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Full Length Research Paper

Utilization of dietary therapies in the alleviation of protein energy malnutrition in kwashiokor-induced rats

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The utilization of diets prepared from available and affordable plants (soya bean, groundnut, maize, and fluted pumpkin green leaves) and animal sources (milk, catfish, and crayfish), in the alleviation of protein energy malnutrition (PEM) was studied, to determine comparative dietary efficiencies of some fortified weaning formulae. The therapeutic diets used in alleviating PEM in the kwashiorkor-induced rats were the (SGMC) (prepared at 20% dietary protein level), (SMC) (prepared at 20% dietary protein), (SGMM) (prepared at 16% dietary protein level), and Rd (reference diet, Nutrend : Nestle ®, 16% protein level) diets. The experimental design for the treatment of kwashiorkor was a Completely Randomized Design (CRD). Feeding of the kwashiokor-induced rats with the test diets caused a reversal of the earlier observed decreases in the parameters (growth performance, serum albumin, pack cell volume, NPU %) measured, in order of consecutive significant increase ($p<0.05$) as follows: SGMM< Rd<SGMC/SMC. Results were recorded as values of mean ± S.D unit, of the test dietary therapy groups, SGMC/SMC, as follows : growth performance : (90.4 ± 0.5/87.0 ± 2.84 grams), serum albumin : (4.11 ± 0.31/4.09 ± 0.61g/l), pack cell volume (PCV%) : (49.05 ± 0.31/50.01 ± 0.21%), aspartate aminotransferase (AST) : (11.1 ± 0.05/10.95 ± 0.05U/l) and net protein utilization (NPU%) : (93.0± 1.96/95.0 ± 0.01%), respectively. SGMC and SMC alleviation diets, elicited significant regression at 5% level, of dietary group, growth performance (grams) and corresponding Net Protein Utilization % values of the weaning formulae, and achieved the most rapid catch-up growth rates in the kwashiorkor-induced rats. SGMC and SMC test diets are effectively, preventive and curative of protein energy malnutrition.

Key words: Protein energy malnutrition, kwashiokor, therapeutic diets, weaning formulae, experimental design.

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). Protein-energy undernutrition (PEU), previously called protein-energy malnutrition, is an energy deficit due to chronic deficiency of all macronutrients (Morley, 2007). PEU can be sudden and total (starvation) or gradual. Severity ranges from subclinical deficiencies to obvious wasting (with edema, hair loss, and skin atrophy) to starvation. Multiple organ systems are often impaired. Diagnosis usually involves laboratory testing, including serum albumin. Treatment consists of correcting fluid and electrolyte deficits with intra-venous solutions, then gradually replenishing nutrients, orally if possible (Morley, 2007).

Etukudo et al. (1999) described the broad spectrum nature of protein energy malnutrition, which ranges from marasmus through marasmic kwashiorkor to kwashiorkor, and is characterized by low weight for age, oedema, dermatitis, hair changes, mental changes, hepatomegaly and diarrhea. The Wellcome 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).

Nutritional status assessment methods used in detecting and monitoring recovery of patients suffering from PEM, include: clinical methods, morphological methods, haematological and biochemical methods,

dietary survey, and anthropometry. Serum albumin and total serum proteins are markedly decreased during protein energy malnutrition (Siddiqui et al., 2007).

A major factor among the dietary causes of PEM, is deficiency in energy intake, kwashiorkor patients have slightly reduced fasting blood sugar and reduced glucose tolerance (Becker, 1983). 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). Hormonal changes, especially in insulin and thyroid hormone secretions, occur in PEM subjects (Abrol et al., 2001). Kwashiorkor subjects have a 65-80% increase in total body water. Protein energy malnutrition results in anemia, detectable, using PCV% and Hb (haemoglobin) count haematological diagnostic tool (Siddiqui et al., 2007). Infections associated with PEM, are largely due to decrease in efficiency of patients immune system, hence the use of the WBC $_{Total}$ (white blood cell) count as a diagnostic tool of PEM (Huang and Fraker, 2003).

