

Nutritional, Hematological and Biochemical Quality of Albino Rat Fed on Complementary Food Produced from Acha (*Digitaria exilis*), Malted Pigeon Pea (*Cajanus cajan*), Grasshopper (*Zonocerus variegatus*) and Beetroot (*Beta vulgaris* L.) Flour Blends

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Abstract

Complementary feeding is the introduction of other foods (solid and liquids) when breast milk alone is no longer sufficient to support the nutritional requirements for the infant. This study evaluated the nutritional and hematological quality of albino rat fed on complementary foods produced from acha, malted pigeon pea, grasshopper, and beetroot flours blends at varied ratios (100:0:0:0; 85:5:5:5; 75:15:5:5; 80:10:5:5; 75:15:5:5 and 70:5:20:5 respectively); Results shows that the incorporation of these ingredients significantly enhanced Wistar rats growth performance 11.40 to 13.94%, nitrogen retention 0.97 to 2.12%, biological value 37.09 to 78.07%, supported by superior protein and carbohydrate digestibility. The WBC 4.45 to 7.30 ($\times 10^9/L$), RBC 5.80 to 6.75 ($\times 10^{12}/L$), HB, PVC, MCV, MCH and MCHC of the blood of rat fed with produced complimentary food showed significant ($p < 0.05$) differences in all the samples. Biochemical indices TP, albumin, Na^+ , K^+ , TC mdl creatinine and urea values ranged 5.52 to 6.30 g/L, 3.07 to 3.45 g/L, 144.15 to 150.85 mEq/L, 4.41 to 5.55 mmol/L, 113.60 to 130.95 TCmdl, and 38.38 to 41.50 mg/dL, 0.58 to 0.65 mg/dL respectively with added malted pigeon pea. These findings suggest that the blends, particularly F3 and F6 (Acha75%+Malted Pigeon pea15%+Grasshopper5%+Betroot5% and Acha70%+Malted Pigeon pea5%+Grasshopper20%+Betroot5%) are nutritionally adequate, safe, and highly acceptable complementary foods with strong potential to address protein-energy malnutrition and micronutrient deficiencies in infants. The study recommends further optimization, vitamin fortification, and strict hygienic processing to enhance the quality and scalability of these complementary foods.

Keywords: Nutritional; Haematological Quality; Albino Rat; Complementary Food

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Introduction

Transitional phase known as the time of complementary feeding is when food other than breast milk is anticipated to provide an increasing percentage of a child's nutritional needs. Lots many babies and inadequate breastfeeding and complementary feeding practices have detrimental short- and long-term health consequences on young children in underdeveloped nations, which further hinders social and economic development [1]. Protein energy deficiency has been found to be highly prevalent in babies older than six months in Africa [1].

Many traditional complementary diets are mainly based on plants; cereals, or roots, and the large amounts of starches in these plant sources result in a thick, gelatinous porridge, which often has a low nutrient content [2]. In addition, mineral bioavailability is poor in many plant-based foods [3]. However, these traditional complementary foods could be improved by combining locally available plant based foods that complement each other in such a way that new patterns of amino acids are created [4]. Enrichment of cereal-based food with other protein source such as legumes, oilseeds etc, have received considerable attentions since investigations have revealed that cereals are deficient in lysine and tryptophan but have sufficient Sulphur containing amino acids which are limiting in legumes [5]. In view of these nutritional challenges, quite a number of studies have investigated ways of formulating quality complementary foods through a combination of available plant based foods to meet the nutritional demands of infants of weaning age [6-8].



Figure 1

Acha (fonio), a tropical millet native to West Africa, one of the most nutritious of all grains rich in methionine and cystine, amino acids vital to human health and deficient in today's major cereals has the advantage to be minimally processed which limited the loss of the native nutritional value during milling [9].

Acha can be used for complementary foods of low dietary bulk and high calorie density. It content 8.5% of protein, fat 3.25%, ash 3.50, fibre 3.71 and 79.00% of carbohydrate [10].

Pigeon pea (*Cajanus cajan*) is an important food legume cultivated mainly as a subsistence crop in the tropics and sub-tropics of Africa [9]. Pigeon pea is rich in protein, calcium, magnesium, crude fibre, fat, trace elements and minerals.

Beside its high nutritional value, pigeon pea is also used as traditional folk medicine in India, China and some other countries. It is capable of preventing and curing a number of human ailments such as cough, pneumonia, respiratory infections, dysentery, tooth ache, wounds among others [11]. Pigeon pea contains 20-26% protein, 1- 2% fat, 53 - 65% carbohydrate, and 7.01% ash [11].



Figure 2

Grasshoppers (*Zonocerus variegatus*) are increasingly recognized as a valuable source of nutrition, particularly as an alternative protein source. Their nutritional content varies depending on species and environmental factors (Ahmed and İnal 2024). Grasshopper contains 36 - 77% protein, 2.5 - 54.9 crude fat, 3-9% ash, 10 - 15% dietary fibre and 2.5 - 16% carbohydrate [12,13]. This makes them comparable to traditional protein sources like beef and chicken, offering about 20 grams of protein per 100 grams of weight [12].



Figure 3

Beetroot (*Beta vulgaris* L.) is a root crop belonging to the family Chenopodiaceae and it is an excellent source of red and yellow pigments [14]. Red beetroot is a rich source of minerals (manganese, sodium, potassium, magnesium, iron, and copper), vitamins (A, C, and B), phenolic compounds and betalain, which have antioxidant properties that help to protect against heart disease and certain type of cancers [15]. Beetroot contains 1.61% protein, 0.17% total fat, 2.8% fibre, 4.9 vitamins C, 23% magnesium [14].



Figure 4

Objective of the Study

The objective of this study was to evaluate the chemical composition of complementary food produced from Acha- malted pigeon pea, grasshopper and Beetroot flour.

Materials and Methods

Materials

Acha grains (*Digitaria exilis*), Pigeon pea (*Phaseolus vulgaris*), Grasshopper (*Zonocerus variegatus*) and Beetroot (*Beta vulgaris*) were purchased from Kaduna and (Jos) Plateau State Nigeria respectively. Wistar Albino rats were purchased from Veterinary Research Institute Vom Jos.

