

Full Length Research Paper

Comparative nutritional evaluation of high quality weaning foods formulated with processed quail (*Cortunix ypsilophora*), guinea fowl (*Numida meleagris*), and domestic poultry hen (*Gallus gallus*) eggs

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Received 20 July, 2016; Accepted 07 August, 2016; Published 15 August, 2016

Weaning formulae, SMQ, SMG, SMD (20% dietary protein) prepared from available and affordable plants (soya bean seeds, maize grains, and fluted pumpkin leaves) and animal (quail, guinea fowl, domestic poultry hen eggs) sources were evaluated for their nutritional efficiency in a comparative study. The experimental design for the animal feeding trial was a single factor, Completely Randomized Design (CRD). Fifty male wistar albino rats were divided into 5 groups of 10 animals each, housed in stainless steel cages and fed *ad-libitum* on 4 different types of weaning formulae (SMQ, SMG, SMD and nutrend (Rd)), and a basal diet, for a period of 28 days. Results were recorded as values of mean \pm S.E unit (range of values): growth performance (GP): (-25.1 \pm 1.02 -130 \pm 0.2 g), Serum albumin (SA) (1.58 \pm 0.1-5.5 \pm 0.1g/dl), Serum total cholesterol (C) (115 \pm 0.01-128.5 \pm 0.01 g/dl), Serum triglyceride (T) (65 \pm 0.02 -80 \pm 0.01g/dl), WBC_{Total} (2754 \pm 2-5205 \pm 0.15 mm³), Aspartate amino transferase (AST): (9.05 \pm 0.16-42.1 \pm 0.16 U/l), PCV% (25.1 \pm 0.2-55.8 \pm 1.2%), Net protein utilization (NPU%) (87.1 \pm 2.1-98.2 \pm 1.0%), True digestibility (TD%) (88.1 \pm 1.1-97.5 \pm 2.0), Biological value (BV): (90.5 \pm 1.0-98.0 \pm 1.02%), and Protein efficiency ratio (PER): (-1.35 \pm 0.5-3.85 \pm 0.16), Histopathology (liver tissues), diet groups : SMQ, SMG, SMD and nutrend (Rd): normal; basal : degenerative changes. The SMQ, SMG, SMD diet groups had the highest mean values of all the parameters measured, except the AST activity of which these groups had the lowest, and C(SMQ had the lowest) and T. The regression and correlation statistical analysis between TD% and SA (g/dl) of the SMQ, SMG, and SMD diet groups were significant ($p < 0.05$) ($r = 0.985, 0.980$ and 0.972 , respectively). The nutritional quality of the weaning formulae listed in sequential order of significant ($p < 0.05$) decrease: SMQ/SMG/SMD > Rd (nutrend). The weaning formulae are preventive and curative dietary therapies of protein energy malnutrition in human infants.

Keywords: Serum albumin, histopathology, protein energy malnutrition.

INTRODUCTION

Protein–energy malnutrition (PEM) or protein–calorie

malnutrition is a disease condition of malnutrition, resulting from coincident lack of dietary protein and calorie deficiency in varying proportions, often associated with diseases, and occurs in infants predomi-

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nantly at the weaning stage, and also in the elderly.

PEM is a spectrum of three disease conditions, which are: Kwashiorkor (protein malnutrition predominant), Marasmus (deficiency in calorie intake), and Marasmic kwashiorkor (marked protein deficiency and marked calorie insufficiency signs present, sometimes referred to as the most severe form of malnutrition) (Franco *et al.*, 1999), distinguishable by the presence or absence of oedema, and some anthropometric indices. PEM accounts for 6 million deaths of both children and adults (WHO, 2002a). Chronic renal disease or cancer cachexia to which PEM may be secondary, is characterized by protein energy wasting [Bosaeus (2008), Muscaritoli *et al.* (2009)].

During the critical period of an infant's growth and development which is the weaning stage in infancy (6 and 24 months), a 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).

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

Cereal-legume-animal supplements mix, possess a great nutritional potential to elicit good biological response, support growth and rehabilitation of protein energy malnutrition subjects (Mosha and Bennink, 2004). Soya bean seeds are a relatively rich source of lysine and tryptophan but are low in sulphur amino acids of which maize protein (though low in lysine and tryptophan) is a good complement because of its possession of fair amounts of sulphur-containing amino acids (methionine and cystine) (Bressani and Elías, 1974). Legume seeds improve nutrient density and nutrient intake qualities of weaning foods, essential to the prevention of protein energy malnutrition (Feyissa, 2009).

