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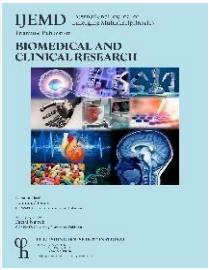


Effects of Complementary Foods Formulation Produced from Malted and Fermented Acha *(Digitaria exilis)* Flours Supplemented with Soybeans *(Glycine max)* Flour on Haematological and Histological Parameters of Albino Rats

Zughumnaan Well Eli [1*], Godwin Oche Obochi [2], Dinnah Ahure [3], Ponan Destiny Alexander [1], Istifanus Gurumtet [4], Nyango Philip [5], Akpensuen Mfe Samuel[1]

1. Centre for Food Technology and Research (CEFTER), Benue State University Makurdi Nigeria.

2. Faculty of Basic and Allied Medical Science, Benue State University Makurdi Nigeria.

3. Department of Food Science and Technology, College of Food Technology and Human Ecology, Federal University of Agriculture, Makurdi, Nigeria.

4. Department of Biochemistry, Faculty of Basic and Allied Medical Science University of Jos Nigeria.

5. Department of Anatomy, Faculty of Basic and Allied Medical Science University of Jos Nigeria.

Abstract

Flours were prepared from malted and germinated acha grains (*Digitaria exilis*) and were supplemented with soybeans *(Glycine max)* flour as the complementary foods. The appropriate ratios of combination of the flours were achieved by material balancing. Four food products were formulated and named as Unmalted Unfermented Acha Soybean (UMUFAS), Malted Unfermented Acha Soybean (MUFAS), Malted Fermented Acha Soybean (MFAS) and Unmalted Fermented Acha Soybean (UMFAS). Thirty-five (35) Wister strain of albino rats were grouped into seven groups of five rats per cage and were fed with the formulated blends. The animals were also fed with (Nestle Cerelac), Unmalted Unfermented Acha flour (UMUFA) and Soybean flour which served as controls. The rats in different groups were placed under the same conditions and were fed with eight hundred (840g) of food for 28 days. Haematological and histological parameters of the kidney and liver of the rats fed were examined to assess the suitability of these formulated diets as a possible substitute for the proprietary infant foods. Analysis of variance (ANOVA) was used to establish any significant difference in the analytical data for formulated and control diets ($p<0.05$). The results also showed that the formulated blends did not impair any significant organ of the rats as indicated by the haematological and histological studies.

Key words: Acha, Complementary Foods, Fermentation, Soybeans, Malting, Haematological

Email Addresses: **elizughumnaanwell@gmail.com (Well), gobochi4@gmail.com (Godwin), dinnahahure@gmail.com (Dinnah), destiny9556@gmail.com (Ponan), istigurumtet@gmail.com (Istifanus), nyangop@unijos.edu.ng (Nyango), mfeakpensuen@gmail.com (Akpensuen).**

INTRODUCTION

Malnutrition in children is a major nutritional problem in developing countries, which leads to morbidity and mortality, retardation in physical growth and mental development, working capacity and increased risk of adult disease [1]. This nutrition problem is due to the low nutritional value of traditional complementary foods, inappropriate complementary feeding practices and high cost of quality protein-based complementary foods [1]. An adequate nutrition within the first 1000 days has been reported to be essential for healthy growth and development in children for their full potential [2]. Complementary foods play a vital role on child growth and development since it complements for both nutritional and developmental needs of the infant when breast milk alone is no longer sufficient [3]. Cereals are generally low in protein, supplementation of cereals with locally available legume that is high in protein increases protein content of cereal-legume blends.

In Nigeria and other west African Countries, the most known complementary food that is used in infant feeding is *ogi*, which is also consumed by adults. *Ogi* is an acid-fermented cereal gruel or porridge made from maize, sorghum, or millet; the choice of cereal depending on preference and ethnicity[3],[4]. Most cereals used in production of *ogi* are limited in protein content especially amino acids notably lysine and methionine [5]. Scientific investigation had reported that over dependence on traditional complementary foods, such as *ogi* and other family diets, without adequate supplementation with high quality protein sources is the main attributable factor for the widespread of protein-energy malnutrition in Nigeria and other developing countries [6].

Fermentation is a desirable process of biochemical modification of primary food matrix brought about by microorganisms and their enzymes [7]. Fermentation is used to enhance the bio-accessibility and bioavailability of nutrients from different crops, improves organoleptic properties as well as extending the shelf life [8]. It makes food safe by not only inhibiting growth of pathogenic bacteria due to antimicrobial activity of lactic acid but also detoxifies aflatoxin [9]. These desirable benefits have made fermentation to be considered as an effective way to reduce the risk of mineral deficiency among populations, especially in developing countries where unrefined cereals and/or pulses are highly consumed [10].

