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Saidu Y
Department of Biochemistry, Usmanu Danfodiyo University, Sokoto, Nigeria

Isa SA
Department of Biochemistry, Usmanu Danfodiyo University, Sokoto, Nigeria

Jibrin B
Department of Paediatrics, Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria

Turaki AA
Department of Biochemistry and Molecular Biology, Federal University, Birnin Kebbi, Kebbi, Nigeria

Yusuf AB
Department of Biochemistry and Molecular Biology, Federal University, Birnin Kebbi, Kebbi, Nigeria

Correspondence
Yusuf AB
Department of Biochemistry and Molecular Biology, Federal University, Birnin Kebbi, Kebbi, Nigeria

Nutritional quality assessment of optimized cereal-based complementary foods fortified with *Moringa oleifera*

Saidu Y, Isa SA, Jibrin B, Turaki AA and Yusuf AB

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Abstract
Childhood malnutrition is a household issue and urgent health challenge in Nigeria. In an attempt to address this challenge, the current study evaluated the effect of optimizing blending ratio of cereals and legumes on nutritional quality, storage stability and determined the most effective blend based on selected nutritional profiles. Optimal design mixture using Nutrisurvey (2007) was used to generate six complementary foods (CF) designated F1, F2, F3, F4, F5, and F6 from yellow maize (*Zea mays*), wheat (*Triticum aestivum*), millet (*Pennisetum glaucum*), groundnut (*Arachis hypogea*), soyabeans (*Glycine max*), and Moringa oleifera to meet nutritional specification in codex guideline for cereal based complementary foods. A basal diet was also formulated as control. Forty nine (49) wistar rats (21–28 days old) were divided into 7 groups and fed with the complementary formulations *ad libitum* for 4 weeks. Feed intake and change in weight were recorded. The urine and faeces were collected for nitrogen content determination. The data obtained was used to calculate various protein quality indices. Upon completion of the feeding trial, the wistar rats were sacrificed, and organs excised for subsequent biochemical analysis. Data was subjected to one way analysis of variance (ANOVA) and results expressed as means± standard error of mean. Protein quality indices revealed that protein efficiency ratio ranged from (2.47-2.69), net protein ratio (3.64-3.79); while the biological value ranged from (93.06-98.21%). The true protein digestibility and net protein utilization were in the range of (76.02-84.46%), (77.85- 85.66%) respectively. The storage stability profile of the formulated CF at baseline and end line were not significantly different ($P > 0.05$). F6 (55% millet based CF) was found to have the highest performance for growth parameters evaluated. Therefore, this study has revealed that with proper blending of local foodstuff, it is possible to prepare nutritionally adequate CF.

Keywords: malnutrition, optimization, blending ratio, nutritional quality and complementary foods

1. Introduction

Malnutrition simply refers to a deviation from adequate or optimal nutritional status which may result from inadequate or excess amount of essential nutrients (Helen and Demewez, 2017) [13]. Most often, malnutrition is used to refer to undernutrition when there is inadequate supply of calories, proteins, or micronutrients. The World Health Organization, defined malnutrition as the cellular imbalance between the supply of nutrients and energy and the body's demand for them to ensure growth, maintenance and specific functions (WHO, 2006) [30]. This implies a disparity between the amount of nutrients that the body needs and the amount that it is receiving. One such form of malnutrition is protein energy malnutrition (PEM). In Nigeria and other low countries, PEM is a problem of public health concern most especially among the indigents (Onofiok and Nnanyelugo, 2012). This problem is persistent because of a faltering economy and low purchasing power of so many who live on the breadline. Literature report indicates that children begin to experience malnutrition shortly after the first six months of exclusive breast-feeding (Akinola *et al*., 2014) [5]. This period is often characterized by growth faltering (Onofiok and Nnanyelugo, 2012; Adeoye *et al*., 2018) [3]. Therefore, quality complementary foods must be introduced to sustain growth and prevent malnutrition (Steve and Olufunke, 2012) [26].
Complementary food is any solid or liquid food with nutritional value other than breast milk, offered to breast-fed infants (Eucharia *et al*., 2020) [10]. In Kebbi and other North-western of Nigeria, poor nutrition during this vulnerable period of childhood is the principal cause of protein energy malnutrition (Joy *et al*., 2012) [16]. The predominant foods used for complementary feeding are mainly gruels from starchy cereals and non-cereal starchy tubers. These complementary foods are grossly inadequate when compared to estimated needs (Joy *et al*., 2012; Adepoju and Etukumoh, 2014) [16, 4].

It is pertinent to note that the quality of weaning foods is among the major drivers of optimal nutrition in children within 6-59 months. Literature report revealed several attempts to improve nutritional value of complementary foods. While some researches arbitrarily varied the ratio of the blends of starchy cereals with different legumes, others evaluated effect of processing methods on nutritional quality (Adeoye *et al*., 2018; Brixi, 2018) [3, 7]. Therefore, the thrust of this study is to enhance nutritional quality by optimizing the blending ratio.