Cereals complement legumes in phosphorus content but legumes complement cereals in calcium 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 protein advisory group (PAG) recommends that the dietary protein contents of weaning foods should be at least 20% (dry weight basis)(FAO/WHO, 1971; WHO, 2001, 2002b).

Cholesterol is the most important steroidal component of animal cell membranes, necessary for proper functioning of cells, tissues and all organism [Accad & Farese, 1998]. Total lipids and cholesterol content of

quail bird egg is significantly lower compared with hen egg and guinea fowl egg [Kaźmierska *et al.* (2005), Polat *et al.* (2013)]. Decrease in the rate of myelination of brain and nervous system before and during the weaning period, may be the result of a low cholesterol intake in infancy, and a cholesterol challenge during infancy may facilitate mechanisms responsible for the degradation and ultimate control of blood cholesterol in adulthood (Fomon, 1971).

Egg is rich in methionine and complements diets formulated with soya bean seeds in which methionine is the limiting amino acid. The ratio of yoke to albumen (Y/A) was higher in guinea fowl egg compared with quail egg (Song *et al.*, 2000). Protein Digestibility Corrected Amino Acid Score (PDCAAS) of whole egg is 1 on a scale of 0 to 1. Whole egg exceeds all other protein foods tested in the Amino Acid Score (AAS) rating system, with a score of 1.21 (above human needs). The Protein Efficiency Ratio (PER) of eggs is 3.8 and is the highest among all dietary proteins. Nitrogen Protein Utilization (NPU) of whole egg is 98%, 1% lower than the NPU of whey protein and casein (both at 99%). The Biological Value (BV) of eggs is rated between 88 and 100, with only whey protein rated higher (100)(NC egg Association, 2016).

Egg yolk contains all of the fat in the egg, a little less than half of the protein, approximately, 55 calories, all of the egg's fat-soluble vitamins, and a higher proportion of the egg's water-soluble vitamins than the white, including vitamins B6 and B₁₂, folic acid, pantothenic acid and thiamin. Egg yolks are one of the few foods naturally containing vitamin D. Calcium, copper, iron, manganese, phosphorus, selenium and zinc content of the yolk are higher than those of the white. Albumen accounts for most of an egg's liquid weight, about 66%. Majority of the egg's niacin, riboflavin, magnesium, potassium and sodium are found in the egg white, and none of the fat. The white of a large egg contains about 17 calories (NC egg Association, 2016).

Vitamins A and B2 content of chicken egg is half that content of quail egg. Quail's eggs were easily the most powerful inhibitor of human trypsin, which plays a major role in the allergic reaction. Quail's eggs contain enzyme inhibitors other than ovomucoid (a fraction of the albumen): ovoidinhibitors, glycoproteins which are also natural de-inhibitors of serine proteases and have a powerful effect on trypsin. Ovomuroid in quail's eggs has a powerful activity on the elastase that acts in a large number of human pathologies, in particular in pulmonary emphysema and psoriasis. Quail egg is the animal product of the most balanced protein content, vitamin – mineral and enzyme able to regulate all these deficiencies, bringing back to normal any human body. Quail eggs: have a positive effect in treating kidney disease, liver and bile, ophthalmic and ENT; are valuable in the growth and development of children;

revitalize the body and prolong life regardless of age; resolve anemia, spasmodophilia, nervous headaches and fatigue; are an excellent anabolic hormone and metabolic regulator with a wide action; restore body and regulates blood sugar levels in diabetes, helps a lot in cases of sexual impotence, asthma, tuberculosis, hypercholesterolemia, allergic rhinitis, allergies and eczema, and strengthens the immune system and regulate the weight and growth disorders (Takahashi *et al.*, 1994).

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 a collective antioxidant biochemical role essential to the inactivation of free radicals in human infants (Obimba *et al.*, 2015).

The nutritional efficiency of traditional weaning formulae is a function of the method(s) employed in the processing of diet components (Akaninwor and Okechukwu, 2004). A significant increase ($p < 0.05$) in some essential amino acids, nutritious minerals and vitamins contents were observed of germinated sorghum and steam-cooked cowpea seeds that were used in the formulation of traditional weaning formulae in which there was increased caloric and nutrient density (Elemo *et al.*, 2011). High values of nutritional and gross energy characterized weaning food prepared from autoclaved, malted cowpea and malted barley flour with true digestibility (TD%) and Biological value (BV) of 87.6% and 90.23%, respectively (Ishfaq *et al.*, 2014). Heat treatment though innocuous in effect to legume proteins, effectively removes antinutritional factors (Kon and Sanshuck, 1981).

