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Formulation and *In-Vivo* Nutritional, Biochemical, and Microbial Quality Assessments of Ready-to-Use Therapeutic Foods for Malnutrition

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Abstract

In Nigeria, factors such as high cost of living, regional insecurity, inflation, poor feeding practices, and high poverty rates have exacerbated the prevalence of Severe Acute Malnutrition (SAM). This study aimed to formulate and evaluate the in-vivo nutritional, biochemical, microbial, and sensory qualities of locally produced therapeutic foods for the management of SAM. The food formulations were coded as PMF – 100% Pearl Millet Flour, PBM1 – 60% Pearl Millet + 20% Bambara Groundnut + 5% Moringa Leaves + 5% Vegetable Oil + 10% Sucrose, PBM2 – 50% Pearl Millet + 30% Bambara Groundnut + 5% Moringa Leaves + 5% Vegetable Oil + 10% Sucrose, PBM3 – 40% Pearl Millet + 40% Bambara Groundnut + 5% Moringa Leaves + 5% Vegetable Oil + 10% Sucrose, CTL – Control (UNICEF standard RUTF product). Nutritional assessments were conducted using rat bioassays, haematological analysis, microbial quality testing, and sensory evaluation for acceptability. Data were subjected to one-way Analysis of Variance (ANOVA) at a significance level of $p< 0.05$. Results indicated that rats fed the formulated pearl millet-based RUTFs demonstrated improved growth performance. The Biological Value (BV) of the samples ranged from 70.36% in PBM1 to 78.50% in PBM3. Net Protein Utilization (NPU) ranged from 58.97% in PBM1 to 70.86% in PBM3, while the Protein Efficiency Ratio (PER) ranged from 2.01 (PBM1) to 2.42 (PBM3). Sensory evaluation revealed that PBM samples exhibited significantly better appearance, texture, taste, and aroma than PMF (100% Pearl Millet), with statistical significance ($p < 0.05$). The study concludes that pearl millet-based ready-to-use therapeutic foods (RUTFs) formulated with indigenous ingredients demonstrate promising nutritional, biochemical, and sensory qualities and may serve as effective functional foods in the management of children with Severe Acute Malnutrition.

Keywords: Severe Acute Malnutrition, Therapeutic Food, Indigenous Foods, Ready-to-Use Therapeutic Food, Nutritional Assessment

INTRODUCTION

A child suffering from Severe Acute Malnutrition (SAM) has approximately nine times the risk of mortality compared to a well-nourished child, making it one of the most life-threatening conditions among under-five children (UNICEF, 2023). SAM is most prevalent in low- and middle-income countries, particularly in Asia and sub-Saharan Africa, where children are especially vulnerable (Otiti & Allen, 2021).

In Nigeria, the situation is further complicated by multiple factors, including the high cost of living, regional insecurity, rising inflation, poor infant feeding practices, and widespread poverty. The nutritional status of a child during early life has a profound influence on their physical, cognitive, and emotional development. Poor nutrition during infancy and childhood not only impairs growth and development but also increases susceptibility to infections and long-term chronic diseases (Victoria et al., 2016; Talabi et al., 2022).

According to the Multiple Indicator Cluster Survey (MICS, 2018), inappropriate feeding practices during infancy and early childhood are major contributors to undernutrition and increased child morbidity and mortality. Furthermore, SAM remains one of the leading causes of death among children under five globally (WHO, 2018).

UNICEF (2012) and FAO/WHO/UNU (2007) emphasized that recovery from SAM requires about 10–15 kg of Ready-to-Use Therapeutic Food (RUTF)—a nutrient-dense formulation designed for the treatment of uncomplicated cases of SAM. Traditional RUTFs typically consist of milk powder, peanut paste, vegetable oil,

sugar, and a vitamin-mineral mix, and are available in solid or semi-solid forms (UNICEF, 2013). However, in many developing countries, traditional complementary foods are predominantly grain-based and often deficient in protein, micronutrients, and energy (Adepoju & Ayenitaju, 2021).

Protein plays a vital role in child development. Deficiencies in protein intake can result in stunted growth, cognitive delays, and weakened immune responses (Saavedra & Prentice, 2023). To improve the accessibility and affordability of RUTFs, the WHO and UNICEF (2011) have recommended using locally available and culturally acceptable ingredients to reduce costs and enhance acceptance.

Pearl millet (*Pennisetum glaucum*) is a climate-resilient cereal widely consumed across Africa. It is a rich source of energy, protein, and essential micronutrients such as calcium, iron, and zinc. Millets are also rich in essential amino acids, such as tryptophan and threonine, and have been shown to offer superior protein quality and digestibility (Kiprotich et al., 2015; Elyas et al., 2002). Laminu et al. (2011) reported that pearl millet-based weaning foods, especially when fermented or combined with legumes like cowpea, exhibit improved nutritional quality.