2. Materials and Methods

2.1 Procurement and Processing of Food Materials

The food materials (Maize, Wheat, Millet, Groundnut, Soya beans, and *Moringa oleifera* leaves) were purchased from Birnin Kebbi New Market, Kebbi State; and authenticated in Department of Biology Federal University, Birnin Kebbi. The maize, wheat, millet, groundnut, and soya beans were manually sorted to remove stones and dirt. This was followed by roasting of the cereals for about 10 to 15 minutes. The groundnuts were dehulled after roasting. Soya beans were soaked for about six hours, then dehulled and blanched for about 15 minutes, dried and roasted. The moringa leaves were manually sorted to remove stones and dirt. The leaves were washed to remove dirt and soaked in 1% saline solution (NaCl) for 5 minutes to get rid of microbes. The leaves were drained of water and shade dried. The dried leaves were ground and sieved.

2.2 Chemicals and reagents

The chemicals and reagents utilized for the current study were of analytical grade and manufactured by Randox Laboratories.

2.3 Experimental Animals

Wistar rats (*Rattus norvegicus*) were used for the study. This species was selected because its adaptability as well as the possibility to determine the nutritional quality of the component studied within few days (Theopihile *et al*., 2017) [27]. All experiment with animals complied with arrive guidelines for care and use of laboratory animals (Oibiokpa *et al*., 2018) [20].

2.4 Determination of amino acid profile

The method of AOAC (2005) [6] was used to determine amino acid profile. Amino acid score was calculated according to FAO (2018) [12] and essential amino acid index was calculated using method of Riza *et al*. (2015) [24].

2.5 Bioevaluation of Protein Quality

The current study used methods described by Oibiokpa *et al*. (2018) [20] to assess growth, nitrogen balance and protein digestibility parameters.

2.5.1 Formulation of the Complementary Foods

Nutrisurvey software (2007) was used to optimize the proportion of various ingredients in the composite blends as indicated in Table 1.

Table 1: Composition of the Formulations per 100g dry weight

Ingredients	F1	F2	F3	F4	F5	F6
Maize	67.00	55.00	-	-	-	-
Wheat	-	-	67.00	55.00	-	-
Millet	-	-	-	-	67.00	55.00
Soybean	8.00	10.00	8.00	10.00	8.00	10.00
Groundnut	22.50	32.50	22.50	32.50	22.50	32.50
Moringa	2.50	2.50	2.50	2.50	2.50	2.50

2.5.2 Experimental Design

The feeding trial was conducted using forty nine (49) apparently healthy wistar rats (3-4 weeks). Each animal was housed individually in cages at ambient temperature. The rats were allowed to acclimatize for 5 days before they were randomly distributed into seven groups. Group F1: animals received (67%) maize based complementary food. Group F2: animals received (55%) maize based complementary food. Group F3: animals received (67%) wheat based complementary food. Group F4: animals received (55%) wheat based complementary food. Group F5: animals were fed received (67%) millet based complementary food. Group F6: animals fed (55%) millet based complementary food. Group F7: animals received basal diet and water *ad libitum* each day for 4 weeks respectively. Upon completion of the feeding trial, the rats were anaesthetized with chloroform and sacrificed. Portions of liver were removed for gene expression analysis.

2.5.3 Determination of daily Feed Intake

The method of Addas *et al*. (2010) was used to determine mean daily feed intake using the formula:

$$\text{MDFI} \ \frac{\text{Total quantity of food consumed}}{\text{Number of days of feeding}}$$

2.5.4 Determination of daily weight gain

Initial weights of the rats were taken at the beginning of the experiment and afterwards on 2 day interval throughout the 4 weeks period of the study. The weight gained was obtained by subtracting the initial weight from the final weights (Addas *et al*., 2010). Average daily weight gain was thus calculated using the formula:

$$\text{DWG} \ \frac{\text{Weight gain during feeding trial}}{\text{Duration of feeding}}$$

2.5.5 Calculation of Protein Efficiency Ratio (PER)

PER was calculated according to Shikiri *et al*. (2015).

$$\text{PER} = \frac{\text{Weight gain}}{\text{Total protein intake}}$$

2.5.6 Calculation of Net Protein Ratio (NPR)

The net protein was calculated according to Oibiokpa *et al*. (2018) [20] using the formula:

$$\text{NPR} = \frac{\text{Weight gain of the test animal (g)} + [\text{Weight loss of the protein free test animal (g)}]}{\text{Weight of test protein consumed (g)}}$$

2.5.7 Calculation of digestibility parameters

$$AD = \frac{\text{Nitrogen in feed} - \text{Nitrogen in faeces}}{\text{Nitrogen in feed}}$$

$$TD = \frac{I - (F - FK)}{I}$$

$$NPU = \frac{I - (F - FK) - (U - UK)}{I}$$

$$BV = \frac{I - (F - FK) - (U - UK)}{I - (F - FK)}$$

Where: I = intake nitrogen, F = Faecal nitrogen, F_K = endogenous faecal nitrogen, U = urinary nitrogen, U_K = endogenous urinary nitrogen (Hsu *et al.*, 2014) [14].
PDCAAS = True Protein Digestibility x limiting Amino Acid Score (FAO, 2011).

