LGJDX ISSN No: 1008-0562 Journal Of Liaoning Technical University Natural Science Edition

GROWTH PERFORMANCE, BIOCHEMICAL MARKERS AND HISTOLOGICAL EVALUATION IN RATS FED OPTIMIZED *LUAM-NAHAN* PORRIDGE FLOUR FORMULATED WITH HIGH-QUALITY PROTEIN MAIZE AND PROVITAMIN-A CASSAVA

DAAGEMA Anastasia Angorkuma
Department of Community Health
Benue State School of Health Technology Agasha, Benue State/
Department of Human Nutrition and Dietetics
College of Medicine and Health Science
Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria

AJAYI Kayode
Department of Human Nutrition and Dietetics
College of Medicine and Health Science
Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria

TALABI Justina Yetunde
Department of Human Nutrition and Dietetics
College of Medicine and Health Science
Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria

OBISESAN Abiola O.
Department of Pharmaceutical Microbiology and Biotechnology
College of Pharmacy
Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria

MOMOH Clement Owoicho
Faculty of Life and Health Science
Nutrition Innovation Centre for Food and Health
Ulster University, BT521SA, UK.

CHIPILI Given
Department of Nutritional Sciences
School of Applied Sciences and Technology
Mukuba University, Kitwe, Zambia.

OLANIYI Kehinde S.
Department of Human Physiology
College of Medicine and Health Sciences
Afe Babalola University, Ado Ekiti, Nigeria.

OYEJIDE Samuel Olarewaju
Department of Clinical Pharmacology and Clinical Pharmacy
Bogomolets National Medical University Kyiv, Ukraine

LGJDX ISSN No: 1008-0562 Journal Of Liaoning Technical University Natural Science Edition

Abstract

Preschooler under nutrition especially in the low and middle-income countries has continued to raise global concern, requiring urgent attention. This study evaluates the growth performance, biochemical and histological parameters of rats feed with an enhanced *luam-nahan* porridge flour developed for preschoolers. Thirty-two male wister rats were acclimatized for seven days, grouped into four groups of eight rats each using complete randomized design for a twenty-eight days feeding experiment. G1=basal diet, G2=traditional diet, G3= Optimized *luam-nahan* diet and G4=commercial rat pellet. Rats were weighed weekly, after sacrifice, reverse HPLC and spectrometry methods were used for serum micronutrient analysis while hematoxylin and eosin staining procedure was used for organ histology. Data was analyzed using one-way ANOVA, result reported as means and standard deviations($p<0.05$). The result indicates that, only rats in groups 3 and 4 showed significant mean weight gain of 7.19g±1.63g and 8.55g±1.06, with the highest mean feed intake of 278.95g±0.15 recorded from rats in G4 compared to other groups. Serum retinol(2.01µmol/L±0.01) and zinc (20.8 µmol/L±0.52) was highest among rats in G3. Serum iron(15.6ng/mL±0.36) was lower in G2 compared to G3(32.9 ng/mL±0.10). Lower organ weight with negative histological changes was observed from rats in G1 and G2 relative to those of G3 and G4 with higher weight and healthy organs. The optimized *luam-nahan* flour showed adequate support for animal growth, higher serum micronutrient bioavailability and consumption safety. The enhanced flour could serve as a viable option for the prevention and reduction of protein and micronutrient deficiencies among preschoolers and other populations.

Keywords: Micronutrients, Malnutrition, Biological Availability, Developing Countries, Organ Size

Introduction

Preschool age is a transitional period when children move from breastfeeding and complementary feeding to eating family meals, which are often primarily composed of starch-based staples resulting to protein and micronutrient malnutrition particularly those of vitamin A, iron and zinc. Preschooler malnutrition has continued to raise global concern, requiring urgent attention especially in the low and middle-income countries like Nigeria where the national prevalence of stunting, wasting and underweight among children under five is currently reported as 40%, 8% and 27% respectively (Nigeria Demographic and Health Survey(NDHS), 2024). Malnourished children have greater risk of infection, morbidity and mortality in addition to long-lasting physical and cognitive impairments compromising a child growth, development, overall survival and future attainments(Mekuria et al., 2022). Culturally, acceptable food-based approaches such as the nutritional enhancement of traditional foods for example traditional porridges such as *luam-nahan* using nutrient rich locally available food materials are advocated for sustainable malnutrition prevention during this critical period of growth and development.

Luam-nahan, is a traditional Nigerian porridge popular in the North Central part of the Nigeria among the Tiv tribe and other ethnic groups. It is a composite flour blend of traditional cereals and cassava; eaten with complementary soups such as okra due to affordability, soft texture and ease of swallowing by young children. *Luam-nahan,* being primarily carbohydrate-based (Dendegh et al., 2021), lacks sufficient protein and micronutrients to adequately support preschooler growth and development. However, given

its widespread consumption, *luam-nahan* presents an opportunity for nutritional enhancement to improve the health and dietary status of preschoolers. By fortifying it with locally available, nutrient-dense ingredients, its nutritional profile can be optimized. In this study, an improved *luam-nahan* flour was developed using high-quality protein maize (QPM), provitamin-A cassava, soybean, and *Jatropha tanjorensis* leaves—a mineral-rich but underutilized vegetable in Nigeria, colloquially known as 'hospital too far.' This fortified formulation aims to provide preschoolers with essential nutrients for growth and development while combating protein-energy malnutrition and micronutrient deficiencies. To rigorously evaluate its growth-promoting potential and safety for human consumption, animal model testing was conducted, as it provides critical preclinical data. This study assessed the growth performance, biochemical markers, and histopathological profiles of rats fed the optimized *luam-nahan* flour formulation. The enhanced flour was developed using nutritionally improved maize-high-quality protein maize (QPM) and improved cassava-provitamin-A cassava, further fortified with defatted soybean and *Jatropha tanjorensis* leaves to address nutritional deficiencies in preschool-aged children.