Annan and Plahar (1995) prepared the FRI weaning formula from processed : soya bean seeds, groundnut seeds, maize grain and milk, and could be used to improve the nutritional status of children and alleviate protein energy malnutrition. Rapid catch-up growth rates were effected in kwashiorkor-induced rodent models using processed soya bean seeds-groundnut seed-maize grains-catfish (SGMC), and soya bean seeds-maize grains-crayfish (SMC) blend of high quality weaning formulae (Obimba, 2012).

Mean values of red blood cells, white blood cells and packed cell volume were numerically, but not significantly higher ($p < 0.05$), 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).

Significant reduction ($p < 0.05$) was observed of the mean values of the aspartate amino transferase (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, compared with those of the reference weaning formulae

groups: nutrend and soya bean seeds- groundnut seeds-maize grains-milk (Obimba, 2011).

Processed cowpea seeds-soya bean seeds-maize grains-crayfish blend of weaning formulae prepared at 20% dietary protein had significantly higher ($p < 0.05$) mean values of net protein utilization (NPU%), biological value (BV), true digestibility (TD%) and protein efficiency ratio (PER) in comparison with the reference diet (nutrend) (Obimba *et al.*, 2015).

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. For this reason among others, the findings in the present study could be extrapolated to the human physiologic conditions.

The aim of the work is to compare the nutritional efficiencies/qualities 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 50 observations per parameter (except NPU%, TD%, and BV, each of which 40 observations were made).

The Linear model is $Y_{ij} = \mu + T_i + e_{ij}$.

Y_{ij} = Individual observations

μ = Overall mean

T_i = Effect of i th level of dietary protein treatment

e_{ij} = 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 were $p < 0.01$, $p < 0.05$.

Processing of diet components

Two hundred g (200 g), each of raw soya bean seeds, and raw maize grains, were washed and soaked, separately, in a liter of water, for 11 hrs, and thereafter, boiled in 800 ml of water, for 2 hrs. Boiled soya bean seeds were dehulled. The samples were dried in the oven for 9 hrs at 105°C, ground and dried for a further 4 hrs, at 105°C. Whole egg was boiled in water for 15 minutes and mashed after the removal of the shell. Albumen (3 parts by weight) was mixed thoroughly with yolk (1 part by weight), and dried for 20 minutes at 105°C (fresh preparations were administered, daily). Fluted pumpkin vegetable leaves were washed in warm water and dried in the oven for 10 minutes at 105°C and

ground. The schematic for diet formulation is shown in Table 1.

Animal feeding trial was conducted in compliance with the guidelines for ethical conduct in the care and use of non-human animals in research (APA, 2010). Fifty male (weanling, 5 weeks old) wistar albino rats were divided into five 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 four different types of weaning diets [soya bean seeds- maize grain-quail egg (SMQ-20%), soya bean seeds- maize grain-guinea fowl egg (SMG-20%), soya bean seeds- maize grain-domestic poultry hen egg (SMD-20%) and Rd (nutrend)], and a basal diet (Bd) (hypothetical protein-free) for a period of 28 days, after 3 days of acclimatization

Daily faecal deposits of the animals were collected during the 28-day period of the feeding trial, pooled, oven dried, and weighed. Euthanizing agents could cause death of experimental rodent model by physical disruption of brain activity which can be produced through physical disruption of brain activity: a blow to the skull resulting in concussive stunning; through direct destruction of the brain with a captive bolt, bullet, or pithing rod (AVMA, 2007). The experimental animals were weighed and sacrificed by a sharp tap on the head with a blunt instrument. Blood collection was carried out via the saphenous vein (Hoff, 2000). 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 carcass were dried for 17 h, in an oven drier at 105°C and stored. 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.

