International Journal of Biochemistry and Biotechnology ISSN: 2169-3048 Vol. 1 (9), pp.230-238, December, 2012. Available online at http://internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Utilization of high quality weaning formulae as dietary therapies of protein energy malnutrition

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Received 19 October 2012; Accepted 11 December 2012

High quality weaning formulae SGMC and SMC (20% protein diets) prepared from available and affordable plants (soya bean, groundnut, maize, and fluted pumpkin green leaves) and animal sources (milk, catfish, and crayfish) were used in rehabilitating kwashiorkor-induced wistar albino rats in a comparative study. The experimental design for the treatment of kwashiorkor was a single factor, Completely Randomized Design (CRD). Administration of the weaning formulae to the kwashiokor-induced rats, caused a reversal of the earlier observed decreases in the parameters [White blood cell count (WBC_{Total}), True Digestibility (TD%) and Protein Efficiency Ratio (PER)], measured, in order of consecutive significant increase ($p<0.05$) as follows: SGMM<Rd<SGMC/SMC; The growth performance change in live weight and Liver weight [SGMM/Rd<SGMC/SMC]. The aspartate amino transferase (AST) enzyme activity, differed significantly ($p<0.05$) in order of sequential increase as follows: SGMM>Rd>SGMC/SMC. Results were recorded as values of mean ± S.D unit, of the SGMC/SMC diet groups, as follows: growth performance: (90.4 ± 0.5/87.0 ± 2.84 g), Liver weight (10.77 ± 1.05/10.47 ± 1.01 g), WBC_{Total} (5156.12 ± 60.3/5150. 2 ± 59.03 mm^3), AST: (11.1 ± 0.05/10.95 ± 0.05 U/l), TD%: (97.0 ± 0.06/96.0 ± 0.07%), and PER: (2.44 ± 0.10/2.45 ± 0.16). The regressions, and correlation between growth performance (grams) and PER of the SGMC and SMC diet groups, were significant ($p<0.05$), with Pearson's product moment correlation coefficients of 0.96 and 0.97, respectively. SGMC and SMC weaning formulae achieved the most rapid catch-up growth rates in the kwashiorkor-induced rats, and serve as prophylaxis and dietary therapy of protein energy malnutrition.

Key words: Protein energy malnutrition, dietary therapy, weaning formulae.

INTRODUCTION

Protein energy malnutrition (PEM) is a range of pathological conditions arising from co-incident lack, in varying proportions, of proteins and calories, occurring most frequently in infants and young children and commonly associated with infections (Roulet, 1994; Muscaritoli et al., 2009). Treatment consists of correcting fluid and electrolyte deficits with intra-venous solutions, then gradually replenishing nutrients, orally if possible (Morley, 2007).

Three types of protein energy malnutrition viz: marasmus, marasmic kwashiorkor, and kwashiorkor have been identified, described and classified (Manary et al., 2009). Kwashiorkor is characterized by low weight for age, oedema, dermatitis, hair changes, mental changes, hepatomegaly and diarrhea. Classification of protein energy malnutrition employs the weight to age percent (%) anthropometric measure, in addition to presence or absence of oedema in distinguishing kwashiorkor (65 to 80% weight for age, oedema present), from marasmic kwashiorkor (<60% weight for age, Oedema present), from marasmus (<60% weight for age, oedema absent) (Grover and Ee, 2009).

Fifty percent reduction in growth performance measured in change in live weight (grams) was observed of kwashiorkor-induced rats compared with control group of experimental animals by Etukudo et al. (1999). Fatty liver and/or atrophy of the liver resulting in increased level of aspartate amino transferase enzyme in the blood, occurs in PEM subjects (Islam et al., 2007; Kayode et al., 2009), and is often characterized by significant reduction ($p<0.05$) in liver organ weight (Tai et al., 2010).

Increased plasma alanine and aspartate amino transferases observed in low protein group of animals probably suggest an important role of free radicals in the

Table 1. Diet formulation.

Diet Components (g/100 g diet)	Kd/basal (3.47% dietary protein level)	SGMC (20% dietary protein level)	SMC (20% dietary protein level)	SGMM (16.5% dietary protein level)
Casein	3.47	-	-	-
Soyabean seed (flour)	-	17.63	21.84	20.00
Groundnut seed (flour)	-	4.41	-	5.00
Maize seed (flour)	-	51.42	50.97	70.00
Powdered cow milk	-	-	-	5.00
Catfish	-	10.20	-	-
Crayfish	-	-	10.31	-
Vegetables (Fluted pumpkin leaves)	-	5.00	5.00	-
Palm oil	8.00	8.00	8.00	-
Vitamin-mineral premix	0.25	0.25	0.25	-
Sucrose	-	1.00	1.00	-
Garri (processed cassava)	88.28	2.09	2.68	-

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. SGMC: 19.71% dietary protein, 64.2% carbohydrates, 9.2% lipids, 3.1% moisture, 3.1% minerals, vitamins ≤ 0.69 g, 437.1 kcal/100g. SMC: 19.71% dietary protein, 64.4% carbohydrates, 9.0% lipids, 3.2% moisture, 3.1% minerals, vitamins ≤ 0.59 g, 432.1 kcal/100g. SGMM: 16% dietary protein, 71.7% carbohydrates, 6% fat, 4.5% moisture, 1.67% minerals, vitamins ≤ 0.13 g, 368.5 kcal/100 g.

aetiopathogenesis of kwashiorkor *(*Etukudo et al., 1999). Protein energy malnutrition causes a significant decrease ($p<0.05$) in white blood cell count, a decrease in number of lymphocytes and a lowering of haemoglobin count (Schaible and Kaufmann, 2007). Infections associated with PEM are largely due to decrease in efficiency of patient's immune system. WBC_{Total} is used as a diagnostic tool in monitoring recovery from PEM (Huang and Fraker, 2003).

