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Full Length Research Paper

Nutritional evaluation of processed Cowpea (*Vigna unguiculata*) seeds-Soya bean (*Glycine max*) seeds-Maize (*Zea mays*) grains-Crayfish blend of weaning formulae

[1*]Obimba, Kelechukwu Clarence, [2]Ihedimbu, Chiamaka Perpetua, [3]Ozougwu, Jevas Chibuike, and [4]Eziuzor, Chukwunyelum Samuel

[1,2]Department of Biochemistry, School of Biological Sciences, Federal University of Technology, Owerri, Nigeria.
[3,4]Physiology and Biomedical Research Unit, Department of Biological Sciences, College of Basic and Applied Sciences, Rhema University Aba, Abia State, Nigeria.

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Weaning formulae, SCMC 20% (20% protein) and SCMC 16% (16% protein) prepared from available and affordable plants (soya bean seeds, cowpea seeds, maize grains, and fluted pumpkin leaves) and animal (crayfish) sources were evaluated for their nutritional efficiency in a comparative study. The experimental design for the animal feeding trial is a single factor, Completely Randomized Design (CRD). Fourty male wistar albino rats were divided into four groups of 10 animals each, housed in stainless steel cages and fed *ad-libitum* on three different types of weaning formulae (SCMC-16%, SCMC-20%, and nutrend (Rd)), and a basal diet, for a period of 28 days. Results were recorded as values of mean ± S.E unit, as follows (range of values): growth performance (GP): (-25.1± 1.02 -86.2 ± 1.0 g), Serum albumin (SA) (1.78 ± 0.2-4.15 ± 0.1g/dl), Serum cholesterol (C) (155 ± 0.02-185 ± 0.02 g/dl), Serum triglyceride (T) (95± 0.04 -125± 0.01g/dl), WBC_{Total} (2754± 12-5183± 25.15 mm), Aspartate amino transferase (AST): (9.1 ± 0.01-18.1 ± 2.16 U/l), PCV% (27.1 ± 0.2-55.8 ± 1.2%), Net protein utilization (NPU%) (87.1 ± 2.1-97.2 ± 1.02%), Biological value (BV) (90.2 ± 1.0-98.2 ± 1. 2), True digestibility (TD%): (90.2 ± 1.0-97.1 ± 1.02%), and Protein efficiency ratio (PER): (-1.35 ± 0.5-3.55± 0.01), Histopathology (liver tissues), diet group : SCMC-20% : normal; basal : degenerative changes. The SCMC-20% diet group had the significantly highest mean values of all the parameters measured, except the AST activity, C and T of which it had the lowest. GP (grams) regressed multiply and significantly ($p<0.05$) with PCV%, SA (g/dl) and TD% of the SCMC-20% diet group. The regression and correlation between NPU% and SA (g/dl) of the SCMC-20% diet group was significant ($p<0.05$) ($r = 0.985$). The nutritional efficiency of the weaning formulae listed in sequential order of significant ($p<0.05$) decrease is: SCMC 20%>SCMC 16%/Rd (nutrend). The weaning formulae could serve as preventive and curative dietary therapies of protein energy malnutrition.

Keywords: Nutritional efficiency, histopathology, protein energy malnutrition.

INTRODUCTION

The critical period for an infant's growth and development is between 6 and 24 months. During this period (weaning stage in infancy), transition in administration of diet occurs from a diet based on mother's milk to another diet which is usually semi-solid (a weaning formula), to a more solid diet (Creed-Kanashiro *et al*., 1990).

Protein energy malnutrition is a disease resulting from the coincident lack of protein and calories in different proportions, and occurs in children primarily at the wearing

Corresponding author. E-mail: kechrisob@yahoo.com

stage in infancy, owing to malnutrition, and is often associated with infections. In developing countries, traditional weaning foods are low in protein content and also deficient in vital nutrients that are required for normal child growth and development (FAO, 2004). Several studies have been carried out on the preparation (formulation and processing) of weaning foods with a view to preventing and/or alleviating protein energy malnutrition in infants [Ijarotimi and Aroge (2005), Obimba (2011)].

Soya bean seeds-groundnut seed-maize grains-catfish (SGMC), and Soya bean seeds-maize grains-crayfish (SMC) blend of high quality weaning formulae were used in effecting rapid catch-up growth rates in kwashiorkor-induced rodent models (Obimba, 2012). SGMC and Nutrend weaning formulae ameliorated the toxic heavy metal effect of Aba River water and bore hole water in experimental wistar albino rats by improving significantly ($p<0.05$) the biochemical and metabolic drinking water qualities of the water sources (Obimba *et al*., 2011). Cereal-legume-animal supplements mix, possess a great nutritional potential to support growth and rehabilitation of protein energy malnutrition subjects (Mosha and Bennink, 2004).