Materials and Method

Source of materials

Male Wister rats were obtained from Lagos State University Teaching Hospital (LUTH); Afe Babalola University Ado-Ekiti (ABUAD) commercial rat pellets and additional materials for animal feed formulation which were cellulose (wheat bran), soya vegetable oil, 'Agriteck' vitamin and minerals pre-mix were obtained from animal feeds shop in Ado-Ekiti. QPM (BR9928-DMR-SRY) and Provitamin-A cassava (TMS01/1371) were obtained from the Institute of Agricultural Research and Training (IAR&T) Ibadan, Oyo State. *Jatropa tanjorensis* leaves, soybeans, white cassava (TME 419) and white corn (TZW 2005) were all obtained from Benue State from a private farm 'Nature's Heritage farms'. All chemicals used for all analyses were of analytical reagent grade.

Processing of food materials

Traditional luam-nahan flour preparation: White maize was cleaned by sorting, washing in tap water, and sun-drying until it reached a constant weight. Mature white cassava roots were peeled with a simple kitchen knife, washed with tap water, sliced into thin chips, and also sun-dried to achieve a constant weight. Both materials were milled separately using a SUMEC 6.5HP disc attrition mill and then sieved with a 1mm fine sieve to obtain fine flours. The two flours were subsequently blended in a 50:50 (w/w) ratio for incorporation into experimental rodent diets

Improved luam-nahan flour preparation: QPM flour was produced following the method described by Beruk *et al.,* (2015) with some modifications. The maize was first cleaned through winnowing, sorting, and washing with tap water. It was then soaked in stainless steel basins for 24 hours, with the water being changed every 6 hours to prevent fermentation. After soaking, the maize was wet-cleaned again with tap water and subsequently oven-dried in a laboratory oven (model TT-9023A) at a constant temperature of 60°C for 6 hours until it reached a consistent weight.

Provitamin-A cassava flour production followed the methods described by Igbua *et al*., (2019) and Toluwalope *et al.,* (2018). Mature cassava roots were first wet cleaned before being peeled with a simple kitchen knife. They were then washed again, sliced into thin

chips, naturally fermented for three days in water, and dried to a constant weight on stainless steel tables under the sun.

Defatted soybean flour production was carried out according to the method of Momoh *et al.,* (2020) with some modifications. The soybean seeds were cleaned through a process of winnowing, sorting, and washing with tap water. The seeds were soaked in tap water for 12 hours in stainless steel basins. After soaking, hand abrasion and flotation techniques were used to separate the seed coat from the seeds. The seeds were then oven-dried in an oven model TT-9023A at 60°C for 4 hours until they reached a constant weight. Following this, the seeds were defatted using a screw press to obtain soy cake.

The production of *Jatropha tanjorensis* leaf flour was carried out according to the method described by Onoja *et al.,* (2014). Freshly harvested mature leaves were washed, then shade-dried indoors on stainless steel tables until they reached a consistent weight.

The four food materials were milled separately using a SUMEC 6.5HP disc attrition mill to produce individual flours. These flours were then sieved through a 1mm fine sieve to obtain a fine texture flour. After flours were then blended at a ratio of 44:44:10:2w/w for utilization in the production of rat feeds.

Animal feeds Formulation

This was done using material balancing as shown in table 1 below. In addition, the improved flour animal feed was formulated by blending the test and basal diet in order to obtain an isonitrogenous diet of 10% protein level using formula 1 below. The feeds prepared were pelleted, and alongside the commercial feeds, they were stored in airtight bags at room temperature for rats' feeding.

$$IN = \frac{a}{100} X\ b = \frac{10}{100} X\ c \qquad (1)$$

Where:

A = the original sample protein content of the improved flour as analyzed

B= required weight of sample for new feed blend

C= total weight of the blend

IN = isonitrogenous

Table 1: Materials used for animal feed preparation

Food component	Basal (g)	Traditional (g)	Improved (g)
Cellulose(5%)	112	112	112
Vegetable oil(8%)	179.2	179.2	179.2
Vitamin mix(1%)	22.4	22.4	22.4

Mineral mix(4%)	89.6	89.6	89.6
Test protein(10%)	-	-	224
Starch(%)	1836.8	1836.8	1580.54
Jatropha Tanjorensis leaf powder(2%)	-	-	32.26
Total	2240	2240	2240

Animal experimentation

Twenty-eight (28) days feeding experiment were carried out with 32 male Wister rats (8 per group). Group One (G1) = basal diet, Group Two (G2) = traditional diet, Group Three (G3) = improved *luam-nahan* flour and Group Four (G4) = commercial rat pellet using the method described by Gernah *et al.,* (2012). Experimental rats of 28- 35 days were obtained from the Lagos University Teaching Hospital (LUTH) animal breeding unit and transported to the ABUAD animal research center before sunrise to reduce stress on the animal. The rats were acclimatized for 7 days, during which commercial rat feeds and water were given ad libitum. At the end of acclimatization, the rats were then grouped into four groups using a complete randomized design (CRD), where the rats were initially weighed to the nearest 0.1g before been allocated to their various groups such that the mean weight of the groups was not more than ± 2g. For all the groups, feeding was done daily and each group was offered 80g of appropriate feeds; daily leftovers were also weighed and documented while water was given ad libitum throughout the experiment period. The rats were housed in a room maintained at a 12-hour light-dark cycle, temperature of 23±2^{o}C, and humidity of 30 - 70%. After the initial weighing and regrouping which was considered as day zero, rats were weighed once a week till the end of the feeding experiment on day 7th, 14th, 21st and 28th respectively, by weighting individual rats in each group using a WANT electronic digital weighing instrument and a light bowl as a bassinet on the weighing scale and summing the total to obtain mean weight gain or loss per week in gramms. The mean feed intake and weight changes of the rats during the study period were used to calculate the following listed below as described by Duodu et al., (2020).