White Blood Cell total (WBC_{Total}) 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 = $\frac{PI - [FP - MFP]}{PI} \times 100$ 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 Util-

ization [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)

$BV = (Nr/Na) \times 100$ Na = nitrogen absorbed in proteins on the test diet (NPU%) Nr = 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 Brozka *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

Shown in Table 2 are the results on the mean values of growth performance (g) of the test and reference weaning formulae, and basal diet group which differed in order of consecutive significant decrease ($p < 0.05$) as follows : SMQ, SMG/SMD, Rd, Basal diet groups. Results on the mean values of the biochemical indices: serum total cholesterol, serum triglyceride, serum albumin, serum aspartate amino transferase (AST) and WBC_{total} of the test and reference weaning formulae, and the basal diet group are shown in Table 3, and are listed in order of sequential significant increase ($p < 0.05$) as follows : serum total cholesterol : SMQ, SMG, Bd/Rd, SMD diet groups; serum triglyceride: Rd, SMD/SMQ/SMG, Bd diet groups; serum albumin: Bd, Rd, SMG/SMD/SMQ diet groups; serum aspartate amino transferase (AST): SMQ/SMG/SMD, Rd, Basal diet groups; WBC_{total} : Bd, Rd, SMD, SMG, SMQ 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 : SMQ/SMG/SMD, Rd 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, SMQ/SMG/SMD had the significantly highest ($p < 0.05$) mean values of PCV% followed by 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 : SMQ/SMG/SMD, 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 cells, and hemorrhage. Plate 2 is the photomicrograph of the lobe section of the liver tissue of a wistar albino rat administered with the SMQ 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 : SMQ, SMG/SMD, Rd, Basal diet groups and is corroborated by the findings of Obimba *et al.* (2011), who recorded significantly higher mean values ($p < 0.05$) of growth performance of rats administered with processed soya bean seeds-groundnut seeds-maize grains-catfish (SGMC 20%) weaning formula blend and treated river water in comparison with the diet group fed on nutrend weaning formula.

The SMQ, SMG, and SMD diet groups had significantly higher mean values ($p < 0.05$) of PCV% (figure 1) and WBC total (Table 3) compared with 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 diet group (Ijarotimi, 2008). PCV% could be used as a haematological diagnostic index of anemia in protein energy malnutrition (Siddiqui *et al.*, 2007). The administration of SMQ, SMG, and SMD diet groups to infants would prevent anemia.

The SMQ, SMG, and SMD diet groups had the significantly highest mean values ($p < 0.05$) of serum albumin (Table 3), and is consistent with the observation that SGMC 20% weaning formula blend diet group had a significantly higher ($p < 0.05$) mean value of serum albumin in comparison with the control, nutrend commercial weaning formula diet group (Obimba, 2011). SGMC 20% was used as dietary therapy to effect rapid catch-

Table 1. Diet Formulation. g/100g diet (%)

S/N	Components	Basal (-ve control)	SMQ	SMG	SMD	*Rd (+ve control)
1.	Quail egg (processed)	-	50.08	-	-	
2.	Guinea fowl egg (processed)	-	-	47	-	
3.	Domestic poultry hen egg (processed)	-	-	-	47.6	
4.	Soya bean seed (flour)	-	36.8	36.8	36.8	
5.	Maize seed (flour)	-	5.0	5.0	5.0	
6.	Vegetable (fluted pumpkin leaves).	-	3.00	3.00	3.00	
7.	Palm oil.	-	4.00	4.00	4.00	
8.	Multivit-multimineral (centrum™)	-	0.5	0.5	0.5	
9.	Garri (<i>Manihot esculenta</i>)	100	0.62	3.7	3.1	

*Reference diet (Rd)(+ve control): 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. SMQ (20%): 20.98% dietary protein, 55.2% carbohydrates, 15.2% lipids, 4.1% moisture, 3.1% minerals, vitamins \leq 1.42 g, 457.1 kcal/100g. SMG (20%): 20.2% dietary protein, 56% carbohydrates, 15.8% lipids, 4.3% moisture, 3.0% minerals, vitamins \leq 0.9 g, 453.2 kcal/100g. SMD (20%): 20.5% dietary protein, 54.99% carbohydrates, 15.5% lipids, 4.2% moisture, 3.05% minerals, vitamins \leq 1.31 g, 457.1 kcal/100g.

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

ΔLive weight (g)	
SMQ	130 \pm 0.2 ^a
SMG	126 \pm 0.2 ^b
SMD	125 \pm 0.1 ^b
Rd	85.1 \pm 2.1 ^c
Basal	-25.1 \pm 1.02 ^d

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

up growth rates in kwashiorkor-induced rats (Obimba, 2012). Protein energy malnutrition decreases markedly, serum albumin and total serum proteins (Siddiqui *et al.*, 2007). SMQ, SMG, and SMD have a good potential to increase serum albumin concentration in human infants. Increased serum aspartate amino transferase (AST) activity (Table 3) observed of basal diet group of animals indicates the important role of free radicals in the aetiopathogenesis of kwashiorkor (Etukudo *et al.*, 1999). The SMQ, SMG, and SMD diet group had the lowest mean values of AST (μ l) and thus have the potential to decrease oxidative stress as antagonists of free radicals production.

