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# Protein Quality and Biochemical Evaluation of Unripe Banana Based Complementary Food Fortified with Crab Meat in Rats

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**Abstract:** Protein-energy malnutrition is continually on the increase among children in developing countries due to low nutrient density of traditional complementary diets. The current cost of commercially prepared complementary food in Nigeria have price out most caregivers resulting in increase formulation of complementary food from local food staples. This study investigated the nutrient and biochemical properties of animal fed infant diets formulated from local food materials, unripe banana and edible crab. The food materials were obtained locally, processed and dried. The processed samples were formulated as unfortified unripe banana, 10% crab meat fortified unripe banana (UBCM1), 15% crab meat fortified unripe banana (UBCM2) and 10% cray fish fortified unripe banana (UBCF). The protein efficiency ratio (PER) of Control (1.81) was higher than those of UBCM1 (1.17), UBCM2 (1.20) and UBCF (1.31). The weight gain/day of the formulated diets were lower than that of Cerelac (2.19g) and significantly difference ($P<0.05$) to the control. The serum total protein of the control group was higher than those on the experimental diets. The serum albumin level of all the samples were within the normal range, 3.5-5.0mg/dL. Serum cholesterol level of UBCM1 (54.14mg/dL) and UBCM2 (62.44mg/dL) were higher than the unfortifed banana group but almost equal that of cerelac. The hematological properties (PCV and hemoglobin) of the Crab meat fortified diets were higher than those of unfortified, but lower than those of control. In conclusion, we established that the crab meat fortified banana diets supported growth and development of weanling rats and it's a suitable replacement for commercial complementary foods.

**Keywords:** Protein Quality, Complementary Food, Food Fortification, Banana Porridge, Infant and Young Child Feeding

## 1. Introduction

Adequate child nutrition and health care during the first several years of life is a fundamental pillar of health, growth and development in children [1]. It is well recognized that the period from birth to two years of age is a "critical window" for the promotion of optimal growth in health, mental and in behavior. The World Health Organization (WHO) recommends exclusive breast feeding till six months of age and continued breastfeeding for at least two years along with timely introduction of adequate amount of complementary foods of suitable nutritional quality [35, 36, 37]. Despite the importance of good nutrition, it is evidence that many families in developing countries are unable to feed their children an appropriate diet [7].

Globally, inappropriate complementary feeding practices have been identified as a determinant of diarrheal disease, malnutrition outcomes and under 5 mortality [33].

It lowers the body's resistance to disease, exposing it to disease causing agents. Several studies support the view that poor nutrition during childhood can have detrimental effect on growth and cognitive development, decrease activity levels, and affect social functioning [32, 11].

In Nigeria, complications from malnutrition remain a major health problem with prominent levels of stunting (37%) wasting (18%) and underweight (12%) in infants and young children [27]. Commercially prepared complementary foods in a developing country like Nigeria are relatively expensive and is out of reach of majority of care givers [7]. However, Mothers and Caregivers usually prepare complementary foods from locally available food staples such as corn, guinea corn, cowpea, Millet, sorghum, banana and these are characterized by low protein and energy density [4, 29].

Unripe banana one of the staples have been used in the local preparation of complementary food in Nigeria especially in communities of Abia, Akwa-Ibom and Cross River State [1]. The unripe banana porridge (*Otor mboro* in Efik/Ibibio language) is usually prepared at the household level by mothers and caregivers [3, 14]. Reports have shown that most of the local complementary foods are in most cases poor in nutrient density. Often times, they are gruels based on cereals and starchy tubers and are poor in protein content [13, 18, 23]. This have been implicated in the etiology of protein energy malnutrition usually manifested as nutritional marasmus and Kwashiorkor [8, 9].

There is, therefore, need to supplement traditional complementary foods with cheap protein sources. Crabs for example has been reported to be very rich in proteins (21.4g/100g) and essentials amino acids [20, 28, 34]. Crab constitutes one of the main sources of animal protein most especially among coastal dwellers [12] and is abundantly available in some parts of Nigeria [26]. But banana has been reported to generally poor in amino acids and protein [7, 30, 31]. This work therefore seek to evaluate the supplementary effects of crab meat in unripe banana based complementary food.