The rationale for the formulation of weaning formulae is founded on the importance of the cereal-legume-animal supplement mix. Maize protein is deficient in lysine and tryptophan but has fair amounts of sulphur-containing amino acids (methionine and cystine). Conversely, the protein of food legumes (soya bean seeds and cowpea seeds is a relatively rich source of lysine and tryptophan but is low in sulphur amino acids (Bressani and Elías, 1974). Legumes in weaning formula are found to improve nutrient density of food and improve nutrient intake, which result in the prevention of protein energy malnutrition (Feyissa, 2009). The limiting amino acid of soya bean seeds is methionine. Animal sourced proteins (fish, meat, egg and milk) are rich in methionine and therefore complement diets formulated with soya bean seeds as component.

Leafy vegetables (e.g fluted pumpkin leaves) offer good sources of soluble crude fiber essential to improving stooling values as well as ensuring moderation of caloric intake by baby (Thomas, 2015). Moreover, leafy vegetables and vitamins-minerals amino acid premix are excellent sources of multi vitamins and multi minerals which in addition to the specific functions of individual vitamins and minerals, play an almost collective anti-oxidant biochemical role essential to the inactivation of free radicals in human infants. Vitamin A inhibits phosphatidylcholine liposomal lipid peroxidation by scavenging lipid peroxyl radicals (Das, 1989).Vitamin D increases the activity of glutathione reductase and simultaneously decreases glutathione peroxidase activity in healthy subjects by which means the pool of glutathione, which is an antioxidant, is enhanced (Wiseman, 1993). Vitamin E (□-Tocopherol) can transfer a hydrogen atom to lipid peroxyl radical forming a stable species which is ultimately recycled back to vitamin E. Vitamin K-hydroquinone, a derivative of vitaminK, is a potent radical scavenging specie (Mukai *et al*., 1993). Calcium-based nutritious minerals such as calcium ascorbate and calcium lactate are antioxidants (Fan *et al*., 2005).

Legumes complement cereals in calcium content but cereals complement legumes in phosphorus content (Gina, 2011). A 20 to 30% addition of animal protein to a 7: 3 (weight to weight) cereal to legume combination improves the nutritive value of foods and induces good and consistent biological responses in experimental animals. The FRI weaning formula prepared from processed : soya bean seeds, groundnut seeds, maize grain; and milk could be used to improve the nutritional status of children and alleviate protein energy malnutrition (Annan and Plahar, 1995). The protein advisory group recommends that the protein contents of weaning foods should be at least 20%, on a dry weight basis (FAO/WHO, 1971; WHO, 2001, 2002). The relevance of using albino rats in nutritional studies, for the purpose of evaluating the nutritional quality of diets is founded on the fact that wistar albino rats have a dietary requirement for the same ten (10) essential amino acids as human infants.

Furthermore, antinutritional factors and therefore, method of food processing of diet components, affect the nutritional efficiency of traditional weaning formulae (Akaninwor and Okechukwu, 2004). Germination of cooked soya bean seeds and cowpea seeds used in the formulation of traditional weaning formulae engendered a significant increase ($p<0.05$) in the essential amino acid (except histidine, sulphur-containing amino acids and tryptophan) content of the legume seeds varieties, and also increased phosphorus, iron, vitamin A, thiamine, riboflavin, niacin and ascorbic acid contents of both legume seeds varieties, thereby increasing caloric and nutrient density of infant diets/weaning formula (Elemo *et al*., 2011).

Weaning food prepared from autoclaved, malted cowpea and malted barley flour had high values of nutritional and gross energy with true digestibility (TD%) and Biological value (BV) of 87.6% and 90.23%, respectively (Ishfaq *et al*., 2014). Heat treatment effectively removes antinutritional factors, but is innocuous in effect to legume proteins (Kon and Sanshuck, 1981).

Mean values of red blood cells, white blood cells and pack cell volume were higher, though not significantly ($p<0.05$), in wistar albino rats fed on traditional weaning formula made of cooked banana fruit and bambara groundnut seeds, compared with control rodent models fed on commercial weaning formula (Nutrend) (Ijarotimi, 2008).

The mean values of the aspartate aminotransferase (AST) enzyme activity (U/l) of each of a soya bean seeds-groundnut seeds-maize grains-catfish weaning

blend and soya bean seeds-maize grains-crayfish weaning blend groups of experimental wistar albino rats were significantly reduced ($p<0.05$) compared with those of the reference weaning formulae groups: nutrend and soya bean seeds- groundnut seeds-maize grains-milk (Obimba, 2011). Experimental rodent models fed on FRI weaner's formula had a mean value of serum albumin concentration which was not significantly different ($p<0.01$) from the normal range of values (4.4-5.3g/dl) of serum albumin concentration (Annan and Plahar, 1995).
The objective of this work is to compare the nutritional efficiencies of some weaning formulae prepared from available and affordable plants and animal sources, essential to the prevention and cure of protein energy malnutrition.