Malting is a process where cereals or legumes are steeped and then germinated. After germination, the seeds are then matured (fermented) by storing away from the sun [8]. Malting is the term used for the preparation of a brewing raw material, employing a controlled germination of grain in moist air [11]. Malting aims to convert or modify the physical structure of the grain and allow synthesis or activation of a series of enzymes such that the final product malt, is more readily used in the subsequent stages of brewing, distilling, or food manufacture[11][12]. During the malting process, hydrolytic enzyme production and/or release is maximized leading to cell-wall degradation and protein solubilization with minimal starch breakdown [11].

Germination and fermentation of cereals are affordable and widely practiced processing method in Africa. The combination of different traditional processing methods such as milling, soaking, drying, dehulling, roasting fermentation, germination, blanching in the production of complementary food with addition of protein from other sources has been observed to improve the nutrient content, palatability, and bioavailability of micronutrients in plant-based diets as well as decrease or remove anti-nutritional factors in food [13].

Acha (*Digitaria exilis*) is a cereal crop of West African origin that can be relied upon during the time of food scarcity or famine due to its short cropping cycle, vital nutritional values and health benefits [14][15]. Research has shown that the methionine content of Acha grain is twice that of egg protein [16]. The nutraceutical potentials of acha (*fonio* and *iburu*) is due to their antioxidant, phenolic, and cholesterol-lowering properties [17]. Acha is recommended to remedy some health challenges. Acha improve blood clotting in women after child birth and also stimulate milk production in breastfeeding

women [16]. Doctors sometimes recommend it for people who want to lose weight [18]. Acha has the medicinal potential in the treatment of diabetic patient [17]. Acha grains have high water absorption capacity due to its appreciable amount of pentosan content[19].

Soybean (Glycine max (L.) Merrill) has become the miracle crop of the 21st century[20]. Soybeans (Glycine max) are cheap source of high-quality proteins with a good balance of amino acids[9]. It is a beneficiary crop, which contains about 40% proteins, possessing high level of essential amino acids except methionine and cystine, 20% oil rich in poly unsaturated fatty acids specially omega-6 and omega-3 fatty acids, 6 to 7% total minerals, 5 to 6% crude fibre and 17 to 19% carbohydrates [20]. Soybean crops provide one of the world's most important sources of protein and oil [21][17]. The digestibility value of soy protein is 91.41%. Soybean has a good source of vitamins and mineral and supply adequate amount of different amino acids required for repairing the damaged body tissue. It could be an essential part of functional foods and could be used for enrichment of product quality [22].

Materials and Methods

Experimental food samples

The study was conducted using formulations and analysis of acha (cereal) and soybean (legumes) supplementary flour. The foodstuff used are acha grains (*Digitaria exilis*) and soya beans (*Glycine max*). Nestle Cerelac was used as the control.

Preparation of products formulation

The entire foodstuffs used in formulating the complementary blends were purchased from local markets in Jos, Plateau State, in adequate quantities and processed as follows:

Acha processing

Whole (undehulled) acha grains were washed in 5% (w/v) sodium chloride (NaCl) solution to disinfect the grains. The washed grains were sun dried dehulled and washed. The washed acha grains were dried, milled and sieved using 0.2 mm sieve.

Malting was carried out using the method described by [4]. 3 kg of whole acha grains were washed in 5% (w/v) sodium chloride (NaCl) solution to disinfect the grains. The grains were then soaked in tap water at ambient temperature (30 + 20 ℃) using a ratio of 1:3 w/v/(grain: water), in a plastic bucket. The steep water was changed every 3 hours for a total steeping time of 6 hours, followed by draining in a plastic basket and the grains were spread in a single layer on a moistened jute bag and allowed to germinate at ambient temperature (30 + 20 ℃) for 48 hours, while spraying with water at intervals of 12 h. The non - germinated and germinated grains were removed after 48 hours. The ungerminated and germinated grains were then sun dried, dried in a confined environment covered with polythene with sunlight as source of drying. The dried malted acha grains were dehulled and winnowed. The winnowed acha grains were washed and sun dried, sieved using 0.2mm size.

The unmalted acha and malted acha flours were then, packaged in low density dark - coloured polyethylene bags, stored in 500 ml plastic containers with airtight lids at ambient temperature (30 +20 ℃) prior to use within 24 hours.

Fermented acha flour were obtained by natural fermentation using the method described by [23]. In this process, 120.0 g each of unmalted and malted acha flours were mixed with 80 ml of distilled water and subjected to natural fermentation in a covered 500 ml glass beaker at ambient temperature (30 + 20 ℃) for 24 hours. At the end of this period, 50% of the fermented mixture was used as starter culture for a new fermentation cycle. During this process, the pH and titratable acidity (an index of lactic acid bacteria activity) were monitored. The fermentation process was continued until the pH of the medium stabilized and remained near or constant. The fermented flours were dried at 80 ℃ in a fan driven electric oven (Genlab Widnes, U.K, model T12 H) to constant weight and milled in a disc attrition mill (Asiko A11, Addis Nigeria) to a particle size of 0.2 mm. The unmalted fermented acha and malted

fermented acha flours were then packaged in low density dark coloured polyethylene bags, stored in 500 ml plastic containers with airtight lids at ambient temperature (30 +20 ℃) prior to use.

