the weaning food studied except the control. Moringa oleifera is an underutilised plant and the whole seeds are highly restricted to eaten green, roasted or powdered and steamed in tea and curries. The pods and seeds often referred to as Moringa kernels have a taste that ranges from sweet to bitter and are most popularly consumed after frying to get a groundnut-like taste. The seeds contain a profile of important minerals, and are good source of protein, vitamins, beta-carotene, amino acids and various phenolics compounds. Information on the possible safety or otherwise of a complementary food made from ogi-moringa seed flour is virtually non-existence, hence the need to ascertain this in view of the likely potential benefit of such products.

Material and Methods

Animal experimentation and ethical approval

Feeding trials were carried out with male wister albino rats using the method describe byGernah et al.[11]after the study was approved by the ethical review committee of the Federal University of Technology, Akure, Ondo State, Nigeria. The thirty rats used were obtained from the rat house University of Ibadan, Oyo State Nigeria and they are randomly distributed to thirty metabolic cages (i.e. individually caged) based on average weight equivalent of 59.10g. The weanling rats were fed a standardised laboratory rat chow for an acclimatization period of ten days in a well-ventilated room at temperature of 30± 4^{0}C and relative humidity of 60 %. Subsequently, the rats were re-wesighed into the individual cage each with mean weight differences of ± 0.20g. Each animal group (in individual cage) was fed the three test diets, control and the basal diets respectively. Experimental and control diets were prepared by incorporating the test diets and casein into the basal diet to achieve an isonitrogenous diet at 10% protein level. The rats were given the formulated diets and water ad-libitum for 28days while unconsumed diets were collected and weighed daily. The animals were weighed twice in a week, while their faeces and urine were collected and pooled for each group. The collected feces and urine were analysed for crude protein using AOAC [12] method. At the end of the test period, the animals were terminated with chloroform and blood samples were taken for haematological analysis while the kidney, liver, spleen and heart were quickly excised and weighed. The protein efficiency ratio (PER), biological value (BV), protein true digestibility (PTD), net protein utilization (NPU), net protein retention (NPR), food efficiency ratio (FER), and protein rating (PR) were calculated according to the procedures ofOsundahunsi&Aworh [13] (Table 1 & 2).

Table 1: The Composition of the Basal Diets.

Samples	Weight (g)
Corn starch	600.5
Glucose	50
Sucrose	150
Non-nutritive cellulose (wheat bran)	50
Vegetable oil	97
Mineral and vitamin premix	20
Oyster shell	10
Bone meal	20
Sodium chloride	2.5
	1000g

Source: Osundahunsi&Aworh[13]

Table 2: Composition of the Various Isonitrogenous Diets at 10% Protein Level.

Group	Feed	Protein Content of the Feed as Analysed (%) A	Weight of Feed Mixture (g) Y	Weight of Basal Diet (g)	Weight of Feed (g) X	Final Protein level (%)
1	Casein	86.18	1000	883.96	116.04	10
2	ORM	15.26	1000	344.69	655.31	10
3	OFM	16.38	1000	389.49	610.5	10
4	OGM	16.58	1000	396.86	603.14	10
5	Basal	-	1000	1000	-	-

ORM: Ogi-Raw moringa flour.

OFM: Ogi-Fermented moringa flour.

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OGM: Ogi-Germinated moringa flour.

$$IN = \frac{a}{100} \times X = \frac{10}{100} \times Y; \ \frac{10}{100} \times Y; \ \frac{10Y}{100} = \frac{aX}{100}; \ \frac{10Y}{a} = X$$

Where: a = original protein content of the sample as analyzed

X = Weight of sample required for the new feed mixture

Y = Total weight of the mixture.

IN = Isonitrogenous

Determination of haematological parameters

At the end of the experiment, all the rats were starved for 3 hours, weighed, anaesthetized and sacrificed. Blood samples with (EDTA) and without anticoagulant were collected from each treatment in triplicate. Blood samples collected with EDTA were sued to determine packed cell volume (PCV), red blood cell counts (RBC), white blood cell (WBC) counts and the haemoglobin in blood samples using the Wintrobe'smicrohaematocrit, improved Neubauer haemocytometer and cyanomethae- moglobin method respectively[14]. The mean corpuscular hemoglobin (MCH) was calculated according to Lamb [15]. The lymphocyte, neutrophil, monocyte, basophill and eosinophil were determined according to[15,16].