Bambara groundnut (*Vigna subterranea*), an indigenous and underutilized legume, contains approximately 32–44% protein and is gaining recognition for its potential in formulating complementary and therapeutic foods (Khan et al., 2021; Alabi et al., 2023). Due to its amino acid profile and sustainability attributes, it has been incorporated into various food products, including biscuits and porridges (Ramatsetse et al., 2023; Talabi et al., 2019; Muhammad, 2021).

Moringa oleifera is another nutrient-dense plant, widely acknowledged for its high content of protein, vitamins, beta-carotene, amino acids, and antioxidants (Sreeja et al., 2021). Its inclusion in therapeutic foods can improve the nutrient density and bioavailability of micronutrients.

In Nigeria, an estimated 2,300 children under the age of five die every day, positioning the country as one of the highest contributors to global under-five mortality (Kalu & Etim, 2018). Undernutrition is implicated in approximately 45% of these deaths, including both direct and indirect consequences of SAM (WHO, 2024). The risk is especially severe among children who are both stunted and wasted.

Given these challenges, this study aimed to formulate and evaluate the in-vivo nutritional, biochemical, hematological, microbial, and organoleptic properties of ready-to-use therapeutic food (RUTF) blends developed from pearl millet, Bambara groundnut, and Moringa oleifera for managing SAM among children aged 6–59 months.

METHODOLOGY

Study Design

This experimental study employed a completely randomized design to formulate and evaluate locally produced Ready-to-Use Therapeutic Foods (RUTFs) using pearl millet, Bambara groundnut, and *Moringa oleifera* leaf powder. The study focused on assessing the in-vivo nutritional, biochemical, hematological, microbial, and organoleptic properties of the formulated RUTFs for the dietary management of Severe Acute Malnutrition (SAM) in children aged 6–59 months, using a rat model as a biological assay system.

Raw Material Procurement and Preparation

Pearl millet, Bambara groundnut, and *Moringa oleifera* leaves were purchased from local markets in Nigeria. All ingredients were cleaned, dried, and milled into fine flour using a hammer mill. *Moringa* leaves were shade-dried to preserve nutrient integrity before milling. The milled ingredients were formulated into five RUTF blends as follows:

- PMF: 100% Pearl millet flour (control 1)
- PBM1: 60% Pearl millet + 20% Bambara groundnut + 5% Moringa leaf + 5% Vegetable oil + 10% Sucrose
- PBM2: 50% Pearl millet + 30% Bambara groundnut + 5% Moringa leaf + 5% Vegetable oil + 10% Sucrose
- PBM3: 40% Pearl millet + 40% Bambara groundnut + 5% Moringa leaf + 5% Vegetable oil + 10% Sucrose
- CTL: Standard UNICEF RUTF (commercial control)

Each formulation was mixed manually until homogenous and packaged in airtight containers.

Animal Feeding Trial

Thirty (30) male Wistar albino rats, aged 3–4 weeks, with initial weights between 35–45 grams, were randomly divided into six groups of five rats each. The animals were housed in well-ventilated cages under controlled laboratory conditions (a 12-hour light/dark cycle and a temperature of 25 ± 2°C) and acclimated for 7 days prior to the feeding trial.

Each group was fed one of the six diets (PMF, PBM1, PBM2, PBM3, CTL, and standard laboratory chow). The feeding trial lasted for 28 days, during which feed intake and body weight were recorded weekly.

Ethical approval

The Ethical Committee at the College of Medicine and Health Sciences, Afe Babalola University Ado Ekiti, Nigeria, approved the animal experiment (permission number: ABUAD/21/05/2024/434), which was conducted in accordance with the established guidelines governing the use of animals.

Determination of Hematological Parameters using blood of fed Rats

Hematological and Biochemical Analysis

At the end of the feeding trial, blood samples were collected via cardiac puncture under anesthesia. Hematological parameters (hemoglobin concentration, packed cell volume, white blood cell count, and red blood cell count) were analyzed using an automated hematology analyzer. Serum biochemical parameters, including total protein, albumin, and lipid profile (HDL, LDL, and total cholesterol), were determined using standard diagnostic kits (Randox Laboratories, UK) and following the protocols outlined in Mohammed et al. (2022).

Biological Evaluation

The **Protein Efficiency Ratio (PER)**, **Net Protein Utilization (NPU)**, and **Biological Value (BV)** were determined using standard methods described by the Association of Official Analytical Chemists (AOAC, 2021). Growth performance indices, including weight gain and feed conversion ratio, were calculated to assess the biological quality of the RUTF formulations.

Microbial Evaluation

The microbiological safety of the RUTF samples was assessed by determining the Total Viable Count (TVC), Total Coliform Count, and the presence of Salmonella and Escherichia coli using standard plate count methods in accordance with ISO 4833-1:2013 and APHA (2020) guidelines. Samples were incubated on selective media, and microbial loads were expressed as colony-forming units (CFU) per gram (g).