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 form kwashiorkor (Etukudo et al., 1999).

The recommended daily allowance for dietary protein is based on the requirement that dietary protein, be provided by a mixture of both animal and plant proteins. The addition of 20 to 30% of animal protein to a 7:3 combination of cereal to legume seed meal, increases, ultimately the nutritive value of the food, and is consistent with the Protein Advisory Group guidelines for weaning foods, which states that dietary protein content of weaning foods, should be at least 20% (on a dry weight basis) (FAO/WHO, 1971). The relevance of wistar albino rats in the measurement of the nutritional quality of dietary protein, as a means of scientific investigations, correlate to the human physiological condition, is founded on the fact that wistar albino rats have a dietary requirement for the same ten (10) essential amino acids as human infants. Variations in performance characteristics could occur, as a result of disease conditions such as protein energy malnutrition (Obimba, 2006). Performance characteristics of dietary protein (Tome and Cecile, 2000), measured in net protein utilization (NPU%) was used in determining the qualities of the diets. The objective of this work is to utilize diets prepared from available and affordable plants and animal sources, in the alleviation of protein energy malnutrition, and also to compare the dietary efficiencies of some dietary therapies/fortified weaning formulae.

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 degree of freedom of error. The Linear model is:

$$Yij = \mu + T_i + e_{ij} .$$

where: 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.

About 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 hours at 105°C, and ground. Moisture-free crayfish, dried in industrial ovens, were ground. Fluted pumpkin vegetable leaves were washed in warm water, and dried in the oven for 1 h, and ground. Table 1 is a schematic for the diet formulation

23 weanling Wister 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. The faecal nitrogen content and the carcass nitrogen content of the experimental animals were determined using the Kjeldahl method (AOAC, 1990).

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 bio-chemical 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).

Kjeldahl Method (AOAC, 1990) was employed for the quantitative determination of nitrogen and crude protein.

The Spun microhaematocrit method of Bull and Hay (2001) was used for the determination of packed cell volume (PCV%).

Quantitative *in vitro* determination of albumin in serum was carried out using the method employed by Qureshi and Qureshi (2001).

Quantitative *in vitro* determination of serum aspartate amino transferase was carried out using the method employed by Pratt and Kaplan (2000).

Performance characteristics analysis of Net Protein Utilization (NPU%) was carried out using the method employed by Pellet and

Table 1. Diet formulation.

Dietcomponents (g/100g diet)	Kd/basal3.47% dietary protein level)	SGMC(20% dietary protein level)	SMC (20% dietary protein level)	SGMM (16% 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 (sourced from *manihot esculenta*)	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.

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	$16.0^{b} \pm 0.05$
Bd/Kd	$4.38^{c} \pm 0.0$

Values are means ± S.D (n = 3).Means in the same column having the same superscripts are not significantly different at 5% level ($p<0.05$).

Young (1980).

Student's t-test, ANOVA (analysis of variance) and regression statistical analytical methods were used in analyzing results.

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 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, PCV%, and the biochemical parameter, serum albumin (g/dl), measured of the weaning formulae diet groups of experimental animals, listed in sequential order of decrease were as follows: SGMC> Rd > SGMM. The mean values of the

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 = 3).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
PCV (%)	38.08^{a}±0.01	49.05^{b} ±0.05	50.01^{b} ±0.01	36.21^{c} ±0.02	28.14^{d} ±0.03
Serum albumin (g/dl)	3.71^{a} ±0.01	4.11^{b} ±0.01	4.09^{b}±0.02	3.62^{c} ±0.03	1.75^{d} ±0.01
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 = 3). Means in the same row having the same superscripts are not significantly different at 5% level (p<0.05).