Material preparation

Preparation of acha flour

Preparation of acha flour was done by the method described by Olapade, *et al.* [16]. The grains of acha were sorted manually before water was used to wash the grains, in order to remove tiny stones, dust and other unwanted items. This was achieved by decanting the grains as they flow on the wash water. The acha was allowed to drain after which it was dried in the oven for 4hrs 50 minutes at 40°C. The milling using attrition mill model. Sieving was done using sieve of 0.3 µm aperture. The flour was packaged in air tight plastic bottles.

Production of malted pigeon pea flour

The method used was as described by Nwosu, *et al.* [17]. The pigeon pea seeds were sorted to remove dirt and other extraneous materials. Approximately 500g of the clean seeds were winnowed and thoroughly washed. These seeds were then steeped in water at ambient temperature of 29 ± 2° for 24 hours. Changing of water at 6 hours interval were observed during steeping. The resultant steeped seeds were spread on jute bag and was covered with white cotton cloth to germinate within 72 hours. The sprouted seeds were oven dried at a temperature of 50°C in order to terminate enzyme activities. The malted seeds were dried and milled into flour with an attrition mill.

Preparation of grasshopper

Nandwani [18] method was used in the preparation of grasshopper. Grasshoppers were washed to remove dirt and any unwanted parts, it was blanched in boiling water to cook them thoroughly and kill any bacteria or parasites. The boiled grasshoppers were dried under the sun and grind into a fine powder before packaging.

Preparation of beet root flour

Beetroot flour was made from fresh beetroot following the method described in the study of Aulia and Saunnurharun [19] with slight modification (blanching before drying). Beetroots were washed and blanched at 85°C for 3 minutes before peeling and slicing using kitchen knife. Beetroot slices were dried using an oven at 60°C for 12h. The dried beetroot chips were ground and sieved to obtain fine flour. The resulting flour was packaged in an airtight container prior to further use.

Production of complementary food from different Acha-pigeon pea-grasshopper-Beetroot blend flours

Complementary food was prepared from different blends of refined Acha flour, malted pigeon pea, grasshopper and beetroot in the respective ratios of 100:0:0:0; 85:5:5:0; 80:10:5:5; 75:15:5:5; 80:5:10:5, 75: 5:15: 5; 70:5:20:5 and 70:25:5 (modified recipe [9]).

Study area

The Animal experimentation was carried out at the Animal Section, Pharmacology Department, university of Jos Plateau State, Nigeria.

Animal experiment

The albino rats were housed in clean metabolic metal cages which is clean and well ventilated. Clean water was given throughout the feeding period. Vita feed Palettes concentrate were used to feed the rats for the four days acclimatization period. A total number of 35 rats (all male and of average weight of 150 - 170g each) and 7 cages were used. Each cage housed five rats. Two group of five rats were used as the control (*ogi* and *cerelac*). A total of 7 treatments of the rats were fed with the different blends of Acha-malted pigeon pea, grasshopper and beetroot flour (Table 1 and 2). At the end of the experiment (28 days), the rats were sacrificed, blood were collected for hematological and biochemical analysis. Organs that is the liver, kidney, carcass weight were determined.

Table 1: Experimental design: Proportion of acha flour, malted pigeon pea flour grasshopper flour and beetroot flour used for complementary food formulation.

Diets	Acha flour (%)	Malted Pigeon pea flour (%)	Grasshopper flour (%)	Beetroot flour (%)
C1	100	0	0	0
F1	85	5	5	0
F2	80	10	5	5
F3	75	15	5	5
F4	80	5	10	5
F5	75	5	15	5
F6	70	5	20	5

Table 2: Composition of experimental diet (g/100g).

Key: F1=85:5:5:5=Acha85%+Malted Pigeon pea5%+Grasshopper5%+Betroot5%.

F2=80:10:5:5=Acha80%+Malted Pigeon pea10%+Grasshopper5%+Betroot5%.

F3=75:15:5:5=Acha75%+Malted Pigeon pea15%+Grasshopper5%+Betroot5%.

F4=80:5:10:5=Acha80%+Malted Pigeon pea5%+Grasshopper10%+Betroot5%.

F5=75:5:15:5=Acha75%+Malted Pigeon pea5%+Grasshopper15%+Betroot5%.

F6=70:5:20:5=Acha70%+Malted Pigeon pea5%+Grasshopper20%+Betroot5%.

Ingredients	F1 85:5:5:5	F2 80:10:5:5	F3 75:15:5:5	F4 80:5:10:5	F5 75:5:15:5	F6 70:5:20:5
Corn Starch	23.73	16.86	10.46	23.73	16.86	10.46
Cerelac	-	-	-	-	-	-
Diet1	48.07	-	-	48.07	-	-
Diet2	-	54.94	-	-	54.94	-
Diet3	-	-	61.34	-	-	61.34
Diet4	48.07	-	-	48.07	-	-
Diet5	-	54.94	-	-	54.94	-
Diet6	-	-	61.34	-	-	61.34
Glucose	5.00	5.00	5.00	5.00	5.00	5.00
Sucrose	10.00	10.00	10.00	10.00	10.00	10.00
Cellulose	5.00	5.00	5.00	5.00	5.00	5.00
Vegetable oil	5.00	5.00	5.00	5.00	5.00	5.00
Min/Vit Premix	3.00	3.00	3.00	3.00	3.00	3.00
Nacl	0.20	0.20	0.20	0.20	0.20	0.20
Total	100	100	100	100	100	100

Ethical clearance certificate

An ethical clearance certificate was obtained from the Ethical committee animal experimental unit University of Jos, Plateau State with the ref. no: F17-00479.

Methods

Determination of nutrient digestibility and utilisation by albino rats fed on the diet

Preparation of Iso-nitrogenous diets for the experimental Wistar rats

The Iso-nitrogenous diet of the produced complementary foods and control samples (cerelac and ogi) were calculated with reference to 10% of protein content Iso-nitrogenous calculation. Iso-nitrogenous (IN) diets were obtained by diluting the analysed protein content of the formulated complementary foods to 10% protein level with basal diet [1].

Equations

$$IN = \frac{\text{Original protein content of food sample}}{100} \times X = \frac{10}{100} \times Y$$

Where:

X = Weight of sample required for the new feed mixture.

Y = Proposed total weight of the basal diet to be added to the feed (e.g. 100%).

The weight gained or loss, excreted faeces and urinary nitrogen was used to evaluate protein qualities of the formulated foods. And would be determined and used to calculate the nutritional quality indices; biological value (BV), nitrogen retention (NR), feed efficiency (FE), net protein utilisation (NPU), true protein digestibility (TPD) and protein efficiency ratio (PER) using the following mathematical equations as described by AOAC [20] and adopted by Ijarotimi [1].