Administration of a hypoproteic (6%) isocaloric diet to wistar albino rats, induced protein energy malnutrition in the rats, with observed liver steatosis and fibrosis. Cirrhosis is associated with liver cells atrophy, and could cause hemorrhage (Conde et al., 1993).

In dietary therapy for optimum recovery, dietary energy intake, treatment of infections, dietary protein intake, correction of electrolyte imbalance, are imperative. Disappearance of apathy, oedema, anorexia, gain in body weight and increase in nervous motor activity, are signs of recovery from kwashiorkor (Etukudo et al., 1999).

The rationale for formulation of diet is founded on the importance of the cereal-legume-animal supplement mix. 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 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, while 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.

This is a comparative study aimed at utilizing weaning formulae prepared from available and affordable plants (soya bean, groundnut, maize, and fluted pumpkin green leaves) and animal sources (milk, catfish, and crayfish) to rehabilitate kwashiorkor-induced wistar albino rats.

MATERIALS AND METHODS

The experimental Design of the treatment of kwashiorkor is a single factor completely randomized design (CRD) of 20 observations per parameter, and 15 degrees of freedom of error. The Linear model is $Yij = \mu + T_i + e_{ij}$. Y_{ij} = Individual observations, μ = Overall mean, T_i = Effect of ith level of dietary protein treatment, e_{ij} = Random error, which is independently, identically, and normally, distributed, with zero mean, and constant variance (Obimba, 2011). Analysis of Variance (ANOVA) and Student t-test were employed in the statistical analysis of the results.

Two hundred grams (200 g), each of raw soya bean seeds, raw groundnut seeds and raw maize seeds, 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 groundnut 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 catfish 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.

Twenty-three (23) weanling Wistar albino rats aged 5 weeks old were weighed and housed in stainless steel cages under 12 h light and dark cycles, under humid tropical conditions, and fed *ad-libitum* on a 3.47% dietary

protein-kwashiokorigenic diet (Kd) for 33 days (the animals were acclimatized to the diet, within the first 3 days), during which period, kwashiorkor was induced in the rats. A control group of experimental rats were fed, during the same period, on conventional feed, prepared at 16% dietary protein level. Daily faecal deposits of the animals were collected during the last thirty days of the feeding trial, pooled, oven dried and weighed. Three experimental animals each of the kwashikor-induced diet group, and the control group 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.

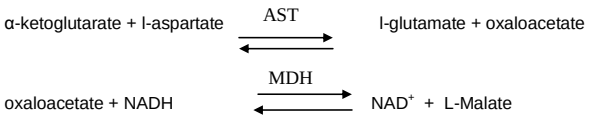
Twenty male kwashiorkor-induced Wister albino rats were divided into five groups of 4 animals each, and housed in stainless steel cages under 12 h light and dark cycles, under humid tropical conditions, and fed *ad-libitum* on four different types of weaning diets (SGMC, SMC, SGMM, and Rd) for a period of 20 days. The kwashiokorigenic diet (basal diet) which served as a control was fed to another group of rat for the same period. Daily faecal deposits of the animals were collected during the 20 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 by the Kjeldahl method was carried out by a modified method, similar to that described by (AOAC, 1990). Samples were digested in 250 ml Kjeldahl flask using concentrated tetraoxosulphate VI acid, sodium tetraoxosulphate VI and selenium containing catalyst. The dilute digest was distilled using 50% sodium hydroxide solution in a distillation apparatus and collected in an alcoholic boric acid solution and titrated against 0.1 M hydrochloric acid. The titre value (t ml) was obtained at endpoint (pink colour of solution). N (%) = [(N acid) (ml acid) − (ml bk) (N NaOH) − (ml NaOH) (N NaOH)] × 1400.67 mg^{-1} sample. Where ml NaOH = milliliters of standard base needed to titrate sample; ml acid = mill liters of standard acid used for that sample; ml bk = milliliters of standard base needed to titrate 1 ml standard acid minus milliliters of standard base needed to titrate reagent blank carried through method and distilled into 1 ml standard acid; N acid = normality of standard acid; N NaOH = normality of standard base.

Crude protein % = N (%) × 6.25

The white blood cell Total count (mm^3) 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 in a counting chamber, and white blood cells were counted.

The IFCC - UV Kinetic Method was used for the quantitative in-vitro determination of aspartate aminotransferase (AST), and measures the rate of catalytic activity of AST as a function of the utilization of oxaloacetate formed by the catalytic activity of AST, according to the equation scheme shown below.



Kee Gad kits, Ver: KGAST102.3/1 (2010) was used for the quantitation (Gaze, 2007). 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.

$$\text{PER} = \frac{\text{Gain/loss in body mass (g)}}{\text{protein intake (g)}}$$

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.