Soybean processing

Soybeans were sorted for stones, rot and other physical defects. The beans were then washed and soaked in distilled water 1:5 w/v for 15 hr according to method proposed by [1]. The soaked beans were then placed in a sieve and allowed to drain. They were then blanched for about 20 min. The hulls were removed manually, then the beans were washed repeatedly using distilled water. The dehulled beans were then dried using tray dryer. Soybeans were milled into flour and sieved through 0.2 mm mesh size screen. The soybeans flours were then packaged in low density dark - coloured polyethylene bags, stored in 500 ml plastic containers with airtight lids at ambient temperature (30 +20 ℃) and utilized for product formulation and analysis within 24 hours.

Formulation of the experimental blends

Four different food formulations were made by blending the different acha flours with the soybeans flour to obtain 16 g protein and 9 g fat/100 g. This was achieved by material balancing from their respective proximate compositions[23].

Diet 1 -Unmalted, acha Unfermented acha: Soybeans (UMUFAS)
Diet 2 - Malted acha Unfermented acha: soybeans (MUFAS)
Diet 3- Malted acha Fermented acha: soybeans (MFAS)
Diet 4- Unmalted acha, Fermented acha: Soy beans (UMFAS)

Animal Experimentation

The study protocol was approved and ethic clearance was given by the Ethical Committee for Laboratory Animals of Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos Nigeria with reference number: UJ/FPS/F17-00379.

Male Winter-strain weanling rats weighing between 30 – 50 g, age 6 to 8 weeks were purchased from the Animal House of the University of Jos, were used in this study. The rats were randomly distributed to seven groups (7) groups of 5 rats each during feeding. They were kept in metabolic cages made of Perspex sheets. The rats were allowed to stabilize on the normal laboratory feed for 3 days and starved for one day before feeding with the experimental diets commenced.

Animal grouping

During the feeding experimentation, rats were allotted the diets as follows:
Group 1- Nestle Cerelac (commercial control)
Group 2 – Unfermented acha Unmalted acha
Group 3- Soybean flour
Group 4 –Unmalted acha, Unfermented acha: Soybeans (UMUFAS)
Group 5 - Malted acha Unfermented acha: soybeans (MUFAS)
Group 6- Malted acha Fermented acha: soybeans (MFAS)
Group 7- Unmalted acha, Fermented acha: Soy beans (UMFAS)

Animal feeding

Day 1 to 14, 20 g of food were given to the rats and was later increased to 40 g at day 15 to 28. Just before every feeding, diet was mixed thoroughly with enough boiling water to a thick paste and allowed to cool before feeding the animals. Rats were given feed and water ad libitum for 28 days. Faeces and urine were collected separately to avoid mixing with the feed.

Collection of Blood

At the 28th day of the feeding experiment period, each rat was anaesthetized with chloroform inside a dessicator before slaughtering. A portion of whole blood was collected from each rat into sample bottles and sample bottles containing EDTA (1 mg/ml) for parameters that required the use of whole

blood. The bottles were immediately capped and the content mixed gently for about 1 min by repeated inversion thereafter used for various haematological studies.

The remaining blood samples were allowed to clot for 20 minutes, before centrifuging at 3000 rpm for 15 minutes in a refrigerated centrifuge (to obtain serum for parameters determined in sera). Serum was carefully transferred with pasteur pipettes into clean, dry labeled light-shielded sample bottles and stored frozen until required.

Determination of Hematological Parameters

Determination of Packed Cell Volume (PCV%)
The packed cell volume (PCV), hemoglobin concentration (HBC), red blood cell count (RBC) and white blood cells (WBC), and thrombolytic indicators, were evaluated as described by Adejuwon *et al*. (2021)[25]. Hemoglobin concentration (HBC) was estimated using the cyanomethemoglobin method.

The whole blood sample was mixed in EDTA anticoagulant. Then 5 ml gently was added and rocking or repeated inversion to ensure proper mixing of the sample and the anticoagulant. A clean microhaematocrit capillary tube was used. The well-mixed anticoagulant sample was allowed to flow by capillary attraction through negative gradient up to ¾ capacity. The lower end of the capillary tube was sealed with plasticine and centrifuged at a predetermined speed f or 5 minutes using the hematocrit centrifuge. The PCV was read using hematocrit centrifuge. The PCV was read using the hematocrit reader and the result was read and reported in percentage.