Nitrogen retention (NR) (dietary nitrogen retained in the body):

$$NR = Ni - (Nf - Nef) - (Nu - Neu)$$

Biological value (BV):

$$BV = \frac{Ni - (Nf - Nef) - (Nu - Neu)}{Ni - (Nf - Neu)} \times 100$$

Food efficiency (FE):

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

Net protein utilisation (NPU):

$$NPU = \frac{Ni - (Nf - Nef) - (Nu - Neu)}{Ni} \times 100$$

Protein efficiency ratio (PER):

$$PER = \frac{\text{Weight gained}}{\text{Protein intake}}$$

True protein digestibility (TD):

$$TPD = \frac{N_i - (N_f - N_{ef})}{N_i} \times 100$$

Protein rating (PR):

$$PR = PER \times \text{daily protein intake (g)}$$

Where: N_i : Nitrogen intake in proteins on the test diet; N_f : Faecal nitrogen on the test diet; N_{ef} : Nitrogen excreted on nitrogen diet (metabolic nitrogen); N_u : Urinary nitrogen whilst on the test diet; N_{eu} : Urinary nitrogen excreted on nitrogen diet (endogenous nitrogen).

Hematological analysis

Blood for haematological analysis collected and drawn by cardiac puncture. After the 28 days of feeding, the animals were sacrificed using chloroform and blood samples were collected using syringe and preserved in tubes containing ethylene diaminetetraacetate (EDTA) solution. Parameters determined include packed cell volume (PCV), red blood cell, RBC, white blood cell (WBC). haemoglobin (Hb), lymphocytes, neutrophils, mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), mean cell volume, were determined using standard laboratory methods of carrying analysis in the Medical Laboratory Unit of the National Research Institute Vom, Jos, Plateau state as described by Paul., *et al.* [21].

Determination of serum biochemical parameters of the blood of rats fed on produced complementary food

Preparation of serum

Serum was separated from the blood samples by centrifugation at $3000 \times g$ for 15 minutes. The separated serum sample were transferred into dry, clean serum tubes, stoppered and stored in the refrigerator at $2-4^{\circ}\text{C}$ till it was used for testing (not more than 24h).

Automatic biochemical analyzer was used to investigate the following: Alanine aminotransferase (ALT), albumin (ALB), alkaline phosphatase (ALP), aspartates aminotransferase (AST), creatinine, globulin (GLO), glucose (GLU), glutenin, total protein (TP), urea and total cholesterol (TC). Total protein was determined calorimetrically, while albumin value was measured using the bromocresol green method. Globulin concentration was calculated as the difference between total protein and albumin. Serum glucose, total cholesterol, urea, creatinine, serum aspartate aminotransferase and alanine aminotransferase were analysed as described by Hassan., *et al.* [22].

Statistical analysis

The data obtained was analysed using Statistical Package for Social Sciences (SPSS, Version 25.0). The mean $\pm$ and standard deviations (SD) of the results was calculated. The data generated was subjected to one way analysis of variance (ANOVA). The significant means was separated using Duncan's New Multiple Range Test (DNMRT) at $p < 0.05$.

Results and Discussion

Protein quality, organ weight and energy of the albino rats fed with the produced complementary food, ogi and cerelac

The weight gained, feed intake, feed efficiency ratio (FER), nitrogen retention (NR), biological value (BV), net protein utilization (NPU), protein efficiency ratio (PER) and true protein digestibility (TPD) of the albino rats fed with the produced complementary food are shown in table 3. There were significant ($p < 0.05$) increase in all parameters. Sample C1 - F3 values ranged from 11.40 to 13.94g, 366.2 to 420.1g, 0.14 to 0.18%, 0.97 to 2.12%, 37.09 to 77.00%, 39.23 to 78.56%, 0.54 to 0.84, 38.56 to 84.44%, respectively, with increase [5

- 15%) malted pigeon pea and (5%) beetroot. Slightly higher values were recorded in sample F4 - F6 values ranged from 13.21 to 13.71g and 410.11 to 422.1g, 0.14 to 0.17, 1.57 to 1.96%, 68.72 to 78.07%, 68.00 to 80.22, 0.67 to 0.80 and 69.01 to 83.23% respectively, with increase in (5 - 20%) grasshopper and (5%) beetroot. The control samples *ogi* and *cereal* recorded values ranged from 10.34 to 14.73g, 354.9 to 513.9g, 0.5 to 0.19%, and 00.00 to 2.67%, 20.64 to 81.71, 16.61 to 83.09, 0.50 to 0.87, 19.23 to 82.24%, respectively. *Ogi* recorded lower values, while, *cerelac* is superior.

Daily weight gain of rats fed with produced samples (C1) 11.40g, (F3) 13.94g and (F6) 13.71g were lower than those fed with *cerelac* 14.73 kg but higher than *ogi* 10.34 kg the traditional gruel. The feed intake of rats fed with produced diets ranged from 366.2 to 422.1g, while *Ogi* and *cerelac* ranged from 354.9 to 513.9g making *cerelac* the most significantly ($p<0.05$) highest consumed among others. *Cerelac* had higher values in all the parameters except for TPD. *Cerelac* is formulated to provide essential nutrients that increase rate of growth, leading to greater weight gain in albino rats.

A significant difference ($P<0.05$) were observed in FER, NR, PER, and TPD with the highest values in F3 (=75:15:5:5=Acha75%+Malted Pigeon15%+Grasshopper5%+Betroot5%) this could be the effect of increased malted pigeon pea (5 - 15%) and (5%) beetroot flour in the current study and could be attributed to the enzyme degradation of macromolecules of legume protein, and carbohydrate into smaller units, thereby increasing in some substances that facilitated digestion, and subsequent absorption by the experimental rats [21]. However BV, NPU recorded higher value in F6=(70:5:20:5=Acha70%+Malted Pigeon5%+Grasshopper20%+Betroot5) due to increased grasshopper (5 -20%) and beetroot (5%). A protein material is said to be of good nutritional quality when its BV is $\geq 70\%$ and BV is 78% in this current study. These observations agreed with the reports of Ijarotimi [1]. The values in (FER), (NR), (NPU), PER, BV, TPD meet the nutrient recommended values by The Protein Advisory Group (PAG) guidelines (1991) [23]. Since the BV and PER obtained in this study is within the ranged of the set standard (BV= 0.5-1.0% and PER = 2.5-3.5), these indicated that the protein contents in the formulated complementary foods would adequately support growth and development in infant.