2.6 Gene Expression analysis

Primers were designed on the primerblast software of the National Center for Biotechnology Information website, using the *Rattus norvegicus* gene sequences. The primers were supplied by Bioneer (Daejeon, South Korea), and diluted using RNAse free water prior to gene expression study.

Table 2: Sequences of the primers used for the real-time RT-PCR gene expression quantification

Gene Name	Left (5'-3')	Right (5'-3')
Retinol Binding Protein	GTTTCTCTGGGCTCTGGTATG	GCGCTCATATGACCCTTCTC
Growth Hormone	GAAACTGAAGGACCTGGAAGAG	GTTGGCGTCAAACTTGTCATAG
GAPDH	GCAAGGATACTGAGAGCAAGAG	GGATGGAATTGTGAGGGAGATG

GAPDH-Glyceraldehyde 3-phosphate dehydrogenase (housekeeping gene)

2.6.1 RNA Extraction

RNA was extracted using the total RNA isolation kit (RBC Biotech Corp., Taipei, Taiwan) according to manufacturer's protocol, and diluted to 10ng/mL. RNA concentration and purity were assessed on a nanospectrophotometer.

2.6.2 Protocol of Polymerase Chain Reaction (PCR)

RT and PCR were done using the NEB Luna Universal one-step qPCR reagent. Briefly, 10ng of RNA was added to 10uL of Sybr mastermix, 0.4uM of each primer (left and right), 1uL of RT enzyme and 7uL of water. The sample was loaded on a Rotorgene Q realtime PCR machine (Qiagen) and the following protocol was used: 55°C for 10 min for RT, and for PCR, an initial denaturation at 95 °C for 5 min, followed by three-step cycles (40 cycles) of 95 °C for 10 s, 56 °C for 5 s and 72 °C for 10 s, ending in a single extension cycle of 74 °C for 5 min. Finally, the gene expression results were finally normalized using GAPDH as reference gene.

2.7 Statistical Analysis

Data were reported as means ± standard error of mean of triplicate determinations. Values were subjected to one way analysis of variance (ANOVA). $P<0.05$ was considered significant. Values were analyzed statistically using Graph Pad PRISM version 6.05 software (Statcon, Witzenhausen, Germany).

3. Results

The result of the amino acid profile was presented in Table 3. Test formulations were found to contain all the 20 standard amino acids; they are therefore sources of both essential and non essential amino acids. The most abundant essential amino acid was arginine; while the least abundant was methionine.
The values of amino acid arginine and histidine are slightly higher than the recommended values of FAO/WHO (2004) [31].

Table 3: Essential Amino acid Profile of the Optimized Cereal-based Complementary Foods

Amino acid	F1(g/100g)	F2(g/100g)	F3(g/100g)	F4(g/100g)	F5(g/100g)	F6(g/100g)
Phenylalanine	4.08±0.01	3.28±0.57	3.55±0.03	3.55±0.06	3.90±0.06	3.37±0.04
Valine	3.97±0.06	3.68±0.01	3.57±0.58	3.68±0.06	4.33±0.19	3.21±0.01
Tryptophan	0.79±0.07	0.94±0.01	0.94±0.23	0.84±0.05	1.10±0.06	0.84±0.02
Histidine	3.00±0.58	2.24±0.14	2.30±0.10	2.81±0.01	2.36±0.21	2.17±0.01
Arginine	4.99±0.02	4.82±0.01	5.16±0.14	4.47±0.23	5.55±0.01	4.99±0.02
Leucine	10.62±0.05	6.30±0.01	8.00±0.02	9.80±0.03	6.83±0.01	7.70±0.04
Lysine	3.02±0.20	3.13±0.10	3.39±0.10	3.55±0.20	3.45±0.57	3.23±0.20
Threonine	3.00±0.10	3.83±0.03	3.39±0.01	3.33±0.01	4.22±0.01	3.27±0.02
Isoleucine	3.40±0.57	3.27±0.03	3.34±0.01	3.14±0.01	3.65±0.01	3.27±0.02
Methionine	1.23±0.01	1.28±0.01	1.28±0.02	1.17± 0.03	1.44±0.02	1.23±0.01

Values are mean ± standard error of mean (SEM) of triplicate determinations. F1, F3, and F5 contain 67% of Maize, Wheat, and Millet respectively; 22.5% of Groundnut, 8% Soybeans and 2.5% *Moringa*. F2, F4 and F6 contain 55% of Maize, Wheat and Millet respectively; 32.5% Groundnut, 10% Soybeans, 2.5% of *Moringa*.

Table 4 present percentage chemical scores of essential amino acids of the optimized cereal-based complementary foods. The score for arginine, histidine leucine and Isoleucine were the highest and methionine recorded the lowest score for all the formulations.

