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Effects of complementary food formulated from millet, soybean, and grasshopper on hematological parameters of malnourished weanling albino rats

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ABSTRACT

Background: Malnutrition in under-five children remains an issue of public health, especially in low and middle-income countries, and is associated with inadequate child development and poor survival. Recent public health intervention strategies for malnourished children allow for localized targeted supplementation with locally available materials to replace the imported Ready-to-Use-Supplementary-Food (RUSF) for the target group. **Aims:** The aim of the study was to develop a local supplementary food and determine its effects on hematological parameters of weanling rats. **Material and Methods:** The experimental study involved thirty weanling albino rats, grouped into groups A, B, and C, each consisting of ten rats. All the groups were acclimatized for fourteen days, thereafter, groups A and B were induced with moderate acute malnutrition (MAM) by feeding with only corn starch for fourteen days. Weight, tail length, and hematological parameters (hemoglobin level, white blood cell count) of all the weanling albino rats were determined and recorded as baseline values on the fourteenth day. Therapeutic food made from millet, soybean, and grasshopper blends was produced and fed to group A as the intervention, Group B was fed with corn starch only and group C was fed on normal rat pellets for twenty-eight days. Weight, tail length, and hematological parameters were determined and recorded at the end of the intervention. **Results:** Experimental group A had the lowest packed cell volume (PCV) value with a difference of 2.20 ± 0.56 %, the lowest hemoglobin (Hb) level with a difference of 0.58 ± 0.02 g/dL, the control group had the highest PCV, Hb, and RBC with values of 4.10 ± 0.5 %, 1.58 ± 0.21 g/dL, and 0.9 ± 0.05 x 106 µ/L respectively. **Conclusions:** With the evidence provided by the parameters assessed in the study, it can be concluded that the developed therapeutic food is efficacious in the treatment of malnutrition especially in weanling albino rats.

Keywords: Moderate Acute Malnutrition (MAM), Ready-to-Use-Supplementary-Food (RUSF) Malnutrition, Complementary feeding, Animal studies.

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1 Introduction

Malnutrition in under-five children has been associated with inadequate child development and poor survival [1, 2]. It has been estimated that over 200 million children in developing countries fail to reach their full development potential as a result of poor nutrition, poverty, and inadequate care [3, 4]. Low-quality local complementary foods, improper feeding techniques, the high cost of animal protein foods, and recurring infections all contribute to the undernourished diet of under-five children thereby increasing the risk of malnutrition. Malnutrition is one of the contributing factors to high morbidity and mortality rates and has also been linked to more than half of cases of deaths among children under-five years, especially in low- and middle-income countries. Recent intervention strategies for treating malnourished children in the public health sector allow for targeted supplementation with enriched maize blends, Ready-to-Use Therapeutic Foods (RUTF), enriched energy drinks, or a combination thereof for the target population. The etiology of protein-energy malnutrition in Nigerian children during the complementary feeding period has been linked to increased consumption of plain cereal pap [5]. Cereals contain low amounts of protein and are deficient in lysine or tryptophan which are essential for the growth and development of under-five children. There is a need to introduce readily available, cost-effective foods to improve the nutrient content of complementary foods given to children.

Millet is one of the numerous kinds of cereal that is cultivated in the northern part of Nigeria and is the most nutrient-dense. It is a rich source of carbohydrates which provides energy and contributes to the calorie requirement of the individual. The germ also contains a higher amount of protein, fatty acids, minerals, and vitamins compared to other cereals [6]. Millets can be prepared into different indigenous delicacies such as pap, pudding, and millet-fufu, giving rise to a diversity of diets [7-9].

Variegated grasshopper (*Zonocerus variegatus*) is one of the insects that is largely consumed especially in Northern Nigeria. It is a rich source of protein and B-vitamin [10]. Generally, insects do not require a special method of cultivation, they are readily accessible and are more economical, thereby providing an alternative source of protein compared with meat and fish [11].

Soybean is a common legume in Nigeria. It contains a high amount of protein and is considered a plant source of protein of high biological value. Further it is rich in some vitamins and minerals [12, 13]. Soybean provides a cheaper alternative source of protein compared to animal protein foods such as meat, fish, eggs, etc. Soybean has been used as part of complementary foods in Nigeria. Cheap and affordable therapeutic food alternatives in the northeastern part of Nigeria compared with the scarce and expensive packaged RUSF have been solicited for by the locals [14, 15]. Therefore, the aim of this study was to produce a complementary food from millet, variegated grasshopper (*Zonocerus variegatus*) and soyabean and test effectiveness of this novel food on the treatment of moderate malnutrition in weanling rats.

2 Material and Methods

2.1 Sample selection

Millet, variegated grasshopper *(Zonocerus variegatus)* and soybean were purchased in four markets out of the seven markets in Maiduguri, Borno State Nigeria using a systematic random sampling technique. Each sample was purchased from different traders randomly to ensure a representation of the samples.

