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Research Article

Evaluation of protein quality and safety of high-fat cookies made from composite flours of *hildegardia barteri*, maize, and plantain using in vivo feeding trials

Josephat Ikechukwu Anyadioha^{1✉}, Roseline Nwabugo Attaugwu², John Ikechukwu Eze³,
Comfort Chinenye Nwagbo⁴

¹Department of Food Science and Technology, Madonna University Nigeria, Akpugo Campus, Enugu State, Nigeria

²Department of Human Nutrition, and Dietetics, State University of Medical and Applied Science, Igbo-eno Enugu State, Nigeria.

³Department of Food Science and Technology, University of Nigeria, Nsukka, Enugu State, Nigeria

⁴Department of Food Science and Technology, Chukwuemeka Odumegwu Ojukwu University, Igbariam Campus, Anambra State, Nigeria

Abstract

This study evaluated the protein quality and safety of high-fat cookies produced via the creaming method from composite flours of maize, (*Hildegardia barteri*) and plantain. In a controlled feeding trial with 42 albino rats, diets formulated with the cookies were assessed for key nutritional and safety parameters. The results indicated that the cookies supported significant rat growth, with high protein quality evidenced by a biological value of 60–84%, net protein utilization of 50–78.6%, and true protein digestibility of 83.6–95%. Rats fed Diets 1, 2, and 3 demonstrated superior nutrient utilization and higher activity levels, with Diet 3 performing most favorably. In contrast, Diets 4, 5, and 6 resulted in lower nutrient absorption and reduced activity. All diets were found to be non-toxic, as confirmed by relative organ weights (kidney, liver, heart) and serum biochemical markers (AST, ALT, ALP) that remained within normal physiological limits. These findings demonstrate that composite flour cookies are a safe and nutritious food option, highlighting their potential to enhance food security and contribute to healthier diets.

Keywords

biological value, composite flour, high-fat cookies, in vivo feeding trials, protein quality evaluation, safety assessment

Abbreviations

ALP – alkaline phosphatase; ALT – alanine aminotransferase; ANOVA – analysis of variance; AST – aspartate aminotransferase; BV – biological value; CHO – carbohydrate; CRD – completely randomized design; DN – digested nitrogen; FER – feed efficiency ratio; FI – food intake; FN – fecal nitrogen; H – *Hildegardia barteri* flour; M – malted maize flour; NA – not assessed; NB – nitrogen balance; NFD – nitrogen free diet; NI – nitrogen intake; NPU – net protein utilization; P – blanched plantain flour; PC – protein consumed; RN – retained Nitrogen; UN – urinary Nitrogen

✉Corresponding author: Dr. Josephat Ikechukwu Anyadioh, Department of Food Science and Technology, Faculty of Engineering and Technology, Madonna University Nigeria, Akpugo Campus, Enugu State, Nigeria
Tel +23408037223698, e-mail: anyadioha@madonnauniversity.edu.ng

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Introduction

Cookies are a globally popular food product, especially in developing countries, due to their convenience, affordability, and long shelf life (Mulak et al. 2020). Traditionally, wheat flour, primarily starch-based, is used in cookie production for its desirable functional and sensory attributes but lacks essential micronutrients (Warrilow, et al. 2019; Biradar et al. 2020). The rising demand for functional foods with health benefits beyond basic nutrition has spurred interest in alternative flours derived from underutilized local cereals, roots, tubers, and legumes, reducing dependence on wheat imports. Composite flours, which blend starchy tubers, protein-rich sources, and cereals, offer tailored functional and nutritional advantages (Akinwotu, et al. 2025). They also promote biodiversity, enhance food security, and provide alternatives for those with wheat allergies or gluten intolerance (Olamiti and Ramashia 2024). Fortifying cookies with legume and oilseed proteins has garnered attention, as Legumes are rich in essential amino acids, particularly lysine and tryptophan – often limited in cereals – and contain higher levels of these nutrients than animal proteins (Kumar et al. 2022). Studies have demonstrated improved nutritional and sensory properties in cookies made with non-wheat composite flours (Olamiti and Ramashia 2024). Such cookies are particularly valuable in regions facing protein-energy malnutrition (Udedeh et al. 2024). However, concerns remain regarding the protein quality, safety, and high-fat content of composite flour-based cookies, underscoring the need for nutritionally balanced and safe products to combat malnutrition in developing countries. Indigenous crops like *Hildegardia barteri*, maize, and plantain remain underutilized in formulating high-protein, nutritionally balanced baked products. *Hildegardia barteri* (“kpaakpa”), a lesser-known legume consumed in Southeastern Nigeria, is notable for its rich protein and mineral content. Currently, it has no established commercial value (Anyadioha et al. 2023). Combining *Hildegardia barteri* with maize and plantain in composite flour blends creates nutritionally balanced products that leverage the strengths of each ingredient. The purpose of this study was to address the existing research gap on the protein quality and safety of cookies produced from *Hildegardia barteri*–maize–plantain flour blends. In vivo feeding trials using albino rats were