Table 4: Percentage Chemical Scores of Essential Amino Acids of the Optimized Cereal-based Complementary Foods

Amino acid	F1	F2	F3	F4	F5	F6
Phenylalanine	64.76	52.06	56.35	56.35	61.90	53.49
Valine	113.43	105.14	102.00	105.14	123.71	91.71
Tryptophan	86.90	85.45	85.45	76.36	100.00	76.36
Histidine	157.89	117.89	121.05	147.89	124.21	114.21
Arginine	249.50	241.00	258.00	223.50	277.50	249.50
Leucine	160.91	95.45	121.21	148.48	100.44	116.67
Lysine	52.07	53.97	58.45	61.21	59.48	55.69
Threonine	88.24	112.65	99.71	97.94	124.12	96.18
Isoleucine	121.43	116.79	119.29	112.14	130.36	116.79
Methionine	49.20	51.20	51.20	46.80	57.60	49.20

Values are mean ± standard error of mean (SEM) of triplicate determinations. F1, F3, and F5 contain 67% of Maize, Wheat, and Millet respectively; 22.5% of Groundnut, 8% Soybeans and 2.5% *Moringa*. F2, F4 and F6 contain 55% of Maize, Wheat and Millet respectively; 32.5% Groundnut, 10% Soybeans, 2.5% of *Moringa*

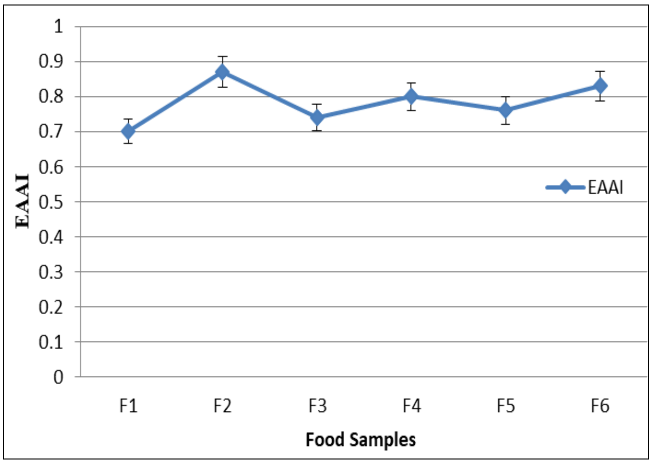

Fig 1: Essential Amino Acid Index (EAAI) of the Optimized Cereal-based Complementary Food Formulations

Values are mean ± standard error of mean (SEM) of triplicate determinations. F1, F3, and F5 contain 67% of Maize, Wheat, and Millet respectively; 22.5% of Groundnut, 8% Soybeans and 2.5% *Moringa*. F2, F4 and F6 contain 55% of Maize, Wheat and Millet respectively; 32.5% Groundnut, 10% Soybeans, 2.5% of *Moringa*. Values can be classified according to EAAI≥0.95: indicate high quality; 0.86-0.95: good quality; 0.75-0.86: useful; ≤0.75: inadequate (Riza *et al*., 2015) [24]. F2 and F1 had the highest and lowest score respectively. F4, F6 and F5 had score within the range of 0.75-0.86

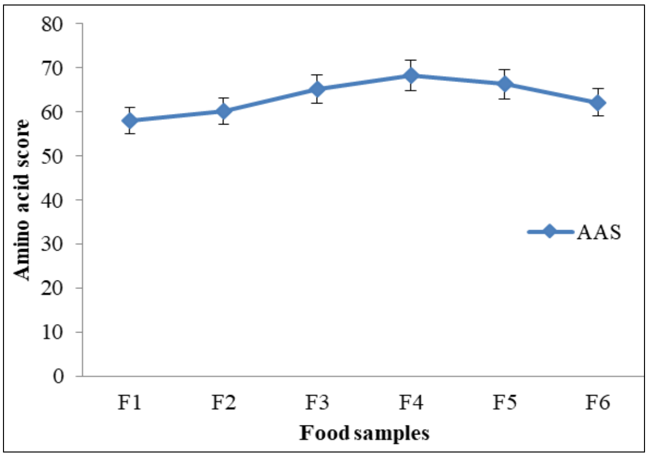

Fig 2: Amino Acid Score of the Optimized Cereal-based Complementary Foods

Values are mean ± standard error of mean (SEM) of triplicate determinations. F1, F3, and F5 contain 67% of Maize, Wheat, and Millet respectively; 22.5% of Groundnut, 8% Soybeans and 2.5% *Moringa*. F2, F4 and F6 contain 55% of Maize, Wheat and Millet respectively; 32.5% Groundnut, 10% Soybeans, 2.5% of *Moringa*. Amino Acid Score (AAS)