MATERIALS AND METHODS

The experimental Design is a single factor completely randomized design (CRD) of 40 observations per parameter (except NPU%, TD%, BV of which 30 observations were made of each). The Linear model is $Yij = \mu + Ti + eij$.

Yij = Individual observations
μ = Overall mean
Ti = Effect of ith level of dietary protein treatment
eij = Random error, which is independently, identically, and normally, distributed, with zero mean, and constant variance.

SPSS for windows (version 17.0, SPSS, Chicago, IL, USA) was used to perform the statistical analyses. The significance level was p value<0.05, $p<0.01$.

Processing of diet components

Two hundred grams (200 g), each of raw soya bean seeds, raw cowpea seeds and raw maize grains, were washed and soaked, separately, in a liter of water, for 11 h, and thereafter, boiled in 800 ml of water, for 2 h. Boiled cowpea seeds and soya bean seeds were dehulled. The samples were dried in the oven for 9 h at 105°C, ground and dried for a further 4 h, at 105°C. Fresh crayfish samples were dried in the oven for 24 h at 105°C and ground. Fluted pumpkin vegetable leaves were washed in warm water and dried in the oven for 1 h and ground. The schematic for diet formulation is shown in Table 1.
Fourty male (weanling, 5 weeks old) wistar albino rats were divided into four groups of 10 animals each, and housed in stainless steel cages under 12 h light and dark cycles, under humid tropical conditions, and fed *ad-libitum* on three different types of weaning diets [soya bean seeds-cowpea seeds-maize grain-crayfish (SCMC-16%), soya bean seeds-cowpea seeds-maize grain-crayfish (SCMC-20%), and Rd (nutrend)], and a basal diet (hypothetical protein-free), for a period of 28 days. Daily faecal deposits of the animals were collected during the 28-day period of the feeding trial, pooled, oven dried, and weighed. The experimental animals were weighed and sacrificed by a sharp tap on the head with a blunt instrument. Blood samples for haematological and biochemical assays were collected in requisite blood sample bottles, and stored in a refrigerator at 4°C. The lean body mass (lungs, liver, heart, kidneys, pancreas, and spleen) were recorded. The carcasses were dried for 17 h, in an oven drier at 105°C and stored. The faecal nitrogen content and the carcass nitrogen content of the experimental animals were determined using the Kjeldahl method (AOAC, 1990). Quantitative determination of nitrogen content of carcass and faecal deposits of experimental animals was carried out by a modified Kjeldahl method, similar to that described by (AOAC, 1990).
The method described by Thavasu *et al*. (1992) was used in obtaining the serum. Whole blood was collected in a covered test tube, and allowed to clot by leaving it undisturbed for 15-30 minutes at room temperature. The clot was removed by centrifuging at 1,000-2,000 x g for 10 minutes in a refrigerated centrifuge, to obtain the blood serum. Citrate phosphate dextrose - adenine 1 (CPDA-1)- stored whole blood was used for whole blood analysis.

Lipid Profile Assays

Serum cholesterol (C), and serum triacylglycerol (TG) were determined using commercial kits (Randox Laboratory Ltd., UK), in conformity with the methods employed by Ibegbulem and Chikezie (2012); Chikezie and Okpara (2013).

Packed Cell Volume (PCV%)

Analysis of packed cell volume (PCV%) was carried out according to the method described by Ovuakporaye (2011). A plain capillary tube was filled with whole blood in an EDTA container by capillary action. It was sealed using plasticine or bunsen burner flame and placed in the haematocrit centrifuge for 10mins and the value of PCV% was obtained using haematocrit reader.

In Vitro Quantitative Analysis of Serum Aspartate Amino Transferase (AST)

Quantitative *in vitro* determination of serum aspartate amino transferase (AST) was carried out using the method employed by Reitman and Frankel (1957). The test based on the reaction in which l-aspartate and α-ketoglutarate are converted to l-glutamate and oxaloacetate by the catalytic activity of AST. The oxaloacetate so formed, forms a complex with 2,4-dinitrophenyl hydrazine, known as oxaloacetate hydrazone which could be measured colorimetrically at 546nm. The intensity of which is proportional to the AST activity of the serum.

Table 1. Diet formulation.