Sensory Evaluation

Organoleptic properties (appearance, aroma, taste, texture, and overall acceptability) were evaluated by a panel of 20 semi-trained adult volunteers using a 9-point Hedonic scale (1 = dislike extremely, 9 = like extremely). Panelists were instructed to rinse their mouths with water between samples. Ethical clearance for sensory evaluation was obtained from the institutional review board, and informed consent was secured.

Statistical Analysis

All data were analysed using SPSS version 26.0 (IBM Corp., 2022). Results were expressed as means ± standard deviation (SD). One-way Analysis of Variance (ANOVA) was used to test for significant differences among treatment groups, with significance accepted at $p< 0.05$. Post-hoc comparisons were made using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Anthropometric Measurement and Nutritional Classification of Albino Wister Rat Fed with Pearl Millet Ready-to-use-therapeutic Food and Control Samples

The growth pattern of Albino Wistar rats fed on the formulated pearl millet ready-to-use-therapeutic food and control sample is presented in Figures 3.1A, 3.1B and 3.1C.

The nutritional status of the experimental rats showed that the rats fed on formulated pearl millet ready-to-use-therapeutic food samples had better growth performance, and were comparable to those rats fed on CTL, but significantly higher in growth performance when compared with those rats fed on PMF (100% pearl millet). However, the rats fed on PBM3 had the highest growth performance among those fed experimental food samples, as indicated by weight-for-age (WFA), length-for-age (LFA), and weight-for-length (WFL) ratios.

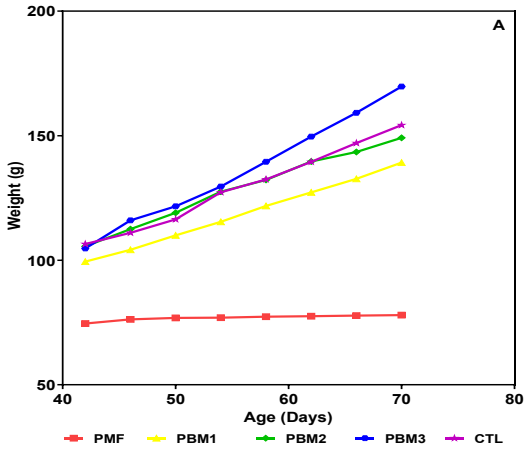

Figure 3.1A: Weight-For-Age (underweight) of Rats Fed ready-to-use- therapeutic food

Key: PMF: (100% Pearl millet flour); PBM1: (60% Pearl millet + 20% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); PMB2: (50% Pearl millet + 30% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); PMB3: (40% Pearl millet + 40% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); CTL: Control (UNICEF product).

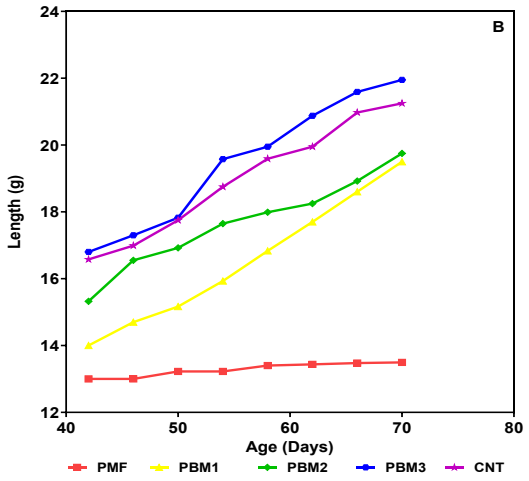

Figure 3.1B: Length-For-Age (stunting) of Rats Fed ready-to-use- therapeutic food

Key: PMF: (100% Pearl millet flour); PBM1: (60% Pearl millet + 20% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); PMB2: (50% Pearl millet + 30% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); PMB3: (40% Pearl millet + 40% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); CTL: Control (UNICEF product).

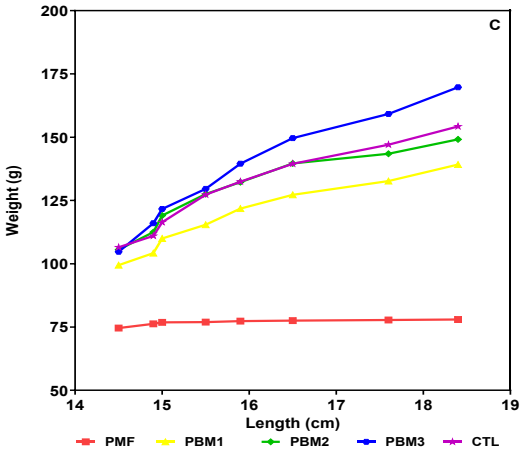

Figure 3.1C: Weight-For-Length (wasting) of Rats Fed on ready-to-use- therapeutic food

Key: PMF: (100% Pearl millet flour); PBM1: (60% Pearl millet + 20% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); PMB2: (50% Pearl millet + 30% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); PMB3: (40% Pearl millet + 40% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); CTL: Control (UNICEF product).