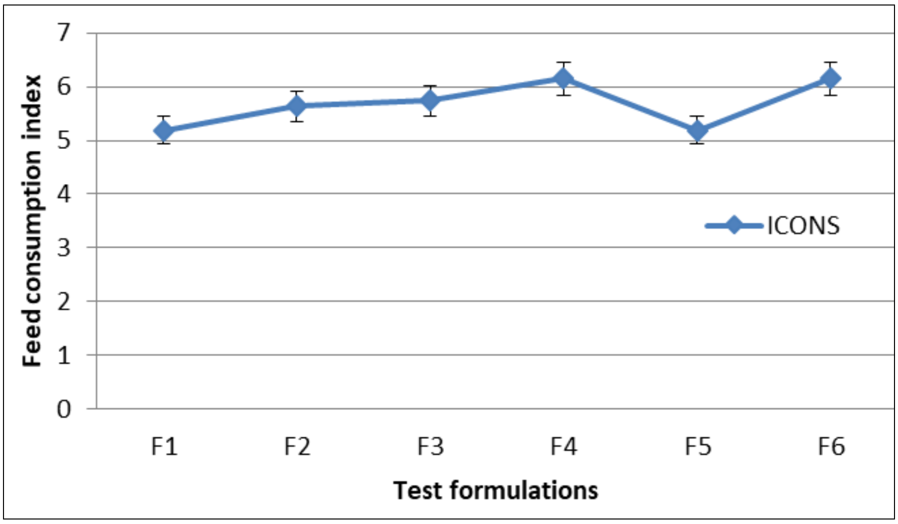

Fig 3: Feed consumption index of the rats fed the Experimental Diets

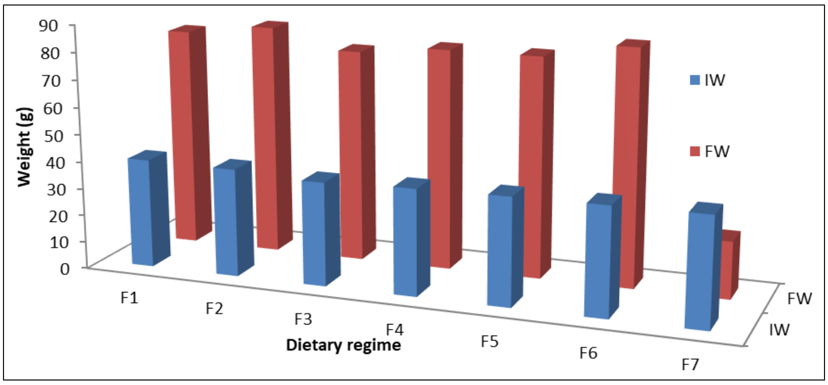

Fig 4: Change in weight of wistar rats fed the experimental diets

Values are mean ± standard error of mean (SEM) of triplicate determinations. F1, F3, and F5 contain 67% of Maize, Wheat, and Millet respectively; 22.5% of Groundnut, 8% Soybeans and 2.5% *Moringa*. F2, F4 and F6 contain 55% of Maize, Wheat and Millet respectively; 32.5% Groundnut, 10% Soybeans, 2.5% of *Moringa*. IW- Initial weight FW- Final weight

Table 5: Growth response of Albino Rats fed with the Optimized Cereal-based Complementary Foods

Groups	WG	PER	C-PER	FER	NPR	PRE
F1	40.90±0.55	2.55±0.18	2.22±0.18	10.27±0.15	3.70±0.12	59.20±0.33
F2	45.00±0.21	2.65±0.20	2.30±0.22	10.56±0.21	3.74±0.26	59.84±0.45
F3	40.00±0.15	2.47±0.16	2.15±0.17	11.18±0.27	3.64±0.31	58.24±0.35
F4	43.00±0.31	2.53±0.23	2.20±0.11	11.33±0.43	3.65±0.29	58.40±0.29
F5	41.50±0.34	2.56±0.15	2.23±0.15	10.14±0.25	3.76±0.21	60.16±0.46
F6	46.65±0.57	2.69±0.19	2.34±0.21	11.38±0.46	3.79±0.33	60.64±0.57

Values are mean ± standard error of mean (SEM) of triplicate determinations. F1, F3, and F5 contain 67% of Maize, Wheat, and Millet respectively; 22.5% of Groundnut, 8% Soybeans and 2.5% *Moringa*. F2, F4 and F6 contain 55% of Maize, Wheat and Millet respectively; 32.5% Groundnut, 10% Soybeans, 2.5% of *Moringa*. WG=Weight gain, PER= Protein efficiency ratio, C-PER PER= Protein efficiency ratio, FER=Feed efficiency ratio, NPR= Net protein ratio, PRE= Protein retention efficiency

Table 6: Biological value and Net protein utilization of rats the Optimized Cereal-based Complementary Foods

Group	NPU (%)	BV (%)
F1	96.96±0.30	84.84±0.51
F2	93.06±0.56	77.85±0.20
F3	94.76±0.27	78.07±0.31
F4	98.21±0.57	81.91±0.28
F5	97.19±0.21	85.66±0.45
F6	96.30±0.36	81.99±0.30

Values are mean ± standard error of mean (SEM) of triplicate determinations. F1, F3, and F5 contain 67% of Maize, Wheat, and Millet respectively; 22.5% of Groundnut, 8% Soybeans and 2.5% *Moringa*. F2, F4 and F6 contain 55% of Maize, Wheat and Millet respectively; 32.5% Groundnut, 10% Soybeans, 2.5% of *Moringa*. NPU= Net protein utilization and BV= Biological value