Liver sections were prepared for histopathological studies consistent with the method described by Brozska et al. (2003) slices off the left liver lobe were fixed in 10% formal saline for 24 h. The fixed tissues were dehydrated and de-alcoholated, in turn, 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

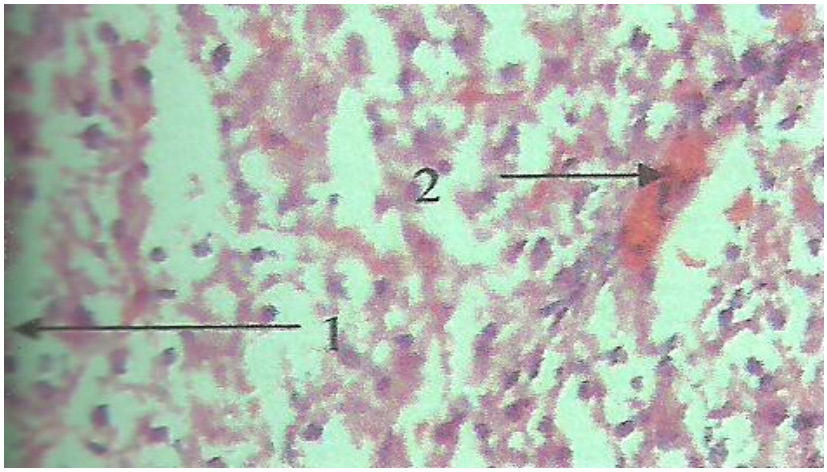


Plate 1. Photomicrograph of the lobe section of the liver tissue of a wistar albino rat administered with the kwashiokorigenic/basal diet. Plate 1 is the Photomicrograph of the lobe section of the liver tissue of a wistar albino rat administered with the kwashiokorigenic/basal diet showing: 1. Atrophic liver cells. 2. Haemorrhage.

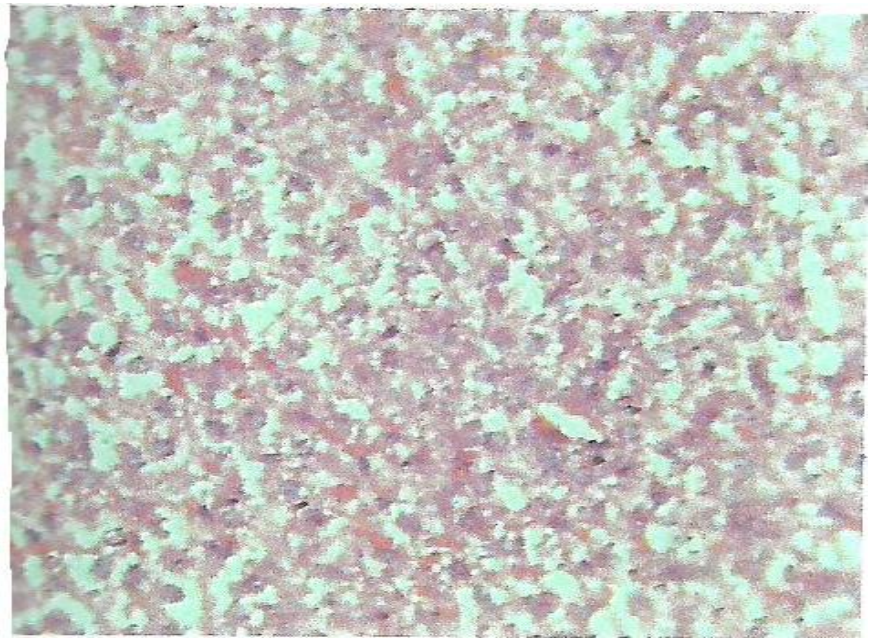


Plate 2. Photomicrograph of the lobe section of the liver tissue of a wistar albino rat administered with the SGMC weaning formula. Plate 2 is the photomicrograph of the lobe section of the liver tissue of a wistar albino rat administered with the SGMC weaning formula, showing normal tissue.

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 are the photomicrographs (Plates 1 and 2).

RESULTS

Table 2 shows the percentage crude protein content of the experimental diets. The percentage crude protein content of the SGMC and SMC weaning formulae, were numerically, and significantly equal ($p < 0.05$), and were

Table 2. Percentage crude protein content of the experimental diets

Diet type	Protein content (%)
SGMC	$19.71^{a} \pm 0.2$
SMC	$19.71^{a} \pm 0.1$
SGMM	$16.5^{b} \pm 0.05$
Bd/Kd	$4.38^{c} \pm 0.0$

Values are means ± S.D (n = 4). Means in the same column having the same superscripts are not significantly different at 5% level (p<0.05).

Table 3. Growth performance of the weaning formulae and basal diet groups of experimental animals.

Diet group	Rd	SGMC	SMC	SGMM	Bd
Gain/loss of live weight (growth performance: grams)	55.00^{b} ±13.80	87.00^{a} ±2.84	90.4^{a} ±0.5	54.80^{b} ±1.85	-30.03^{c} ±6.28

Values are means ± S.D (n = 4). Means in the same row having the same superscripts are not significantly different at 5% level (p<0.05).

Table 4. Haematological and biochemical parameters assayed of the weaning formulae and basal diet groups of experimental animals.