Histopathological Analysis

The liver and kidney functions were analyzed using the method described by [27].The processing of liver and kidney tissue for histology were carried as stated below, formalin-fixed liver and kidney (about 5 mm thick) were consistently excised from the kidney and liver equator and processed using the protocol of [28]. The excised formalin-fixed kidney and liver were subjected to dehydration in grades of ascending alcohol concentrations (70 –100%), cleared in xylene, embedded in paraffin, and sectioned at 5 µm. Sections of the kidney and liver tissues were stained with hematoxylin-eosin and viewed with light microscope (Olympus BX3-CBH, USA) for histoarchitectural differences in the liver and kidney of the albino rats.

Statistical Analysis

All data each determination was carried out in 3 replicates and results were reported as an average value (mean ± standard deviation). Data were analyzed by Analysis of Variance (ANOVA) model using SPSS Version 20. The means where significantly different were separated by Duncan multiple range test. Statistical significance was accepted as $p<0.05$.

Haematological indices

The haematological parameters of animals fed with the control and formulated diet are White blood cell (WBC), Neutrophils (NEU), Lymphocytes (LYM) and Eosinophil (EOS) are presented in Table 1. While Red blood cell (RBC), haemoglobin (Hb), Packed Cell Volume (PCV) and Platelets (PLT) of the rats are shown in Table 2. The statistical difference ($P>0.05$) existed among the formulated foods compared to the controls

Table 1: Haematological Parameters of Rats Fed with the Formulated and Control Diets

Group	WBC($X10^3mm^3$)	Neutrophils (%)	Lymphocytes (%)	Eosinophil (%)
CERELAC	5.47 ± 0.502	32.40 ± 1.965	38.20 ± 3.891	2.58 ± 0.516
UMUFA	8.96 ± 0.977	27.16 ± 1.085	42.70 ± 1.351	2.60 ± 0.518
SOYBEAN	8.54 ± 1.350	35.38 ± 0.909	41.00 ± 0.701	2.72 ± 0.611
UMUFAS	5.97 [bce] ± 0.747	31.08 [ade] ± 2.864	41.94 [bcf] ± 2.155	3.96 [bdf] ± 1.028
MUFAS	8.01 [bce] ± 1.115	45.48 [bdf] ± 1.533	26.36 [ace] ± 1.164	3.14 [bdf] ± 1.273
MFAS	5.92 [bce] ± 0.895	39.70 [bdf] ± 2.148	31.86 [ace] ± 2.538	1.84 [ace] ± 0.229
UMFAS	6.85 [bce] ± 1.081	37.20 [bdf] ± 2.834	24.04 [ace] ± 4.336	1.56 [ace] ± 0.411
Ranges	4 – 10	50 – 70	20 – 40	0.5 – 5.0
p-values	0.0963	<0.0001	0.0009	0.3515

Values are expressed as mean ± SEM (n = 5).

If p value is less than or equal to 0.05, mean values are statistically significant.

[a] Values are significantly low when compared with cerelac ($p < 0.05$)

[b] Values are significantly high when compared with cerelac ($p < 0.05$)

[c] Values are significantly low when compared with normal acha ($p < 0.05$)

[d] Values are significantly high when compared with normal acha ($p < 0.05$)

[e] Values are significantly low when compared with soybean ($p < 0.05$)

[f] Values are significantly high when compared with soybean ($p < 0.05$)

[g] Value is not statistically significant ($p < 0.05$)

Table 2: Haematological Parameters of Rats Fed with the Formulated and Control Diets

Group	RBC($X10^3mm^3$)	HGB(g/dL)	PCV%	PLT($X10^9/L$)
CERELAC	3.49 ± 0.460	10.16 ± 0.663	27.20 ± 2.059	134.40 ± 26.349
NORMAL ACHA	5.42 ± 0.835	10.82 ± 0.667	37.20 ± 8.021	194.40 ± 13.071
SOYBEAN	4.89 ± 0.789	12.98 ± 0.599	41.76 ± 2.172	205.20 ± 50.935
UMUFAS	4.83 [bcf] ± 0.629	12.46 [bde] ± 0.213	35.80 [bce] ± 1.744	109.00 [ace] ± 7.141
MUFAS	5.73 [bdf] ± 0.344	12.38 [bde] ± 0.302	39.40 [bde] ± 1.778	225.00 [bdf] ± 10.169
MFAS	4.17 [bcf] ± 0.761	11.30 [bde] ± 0.864	34.76 [bce] ± 3.749	183.60 [bce] ± 42.418
UMFAS	5.63 [bdf] ± 0.792	12.44 [bde] ± 0.231	40.16 [bde] ± 1.663	228.60 [bdf] ± 3.460
p-values	0.2357	0.0110	0.1594	0.1950
Ranges	3.50 - 5.50	11 - 16	37 – 54	100 - 300

Values are expressed as mean ± SEM, (n = 5).