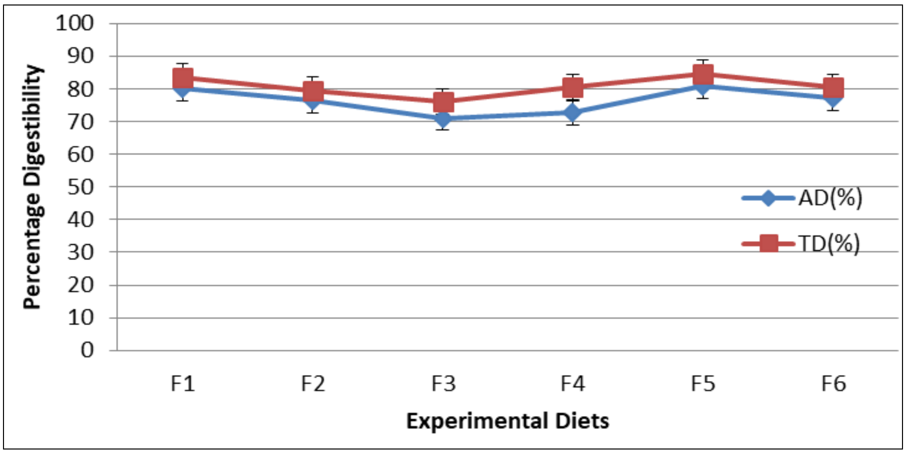

Fig 5: Variations in Apparent and Real Digestibility of the Optimized Cereal-based Complementary Foods

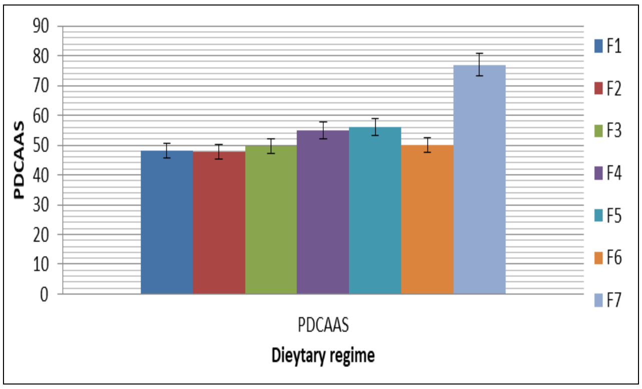

Fig 6: Comparison of PDCAAS of the test formulations with RUTF

Values are mean ± standard error of mean (SEM) of triplicate determinations. F1, F3, and F5 contain 67% of Maize, Wheat, and Millet respectively; 22.5% of Groundnut, 8% Soybeans and 2.5% *Moringa*. F2, F4 and F6 contain 55% of Maize, Wheat and Millet respectively; 32.5% Groundnut, 10% Soybeans, 2.5% of *Moringa*. F7- Recommended value for Cereal-based RUTF (FAO/WHO, 2018) [12]

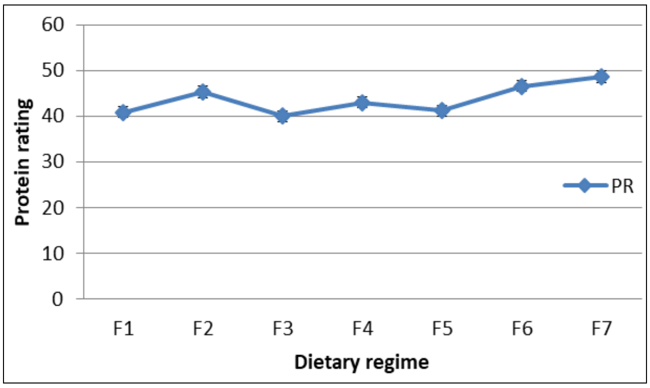

Fig 7: Comparison of Protein rating of the Optimized cereal-legume based complementary foods with Casein

Values are mean ± standard error of mean (SEM) of triplicate determinations. F1, F3, and F5 contain 67% of Maize, Wheat, and Millet respectively; 22.5% of Groundnut, 8% Soybeans and 2.5% *Moringa*. F2, F4 and F6 contain 55% of Maize, Wheat and Millet respectively; 32.5% Groundnut, 10% Soybeans, 2.5% of *Moringa*. F7- Casein; PR- protein rating

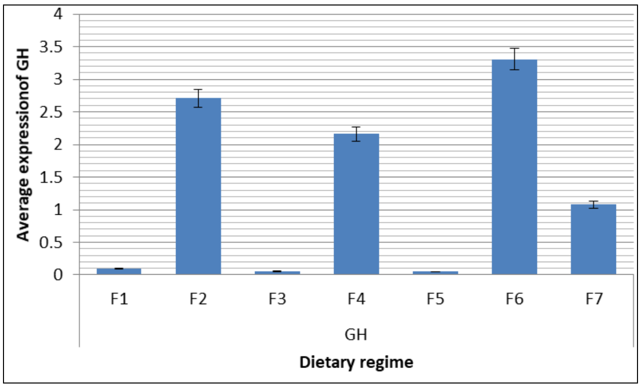

Fig 8: Expression of Growth hormone in Rats fed Optimized Cereal based Complementary Foods