## 2. Materials and Methods

### *2.1. Collection and Treatment of Samples*

Fresh matured lively specimens of *Callinetes amnicola* (blue crab) were purchased from fishermen at the landing site of the Great Kwa River in April 2017 and placed in an ice chest, taken to the Department of Zoology and Environmental Biology, University of Calabar for authentication and immediately transported to the Research laboratory in the Department of Medical Biochemistry, University of Nigeria, Enugu Campus. The unripe banana (*Musa sapientum*) fingers were procured from farmers in Atan Offot, Uyo and identification was done in the department of Botany, university of calabar, The banana fingers were washed with distilled water, peeled, grated, boiled and dried at 51°C for 24hrs, then milled and stored in a refrigerator at -4°C prior analyses. Crab samples was stepped in hot distilled water at a temperature of 60°C for 5min to remove all dirt. The appendages and digestive system will be removed from the body and the edible portion will be taken separately. The edible portion (crab meat) will be kept in an electric oven at 51°C for 24hrs for proper drying. The dried samples was milled and stored in a refrigerator at -4°C until analyses. Cerelac®, a commercial weaning food, was procured from a super market in Enugu metropolis.

### *2.2. Formulation of Diet*

The experimental diets were formulated as follows:

Diet I: Cerelac® as Standard

Diet II: Unfortified Unripe banana

Diet III: 10% Crab meat fortified Unripe banana meal (UBCM1)

Diet IV: 15% Crab meat fortified Unripe banana meal (UBCM2)

Diet V: 10% Cray fish fortified Unripe banana meal (UBCF)

### *2.3. Animal Husbandry and Experimental Design*

20 Wistar rats weighing between 60-65g obtained from the animal house, College of medicine, University of Nigeria Enugu Campus were used for the study. The rats were acclimatized on a normal rat chow or 7 days. The rats were divided into five groups of 4 animals each. Each rat cage were numbered 1 – 5.

#### *2.3.1. Feeding Regime*

The rats in each group were fed commercial complementary food (Cerelac), unripe banana, 10% crab meat fortified unripe banana, 15% crab meat fortified unripe banana and 10% cray fish fortified unripe banana respectively. The groups of animals were fed with the food sample and distil led water *ad libitum* for 21 days. Weighed foods were placed in small containers. The Spilled foods were collected and combined with the unconsumed food for the determination of total amount of food actually consumed by each rat daily. The daily food consumption was determined by weight difference between the served food and unconsumed food in addition to split food. The body weights of the animal were measured at two days interval for 21 days using digital electronic weighing scale no scout pro sp 401 (china) calibrated to the nearest 0.1kg.

#### *2.3.2. Collections of Fecal Matters*

The fecal samples were collected daily, bulked for each rat, weighed, dried and milled prior to laboratory after 21 days. The daily feed consumed were also collected. Triplicate samples of feces and diets were obtained for nitrogen determination by the kjedahl method [2].

#### *2.3.3. Nutritional Evaluation of Diets*

The protein quality of the experimental diets was evaluated biologically, based on their ability to promote growth and nitrogen retention in the albino rats. The weight gained by the animals and nitrogen were used to calculate the following parameters:

1) Feed Efficiency (FE):

$$\mathrm{FE} = \frac{\text{Weight gained}}{\text{Food intake}}$$

2) Protein Efficiency Ratio (PER)

$$\mathrm{PER} = \frac{\text{Weight gain}}{\text{Protein intake}} \times 100$$

3) Apparent digestibility (AD %)

$$\mathrm{AD} = \frac{\mathrm{Ni} - \mathrm{Fn}}{\mathrm{Ni}} \times 100$$

Where Ni = Nitrogen intake
Fn = Fecal nitrogen
The nitrogen intake and fecal nitrogen were determined by microkjeldahl method previously described [2, 22, 25].

#### *2.3.4. Blood Collection*

On the 21 days of the experimental period all rats were starved for about 3 hours and weighed. Each rat was anaesthetized with chloroform inside desiccators before been sacrificed. Blood was collected into sample bottles containing a few milligram of EDTA prior to analysis.

#### *2.3.5. Determination of the Organ Weight*

The heart, lungs, kidneys and liver were separated blotted free of blood, oven dried and weighed. The values were subsequently expressed in g/kg of body weight.