The significant differences observed in organ weights (heart 1.09 kg, kidney 0.8 kg, and liver 2.01 kg) of albino rats fed with different produced samples of malted pigeon pea, grasshopper and beetroot flour could be due to several factors related to nutritional composition, method of processing, bioactive compounds, and metabolic responses induced by the diets. The formulation with Acha75%+Malted Pigeon15%+Grasshopper5%+Betroot5% provide different balance of protein and energy compared to the other produced complementary food. Higher protein content can lead to increased organ growth and overall body weight due to enhanced metabolic activity and tissue synthesis [21,24]. Grasshopper contributes essential fatty acids, high quality protein and other nutrients which may influence organ weight. Beetroot contain antioxidants, rich in fibre vitamin and minerals that can impact digestion and organ health positively. Increased fibre can improve digestive health and nutrients absorption into the bloodstream, potentially impacting organ size [21,25]. Diets rich in certain nutrients can stimulate hormonal responses that promote growth in specific organs.

The weight gained of the organs of animals fed with the experimental complementary food samples were observed to be significantly higher than those animals fed on *ogi*, but found to be lower than those animals fed with *Cerelac* ($p<0.05$).

Protein and carbohydrate digestibility

Table 4 presents the protein digestibility (non-fat free nitrogen) and weight of hydrolysate. Significant differences ($p<0.05$) were observed. Non-fat free nitrogen ranged from 1.99% in C1 to 3.36% in F6, indicating enhanced digestibility with increased insect protein.

Table 3: Protein quality and energy of the albino rats produced complementary food, ogi and cerelac.

Data presented as the mean $\pm$ standard deviation with different alphabetical superscripts in the same column significantly different ($p < 0.05$).

C1=100:0:0:0 Acha.

F1=85:5:5:5=Acha85%+Malted Pigeon pea5%+Grasshopper5%+Betroot5%.

F2=80:10:5:5=Acha80%+Malted Pigeon pea 10%+Grasshopper5%+Betroot5%.

F3=75:15:5:5=Acha75%+Malted Pigeon pea 15%+Grasshopper5%+Betroot5%.

F4=80:5:10:5=Acha80%+Malted Pigeon pea5%+Grasshopper10%+Betroot5%.

F5=75:5:15:5=Acha75%+Malted Pigeon pea5%+Grasshopper15%+Betroot5%.

F6=70:5:20:5=Acha70%+Malted Pigeon pea5%+Grasshopper20%+Betroot5%.

Parameters	C1	F1	F2	F3	F4	F5	F6	Ogi	Cerelac	R-value
Weight gained (kg)	11.40 $\pm$ 0.07 ^d	12.80 $\pm$ 0.15 ^c	13.11 $\pm$ 0.65 ^{bc}	13.94 $\pm$ 0.15 ^b	13.21 $\pm$ 0.32 ^{bc}	13.49 $\pm$ 0.03 ^b	13.71 $\pm$ 0.11 ^b	10.34 $\pm$ 0.35 ^e	14.73 $\pm$ 0.09 ^a	
Feed intake(g)	366.2 $\pm$ 0.45 ^c	387.1 $\pm$ 0.54 ^c	403.6 $\pm$ 0.39 ^b	420.1 $\pm$ 0.92 ^b	410.11 $\pm$ 0.11 ^b	420.23 $\pm$ 0.06 ^b	422.1 $\pm$ 0.08 ^b	354.9 $\pm$ 0.00 ^c	513.9 $\pm$ 0.23 ^a	
FER (%)	0.14 $\pm$ 0.56 ^c	0.16 $\pm$ 0.34 ^b	0.17 $\pm$ 0.91 ^b	0.18 $\pm$ 0.34 ^b	0.14 $\pm$ 0.08 ^c	0.15 $\pm$ 0.23 ^c	0.17 $\pm$ 0.83 ^b	0.15 $\pm$ 0.45 ^c	0.19 $\pm$ 0.56 ^a	0.1 -0.5
NR (%)	0.97 $\pm$ 0.91 ^f	1.12 $\pm$ 0.71 ^e	1.50 $\pm$ 0.56 ^d	2.12 $\pm$ 0.78 ^b	1.57 $\pm$ 0.76 ^d	1.71 $\pm$ 0.23 ^c	1.96 $\pm$ 0.34 ^b	00. $\pm$ 0.00 ^g	2.67 $\pm$ 0.53 ^a	
BV (%)	37.09 $\pm$ 0.78 ^c	72.03 $\pm$ 0.45 ^a	75.12 $\pm$ 0.26 ^a	77.00 $\pm$ 0.99 ^a	68.72 $\pm$ 0.45 ^b	74.00 $\pm$ 0.56 ^a	78.07 $\pm$ 0.35 ^a	20.64 $\pm$ 0.15 ^d	81.71 $\pm$ 0.58 ^a	70 -80
NPU (%)	39.23 $\pm$ 0.56 ^c	65.78 $\pm$ 0.51 ^b	70.34 $\pm$ 0.78 ^b	78.56 $\pm$ 0.34 ^a	68.00 $\pm$ 0.23 ^b	72.43 $\pm$ 0.23 ^b	80.22 $\pm$ 0.12 ^a	16.61 $\pm$ 0.01 ^d	83.09 $\pm$ 0.06 ^a	
PER (%)	0.54 $\pm$ 0.34 ^c	0.60 $\pm$ 0.37 ^b	0.76 $\pm$ 0.39 ^a	0.84 $\pm$ 0.56 ^a	0.67 $\pm$ 0.21 ^b	0.72 $\pm$ 0.21 ^a	0.80 $\pm$ 0.34 ^a	0.50 $\pm$ 0.45 ^c	0.87 $\pm$ 0.57 ^a	2.10
TPD (%)	38.56 $\pm$ 0.21 ^c	67.23 $\pm$ 0.12 ^b	70.56 $\pm$ 0.45 ^b	84.44 $\pm$ 0.19 ^a	69.01 $\pm$ 0.09 ^b	75.26 $\pm$ 0.29 ^a	83.23 $\pm$ 0.43 ^a	19.23 $\pm$ 0.57 ^d	82.24 $\pm$ 0.35 ^a	
Organ weight (kg)										
Liver	0.90 $\pm$ 0.11 ^d	1.32 $\pm$ 0.58 ^c	1.76 $\pm$ 0.45 ^b	2.01 $\pm$ 0.67 ^a	1.38 $\pm$ 0.00 ^c	1.74 $\pm$ 0.56 ^b	1.92 $\pm$ 0.15 ^a	0.80 $\pm$ 0.23 ^d	2.10 $\pm$ 0.32 ^a	
Kidney	0.62 $\pm$ 0.34 ^c	0.70 $\pm$ 0.24 ^b	0.72 $\pm$ 0.12 ^b	0.85 $\pm$ 0.35 ^a	0.71 $\pm$ 0.01 ^b	0.79 $\pm$ 0.11 ^a	0.83 $\pm$ 0.09 ^a	060 $\pm$ 0.09 ^c	0.85 $\pm$ 0.02 ^a	
Heart	0.78 $\pm$ 0.26 ^c	0.89 $\pm$ 0.23 ^b	0.97 $\pm$ 0.67 ^b	1.09 $\pm$ 0.23 ^b	0.90 $\pm$ 0.10 ^b	0.96 $\pm$ 0.67 ^b	1.00 $\pm$ 0.34 ^b	076 $\pm$ 0.58 ^c	1.23 $\pm$ 0.05 ^a	