2.2 Sample preparation

Millet preparation

The millet was mixed to have a homogenous mixture of grains. Thereafter, the homogenous millet sample was winnowed to separate the chaff from the grains. The grains were rinsed in warm water to remove the dirt and debris on the grain before it was milled using a disc attrition mill. The gruel was dried in the hot air oven at 55 ºC for 90 minutes, the drying process was monitored by checking the samples every 20 minutes till the end of drying to obtain a flour sample and finally packed in air-tight Ziploc bags.

Soybean preparation

The soybean was mixed to obtain a homogenous sample. The homogenous soybean sample was handpicked to separate the seeds from stones and other debris. The clean soybean was washed and soaked in water at room temperature (22 ºC) for 12 hours. Thereafter it was washed by rubbing between the palms to remove the testa (skin) and rinsed several times with more water to ensure that there were no more seed coats. The clean soybean was then soaked in hot water at 85 ºC for 15 minutes, air-dried for 48 hours, and then roasted in an oven at 70 ºC for 30 minutes. The dried sample was milled using a locally fabricated disc attrition mill to obtain a flour sample which was packed in air-tight Ziploc bags.

Variegated grasshopper (Zonocerus variegatus) preparation

The Variegated grasshopper (*Zonocerus variegatus*) was winnowed to remove physical debris and poured into a clean bowl. Thereafter, hot boiling water (100 ºC) was added to the grasshopper to remove undesirable flavors and other forms of dirt. The water was sieved out and the variegated grasshopper (*Zonocerus variegatus*) was oven-dried using a hot air oven for 12 hours at 60 ºC. The dried sample was milled into powder using the locally fabricated disc attrition mill and packed in air-tight Ziploc bags.

Therapeutic diet processing

The flour food samples were mixed in a ratio of 70:20:10 (Millet: 70 %, Soybean: 20 %, and Variegated grasshopper (*Zonocerus variegatus*): 10 %) and produced into pellets of different sizes which were fed to the rats at different stages of development. The sizes of the pellets were increasing as the rats were growing and increasing in size.

Nutrient analysis

The proximate composition of the therapeutic diet produced was carried out using AOAC methods [16]. The food was analyzed for moisture content, crude fat, fiber, and ash. Nitrogen content was determined using the Kjeldahl method and the resulting value was multiplied by a factor of 6.25 to estimate the protein content. Carbohydrate was determined by a method that involves measuring the total weight of a sample before and after removing non-carbohydrate components, such as proteins, lipids, and minerals. The difference in weight corresponds to the carbohydrate content in the sample. Total energy was calculated by multiplying the resulting carbohydrate content by a factor of 4 with the knowledge that carbohydrates provide approximately 4 kilocalories of energy per gram.

2.3 Animal experimentation

Thirty weanling albino rats at 21 days old were obtained from the Department of Veterinary Medicine, University of Ibadan. The rats were divided into three groups A, B, and C of ten weanling albino rats in each group. Each group was kept in a well-ventilated cage, and each of the rats was given an identification mark. Their weight and tail length were recorded at the entry point. They were all acclimatized for 14 days, feeding with regular pelletized feed and water. Their weight and tail length were measured weekly.

Baseline

Group A and B were used as the experimental groups while group C was the control group. Rats in groups A and B were induced with moderate acute malnutrition (MAM) by feeding them with corn starch (*ogi*) only for fourteen days while group C continued feeding on commercial rat pelletized feed.

Intervention

Rats in group B were fed with the therapeutic food while group A continued feeding on corn starch (*ogi*) and group C continued on the commercial rat pelletized feed. The intervention period lasted for 28 days.

The initial and final results were compared to determine the effect of the therapeutic diet on the nutritional status of the weanling albino rats induced with moderate acute malnutrition.

Anthropometric measurement

At baseline, each group was measured for weight and tail length following standard procedures and recorded as the initial anthropometric status. All measurements were carried out very early in the morning between 7 am – 9 am. A digital weighing scale was used to measure the weight of the rats to the nearest 0.1 kg. The tail length was measured with a meter ruler. The rats were stretched on the ruler. The measurements were taken to the nearest 0.1 cm.

After the intervention, the measurements of weight and tail length were repeatedly measured and recorded as the final anthropometric status.

Blood sample collection

Blood samples were collected through the ocular vein. The ocular vein was punctured using a heparinized capillary tube while the rats were held down not to affect the flow of blood. The blood samples were collected in a heparinized test tube. At the end of the experimental period, each animal was sacrificed by decapitation. Blood samples were collected into (i) EDTA bottles for hematological analysis and (ii) plain bottles for biochemical parameters. After 10 minutes, the blood sample for biochemical parameters was centrifuged for 10 minutes. The serum was then carefully transferred with Pasteur pipettes into cleaned labeled sample bottles and stored at 20 ºC until required [17].

