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Efficiency Ratios of Protein and Selected Dietary Elements of A Locally Formulated Complementary Food In Male Wistar-Strain Weaning Rats

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Abstract: *Breast milk does not provide adequate amount of nutrient for infant beyond six month of age. Malnutrition during infancy, an endemic public health nutritional challenge in Africa at large and Nigeria in particular, presents proportionate health challenges during adulthood. Such nutritional challenges do not only arise from inadequate intake of food nutrients, but also poor absorption that lead poor efficiency ratio. Therefore, the amount of selected dietary elements obtained from a legume-cereal based complementary food required to bring about a unit growth was studied in 45 Male Wistar-strain weaning rats weighing 40 - 55g. The result showed that CF has a significantly higher PER when compared with a proprietary complementary formula. N, P, K, Na, Ca, Fe, Cu, Mg, Mn, Zn obtained from CF are of high efficiency ratio. Also, it was observed that the animals placed on CF showed higher weight gain than those placed on BF. This suggests that locally sourced foodstuff of the kinds used in this study could be used to formulated complementary food with high PER and appreciable MER that could also support healthy growth and development in infants.*

Keywords*: Mineral efficiency Ratio, Protein Efficiency Ratio, Complementary Food, Bioavailability, Dietary Element*

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I. Introduction

Healthy nutrition during infancy has been asserted to be critical to healthy system function, growth and development in adulthood. Healthy complementary nutrition is, therefore, encouraged since from six months of age, infants are predisposed to malnutrition and its concomitant downstream effects. It has been demonstrated that breast milk alone can no longer adequately provide for the total nutritional requirements of infant after six months of age; hence, the need for complementary feeding afterwards. Complementary foods have been shown to exhibit less significant nutritional value compared to breast milk and deficit amounts are given in most cases (WHO, 2001). This suggests that such deficit could be complemented by the breast milk; hence, the term ‘complementary food’. Over 50% of all children below the age of 5 (in Nigeria) have been reported to be malnourished and exhibit stunted growth. Stunting has been reported to blight neurological development, immunological vigour and proportionate ‘height to age’ ratio. Also, in Nigeria, about one million death cases was reported for children (under the age of 5) each year and malnutrition accounts for 35% of those deaths (DHS, 2008; Federal Ministry of Health, 2011).

Nutritional needs will only be met at this age if deliberate and sufficient attention is given to young children. Hence, complementary feeding is an essential element of care giving at infancy. Complementary feeding ought to be micronutrient-rich, timely, adequate, safe, fed properly and counseling based (Santos *et a*l., 2001).

Between birth and weaning period, the infant’s body synthesizes protein over three times more than the protein consumed. This is as a result of the fact that approximately 90% of the protein consumed is deployed for tissue synthesis, which is crucial for development and growth and key for healthy adulthood (Young *et al*., 1991; Dupont, 2003). Deficiency of iron has been reported to be the most prevalent dietary element deficiency among children in the world. Iron is essential for adequate development of hippocampus (learning and memory site) and cognitive function (Georgieff, 2008; Fretham *et al*., 2011).

Studies have reported that in extended lactation, calcium percentage composition decreases in breast milk (Fomon and Nelson, 1993; Koo and Warren, 2003). Calcium deficiency impairs skeleton growth and leads to low bone mass; which can alter growth, with increased risk of fracture in infancy (Goulding *et al*., 2000; Goulding *et al*., 2001), osteopenia and ricketism (Koo and Steichen, 1998; Thacher *et al*., 1999). In severe cases, hyperparathyroidism could result (National Institutes of Health, 1994).

Copper (Cu), zinc (Zn), and iron (Fe) are elemental nutrients needful in infant growth and development, as they are involved in several intricate enzyme coordination functions in biological processes in the body (Burjonrappa and Miller, 2012; Voskaki *et al*., 2010). In underdeveloped and developing countries, infants are predominantly at risk of Iron (Fe) and Zinc (Zn) deficiency because of increased requirements at that age, low bioavailability, and recurrent infections (Zlotkin *et al*., 2006). At about 6 months of age, infants could lose autogenous zinc via urine and/or body surface (Özden *et al*., 2015). Lack of Cu has been reported to leads to anemia, neutropenia, impairment of growth, abnormalities in glucose and cholesterol metabolisms, and increased rates of infection (Shazia *et al*., 2012). Nutrient deficiencies are therefore very common in children (Özden *et al*., 2015) and deficiency in one trace element possibly will impair absorption of another (Özden *et al*., 2015), which may overall cause stunting, reduced immune function, skin lesion, depression, ineffectual cognitive function; hence the need for nutritional adequacy of these dietary elements and needful supplementation as the case may arise. Most legumes contain anti-nutrients that reduce the bioavailability of especially bivalent cation dietary elements, thereby reduce the nutritional efficiency of such foodstuff. Such reduced or poor mineral efficiency ratio (MER) further compounds the public heath nutritional challenges confront children in developing countries. To this end, this study was carried to investigate the MER of some dietary elements in complementary compounded with the use of certain legumes and cereals.

