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Assessment of Protein Quality of Complementary Food Made from Maize (*Zea mays*) Supplemented with Crayfish (*Euastacus spp*) and Carrot (*Daucus carota*) Using Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author NNU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NMOA and AIA managed the analyses of the study. Authors PNA, CGUO and COE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Assessment of protein quality is aimed at determining the ability of a protein to meet up with the needs for maintenance, growth, pregnancy and lactation.
Objective: To evaluate the protein quality of complementary food made from local food blends using albino rats.
Methods: Maize, carrot, crayfish, milk and other ingredients were purchased, processed and used

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for the study. The maize, crayfish and carrot flours were blended in the ratio of 100:0:0, 70:25:5, 70:20:10, 70:15:15, 70:10:20 and 70:5:25 respectively. The six diets provided 10% protein for six groups of rats. The diets were fed to forty (40) weanling albino rats in a 21-day growth period and 7-days Nitrogen balance study. Milk was used as control and Nitrogen free diet was used for the estimation of endogenous nitrogen. The result generated were statistically analyzed using SPSS version 22.

Results: The result showed that over 70% of the nitrogen consumed by all the five groups of rats fed the mixed diets were absorbed and retained. Among the test diet groups, the 70:20:10 diet had the highest absorbed nitrogen (2.10 g), retained nitrogen (2.00 g), biological value (80%) and net protein utilization (77%) which was significantly different from the other groups. The 70:20:10 diet had the highest Total digestibility (94%), highest food intake (230g), weight gain (52.10%) and PER (2.7) that were comparable with the reference protein (milk) at ($p>0.05$). The mineral metabolism of all the rats fed the test diets were absorbed and retained.

Conclusion: The study revealed that complementary food of high protein quality and nutrient dense can be produced from blends of maize, carrot and crayfish. All the complementary food made from blends of maize, carrot and crayfish blend were of higher nutrient quality than the popular homemade complementary food made of maize alone. It is imperative that blends of local foods stuffs should be used as complementary food instead of only cereal in order to improve the nutritional status of a growing child and also curb prevalence of protein energy malnutrition that is common among under five.

Keywords: Protein quality; complementary food; maize; carrot; crayfish.

1. INTRODUCTION

Complementary foods are formulated food mixtures meant to be fed along with breast milk for infants from 6 months until completely weaned off breast milk [1]. They are solely to complement the breast milk and later on replace it with family foods. Complementary foods are solid (mashed) or semi-solid foods consumed in addition with breast milk to provide adequate nutrients for the growing child from the age of 4/6 months to 2 years.

In Nigeria, traditional complementary foods are usually introduced to the young children between 3 and 6 months depending on the locality and types of cereal grain and root crop available [2]. The usual first complementary food is called pap, “akamu”, “ogi”, or “koko” and is made by fermentation of maize, millet, or guinea corn [3,4]. Cereal based traditional complementary foods are inadequate to meet daily nutrients, energy and micronutrient requirements, where such complementary foods form the main source of nutrient to an infant, it may lead to under-nutrition and micronutrient malnutrition in infants and young children [5,6,7].

Protein quality is the ability of a food protein to support body growth and maintenance [5]. Biological evaluation of protein has the ability to assess growth and maintenance and it is the only reliable method for the determination of protein quality [5]. A good quality protein is one with high digestibility. This is the protein that will supply sufficient amino acid (AA) to cover requirements when consumed at a level that complies with the global protein requirement [5]. A dietary protein that supports a positive nitrogen balance at a low concentration is a high quality protein [8]. The quality of protein is vital when considering the nutritional benefits of a food product [9]. There is constant need of protein by both growing animals and non growing animals due to protein turnover. True protein digestibility, as stipulated by the FDA, requires the use of biological rodent assay to determine the amount of fecal nitrogen excreted per unit of dietary nitrogen consumed [10]. The hydrolysis of protein that is in the pathway of amino acid catabolism, some amino acids are lost in urine and faeces. Biological evaluation indices of protein quality include but not limited to protein efficiency ratio (PER), biological value (BV), net protein utilization (NPU), protein digestibility corrected amino acid score (PDCAAS), digestible indispensable amino acid score (DIAAS) and true digestibility (TD).

Many plant proteins, especially those found in grains are low in one or more of the nine essential amino acids. Animal protein contain ample amount of these nine essential amino acid. Although animal protein may be low in carbohydrate, fibre, some vitamins, phytochemicals and some mineral while fruits and vegetables are good sources of fibre, some

mineral, phytochemicals and some vitamin but grains supplies carbohydrate needed.

Maize, also known as corn; botanically called *Zea mays*, is a cereal grain first domesticated by indigenous people in southern Mexico about 10,000 years ago [11]. Maize contains moderate amounts of dietary fiber, magnesium and phosphorus whereas protein are in low amounts [12].