Diet group	Rd	SGMC	SMC	SGMM	Bd
WBC_{Total} (mm^3)	4607.41^{d}±50	5156.12^{b}±60	5150.2^{b}±58	4528.97^{a}±59	2550^{c} ±35
AST (U/l)	12.05^{a} ±0.05	11.1^{b}±0.05	10.95^{b} ±0.05	12.4^{c}±0.05	15.4^{d} ±0.05

Values are means ± S.D (n = 4). Means in the same row having the same superscripts are not significantly different at 5% level (p<0.05).

not significantly different ($p < 0.05$), from the calculated value of 20% , substituted in the formulae derived by the author, for the diet formulation. The percentage protein content of each of the SGMC and SMC weaning formulae, differed significantly ($p < 0.05$), from those of the Rd (reference) weaning formula, and the basal diets, in consecutive order of significant decrease. The significant differences ($p < 0.05$), recorded of the quantitative percentage crude protein content of the weaning formulae, and the basal diet, listed in descending order, are as follows : SGMC/SMC> Rd >SGMM > Bd. Table 3 shows the growth performance of the weaning formulae and basal diet groups of experimental animals. The growth performance recorded of the diet groups of experimental animals, in order of consecutive decrease were as follows: SMC/SGMC> Rd/SGMM. The mean value of growth performance of the SMC diet group of experimental animals was significantly higher ($p<0.05$) than those of the SGMM and Rd diet groups of experimental animals. The basal diet group of experimental animals suffered significant loss of weight ($p<0.05$).

Table 4 shows the hematological and biochemical parameters measured of the weaning formulae and basal diet groups of experimental animals. The mean values of the haematological parameter, WBC_{Total} (mm^3), measured of the weaning formulae diet groups of experimental animals, listed in sequential order of decrease were as follows: SGMC/SMC> Rd>SGMM. The mean values of the WBC_{Total} (mm^3) of each of the SGMC and SMC diet groups of experimental animals were not significantly different ($p<0.05$), but were both significantly different ($p<0.05$) from the mean values of the WBC_{Total} (mm^3) of each of the Rd and SGMM diet groups of experimental animals. The mean values of the aspartate aminotransferase (AST) enzyme activity (U/l) of each of the SGMC and SMC diet groups of experimental animals were significantly reduced ($p<0.05$) compared with those of the Rd and SGMM. The basal diet group of experimental animals suffered the most significant decrease ($p<0.05$) of mean values of WBC_{Total} (mm^3) and the most significant increase ($p<0.05$) in AST enzyme activity.

Figures 1 and 2 show the quantitative values of the performance characteristic, True Digestibility (TD%) and Protein Efficiency Ratio (PER), respectively, of the weaning formulae and basal diet. The TD% and PER were used in assessing the nutritional efficiency and dietary protein quality of the dietary therapies/weaning formulae. The mean values of the TD% and PER of the weaning formulae listed in sequence of significant decrease ($p<0.05$) were as follows: SGMC/SMC>Rd

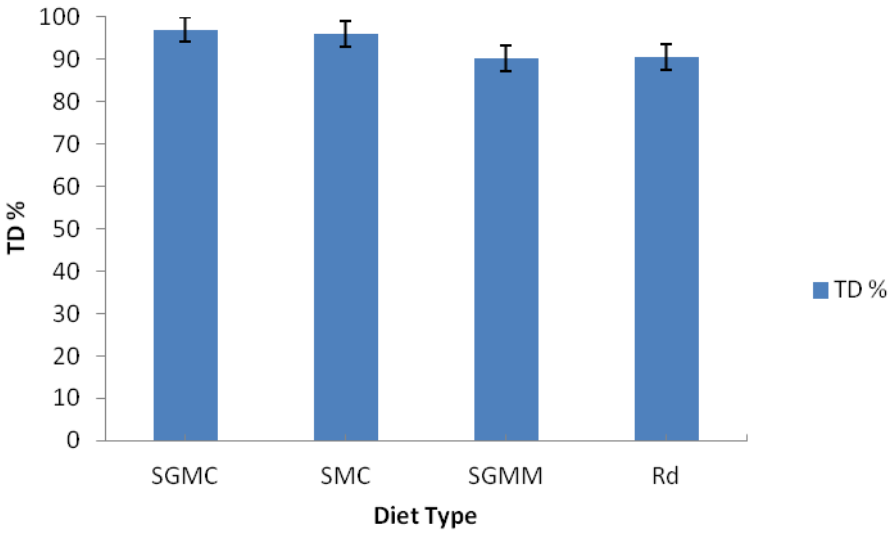


Figure 1. True digestibility (TD%) of dietary therapies/ weaning formulae.

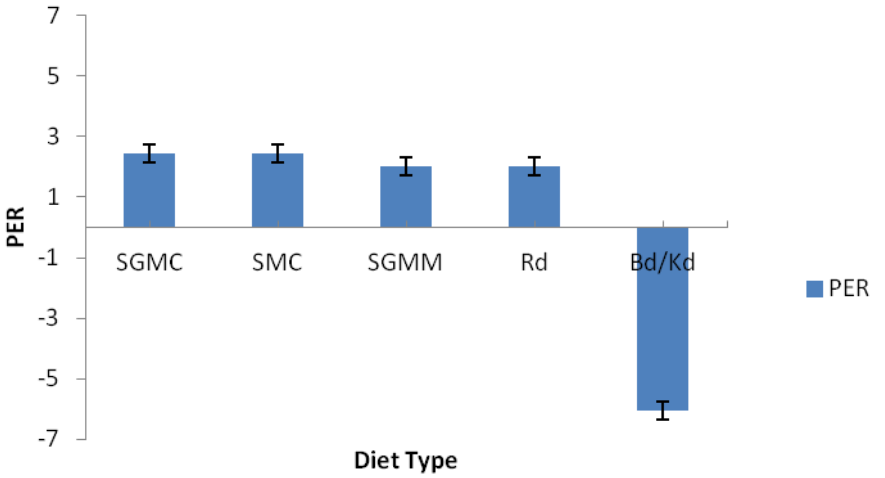


Figure 2. Protein efficiency ratio (PER) of dietary therapies/ weaning formulae.