Nutritionally, this study suggests that the formulated food samples, particularly PBM3, may be suitable as a functional food, particularly for supporting infant growth and development, as well as preventing malnutrition among young children and adults. Studies have reported an increase in protein-energy malnutrition (PEM) from many parts of developing countries due to the low nutritional quality of traditional foods (Hurrell, 2003; Adepoju and Etukumoh, 2014). Hence, a low-cost food that is high in protein and energy-dense, such as PBM3, is a desirable substitute for expensive imported foods and low-quality local foods (Ijarotimi and Keshinro, 2012).

Protein Quality and Relative Organ Weight of Experimental Rats Fed with Pearl Millet Ready-to-use-therapeutic Food and Control Samples

Table 1: Nutritional Quality and Relative Organ Weight of Rat Fed with ready-to-use-therapeutic food

Parameters	PMF	PBM1	PBM2	PBM3	CTL
Weight gained (g)	3.35^{e}	39.75^{d}	43.57^{c}	65.02^{a}	47.75^{b}

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Table 1 Continued

Parameters	PMF	PBM1	PBM2	PBM3	CTL
Food intake (g)	495.70^{a}	482.51^{c}	483.55^{b}	460.40^{e}	461.53^{d}
Feed Efficiency Ratio	0.01^{e}	0.08^{d}	0.09^{c}	0.14^{a}	0.10^{b}
Nitrogen Retention (%)	0.94^{e}	15.68^{d}	16.28^{c}	17.30^{a}	16.53^{b}
Biological Value (%)	56.71^{e}	70.36^{d}	71.38^{b}	78.50^{a}	70.54^{c}
Net Protein Utilization (%)	39.99^{e}	58.97^{d}	68.73^{c}	70.86^{a}	69.86^{b}
Protein Efficiency Ratio	1.97^{d}	2.01^{e}	2.06^{c}	2.42^{a}	2.35^{b}
Relative organs weight (%)					
Kidney	0.56^{e}	0.77^{d}	1.43^{c}	1.86^{a}	1.61^{b}
Liver	2.17^{e}	2.61^{d}	3.38^{b}	3.54^{a}	3.16^{c}
Heart	0.35^{e}	0.49^{d}	0.72^{c}	0.78^{a}	0.75^{b}

Mean values with the same superscript are not significantly different at p>0.05.
Key: PMF: (100% Pearl millet flour); PBM1: (60% Pearl millet + 20% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); PMB2: (50% Pearl millet + 30% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); PMB3: (40% Pearl millet + 40% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); CTL: Control (UNICEF product).

The weight gain in rats fed on formulated pearl millet ready-to-use-therapeutic food was highest in those fed PBM3, but it was comparatively lower than that of CTL (47.75 g). The relative organ weight of the experimental rats, that is, kidney, liver and heart, fed on formulated pearl millet ready-to-use-therapeutic food was highest on PBM3. These observations further demonstrated that the formulated pearl millet ready-to-use therapeutic food samples were of higher protein quality and may be suitable as a functional food to support growth and body maintenance.

This finding aligns with other studies that reported on the nutritional qualities of foods formulated from combinations of two or more plant-based food materials (Ijarotimi and Keshinro, 2012).

The biological values (BV) of the prepared pearl millet ready-to-use therapeutic food samples exhibited variability, with PBM3 demonstrating the highest biological value, followed by PBM1.

The net protein utilization (NPU) values ranged from 58.97% in PBM1 to 70.86% in PBM3. Furthermore, the protein efficiency ratio (PER) was noted to be between 2.01 for PBM1 and 2.42 for PBM3. A comparative analysis indicates that the biological value (BV), net protein utilization (NPU), and protein efficiency ratio (PER) of the formulated pearl millet ready-to-use therapeutic food surpassed those of 100% pearl millet (PMF), which had a BV of 56.71%, NPU of 39.99%, and PER of 1.97. In contrast, these values were comparable to those of the UNICEF product (CTL), which recorded BV of 70.54%, NPU of 69.86%, and PER of 2.35. Biological value (BV) serves as an indicator of the proportion of absorbed protein from food that is incorporated into the body's proteins, as well as the efficiency with which digested protein is utilized for cellular protein synthesis. Notably, the BV and PER of the formulated pearl millet ready-to-use therapeutic food samples exceeded the FAO/WHO (2007) recommended thresholds of 70% and 2.0, respectively, for an ideal food.

This finding suggests that the protein content in the formulated pearl millet ready-to-use therapeutic food is likely sufficient to meet the protein requirements necessary for growth and development in both infants and adults (NPU), and protein efficiency ratio (PER) among the formulated Pearl millet ready-to-use-therapeutic food samples were higher than 100% pearl millet (PMF) (BV= 56.71%; NPU = 39.99% and PER = 1.97). However, these values were similar to those of the UNICEF product, (CTL) (BV = 70.54%; NPU = 69.86%; PER = 2.35). Biological value (BV) serves as an indicator of the fraction of protein absorbed from dietary sources that are subsequently utilized in the synthesis of body proteins. It also reflects the efficiency with which digested protein can be employed in cellular protein synthesis.