Grams/100grams diet (%)

Components		Basal	SCMC (20%)	SCMC (16%)	*Rd (16%)
1.	Soya bean seed (flour)	-	17.63	14.55	
2.	Cowpea seed (flour)	-	4.41	3.5	
3.	Maize seed (flour)	-	51.42	50.1	
4.	Crayfish	-	10.20	7.35	
5.	Vegetable (fluted pumpkin leaves)	-	5.00	5.00	
6.	Palm oil.	-	8.00	8.00	
7.	Vitamins –minerals-amino acid complex	-	0.25	0.25	
8.	Garri (*Manihot esculenta*)	100	3.09	11.25	

*Reference diet (Rd): Nutrend prepared industrially by Nestle®, of nutritional value- 16% dietary protein, 63.7% carbohydrates, 9% fat, 4% moisture, 2.3% minerals, 417.5 kcal/100g. SCMC (20%): 19.98% dietary protein, 64.2% carbohydrates, 9.2% lipids, 3.1% moisture, 3.1% minerals, vitamins ≤ 0.69 g, 437.1 kcal/100g. SCMC (16%): 16.1% dietary protein, 71.7% carbohydrates, 6% fat, 4.5% moisture, 1.67% minerals, vitamins ≤ 0.13 g, 368.5 kcal/100 g.

White Blood Cell total (WBCTotal) Assay

The white blood cell total count (mcl) was determined according to the method described by Annan and Plahar (1995). Blood samples (0.02 ml) were mixed with sequesterine and diluted in 0.38 ml diluting fluid (1.5 ml glacial acetic acid, 0.5 ml malachite green, 98.0 ml water). The diluted blood was mounted on a counting chamber, and white blood cells were counted.

Quantitative *in Vitro* Determination of Serum Albumin

Quantitative *in vitro* determination of serum albumin was carried out consistent with the method described by Qureshi and Qureshi (2001) and Huang and Fraker (2003). Serum albumin was determined using human albumin standards and sigma diagnostics albumin reagent (Sigma, St. Louis, MO) containing bromocresol green. The absorbance of the mixture of the reagent and serum albumin was measured at 578 nm against a reagent blank.

Protein Efficiency Ratio (PER)

Protein Efficiency Ratio (PER) was determined by the method described by Sarwar and Peace (1994). Protein efficiency ratio (PER) is based on the weight gain or loss of a test subject divided by its intake of a protein-in-food during the test period.

True digestibility (TD%)

True digestibility (TD%) was determined by the method described by Sarwar and Peace (1986). To determine protein digestibility, measurements of the nitrogen in food and faeces are made. True protein (N) digestibility is calculated as follows: True Digestibility = PI – [FP - MFP]/PI × 100 Where PI = protein intake, FP = fecal protein and MFP = metabolic fecal protein. The amount of protein in the feces of rats fed the protein-free diet was used as the estimate for MFP.

Net Protein Utilization [NPU (%)]

Performance characteristics analysis of Net Protein Utilization [NPU (%)] was carried out using the method employed by Pellet and Young (1980). The slope of the regression line of N intake on N retention is related to net protein utilization. The correlation coefficient of the regression is a measure of the net protein utilization [NPU (%)].

Biological Value (BV)

$BV = (N_r/N_a) \times 100$
N_a = nitrogen absorbed in proteins on the test diet (NPU%)
N_r = nitrogen incorporated into the body on the test diet (TD%) (Mitchell, 1923).

Histopathology

Liver sections were prepared for histopathological studies according to the method described by Brozska *et al*. (2003), slices of the left liver lobe were fixed in 10% formal saline for 24 h. The fixed tissues were dehydrated and de-alcoholated using increasing concentrations of alcohol and xylene, respectively. Infiltration and embedding of the infiltrated tissues were carried out using paraffin wax. Sections (5 to 6 µm) of the liver were obtained using the

microtome (MR 2) (Boeckeler Instruments Inc., USA), and routinely stained with haematoxylin and eosin and the stains differentiated, using 1% hydrochloric acid ethanol. The stained sections were examined in a Digital microscope (Motic DMIII) (Motic China Group Co. Ltd). The magnified images of the liver sections taken include the photomicrographs (Plates 1 and 2).

RESULTS

Table 2 shows the results on the mean values of growth performance (grams) of the test and reference weaning formulae, and basal diet group which differed in order of consecutive significant decrease ($p<0.05$) as follows : SCMC-20%, SCMC-16%/Rd, and Basal diet groups.

Results on the mean values of the biochemical indices: serum cholesterol, serum triglyceride, serum albumin, serum aspartate aminotransferase (AST) and WBC_{total} of the test and reference weaning formulae, and the basal diet group are shown on Table 3, and are listed in order of consecutive significant increase as follows : serum cholesterol : SCMC-20%, SCMC-16%/Basal and Rd diet groups; serum triglyceride: SCMC-20%, SCMC-16%/Rd and Basal diet groups; serum albumin: Basal, Rd/SCMC-16%/SCMC-20% diet groups; serum aspartate aminotransferase (AST): SCMC-20%/SCMC-16%/Rd, and Basal diet groups; WBC_{total}: Basal, SCMC-16%/Rd and SCMC-20% diet groups.

The mean values of the performance characteristics NPU%, TD%, BV and PER varied as listed in order of consecutive significant decrease ($p<0.05$), PER ($p<0.01$), as follows : SCMC-20%, SCMC-16%/Rd and Basal (PER) diet groups as shown in Table 4. The basal diet group had a negative (-) PER because they suffered significant loss of weight.