Histopathological studies

The kidney, liver, spleen and heart were separated, blotted free of blood, oven dried at 40°c for 5h and weighed. The values were subsequently expressed in g/kg body weight. After the induced bleeding, rats from each group were sacrificed through vascular dislocation and livers were removed from the animals. They were weighed and subjected to histopathology analysis (toxicity signs) after fixation in slides using method of Adesiji [17]. The tissue was dehydrated in graded levels of ethanol, cleared in xylene, and embedded in paraffin wax for sectioning. The 5µm thick sections were cut, mounted on glass slides and stained with haematoxylin and eosin for light microscopy. The photomicrographs of slides were observed under the microscope by pathologist with magnifications of x400 and x100. Selected images were captured using a moticam 2.0 digital camera attached to a computer.

Statistical analysis

Data were collected as means of three separate determinations and subjected to one-way analysis of variance using Statistical Package for Social Statistics (SPSS 20.0). The significant differences (p≤0.05) between the mean values were determined using the Duncan's Multiple Range Test.

Results and discussion

Protein quality and growth performance of the experimental animals during feeding trials

The feeding experiment showed that the mean food intake (Table 3) of the experimental animals kept on various formulated diets varied from 805.00g Ogi-raw moringa flour (ORM) to 980.00 g casein (CAS). The food intake of the animals fed with casein and cerelac were significantly higher however, rats fed with ogi-fermented moringa flour (OFM) and ogi-germinated moringa flour (OGM) were significantly higher than the Ogi-raw moringa flour (ORM). A similar trend was observed in the protein intake of the experimental animals. The mean weight gain of the animals ranged from 35.55g (ORM) to 46.65g (CAS). The weight gain was influenced by the quality of the protein constituents and the quantity of diet consumed. It was observed that the weight gain of animals placed on experimental diets (OFM and OGM) were lower than those of animals fed with cerelac and casein diets but were higher than those of animals fed with ogi-raw moringa flour (ORM). Protein is required for good growth, healthy living, maintenance and production of tissues and cells of the body. Among the formulated diets, the ogi-feremented moringa seed flour (OFM) supported the best weight gain while the ogi-raw moringa flour (ORM) supported the least weight gain.

Table 3: Protein Quality of Ogi-moringa Seed Flour Complementary and Control Diets.

Samples	ORM	OFM	OGM	CRL	CAS
Food intake (g)	805.00^{d}	977.00^{b}	969.00^{c}	980.00^{a}	980.00^{a}
Weight gain (g)	35.55^{e}	45.01^{b}	40.70^{d}	43.90^{c}	46.65^{a}
Protein intake (g)	16.10^{c}	19.40^{b}	19.38^{b}	19.60^{a}	19.60^{a}
PER	2.20^{d}	2.32^{b}	2.10^{e}	2.24^{c}	2.38^{a}
BV (%)	60.01^{e}	65.01^{c}	62.01^{d}	82.01^{b}	89.01^{a}
NPU	33.47^{e}	44.12^{c}	38.54^{d}	59.18^{b}	70.52^{a}
NPR	1.00d	1.18^{b}	1.17^{b}	1.16^{c}	1.29^{a}
FER	0.04^{b}	0.05^{a}	0.04^{b}	0.04^{b}	0.05^{a}
PTD (%)	55.79^{e}	67.88^{c}	62.17^{d}	72.18^{b}	79.25^{a}
PR	35.42^{e}	49.95^{c}	41.68^{d}	45.87^{b}	48.61^{a}

Each value is a mean of triplicate determination. Values in the same row with different letters are significantly different (p<0.05). ORM: Ogi-raw moringa seed flour; OFM: Ogi-fermented moringa seed flour; OGM- Ogi-germinated moringa seed flour; CRL- Cerelac (Control); BAS: Basal diet; CAS: Casein diet. PER: Protein efficiency ratio; BV: Biological value; NPU: NPR-Net protein utilization; FER: Feed efficiency ratio; TD: True digestibility; PR: Protein rating.

The protein efficiency ratio of the formulated diets ranged from 2.10 (OGM) to 2.38 (CAS). The results of the protein efficiency ratio obtained was higher than the value reported by Olapade&Aworh[18] in extruded complementary foods from blends of fonio and cowpea 0.08-0.32 and 1.14-2.15 for weaning food from pigeon pea and millet. However, these values were lower than those reported by [19] in the complementary foods produced from fermented popcorn, African locust bean and bambara groundnut (2.80-4.88). Protein Advisory Group [20] guidelines recommended a protein efficiency ratio of not less than 2.10 and preferably greater than 2.30 for weaning food. A similar recommendation was made by the U.S Department of Agriculture guidelines for corn-based blends [21,22].