1. Feed intake for the feeding period(g) = total amount of feed offered – total amount of feed consumed. (2)
2. Daily mean feed consumed per week(g/day) $= \frac{total\ feed\ intake\ per\ group}{number\ of\ days}$ (3)
3. Protein intake per group for the feeding period(g) $= \frac{protein\ content\ of\ food\ x\ quantity\ consumed}{total\ quantity\ given\ to\ the\ animals}$ (4)

4. Total mean weight gain(s) for the feeding period(g) = final mean weight – initial mean weight (5)
5. Daily mean growth rate per week(g/day) = $\frac{final\ weight - initial\ weight}{numbers\ of\ days}$ (6)
6. Feed efficiency for the feeding period = $\frac{final\ weight - initial\ weight}{amount\ of\ feeds\ consumed}$ (7)
7. Mean feed intake/group = $\frac{Total\ feed\ intake}{No\ of\ animals}$ (8)
8. Mean weight gain/group = $\frac{Total\ weight\ gain}{No\ of\ animals}$ (9)

Animal sacrifice

At the end of the experiment, the rats were euthanized with a single dose of 100mg/kg Ketamine hydrochloride(Overmyer et al., 2015). Blood was collected by a ventricular puncture to the heart into plain sample bottles covered with aluminum foil, allowed to stand for 10 minutes away from light and centrifuged at 3000rpm for 30 minutes to obtain serum, that was transferred carefully with pasture pipettes into new sets of well labeled plain sample bottles covered with aluminum foil and stored at -5^{o}c away from light until analysis within 48 hours. The liver, kidney, spleen and gut were collected, quickly weighed and post-fixed into 10% BFS (Buffered Formaldehyde Solution) for 24hrs for histology.

Serum vitamin A analysis

Serum retinol was done using the reverse phase HPLC method described by Fares *et al.,* (2011). 0.5ml serum and retinol acetate used as an internal standard were deproteinized in the presence of 0.5ml ethanol containing 0.1ml butylated hydroxytoluene. The organic layer containing retinol was extracted with 0.1ml hexane and evaporated to dryness under nitrogen steam. The residue was re-dissolved in 0.25ml ethanol, and 8µml of the sample was injected into a C18 reversed-phase column (Shimpack ODS-M, Shimadzu, Japan); the mobile phase consisted of methanol gradient grade (Merk kGaA, and Germany) at a flow rate of 1.5ml/min for 10mins run. Vitamin A and retinol acetate peaks were dictated at 290nm. Linearity was achieved in the range of 0.175-4.24µmol/L. The accuracy and precision of this method were tested with two control levels of 6.1% and 5.6% at the concentrations of 1.65 and 26.7 µmol/L.

Serum iron and zinc determination

This was done using the spectrometry method described by Rolf *et al.,* (2021). Sample decomposition was done by adding 1ml of HNO_3 and 250µl H_2O_2 to 500 µl serum in a 15ml vials and allowed to rest for 30mins. After the rest, the vial was heated in a domestic microwave according to the following programme: 6 times of 50sec at 420W, 5 times of 5mins at 90W and 4 times of 50sec at 420W with a 2min cooling down step at -4^{o}C after each individual step. Finally, Milli-Q water was added in the same vial until a final volume of 10ml was obtained. Levels of iron and zinc in the samples were determined using flame atomic absorption spectrometry(FAAS) using UNICAM 989 SOLLAR (UK) spectrophotometer. Standard curve for determining of iron and zinc were prepared by diluting iron (Merc 1.19781) and zinc (Merck 1.19806) standard reference materials in a range from 0 to 5.0µg/cm^3. To test the accuracy of the method serum certified reference material (Seronorm^{TM} Trace Element Serum L-1 JL4409) was used.

Organ histology

The liver, kidney, spleen and gut tissue post-fixed in 10% BFS were removed and dehydrated in graded levels of alcohol, cleared in xylene, embedded in molten paraffin wax for infiltration and sectioned into 5 microns using rotary microtone then placed on slides. Satisfactory tissue sectioned slides were then stained with hematoxylin and eosin, according to the procedure of Ankle and Joshi, (2011) for easy observation of the tissue elements under the microscope. Photomicrographs were taken with Moticam image plus a 2.0 digital camera (Motif China Group Ltd. 1999-2004) at X100 magnification.

Statistical analysis

Statistical Package for Social Sciences (SPSS) V21 and Graphpad Prism computer software V10 were used to analyze the data. Means, frequency and Standard deviation were calculated where appropriate. Analysis of variance (ANOVA) and Fisher's least significant difference (LSD) was used to determine the mean difference between the groups post hoc; differences were considered significant at ($P<0.05$) significance level.

Result

Feed intake, weekly weighing and evaluation

Weekly weight monitoring and evaluation results (Table 2) revealed that animals fed the standard rat diet (G4) demonstrated the highest feed intake (2231.60g of 2240g provided). However, intergroup differences in feed consumption remained minimal (<10g). Protein intake and feed efficiency followed a similar pattern, with G4 showing the highest values, followed by progressively lower values through G3 to G1. The weekly mean feed consumption data (Figure 1a) revealed that all experimental groups maintained consistent daily intake (80g) during the first two weeks. However, consumption declined in weeks 3-4, with all groups consuming <80g/day. Growth performance analysis demonstrated distinct patterns among groups (Figures 1b-1d). While G2 (traditional *luam-nahan*), G3 (enhanced *luam-nahan*), and G4 (commercial feed) groups showed net weight gain during the study period, only G3 (57.48g) and G4 (68.42g) exhibited significant increases ($p<0.05$). In contrast, G1 (basal diet) showed a net weight loss (-15.16g).