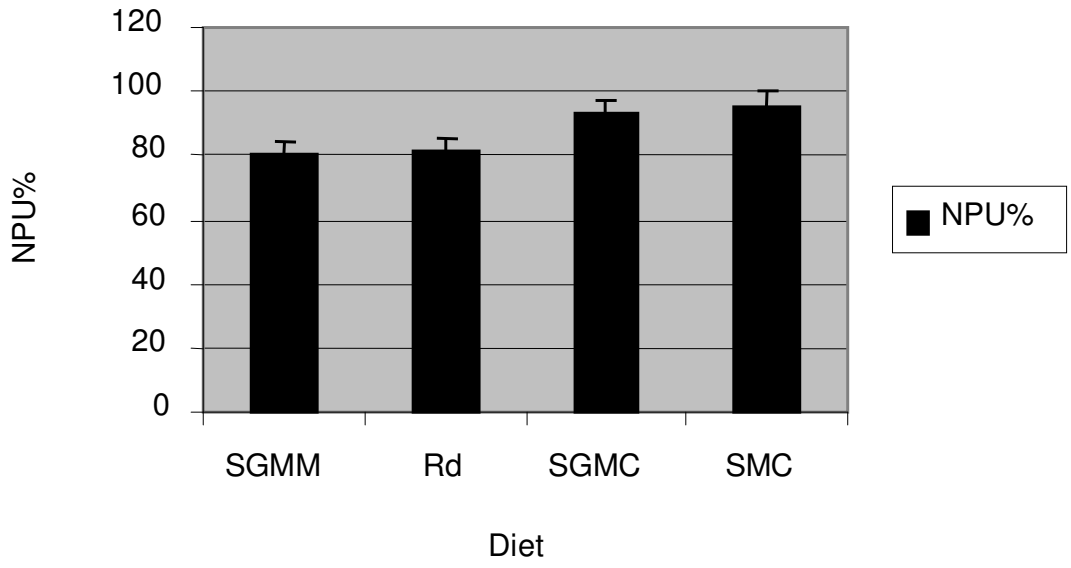

Figure 1.Net protein utilization (NPU%) of dietary therapies/weaning formulae.

PCV%, and the serum albumin of each of the SGMC and SMC diet groups of experimental animals were not significantly different (p<0.05). 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 PCV%, and serum albumin (g/dl), and the most significant increase (p<0.05) in AST enzyme activity.

Figure 1 shows the quantitative values of the performance characteristic, net protein utilization (NPU%) of the weaning formulae and basal diet. The NPU% was used in assessing the nutritional efficiency and quality of the dietary therapies/weaning formulae. The mean values of the NPU% of the weaning formulae listed in sequence of significant decrease (p<0.05) were as follows: SGMC/SMC>Rd /SGMM.

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).

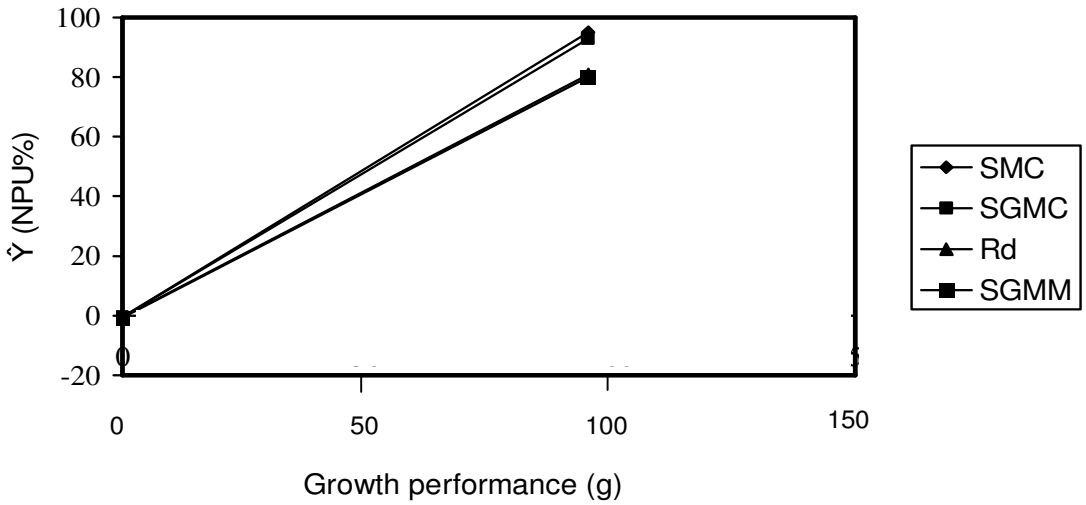

Figure 2. Regression curves of (growth performance [g]) and NPU%.