### *2.4. Data Analysis*

All data were expressed as Mean ± SD. The data was analyzed by one way ANOVA with post hoc least significant difference equal variances assumed using the IBM SPSS statistic software version 22 (SPSS: Statistical Package for Social Sciences) Differences at p < 0.05 were considered significant.

### *2.5. Ethical Considerations*

Ethical approval was obtained from the University of Nigeria Teaching Hospital (UNTH) Ituku – Ozalla, Enugu.

## 3. Results and Discussions

Table 1 presents the nutritional indices of the rats fed the formulated food sample and the control. The results showed that there is no consistent pattern in feed efficiency trend, though non-significant increase in feed intake were exerted by the test diets (P < 0.05).

Table 2 presents the influence of the diets on the organs of the animals fed the formulated food samples. Table 3 present the effect of Crab meat fortified Unripe banana meal on biochemical parameters of the blood.

Table 4 present the effect of Crab meat fortified unripe banana meal on hematological parameters of blood. The results showed no significant differences between the heamoglobin, WBC, eosinophils and neutropils in animal fed any the diets.

***Table 1.*** *Nutritional indices of Crab meat fortified unripe banana-fed Rats.*

| | Feed Efficiency (%) | Feed Intake (g) | PER | Weight gain/day (g) | Apparent Digestibility (%) |
|---|---|---|---|---|---|
| Cerelac | $0.27 \pm 0.04^a$ | $8.71 \pm 1.31^a$ | $1.81 \pm 0.29^a$ | $2.19 \pm 0.32^a$ | $94.4 \pm 0.43^a$ |
| Unripe banana | $-0.04 \pm 0.00^b$ | $6.45 \pm 0.36^a$ | $-1.09 \pm 0.13^b$ | $-0.29 \pm 0.36^b$ | $60.8 \pm 0.28^a$ |
| UBCM1 | $0.07 \pm 0.01^c$ | $11.56 \pm 1.60^a$ | $1.17 \pm 0.18^a$ | $0.81 \pm 0.10^b$ | $54.89 \pm 0.21^a$ |
| UBCM2 | $0.09 \pm 0.02^d$ | $13.15 \pm 2.00^a$ | $1.20 \pm 0.30^a$ | $1.13 \pm 0.17^b$ | $66.92 \pm 0.30^b$ |
| UBCF | $0.12 \pm 0.03^e$ | $10.58 \pm 0.09^a$ | $1.31 \pm 0.34$ | $1.17 \pm 0.24^a$ | $65.23 \pm 0.06$ |

Mean±SD (Standard deviation) UBCM1, 90% unripe banana + 10% crab meat; UBCM2, 85% unripe banana + 15% crab meat; UBCF, 90% unripe banana + 10% cray fish, PER= Protein Efficiency Ratio. Group with superscript 'a' are significantly different from the control (P< 0.05).

***Table 2.*** *Effect of Crab Meat fortified unripe banana Meal on Organ Weight (g).*

| | Heart | Kidney | Liver | Lungs |
|---|---|---|---|---|
| Cerelac | $50.89 \pm 0.50^a$ | $13.98 \pm 1.83^a$ | $4.13 \pm 0.62^a$ | $10.31 \pm 7.80^a$ |
| U. Banana | $27.50 \pm 2.43^b$ | $6.69 \pm 0.40^b$ | $2.46 \pm 0.15^b$ | $6.91 \pm 0.97^b$ |
| UBCM1 | $33.47 \pm 0.66^c$ | $9.00 \pm 0.53^c$ | $3.79 \pm 0.39^a$ | $7.90 \pm 0.73^a$ |
| UBCM2 | $35.97 \pm 1.41^d$ | $9.93 \pm 0.81^d$ | $4.13 \pm 0.37^a$ | $8.71 \pm 0.46^a$ |
| UBCF | $36.31 \pm 1.91^e$ | $10.25 \pm 0.40^a$ | $4.12 \pm 0.37^a$ | $8.91 \pm 0.79^a$ |

Mean ± SD (Standard Deviation)
The groups with superscript of "a" are significantly different from the control ( p< 0.05).