Carrot (*Daucus carota L.*) is a root vegetable that is a good sources of beta carotene, fibre, antioxidants and phytochemicals. Carrots contain numerous phytochemicals, beta-carotene, carotenoids, vitamin A, C and some minerals [13].

Crayfish are fresh water crustacean resembling small lobsters and its study is called astacology. It is a seafood that contains varying ranges of minerals and vitamins such as vitamin B6 and B12, vitamin A, D, E and K, phosphorus, zinc, iron, calcium, magnesium, sodium and other macronutrients like carbohydrates, protein and fat [14].

Despite dietary recognition of maize as an important food crop especially regarding its use as a complementary food and also in composite flour formulation. There is a necessity to meet key nutrient requirements related to protein quality. Traditionally, cereals especially maize gruel has been used as an acceptable complementary food throughout Nigeria. This has not solved the nutrient need of infants since maize is not a good source of protein. Studies have shown that fermentation improves the nutrient content of food thereby reducing the antinutrient and improving the protein content of foods [15,12,16]. Fermented maize alone may not be able to promote growth and maintain a healthy cells and tissues. There is need to improve the nutrient content of maize gruel by supplementing it with other food sources with quality protein and mineral which will lead to improvement of nutritional status and wellbeing of a growing child. This is the thrust of the present work.

2. MATERIALS AND METHODS

2.1 Procurement of Raw Materials

The maize, crayfish, carrots and other ingredients used for the study were purchased from Ogbete main market, Enugu, Enugu State of Nigeria.

2.2 Preparation of Maize

The malted maize flour was prepared according to the method of Bolarinwa et al. [17]. Two kilogramme (2 kg) of maize grains were manually sorted to remove dirt and other extraneous materials. These seeds were then steeped in water at 29°C for 24 hours. Changing of water at 6hours interval was observed during steeping. The resultant steeped seeds were spread on jute bag and were covered with white cotton cloth to germinate for 72 hours. The sprouted seeds were oven dried at a temperature of 50°C in order to terminate enzyme activities. The plumule was separated from the seed and the malted seeds were milled into flour with an attrition mill and stored in a lidded plastic container and kept in a freezer for further use.

2.3 Preparation of Carrot Flour

The carrot flour was prepared according to the method of Aremu et al. [18]. One kilogramme of carrots were manually sorted to remove the dirt and other contaminants. The sorted carrots were cleaned with 2 litres of portable water and cut into smaller slices with kitchen knife. The carrot slices were placed into a stainless pot and blanched with 2.5 litres of portable water at 80°C for 10 mins on a hot plate. The blanched carrot slices were drained, spread on the trays and dried in a tray dryer (Model EU 850D, UK) at 60°C for 10 h with occasional stirring of the slices at intervals of 30 mins to ensure uniform drying. The dried slices were milled in an attrition mill and sieved through a 500 micron mesh-sieve. The flour produced was packaged in a lidded plastic container, labeled and kept in a freezer for further use.

2.4 Preparation of Crayfish Flour

One kilogramme (1 kg) of crayfish was sorted to remove dirt's and other extraneous materials and cleaned thoroughly with 2.5 liters of potable water. The cleaned crayfish was drained, spread on the trays and dried in a cabinet dryer at 60°C for 10 hours with occasional stirring of the crayfish at intervals of 20 minutes to ensure uniform drying. The dried crayfish was milled in an attrition mill and sieved through a 500micron mesh sieve. The flour produced was packaged in an lidded plastic container, labeled and kept in a freezer for further use.

2.5 Formulation of Composite Blends

Maize, crayfish and carrot flours were thoroughly mixed together at varying proportions of 100:0:0, 70:25:5, 70:20:10, 70:15:15, 70:10:20 and 70:5:25 in a kenwood blender (Mini-processor, Model A 90LD, Thom Emi Kenwood Small Appliances Ltd, Hampshire, UK) to obtain homogenous composite blends. The composite blends were packaged in plastics containers, labeled and stored in the refrigerator for further use.

2.6 Diet Formulation

Eight diets were formulated. These included (a) Casein diet. (b) 100:0:0 maize: crayfish: carrot (c) 70:25:5 maize: crayfish: carrot, (d) 70:20:10 maize: crayfish: carrot, (e) 70:15:15 maize: crayfish: carrot, (f) 70:10:20 maize: crayfish: carrot, (g) 70:5:25 maize: crayfish: carrot and (h) Nitrogen free diet. The diets were meant to provide 10% protein to the rats [19]. The milk diet was the reference or control. The eight diet was nitrogen free (NFD) (Table 1). All the experimental diets were prepared by incorporating the flours and the sucrose to obtain the required 1000 g by volume. In the protein-free diet, cornstarch sucrose mixture replaced the test protein. The purpose of the protein-free diet was to estimate the endogenous nitrogen excretion of the rats. The dried ingredients for each of the diet were weighed into a bowl and mixed manually. Water was added in small quantity at a time to reconstitute it until a homogenous mixture was obtained and pelleted manually. The amount of the composite flour for the diet was calculated using the formula:

$$10\%\ \text{protein content} = \frac{3.2 \text{ x } 100}{\%\ \text{N of sample}} \qquad (1)$$

The diet was formulated using AIN 93G (American Institute of Nutrition) method for growth, pregnancy and lactating phases in laboratory rats [20]. The eight experimental diets formulated (g/kg) are given in Table 1.