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Biological value of the complementary diets ranged from 60.01% (ORM) to 89.01% (CAS).The results showed that the ogi-fermented moringa seed flour and ogi-germinated moringa seed flour gave better biological values than the ogi-raw moringa seed flour, but cerelac and casein gave much better biological values than others. Biological value evaluates the competence of protein to support growth through nitrogen holding in the body. It is the assessment of the absorbed protein from food that becomes part of the body [23]. It was observed that the formulated diets may support the growth of children. Net protein utilization ranged from 33.47 (ORM) to 70.52 (CAS). The net protein utilization of the formulated diets, that is ogi-fermented moringa seed flour and ogi-germinated moringa seed flour was significantly higher than ogi-raw moringa seed flour. The lower net protein utilization recorded by the ogi-raw moringa seed flour may be due to enhanced nitrogen excretion and hence relative reduction of the nitrogen in body. Higher values for the net protein utilization were observed for cerelac and casein and this reflects that higher nitrogen was retained and utilized by the body of the experimental rats. The net protein ratio of the diets ranged from 1.00 (ORM) to 1.29 (CAS). The net protein ratio of ogi-fermented moringa seed flour and ogi-germinated moringa seed flour were higher than that of ogi-raw moringa seed flour and commercial diet (cerelac) and this indicates that the diets are efficient in supporting the growth and maintenance of the wealing rats. The feed efficiency ratio is measured as a function of gain in body weight and food intake. Foods with high feed efficiency ratio tends to add to weight gain while low feed efficiency ratio are prone to be used as energy rather than stored as body weight Bryrd-Brdbenner et al.[24]. The value of fed efficiency ratio of the formulated diets (ogi-fermented moringa seed flour and ogi-germinated moringa seed flour) was higher than ogi-raw moringa seed flour and compared favourably with commercial (cerelac) and casein diets. The true protein digestibility of the samples ranged from 55.79 % (ORM) to 79.25 % (CAS). True protein digestibility of complementary foods specifies lower faecal nitrogen output and higher retention of nitrogen in animal bodies. The lowest true digestibility in ogi-raw moringa seed flour was possibly owing to the present of the high antinutritional factors in the sample which may inhibit proper digestibility of protein. The reduction in the antinutritional values observed in ogi-fermented moringa seed flour and ogi-germinated moringa seed flour may influence the high true digestibility of the complementary diets as observed in the study. The protein rating of the formulated diets ogi-fermented moringa seed flour and ogi-germinated moringa seed flour compared favorably with cerelac and casein. The ogi-fermented moringa seed diet have better growth promoting quality than the ogi-germinated moringa diet and the superiority of the ogi-fermented moringa seed flour diet over ogi-germinated moringa agrees with the report of Ikujenlola & Fashakin [25].

The growth performance of the rats fed with formulated and control diets are presented in Figure 1. The results showed that the growth rate of the rats fed with control (cerelac) and casein (casein) were significantly higher compared to the formulated diets. However, the rats fed with ogi-fermented moringa seeds diet (ogi-fermented moringa seed flour) and (ogi-germinated moringa seeds flour) was significantly higher than ogi-raw moriga seeds flour and basal (BAS) diets.