Table 2: Total protein intake, feed efficiency, Mean feed intake and weight gain of experimental rats per group

	Groups			
Parameter	**G1**	**G2**	**G3**	**G4**
Total feed intake (g)	2225.48	2227.32	2230.62	2231.60
Total protein intake(g)	0	4.6	11.37	14.94
Feed efficiency	-0.681	0.143	2.577	3.066
Mean feed intake (g)	278.19 ± 0.02^{a}	278.42 ± 0.00^{b}	278.83 ± 0.10^{c}	278.95 ± 0.15^{d}
Mean weight gain(g)	-1.90 ± 3.56^{a}	0.40 ± 2.23^{b}	7.19 ± 1.63^{c}	8.55 ± 1.06^{d}

Values are presented as means and standard deviations(n=8), means with the different superscript on the same row are significantly different ($p<0.05$); G1-Basal diet, G2-Traditional *Luam-nahan*, G3 – Improved *Luam- nahan* and G4 – commercial rat feed.

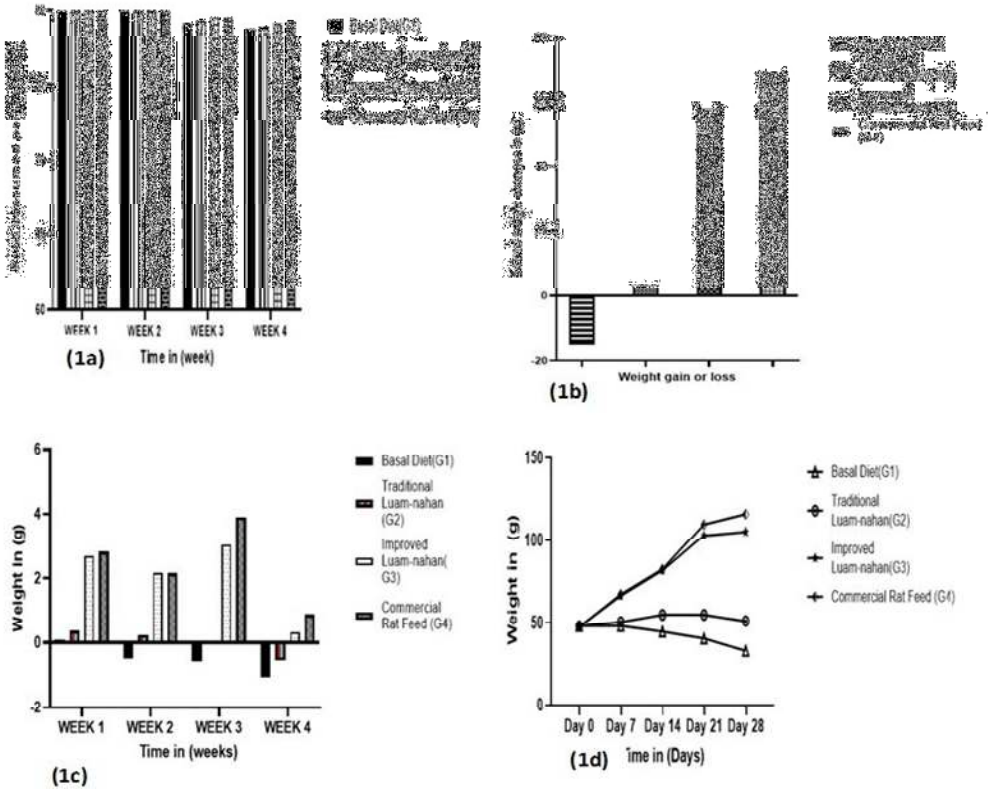

Figure 1 (1a) Daily mean feed consumed per week(g/day), **(1b)** Total mean weight changes for the feeding period(g), **(1c)** Daily mean growth rate per week(g/day) and **(1d)** Growth pattern of experimental rats for the feeding period

Serum vitamin A, iron and zinc

Serum analysis revealed distinct patterns in micronutrient status across groups (Figures 2a-2c). For serum retinol (Figure 2a), rats fed the improved *luam-nahan* diet (G3) showed the highest concentrations, followed by the commercial feed group (G4), while both the basal diet (G1) and traditional *luam-nahan* (G2) groups exhibited significantly lower levels ($p<0.05$), with G1 demonstrating the poorest retinol status. A similar trend was observed for serum zinc (Figure 2b), where G3 maintained superior zinc levels compared to all other groups, including G4, with G1 and G2 again showing the lowest concentrations. Regarding serum iron (Figure 2c), both G3 and G4 displayed enhanced iron status relative to G1 and G2, though interestingly, G4 (commercial feed) achieved slightly higher iron levels than the improved *luam-nahan* group (G3). These findings collectively demonstrate that the nutritionally enhanced *luam-nahan* formulation (G3) effectively improved micronutrient status, performing comparably to or exceeding the commercial feed (G4) in retinol and zinc provision, while the basal (G1) and traditional (G2) diets consistently showed inferior micronutrient profiles

LGJDX ISSN No: 1008-0562 Journal Of Liaoning Technical University Natural Science Edition