The weight of hydrolysate decreased with formulation, from 1.03 (C1) to 0.61 (F6), suggesting better digestion and absorption of protein and carbohydrate in blended samples. The data imply that the formulated foods were more digestible than the control.

Digestibility is a measure of how well nutrients are broken down and absorbed. The study revealed that protein digestibility was highest in F3 and F6 (88.4% and 90.1%, respectively), attributed to the high-quality proteins from grasshopper and malted pigeon pea. These values exceeded the FAO/WHO [26] recommended minimum of 85% for complementary foods, hence could improve the availability of nutrient particularly that of protein.

Carbohydrate digestibility was also high across all blends (>90%), likely due to processing methods such as malting and roasting, which break down complex carbohydrates into absorbable forms. These findings are consistent with Mensah and Tomkins [27], whose results showed that malting improves starch and protein digestibility in complementary diets.

Table 4: Protein and carbohydrates digestibility of Wister Albino rats fed with complementary foods produced from Acha malted pigeon pea grasshopper and beetroots flour blends for 28 days.

Means ($\pm$ SEM) with different alphabetical superscripts in the same column are significantly different at ($P<0.05$).

C1=100:0:0:0 Acha.

F1=85:5:5:5=Acha85%+Malted Pigeon pea 5%+Grasshopper5%+Betroot5%.

F2=80:10:5:5=Acha80%+Malted Pigeon pea 10%+Grasshopper5%+Betroot5%.

F3=75:15:5:5=Acha75%+Malted Pigeon pea 15%+Grasshopper5%+Betroot5%.

F4=80:5:10:5=Acha80%+Malted Pigeon pea 5%+Grasshopper10%+Betroot5%.

F5=75:5:15:5=Acha75%+Malted Pigeon pea 5%+Grasshopper15%+Betroot5%.

F6=70:5:20:5=Acha70%+Malted Pigeon pea 5%+Grasshopper20%+Betroot5%.

Parameters	Samples						
	C1	F1	F2	F3	F4	F5	F6
Non-fat free	1.99 $\pm$ 0.04 ^a	2.53 $\pm$ 0.04 ^{bc}	2.46 $\pm$ 0.04 ^b	2.68 $\pm$ 0.05 ^c	3.09 $\pm$ 0.04 ^d	2.67 $\pm$ 0.04 ^c	3.36 $\pm$ 0.05 ^e
Weight of hydrolysate	1.03 $\pm$ 0.02 ^g	0.81 $\pm$ 0.01 ^{bc}	0.83 $\pm$ 0.01 ^c	0.77 $\pm$ 0.01 ^b	0.66 $\pm$ 0.01 ^a	0.77 $\pm$ 0.01 ^b	0.61 $\pm$ 0.01 ^a

Haematological parameters of albino rats fed with complementary food blends

Table 5 presents the haematological parameter of albino rats fed with complementary food and control samples. The volume of blood components is an indication of availability of nutrients for synthesis of blood cells. Pack cell volume (PCV) of albino rats fed with diet with increased (5 - 15%) malted pigeon pea samples C1 -F3 ranged from 35.00 to 42.00%, while those fed with increased (5 -20%) grasshopper (F4 - F6) range showed values from 38.0 0 to 41.00%, higher than ogi 36.00% but lower than cerelac 47%. C1-F3 recorded higher Haemoglobin concentration (Hb) of 12.85 to14.60 g/dl while F4-F6 showed values 12.40 to 13.65 g/dl respectively. However, there were significant increase in Red blood cell concentrations (RBC),white blood concentration (WBC), values ranged from 5.80 to 6.75 ($\times 10^{12}$ /L), 4.45 to 7.30($\times 10^9$ /L) respectively, while decrease values were observed in Neutrophils 68.50 to 45.00%, MCV 74.50 to 65.00(fL) in samples with increased (5 -20%) grasshopper; and it is interesting to note samples with added malted pigeon pea (5-15%) significantly increased from 72.00 to 85.00 (fL) in MCV.

An increased value was observed in Lymphocytes 26.50 to 64.50%, MCH 20.50 to 27.50 pg, MCHC 26.50 to 40.00 g/dL, decreased values were observed in neutrophil 78.50 to 38.50% respectively, with increased (5-15%) malted pigeon pea. Rats fed with Ogi recorded lower values in all the parameters while those fed with cerelac recorded significant ($p < 0.05$) values ranged PCV 37.00 to 47.00%, Hb 10.30 to 12.30 g/dL, RBC 6.00 to 7.45 ($\times 10^{12}/L$), WBC 1.05 to 13.90 ($\times 10^9/L$), neutrophils 42.00 - 52.00%, lymphocytes 24.00 - 72.00%, MCHC 23.70 - 33.10 g/dL, MCH 29.70 to 30.00 pg, and MCV 65.00 to 85.00 fL respectively.

Table 5: Haematological indices of Wister Albino rats fed with complementary foods produced from Acha malted pigeon pea grasshopper and beetroots flour blends for 28 days.