Effect of Pearl Millet Ready-to-use-therapeutic Food and Control Samples on Hematological Parameters in Diabetic Albino Wistar Rat

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Table 2: Effects of pearl millet ready-to-use- therapeutic food and control samples on Hematological Parameters in Wister Rat

Parameters	PMF	PBM1	PBM2	PBM3	CTL	*NR
PVC%	$31.00^{e}\pm0.21$	$37.06^{d}\pm0.28$	$40.09^{c}\pm0.15$	$44.99^{a}\pm0.94$	$42.00^{b}\pm0.65$	37.6-50.6
Hb (g/dl)	$10.02^{e}\pm0.02$	$11.80^{d}\pm0.30$	$12.50^{c}\pm0.09$	$14.98^{a}\pm0.31$	$14.40^{b}\pm0.11$	11.5-16.1
WBC ($x10^{3}mm^{-3}$)	$5.91^{d}\pm0.31$	$6.60^{c}\pm0.28$	$7.08^{c}\pm0.15$	$11.55^{a}\pm0.62$	$9.30^{b}\pm0.81$	6.6-12.6
RBC ($x10^{3}mm^{-3}$)	$5.91^{e}\pm0.30$	$6.54^{d}\pm0.71$	$7.99^{b}\pm0.05$	$8.04^{a}\pm0.03$	$7.81^{c}\pm0.24$	6.76-9.75
MCHC (g/dL)	$27.69^{e}\pm0.50$	$30.48^{d}\pm0.11$	$32.80^{c}\pm0.30$	$34.00^{a}\pm0.08$	$33.06^{b}\pm0.07$	28.2-34.1
MCH (pg)	$10.95^{e}\pm0.05$	$14.64^{d}\pm0.08$	$17.84^{c}\pm0.13$	$19.76^{b}\pm0.13$	$20.00^{a}\pm0.03$	16.0-23.1
MCV (fl)	$41.73^{e}\pm0.07$	$49.38^{d}\pm0.91$	$52.53^{c}\pm0.13$	$68.72^{a}\pm0.08$	$59.06^{b}\pm0.10$	50.0-77.8
Neutrophils (%)	$8.33^{e}\pm0.03$	$13.60^{d}\pm0.50$	$28.10^{c}\pm0.90$	$35.11^{a}\pm0.15$	$33.10^{b}\pm0.15$	5.3-38.1
Lymphocytes (%)	$53.10^{e}\pm0.50$	$62.40^{d}\pm0.45$	$75.60^{b}\pm0.35$	$86.90^{a}\pm0.07$	$74.30^{c}\pm0.35$	56.7-93.1
Monocytes (%)	$0.00^{c}\pm0.00$	$0.00^{c}\pm0.00$	$1.00^{b}\pm0.03$	$2.00^{a}\pm0.00$	$0.00^{c}\pm0.00$	0.00-7.7
Eosinophils (%)	$0.18^{c}\pm0.03$	$1.70^{b}\pm0.02$	$1.99^{a}\pm0.05$	$2.00^{a}\pm0.02$	$2.00^{a}\pm0.04$	0.0-3.4
Basophils (%)	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	0.0-0.4

Mean ± SD. Values with the same superscript are not significantly different at p>0.05.
Key: PMF: (100% Pearl millet flour); PBM1: (60% Pearl millet + 20% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); PMB2: (50% Pearl millet + 30% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); PMB3: (40% Pearl millet + 40% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); CTL: Control (UNICEF product).*Normal Range.

Packed cell volume (PCV) of the experimental rat fed on formulated pearl millet ready-to-use-therapeutic food ranged from 37.06% in PBM1 to 44.99% in PBM3, while that of control sample (PMF) and CTL was 31.00% and 42.00% respectively. The hemoglobin (Hb) concentration ranged from 11.80 g/dL in PBM1 to 14.98 g/dL in PBM3. The hematological characteristics of Albino Wistar rats that were administered a formulated pearl millet ready-to-use therapeutic food, alongside a control sample, are detailed in Table 2. The rats fed on CTL (14.40 g/dL) but had hematological values higher than 10.02 g/dL (PMF). For white blood cells, the values ranged between 6.60x $10^{3}mm^{-3}$and 11.55 x $10^{3}mm^{-3}$in PBM1 and PBM3, respectively. More so, they exhibited comparable similarities to those rats fed on CTL (9.30 x $10^{3}mm^{3}$) but higher than 5.91 x $10^{3}mm^{-3}$in PMF. The red blood cells concentrations ranged between 6.54 x $10^{3}mm^{-3}$ and 8.04 x $10^{3}mm^{-3}$ for PBM1 and PBM3, respectively. These values shared seminaries to CTL (7.81 x $10^{3}mm^{-3}$) and higher than 5.91 x $10^{3}mm^{-3}$in PMF. Lymphocytes ranged from 62.40 to 86.90% for rats in the PBM1 and PBM3 groups, respectively, and were comparable to CTL (74.30%) but higher than the 53.10% obtained in PMF.