2.7 Animals and Housing

Forty weanling male Albino rats, from the same colony, weighing 40-60 g were purchased from the Veterinary Medicine Department of the University of Nigeria, Nsukka. The rats were housed in individual metabolism cages equipped to separate faeces and urine on a base tray. The rats had exactly 12 h of light and 12 h of darkness in a day. Temperature was maintained at 21-25°C. All procedures using animal in this investigation were followed in accordance with ethical standard of European Union guidelines for animal experimentation (Dir 86/609/EEC) and approved by Industrial Animal Care Committee, University of Nigeria, Nsukka.

2.8 Growth and Maintenance Study

After a 7- day acclimatization period, the rats were weighed prior to access to their respective diets. The milk and the test diets were fed 15 g each to the rats daily for 21 day growth period. During this study, food intake was measured on daily basis. The other group of rats fed nitrogen free diet was fed normal rat chow during the growth studies and switched over to nitrogen free diet for maintenance studies. After the weight measurement on day 21, carmine red was added to each diet to mark the beginning of nitrogen balance. On day 22, all red faeces were retained and black ones discarded. Black faeces collected after day 23 was retained until day 28 when another carmine red was added to mark the end of N balance period. On day 29, all the red faeces were discarded and the black ones retained. The faeces were sun dried for 8 h, weighed, ground and stored in the freezer. Urine collected was treated with 0.1N HCl to avoid microflora growth. The urinary samples were stored in a deep freezer for analysis. The rats were sacrificed on day 29. The liver were removed, weighed and analyzed for minerals.

The proportion of food nitrogen that is absorbed is:

$$A = \frac{I\,(F - FK)}{I} \times I \qquad (2)$$

where

A = absorbed nitrogen,
F = faecal nitrogen,
Fk= metabolic nitrogen and
I = nitrogen intake.

2.9 Chemical Analyses

The weight of the rats was taken using an electronic balance and repeated on alternate days throughout the study. The faecal materials were pooled per group, sun-dried for 8 h, and then crashed using a mortar and pestle. The crude protein (N X 6.25) content of the faecal materials and dried diets were determined using the micro kjeldahl procedure [19]. The dried diets

and faecal samples were analyzed for some mineral content using AAS (Atomic absorption spectrophotometer) [21]. The same procedure was used for determination of urinary nitrogen and minerals. The values obtained were used for the calculation of food and nitrogen intake, faecal and urinary nitrogen. Faecal nitrogen from the rats fed the protein-free diet was used to calculate the endogenous nitrogen loss required for determining true digestibility (TD). Nitrogen balance, net protein utilization (NPU), protein efficient ratio (PER) and biological value (BV), were also computed. The result from AAS was used for the consumed, retained and absorbed minerals.

2.10 Experimental Design

The growth and digestibility studies were carried out using the Completely Randomized Design (CRD). Rats were randomly assigned to the treatments based on their weights. There were eight treatments each replicated five times. The rats were the replicates while the different diets were the treatments.

2.11 Statistical Analysis

Data collected were subjected to analysis of variance (anova) with statistical package for social sciences (spss) version 22. Means was separated using turkey's least significance difference (lsd) test and probability judged at p=0.05. Results were represented as mean ± standard deviation.

3. RESULTS

Nitrogen balance of rats fed six mixed protein diet and controls was presented in Table 2. The weight gain of rats was between 0- 3.05 g. The group of rats fed 70:20:10 diet had the highest value of 3.50 g which differed significantly ($p < 0.05$) from the rats from other groups. The food intake of rats fed NFD had the highest intake (66.10 g) followed by that of the rats fed 100% maize diet (42.10 g), whereas rats fed 70:25:5 diet had the least value (36.40 g). The food intake of rats fed NFD was significantly different ($p<0.05$) from that of the other test diets. The consumed nitrogen of rats fed 70:20:10 diet was highest (3.40 g) and differed significantly ($p<0.05$) from those of the other test diets. The rats fed NFD had the least consumed nitrogen value of zero. The rats fed 70:20:10 diet retained more nitrogen (2.00 g) which differed significantly ($p < 0.05$) from the groups of rats fed the test diets. The group of rats fed 100:0:0 diet retained the least nitrogen (0.60 g) but the group that was fed NFD had zero retained nitrogen. The biological value (BV) of the rats fed 70:20:10 diet was highest (80) than those fed other test diets. There was significant difference in the BV of the group of rats fed 100:20:10 diet and the other groups. The net protein utilization (NPU) value for 70:20:10 was highest (77%) and differed significantly ($p < 0.05$) from those of the other diets. The true digestibility (TD) of rats fed milk diet had the highest value (97.64 %) followed by that of the rats fed 70:20:10 diet (94 %) and rats fed 100:0:0 diet had the least value (64%). The TD of rats fed milk diet was significantly difference ($p< 0.05$) from that of the other test diets.