2.4 Biological parameters analysis

The Biuret method for estimating serum total protein was employed [18]. BCG (Bromocresol green) was used to measure serum albumin content according to Doumas and Biggs [19]. Globulin was calculated by subtracting total protein from albumin according to Whiteside et al. [20]. Serum urea was assessed using the colorimetric method [21] while flame photometry and atomic absorption spectrophotometry analyzed serum mineral components [22]. Cynomethemoglobin was used to analyze Hb [23]. Packed cell volume (PCV) and white blood cells (WBC) were measured using Linne and Ringsrud's techniques [24].

2.5 Ethical consideration

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed and the study was approved by a research ethics committee at the institution or practice at which the studies were conducted. The ethical approval obtained from the study was obtained from the Animal Care Use and Research Ethics Committee (ACUREC). University of Ibadan. With the assigned number: UI-ACUREC/17/0040.

2.6 Statistical Analysis

The data were analyzed using Statistical Package for Social Sciences (SPSS version 20.0). Samples were analyzed in triplicate. Descriptive statistics such as range, mean, standard deviations, and percentages were carried out, and the inferential statistics t-test was considered significant at $p < 0.05$.

3 Results

The nutritional composition of the developed therapeutic food is presented in Table 1. The calorific value of the developed therapeutic food was ≈ 390 ± 29 Kcal, with a protein value of 20.83 ± 3.68 %. The fat and carbohydrate contents represented 7.20 ± 1.93 % and 60.56 ± 12.73 % of the total calories respectively. Furthermore, the Na, Mg and K have 235.5 ± 2 mg/100 g, 290.5 ± 2 mg/100 g and 827.5 ± 3mg/100 g contents respectively.

Table 1. Proximate analysis and mineral composition of therapeutic diet produced

Sample ID	% Moisture Content	% Protein	% Fat	% Fiber	% Ash	% Carb	Energy (Kcal)	Na (mg/100g)	Mg (mg/100g)	K (mg/100g)
Formulated therapeutic food	8.82 ± 1.63	20.83 ± 3.68	7.20 ± 1.93	2.61 ± 0.33	2.59 ± 0.12	60.56 ± 12.73	390.36 ± 28.56	235.5 ± 2	290.5 ± 2	827.5 ± 3

Abs: absence; Carb: Carbohydrates

The anthropometric measurement of weight and tail length are presented in Tables 2 and 3. The weight gain of the weanling albino was monitored over time every 7 days till the end of the study. Group C fed with the normal rat pellets had a weight gain of 47.40 ± 1.60 g, group B fed with the corn starch only had the least weight gain of 42.90 ± 2.70 g. A statistically significant difference (p = 0.040) was observed in the weight gain of group A which was fed with the therapeutic food as they had the highest weight gain (83.16 ± 9.29 g) across the three groups. The tail length as a parameter for growth was assessed in the rats. Group A had a longer tail length with a difference of 0.66 ± 0.34 cm compared with group B's tail length difference of 0.45 ± 0.02 there is no statistical significance between the tail lengths of each group.

Table 2. Weight gain of the weanling Albino rats after feeding with formulated food

Groups	Weight	Weight gain	p-value
Group A			
- Initial	35.34 ± 8.54	83.16 ± 9.29	0.042*
- Final	118.50 ± 13.83		
Group B			
- Initial	39.10 ± 8.30	42.90 ± 2.70	0.040*
- Final	82.00 ± 11.00		
Group C			
- Initial	68.60 ± 3.80	47.40 ± 1.60	0.029*
- Final	116.00 ± 2.20		

**Values are mean of 2 groups of 5 weanling albino rats each*
**Paired sample T-test Significant at the p<0.05 level*

Table 3. Tail length per group before feeding with formulated food and after feeding with formulated food

Groups	Tail length (cm)	Difference (cm)	p-value
Group A			
- Initial	11.77 ± 1.37	0.66 ± 0.34	0.651*
- Final	12.43 ± 2.47		
Group B			
- Initial	11.38 ± 0.61	0.45 ± 0.02	0.445*
- Final	11.43 ± 0.58		
Group C			
- Initial	13.50 ± 0.9	0.55 ± 0.89	0.316*
- Final	14.05 ± 0.9		

**Values are mean of 2 groups of 5 weanling albino rats each*
**Paired sample T-test Significant at the p<0.05 level*

Hematological parameters

The results of packed cell volume, red blood cells (RBC), WBC, lymphocytes, neutrophils, monocytes, eosinophils, and hemoglobin levels of the weanling albino rats before and after the intervention are presented in Table 4.