>SGMM.

Plate 1 is the Photomicrograph of the lobe section of the liver tissue of a wistar albino rat administered with the kwashiokorigenic/basal diet showing some degenerative changes resulting in the production of necrotic and atrophic cells, and haemorrhage. Plate 2 is the photomicrograph of the lobe section of the liver tissue of a wistar albino rat administered with the SGMC weaning formula, showing normal tissue.

DISCUSSION

Kwashiorkor is an acute form of childhood protein-energy malnutrition characterized by edema, irritability, anorexia, ulcerating dermatoses, and an enlarged liver with fatty infiltrates. The presence of edema caused by poor nutrition defines kwashiorkor (Ciliberto et al., 2005).

The age effect on the onset of marasmic kwashiorkor is distinct. It takes about 30 days for the induction of marasmic kwashiorkor on weanling Wister albino rats, using a dietary regimen of 3.47% of dietary protein, in keeping with the method of induction employed by Akinola et al. (2010). The significant reductions ($p<0.05$) observed of the growth performance (-12.5 ± 3.3 grams), lean body mass, PCV% (30.2 ± 0.24%), serum albumin (1.77 ± 0.97 g/l) , PER (-1.21 ± 0.04), and significant increase observed of the AST (15.53 ± 5.95 U/l) of the kwashiorkor induced rats, compared with those of the control and weaning formulae diet groups, reflect poor

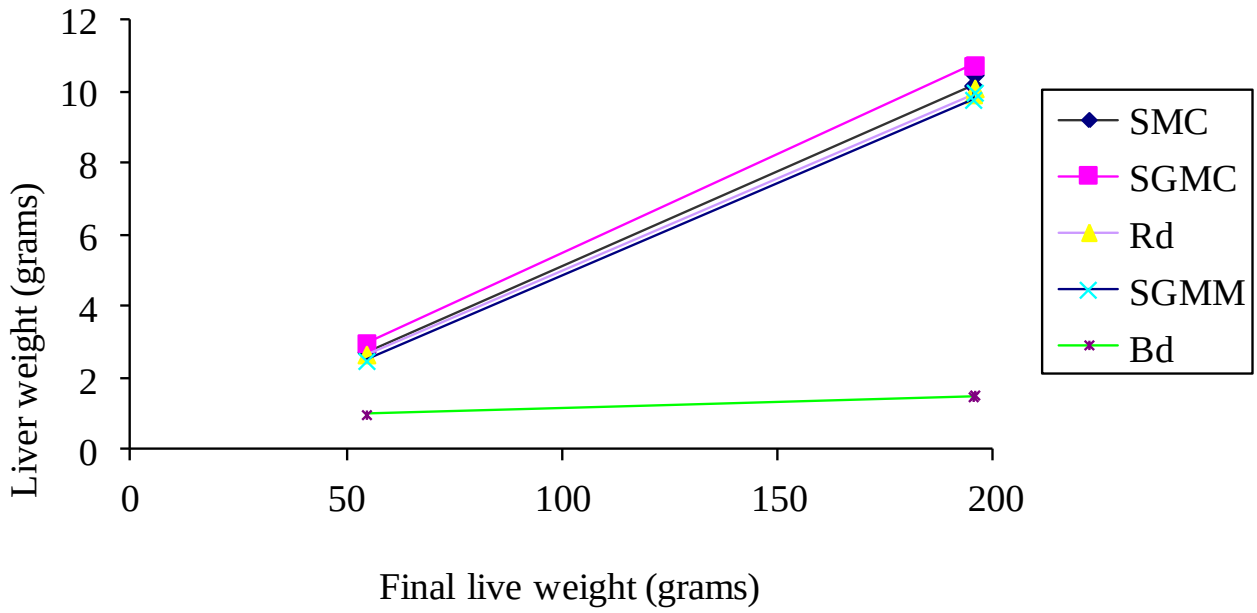


Figure 3. Relative Liver Organ Weight Ratio of the weaning formulae and basal diet groups of experimental animals. Significant differences observed of the relative Liver organ weight ratios were as follows: SGMC ($0.0551^{a} \pm 0.0001$) / SMC ($0.0549^{a} \pm 0.0001$) > Rd ($0.0517^{b} \pm 0.0001$) / SGMM ($0.0515^{b} \pm 0.0001$) > Bd ($0.0101^{c} \pm 0.0001$). Values in the inequality equation/expression are mean ± S.D (n = 4). Means having the same superscript are not significantly different at 5% confidence level ($p<0.05$).

nutritional status at various stages of protein energy malnutrition and correspond with the findings of Collins (2003). The kwashiorkor–induced group of experimental animals was characterized by retarded growth, dermatitis, oedema, hair loss, physical inactivity, observable loss of motor co-ordination and apathy.