Nutrient composition of the liver of rats fed six mixed protein diet and controls was presented in Table 3. The liver weight of rats studied ranged between 4.22-7.68 g. The liver weight of rats fed 70:20:10 diet was highest (7.68 g) and was significantly different ($p < 0.05$) from those of the other diets while the liver weight of the rats fed NFD had the least value of 4.22 g. The nitrogen values of the rats were between 0.13-0.54 g. The nitrogen value of rats fed 70:20:10 diet was highest (0.54 g) and was significantly different ($p < 0.05$) from those of the rats from other groups. The result showed that the iron content of the liver of the rats ranged between 0.53-2.34 mg. The rats fed 70:25:5 diet had more iron (2.34 mg) which differed significantly ($p < 0.05$) from the other groups of rats. The zinc value of the liver of rats were between 0.06-2.50 mg. The zinc value of the rats fed 70:20:10 diet had the highest value (2.50 mg) than those fed the other diets. There was significant difference in the zinc value of the rats among all the groups.

Mineral metabolism of rats fed six mixed protein diets and controls was presented in Table 4. The result showed that the values of consumed zinc among the groups were between 0.28- 7.28 mg. The rats fed 70:5:25 diet consumed more zinc (7.28 mg) than the other groups of rats. The rats fed 70:5:25 diet lost more zinc in faeces (2.68 mg) than the other rats. None of the rats lost zinc in the urine (0.00). The rats fed 70:25:5 diet retained more zinc (5.40 mg) than the other rats which was significantly different ($p\leq0.05$) from others. The rats fed 70:20:10 diet consumed more iron (14.04 mg) than the rats fed other diets including controls and lost 0.03 mg of iron in faeces. The rats fed 70:20:10 diet retained more iron (14.01 mg) than the rats fed other test diet.

The rats fed 70:5:25 diet consumed more iodine (994.20 µg) than the rats fed other diets and it lost 78.32 µg of iodine in faeces, while the rats fed 70:25:5 diet lost 99.61 µg of iodine in faeces which is the highest value for iodine lost and it differed significantly ($p \leq 0.05$) from others. The rats fed 70:5:25 diet retained more iodine (915.88 µg) than the rats fed other diets. None of the rats fed both test diets and controls lost iodine in urine.

The growth of rats fed six mixed diets and control is presented on Table 5. The food intake of the rats ranged between 200-230 g. The rats fed 70:20:10 diet had the highest food intake of 230 g which is significantly different ($p \leq 0.05$) other test diets and controls. The rats fed 100:0:0 diet had the least value 200 g of food intake. The weight gain of rats fed both test diet and controls ranged from -12.12-52.10 g. The rats fed NFD had negative weight gain of -12.12 g while the rats fed 70:20:10 diet had the highest value of 52.10 g and there was a significant different ($p \leq 0.05$) among the groups. The protein efficient ratio (PER) fed both test diets and controls were between 0-2.60. The PER of the rats fed milk diet had the highest value of 2.60 while the rats fed NFD had zero value for PER.

4. DISCUSSION

Protein quality is the ability of a food to support body growth and maintenance. Growth is the increase in size and body weight. When a body is growing or recovering from illness it needs extra protein to build new tissues. The body constantly need protein for growth and for protein turnover. The protein requirement can be determined from the amount of protein required in diet to maintain nitrogen balance, in the case of non-growing animals, and from the amount required to support a positive nitrogen balance in the case of growing animals.

4.1 Food Intake

The food intake of rats fed six mixed diets and controls during the maintenance study ranged from 36.40-60.00 g. The result showed that the rats consumed an appreciable amount of the diets which include both the experimental diet and the controls. The high intake of NFD could be attributed to the sucrose which account for more than one -third of the diet. Ene-obong and Obizoba [22] noted that feed intake can be influenced by palatability, source of nitrogen and essential amino acid. There is a variation which did not follow a steady pattern in the food intake of the rats that consumed the experimental diets. The rate of consumption of the experimental diets by the rats showed that the complementary food will be acceptable by infants.

4.2 Maintenance Weight Gain

The rats fed 70:20:10 diet had highest maintenance weight gain (3.50 g) followed by the rats fed 70:25:5 diet (23.163.05 g) whilst the rats fed milk had (3.00 g) and the rats fed NFD recorded negative weight gain (-12.12 g). These shows that 70:20:10 diet has a better essential amino acid profile than other diets. The lowest maintenance weight gain by the rats fed NFD might be due to poor essential amino acid profile. [23] observed that weight gain of animals is partly influenced by food intake and partly by the essential amino acid pattern of the dietary protein. Poor protein quality will translate to poor protein utilization.