II. Materials And Methods

Animals

45 Male Wistar-strain weaning rats weighing 40 - 55g purchased from the animal facility of Babcock University Ilishan, Ogun State, Nigeria, provided the *in vivo* system for this study. The experimental animals were randomized into 3 groups of 15 rats each, housed in standardized metabolic cages. The animals were allowed to acclimatize on rat chow for 7 days, and fed *ad libitum*. They were then fasted for 24hours before commencing feeding experiment on them.

Experimental Food Samples

The formulated complementary formula (CF) was made of wheat (Triticum spp.), groundnut (Arachis hypogea), soya bean (Glycine max) and guinea corn (Sorghum bicolor) in ratio 1:1:2:1. The foodstuffs and the proprietary branded formula, BF, were purchased from Ilishan main market, Ogun state, Nigeria. The soybeans and groundnut were to complement the nutrients deficient in the grains. The cereals and legume were respectively sorted to remove the defective grains, sand granules, and other extraneous matters, followed by washing and drying at room temperature. Care was taken to avoid prolonged stay in water during washing so as to forestall the grains absorbing much water. The grains were dried in dry air Electrothermal oven, proportionately pulled together and milled into finely particulate texture similar to that of 'powder', using a laboratory hammer mill. The flour particles were sizes less than 300µm. The consistency of the flour particular size was ensured by sifting through a 300µm screen. Milled mixed grains and legumes of the combination thereof is what are referred to as CF in this study. The CF was sealed in transparent polyethylene packs of 25g and stored in air tight container at room temperature for both the feeding experiment and required chemical analyses. The following were the respective animal groups placed on the food types investigated in this study:

Group 1- fed CF - Soya bean: Groundnut: Wheat: Guinea corn (40:20:20:20%w/w)
Group 2 - fed Nutrilac® (BF), a proprietary standard formula
Group 3 - fed Rat Chow (Control diet)

The foods were respectively analyzed for their proximate nutrient composition and then fed to experimental animals. Their nutritional parameters such as feed intake, growth rate, and efficiency ratios were assessed and results obtained from CF group compared against those obtained from the group fed the standard formula, BF.

Collection of the fecal matter

Fecal matter was collected on a daily basis from the litter compartment of the cage, and weighed using top loading balance. It was then homogenized and preserved for further analysis.

Dietary element Analyses

Wet digestion of samples was performed using mixtures of acids: HNO_3: HCl (3:1), by the modified method of Demirel *et al*. (2008). The digest was appropriately constituted into solution and subjected to acetylene flame

Atomic Absorption Spectrophotometry to quantify the dietary elements of interest in the respective sample. The recovery study of the analytical procedure was carried out by spiking and harmonizing several already analyzed food samples with varied amounts of standard solutions of the respective dietary elements (Onianwa *et al.*, 2001). The Method of Association of Official Analytical Chemist (2005) was adopted in determining the nitrogen and protein contents of the samples.

Biological Determinations

Weight gain of the animals placed on each of the foods was determined by subtracting the initial weight of the animal from the final weight. Faecal nitrogen was determined according to the Method of Association of Official Analytical Chemist (2005), with slight modification. Efficiency Ratio of each nutrient was estimated by accurately measuring its dietary intake (amount consumed) and related weight gained by the animal, and then finding the ratio thereof.

III. Results

Table 1: Nitrogen Efficiency Ratio (NER) of the studied complementary Formula and Branded Formula in Weaning Male Wister Rat Model.