Significantly lower serum cholesterol concentrations were observed of infants fed commercially prepared formulae compared with those fed on breast milk (Friedman and Goldberg, 1976). The lowest mean value of serum total cholesterol was recorded of the

SMQ diet group (Table 3): Quail eggs contain good fat/cholesterol because they have a high HDL cholesterol content. Furthermore, the high quality weaning formulae contain significant amounts of the phytosterol, β -sitosterol, sourced from their plant components (soya bean seeds, maize grains, fluted pumpkin leaves, palm oil). β -sitosterol, a structural analogue of cholesterol, mimics the regulatory actions of cholesterol thus inhibiting the metabolism of cholesterol to a large extent by inhibiting the absorption of cholesterol in the intestine (Matsuoka *et al.*, 2008). This could account for the moderate serum total cholesterol content of these diet groups.

SMQ/SMG/SMD, Rd diet groups 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

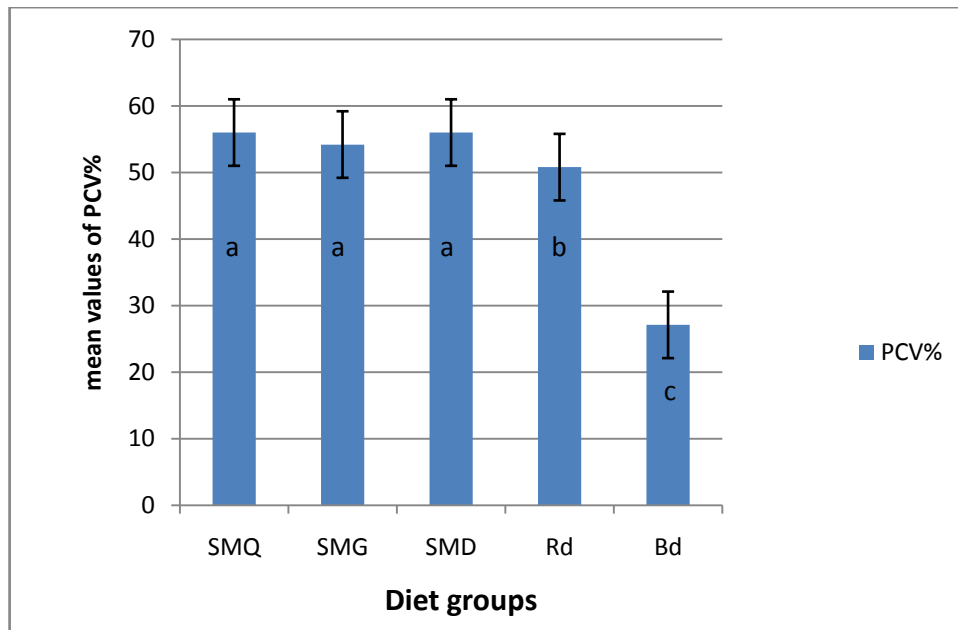


Figure 1. Graphical results on the packed cell volume (PCV%) of the test and reference weaning formulae, and the basal diet group. Statistical results are expressed as mean \pm standard error (%) ($n = 10$). Error bars represent values of standard error (0.2 – 1.2%). Bars labeled with the same letters represent mean values of PCV% which are not significantly different ($p < 0.05$).

consonance with the postulates of Mosha and Bennink (2004).

The basal diet group of experimental animals suffered the most significant decrease ($p < 0.05$) in mean values of the growth performance, hematological, biochemical and performance characteristic [PER ($p < 0.01$)] indices measured except the AST enzyme activity and serum triglyceride of which it had the most significant increase ($p < 0.05$): Degenerative changes had occurred in the basal diet group.

Liver steatosis and fibrosis, culminating in cirrhosis, which is associated with necrotic hepatocytes, and hemorrhage was the result of the administration of a 6% hypoproteic to wistar albino rats. This isocaloric diet induced protein energy malnutrition in the rats (Conde *et al.*, 1993). Groups of experimental rodent models fed on 15.5% dietary protein formulated cerelac and 20% dietary protein formulated weaning formula had normal histopathological features but those of a 10% dietary protein weaning formulae showed cytoplasmic granulation and vacuolation (Mahgoub, 1999). 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, and necrotic cells, as shown in Photomicrograph/Plate 1 (compare with Plate 2).