If p value is less than or equal to 0.05, mean values are statistically significant.

[a] Values are significantly low when compared with cerelac ($p < 0.05$)

[b] Values are significantly high when compared with cerelac ($p < 0.05$)

[c] Values are significantly low when compared with normal acha ($p < 0.05$)

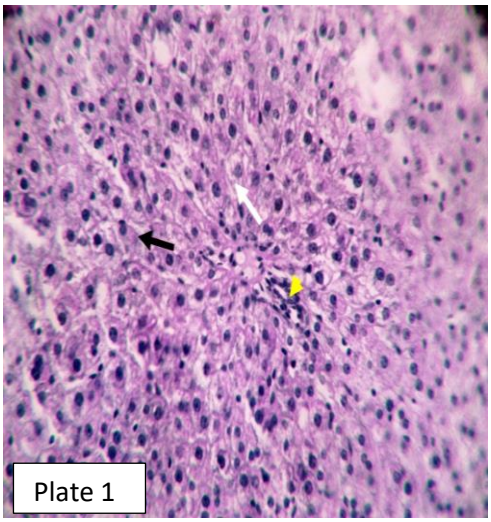
[d] Values are significantly high when compared with normal acha ($p < 0.05$)

[e] Values are significantly low when compared with soybean ($p < 0.05$)

[f] Values are significantly high when compared with soybean ($p < 0.05$)

[g] Value is not statistically significant ($p < 0.05$)

Histology Results of Kidney and Liver of Animals Fed with Formulated Blends and Controls



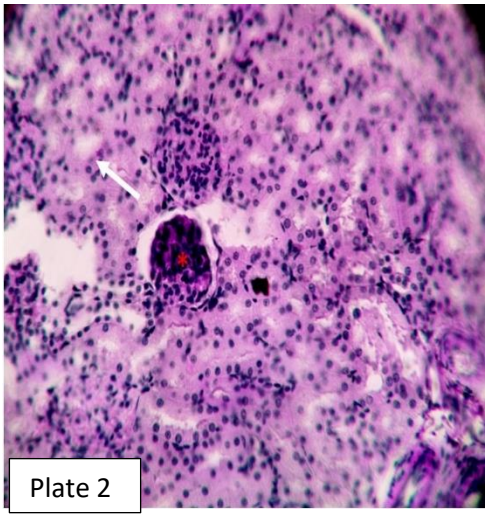
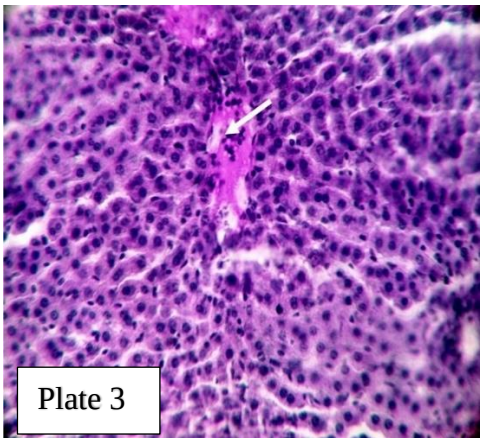


Plate 1: Photomicrograph of the liver of rat fed with UMUFAS. There is mild cellular infiltration (yellow arrowhead), hepatocyte nuclear degeneration (black arrow) and cytoplasmic vacuolations (white arrow). Stain: Haematoxylin and Eosin; Magnification: x400.

Plate 2: Photomicrograph of the kidney of rat fed with UMUFAS. There is no visible lesion in the renal histoarchitecture as revealed by prominent glomerulus and renal tubules. Stain: Haematoxylin and Eosin; Magnification: x400.



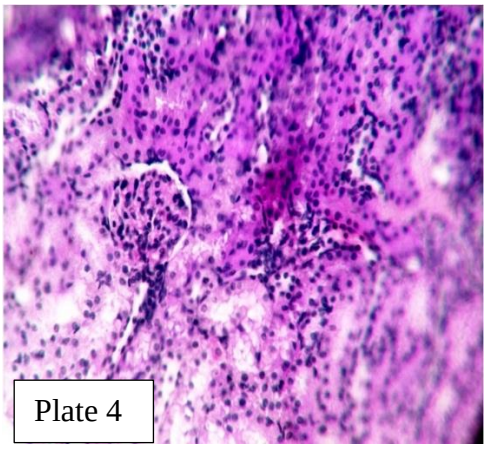
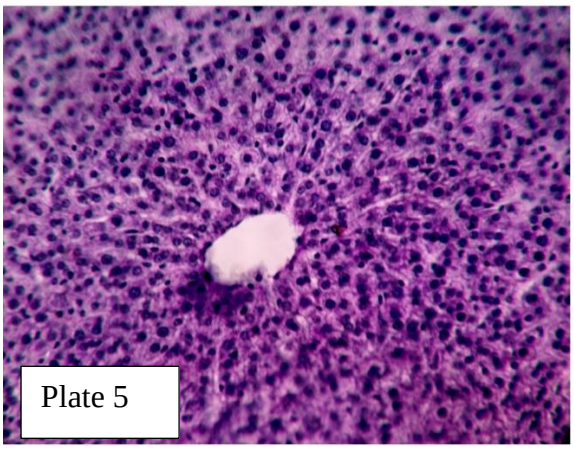