The elevated levels of packed cell volume (PCV), hemoglobin (Hb), red blood cells (RBC), and lymphocytes noted in rats administered a formulated pearl millet ready-to-use therapeutic food further underscore the nutritional benefits of these food samples. Research has established that diets deficient in protein typically lead to diminished hemoglobin synthesis and compromised immune function. Conversely, the low PCV and Hb levels observed in rats consuming a diet exclusively based on pearl millet suggest inadequate protein quality and reduced blood production (Ijarotimi and Keshinro, 2012).

Effect of Pearl Millet Ready-to-use-therapeutic Food and Control Samples on Bchemical Parameters of Diabetic Albino Wistar Rat

The creatinine levels in rats consuming the formulated pearl millet ready-to-use therapeutic food varied from 27.12 mg/dl in the PBM1 group to 14.00 mg/dl in the PBM3 group, while the control samples exhibited values of 36.03 mg/dl (PMF) and 17.22 mg/dl (CTL). Urea concentrations in rats fed the experimental pearl millet formulation ranged from 8.93 mg/dL in the PBM1 group to 18.31 mg/dL in the PBM3 group, showing comparability to the control group (15.22 mg/dL) but remaining lower than the 6.30 mg/dL threshold.

Research indicated that serum creatinine levels are influenced by its production, glomerular filtration, and tubular secretion, which can serve as indicators of renal function. It is a byproduct of dietary protein metabolism and is excreted in urine by the kidneys (Akbar *et al.*, 2013). Urea nitrogen, a typical nitrogenous waste product present in the bloodstream, results from the degradation of dietary proteins.

In healthy individuals, the kidneys effectively eliminate urea nitrogen from the blood; however, elevated blood urea levels are indicative of renal impairment, with increased creatinine and urea levels reflecting diminished kidney function (Haider *et al.*, 2020). In a comparative analysis, the creatinine levels in rats consuming the formulated pearl millet ready-to-use therapeutic food remained within the normal range, as reported by Iseki *et al.* (1997).

Therefore, it is suggested that these food samples did not adversely affect the kidney's glomerular filtration rate or overall functionality.

The albumin concentrations in the developed pearl millet ready-to-use therapeutic food varied from 3.79 g/dL in PBM1 to 4.40 g/dL in PBM3. In contrast, the albumin level for the control sample was recorded at 4.41 g/dL (CTL) and 3.15 g/dL in PMF. Notably, the albumin level in rats consuming the control sample (CTL) was significantly elevated ($p<0.05$) compared to those who fed the formulated pearl millet ready-to-use therapeutic food and PMF samples. Regarding globulin levels, the findings indicated a range from 3.30 g/dL in PBM1 to 3.61 g/dL in PBM3, while the control sample (CTL) exhibited values of 2.51 g/dL and 2.11 g/dL in PMF. A significant difference ($p<0.05$) was observed between the globulin levels of the control sample and those of the experimental food samples.

This study revealed that the total blood protein and serum albumin concentrations in rats fed the developed pearl millet ready-to-use therapeutic food were comparatively below the normal ranges for serum protein (5.6 to 7.6 g/dL) and albumin (3.8 to 4.8 g/dL). This phenomenon may be linked to the plant-based nature of the protein source utilized. It is a well-documented fact that the bioavailability of proteins from plant sources is often lower than that of animal proteins, and the levels of plasma proteins, particularly albumin, are dependent on both the intake quantity and the quality of the protein consumed.

Table 3: Effects of ready-to-use- therapeutic food on Biochemical Parameters in Wistar Rat

Parameters	PMF	PBM1	PBM2	PBM3	CTL	*NR
Creatinine (mg/dl)	$36.03^{a}\pm0.04$	$27.12^{b}\pm0.05$	$20.85^{c}\pm0.13$	$14.00^{e}\pm0.31$	$17.22^{d}\pm0.08$	0.2-0.8
Urea (mg/dl)	$6.30^{e}\pm0.03$	$8.93^{d}\pm0.05$	$10.45^{c}\pm0.14$	$18.31^{a}\pm0.09$	$15.22^{b}\pm0.11$	7 -20
Total protein(g/dL)	$5.26^{e}\pm0.04$	$7.09^{c}\pm0.03$	$7.54^{b}\pm0.02$	$8.01^{a}\pm0.03$	$6.92^{d}\pm0.22$	5.6-7.6
Albumin (g/dL)	$3.15^{d}\pm0.07$	$3.79^{c}\pm0.10$	$4.01^{b}\pm0.06$	$4.40^{a}\pm0.02$	$4.41^{a}\pm0.06$	3.8-4.8
Globulin (g/dL)	$2.11^{e}\pm0.02$	$3.30^{c}\pm0.03$	$3.53^{b}\pm0.09$	$3.61^{a}\pm0.10$	$2.51^{d}\pm0.03$	-
AST (µ/L)	$30.21^{e}\pm0.12$	$46.11^{d}\pm0.14$	$50.12^{c}\pm0.61$	$57.15^{b}\pm0.82$	$60.11^{a}\pm0.41$	45.7-80.8
ALT (µ/L)	$16.22^{e}\pm0.82$	$52.41^{d}\pm0.16$	$57.87^{c}\pm0.41$	$69.15^{b}\pm0.82$	$78.17^{a}\pm0.34$	17.5-30.2
ALP (µ/L)	$89.00^{d}\pm1.30$	$95.11^{c}\pm1.05$	$106.66^{b}\pm0.94$	$116.83^{a}\pm1.46$	$105.77^{b}\pm1.65$	56.8-128
AST/ALT ratio	$1.86^{a}\pm0.00$	$0.88^{b}\pm0.01$	$0.86^{b}\pm0.01$	$0.83^{c}\pm0.00$	$0.77^{d}\pm0.01$	<1.0