The mean values of the relative liver organ weight ratio indicate that the potential of the SMQ/SMG/SMD weaning formulae to effect proportionate increases in the body weight (gram) and liver weight (gram) of the experimental group of animals is approximately equal (as shown by the co-incident lines in figure 2), but significantly higher ($p < 0.05$) than that potential of the nutrend (Rd) weaning formulae.

Multiple regression studies revealed that TD% regressed significantly ($p < 0.05$) with PCV%, and serum albumin (g/dl) of the SMQ, SMG, and SMD diet groups. The regression and correlation statistical analysis between TD% and serum albumin (g/dl) of the SMQ, SMG, and SMD diet groups were significant ($p < 0.05$) with Pearson's product moment correlation coefficients of 0.985, 0.980 and 0.972, respectively. The TD% could be predicted from the regression curves: $\hat{Y} = 7.36x_i + 57(\%)$:SMQ, $\hat{Y} = 7.0x_i + 59.8(\%)$:SMG, $\hat{Y} = 7.1x_i + 59.37(\%)$:SMD, using observed values (x_i) of serum albumin (g/dl) (figure 3).

Initially, the lower protein (16% dietary protein) diet (Rd: Nutrend) effected a slightly more rapid increase in TD% per serum albumin concentration (g/dl), up to a certain high, 88.1% of TD% and 3.75 g/dl of serum albumin as shown by the gradient or slope of the regression curve (figure 3) which is slightly more steep than those of the SMQ, SMG, and SMD high quality weaning formulae.

Table 3. Results on the biochemical indices: Serum total 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 total cholesterol (mg/dl)	Serum triglyceride (mg/dl)	Serum albumin (mg/dl)	Serum aspartate aminotransferase (U/l)	WBC _{total} (mm ³)
SMQ	115 ± 0.01 ^a	75.5 ± 0.04 ^a	5.50 ± 0.1 ^a	9.05 ± 0.16 ^a	5205 ± 0.15 ^a
SMG	120 ± 0.01 ^b	76.5 ± 0.04 ^a	5.2 ± 0.2 ^a	9.15 ± 0.16 ^a	5200 ± 0.15 ^b
SMD	128.5 ± 0.01 ^c	75 ± 0.04 ^a	5.3 ± 0.1 ^a	9.25 ± 0.16 ^a	5185 ± 0.10 ^c
Rd	124.5 ± 0.02 ^d	65 ± 0.02 ^b	3.75 ± 0.2 ^b	15.5 ± 0.15 ^b	5108 ± 0.14 ^d
Basal	124 ± 0.02 ^d	80 ± 0.01 ^c	1.58 ± 0.1 ^c	42.1 ± 0.16 ^c	2754 ± 2 ^e

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 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
SMQ	98.2 ± 1.0 ^a	97.5 ± 2.0 ^a	98.0 ± 1.0 ^a	3.85 ± 0.16 ^a
SMG	97.2 ± 1.0 ^a	96.2 ± 1.0 ^a	96.2 ± 1.1 ^a	3.65 ± 0.10 ^a
SMD	97.5 ± 1.0 ^a	97.0 ± 1.0 ^a	96.0 ± 1.0 ^a	3.75 ± 0.16 ^a
Rd	87.1 ± 2.1 ^b	88.1 ± 1.1 ^b	90.5 ± 1.1 ^b	2.61 ± 0.15 ^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<0.01)*, (p<0.05).