The significant increases ($p<0.05$) in growth performance, and WBC_{Total}, and the significant decreases ($p<0.05$) in AST enzyme activity shown in Tables 3 and 4, recorded of the SGMC and SMC diet group of experimental animals, indicate the efficacy of the SGMC and SMC weaning formulae in promoting good biological responses in rodent models, consistent with the findings of Obatolu et al. (2003).

Figures 1 and 2 are the evidence to show that cereal-legume and animal supplements mix possess a great nutritional potential, measured in the performance characteristics, TD% and PER, respectively, to support growth and rehabilitation of protein energy malnutrition subjects, and corroborates the findings of Mosha and Bennink (2004).

Upon rehabilitation, the kwashiorkor induced group of experimental animals, were characterized by a restoration to normal dermal conditions, loss of oedema, hair growth, noticeable restoration to normal physical activities, motor co-ordination, and weight gain.

The relative liver weight ratios are the respective slopes of the curves/straight line graphs shown in Figure 3, and are fairly constant, without significant difference ($p<0.05$), in each diet group, but vary significantly ($p<0.05$) among the diet groups as follows : SGMC/SMC > Rd/SGMM > Bd, in order of consecutive significant decrease. The values of the relative liver weight ratios indicate that the potential of the SGMC and SMC weaning formulae to effect proportionate increases in body weight (grams) and liver weight (grams) of the corresponding experimental group of animals is significantly higher ($p<0.05$) than that potential of the Rd and SGMM weaning formulae.

The regressions, shown in Figure 4, and correlation between growth performance (grams) and PER of the SGMC and SMC diet groups, were significant ($p<0.05$), with Pearson's product moment correlation coefficients of 0.96 and 0.97, respectively. The growth performance of the experimental groups of animals fed on the SGMC and SMC weaning formulae are functions of their various dietary protein qualities.

Figure 5 shows the respective regressions between liver organ weight (grams) and AST(u/l) enzyme activity of the SGMC and SMC diet groups were significant ($p<0.05$). The Pearson's product moment correlation coefficients of the SGMC and SMC diet groups were 0.95 and 0.96, respectively. The negative gradients of the curves indicate that the liver organ weight increase as the AST(u/l) enzyme activities decrease, proportionately. The enzyme activity can be predicted given the liver organ weight.

Degenerative changes resulting in the production of

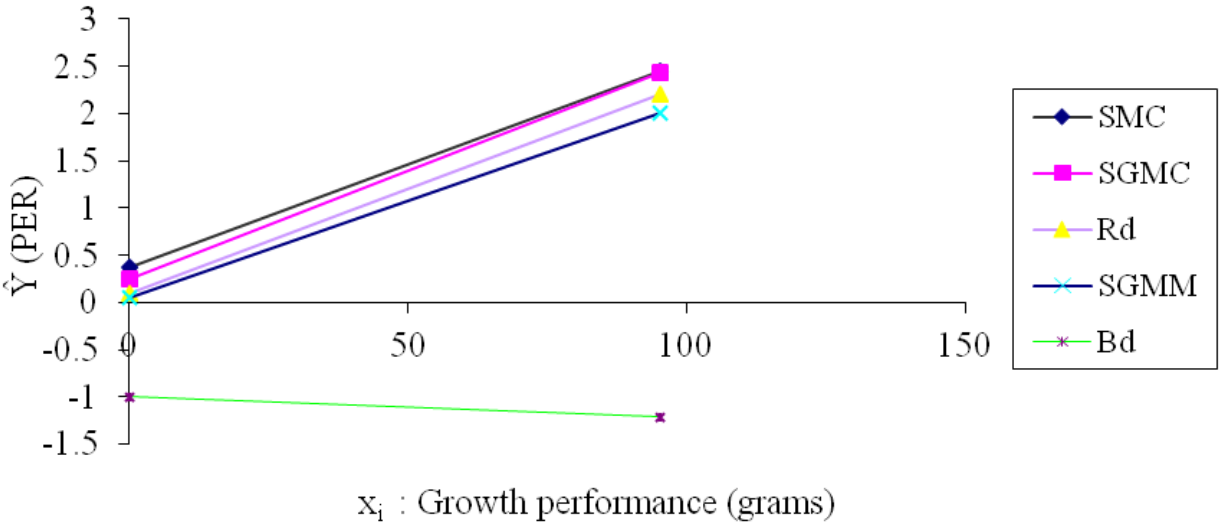


Figure 4. Regression curve of Growth performance (grams) and PER. The regression equations of Growth performance (grams) vs PER are: (SGMC): $\hat{Y} = 0.25 + 0.10\ g^{-1}\ x_i\ g$; (SMC): $\hat{Y} = 0.376 + 0.12g^{-1}\ x_i\ g$; (Rd): $\hat{Y} = 0.1 + 0.10g^{-1}\ x_i\ g$; (SGMM): $\hat{Y} = 0.05 + 0.11g^{-1}\ x_i\ g$; (Bd): $\hat{Y} = -1 - 0.13\ g^{-1}\ x_i\ g$.