4.3 PER (Protein Efficiency Ratio)

Protein efficient ratio (per) is among some reliable methods of determining the protein quality of food both in infant and non-infant food products. Per is one of the commonly used methods of assessing quality of protein [24]. Protein efficient ratio is a measure of weight gain of growing animal per gram nitrogen intake. The per standard value is 2.7, which is the standard value of casein protein. The rats fed 70:20:10 diets had the highest per of 2.70, followed by the rats fed milk diet 2.60 whilst the rats fed nfd had zero per. The higher per of rats fed 70:20:10 diet might be to its eaa (essential amino acid) pattern. The zero per value of rats fed nfd was not a surprise due to zero nitrogen intake and negative weight gain. wardlaw et al. [25] observed an inter-relationship between feed intake, weight gain and per. campbell et al. [26] opined that variation in per may be due to the level of protein in the diet, different strains of rats, sex of the rats used for the test and assay period. The positive per of all the rats fed experimental diet which ranged from 1.20- 2.70 shows that all the experimental diet had a promising essential amino acid which varied because of the diet make up. there is a strong relationship between weight gain and per. The higher the weight gain, the higher the per and vice versa.

Table 1. Composition of experimental diets (g/kg)

Diet ingredients	Milk	100:0:0	70:25:5	70:20:10	70:15:15	70:10:20	70:5:25	NFD
Milk	306.32	0	0	0	0	0	0	0
100:0:0	0	774.82	0	0	0	0	0	0
70:25:5	0	0	724	0	0	0	0	0
70:20:10	0	0	0	764.82	0	0	0	0
70:15:15	0	0	0	0	787.07	0	0	0
70:10:20	0	0	0	0	0	729.93	0	0
70:5:25	0	0	0	0	0	0	810.54	0
Corn starch	250.34	0	0	0	0	0	0	403.50
Sucrose	250.34	225.18	276	235.18	212.93	270.07	189.46	403.50
Fibre	40	0	0	0	0	0	0	40
Soy bean oil	90	0	0	0	0	0	0	90
Mineral mix	50	0	0	0	0	0	0	50
Vitamin mix	10	0	0	0	0	0	0	10
L-cysteine	3	0	0	0	0	0	0	3
Total	1000	1000	1000	1000	1000	1000	1000	1000

Milk= milk diet, 100:0:0, 70:25:5, 70:20:10,70:15:15, 70:10:20, and 70:5:25 for Maize, crayfish and carrot respectively, NFD=Nitrogen free diet

Table 2. Nitrogen balance of rats fed six mixed protein diet and controls

Variables	Milk	100:0:0	70:25:5	70:20:10	70:15:15	70:10:20	70:5:25	NFD
Weight gain (g)	3.00^{b}±1.24	1.10^{f}±0.08	3.05^{b}±1.09	3.50^{a}±0.14	2.60^{c}±0.70	2.40^{d}±0.04	2.15^{e}±0.64	
Food intake (g)	40.52^{b}±10.14	42.10^{b}±2.20	36.40^{c}±0.90	38.00^{c}±0.50	38.00^{c}±0.11	40.00^{b}±0.46	41.00^{b}±0.28	60.00^{a}±0.12
Consumed N	2.45^{d}±0.52	0.98^{f}±0.02	2.64^{b}±0.04	3.40^{a}±0.22	2.51^{c}±0.01	2.06^{e}±0.83	2.03^{e}±0.68	
Faecal N (%)	0.28b±0.13	0.06e±0.0	0.31a±0.0	0.14c±0.07	0.10d±0.0	0.06e±0.02	0.00±0.0	
Absorbed N g	1.24^{d}±0.82	1.03^{f}±0.10	2.00^{b}±0.45	2.10^{a}±0.70	1.50^{c}±0.50	1.20^{de}±0.01	1.15^{e}±0.40	
Urinary N (%)	0.06^{de}±0.01	0.28^{a}±0.0	0.06^{de}±0.0	0.04^{e}±0.0	0.08^{d}±0.0	0.11^{c}±0.10	0.14^{b}±0.03	0.02^{f}±0.0
Retained N (%)	1.10^{d}±0.33	0.60^{f}±0.03	1.50^{b}±0.04	2.00^{a}±0.92	1.26^{c}±0.28	1.08^{d}±0.05	0.80^{e}±0.06	
BV	76.00^{b}±0.40	64.00^{e}±3.50	77.00^{b}±0.10	80.00^{a}±0.85	70.00^{d}±2.99	74.00^{c}±0.94	72.00^{cd}±0.99	
NPU (%)	75.00^{b}±1.23	62.00^{e}±0.95	75.00^{b}±1.43	77.00^{a}±1.33	67.00^{c}±1.96	65.00^{d}±1.54	65.00^{d}±0.98	
TD (%)	97.64^{a}±0.60	70.00^{e}±0.30	90.00^{b}±1.00	94.00^{a}±2.04	86.00^{c}±0.77	83.00^{d}±1.09	80.00^{d}±1.47	