Plate 3: Photomicrograph of the liver of rat fed with MUFAS. There is no visible lesion in the hepatic parenchyma except for mild portal congestion (white arrow). Stain: Haematoxylin and Eosin; Magnification: x400

Plate 4: Photomicrograph of the kidney of rat fed with MUFAS. There is no visible lesion in the kidney histoarchitecture. Stain: Haematoxylin and Eosin; Magnification: x400



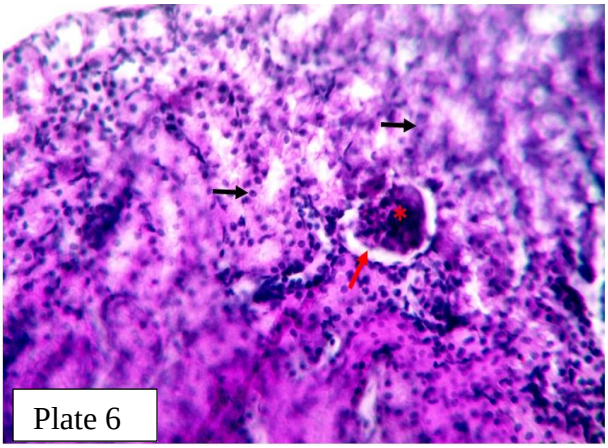
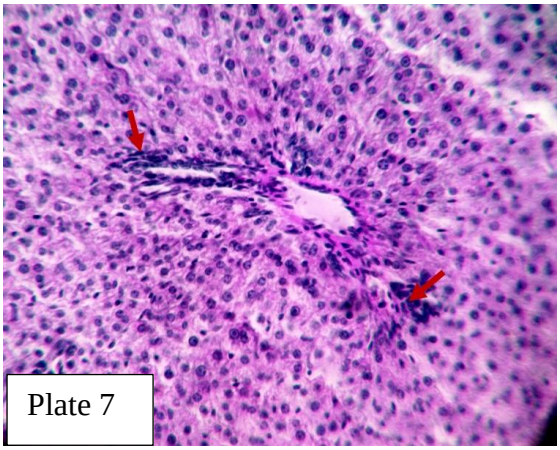


Plate 5: Photomicrograph of the liver of rat fed with MFAS. The histoarchitecture of the liver is devoid of visible lesion. Stain: Haematoxylin and Eosin; Magnification: x400

Plate 6: Photomicrograph of the kidneys of rat fed with MFAS. The kidney parenchyma is devoid of visible lesion as shown by the presence of intact glomerulus (asterick), urinary space (red arrow) distinct renal tubular architecture (black arrows). Stain: Haematoxylin and Eosin; Magnification: x400



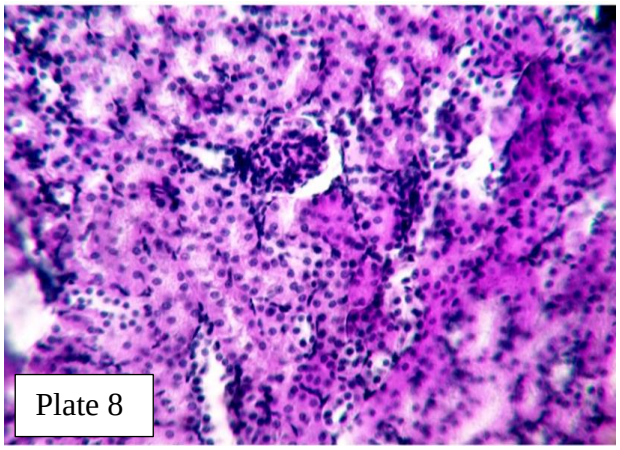
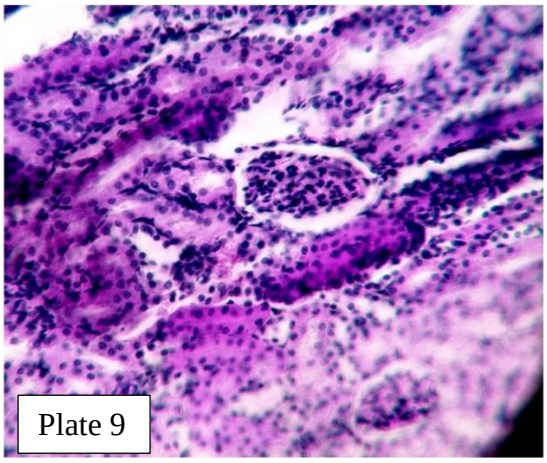