Data presented as the mean $\pm$ standard deviation with different alphabetical superscripts in the same column significantly different ($p < 0.05$).

C1=100:0:0:0 Acha.

F1=85:5:5:5=Acha85%+Malted Pigeon5%+Grasshopper5%+Betroot5%.

F2=80:10:5:5=Acha80%+Malted Pigeon10%+Grasshopper5%+Betroot5%.

F3=75:15:5:5=Acha75%+Malted Pigeon15%+Grasshopper5%+Betroot5%.

F4=80:5:10:5=Acha80%+Malted Pigeon5%+Grasshopper10%+Betroot5%.

F5=75:5:15:5=Acha75%+Malted Pigeon5%+Grasshopper15%+Betroot5%.

F6=70:5:20:5=Acha70%+Malted Pigeon5%+Grasshopper20%+Betroot5%.

Sample	PCV (%)	HB (g/dL)	RBC ($\times 10^{12}/L$)	WBC ($\times 10^9/L$)	Neutrophils (%)	Lymphocytes (%)	MCH (pg)	MCHC (g/dL)	MCV (fL)
C1	35.00 $\pm$ 2.83 ^a	12.85 $\pm$ 0.92 ^a	4.55 $\pm$ 0.35 ^a	3.15 $\pm$ 0.35 ^a	62.50 $\pm$ 3.54 ^a	26.50 $\pm$ 2.12 ^a	20.50 $\pm$ 2.12 ^a	26.50 $\pm$ 2.12 ^a	72.00 $\pm$ 4.24 ^a
F1	39.00 $\pm$ 2.83 ^a	13.40 $\pm$ 1.13 ^a	5.25 $\pm$ 0.35 ^a	5.25 $\pm$ 0.49 ^b	78.50 $\pm$ 4.95 ^b	41.50 $\pm$ 2.12 ^b	25.00 $\pm$ 2.83 ^a	32.50 $\pm$ 2.12 ^b	75.00 $\pm$ 4.24 ^b
F2	40.00 $\pm$ 2.83 ^a	14.45 $\pm$ 0.92 ^a	5.75 $\pm$ 0.35 ^a	5.40 $\pm$ 0.42 ^a	55.00 $\pm$ 4.24 ^a	50.00 $\pm$ 2.83 ^a	26.50 $\pm$ 2.12 ^a	39.50 $\pm$ 2.12 ^a	78.50 $\pm$ 4.95 ^c
F3	42.00 $\pm$ 2.83 ^a	14.60 $\pm$ 0.99 ^a	5.95 $\pm$ 0.35 ^a	6.75 $\pm$ 0.49 ^c	38.50 $\pm$ 4.95 ^c	64.50 $\pm$ 3.54 ^c	27.50 $\pm$ 2.12 ^a	40.00 $\pm$ 2.83 ^a	85.00 $\pm$ 4.24 ^a
F4	38.00 $\pm$ 2.83 ^a	12.65 $\pm$ 1.20 ^a	5.80 $\pm$ 0.42 ^a	4.45 $\pm$ 0.49 ^c	68.50 $\pm$ 4.95 ^b	34.50 $\pm$ 2.12 ^b	23.00 $\pm$ 2.83 ^b	31.50 $\pm$ 2.12 ^b	74.50 $\pm$ 4.95 ^a
F5	40.00 $\pm$ 2.83 ^a	12.40 $\pm$ 1.13 ^a	6.15 $\pm$ 0.35 ^a	6.80 $\pm$ 0.57 ^c	61.00 $\pm$ 4.24 ^a	41.50 $\pm$ 2.12 ^a	21.50 $\pm$ 2.12 ^c	32.50 $\pm$ 2.12 ^b	68.50 $\pm$ 4.95 ^c
F6	41.00 $\pm$ 2.83 ^a	13.65 $\pm$ 1.06 ^a	6.75 $\pm$ 0.35 ^a	7.30 $\pm$ 0.42 ^b	45.00 $\pm$ 4.24 ^c	42.00 $\pm$ 2.83 ^c	21.50 $\pm$ 2.12 ^c	31.50 $\pm$ 2.12 ^b	67.50 $\pm$ 4.95 ^c
Ogi	36.00 $\pm$ 1.15 ^e	10.30 $\pm$ 0.58 ^d	6.00 $\pm$ 0.57 ^e	1.05 $\pm$ 0.57 ^e	42.00 $\pm$ 0.05 ^c	24.00 $\pm$ 0.03 ^f	29.00 $\pm$ 1.73 ^a	23.70 $\pm$ 1.73 ^d	65.00 $\pm$ 1.73 ^a
Cerelac	47.00 $\pm$ 1.15 ^c	12.30 $\pm$ 0.57 ^c	7.45 $\pm$ 0.58 ^c	13.90 $\pm$ 0.57 ^a	52.00 $\pm$ 0.26 ^d	72.00 $\pm$ 0.20 ^a	30.70 $\pm$ 0.58 ^a	33.10 $\pm$ 1.73 ^a	89.00 $\pm$ 1.15 ^a
R-value	37.6-50.6	11.5-16.1	6.76-9.75	6.6-12.6	45 - 75	20 - 40	16.0-23.1	28.2-34.1	50.0-77.8

The haematological variation between rats fed on samples with increase (5-15%) added malted pigeon pea, and those fed on samples with increased (5 -20%) grasshopper and control samples (ogi and cerelac) could be attributed to ingredients used, method of processing (malting and fermentation), which might have increased the bioavailability of essential nutrients such as protein and iron that are required for blood formations.

Rats fed on F1 and F3 had significantly higher PCV, Hb 42.00 and 41.00%, 14.60 and 13.65% respectively values compared to those fed with control 10.30%, suggesting improved oxygen-carrying capacity and haematopoiesis. Packed cell volume also known as haematocrit, measure the proportion of blood volume and helping in diagnosing and monitoring various condition related to blood volume [1] Hb values (13.8-14.2 g/dL) exceeded those in the control group (10.30 g/dL), indicating the blend's efficacy in preventing anaemia. These results align with Onuorah., *et al.* [28], who observed improved haematological profiles in rats fed with grasshopper-fortified diets. The WBC and neutrophil recorded higher values in samples with increase grasshopper (5-20%), implying enhanced immune status. This agrees with FAO [29] findings on the immunomodulatory effects of insect-based proteins. White blood cell play a vital role in protecting the body against diseases and infections [21]. Lymphocytes, RBC, MCH, MCHC and MCV recorded a significant ($p < 0.05$) higher values in samples with increase malted pigeon pea. The low and higher values recorded could be due to nutritional, environmental and hormonal factors. Red blood cells are essential for maintaining oxygen delivery to the body tissues [1]. Lymphocytes are a type of white blood cell that plays a key role in the immune system while, MCH measures the average amount of haemoglobin in a single red blood cell. However, rat fed with cerelac showed significant ($p < 0.05$) differences in the haematological parameters while those fed with ogi showed lower values than the produced complementary foods.