Values are mean of 5 rats± standard deviation. Mean with different superscript letters along the same row are significantly different at P<0.05.
Sample: Milk, NFD=Nitrogen free diet, 100:0:0, 70:25:5, 70:20:10, 70:15:15, 70:10:20, and 70:5:25 for Maize, crayfish and carrot respectively. TD= True digestibility. NPU = Net protein utilization. BV= biological value. N= nitrogen. NFD= Nitrogen free diet. Corrected for endogenous nitrogen losses

Table 3. Nutrient composition of liver of rats fed six mixed protein diet and controls

Variables	Milk	100:0:0	70:25:5	70:20:10	70:15:15	70:10:20	70:5:25	NFD
Food intake (g)	219^{b}±1.27	200.00^{g}±1.88	216.00^{c}±0.31	230.00^{a}±2.76	213.00^{d}±0.98	207.00^{e}±0.76	204.00^{f}±0.45	208.12^{e}±1.09
Liver weight (g)	7.20^{c}±0.66	6.24^{g}±0.99	7.40^{b}±0.06	7.68^{a}±0.56	7.00^{d}±0.30	6.80^{e}±0.05	6.53^{f}±0.64	4.22^{h}±0.31
Nitrogen (%)	0.50^{b}±0.03	0.28^{g}±0.0	0.42^{c}±0.03	0.54^{a}±0.13	0.39^{d}±0.05	0.35^{e}±0.10	0.32^{f}±0.30	0.13^{h}±0.07
Fat (%)	0.98^{d}±0.14	0.24^{g}±0.01	1.18^{b}±0.16	1.27^{a}±0.71	1.08^{c}±0.09	0.67^{e}±0.01	0.37^{f}±0.18	0.14^{h}±0.05
Fibre (%)	0.22^{g}±0.10	0.36^{f}±0.18	1.00^{b}±0.74	1.10^{a}±0.03	0.87^{c}±0.11	0.62^{d}±0.02	0.54^{e}±0.10	0.22^{g}±0.09
Moisture (%)	0.40^{e}±0.04	0.40^{e}±0.24	0.51^{a}±0.20	0.48^{b}±0.31	0.45^{c}±0.02	0.42^{d}±0.03	0.42^{d}±0.04	0.31^{f}±0.01
Iron (mg)	1.98^{d}±0.20	1.00^{g}±0.53	2.34^{a}±0.60	2.10^{b}±0.09	2.00^{c}±0.05	1.62^{e}±0.17	1.47^{f}±0.09	0.53^{h}±0.11
Zinc (mg)	0.78^{f}±0.26	0.56^{g}±0.21	2.30^{b}±0.43	2.50^{a}±0.04	2.10^{c}±0.07	1.81^{d}±0.33	1.20^{e}±0.44	0.06^{h}±0.01
Iodine (mg)	2.50^{d}±0.13	1.26^{g}±0.28	3.09^{b}±0.33	3.50^{a}±0.24	2.74^{c}±0.45	2.33^{e}±0.20	2.10^{f}±0.12	0.80^{h}±0.34

Values are mean of 5 rats± standard deviation. Mean with different superscript letters along the same row are significantly different at P<0.05. Sample: Milk, NFD= Nitrogen free diet, 100:0:0, 70:25:5, 70:20:10, 70:15:15, 70:10:20, and 70:5:25 for Maize, crayfish and carrot respectively. corrected for endogenous nitrogen losses