Values were expressed as mean ± SEM. F1, F3, and F5 contain 67% of Maize, Wheat, and Millet respectively; 22.5% of Groundnut, 8% Soybeans and 2.5% *Moringa*. F2, F4 and F6 contain 55% of Maize, Wheat and Millet respectively; 32.5% Groundnut, 10% Soybeans, 2.5% of *Moringa*. F7- protein free diet, GH- Growth hormone

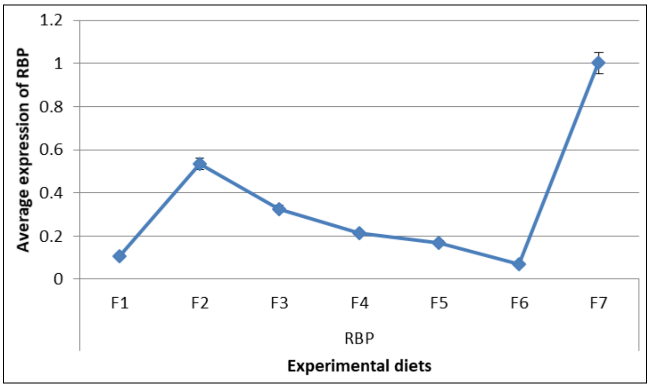

Fig 9: Expression of RBP Gene in Rats fed Optimized Complementary Foods

F1, F3, and F5 contain 67% of Maize, Wheat, and Millet respectively; 22.5% of Groundnut, 8% Soybeans and 2.5% *Moringa*. F2, F4 and F6 contain 55% of Maize, Wheat and Millet respectively; 32.5% Groundnut, 10% Soybeans, 2.5% of *Moringa*. F7- protein free diet, RBP- Retinol binding protein, values were expressed as means± SEM

Table 7: Nutritional ranking of the test formulations using selected parameters

Parameter	F1	F2	F3	F4	F5	F6
PER	4	3	6	5	2	1
NPR	4	3	6	5	2	1
PRE	4	3	6	5	2	1
C-PER	4	2	6	5	2	1
NPU	2	6	5	4	1	2
GH	3	3	3	2	3	1
TD	2	3	4	3	1	2
PDCAAS	5	6	4	2	1	3
AAS	6	5	2	1	3	4
EAAI	6	1	5	3	4	2
Total	40	35	47	35	21	19

Most desirable (1) to least desirable (6)

4. Discussion

In formulating complementary foods, the guiding principles are: it should be calorie dense to complement breast milk, acceptable, affordable, as well accessibility (Dewey and Brown, 2003) [9]. This is very crucial for countries like Nigeria where malnutrition in children is precipitated by low income, poor dietary choices, inadequate health care facilities as well as diseases which may impair adequate utilization of nutrients in food substances (Adeoye *et al.*, 2018) [3].

Nnam (2002) [18] suggested the use of selected inexpensive cereals and legumes in the formulation of composite blends of complementary foods for children in the complementary feeding age bracket. Literature report indicates that cereals are limiting in some basic amino acid like lysine as well as aromatic amino acid like tryptophan which are essential and must be provided via dietary intake. However, cereals contain appreciable amount of cysteine and methionine. Hence cereals may be taken in combination legumes which are plant sources of cheap proteins as well as better sources of tryptophan and threonine. Therefore, a blend of cereal and legume would produce CF with superior amino acid profile than only cereal based CF. However, double mix of cereals and legumes may still be nutritionally inadequate because of their micronutrients (Osagie and Eka, 1998) [22].

Children need to be fed the right quality and quantity of nutrients. With proper selection and combination, fruits and vegetables could lead to significant improvement in the micronutrient content of CF. In Nigeria, composite blends of cereals and legumes have been formulated and utilized for complementary feeding. However, these cereal-legume blends are inadequate in some minerals and vitamins (Osagie and Eka, 1998) [22]. Hence, the need for triple or quadrimix consisting of cereals, legumes, and vegetables like moringa that would provide a superior blend of macro and micronutrients.

4.1 Amino acids content of the formulations

Table 3 presents the amino acid content of the formulations under investigation. The current study reveals that all the essential amino acids are present in the six CF formulations and they met the FAO/WHO reference values. Similar findings were reported by Mansour (1996) [17] and Hussein (2000) [15]. The selection and combination of selected cereals, legumes, and moringa to formulate the CF under investigation gave efficient complementation of most of the essential amino acids. The CF formulations were not deficient in any of the essential amino acid. Even though literature report indicates that cereals have limiting in some essential amino acid like tryptophan and lysine. The inclusion of legumes (soya beans and ground nut) in the blends resulted in better amino acid profile of the formulations under investigation.

4.2 Weight Gain

The mean weight gain per group of rats fed the test formulations were within 40.0g and 46.65 which correspond to mean daily weight in range 1.43 and 1.67g respectively after 28 days of feeding trial. Whereas the group on protein free diet recorded weight loss of 21g which translates to mean daily weight loss of 0.69g during the trial. These values are similar to the values reported by a previous study (Adeoti *et al*., 2018) [2]. Overall; the groups receiving the test formulations recorded over 50% weight gains during the feeding trial. This finding reflects the preliminary phase of our research that indicated that gruel from the composite blends under investigation supported growth and recovery in moderately malnourished children. Worthy of note, is that the test groups did not differ ($P > 0.05$) in weight gain during the feeding trial. However, F6 had the highest value.