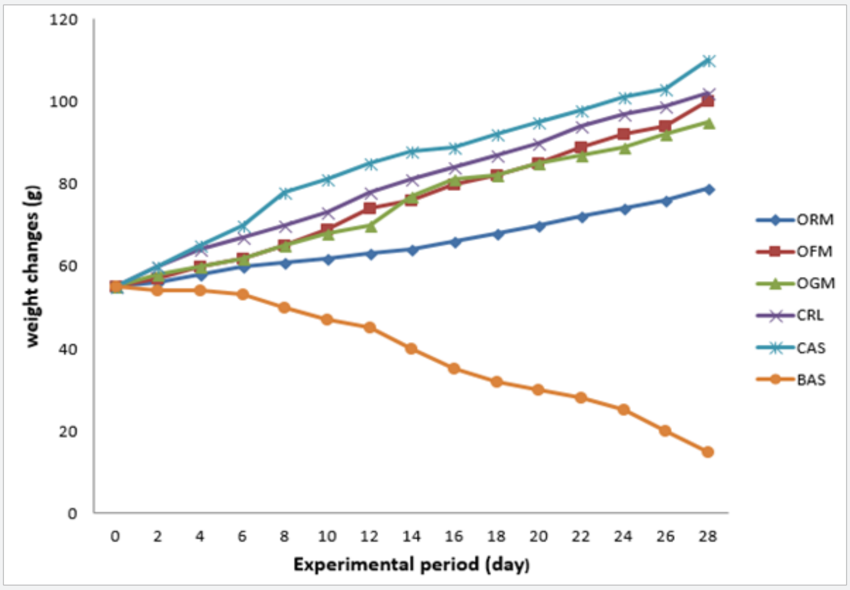

Figure 1: Growth Performance of the Experimental Animals Fed with Formulated, Basaland Control Diets.

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Organ weights and haematological parameters of animals fed with formulated, basal and control Diets

The weight of some vital organs of animals fed with formulated, basal and control diets are shown in Table 4. The formulated complementary diets more importantly the ogi-fermented moringa seed flour and ogi-germinated moringa seed flour promoted growth and development of the organs (kidney, liver, spleen and heart) of animals better than ogi-raw moringa seed flour due to increase in the tissue nitrogen of the experimental animals, but not as well as casein diet. The liver is one of the major important organs of the body that has enzyme to metabolise amino acids and it acts as the body's chemical factory. It regulates the levels of most of the main blood chemicals and acts with the kidney to clear the blood of drugs and toxic substances. Therefore, it can be said that the weight and general appearance of the liver and other body organs could tell a lot on the health status of the experimental animals fed on the formulated diets. The results obtained in relation to organs weight of the experimental rats agree with the findings of Ibironke et al. [26].

Table 4: Relative Organs Weight (grammes) of Rats Fed with Formulated, Basal and Control Diets.

Samples	ORM	OFM	OGM	CRL	BAS	CAS
Kidney	0.63d	0.73b	0.70c	0.73b	0.57e	0.76a
Liver	3.47d	3.60a	3.53c	3.55b	2.87e	3.58a
Spleen	0.33d	0.57b	0.54c	0.57b	0.23e	0.67a
Heart	0.37d	0.44b	0.40c	0.47b	0.33e	0.57a

Each value is a mean of triplicate determination. Values in the same row with different letters are significantly different (p<0.05).

Haemotological indices of albino rats fed with formulated, basal and control diets

Table 5: Haematological Indices of the Rats Fed with Formulated, Basal and Control Diets.

Samples	ORM	OFM	OGM	CRL	BAS	CAS
PCV (%)	36.67^b	37.00^b	40.33^a	40.67a	39.33^a	40.33^a
RBC (X10^6 mm^3)	5.15^a	5.19^a	5.33^a	5.93^a	5.00^a	5.84^a
WBC (X10^3 mm^3)	3.80^a	3.87^a	3.88^a	3.88^a	3.47^a	3.98^a
Hb (g/dl)	13.23^b	13.33^b	13.43^a	13.57^a	13.10^a	13.49^a
MCH (Pg)	10.13^b	10.65^b	10.68^b	10.94^b	10.04^b	11.71^a
MCHC (%)	33.30^a	33.33^a	33.37^a	33.35^a	33.10^a	33.45^a
MCV (µm3)	46.87^a	44.02a	30.51^b	33.27^b	32.87^b	35.17^b
LYM (%)	63.33^a	63.00^a	64.00^a	64.00^a	65.00^a	66.00^a
NEU (%)	24.00^b	26.00^a	25.33^a	25.00^a	23.67^b	23.00^b
MON (%)	7.67^a	6.67^a	6.67^a	7.00^a	7.00^a	7.00^a
EOS (%)	3.33^a	3.00^a	2.33^a	3.33^a	3.33^a	2.67^a
BAS (%)	1.67^a	1.33^b	1.33^b	1.67^a	1.33^b	1.67^a

Each value is a mean of triplicate determination. Values in the same row with the different letters are significantly different (p<0.05).