conducted to evaluate the cookies, focusing on nutritional parameters such as biological value (BV), net protein utilization (NPU), and true protein digestibility.

Materials and methods

Materials. The materials used for this research *Hildegardia barteri* seeds sourced from the Independent Layout area of Enugu, Nigeria, while maize, unripe plantain, casein, sucrose (St Louis, Senegal West Africa), vegetable oil (Lziz pure vegetable oil (Kajola village Ogun State, Nigeria), and Kingsford corn starch (Lagos Nigeria) were purchased from Ogbete Market, Enugu, and the vitamin and mineral premix was obtained from Bio-organics Lagos, Nigeria.

Reagents and equipment used. Reagents and equipment used for proximate and HPLC analyses included standard laboratory-grade chemicals and instruments. For proximate analysis, reagents such as sulfuric acid, hydrochloric acid, sodium hydroxide, petroleum ether or diethyl ether, boric acid, Kjeldahl catalyst tablets, mixed indicators, acetone, and desiccants (silica gel or Drierite) were employed, all of ACS or equivalent analytical grades. The major equipment included an analytical balance, hot-air oven, muffle furnace, Soxhlet extraction apparatus, Kjeldahl digestion and distillation system, fiber analyzer, and moisture dishes or crucibles made of porcelain, silica, or platinum. For HPLC analysis, HPLC-grade water, acetonitrile, methanol, and high-purity acids such as trifluoroacetic, phosphoric, and formic acids were used, along with HPLC-grade buffer salts (e.g., ammonium acetate, ammonium formate, potassium phosphate) and certified analytical standards. The key instruments included a complete HPLC system (pump, autosampler, column oven, and detector), analytical and guard columns (C18, C8, or HILIC types), solvent filtration units with 0.22 µm or 0.45 µm membrane filters, an ultrasonic bath.

Preparation of *Hildegardia barteri* seed flour. The dried *Hildegardia barteri* seeds were cleaned, and sorted. Two kilograms (2 kg) seeds were soaked for 36 h with 4 h air-resting, germinated for 96 h, kilned, dry milled (Panasonic MX-AC 2105 Jhajjar Haryana India), and sieved using a 400 µm mesh sieve. The flour was packaged in an airtight plastic container and stored in a refrigerator at 4°C before use (Anyadioha and Attaugwu 2023).

Preparation of germinated maize flour. The method of (Liu et al. 2022) with minor modifications was used. Two kilograms (2 kg) of maize grains were sorted, cleaned, and steeped in excess water at $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 24 h. The steeped grains were germinated for 96 h. The germinated grains were dried in a hot air oven (Gulfex Scientific DHG 9202, England) at a temperature of 50°C for 48 h, milled into flour using an attrition mill (Panasonic MX-AC 2105 Jhajjar Haryana India), and sieved using a 0.45 mm mesh size. The flours obtained were packaged in plastic containers with lids and stored at 4°C in a refrigerator before use.

Preparation of unripe plantain flour. The matured unripe plantain was washed in tap water and peeled, and 2 kg of edible portion was weighed and sliced into cylindrical pieces of 2 cm thickness with a stainless knife. The sliced samples were blanched in hot water (80°C) for 5 min. and dried in a hot air oven at 60°C (Gulfex Scientific DHG 9202, England) for 24-48 h. The dehydrated plantain

sample were milled using an attrition mill (Panasonic MX-AC 2105 Jhajjar Haryana India) and passed through a 0.45 mm mesh sieve to obtain the flour. The flours were packaged in plastic containers with lids and stored at 4°C in a refrigerator before further use.