Mean ± SD. Values with the same superscript are not significantly different at $p>0.05$.
Key: PMF: (100% Pearl millet flour); PBM1: (60% Pearl millet + 20% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); PMB2: (50% Pearl millet + 30% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); PMB3: (40% Pearl millet + 40% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); CTL: Control (UNICEF product). *Normal Range.

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) serve as critical enzymatic markers for assessing liver functionality. In the context of the formulated pearl millet ready-to-use therapeutic food, AST levels were observed to range from 46.11 µ/L in PBM1 to 57.15 µ/L in PBM3, with these values being significantly elevated ($p<0.05$) compared to both the 100% pearl millet (PMF) at 30.21 µ/L and the control sample (CTL) at 60.11 µ/L. Notably, the AST levels in the experimental food samples remained within the normal range of 45.70-80.80 µ/L. For ALT, values varied from 52.41 µ/L in PBM1 to 69.15 µ/L in PBM3, while the CTL recorded 78.17 µ/L and PMF showed a lower value of 16.22 µ/L. Statistically, the ALT levels in rats consuming 100% pearl millet (PMF) were significantly lower than those in the formulated pearl millet ready-to-use therapeutic food and the UNICEF sample (CTL). The ALP values for the formulated pearl millet ready-to-use therapeutic food ranged from 95.11 µ/L in PBM1 to 116.83 µ/L in PBM3, contrasting with PMF and CTL values of 89.00 µ/L and 105.77 µ/L, respectively. Statistically, the ALP value for the PMF sample was significantly lower than that of the other samples. The AST/ALT ratios for the experimental, formulated pearl millet ready-to-use therapeutic food ranged from 0.88 in PBM1

to 0.83 in PBM3, exceeding the CTL ratio of 0.77 but remaining within the normal range of less than 1.0. These findings suggest that the formulated pearl millet ready-to-use therapeutic food is safe for consumption and does not pose a risk of liver toxicity. Previous scientific studies have indicated that elevated levels of AST or ALT in the bloodstream are indicative of liver dysfunction and damage (Al-Mamary *et al.*, 2002Aliyu *et al.*, 2022).

4. Microbial, and Aflatoxin Status of Pearl Millet Ready-to-use-therapeutic Food and Control Samples

Table4: Microbial and aflatoxin status of ready-to-use- therapeutic food

Samples	PMF	PBM1	PBM2	PBM3	CTL	*SON
Total Viable count (*cfu/g*)	1.9×10^2	1.5×10^2	1.4×10^2	1.3×10^2	1.0×10^2	$> x10^5$
Mould/yeast (*cfu/g*)	2.5×10^2	2.0×10^1	1.2×10^2	1.3×10^2	1.0×10^2	$> x10^5$
Coliform (*cfu/g*)	Nil	Nil	Nil	Nil	Nil	0
Escherichia coli (*cfu/g*)	Nil	Nil	Nil	Nil	Nil	0
AFB_1	3.90	2.85	2.30	2.00	1.00	<5

Key: PMF: (100% Pearl millet flour); PBM1: (60% Pearl millet + 20% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); PMB2: (50% Pearl millet + 30% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); PMB3: (40% Pearl millet + 40% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); CTL: Control (UNICEF product); *SON: Standard organization of Nigeria recommendation.