Biochemical parameters

Table 6 shows the serum biochemical indices of rats after 28 days of feeding. The parameters assessed included liver marker enzymes (AST, ALT, and ALP), total protein (TP), albumin (ALB), glucose (Glu), sodium ion (Na^+), potassium ion (K^+), total cholesterol (TCmdl), urea and creatinine. AST and ALT level showed significant ($P < 0.05$) increase in both samples with increase malted pigeon pea, and samples with increased grasshopper ranged from 33.12 to 37.26 U/L and 33.19 to 36.23 (AST) and 16.56 to 17.60 and 7.05 to 18.63 U/L (ALT) respectively, with values higher than *ogi* but low than cerelac. It was observed that ALP recorded decreased values from 77.69 to 68.56 U/L, with increased grasshopper, while samples with added malted pigeon pea recorded decrease values in glu 5.16 to 4.94 mmol/L respectively.

TP, albumin, Na^+ , K^+ , TCmdl creatinine and urea values ranged 5.52 to 6.30 g/L, 3.07 to 3.45 g/L, 144.15 to 150.85 mEq/L, 4.41 to 5.55 mmol/L, 113.60 to 130.95TCmdl, and 38.38 to 41.50 mg/dL, 0.58 to 0.65 mg/dL respectively with added malted pigeon pea. While an increase from added grasshopper ranged from 5.70 6.24 g/L, 3.59 to 3.65 g/L, 146.45 to 155.15 mEq/L, 4.25 to 5.28 mmol/L, 134.50 to 137.35TCmdl, 38.35 to 39.99 mg/dL and 0.51 to 0.62 mg/dL respectively. However, the control sample ogi recorded lower values ranged 3.58 g/L, 3.01 g/L, 82.00 mEq/L, 4.0 mmol/L, 120.00 TCmdl, 23.94 mg/dL, and 0.42 mg/d respectively. Cerelac recorded higher values from 6.4 g/L, 3.62 g/L, 143.91 mmol/L, 165.9TCmdl, 34.68 mg/dL and 0.69 mg/dL respectively.

The levels of ALT and AST, which are liver enzymes and biomarkers for liver cell health, were within normal physiological ranges in rats fed with the produced blends showing no signs of liver toxicity. These values are consistent with reference values and in line with report by Murray., *et al.* [30] and fall within acceptable limits outlined by FAO/WHO [31] for non-toxic food formulations. Studies by Akinola., *et al.* [32] and Okoye., *et al.* [33] also confirmed that legumes and insect-enriched flours do not elevate liver enzymes, demonstrating hepatic safety.

Alkaline phosphatase (ALP) increased values were observed in samples F1 - F3 ranges from 67.30 to 84.31 IU/L with increased in malted pigeon peas (5 - 15%) and beetroot (5%) blends, but decreased from 77.69 to 68.56IU/L in F4-F6 with increase in grasshopper, but still within tolerable biochemical limits. ALP is a sensitive indicator of gallbladder function and bone growth in infants.

Citation: Ayo Jerome Adekunle., *et al.* "Nutritional, Hematological and Biochemical Quality of Albino Rat Fed on Complementary Food Produced from Acha (*Digitaria exilis*), Malted Pigeon Pea (*Cajanus cajan*), Grasshopper (*Zonocerus variegatus*) and Beetroot (*Beta vulgaris* L.) Flour Blends". *EC Nutrition* 20.3 (2025): 01-17.

The levels of total protein (TP) (ranging from 5.6.2 to 6.30 g/dL) and albumin (ALB) (3.07 to 3.65 g/dL) indicate good protein nutritional status across all formulations. These values are comparable to control sample cerelac 6.40 and 3.62, however, higher than *ogi* and it supports efficient hepatic protein synthesis. The observed improvements, especially in F4-F6 (ALB), suggested a positive nitrogen balance due to high-quality proteins from edible insects (grasshopper). According to FAO/WHO [34].

Globulin (GLO) values followed the expected physiological pattern and reflect adequate immune protein synthesis. This suggests the potential immune nutritive benefit of the blends, especially those containing increased (5 -15%) malted pigeon pea and (5%) beetroot flour are known for their phytochemicals and antioxidant properties, this finding is in line with findings of Onimawo and Eboh [35] who stated that legume-based complementary foods promote immunoglobulin production in growing children. Glucose (Glu) levels decrease from 5.16 to 4.97 mg/dL) in samples C1- F3 (with increase 5-15% malted pigeon pea) due to fibre content which might slow down carbohydrate digestion and absorption hence stabilized glucose level, while samples with increased grasshopper (5-20%) F4 -F6 increased from 4.71 to 6.77. Grasshopper contain chitin which may affect gut and glucose metabolism. The moderate glycaemic response may be due to the inclusion of complex carbohydrates from acha and fiber-rich ingredients like beetroot. This agrees with reports by Adeyemi., *et al.* (2021). Serum sodium ion (Na⁺) and potassium ion (K⁺) levels remained within physiological limits, showing no electrolyte imbalance. Sodium ranged from 144.15 to 155.15 mmol/L, and potassium ranged from 4.41 to 5.35 mmol/L, aligning with WHO's ideal serum levels (Na: 4.5-5.6 mmol/L, K: 3.5-5.40 mol/L). The blends maintained electrolyte homeostasis, which is essential for neuromuscular and cardiovascular functions in infants. These findings are supported by Fayemi., *et al.* [36] and Adebayo., *et al.* [37]. Total cholesterol (TC) levels recorded lower values from 113.60 to 130.95 mg/dL) in samples C1- F3 (with increase 5-15% malted pigeon pea) due to fibre and carbohydrate content digestion and absorption hence stabilized glucose level finding in line with Ogunbanwo., *et al.* [38] and Eyinla., *et al.* [39]. According to FAO [40], acceptable cholesterol levels in infants and young children should remain below 170 mg/dL, placing the blends safely within range. Creatinine and urea are markers of kidney function. Creatinine levels ranged from 38.38 to 41.50 mg/dL, and creatinine from 0.58 to 0.65 mg/dL, suggesting that the formulated diets do not adversely affect renal function. These findings correspond to reference range established by SON [41].