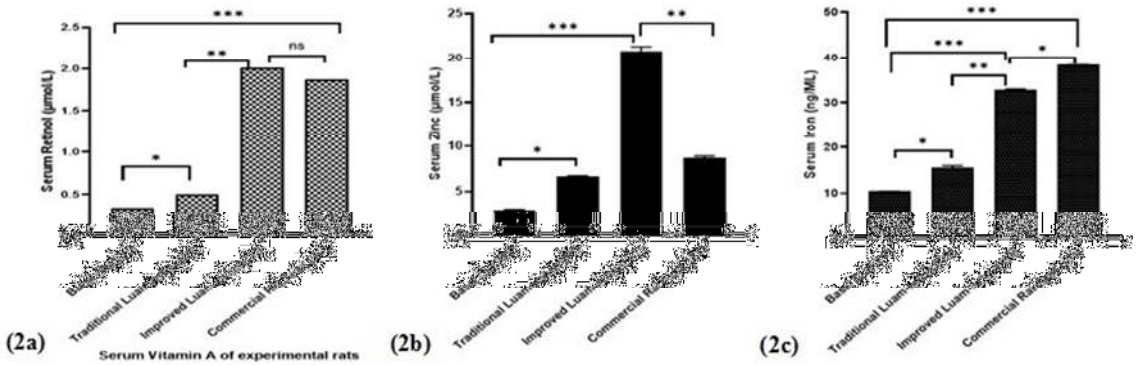

Figure 2 (2a) Serum vitamin A of experimental rats: (*P<0.01) There is a significant increase in in G2 compared to G1. (**P<0.001) There is a highly significant increase in G3 compared to G2. (***P<0.0001) There is a highly significant increase in G4 compared to G1. (^{ns}P>0.01) no significant difference between G3 and G4. **(2b)** Serum zinc of experimental rats: (*P<0.01) There is a significant increase in G2 compared to G1. (**P<0.001) There is a highly significant increase in G3 compared to G4. (***P<0.0001) There is a highly significant increase in G3 compared to G1. **(2c)** Serum iron of experimental rats: (*P<0.01) There is a significant increase in G2 compared to G1. (*P<0.01) There is a significant increase in G4 compared to G3. (**P<0.001) There is a highly significant increase in G3 compared to G2. (***P<0.0001) There is a highly significant increase in G3 compared to G1. (***P<0.0001) There is a highly significant increase in G4 compared to G1.

Organ weight analysis

The analysis of essential organ weights revealed distinct patterns among the experimental groups (Table 3). The improved *luam-nahan* formulation (G3) supported better organ development than both the traditional *luam-nahan* (G2) and basal diet (G1), though the commercial feed (G4) showed optimal effects on kidney, liver and spleen growth. Notably, G3 demonstrated particular efficacy in supporting gut development. Statistical comparisons showed no significant differences ($p>0.05$) in kidney weights among G2, G3 and G4, nor in liver/spleen weights between G1 and G2. However, gut weights differed significantly across all groups ($p<0.05$), with G1 exhibiting the highest values and G2 the lowest. These findings suggest that while the commercial diet promoted general organ growth, the enhanced *luam-nahan* formulation specifically enhanced gut development, potentially indicating improved nutrient absorption capacity compared to the traditional formulation.

Table 3: Relative organ weight(g) of experimental rats from the four groups

	Groups			
Organ	**G1(g)**	**G2(g)**	**G3(g)**	**G4(g)**
Kidney	1.04 ± 0.13^{a}	1.12 ± 0.28^{b}	1.14 ± 0.10^{b}	1.16 ± 0.07^{b}
Liver	1.60 ± 1.01^{a}	1.62 ± 0.53^{a}	2.50 ± 0.28^{b}	2.69 ± 1.19^{c}
Gut	4.17 ± 1.56^{a}	1.94 ± 0.43^{b}	2.70 ± 1.02^{c}	2.59 ± 0.64^{d}

Spleen	0.33 ± 0.76^{a}	0.36 ± 0.93^{a}	0.42 ± 0.90^{b}	0.68 ± 0.75^{c}

Values are presented as means and standard deviations(n=3), means with the same superscript on the same row are NOT significantly different ($p<0.05$). G1-Basal diet, G2-Traditional *Luam-nahan*, G3 – Improved *Luam- nahan* and G4 – commercial rat feed.

Histopathological Analysis

Microscopic evaluation of organ tissues revealed significant differences among experimental groups (Figures 3a-3d). Renal histology (Figure 3a) demonstrated marked pathological changes in G1, including glomerular enlargement with narrowed urinary spaces (US) and severe tubular hypertrophy. G2 kidneys exhibited glomerular shrinkage, reduced US, and minor tubular nuclear degeneration. In contrast, both G3 and G4 maintained normal renal architecture with preserved glomeruli (G), Bowman's capsules (BC), and distinct proximal (P) and distal (D) convoluted tubules. Hepatic examination (Figure 3b) showed severe cellular atrophy and apoptotic hepatocytes in G1, while G2 displayed mild mononuclear infiltration with focal necrosis. The improved *luam-nahan* group (G3) demonstrated normal hepatic cytoarchitecture with well-organized hepatocyte arrangement, comparable to the commercial feed group (G4) which exhibited typical liver histology including intact sinusoids and portal triads (PT). Splenic morphology (Figure 3c) revealed architectural disruption in G1, characterized by indistinct red/white pulp boundaries and increased megakaryocytes (arrowheads). G2 spleens showed moderate architectural disorganization with vacuolation, whereas G3 and G4 maintained normal splenic structure with clearly demarcated red (RP) and white pulp (WP) compartments. Intestinal evaluation (Figure 3d) indicated mucosal alterations in G1, including villous flattening, lamina propria inflammation, and prominent goblet cells. While G2 displayed near-normal villous architecture with mild lamina propria infiltration, both G3 and G4 exhibited optimal intestinal histology with intact mucosal layers, normal villous structure, and properly organized epithelium and lamina propria. These findings collectively demonstrate that the improved *luam-nahan* formulation (G3) effectively preserved tissue integrity across all examined organs, performing comparably to the commercial feed (G4) and markedly superior to both the basal (G1) and traditional (G2) diets.