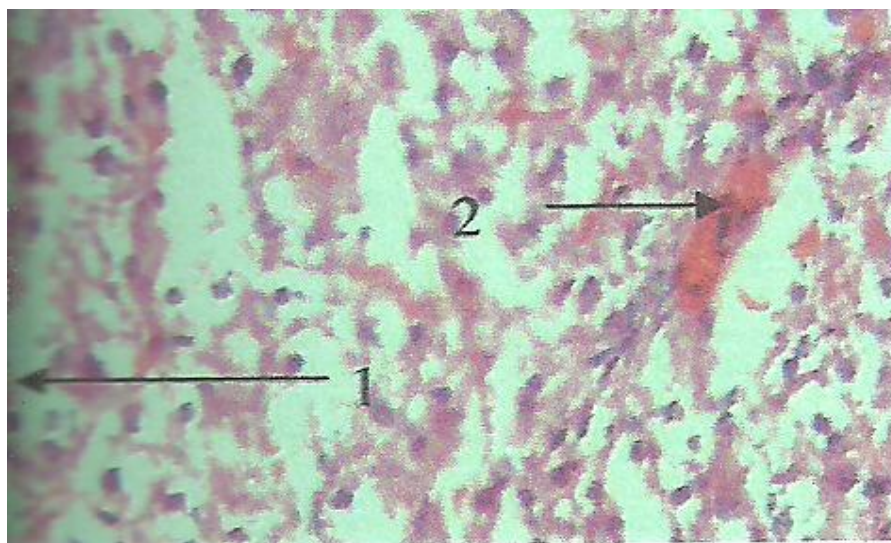


Plate 1. Photomicrograph of lobe section of the liver tissue of a wistar albino rat, administered basal diet. showing degenerative changes: 1. Necrotic and atrophic cells (sparsely distributed black dots). 2. Hemorrhage.

Beyond the values: 88.1% of TD% and 3.75 g/dl of serum albumin, the nutrend diet ceased to effect further

increase in both TD% and serum albumin: an indication that the reference diet, nutrend, possesses the potential

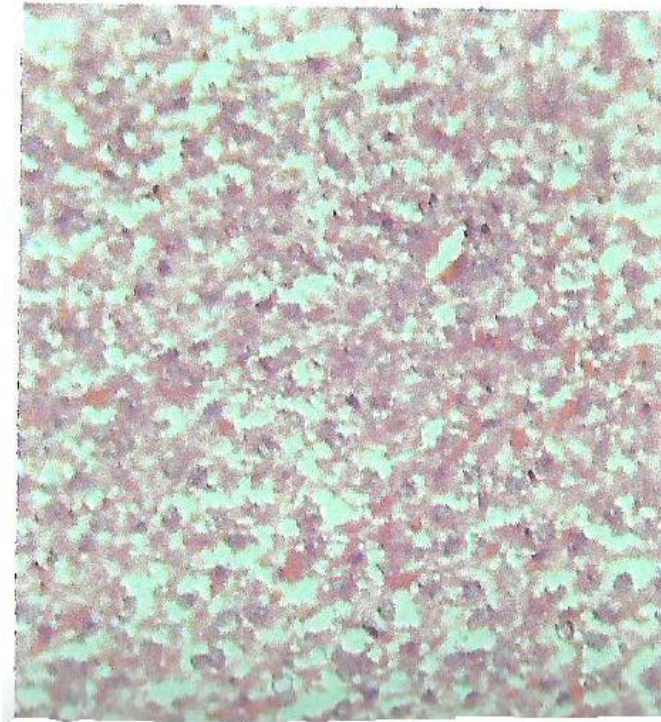


Plate 2. Photomicrograph of lobe section of the liver tissue of a wistar albino rat fed on SMG weaning formula showing normal tissue.