Among the diet groups, SCMC-20% had the significantly highest ($p<0.05$) mean value of PCV% followed by SCMC-16%/Rd and Basal as shown in figure 1.

The mean values of the relative liver organ weight ratio given by the slope (gradient) of each of the straight line graphs in figure 2, are fairly constant within each weaning formula diet group, but differed among the diet groups, in order of consecutive significant decrease as follows : SCMC-20%, SCMC-16%/Rd.

Plate 1 is the Photomicrograph of the liver tissue of a wistar albino rat administered with the basal diet showing some degenerative changes characterized by necrotic and atrophic cells, and hemorrhage. Plate 2 is the photomicrograph of the lobe section of the liver tissue of a wistar albino rat administered with the SCMC-20% weaning formula, showing normal tissue.

DISCUSSION

The mean values of growth performance of the diet groups (Table 2) differed in sequential order of significant decrease ($p<0.05$) as follows : SCMC-20%, SCMC-16%/Rd, and Basal diet groups, and is consistent with the observation that rats administered with processed soya bean seeds-groundnut seeds-maize grains-catfish (SGMC 20%) weaning formula blend and treated river water had significantly higher mean values of growth performance than the diet group fed on nutrend weaning formula (Obimba *et al*., 2011).

The SCMC-20% diet group had significantly higher values ($p<0.05$) of PCV% (figure 1) and WBC_{total} (Table 3) than the nutrend (Rd) diet group and is in keeping with the finding that rats fed on weaning formula prepared with banana fruit and fermented bambara groundnuts had higher values of PCV% and WBC_{total} than the control, nutrend commercial weaning formula group (Ijarotimi, 2008). Protein energy malnutrition results in anemia, detectable, using PCV% haematological diagnostic index (Siddiqui *et al*., 2007). The occurrence of anemia and its ill-consequencies could be circumvented by the administration of SCMC-20% to infants.

The SCMC-20% diet group had the highest mean value of serum albumin (Table 3), and corroborates the findings of Obimba (2011), who recorded a significantly higher ($p<0.05$) mean value of serum albumin in SGMC 20% weaning formula blend diet group in comparison with the control, nutrend commercial weaning formula group. SGMC 20% was used as dietary therapy to effect rapid catch-up growth rates in kwashiorkor-induced rats (Obimba, 2012). Serum albumin and total serum proteins are markedly decreased during protein energy malnutrition (Siddiqui *et al*., 2007). SCMC-20% has a good potential to increase serum albumin concentration in human infants.

Increased plasma aspartate amino transferases (AST) (Table 3) observed in basal diet group of animals indicates the important role of free radicals in the aetiopathogenesis of kwashiorkor (Etukudo *et al*., 1999). The SCMC-20% diet group had the lowest mean value of AST (µ/l) and would decrease the capacity of the weanling system to produce free radicals.

Significantly lower serum cholesterol concentrations were observed in infants fed commercially prepared formulae compared with those fed on breast milk (Friedman and Goldberg, 1976). A low cholesterol intake in infancy may decrease the rate of myelination of brain and nervous system before and during the weaning period, and a cholesterol challenge during infancy may initiate mechanisms responsible for the degradation and ultimate control of blood cholesterol in adulthood (Fomon, 1971). The lowest value (though moderate), of serum cholesterol was observed of the SCMC 20% diet group (Table 3).

SCMC-20%, SCMC-16%/Rd, listed in order of consecutive significant decrease ($p<0.05$), shown in Table 4, possess great nutritional potentials, measured in the mean values of performance characteristics, NPU%, TD%, BV and PER ($p<0.01$), to support growth and rehabilitation of protein energy malnutrition subjects, in keeping with the findings of Mosha and Bennink (2004).

Table 2. Results on the Growth performance (grams) of the test and reference weaning formulae, and basal diet group.

	ΔLive weight (grams)
SCMC-16%	86.2 ± 1.0[a]
Rd	85.1 ± 2.1[a]
SCMC-20%	100.2 ± 1.02[b]
Basal	-25.1± 1.02[c]

Results are expressed as mean ± standard error (S.E) (unit) (n = 10). Values that are labeled, in the same column, with the same superscripts, are not significantly different (p<0.05).

Table 3. Results on the biochemical indices: Serum cholesterol, serum triglyceride, serum albumin, serum aspartate aminotransferase (AST) and WBC_{total} of the test and reference weaning formulae, and the basal diet group.