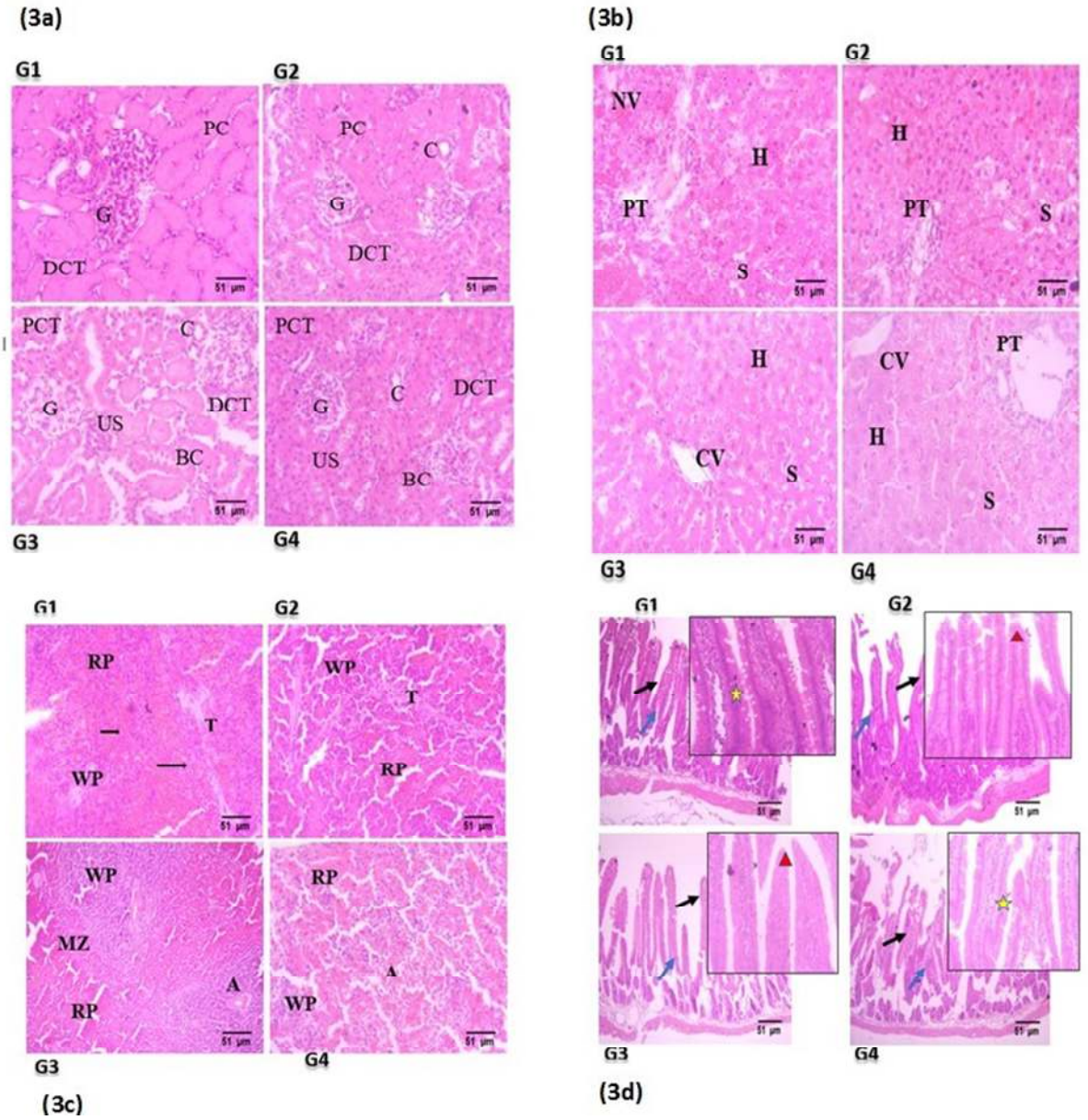

Figure 3 (3a) Photomicrograph of kidney (H & E stain) showing G: Glomerulus, PCT: Proximal convoluted tubules, DCT: Distal convoluted tubules, C: Capillaries, Bowman's capsule (BC), urinary space (US). **(3b)** Photomicrograph of liver (H & E stain), showing the Hepatocytes (H), Portal triad (PT), Central vein (CV), Nuclear vacuolization (NV), Sinusoids (S). **(3c)** Photomicrograph of spleen (H & E stain), the spleen's parenchyma showing the red pulp (RP), white pulp (WP), trabecular (T), marginal zone (MZ) and arteriole (A). **(3d)** Photomicrograph of intestine (H & E stain), black arrow = the epithelial lining, blue arrow = lamina propria, =goblet cells, = villi.

Discussion

The present study evaluated the nutritional efficacy of an improved *luam-nahan* formulation through comprehensive growth performance, biochemical, and histological assessments in a rat model. Our findings demonstrate that the enhanced flour (G3), formulated with high-

quality protein maize (QPM), provitamin-A cassava, soybean, and *Jatropha tanjorensis* leaves, significantly improved growth parameters, micronutrient status, and organ health compared to traditional *luam-nahan* (G2) and basal diets (G1), performing comparably to commercial feed (G4).

Feed intake and growth performance

The observed decline in feed intake after the initial two weeks aligns with established rodent growth patterns, where consumption peaks during early development (0–35 days) before stabilizing(Ghasemi *et al.,* 2021). The superior weight gains in G3 and G4 rats underscores the critical role of protein quality and bioavailability in growth, consistent with prior studies(Adeoti *et al.,* 2018). Notably, the improved *luam-nahan* (G3) supported growth nearly equivalent to commercial feed (G4), likely due to optimized nutrient composition and processing methods that enhanced digestibility. In contrast, the negative growth trajectory in G1 and G2 reflects protein-energy malnutrition, characterized by muscle wasting and hypoproteinemia(Adejuwon *et al.,* 2021; NRC, 1995). These outcomes mirror the challenges faced by Nigerian preschoolers consuming nutrient-poor diets, emphasizing the need for fortified traditional foods.