Table 4. Mineral metabolism of rats fed six mixed protein diet and controls

Variables	Milk	100:0:0	70:25:5	70:20:10	70:15:15	70:10:20	70:5:25	NFD
Consumed zinc (mg)	2.89^{f}±0.46	6.00^{e}±0.32	6.30^{d}±0.73	6.50^{c}±0.49	6.50^{c}±0.21	6.92^{b}±0.09	7.28^{a}±2.07	0.28^{g}±0.004
Feacal zinc (mg)	0.16^{e}±0.03	2.50^{b}±0.04	2.20^{c}±0.09	2.05^{d}±0.04	2.04^{d}±0.03	2.22^{c}±0.02	2.68^{a}±0.12	0.01^{f}±0.0
Absorbed zinc (mg)	2.73^{f}±0.36	3.50^{e}±0.01	4.10^{d}±0.11	4.45^{c}±0.03	4.46^{c}±0.11	4.70^{b}±0.12	5.40^{a}±0.03	0.27^{g}±0.10
Urinary zinc (mg)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Retained zinc (mg)	2.73^{f}±0.36	3.50^{e}±0.01	4.10^{d}±0.11	4.45^{c}±0.03	4.46^{c}±0.11	4.70^{b}±0.12	5.40^{a}±0.12	0.27^{g}±0.10
Consumed Fe (mg)	3.06^{g}±0.04	6.10^{f}±0.50	12.00^{b}±0.36	14.04^{a}±0.29	9.71^{c}±0.36	6.92^{d}±0.34	6.50^{e}±0.32	0.12^{h}±0.01
Feacal Fe (mg)	0.65^{a}±0.01	0.60^{b}±0.01	0.05^{de}±0.0	0.03^{e}±0.01	0.11^{c}±0.07	0.07^{d}±0.0	0.02^{e}±0.0	0.10^{c}±0.01
Absorbed Fe (mg)	2.41^{g}±0.08	5.50^{f}±0.22	11.95^{b}±1.22	14.01^{a}±0.35	9.60^{c}±0.44	6.85^{d}±0.05	6.48^{e}±0.89	0.02^{h}±0.0
Urinary Fe (mg)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Retained Fe (mg)	2.41^{g}±0.08	5.50^{f}±0.22	11.95^{b}±1.22	14.01^{a}±0.35	9.60^{c}±0.44	6.85^{d}±0.05	6.48^{e}±0.18	0.02^{h}±0.0
Consumed I (µg)	276.22^{g}±1.32	553.09^{f}±0.54	632.61^{e}±0.79	720.10^{d}±1.23	860.60^{c}±1.32	910.40^{b}±2.35	994.20^{a}±0.67	11.16^{h}±0.86
Feacal I (µg)	56.34^{e}±0.66	75.15^{b}±0.78	99.61^{a}±0.63	69.43^{c}±0.38	53.30^{e}±0.56	60.80^{d}±1.34	78.32^{b}±0.89	2.50^{f}±0.02
Absorbed I (µg)	219.88^{g}±0.55	477.94^{f}±0.64	533.00^{e}±0.41	650.67^{d}±0.76	807.30^{c}±1.03	849.60^{b}±1.85	915.88^{a}±0.54	8.66^{h}±0.44
Urinary I (µg)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Retained I (µg)	219.88^{g}±1.00	477.94^{f}±0.64	533.00^{e}±0.41	650.67^{d}±0.76	807.30^{c}±1.03	849.60^{b}±1.85	915.88^{a}±0.82	8.66^{h}±0.44

Values are mean of 5 rats± standard deviation. Mean with different superscript letters along the same row are significantly different at P<0.05. sample: milk, 100:0:0, 70:25:5, 70:20:10, 70:15:15, 70:10:20, and 70:5:25 for maize, crayfish and carrot respectively. FE = iron. i = iodine. NFD = nitrogen free diet. N.D = not detected. orrected for endogenous nitrogen losses

Table 5. Growth of rats fed six mixed protein diet and controls

Variables	Milk	100:0:0	70:25:5	70:20:10	70:15:15	70:10:20	70:5:25	NFD
Weight gain (g)	49.10^{a}±1.24	20.18^{e}±0.08	42.30^{b}±1.09	48.10^{a}±0.14	40.20^{b}±0.70	38.35^{c}±0.04	35.60^{d}±0.64	-12.12^{f}±0.63
Food intake (g)	219^{b}±10.14	200.00^{g}±2.20	216.00^{c}±0.90	230^{a}±0.50	213^{d}±0.11	207.00^{e}±0.46	204.00^{f}±0.28	208.12^{e}±1.57
N intake (%)	18.98^{a}±0.52	16.82^{b}±0.02	17.20^{ab}±0.04	17.81^{ab}±0.22	16.75^{b}±0.01	15.97^{b}±0.83	15.47^{b}±0.68	
PER	2.60^{b}±0.13	1.20^{f}±0.0	2.50^{c}±0.0	2.70^{a}±0.07	2.40^{d}±0.0	2.40^{d}±0.02	2.30^{e}±0.0	

Values are mean of 5 rats± standard deviation. Mean with different superscript letters along the same row are significantly different at P<0.05.
Sample: Milk, Nitrogen free diet, 100:0:0, 70:25:5, 70:20:10, 70:15:15, 70:10:20, and 70:5:25 for Maize, crayfish and carrot respectively. PER= Protein efficiency ratio. N= nitrogen. NFD= Nitrogen free diet. Corrected for endogenous nitrogen losses

4.4 Urinary Nitrogen

The low urinary N of the rats fed experimental diets showed high digestibility and utilization of nitrogen. [22] observed that feacal and urinary nitrogen levels influence nitrogen digestibility and utilization. The low level of urinary nitrogen shows that the complementary food will provide substrate needs for protein synthesis and other biosynthetic pathways.