***Table 3.*** *Effect of Crab Meat fortified Unripe banana Meal on Biochemical parameters of the blood (mg/dL).*

| | Albumin | Total Protein | HDL | LDL | Total Cholesterol | Triacylglyceride |
|---|---|---|---|---|---|---|
| Cerelac | $4.30 \pm 0.29^a$ | $66.75 \pm 11.90^a$ | $13.18 \pm 3.71^a$ | $15.44 \pm 5.48^a$ | $63.07 \pm 2.09^b$ | $99.86 \pm 33.97^a$ |
| Unripe | $3.85 \pm 0.66^a$ | $52.69 \pm 4.91^a$ | $26.04 \pm 6.43^a$ | $40.62 \pm 8.74^a$ | $53.67 \pm 1.87^b$ | $120.86 \pm 17.96^a$ |
| UBCM1 | $4.18 \pm 0.16^a$ | $57.82 \pm 5.30^a$ | $13.20 \pm 2.84^a$ | $53.38 \pm 22.31^a$ | $56.14 \pm 21.68^b$ | $104.97 \pm 56.51^a$ |
| UBCM2 | $4.63 \pm 0.59^a$ | $59.40 \pm 6.77^a$ | $12.42 \pm 6.23^a$ | $51.60 \pm 21.74$ | $62.44 \pm 17.79^b$ | $39.36 \pm 18.57^a$ |
| UBCF | $4.79 \pm 0.78^a$ | $63.31 \pm 13.54^a$ | $13.30 \pm 5.38^a$ | $48.76 \pm 27.04^a$ | $56.85 \pm 29.88^b$ | $63.91 \pm 19.64^a$ |

Mean ± SD (Standard Deviation). Mean value with different superscripts in a row are significantly different (P<0.05).
HDL, High density lipoprotein; LDL, low density lipoprotein.

***Table 4.*** *Effect of Crab Meat fortified unripe banana Meal on Haematological Parameters.*

| Group | E (%) | Hb (g/100ml) | L (%) | N (%) | PCV (%) | WBC (*$10^3$ $mm^3$) |
|---|---|---|---|---|---|---|
| Cerelac | 0.50±0.29[a] | 8.53±0.56[a] | 53.75±3.77[a] | 45.50±4.03[b] | 26.75±1.31 | 9775.00±225.00[a] |
| Unripe banana | 0.75±0.25[a] | 8.38±0.56[a] | 61.50±1.19[a] | 37.50±1.04[a] | 24.50±1.50[b] | 10100.00±837.66[a] |
| UBCM1 | 1.75±0.25[a] | 8.83±0.59[b] | 67.00±5.05[b] | 31.25±4.94[a] | 25.25±1.49[b] | 11050.00±1152.17[a] |
| UBCM2 | 0.75±0.25[a] | 7.93±0.46[b] | 57.50±5.33[a] | 42.25±5.33[a] | 26.25±1.11[a] | 10100.00±393.70[a] |
| UBCF | 1.25±0.25[a] | 7.95±0.61[a] | 57.50±1.26[a] | 41.25±1.31[a] | 27.00±1.41[a] | 9,275.00±996.14[a] |

The groups with superscript of "a" are significantly different from the control (P<0.05). E, Eosinophils; Hb, Heamoglobin; L, Lymphocytes; N, Neutrophils; PCV, packed cell volume; WBC, white blood cell.

The Nutritional indices of the rats fed the formulated food sample and the control are shown in Table 1. All the diets showed positive PER values except unfortified unripe banana. The PER of UBCF (1.31) was higher than that of UBCM1 (1.17) and UBCM2 (1.20) but not significantly (P<0.05) to the cerelac diet (1.81). The weight gain/day of the formulated diets were lower than that of cerelac (2.19g) and significantly difference (P<0.05) to the control.

The effects of crab meat fortified unripe banana diets on nutritional indicies shows that there is no consistent pattern in feed efficiency trend, though non significant increase in feed intake and protein efficiency ratio (PER) were exerted a meal of low quality of protein. This was found in the unfortified banana meal (-1.06) but the PER was increased with crab meat fortification up to 1.17 and 1.20 at 10% and 15% fortification. This could be because of the increase in protein and micronutrient content of the food formulations, which were utilized by the experimental animals. However, all other PER values, including that of Nestle Cerelac (1.81) were lower than, though within the same range with the value of 2.10 recommended by the Protein Advisory Group (PAG 1971) for complementary foods. Both PER and apparent digestiblility are indicies of protein quality [16]. Rats on UBCM1 and UBCM2 and UBCF recorded an appreciable gain in body weight 0.81, 1.13, and 1.17g/day respectively. Though not as a high as cerelac (2.19g/day), this is attributed to the fact that protein quality of the test diets probably exerteds direct influence on the weight gain per day. However, this study seem to agree with [19] who reported a similar weight gain in rats fed bambara groundnut mix complementary food. [7] argues that malnutrition during infancy permanently changes the body's structure, physiology, and metabolism, leading to coronary heart disease and stroke later in life.