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 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.

Dietary therapy for kwashiorkor has two primary aims which are:

(a). To reduce mortality to a minimum, achieved, partly, by controlling infections which frequently accompany PEM, with a view to affecting the most rapid recovery possible.

(b). To use therapeutic diets of PEM, which are relatively high in protein, and energy, with special emphasis placed on fluid intake, with a view to avoiding electrolyte imbalance.

The significant increases ($p<0.05$) in growth performance, PCV%, serum albumin, and AST activity shown in Tables 3 and 4, recorded the SGMC and SMC diet group of experimental animals, which correspond with the findings of Etukudo et al. (1999), Obatolu et al. (2003). The efficiency of the SGMC and SMC weaning formulae in effecting rehabilitation and promoting good biological responses is consistent with the research findings of Mosha and Bennink (2004), Obatolu et al. (2003). Comparative analysis of the present study and the work of Annan and Plahar (1995), shows that protein malnutrition reduces significantly ($p<0.05$), the performance characteristics (NPU%) of therapeutic diets. In keeping with the observations made of the performance characteristics of the dietary therapies/weaning formulae, used in the present study, shown in Figure 1 is the evidence that cereal-legume mix and animal supplements possess a great nutritional potential to support growth and rehabilitation of protein energy malnutrition subjects (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 regression between growth performance (grams) of the SGMC and SMC diet groups of experimental animals and their corresponding NPU% values of the weaning formulae were significant at 5% level ($p<0.05$), with Pearson's product moment correlation coefficient of 0.96 and 0.97, respectively. These figures indicate that the growth performance of the SGMC and SMC diet groups of experimental animals were a function of the respective dietary protein qualities of the SGMC and SMC weaning formulae. The regression curves are shown in Figure 2.

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).

Conclusion

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.

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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); **PCV,**pack cell volume; **AST**, aspartate amino transferase.

REFERENCES

Abrol P, Verma A, Hooda HS (2001). Thyroid hormone status in protein energy malnutrition in Indian children. Indian J. Clin. Biochem. 16 (2): 221-223.

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.

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

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.

Becker DJ (1983). The Endocrine Responses to Protein Calorie Malnutrition. Ann. Rev. Nutr. 3: 187-212.

Bull SB, Hay KL (2001). Is the Packed Cell Volume (PCV) Reliable? Lab. Hem. Carden Jennings Publishing Co. Ltd. 7: 191–196.

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.

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): 43-7.

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.

Grover Z, Ee LC (2009). Protein energy malnutrition. Pediatr. Clin. North. Am. 56(5): 1055-68.

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. Physio. 2: 17-19.

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.

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 : 145 pages.

Pellet PL, Young VR (1980). Evaluation of Protein Quality in Experimental Animals. In: Nutritional Evaluation of Protein Foods. The United Nations University, Food Nutr. Bull. Supp. 4: 41-57.

Pratt DS, Kaplan MM (2000). Evaluation of abnormal liver-enzyme results in asymptomatic patients. N. Engl. J. Med. 342(17): 1266-1271.

Qureshi MI, Qureshi Z (2001). Effect of Protein Malnutrition on the Weight and Serum Albumin of Albino Rats. J. Ayub. Med. Coll. Abottabad. 13: 8-10.

Roulet M (1994). Protein-energy malnutrition in cystic fibrosis patients. Acta Paed. 83 (395): 43-81.

Siddiqui AU, Halim A, Hussain T (2007). Nutritional Profile And Inflammatory Status Of Stable Chronic Hemodialysis Patients At Nephrology Department, Military Hospital Rawalpindi. J. Ayub Med. Coll. Abbottabad. 19(4): 29-31.

Tome D, Cecile B (2000). Dietary Protein and Nitrogen Utilization. J. Nutr. 130: 1868S-1873S.