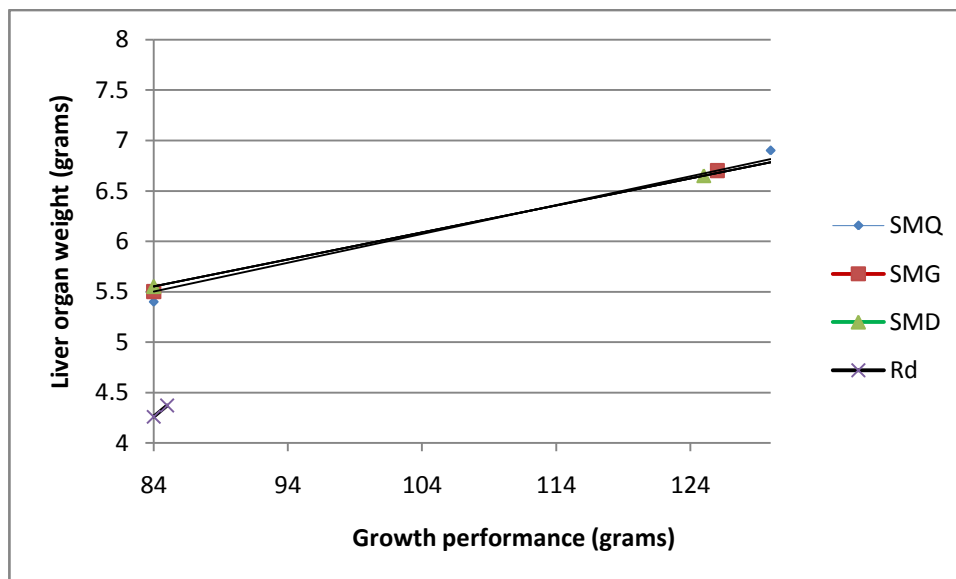


Figure 2. Relative Liver organ weight ratio of the test and reference weaning formulae diet groups. Significant differences observed of the relative liver organ weight ratios were as follows: SMQ (0.0531 ± 0.0001^a)/ SMG (0.0532 ± 0.0001^a)/ SMD (0.0532 ± 0.0001^a) > Nutrend (Rd) (0.0514 ± 0.0001^b). Values in the inequality equation/expression are mean \pm S.E (n = 10). Means having the same superscript are not significantly different ($p < 0.05$).

for adequate supply of the essential amino acid to the weanling animals, only during the initial period of the

feeding trial. Whereas, it is evident from the same figure (figure 3) that the high quality weaning formulae were of

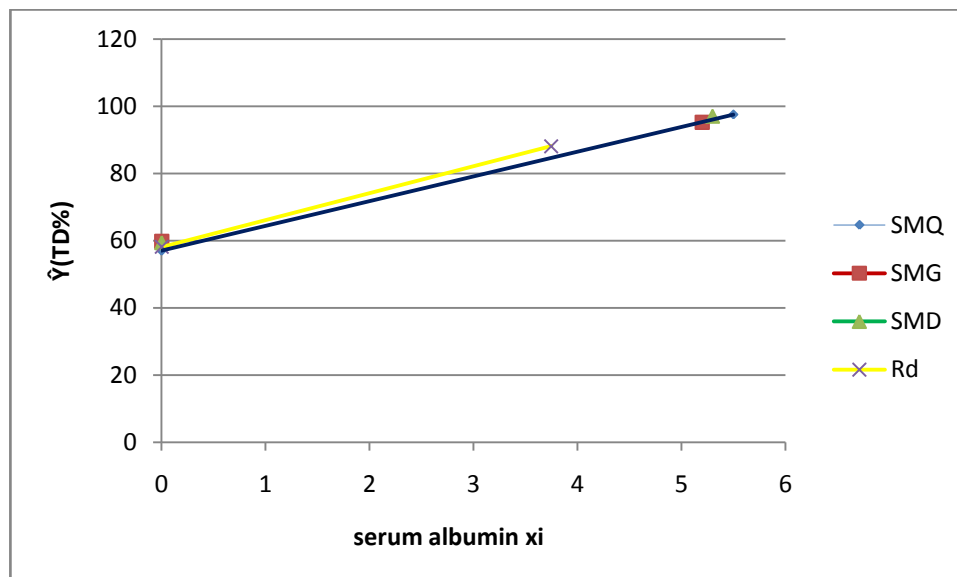


Figure 3. Regression curve of TD% and serum albumin (g/dl) of the high quality weaning formulae and reference diet (nutrend): $\hat{Y} = 7.36x_i + 57(\%)$:SMQ, $\hat{Y} = 7.0x_i + 59.8(\%)$:SMG, $\hat{Y} = 7.1x_i + 59.37(\%)$:SMD, $\hat{Y} = 8x_i + 58.1(\%)$:Rd.

better nutritional value given their nutritional capacity to effect significant increase in TD% ($p < 0.05$), and serum albumin ($p < 0.01$) beyond values of 88.1% and 3.75 g/dl, reaching values of 97.5% and 5.5g/dl, respectively, consistent with the observation that the SMQ, SMG, and SMD diet groups outgrew the former (nutrend diet group) with a large and significant ($p < 0.05$) margin of growth performance (g). The more digestible, dietary protein is (a function of high value of true digestibility), the more efficient it is in building up body proteins (a function of high value of serum albumin).

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). SMQ, SMG, and SMD high quality weaning formulae possesses the most potential to prevent kwashiorkor and effect rapid catch-up growth rates in protein energy malnutrition subjects.

CONCLUSION

SMQ (14.00 g), SMG (15.00 g), SMD (15.05 g), or Rd (Nutrend) (25g)/4.0 g of dietary protein/(solubilized in 100 ml of water)/100 kcal/kg body weight, 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 quality/efficacy of the various weaning formulae, listed in sequential order of

significant ($p < 0.05$) decrease is as follows: SMQ/SMG/SMD > Rd (nutrend).

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