Table 6: Serum biochemistry parameters of Wister Albino rats fed with complementary foods produced from Acha malted pigeon pea grasshopper and beetroots flour blends for 28 days.

Data presented as the mean ± standard deviation with different alphabetical superscripts in the same column significantly different ($p < 0.05$).

C1=100:0:0:0 Acha.

F1=85:5:5:5=Acha85%+Malted Pigeon pea 5%+Grasshopper5%+Betroot5%.

F2=80:10:5:5=Acha80%+Malted Pigeon pea 10%+Grasshopper5%+Betroot5%.

F3=75:15:5:5=Acha75%+Malted Pigeon pea 15%+Grasshopper5%+Betroot5%.

F4=80:5:10:5=Acha80%+Malted Pigeon pea 5%+Grasshopper10%+Betroot5%.

F5=75:5:15:5=Acha75%+Malted Pigeon pea 5%+Grasshopper15%+Betroot5%.

F6=70:5:20:5=Acha70%+Malted Pigeon pea 5%+Grasshopper20%+Betroot5%.

Sample	AST (U/L)	ALT (U/L)	ALP (U/L)	TP (g/L)	ALB (g/L)	Glu (mmol/L)	Na ⁺ (mEq/L)	K ⁺ (mmol/L)	TCmdl (mg/dL)	Urea (mg/dL)	Creat (mg/dL)
C1	33.21 ± 1.71 ^a	14.56 ± 0.00 ^a	67.30 ± 0.00 ^a	56.99 ± 0.00 ^a	30.76 ± 0.00 ^a	5.16 ± 0.00 ^a	144.15 ± 0.00 ^a	4.41 ± 0.00 ^a	133.60 ± 0.00 ^a	38.38 ± 0.00 ^a	0.58 ± 0.00 ^a

F1	34.16 ± 1.63 ^a	15.60 ± 0.00 ^a	72.80 ± 0.00 ^b	58.21 ± 0.00 ^a	31.84 ± 0.00 ^a	5.73 ± 0.00 ^a	146.70 ± 0.00 ^a	5.26 ± 0.00 ^a	134.72 ± 0.00 ^a	39.46 ± 0.00 ^a	0.60 ± 0.00 ^b
F2	35.26 ± 1.78 ^a	16.56 ± 0.00 ^a	74.35 ± 0.00 ^b	60.70 ± 0.00 ^a	32.46 ± 0.00 ^a	5.19 ± 0.00 ^a	148.70 ± 0.00 ^a	5.91 ± 0.00 ^a	135.25 ± 0.00 ^a	39.75 ± 0.00 ^a	0.63 ± 0.00 ^a
F3	37.23 ± 1.73 ^a	17.60 ± 0.00 ^a	84.31 ± 0.00 ^c	63.00 ± 0.00 ^a	34.56 ± 0.00 ^a	4.94 ± 0.00 ^a	150.85 ± 0.00 ^a	6.65 ± 0.00 ^a	136.95 ± 0.00 ^a	41.50 ± 0.00 ^a	0.65 ± 0.00 ^b
F4	33.19 ± 1.68 ^a	17.05 ± 0.00 ^a	77.69 ± 0.00 ^b	57.14 ± 0.00 ^a	33.59 ± 0.00 ^a	4.71 ± 0.00 ^a	146.45 ± 0.00 ^a	4.25 ± 0.00 ^b	134.50 ± 0.00 ^a	38.35 ± 0.00 ^a	0.51 ± 0.00 ^a
F5	35.12 ± 1.58 ^a	17.63 ± 0.00 ^a	71.00 ± 0.00 ^b	60.15 ± 0.00 ^a	33.22 ± 0.00 ^a	5.04 ± 0.00 ^a	149.25 ± 0.00 ^a	5.24 ± 0.00 ^a	135.10 ± 0.00 ^a	39.27 ± 0.00 ^a	0.53 ± 0.00 ^a
F6	36.23 ± 1.73 ^a	18.60 ± 0.00 ^a	68.56 ± 0.00 ^a	62.42 ± 0.00 ^a	34.85 ± 0.00 ^a	26.77 ± 0.00 ^b	155.15 ± 0.00 ^a	6.28 ± 0.00 ^a	137.35 ± 0.00 ^a	39.99 ± 0.00 ^a	0.62 ± 0.00 ^a
Ogi	16.09 ± 0.58 ^b	1.06 ± 0.58 ^b	16.30 ± 0.57 ^d	3.87 ± 0.58 ^b	3.00 ± 0.57 ^c	4.44 ± 0.09	82.00 ± 0.34	4.0 ± 0.09	120.00 ± 0.19	1.94 ± 0.58 ^b	24.23 ± 2.29 ^b
Cerelac	55.17 ± 0.60 ^a	7.55 ± 0.58 ^a	121.32 ± 0.65 ^c	4.06 ± 0.57 ^b	3.22 ± 1.15 ^c	5.00 ± 0.07	143.91 ± 0.00	5.6 ± 0.87	165.97 ± 0.76	1.86 ± 0.57 ^b	25.20 ± 1.17 ^b
R-value	45.7 - 80.5	17.5 - 30.2	56.8 - 128	5.6 - 7.6	3.8 - 4.8	3.9-6.1	135 - 145	3.5 -5.0	<200	1.5 -4.0	7.0-20.0

Conclusion

The study demonstrated that incorporating malted pigeon pea, grasshopper, and beetroot into acha-based blends significantly improved nutritional parameters, the blends supported growth, protein efficiency ratio, BV, TP, NR and nutrient utilization in Wistar rats, with protein and carbohydrate digestibility surpassing FAO/WHO standards. Haematological and biochemical assessments confirmed improved blood indices, normal organ function, and absence of toxicity, validating the nutritional safety of the blends. Animals fed with Cerelac exhibited higher values than those fed with produced diet, but ogi recorded lower values.

Overall, the formulated blends, particularly F3 and F6, emerged as nutritionally adequate, safe, and highly acceptable complementary foods with strong potential to combat protein-energy malnutrition and micronutrient deficiencies in infants.

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