The result showed that the average PCV value and the hemoglobin value (Hb) before and after the intervention across the groups had uniform progressions in value. However, the experimental group B fed with the corn starch had the least PCV count with a value of 34.60 ± 0.67 % from 33.80 ± 1.10 % after being fed with the corn starch. The changes in the RBC values were more prominent in the control group from 5.60 ± 0.15 x106 µ/L to 6.45 ± 0.20 x106 µ/L followed closely by the experimental group B fed with corn starch only with a value of 5.50 ± 0.24 x106 µ/L, to 5.92 ± 0.20 x106 µ/L; while the experimental group B fed with the corn starch had the least increase in the RBC value. The difference in the RBC count across the groups was more notable among the Control group and experimental group A while the experimental group B fed with the cornstarch had the highest RBC count. The Control group had the least increase in the platelet value from 199720.71 ± 37470.74 x109/L to 221000.00 ± 10946.33 x109/L while the experimental group B had the highest platelet count after feeding on corn starch for 28 days with values from 124700.08 ± 13174.93 x109/L to 170900.00 ± 12537.46 x 109/L, for the eosinophils, it was observed that the experimental group B had a reduction in the values from 1.80 ± 2.00 % to 1.50 ± 0.22 % while the control group and the experimental group fed with the developed therapeutic food both had an increase in the values from 2.20 ± 0.33 % to 2.4 ± 0.40 % and from 2.33 ± 0.29 % to 2.83 ± 0.31 % respectively.

Table 4. Comparison of hematological parameters across the groups

Parameters	Group C (Mean ± SEM)	Group C (Mean ± SEM)	Group C (Mean ± SEM)
PCV (%)			
- Initial	34.20 ± 0.87	32.30 ± 1.56	33.80 ± 1.10
- Final	38.30 ± 1.36	34.50 ± 1.82	34.60 ± 0.67
HB (g/dL)			
- Initial	11.20 ± 0.28	10.90 ± 0.65	11.20 ± 0.24
- Final	12.78 ± 0.49	11.68 ± 0.63	12.26 ± 0.27
RBC (x10^6 µ/L)			
- Initial	5.60 ± 0.15	5.20 ± 0.33	5.50 ± 0.24
- Final	6.45 ± 0.20	5.71 ± 0.35	5.92 ± 0.20
WBC (10^3 µ/L)			
- Initial	6620 ± 292	7056 ± 780	6056 ± 240
- Final	2960 ± 132	2767 ± 154	3266 ±154
PLATELET (x10^9/L)			
- Initial	199720 ± 37470	142000 ± 18111	124700 ± 13175
- Final	221000 ± 10946	200166 ± 14321	170900 ± 12537
LYM (%)			
- Initial	63.40 ± 1.69	64.56 ± 1.74	65.10 ± 1.52
- Final	58.30 ± 1.42	61.83 ± 2.414	58.60 ± 1.51
NEUT (%)			
- Initial	31.70 ± 1.80	31.33 ± 1.86	31.00 ± 1.51
- Final	37.1 ± 1.35	34.33 ± 2.86	37.00 ± 1.65
MON (%)			
- Initial	1.70 ± 0.21	1.78 ± 0.36	2.10 ± 0.23
- Final	2.2 ± 0.25	2.0 ± 0.37	1.80 ± 0.20
EOS (%)			
- Initial	2.20 ± 0.33	2.33 ± 0.29	1.80 ± 2.00
- Final	2.4 ± 0.40	2.83 ± 0.31	1.50 ± 0.22

PCV packed Cell Volume, *RBC* red Blood Cell, *WBC* white Blood Cell, *LYM* lymphocytes, *NEUT* neutrophils, MON monocytes, *EOS* eosinophils, *HB* hemoglobin

4 Discussion

The aim of this study was to produce a complementary food from millet, variegated grasshopper (*Zonocerus variegatus*) and soyabean and test the effectiveness of this novel food on the treatment of moderate malnutrition in weanling rats.

In comparison with the UNICEF standard RUSF, the developed therapeutic food had more moisture content and higher protein value; while the UNICEF RUSF standard has a moisture content of less than 2.5 %, the higher fat content of 26 – 36 % and calorific value of 510 – 560 Kcal while the developed therapeutic diet has values of 8.82 ± 1.63 %, 7.20 ± 1.93 %, and ≈ 390 ± 29 Kcal respectively. While the Standard RUSF has a Sodium value of 270 mg/day and potassium value of 900 – 1400 mg/day higher in value than the developed formulated food sodium content of 235.5 ± 2 mg/100g and potassium value of 827.5 ± 3 mg/100g.