Plate 7: Photomicrograph of the liver of rat exposed to UMFAS. Except for mild periportal cellular infiltration (red arrow), there is no visible lesion in the hepatocytes. Stain: Haematoxylin and Eosin; Magnification: x400

Plate 8: Photomicrograph of the kidneys of rat fed with UMFAS. There is no visible lesion in kidney histological appearance. Stain: Haematoxylin and Eosin; Magnification: x400



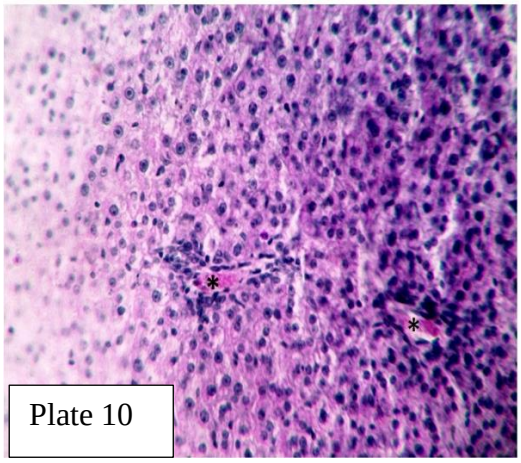
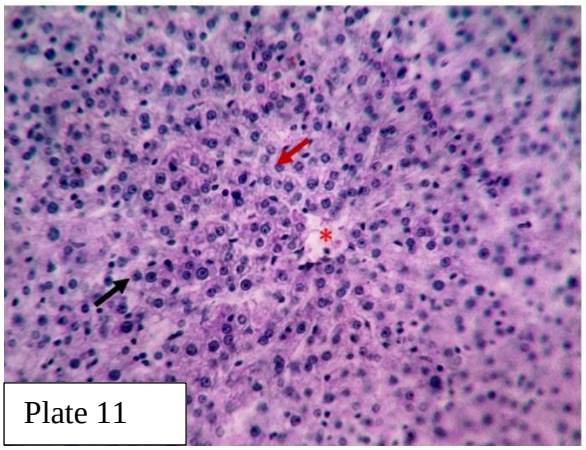


Plate 9: Photomicrograph of the kidneys of rat fed with cerelac. There is no visible lesion in the renal histo-architecture. Stain: Haematoxylin and Eosin; Magnification: x400

Plate 10: Photomicrograph of the liver of rat fed with cerelac. With the exception of mild central veinous congestion (asterick), there is no visible lesion in the hepatic parenchyma. Stain: Haematoxylin and Eosin; Magnification: x400



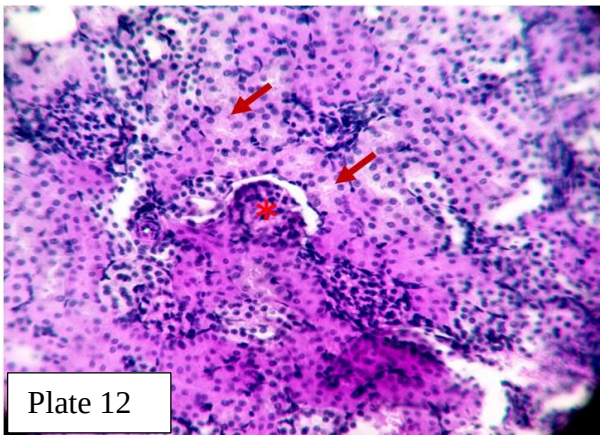


Plate 11: Photomicrograph of the liver of rat fed with UMUFA flour. There is no visible lesion in the hepatic parenchyma as revealed by the prominent presence of roundish nuclei (red arrow) and clearly defined cytoplasm (black arrow) in the hepatocytes as well as distinct central vein (asterick). Stain: Haematoxylin and Eosin; Magnification: x400

Plate 12: Photomicrograph of the kidney of rat fed with UMUFA flour. The kidney histoarchitecture is devoid of lesion as typified by intact glomerulus (asterick) and renal tubules (red arrow). Stain: Haematoxylin and Eosin; Magnification: x400