	Serum cholesterol (mg/dl)	**Serum triglyceride** (mg/dl)	**Serum albumin** (mg/dl)	**Serum aspartate aminotransferase (U/l)**	**WBC_{total}** (mm)
SCMC-16%	175 ± 0.01[a]	113± 0.03[a]	4.15 ± 0.1[a]	12.05 ± 0.16[a]	5005± 12.15[a]
Rd	185 ± 0.02[b]	114± 0.02[b]	4.05 ± 0.2[a]	13.1 ± 0.15[a]	5008± 11.15[a]
SCMC-20%	155 ± 0.02[c]	95 ± 0.04[b]	6.1 ± 0.2[a]	9.1 ± 0.01[a]	5183± 25.15[b]
Basal	174± 0.02[a]	125± 0.01[c]	1.78 ± 0.2[b]	18.1 ± 2.16[b]	2754± 12[c]

Results are expressed as mean ± standard error (S.E) (unit) (n = 10).
Values that are labeled, in the same column, with the same superscripts, are not significantly different (p<0.05).

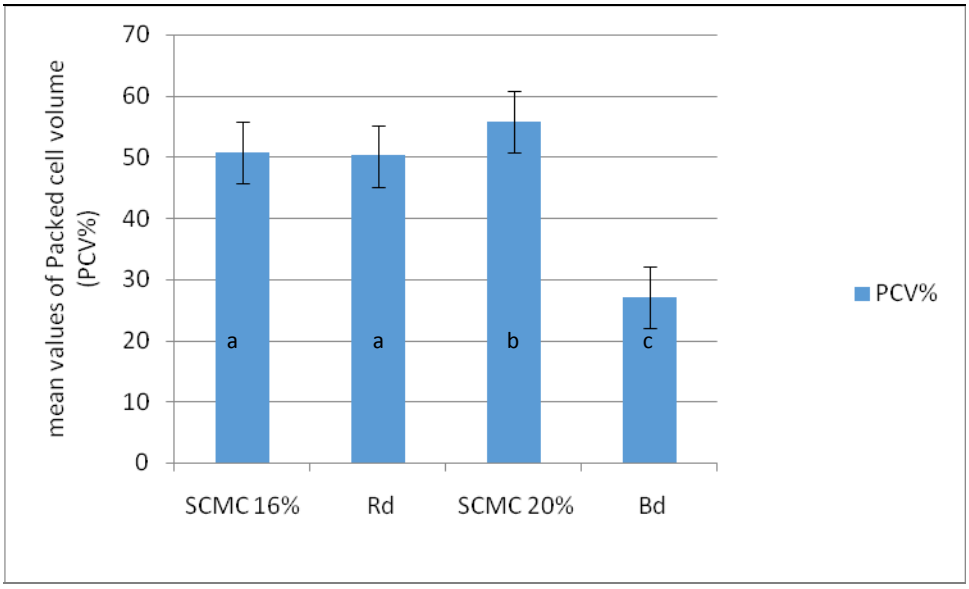

Figure 1. Graphical results on the packed cell volume (PCV%) of the test and reference weaning formulae, and the basal diet group.

Statistical results are expressed as mean ± standard error (%) (n = 10). Error bars represent values of standard error (0.2 – 1.2%). Bars labeled with the same letters represent mean values of PCV% which are not significantly different (p<0.05).

The mean values of the relative liver organ weight ratio indicate that the potential of the SCMC-20% weaning formula to effect proportionate increases in the body weight (gram) and liver weight (gram) of the experimental

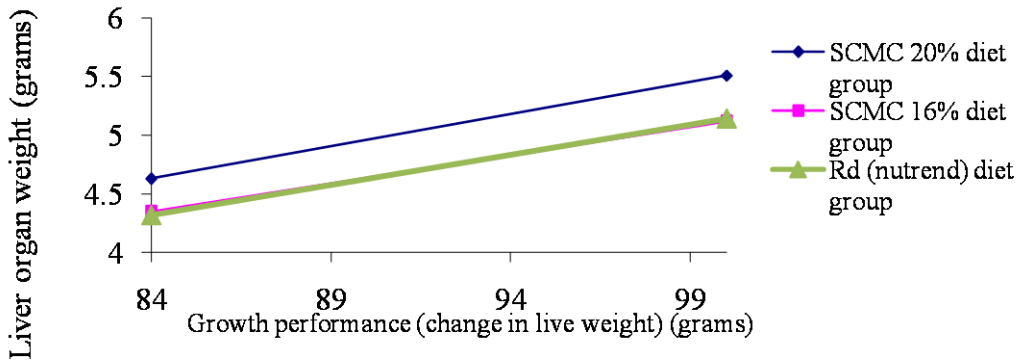

Figure 2 : Relative liver organ weight ratios of weaning formulae diet groups of experimental wistar albino rats.

Significant differences observed of the relative liver organ weight ratios were as follows: SCMC 20% (0.05515 ± 0.0001[a]) > SCMC 16% (0.0515± 0.0001[b])/Nutrend (Rd) (0.0514 ± 0.0001[b]). Values in the inequality equation/expression are mean ± S.E (n = 10). Means having the same superscript are not significantly different (p<0.05).