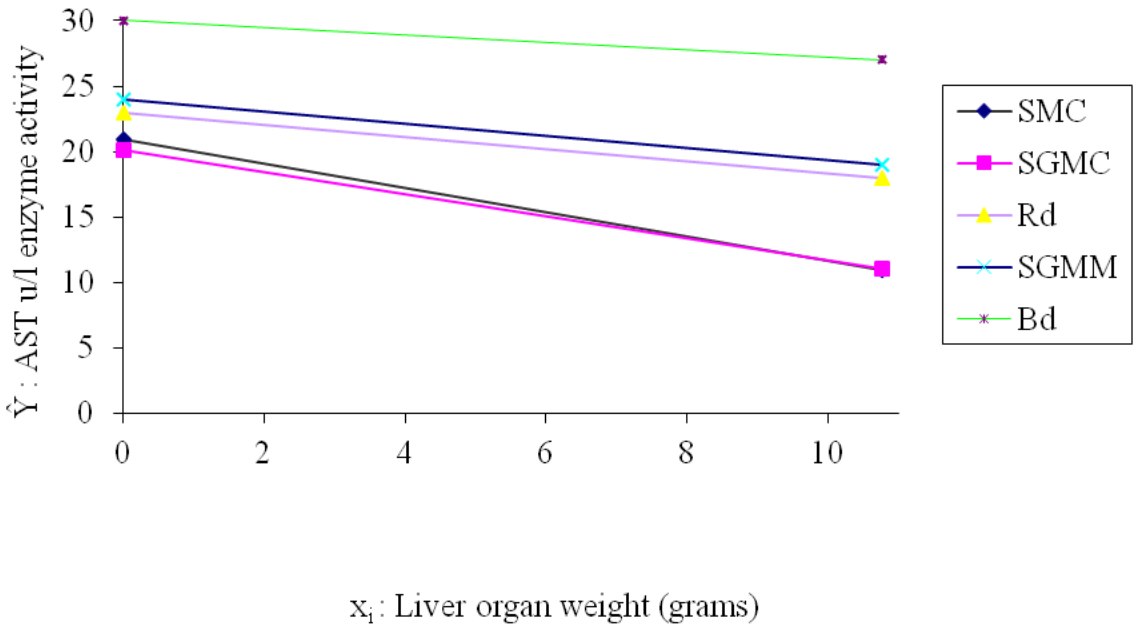


Figure 5. Regression curve of AST (u/l) enzyme activity and Liver organ weight (grams). The regression equations of AST (u/l) enzyme activity vs Liver organ weight (grams) are: (SGMC): $\hat{Y} = 20.10 - 0.885g^{-1}u/l\ x_{i;}$ (SMC): $\hat{Y} = 20.90 - 0.995g^{-1}u/l\ x_{i;}$ (Rd): $\hat{Y} = 23.00 - 1.14^{-1}u/l\ x_{i;}$ (SGMM): $\hat{Y} = 24.00 - 1.2g^{-1}u/l\ x_{i;}$ (Bd): $\hat{Y} = 30.00 - 1.5g^{-1}u/l\ x_{i.}$

necrotic and atrophic cells, and haemorrhage, seen in the liver tissue of a wistar albino rat administered with the kwashiokorigenic/basal diet (Photomicrograph/Plate 1), corroborates the findings of Conde et al. (1993), who posited that the administration of a hypoproteic (6%) isocaloric diet to wistar albino rats, induced protein energy malnutrition in the rats, caused liver steatosis and fibrosis. Cirrhosis which is the result, is associated with liver cells atrophy, and could cause hemorrhage.

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 rat model is 4:7 (Obimba, 2006).

SGMC (18.09 g), SMC (18.3 g), SGMM (28.09 g), or Rd (Nutrend) (25 g), respectively, /4.0 g of dietary protein/100 ml of water /100 kcal/day, is sufficient treatment for preventing and/or curing PEM in human infants. SGMC and SMC weaning formulae meet the reduced bulk size, and capacity to effect high rapid catch-up growth rates, requirements for PEM dietary therapy (Obimba, 2011).

CONCLUSION

The regressions, and correlation between growth performance (grams) and PER of the SGMC and SMC diet groups, were significant ($p<0.05$), with Pearson's product moment correlation coefficients of 0.96 and 0.97, respectively. SGMC and SMC weaning formulae achieved the most rapid catch-up growth rates in the kwashiorkor-induced rats, and serve as prophylaxis and dietary therapy of protein energy malnutrition. The protein value, and efficiency of the various weaning diets, listed in sequential order of significant ($p<0.05$) decrease were as follows: SGMC/SMC> Rd> SGMM.

ACKNOWLEDGEMENT

The author is grateful to Prof. O.C. Onwudike for his support and assistance.

Abbreviations: SGMC, weaning formulae prepared at 20% dietary protein, with processed soya bean seeds, groundnut seeds, maize seeds and catfish, diet group; **SMC,** weaning formulae prepared at 20% dietary protein, with processed soya bean seeds, maize seeds and crayfish, diet group; **Rd,** Nutrend-Nestle®, weaning formulae industrially prepared at 16% dietary protein, diet group; **Bd,** basal diet (hypothetical protein-free diet), diet group; **Kd,** kwashiokorigenic diet (prepared at 3.47% dietary protein level); **AST**, aspartate amino transferase; **WBC_{Total},** white blood cell total.

REFERENCES

Akinola FF, Oguntibeju OO, Alabi OO (2010). Effects of severe malnutrition on oxidative stress in Wistar rats. Sci. Res. Essays. 5(10): 1145-1149.

Annan TA, Plahar WA (1995). Development and quality evaluation of a soy-fortified Ghanaian Weaning Food. Food Nutr. Bull., 16(3): 263-269. http://www unu.edu/unu press/food 18F163e/8F163E of htm.