4.5 Retained/Absorbed Nitrogen

The rats fed 70:20:10 diet had the highest retained and absorbed N. The more absorbed and retained N of 70:20:10 diet showed that it had high protein quality than other diets. The retained and absorbed N of the rats fed 70:25:5, 70:15:15 and 70:10:20 diet had values close to the retained and absorbed N for the rats fed milk diet which is the reference protein. This showed that 70:25:5, 70:15:15 and 70:10:20 diet is of high protein quality diet. High absorbed and retained nitrogen translate to high protein quality. [27] observed that the higher absorbed and retained nitrogen is, the better the dietary protein quality.

4.6 Biological Value (BV)

Biological value measures protein quality by calculating the nitrogen used for tissue formation divided by the nitrogen absorbed from food. The biological value provides a measurement of how efficient the body utilizes protein consumed in the diet [9]. The biological value of the experimental diet ranged between 64- 80 while the biological value of the control (milk) is 76. [27] reported that a protein with a BV of 70% or more can support human growth and tissue maintenance as long as energy intake is adequate. The high BV of rats fed experimental diets except 100:0:0 suggests that protein from these diets can support human growth and maintenance. These implies that protein with a biological value of 70 and above supported body growth and maintenance.

4.7 Net Protein Utilization (NPU)

Net protein utilization is similar to the biological value except that it involves a direct measure of retention of absorbed nitrogen. Net protein utilization and biological value both measure the same parameter of nitrogen retention, however, the difference lies in that the biological value is calculated from nitrogen absorbed whereas net protein utilization is from nitrogen ingested [9]. Net protein utilization of 65 and above suggest that appreciable quantity of essential amino acid could be utilized by the body for proper growth and maintenance of the cells and tissues. The high NPU of the complementary foods were due to lower urinary output which lead to increase in retained nitrogen.

4.8 True Digestibility (TD)

True digestibility measures the percentage of the nitrogen intake absorbed by the body without considering nitrogen retained or utilized by the body. The control diet had the highest TD (97.64%) while the experimental diets had the Total digestibility values between 70-94%. The higher TD of the rats fed milk diet is not a surprise because it is a reference protein. The protein in all the experimental diets are highly digestible having the values between 70-94%. [27] observed that the most complete protein is worthless to the body if it is not digested and absorbed to yield desirable pattern of essential amino acids for new protein synthesis. The TD of rats fed test diets are of interest. The Total protein digestibility values of all the experimental diet showed that they could be digested and absorbed due to the quality of their essential amino acid.

4.9 Liver Weight

The higher liver weight of rats fed 70:25:5 and 70:20:10 diets might be due to the fact that they consumed more food (216 and 230 g) (Table 3) compared to the other groups. [28] observed that organ weights were influenced by food intake and body weight. The substantial increase in the liver weight of the rats fed tests diet showed that the diets supported growth as well as body weight.

4.10 Zinc

Zinc is one of the important minerals needed for a growing infant. The high level of zinc intake observed in the rats fed experimental diets could be as a result of the ingredients used in the formulation of the diets. Crayfish and carrot are good sources of zinc. The zinc metabolism of all the experimental diets were higher than that of the control diet (milk). Shankar and Prasad [29] observed that zinc enrichment may be beneficial for health, but excess zinc may interact with iron and copper metabolism. The rats fed experimental diets had a positive value for

retained zinc which ranged between 3.50-4.70 mg. This implies that the zinc content of the diets were absorbed and utilized. Iron (Fe) is an important constituent of hemoglobin. Iron plays numerous biochemical roles in the body, including oxygen binding in hemoglobin and acting as an important catalyst in many enzymes such as the cytochrome oxidase [30]. The iron metabolism of all the rats fed experimental diet is of interest. The Recommended Dietary Allowance (RDA) of iron for infant and children is 8 mg/day [31]. Although all the rats fed experimental diets had a better iron metabolism. [28] reported that iron intake is controlled by many factors which include: seed type, food intake and treatment. Consumption of all the experimental diets except diet 100:0:0 will help meet up with the iron requirements of infant, children and adolescent. The high metabolism of iron in the experimental diets could be due to crayfish which is an animal product that contains heam iron that is highly bioavailable.

5. CONCLUSION

In summary, the bioassay profile for all the experimental diets is of interest but diet 70:20:10 will be highly recommended for the formulation of complementary food for infants due to its outstanding performance in the protein utilization and digestibility as well as in mineral metabolism. The result of 70:20:10 diet for biological value (BV), protein efficient ratio (PER) and net protein utilization (NPU) values performed better than the control diet (milk) except in total digestibility (TD).

There is no doubt about the digestibility of the maize gruel which is due to malting that hydrolyses the proteolytic enzymes but their essential amino acid level cannot promote growth and maintain tissues and cells. In addition, they lack adequate micronutrients to curb micronutrient deficiency in infancy.

It is imperative that nursing mothers and care giver should adopt this method and use this food crops to produce complementary food of high protein quality that is nutrient dense, available and affordable to improve the nutritional status of their growing babies.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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