The influence of the diets on the organs of the animals shows that the formulated diets promote growth and development of the organs (heart, kidney, lungs and liver) of animals better than unripe banana but not as well as cerelac (P<0.05). This is similar to the growth pattern reported by [19] using fermented popcorn and bambara groundnuts. However, the UBCM2 meal promoted growth and development of these organs better than that of the other formulated diets.

Serum biochemical parameters showed that the rats on cerelac diet recorded a higher total serum protein (66.75mg/dL) than those on the experimental diets which ranges (52.69 – 59.40mg/dL) [15] reported a similar serum protein in boobab seed protein utilization in young albino rats. A total serum protein in liver can suggest a disorder in which protein is not digested or properly absorbed [24]. Low levels of serum protein may be seen in severe malnutrition [10, 16] and with condition that cause malabsortion [21]. Serum Albumin level in all the experimental diets were within the normal range 3.5 – 5.0g/dL. Albumin is the most abundant serum protein representing 55-65% of the total protein. It is synthesized in the liver and has a half-life of 2 to 3 weeks. The main biological functions of albumin are to maintain the water balance in serum and plasma and to transport and store a wide variety of ligands e.g fatty acids, calcium, bilirubin and hormones such as thyroxine. Albumin also provides endogenous source of amino acids. Hypoalbuminaemia is assocated with the following conditions: analbuminaemia; impaired albumin synthesis in the liver, liver disease; malnutrtion or malabsorption; generalized shock; burns or dermatitis; kidney disease and intestinal disease. Hyperalbuminemia ahs little diagnostic relevance except, perhaps in dehyration. Again, the low level of albumin in the blood (hypoalbuminemia) suggests protein energy malnutrition and sometimes can be life threatening.

However, serum cholesterol of rats on crab meat fortified unripe banana were elevated but only significantly (P<0.05) for blood cholesterol, compared to controls and this may have some physiological signifance [17]. This observation, however, is in conflict with the hypocholesterolaemic effect of plant protein compared to caesin diet [15]. Plants have been reported to have lipid- lowering action [5]. Again, studies in experimental animal have demonstrated that presence of certain plant fibres in the diet is accompanied by significant lowering of serum and tissue cholesterol levels [21]. This is in tandem with this study of which the rats on unripe banana recorded a total cholesterol level of 53.67mg/dl lower than the fortified groups [6]. Dietary fibre adsorbs bile salts and this is excreted. Again, recycling of bile is diminished resulting in the lowering of serum cholesterol level [21].

The results showed no significant differences between the heamoglobin, WBC, eosinophils and neutropils in animal fed any the diets. This is similar to hematological properties reported by [19] in fermented popcorn and African locust bean complementary food blends. A significant fall in heamoglobin and RBC reflects erthropenia [17]. Inadequate intake of rich meal could lead to low heamoglobin count. [17] also reported no significant difference in these hematological variables (neutrophils, eosinophils and WBC) between formulated diets of cooking banana (60%) and bambara groundnut (40%) and that of Nutrend, Ogi and Caesin. However, Heamoglobin, Packed Cell Volume and Platelet were generally high, thus indicating the adequacy of the formulated diets as a supplement to commercial weaning foods in a manner that promotes similar hematopoiesis. PCV, heamoglobin and platelet of the animal fed the UBCF and control were significantly higher when compared with those fed the UBCM1 and UBCM2 samples ($P<0.05$). These high values indicate the adequacy of the formulated diets for promoting good blood health status.

## 4. Conclusion

Unripe banana based complementary foods are of low protein content and may result in increased childhood malnutrition. However, supplementing with crab meat increases the protein and micronutrient contents required for optimal growth and development for infants. Crab is cheaper and affordable and when added in the adequate proportion can compare positively with the commercially available complementary foods among caregivers both in nutrient quality and acceptability. Efforts should therefore be made to popularize the use of crabmeat as supplement in local unripe banana complementary foods used in infant feeding.

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