Day	Weight gain (g)		Nitrogen consumed		NER	
	CF	BF	CF	BF	CF	BF
1	2.00	1.25	0.7045	0.1371	0.35	0.11
2	0.75	0.90	0.7459	0.1621	0.10	0.18
3	1.00	0.85	0.7252	0.1656	0.73	0.20
4	1.25	0.50	0.7666	0.1591	0.61	0.32
5	0.75	0.84	0.6838	0.1685	0.91	0.20
6	1.50	0.79	0.7459	0.1568	0.50	0.13
7	1.00	0.96	0.7874	0.1884	0.79	0.19
Mean	1.18[a] ±0.17	0.87[b] ±0.08	0.7370[c] ±0.013	0.1625[d] ±0.006	0.57[e] ±0.11	0.19[f] ±0.03

Note: Different suffix alphabet in cell indicates values are significantly different at $p \leq 0.05$.

Table 2: Protein Efficiency Ratio (PER) of the studied complementary Formula and Branded Formula in Weaning Male Wister Rat Model.

Day	Weight gain (g)		Protein consumed (g)		PER	
	CF	BF	CF	BF	CF	BF
1	2.00	1.25	4.40	0.86	2.20	0.69
2	0.75	0.90	4.66	1.01	6.21	1.12
3	1.00	0.85	4.53	1.04	4.53	1.22
4	1.25	0.50	4.79	0.99	3.83	1.98
5	0.75	0.84	4.27	1.33	5.69	1.58
6	1.50	0.79	4.66	1.28	3.11	1.62
7	1.00	0.96	4.92	1.98	4.92	2.06
Mean	1.18[a] ±0.17	0.87[b] ±0.08	0.46[g] ±0.08	1.21[h] ±0.14	4.36[i] ±0.54	1.47[j] ±0.18

Note: Different suffix alphabet in cell indicates values are significantly different at $p \leq 0.05$.

Table 3: Mineral Efficiency Ratio (MER) of the studied complementary Formula and Branded Formula in Weaning Male Wister Rat Model.

Day	P (mg)	N (mg)	Ca (mg)	Mg (g)	Na mg)	K (mg)	Mn (µg)	Fe (µg)	Cu (µg)	Zn (µg)
1	0.11	0.35	0.04	0.03	4.26	0.16	0.23	28.9	0.21	0.58
2	0.31	0.10	0.10	0.09	12.03	0.45	0.63	81.6	0.60	1.65
3	0.22	0.73	0.07	0.06	8.77	0.33	0.46	59.5	0.44	1.20
4	0.19	0.61	0.06	0.05	7.42	0.28	0.39	50.32	0.37	1.02
5	0.28	0.91	0.09	0.08	11.03	0.41	0.57	74.8	0.55	1.51
6	0.15	0.50	0.05	0.04	6.01	0.22	0.31	40.8	0.46	0.83
7	0.24	0.79	0.07	0.07	9.52	0.35	0.50	64.6	0.48	1.31

IV. Discussion

Nitrogen Efficiency Ratio (NER) of the locally formulated complementary food, CF, was studied against that of the proprietary branded complementary formula, BF. The result obtained (shown on Table 1) demonstrated that the NER of CF is significant higher than that of BF. This is most likely due to the significantly higher amount of nitrogen consumed by experimental animals fed CF when compared with that consumed by the group fed BF (Table 1). The increase in both NER and amount of nitrogen consumed observed in the group fed CF relative to that fed BF was asserted to undertone the significantly higher weight gained by the experimental group than the standard group (Table 1).

The tendency for increased amount of nitrogen consumed to give rise to increased amount of protein consumed and increased NER to lead to high Protein Efficiency Ratio (PER) holds in this study (cross reference Table 1 and Table 2). A prime observation is that the experimental animals seemed to prefer CF to BF. Thus, they consumed more of CF compared to how much of BF they consumed. A fundamental assertion of this investigation is that both the protein contained in CF seemed optimally digestible in the gastrointestinal tract of the animals and therefore, was highly bioavailable; besides, the protein thereof seemed to be of such high quality that supported healthy growth and development in the animals.

The amount of each of the dietary element in the food consumed that is able to bring about 1g of weight gain in the experimental animal connote Mineral Efficiency Ratio, MER, in this study. The MER of the dietary elements investigated is presented on Table 3. With respect to days there seemed not to be any particular of relationship with the MER of the food in the animals. However, the result shows that dietary elements in CF was absorbed from the food matrix by the digestive system and made available to body tissues to serve their various as such border on growth, development and body maintenance during the study. This suggests that locally sourced foodstuff of the kinds used in this study could be used to formulated complementary food with high PER and appreciable MER that could also support healthy growth and development in infants. However, clinical investigation is recommended to assess the nutrient adequacy and biological values of CF before adopting it as complementary formula in human.

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