The mean total viable count of pearl millet ready-to-use-therapeutic food ranged between 1.5 x 10^2 cfu/g in PBM1 to 1.30 x 10^4 cfu/g in PBM3, while the controls varied from 1.90 x 10^2 cfu/g in PMF and 1.0 x 10^2 cfu/g CT. Compared to the values obtained in the pearl millet ready-to-use therapeutic food and CTL, those in PMF are significantly lower. However, all experimental samples are within the recommended values (<10 *x* 10 ^{5}) by Standard Organization of Nigerian for quality for food check. For the mean mould/yeast count of pearl millet ready-to-use-therapeutic food ranged between 2.0 x 10^2 cfu/g in PBM1 to 1.3 x 10^4cfu/g in PBM3, while the controls varied from 2.5 x 10^2 cfu/g in PMF and 1.0 x 10^2 cfu/g CTL. Compared to the values obtained in the pearl millet ready-to-use therapeutic food and CTL, those in PMF are significantly lower. However, all experimental samples are within the recommended values (<10 *x* 10 ^{5}) by Standard Organization of Nigerian for food safety. However, neither coliform bacteria nor Escherichia coli were detected in any of the prepared pearl millet ready-to-use therapeutic foods or in the control samples. The aflatoxin content of the formulated pearl millet ready-to-use therapeutic samples and the controls ranged from 3.90 in PMF to 1.0 in CTL. All experimental samples are within the recommended values (<10 *x* 10 ^{5}) by Standard Organization of Nigerian for food safety. Hence, the formulated pearl millet ready-to-use therapeutic samples and the controls may pose no adverse effects on consumption.

5. Sensory Attributes of Pearl Millet Ready-to-use-therapeutic Food and Control Samples

Table 5. Sensory attributes of ready-to-use- therapeutic food

Sample	PMF	PBM1	PBM2	PBM3	CTL
Appearance	$5.43^{e}\pm1.21$	$6.90^{d}\pm1.02$	$6.98^{c}\pm1.26$	$7.50^{b}\pm1.54$	$8.05^{a}\pm1.00$
Texture	$6.38^{e}\pm1.27$	$6.85^{c}\pm1.53$	$6.80^{d}\pm1.47$	$6.89^{b}\pm1.68$	$8.00^{a}\pm0.77$
Taste	$5.99^{d}\pm1.57$	$7.12^{b}\pm1.59$	$6.76^{c}\pm1.66$	$6.79^{c}\pm1.88$	$7.59^{a}\pm1.50$
Aroma	$6.56^{d}\pm1.58$	$6.59^{d}\pm1.90$	$6.99^{b}\pm1.55$	$6.65^{c}\pm1.29$	$8.22^{a}\pm0.99$
Overall acceptability	$6.70^{e}\pm1.56$	$7.78^{c}\pm0.85$	$6.89^{d}\pm1.22$	$7.99^{b}\pm1.74$	$8.93^{a}\pm1.65$

Mean ± SD. Values with the same superscript are not significantly different at p>0.05.
Key: PMF: (100% Pearl millet flour); PBM1: (60% Pearl millet + 20% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); PMB2: (50% Pearl millet + 30% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); PMB3: (40% Pearl millet + 40% Bambara groundnut + 5% Moringa leave + 5% Vegetable oil + 10% Sucrose); CTL: Control (UNICEF product).

The formulated pearl millet product exhibited significantly higher ratings (p<0.05) in terms of appearance, texture, taste, and aroma compared to PMF (100% pearl millet), although it received lower scores when juxtapose with the control sample (CTL). In terms of overall

acceptability, the CTL was rated insignificantly ($p < 0.05$) higher than the other experimental food samples, yet it was significantly lower than the CNT (a UNICEF standard). The observed differences between the formulated pearl millet ready-to-use therapeutic food and CNT may be attributed to variations in food composition, processing methods, and the panelists' familiarity with the control sample.

CONCLUSION

This study successfully formulated and evaluated the nutritional, hematological, microbial, and sensory properties of locally produced Ready-to-Use Therapeutic Foods (RUTFs) using readily available ingredients, including pearl millet, Bambara groundnut, and *Moringa oleifera* leaf powder. The findings demonstrated that the formulated RUTFs—particularly PBM3 (40% Pearl millet, 40% Bambara groundnut, 5% *Moringa* leaf, 5% vegetable oil, and 10% sucrose)—exhibited superior nutritional performance compared to both the 100% pearl millet formulation (PMF) and other experimental blends. Hematological indices, including packed cell volume (PCV), red blood cell count, and hemoglobin concentration, showed significant improvement in rats fed the formulated RUTFs, especially PBM3. Growth performance in these groups also surpassed that of animals fed PMF, indicating improved protein quality and utilization. Notably, the performance of PBM3 was comparable to that of the commercial UNICEF RUTF (CTL), suggesting its potential as an effective alternative for managing severe acute malnutrition. In terms of organoleptic properties (appearance, texture, taste, aroma, and overall acceptability), the formulated RUTFs received higher ratings than PMF but slightly lower than CTL. This may reflect the panelists' greater familiarity with the commercial product. Importantly, microbial analysis confirmed the safety of the formulated products, with no detectable *Escherichia coli* or coliform bacteria, and total viable counts within acceptable limits.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTIONS

Junaidu MS, Talabi JY, Alebiosu I, and Ajayi K designed research; Junaidu MS conducted research; Junaidu MS analyzed data; and Junaidu MS, Talabi JY, Alebiosu I, and Ajayi K wrote the paper. Junaidu MS had primary responsibility for the final content. All authors read and approved the final manuscript.

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