The moisture content reported by our study was 8.82 ± 1.63 %, this value is similar to that reported by Yazew [25] for the developed RUTF2 which was 9.6 % Mazgebo et al. [26], reported a moisture content of 8.38 %, 5.2 %, and 6.8 %, for their samples. Our reported value was higher than the range reported by Gamede et al., [27] 4.78 % to 5.31 %. It is essential to monitor the moisture level of meals and food products because excessive moisture contents can shorten the shelf life of the product by increasing the activity of microorganisms that cause microbial deterioration, which in turn results in an unpleasant odor and an unsatisfactory taste [26]. The developed therapeutic food was reported to have a calorific value of 390.36 ± 28.56 Kcal; a value lower than that of Yazew [25] who reported 642.4 kcal/100 g, 532.3 kcal/100 g, and 513.2 kcal/100 g of the developed therapeutic food. The high protein content of the variegated grasshopper (*Zonocerus variegatus*) and the soybean could be attributed as the reason the protein value of the food was high. This indicates that the developed therapeutic food meets the protein requirement of children 1-3 years. Our study reported a crude fat content of 7.20 ± 1.93 %. The findings align with those of Gamede et al. [27] who reported a range of 5.95 % to 9.94 % for crude fat content. These authors recorded the highest crude fat content in diet 6, which consisted of 60.0 % maize, 23.0 % pea, and 17.0 % *anchote*, while the lowest content was found in diet 3, a blend of 45.0 % maize, 30.6 % pea, and 24.4 % *anchote*. The reported values in this study were lower compared to Solomon [28] findings, which ranged from 11.5 % to 24.8%. Solomon conducted research on complementary diets using various cereal crops and vegetable products, including maize, rice, soya beans, acha grains, benniseed, crayfish, carrot, Bambara groundnut, and garden egg. A standard RUSF should have a fat value of 26 – 36 % however our reported value was less than the accepted standard; 2.59 ± 0.12 % was the crude ash content of our formulated therapeutic food, and it is similar in value to the figures reported by Gamede et al.[27], who reported that the crude ash content of the six formulated complementary diets ranged from 1.98 % to 2.99 %. Diet 1 (27.7 % anchote, 45.5 % maize, and 26.8 % pea) contained the highest total ash content while diet 2 (13.5% anchote, 28.7 % pea, and 57.8 % maize) contained the least ash content. As per the WHO/FAO guidelines [29], all the formulated diets examined in this study adhere to the recommended standards, which specify that the ash contents of complementary foods should not exceed five percent.

As reported in the current study, the minerals assessed for in the therapeutic food developed were 235.5 ± 2 mg/100 g, 290.5 ± 2 mg/100 g, and 827.5 ± 3mg/100 g. In comparison with the figures reported by Abeshu et al. [30], for calcium, iron, zinc, phosphorous, potassium, sodium, and magnesium, the formulated diet's mineral contents ranged from 225.45 mg/L to 261.32 mg/L, 11.48 mg/L to 12.61 mg/L, 2.73 mg/L to 3.00 mg/L, 357.92 mg/L to 391.13 mg/L, 298.55 mg/L to 332.63 mg/L, and 44.26 mg/L to 51.56 mg/L, respectively. Starting at six months of age, it is essential to supplement complementary foods with iron, zinc, phosphorus, magnesium, and calcium, as these nutrients have been identified as areas of concern [31-33].

Phytate contents of the formulated diets ranged from 64.74 to 72.15 mg/100 g. The oxalate contents of the formulated diets ranged from 37.46 to 44.82 mg/100 g. Tannin contents of the formulated diets ranged from 10.41 to 11.77 mg/100 g reported by Gamede et al. [27] shows lower figures compared with the developed therapeutic food, 305 ± 25 mg/100 g, 119.5 ± 20 mg/100 g, and 103.5 ± 20 mg/day for the tannin, phytate, and oxalate content respectively with only tannin values higher than the recommended value of 150 – 200 mg/day.

The weight gain of the experimental group is attributed to the quality of protein contained in the developed therapeutic food. A rat that had the highest weight gain after being induced with moderate acute malnutrition.

According to the findings of quite a few nutritional studies, the majority of traditional complementary foods found in developing countries are low in the amount of protein and energy that they contain. Due to the prevalence of protein-energy malnutrition, which has increased as a result of this nutrition issue, the rate of morbidity, mortality, reduced level of intellectual development has increased significantly [34-36]. Therefore, to effectively combat protein-energy malnutrition, affordable, protein-rich, and energy-dense complementary food is a preferable alternative to costly imported complementary foods. As a result, the formulated diet that was used in this study can be considered appropriate for protecting children from suffering from protein-energy malnutrition.