Table 4. Results on the performance characteristics : Net protein utilization (NPU%), True digestibility (TD%), Biological value (BV), and Protein Efficiency ratio (PER) of the test and reference weaning formulae, and the PER of the basal diet group.

	NPU%	TD%	BV	*PER
SCMC-16%	88.2 ± 1.0[a]	90.2 ± 1.0[a]	90.2 ± 1.0[a]	2.5 ± 0.16[a]
Rd	87.1 ± 2.1[a]	88.1 ± 2.1[a]	90.5 ± 2.1[a]	2.45 ± 0.15[a]
SCMC-20%	97.2 ± 1.02[b]	97.1 ± 1.02[b]	98.2 ± 1. 2[b]	3.55± 0.01[b]
Basal	----------	----------	----------	-1.35 ± 0.5[c]

Results are expressed as mean ± standard error (S.E) (unit) (n = 10). Values that are labeled, in the same column, with the same superscripts, are not significantly different (p<0.05).*(p<0.01).

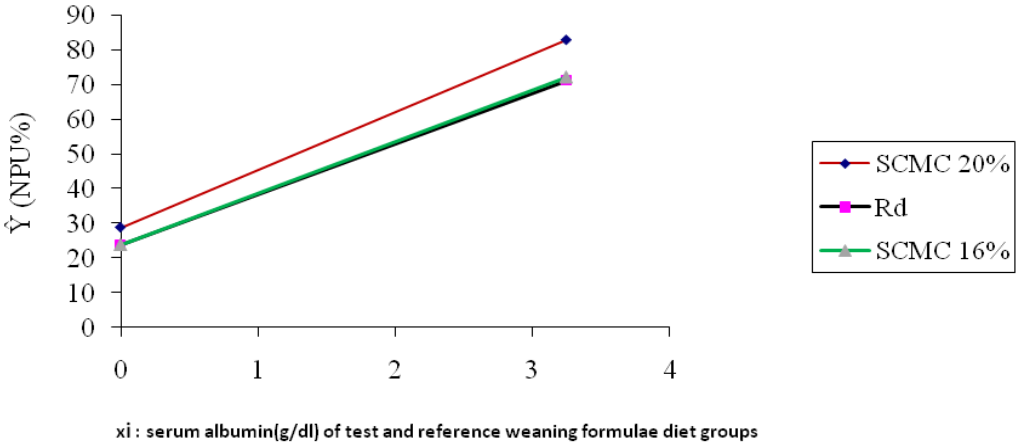

Figure 3 : Regression curve of NPU% and serum albumin (g/dl) :
(SCMC 20%) : Ŷ (%) = 28.73 (%) + 16.69 (% * dl/g)xi g/dl.
(SCMC 16%) : Ŷ (%) = 28.94 (%) + 14.97 (% * dl/g)xi g/dl.
(Rd) : Ŷ (%) = 23.73 (%) + 14.53(% * dl/g)xi g/dl

group of animals is significantly higher ($p<0.05$) than that potential of the SCMC-16% and nutrend (Rd) weaning formulae (figure 2).

The basal diet group of experimental animals suffered the most significant decrease ($p<0.05$) in mean values of all the growth performance, hematological, biochemical and

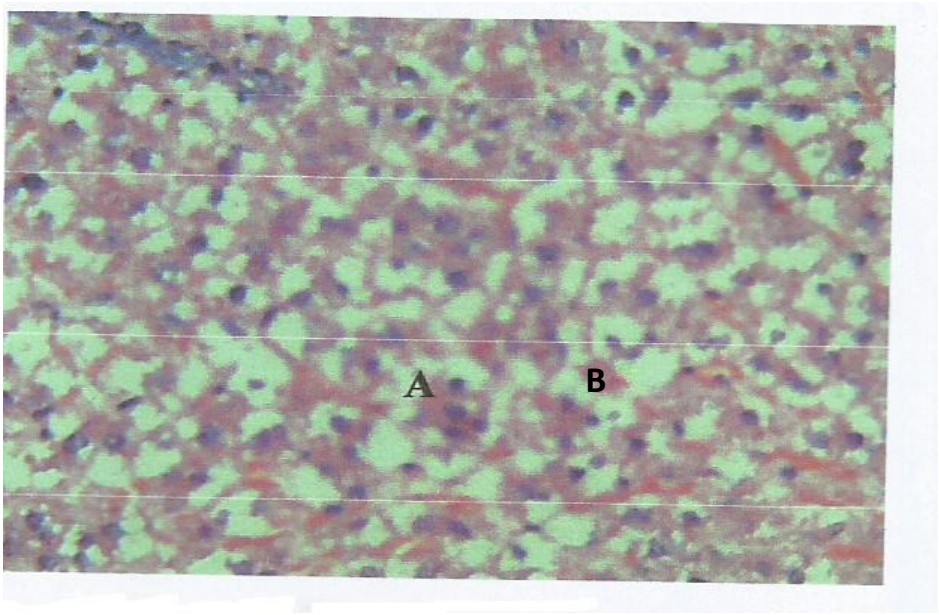

Plate 1. Photomicrograph of lobe section of the liver tissue of a wistar albino rat, administered basal diet. showing degenerative changes: **A**. Necrotic and atrophic cells (sparsely distributed black dots). B. Hemorrhage.