Micronutrient bioavailability

The significantly higher serum retinol in G3 and G4 rats validates the efficacy of provitamin-A cassava and *Jatropha tanjorensis* as sustainable sources of β-carotene, consistent with findings from Afolami et al., (2021). This supports biofortification as a cost-effective strategy to combat vitamin A deficiency (VAD), which contributes to childhood blindness and immune dysfunction (Abdullahi, 2024; Maziya-Dixon et al., 2006). Similarly, elevated serum zinc and iron levels in G3 and G4 highlight the synergistic relationship between dietary protein and mineral absorption(Willoughby & Bowen, 2014). The inclusion of soybean and *Jatropha tanjorensis*—rich in iron and zinc coupled with antinutrient reduction during processing, likely enhanced micronutrient bioavailability(Castro-Alba et al., 2019). These results are particularly relevant for plant-based diets in low-income settings, where mineral deficiencies impair cognitive and immune development(WHO, 2023).

Organ development and histopathology

The improved *luam-nahan* (G3) promoted organ growth comparable to commercial feed, with no histopathological abnormalities in liver, kidney, spleen, or gut tissues. In contrast, G1 and G2 rats exhibited severe architectural disruptions, including glomerular hypertrophy (kidney), hepatocyte atrophy (liver), and villous flattening (gut)—hallmarks of malnutrition-associated organ dysfunction (Dipasquale *et al.,* 2020). The gut inflammation and dysbiosis observed in G1 align with clinical reports of malnourished children failing to thrive despite therapeutic feeding(Madhusoodanan, 2021). These findings underscore the long-term risks of early-life malnutrition, including cardiometabolic and cognitive deficits in adulthood(Kirolos *et al.,* 2024).

Limitations of the study

While the study highlights the feed intake, growth performance, Serum retinol, iron and zinc as well as the histopathological of the liver, kidney, spleen, and gut tissues of the experimental animals, it is essential that, future research should evaluate the hematological parameters, serum bioavailability of more micronutrient and also the histopathological of more organ tissues.

Conclusion

This study provides robust preclinical evidence that fortifying traditional staples with locally available, nutrient-dense ingredients (e.g., QPM, provitamin-A cassava) can address protein and micronutrient gaps in at-risk populations. The 44:44:10:2 formulation ratio effectively balanced nutrient density and safety, offering a scalable solution to mitigate childhood malnutrition. However, human trials are warranted to validate efficacy in target populations. The improved *luam-nahan* flour therefore, demonstrates significant potential as a sustainable, culturally acceptable intervention to combat malnutrition among Nigerian preschoolers. Its integration into national supplementation programs could reduce the burden of protein-energy malnutrition and micronutrient deficiencies, aligning with global efforts to achieve Sustainable Development Goal 2 (Zero Hunger).

Ethical approval

Ethical approval for this research was obtained from the ethics committee of Afe Babalola University Ado-Ekiti(ABUAD) with No: ABUADHREC/26/04/2024/514.
Experimental animals were handled in strict accordance with the internationally accepted principles and recommendations in the revised guide for the care and use of laboratory animals of the National Research Council of the United States of America National Institute of Health (NRC, 2011).

1. **Source(s) of support (funding):** No funding was received for this study.
2. **Acknowledgment (if applicable):** None.
3. **Previous presentations:** None

4. **Author Contribution**: **Credit authorship contribution statement**

A. O., C. O., G., K. S., S. O. - Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Software, Visualization, Writing - Original Draft, Writing - Review & Editing.
A. A., K., J. Y. - Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Software, Visualization, Resources, Validation, Writing - Original Draft, Writing, Review & Editing, Project Administration and Supervision.

5. **Conflicts of Interest**: The authors declared no conflict of interest

Preprint deposit: No

References

Abdullahi, S. M. (2024). Relationship between serum vitamin A levels and maternal education and social status among undernourished children in Zaria. *Nigerian Journal of Paediatrics, 51*(1), 1–9. https://doi.org/10.4314/njp.v51i1.01

Adejuwon, K. P., Osundahunsi, O. F., Akinola, S. A., Oluwamukomi, M. O., & Mwanza, M. (2021). Effect of fermentation on nutritional quality, growth and hematological parameters of rats fed sorghum-soybean-orange flesh sweet potato complementary diet. *Food Science and Nutrition, 9*(2), 639–650. https://doi.org/10.1002/fsn3.2013

Adeoti, O. A., Osundahunsi, O. F., & Salami, A. A. (2018). Protein quality, haematological and histopathological studies of rats fed with maize-based complementary diet enriched with fermented and germinated Moringa oleifera seed flour. *Nutrition and Food Science International Journal, 7*(1), 1–7. https://doi.org/10.19080/NFSIJ.2018.07.555705

Afolami, I., Mwangi, M. N., Samuel, F., Boy, E., Ilona, P., Talsma, E. F., Feskens, E., & Melse-Boonstra, A. (2021). Daily consumption of pro-vitamin A biofortified (yellow) cassava improves serum retinol concentrations in preschool children in Nigeria: A randomized controlled trial. *American Journal of Clinical Nutrition, 113*(1), 221–231. https://doi.org/10.1093/ajcn/nqaa290

Ankle, M. R., & Joshi, P. S. (2011). A study to evaluate the efficacy of xylene-free hematoxylin and eosin staining procedure as compared to the conventional hematoxylin and eosin staining: An experimental study. *Journal of Oral and Maxillofacial Pathology, 15*(2), 161–167. https://doi.org/10.4103/0973-029X.84482

Beruk, B., Kebede, A., & Esayas, K. (2015). Effect of blending ratio and processing technique on physiochemical composition, functional properties and sensory acceptability of quality protein maize (QPM) based complementary foods. *International Journal of Food Science and Nutrition Engineering, 5*(3), 121–129. https://doi.org/10.5923/j.food.20150503.03

Castro-Alba, V., Lazarte, C. E., Bergenståhl, B., & Granfeldt, Y. (2019). Phytate, iron, zinc, and calcium content of common Bolivian foods and their estimated mineral bioavailability. *Food Science and Nutrition, 7*(9), 2854–2865. https://doi.org/10.1002/fsn3.1127