Umar et al. [17] reported that when compared, the Hb values for rats fed the MLMX; a diet composed of millet (60 %), groundnut (16 %), soybeans (16 %), crayfish (5 %) and palm oil (3 %) and MZMX; a diet composed of maize (60 %), groundnut (16 %), soy beans (16 %), crayfish (5 %) and palm oil (3 %) diet groups differ significantly ($p < 0.05$), and they are slightly identical to those for rats fed PC diets (Friscocream diet). Rats fed PC, MLMX, and MZMX diets had Hb levels between 12.0 g/dL and 17.5 g/dL, the higher iron content in the food component may be the cause of the higher Hb levels observed in rats fed PC, MLMX, and MZMX diets. Rats fed the MLA (100% millet) and MZA (100% maize) diets had Hb levels that were noticeably lower. This is consistent with the report from Umar, et al. [17]. Rats fed the MLMX and MZMX diets had significantly ($p < 0.05$) higher PCV values than rats fed the PC diet. The results are within the expected range, according to Umar 17 it was also reported that rats fed MLMX and MZMX had higher PCV values than those fed MLA and MZA which may be related to the improved nutritional components. Similarly, the average PCV and Hb values in each group increased uniformly before and after the intervention. However, the experimental group B given corn starch had the lowest PCV count, with a value of 34.60 ± 0.67 % compared to 33.80 ± 1.10 % after being fed corn starch. This can be ascribed to maize starch's low nutritional content. The changes in RBC values were more evident in the control group, from 5.60 ± 0.15 x106 µ/L to 6.45 ± 0.20 x106 µ/L, followed closely by the experimental group B fed with maize starch exclusively, with a value of 5.50 ± 0.24 x106 µ/L, to 5.92 ± 0.20 x106 µ/L. The difference in RBC count between groups was more pronounced in the control group and experimental group A, whereas the experimental group B fed with cornstarch had the greatest RBC count. The Control group had the smallest increase in platelet count from 199720.71 ± 37470.74 x 109/L to 221000.00 ± 10946.33 x109/L, whereas the experimental group B had the highest platelet count after 28 days of feeding on corn starch, with values ranging from 124700.08 ± 13174.93 x 109/L to 170900.00 ± 12537.46 x109/L. The indices for neutrophils, lymphocytes, and monocytes all followed similar trends; however, for Eosinophils, the experimental group B had a decrease in values from 1.80 ± 2.00 % to 1.50 ± 0.22 %, whereas the control group and the experimental group fed with the developed therapeutic food both had an increase in values from 2.20 ± 0.33 % to 2.40 ± 0.40 % and from 2.33 ± 0.29 % to 2.83 ± 0.31 %, respectively.

The strength of the study includes the utilization of a controlled experimental design, which helps establish a cause-and-effect relationship between the complementary food formulation and the observed changes in hematological parameters. This design allows for better control of confounding factors and increases the internal validity of the findings also the study used a specific and standardized formulation of complementary food, consisting of millet, soybean, and grasshopper. This consistency in the composition of the food ensures that the observed effects can be attributed to the specific ingredients and their combination. Some limitation of the study includes the fact that the study provides insights into the effects on albino rats, direct human trials are necessary to determine the applicability and potential benefits or risks of the complementary food formulation for human consumption also the use of a limited number of animals, which could impact the statistical power and generalizability of the results. Larger sample sizes are generally preferred to enhance the reliability and representativeness of the findings. The results obtained from the analysis are with animal models therefore it cannot be confirmed to be equivalent to the human population. The result provides with the evidence that local alternative therapeutic foods can be a viable alternative to locally address malnutrition in children under the age of five years as simulated with the weanling albino rats. Internal validity was ensured firstly by using a control group receiving an alternative diet, this helps to isolate the effects of the complementary food formulated by comparing to a reference group; Also, randomization was employed to assign the animals to different treatment groups this helps to reduce selection bias and ensure that any differences observed between groups are likely due to the intervention rather than pre-existing characteristics. The food samples used in the formulation of the diet is unique to the target population in the insurgency affected part of Nigeria this ascertain an aspect of the external validity of the study. The result cannot be generalized to the human population yet until further trials are conducted, hence the study would require further clinical investigation before being adapted to the human population.

5 Conclusion

With the evidence provided by the parameters assessed in the study, it can be concluded that the developed therapeutic food is efficacious in the treatment of malnutrition especially in weanling albino rats.

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References

[1] Mzumara, B., Bwembya, P., Halwiindi, H., Mugode, R., & Banda, J. (2018). Factors associated with stunting among children below five years of age in Zambia: Evidence from the 2014 Zambia demographic and health survey. *BMC Nutrition, 4*(1). https://doi.org/10.1186/s40795-018-0260-9

[2] Mkhize, M., & Sibanda, M. (2020). A review of selected studies on the factors associated with the nutrition status of children under the age of five years in South Africa. *International Journal of Environmental Research and Public Health, 17*(21), 7973. https://doi.org/10.3390/ijerph17217973

[3] Grantham-McGregor, S., Cheung, Y. B., Cueto, S., Glewwe, P., Richter, L., & Strupp, B. (2007, January). Developmental potential in the first 5 years for children in developing countries. *The Lancet, 369*(9555), 60–70. https://doi.org/10.1016/s0140-6736(07)60032-4