4.3 Evaluation of Protein Nutritive Quality

The nutritive quality of protein can be assessed by several methods. One of such is protein efficiency ratio. Protein efficiency ratio measures gain in weight of growing animals in relation nitrogen intake. Oibiokpa *et al.* (2018) [20] opined that it reflects the capacity to digest a test protein as well as the availability upon absorption. PER is among the reliable methods for estimating the nutritional value of sources of proteins infants as well as non-infant food products.

Results obtained for the PER of the test formulations revealed F6 could be of greater nutritional significance in comparison with the other formulations. There is a strong relationship between weight gain and PER (Shikiri *et al*., 2015; Adeoti *et al*., 2018) [2]. Protein Advisory Group (1971) [23] guidelines recommended a protein efficiency ratio of not less than 2.10 and preferably greater than 2.30 for weaning food. This study recorded greater than 2.30 recommended by PAG. Even though our values were lower than the values for weaning food formulated from fermented popcorn by Steve and Olufunke (2012) [26].

Feed efficiency ratio (FER) is another method that assesses gain in weight of growing animals in relation to feed intake. While feeds with efficiency ratio support gain in weight of growing animals; the reverse is obtainable with feeds with low values for efficiency ratio. Feeds with low efficiency ratio often support energy expenditure rather gain in weight (Bryrd-Brdbenner *et al*., 2009) [8]. The FER of the formulations under investigation showed similar trend with the PER. Hence as obtained for PER, F6 also had the highest value.

4.4 True protein digestibility (TPD)

True digestibility measures the amount of nitrogen ingested that is absorbed without putting into consideration the amount of nitrogen the body made use of or keep possession. The experimental diets had true protein digestibility values between 70-94%. The high TPD of the rats fed the experimental diets is an indication that proteins in these diets are highly digestible. It is pertinent to note that a protein source may contain all the essential amino acids; but if it cannot be digested and absorbed for protein biosynthesis then it is worthless (Umerah *et al*., 2020) [28].

4.5 Biological value and Net protein utilization

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 (Umerah *et al*., 2020) [28]. Biological value evaluates the competence of protein to support growth through nitrogen holding in the body. It is the assessment of the absorbed protein from food that becomes part of the body. The biological value of the experimental diet ranged between 93.06- 98.21%. Whitney and Rolfes (1996) [29] 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 suggests that protein from these diets supported growth and maintenance. This also confirms report from the previous phase of this study that the test diets supported growth and recovery in children with PEM.

Umerah *et al*. (2020) [28] opined that NPU and biological value are alike. The duo measures the same parameter of nitrogen retention. However, the disparity lies in the fact that while biological value is calculated from the amount of nitrogen absorbed; net protein utilization is estimated from nitrogen ingested (Umerah *et al*., 2020) [28]. Net protein utilization of 65 and above suggests that appreciable quantity of essential amino acid which is crucial for the body for proper growth of cells and tissues. The high NPU of the complementary foods were due to lower urinary outputs which lead to increase in retained nitrogen. However, our study recorded values of NPU which were higher than the values in a previous study for NPU by Obatolu *et al*. (2000) [19].

4.6 Expression of growth hormone and retinol binding protein

Under the conditions of this study, there was an up-regulation of gene of GH in the liver of the rats that received 55% cereal-legume based complementary foods. Group F6, F2 and F4 had the highest average expression respectively. However, as for retinol binding protein, visible expression was observed for all the experimental groups fed the optimized complementary food formulations. However, the current study revealed that the gene of RBP in the liver of the rats that received the test formulations was down regulated.

5. Conclusion

Nigeria and other low income countries are facing problems of childhood malnutrition. This may be attributed to the quality of complementary foods which constitute one of the major drivers of optimal nutrition in children within 6-36months of age. In Nigeria, so many families live on the bread line; hence cannot buy fortified commercially available complementary foods. Therefore, they prepare porridge from only cereals which are grossly inadequate when compared to estimated needs.

The current study found that the nutritional quality of cereal based complementary foods may be improved by optimizing the cereal legume ratio and supplementing with moringa leaf powder. The bioassay profile for all the experimental diets is of interest but diet F6 happen to be the most effective due to its outstanding performance in the protein utilization, biological value, digestibility as well as expression of growth hormone. Diet F6 also recorded the highest score for parameters of growth performance.

Therefore on the basis of the findings of this study, nutritionally adequate complementary foods can be formulated from locally available and inexpensive food materials that support growth, physical activity and prevent protein energy malnutrition. It is therefore imperative that care givers should adopt this method to produce weaning foods for infants and young children particularly in low income countries.

6. Authors' Contribution

This study is a product of the collective efforts of the all authors. YS, SAI and JB participated in conceptualization, designing, supervising and coordinating the study. TAA and YAB conducted the experimental studies, analyzed, interpreted data and wrote the manuscript. YS, SAI and JB read and approved the final manuscript.

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9. Conflicts of Interest

The authors hereby declare no conflict of interest.

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