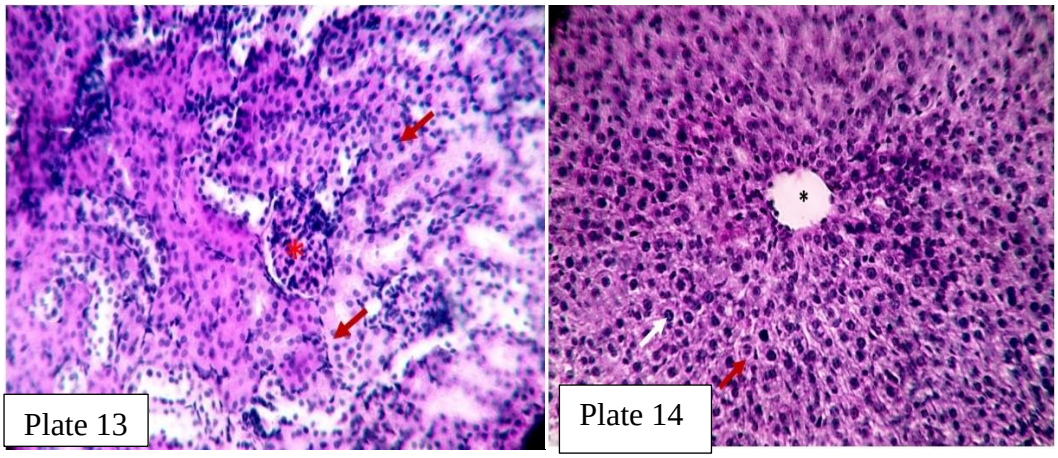


Plate 13: Photomicrograph of the liver of rat fed with Soybean flour. The liver parenchyma is devoid of histopathological lesion as evidenced by distinct central vein (asterick), prominent hepatocytes with somewhat roundish nuclei (white arrow) and intact cytoplasm (red arrow). Stain: Haematoxylin and Eosin; Magnification: x400.

Plate 14: Photomicrograph of the kidneys of rats fed with to Soybean flour. There is no visible lesion in the renal parenchyma as revealed by intact glomerulus (asterick) and renal tubular epithelia cells (red arrow). Stain: Haematoxylin and Eosin; Magnification: x400.

Discussion

Haematological parameters

Haematological parameters are those parameters that are related to the blood and blood-forming organs. The haematological and serum examination is among the methods which may contribute to the detection of some changes in health status, which may not be apparent during physical examination but which affect the fitness of the animals [29]. In addition, haematological indices in animals are important to determine the toxicity risk since the changes in the blood system have a higher predictive value for human toxicity. The comparable level of rats fed with the formulated blends and control diets suggested that consumption of the composite flours did not induce an immune response in the rats' bodies. Blood platelets are a vital element of blood, in that, they ensure vascular integrity and prevent bleeding. An insufficient number of platelets or the presence of non-functional platelets may be responsible for bleeding and could constitute a health risk. Lymphocytes, neutrophils, monocytes, eosinophils, basophils and leukocytes are obtained from splitting of white blood cells and their general function is to help the body fight infections [29], Adeoti *et al*., 2018). The white blood cells and some stated parameters above were comparable with those of the CERELAC control and was within the clinical reference range, indicates that the antinutritional factors in the formulated diet was not significant to cause harm to humans. Physiological parameters such as heamoglobin (Hb); packed cell volume (PCV) are used to assess iron intake, metabolism and deficiency [30]. Results obtained from the rats feeding experiment showed that the formulated blends supported normal iron metabolisms since there was no marked difference in physiological response to iron intake. Packed cell volume also known as haematocrit (Ht or Hct) is the percentage of red blood cells in the whole blood. 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 [31].

The Haemoglobin concentration and haematocrit are values that show the degree of anemia. 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 [31] [25]. The values obtained from above stated parameters showed that the formulated diets were not toxic to the red blood cells.

Histological study of livers and kidney

Histology is a discipline that aims to study the morphology and functioning of tissues. It therefore constitutes a fundamental basis for the diagnosis of pathology on a tissue scale [29]. The Photomicrograph of histological study carried out on kidneys and liver of young rat fed with various formulated diets and the control. Showed no abnormalities or dysfunctions in these organs of the rats.

The hepatocytes in the liver showed no form of necrosis and no histopathological alterations, distortions such as midcell edema, degeneration of the hepatocytes, enlargement of the alveoli and alveoli hemorrahage. This fact indicated that consumption of formulated diets and the control had no negatively impact on the liver of the rats[31]. The liver is one of the most important organs of the body, since it provides many functions, in particular the synthesis and secretion of bile, the synthesis of proteins such as albumin, fibrinogen and coagulation factors. It is also involved in the metabolism of sugars and lipids, the synthesis of glycogen and the storage of vitamin B_{12} and iron [29].

The result of kidney Photomicrograph showed that there were no degeneration of glomerulus and congestion of filtration chambers in kidneys of all the animals. This fact simply implied that the cells involved in renal filtration were normal and can purify the blood by eliminating the waste that comes from functioning of the body and maintain the chemical balance of blood [32].

Conclusion

The absence of toxic substances in the formulated diets as evidenced in the haematological and histological findings indicated that the formulated foods were suitable and also safe for infant consumption.

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