[4] Leroy, J. L., & Frongillo, E. A. (2019). Perspective: What does stunting really mean? A critical review of the evidence. *Advances in Nutrition, 10*(2), 196-204. https://doi.org/10.1093/advances/nmy101

[5] von Grebmer K., Saltzman A., Birol E., Wiesmann D., Prasai N., Yin S., Yohannes Y., Menon P., Thompson J., & Sonntag A. 2014. *2014 Global Hunger Index: The Challenge of Hidden Hunger. Bonn, Washington, D.C., and Dublin: Welthungerhilfe, International Food Policy Research Institute, and Concern Worldwide*. http://dx.doi.org/10.2499/9780896299580

[6] Adebiyi, J. A., Obadina, A. O., Adebo, O. A., & Kayitesi, E. (2016). Fermented and malted millet products in Africa: Expedition from traditional/ethnic foods to industrial value-added products. *Critical Reviews in Food Science and Nutrition*, 1-12. https://doi.org/10.1080/10408398.2016.1188056

[7] Joseph, B., Matilda Ntowa, B., Edmund Osei, O., Rashied, T., Richard, A. A., Yaw, K., Lawrence Misa, A., Stephen, N., & Daniel Ashie, K. (2023). Sustainable food system in Ghana: Role of neglected and underutilized crop species and diversity. *Anthropocene Science*, *2*(1), 62-70. https://doi.org/10.1007/s44177-023-00049-1

[8] Eliazer Nelson, A. R., Ravichandran, K., & Antony, U. (2019). The impact of the Green Revolution on Indigenous crops of India. *Journal of Ethnic Foods*, *6*(1). https://doi.org/10.1186/s42779-019-0011-9

[9] Takhellambam, R. D., Chimmad, B. V., & Prkasam, J. N. (2015). Ready-to-cook millet flakes based on minor millets for modern consumer. *Journal of Food Science and Technology*, *53*(2), 1312-1318. https://doi.org/10.1007/s13197-015-2072-0

[10] Berasategui, A., Shukla, S., Salem, H., & Kaltenpoth, M. (2015). Potential applications of insect symbionts in biotechnology. *Applied Microbiology and Biotechnology*, *100*(4), 1567-1577. https://doi.org/10.1007/s00253-015-7186-9

[11] Onwezen, M., Bouwman, E., Reinders, M., & Dagevos, H. (2021). A systematic review on consumer acceptance of alternative proteins: Pulses, algae, insects, plant-based meat alternatives, and cultured meat. *Appetite*, *159*, 105058. https://doi.org/10.1016/j.appet.2020.105058

[12] Langyan, S., Yadava, P., Khan, F. N., Dar, Z. A., Singh, R., & Kumar, A. (2022). Sustaining protein nutrition through plant-based foods. *Frontiers in Nutrition*, *8*. https://doi.org/10.3389/fnut.2021.772573

[13] Samtiya, M., Aluko, R. E., & Dhewa, T. (2020). Plant food anti-nutritional factors and their reduction strategies: An overview. *Food Production, Processing and Nutrition*, *2*(1). https://doi.org/10.1186/s43014-020-0020-5

[14] Schoonees, A., Lombard, M. J., Musekiwa, A., Nel, E., & Volmink, J. (2019). Ready-to-use therapeutic food (RUTF) for home-based nutritional rehabilitation of severe acute malnutrition in children from six months to five years of age. *Cochrane Database of Systematic Reviews*, *2019*(5). https://doi.org/10.1002/14651858.cd009000.pub3

[15] Dibari, F., Diop, E. H., Collins, S., & Seal, A. (2012). Low-cost, ready-to-Use therapeutic foods can be designed using locally available commodities with the aid of linear programming. *The Journal of Nutrition,* *142*(5), 955-961. https://doi.org/10.3945/jn.111.156943

[16] Appendix F: Guidelines for Standard Method Performance Requirements. (2016). *AOAC Official Methods of Analysis*.

[17] Umar, L., Saidu, Y., Lawal, M., & Maigandi, S. (2010). Biochemical and Haematological indices of Weanly albino rats fed millet and maize based complementary weaning food. *Nigerian Journal of Basic and Applied Sciences*, *18*(1). https://doi.org/10.4314/njbas.v18i1.56840

[18] Fine, J. (1935). The biuret method of estimating albumin and globulin in serum and urine. *Biochemical Journal*, *29*(3), 799-803. https://doi.org/10.1042/bj0290799

[19] Doumas, B. T., Biggs, H. G., Arends, R. L., & Pinto, P. V. (1972). Determination of serum albumin. *Standard Methods of Clinical Chemistry,* 175-188. https://doi.org/10.1016/b978-0-12-609107-6.50022-2