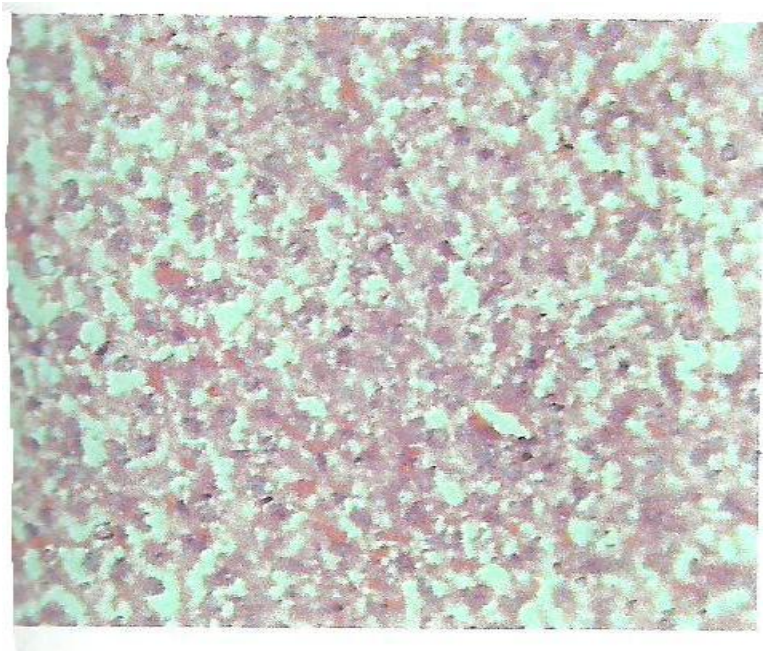

Plate 2. Photomicrograph of lobe section of the liver tissue of a wistar albino rat fed on SCMC-20% weaning formula showing normal tissue.

performance characteristic [PER ($p<0.01$)] indices measured except the AST enzyme activity and serum triglyceride of which it suffered the most significant increase ($p<0.05$). This observation indicates that degenerative changes had occurred in the basal diet group.

Liver section photomicrographs of 15.5% dietary protein formulated cerelac and 20% dietary protein formulated weaning formula groups of experimental rodent models showed normal histopathological features but those of 10% dietary protein formulated weaning formulae showed cytoplasmic granulation and vacoulation (Mahgoub, 1999).

Administration of a hypoproteic (6%), isocaloric diet to wistar albino rats, induced protein energy malnutrition in the rats, caused liver steatosis and fibrosis, culminating in cirrhosis, which is associated with liver cells atrophy, and hemorrhage (Conde *et al*., 1993). The basal diet used in the present study was hypoproteic (<3.47% dietary protein). The liver tissue of a wistar albino rat administered with the basal diet was characterized by haemorrhage, necrotic and atrophic cells, as shown in Photomicrograph/Plate 1.

Multiple regression studies revealed that growth performance (grams) regressed significantly ($p<0.05$) with PCV%, serum albumin (g/dl) and TD% of the SCMC-20% diet group. The regression and correlation statistical analysis between NPU% and serum albumin (g/dl) of the SCMC-20% diet group was significant ($p<0.05$) with a Pearson's product moment correlation coefficient of 0.985. The net protein utilization (NPU%) could be predicted from the regression curve: [$\hat{Y}$ (NPU%) = $\hat{Y}$ (%) = 28.73 (%) + 16.69 (% * dl/g)xi g/dl.], xi is observed value of serum albumin(g/dl) (figure 3).

The ratio of the weight of dietary protein required to alleviate kwashiorkor per unit weight of human subject to the weight of dietary protein required to alleviate kwashiorkor per unit weight of wistar albino rat model is 4:7 (Obimba, 2006).

SCMC-16% and nutrend have comparable nutritional efficiencies. SCMC-20% possesses the most potential to effect rapid catch-up growth rate in protein energy malnutrition subjects.

CONCLUSION

SCMC-20% (18.00 g), SCMC-16% (23g), or Rd (Nutrend) (25g), respectively, /4.0 g of dietary protein/100 ml of water /100 kcal/day, is sufficient for the prevention of PEM, and could also serve as dietary therapy essential to effecting rapid catch-up growth rates in protein and calorie-deficient human infants. The nutritional efficiency of the various weaning formulae, listed in sequential order of significant ($p<0.05$) decrease is as follows: SCMC 20%>SCMC 16%/Rd (nutrend).

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