Dendegh, T. A., Yelmi, B. M., & Dendegh, R. A. (2021). Evaluation of stiff porridge (Ruam-nahan) produced from composite flour blends of pearl millet (Pennisetum glaucum) and African yam bean (Sphenostylis stenocarpa). *Asian Food Science Journal, 20*(9), 63–77. https://doi.org/10.9734/AFSJ/2021/v20i930343

Dipasquale, V., Cucinotta, U., & Romano, C. (2020). Acute malnutrition in children: Pathophysiology, clinical effects and treatment. *Nutrients, 12*(8), 1–9. https://doi.org/10.3390/nu12082413

Duodu, C., Boateng, D., & Edziyie, R. (2020). Effect of pond fertilization on productivity of tilapia pond culture in Ghana. *Journal of Fisheries and Coastal Management, 2*(1), 56–64. https://doi.org/10.5455/jfcom.20190722060436

Fares, S., Chahed, M. K., Feki, M., Beji, C., Traissac, P., El Ati, J., & Kaabachi, N. (2011). Status of vitamins A and E in schoolchildren in the centre west of Tunisia: A population-based study. *Public Health Nutrition, 14*(2), 255–260. https://doi.org/10.1017/S1368980010001631

Gernah, D. I., Ariahu, C. C., & Ingbian, E. K. (2012). Nutritional and sensory evaluation of food formulations from malted and fermented maize (Zea mays L.) fortified with defatted sesame (Sesamum indicum L.) flour. *African Journal of Food, Agriculture, Nutrition and Development, 12*(6), 6614–6631.

Ghasemi, A., Jeddi, S., & Kashfi, K. (2021). The laboratory rat: Age and body weight matter. *EXCLI Journal, 20*, 1431–1445. https://doi.org/10.17179/excli2021-4072

Igbua, F. Z., Adejo, S. O., Igoli, N. P., & Daagema, A. A. (2019). Production and quality evaluation of maize, cassava and soybeans composite flour. *International Journal of Science Research, 8*, 798–803. https://doi.org/10.21275/ART20201767

Kirolos, A., Harawa, P. P., Chimowa, T., Divala, O., Freyne, B., Jones, A. G., ... Wells, J. C. (2024). Long-term outcomes after severe childhood malnutrition in adolescents in Malawi (LOSCM): A prospective observational cohort study. *The Lancet Child and Adolescent Health, 8*(4), 280–289. https://doi.org/10.1016/S2352-4642(23)00339-5

Madhusoodanan, J. (2021). Inner workings: Can feeding the gut microbiome treat malnutrition? *Proceedings of the National Academy of Sciences of the United States of America, 118*(50). https://doi.org/10.1073/pnas.2120478118

Maziya-Dixon, B. B., Akinyele, I. O., Sanusi, R. A., Oguntona, T. E., Nokoe, S. K., & Harris, E. W. (2006). Vitamin A deficiency is prevalent in children less than 5 y of age in Nigeria. *Journal of Nutrition, 136*(8), 2255–2261. https://doi.org/10.1093/jn/136.8.2255

Mekuria, S. A., Kinyuru, J. N., Mokua, B. K., & Tenagashaw, M. W. (2022). Growth performance, biochemical and haematological parameters of BALB/c mice fed on staple grains and bee larvae (Apis mellifera) blended complementary foods. *Heliyon, 8*(2). https://doi.org/10.1016/j.heliyon.2022.e09003

Momoh, C. O., Abu, J. O., & Yusufu, M. I. (2020). Production of noodles from rice (Oryza sativa), African yam bean (Sphenostylis stenocarpa) and rice bran: A tool for ameliorating PEM and hidden hunger in Nigeria. *International Journal of Food Engineering and Technology, 4*(2), 25–31. https://doi.org/10.11648/j.ijfet.20200402.13

National Research Council. (2011). *Guide for the care and use of laboratory animals* (8th ed.). The National Academies Press. https://doi.org/10.17226/12910

NDHS. (2024). *Report on the nutrition and health situation of Nigeria 2023–2024: Key indicators report*.

NRC. (1995). *Nutrient requirements of laboratory animals* (4th ed.). National Academies Press (US). https://doi.org/10.17226/4758

Onoja, U. S., Akubor, P. I., Gernah, D. I., & Chinmma, C. E. (2014). Evaluation of complementary food formulated from local staples and fortified with calcium, iron and zinc. *Journal of Nutrition and Food Science, 4*, 1–6. https://doi.org/10.4172/2155-9600.1000326

Overmyer, K. A., Thonusin, C., Qi, N. R., Burant, C. F., & Evans, C. R. (2015). Impact of anesthesia and euthanasia on metabolomics of mammalian tissues: Studies in a C57BL/6J mouse model. *PLoS ONE, 10*(2). https://doi.org/10.1371/journal.pone.0117232

Rolf, K., Januszko, O., Frąckiewicz, J., Madej, D., & Kaluza, J. (2021). The influence of iron and zinc supplementation on iron apparent absorption in rats fed vitamins and minerals reduced diets. *Biological Trace Element Research, 199*(8), 3013–3020. https://doi.org/10.1007/s12011-020-02433-z

Toluwalope, E., Sanusi, R., Alamu, E., & Maziya-Dixon, B. (2018). Variations of β-carotene retention in a staple produced from yellow fleshed cassava roots through different drying methods. *Functional Foods in Health and Disease, 8*(7), 372–384. https://doi.org/10.31989/ffhd.v8i7.524

WHO. (2023). *Infant and young child feeding: Key facts*. Fact Sheets. https://www.who.int/news-room/fact-sheets/detail/infant-and-young-child-feeding

Willoughby, J. L., & Bowen, C. N. (2014). Zinc deficiency and toxicity in pediatric practice. *Current Opinion in Pediatrics, 26*(5), 579–584. https://doi.org/10.1097/MOP.0000000000000132