Association of Official Analytical Chemists (AOAC) (1990). Protein (Crude) Determination in Animal Feed: Copper Catalyst Kjeldahl Method. (984.13) Official Methods of Analysis. 15th Edition.

Brzoska MM, Moniuszko-Jakoniuk J, Pilat-Marcinkiewcz B, Sawicki B (2003). Liver and Kidney Function and Histology in Rats exposed to Cadmium and Ethanol. J. of Alc. Alc., 38(1): 2-10.

Ciliberto H, Ciliberto M, Briend A, Ashorn P, Bier D, Manary M (2005). Antioxidant supplementation for the prevention of kwashiorkor in Malawian children: randomised, double blind, placebo controlled trial. BMJ, 330(7500): 1109.

Collins N (2003). Protein-energy malnutrition and involuntary weight loss: nutritional and pharmacological strategies to enhance wound healing. Expert Opin. Pharmacother., 4(7): 1121-1140.

Conde MA, González RE, Santolaria FF, Castro AV, Marchena GJ, Martínez RA (1993). Liver changes in protein malnutrition. An experimental study in rats. Nutr. Hosp., 8(6): 358-363.

Etukudo M, Agbedana O, Akang E, Osifo B (1999). Biochemical Changes and Liver Tissue Pathology in Weanling Wistar Albino Rats with Protein Energy Malnutrition. (PEM). Afr. J. Med. Sci., 28(1-2): 43-47.

FAO/WHO (1971). Protein Advisory Group (PAG) of the United Nations. PAG Guideline No 8. Protein – Rich Mixtures for Use as Weaning Food., New York: FAO / WHO/ UNICEF.

Gaze DC (2007). "The role of existing and novel cardiac biomarkers for cardioprotection". Curr. Opin. Invest. Drugs, 8(9): 711–717.

Grover Z, Ee LC (2009). Protein energy malnutrition. Pediatr. Clin. North Am., 56(5): 1055-1068.

Huang ZL, Fraker PJ (2003). Chronic Consumption of a Moderately Low Protein Diet does not alter Hematopoetic Processes in young adult mice. J. Nutr. Sci., 133: 1403-1408.

Islam MS, Chowdhury ABM, Rahman Z, Haque M, Nahar N, Taher A (2007). Serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) Levels in Different Grades of Protein Energy Malnutrition. J. Bangladesh Soc. Phys., 2: 17-19.

Kayode OT, Kayode AA, Odetola AA (2009). Therapeutic Effect of *Telfairia Occidentalis* on Protein Energy Malnutrition-Induced Liver Damage. Res. J. Med. Plant, 3: 80-92.

Manary JM, Heikens GT, Golden M (2009). Kwashiokor: More Hypothesis Testing is needed to understand the Aetiology of Oedema. Malawi Med. J. 21(3): 106-107.

Morley JE (2007). Protein-Energy Malnutrition Definition. In: The Merck Manual of Diagnosis and Therapy. Porter R (ed.). 18th Edition. Merck & Co. Inc., New Jersey.

Mosha CET, Bennink MR (2004). Protein Quality of Drum Processed Cereal–Bean–Sardine Composite Supplementary Foods for Preschool–age Children. J. Sci. Food Agric., 84(10): 1111-1118.

Muscaritoli M, Molfino A, Bollea MR, Fanelli FR (2009). Malnutrition and wasting in renal disease. Curr.

Opinion Clin. Nut. Metab. Care, 12 (4): 378–383.
Obatolu VA, Ketiku A, Adebowale EA (2003). Effect of Feeding Maize / Legume Mixtures on Biochemical Indices in Rats. Ann. Nutr. Metab., 47: 170-175.
Obimba KC (2006). Utilization of Some Dietary Therapies in the Alleviation of Protein Energy Malnutrition. M.Sc. Thesis, University of PortHarcourt. PortHarcourt. Nigeria: p. 145.
Obimba KC (2011). Utilization of Dietary Therapies in the Alleviaton of Protein Energy Malnutrition in Kwashiokor-induced Rats. Afr. J. Biochem. Res., 5(4): 137-142.
Roulet M (1994). Protein-energy malnutrition in cystic fibrosis patients. Acta. Paed., 83(395): 43-81.
Sarwar G, Peace RW (1986). Comparisons between true digestibility of total nitrogen and limiting amino acids in vegetable proteins. J. Nutr., 116: 1172–1184.
Sarwar G, Peace RW (1994). The protein quality of some enteral products is inferior to that of casein as assessed by rat growth methods and digestibility-corrected amino acid scores. J. Nutr., 124: 2223–2232.
Schaible UE, Kaufmann SHE (2007). Malnutrition and Infection: Complex Mechanisms and Global Impacts. PLoS Med., 4(5): e115.
Tai MS, Goh K, Mohd-Taib SH, Rampal S, Mahadeva S. (2010). Anthropometric, biochemical and clinical assessment of malnutrition in Malaysian patients with advanced cirrhosis. Nut. J., 9: 27.
WHO (2001). Global Strategy for Infant and Young Child Feeding (A54/INF.DOC./4). Geneva: World Health Organization, pp. 1-5.
WHO (2002). Global strategy for Infant and young child feeding. WH/A55/2002/REC/1 Annex 2. Geneva.