[20] Whiteside, P., Stockdale, T., & Price, W. (1980). Signal and data processing for atomic absorption spectrophotometry. *Spectrochimica Acta Part B: Atomic Spectroscopy*, *35*(11-12), 795-806. https://doi.org/10.1016/0584-8547(80)80018-8

[21] Cardoso, E. M., Arregger, A. L., Tumilasci, O. R., Elbert, A., & Contreras, L. N. (2009). Assessment of salivary urea as a less invasive alternative to serum determinations. *Scandinavian Journal of Clinical and Laboratory Investigation*, *69*(3), 330-334. https://doi.org/10.1080/00365510802588076

[22] Drabkin, D. L., & Austin, J. H. (1932). Spectrophotometric studies. *Journal of Biological Chemistry*, *98*(2), 719-733. https://doi.org/10.1016/s0021-9258(18)76122-x

[23] Calvaresi, E. C., La'ulu, S. L., Snow, T. M., Allison, T. R., & Genzen, J. R. (2021). Plasma hemoglobin: A method comparison of six assays for hemoglobin and hemolysis index measurement. *International Journal of Laboratory Hematology*, *43*(5), 1145–1153. https://doi.org/10.1111/ijlh.13457

[24] Adewumi, O. O., Felix-Minnaar, J. V., & Jideani, V. A. (2022). Physiochemical and nutritional characteristics of ready-to-Use therapeutic food prepared using Bambara groundnut-moringa oleifera leaf protein complex. *Foods*, *11*(12), 1680. https://doi.org/10.3390/foods11121680

[25] Yazew, T. (2022). Therapeutic Food Development from Maize Grains, Pulses, and Cooking Banana Fruits for the Prevention of Severe Acute Malnutrition. *The Scientific World Journal*, *2022*, 1–7. https://doi.org/10.1155/2022/3547266

[26] Mezgebo, K., Belachew, T., & Satheesh, N. (2018). Optimization of red teff flour, malted soybean flour, and papaya fruit powder blending ratios for

better nutritional quality and sensory acceptability of porridge. *Food Science & Nutrition*, *6*(4), 891-903. https://doi.org/10.1002/fsn3.624

[27] Gamede, M., Mabuza, L., Ngubane, P., & Khathi, A. (2020). Plant-derived oleanolic acid ameliorates markers of subclinical inflammation and innate immunity activation in diet-induced pre-diabetic rats. *Therapeutic Advances in Endocrinology and Metabolism*, *11*, 204201882093577. https://doi.org/10.1177/2042018820935771

[28] Solomon, M. (2005). Nutritive value of three potential complementary foods based on cereals and legumes. *African Journal of Food, Agriculture, Nutrition and Development*, *5*(9), 01–14. https://doi.org/10.18697/ajfand.9.1690

[29] World Health Organization (WHO) (1999). *Management of Severe Malnutrition: A Manual for Physicians and Other Senior Health Workers*.

[30] Abeshu, M. A., Lelisa, A., & Geleta, B. (2016). Complementary feeding: Review of recommendations, feeding practices, and adequacy of homemade complementary food preparations in developing countries – Lessons from Ethiopia. *Frontiers in Nutrition*, *3*. https://doi.org/10.3389/fnut.2016.00041

[31] Kathryn G., D. (2013). The challenge of meeting nutrient needs of infants and young children during the period of complementary feeding: An evolutionary perspective. *The Journal of Nutrition*, *143*(12), 2050-2054. https://doi.org/10.3945/jn.113.182527

[32] Lutter, C. K., Grummer-Strawn, L., & Rogers, L. (2021). Complementary feeding of infants and young children 6 to 23 months of age. *Nutrition Reviews*, *79*(8), 825-846. https://doi.org/10.1093/nutrit/nuaa143

[33] Mosha, T., Laswai, H., & Tetens, I. (2000). Nutritional composition and micronutrient status of home made and commercial weaning foods consumed in Tanzania. *Plant Foods for Human Nutrition*, 55(3), 185-205. https://doi.org/10.1023/a:1008116015796

[34] Bhutta, Z. A., & Salam, R. A. (2012). Global nutrition epidemiology and trends. *Annals of Nutrition and Metabolism*, *61*(Suppl. 1), 19-27. https://doi.org/10.1159/000345167

[35] Duke, J. A. (2012). *Handbook of LEGUMES of world economic importance*. Springer. https://doi.org/10.1007/978-1-4684-8151-8

[36] Mbithi-Mwikya, S., Van Camp, J., Mamiro, P. R., Ooghe, W., Kolsteren, P., & Huyghebaert, A. (2002). Evaluation of the nutritional characteristics of a finger millet based complementary food. *Journal of Agricultural and Food Chemistry*, *50*(10), 3030-3036. https://doi.org/10.1021/jf011008a