The haematological property of the maize-based complementary food enriched with fermented and germinated moringa oleifera seed flour is presented in Table 5. The packed cell volume of the diets ranged between 36.67 and 40.67% for (ogi-raw moringa seed flour) and (cerelac) respectively. The values of the red and white blood cells ranged between 5.00-5.84 (×10^6 mm^3) and 3.47-3.98 (×10^6 mm^3) respectively for basal (BAS) and casein (CAS). The haemoglobin concentration of the samples ranged from 13.10 and 13.57 g/dl. The mean concentration haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and mean cell volume (MCV) values for the complementary diets ranged from 10.04 and 11.71%, 33.10 and 33.45%, and 30.51 and 46.87% respectively. The differential counts lymphocytes, neutrophils, monocytes, eosinophils and basophils of the samples values ranged between 63.00 and 66.00%, 23.00 and 26.00%, 6.67 and 7.67%, 2.33 and 3.33% and 1.33 and 1.67% respectively. Packed cell volume also known as haematocrit (Ht or Hct) is the percentage of red blood cells in the whole blood [27]. According to Isaac et al.[28] packed cell volume is involved in the transportation of oxygen and absorbed nutrients, hence increased packed cell volume shows a better transportation and thus results in an increased primary and secondary polycythemia. It can then be observed from the present study that the developed samples show high percentage of packed cell volume when compared with reference value of 24-34 % recommended for healthy rat [29]. There was no significant difference (p<0.05) in the haemoglobin concentration of the samples. The protein-free-diet had the lowest haemoglobin concentration while the developed, commercial samples and casein had the highest haemoglobin concentration. However, the haemoglobin concentrations of all samples were in the range of 10-15g/dl recommended for haemoglobin concentration of healthy rats[29,30]. Haemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates and it has the physiological function of transporting oxygen to tissues of the animals for oxidation of ingested foods so as to release energy for other body functions as well as transport carbon dioxide out of the body of animal [31]. The Haemoglobin concentration and haematocrit are values that show the degree of anemia. The high values of Haemoglobin concentration and haematocrit in the present study suggested adequate iron status and this could be associated to the iron content of the moringa seeds which is a good source of non-heme iron. The mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) values are major indicators of assessment of tendency toward anaemia and a low level is an indication of anaemia [32]. The values obtained for Mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and Mean corpuscular volume in this study showed that the diets were not toxic towards the red blood cells. The differential counts i.e. lymphocytes, neutrophils, monocytes, eosinophils and basophils values for the samples showed no pattern of

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increase or decrease. White blood cell counts are important in defending the body against infections, however, white blood cell counts cannot give definite or specific information, but the results of differential counts narrow down to give specific information about infections, toxicity allergy and immune-suppression and poisoning [33].

Histological findings on the livers of rats fed with formulated basal and control diets

The hepatocytes in the liver showed no form of necrosis and no histopathological alterations, distortions such as mid-cell edema, degeneration of the hepatocytes, enlargement of the alveoli and alveoli hemorrhage as shown in Plate 1. This suggested a high margin of safety of the complementary diets following adequate consumption of the diets. The mild fatty liver observed in the ogi-fermented Moringa oleifera complementary diet may be as a result of overconsumption of the diet and presence of too much fat in the diet which may cause fatty infiltration and could possibly lead to liver damage.

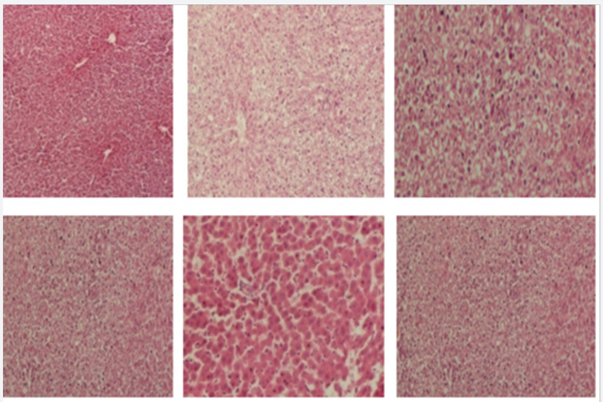

Plate 1: Photomicrographs of the livers of rats fed with ORM, OFM, OGM, CRL,BAS and CAS dietsshowing no form of necrosis.

Conclusion

Based on the protein quality evaluation of the diets, ogi-fermented moringa seed flour complementary diets might be considered suitable for infant growth and development. The absence of toxic substances in the formulated diets as evidenced in the haematological and histological findings could as well adjudge the diets to be suitable and also safe for infant consumption.

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