Formulation of flour blends. Flour blends of germinated maize (GM), *Hildegardia barteri* (HB), and blanched unripe plantain (BP) were prepared to fit into the formulation as shown in Table 1. To obtain a homogenous blend, the flours were thoroughly mixed at predetermined ratios using a blending machine (Panasonic MX-AC 2105 Jhajjar Haryana India). Diets formulated from composite cookie blends were evaluated using albino rats to assess protein utilization, growth, and safety, with a focus on nutritional balance, comparison with wheat-based control diets, and diverse ingredient ratios to analyze biological value, net protein utilization, digestibility, and biochemical indices, highlighting their potential as alternative dietary sources.

Table 1. Percentage of germinated *Hildegardia barteri*, maize, and blanched plantain flours used for the blends

Blend no.	<i>Hildegardia barteri</i> (gram)	Germinated Maize (gram)	Plantain (gram)
1.1	33.3	33.3	33.3
2.2	16.7	16.7	66.7
3.3	66.7	16.7	16.7
4.4	16.7	66.7	16.7

Cookies Production. The proportions of ingredients used in cookie preparation are presented in Table 1, and the corresponding cookie formulations are detailed below. The percentage of the major ingredients used included the following: Flour 46.7%, Fat 18.7%, sugar 11.7%, Milk powder 7.0%, Baking powder 0.5%, Vanilla flavor 0.1%, Nutmeg 0.1%, Salt 0.5% and Egg 14.6%. The fat and sugar were mixed thoroughly until both formed a homogenous mixture (fluffy). The beaten egg was added while mixing and mixed for 30 min. Other ingredients (flour, milk powder, salt and baking powder, Vanilla, and Nutmeg) were thoroughly mixed in a separate bowl and added to the cream mixture to form a dough. The dough was rolled and cut into circular shapes 5 cm in diameter. Baking

was carried out at 185°C for 15-25 min. Baking was terminated when the cookies developed a golden brown color. Cookie samples were removed from the oven, allowed to cool at ambient temperature (29°C) for 30 min, and stored in cellophane bags before evaluation. Formulation of diet. Each cookie sample's crude protein ($\text{N} \times 6.25$) was used to formulate the test diets. The diets were adjusted to 10% protein for those cookies with protein content above 10%. A reference protein diet of casein with a 10% protein level was provided to compare the protein quality of the formulated test diets (Jeong et al. 2021). The test diet was balanced by adding vegetable oil, minerals, vitamins, and corn starch. All these were properly mixed and packaged in an airtight container before use.

Table 2. Composition of diet used for nutritional studies

Ingredients, g	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Casein	150	-	-	-	-	-	-
Skim milk	-	160	-	-	-	-	-
Cookies	-	-	1056	759.5	504.5	717.5	-
Veg. oil	66.75	66.75	66.75	66.75	66.75	66.75	66.75
Vit/Min.	37.5	37.5	37.5	37.5	37.5	37.5	37.5
Sucrose	62.5	62.5	62.5	62.5	62.5	62.5	705
Maize starch	1175	1165	265	565.5	820.5	607.5	682
Total	1500	1500	1500	1500	1500	1500	1500

Key: Diet 1 = Casein-based diet-(positive control), Diet 2= Diet of skim milk-based diet, Diet 3 = Diet from H_{33.3}M_{33.3}P_{33.3}, (33.3: 33.3:33.3 ratios of *H. barteri*- Malted Maize-Blanched Plantain), Diet 4 = Diet from H_{16.7}M_{16.7}P_{66.7} (16.7: 16.7:66.7: ratios of *H. barteri*- Malted Maize-Blanched Plantain), Diet 5 = Diet from H_{66.7}M_{16.7}P_{16.7} = (66.7: 16.7:16.7: ratios of *H. barteri*- Malted Maize-Blanched plantain), Diet 6 = Diet from H_{16.7}M_{66.7}P_{16.7} (16.7: 66.7:16.7: ratios of *H. barteri*- Malted Maize-Blanched Plantain), Diet 7 = Nitrogen free diet (NFD) (Negative Control). H =*Hildegardia barteri* flour; M= Malted Maize flour; P= Blanched Plantain flour

Animal Housing and Experimental Design. A completely randomized design (CRD) was employed for this study. Forty-two weaning male albino rats (*Rattus norvegicus*) of the Wistar strain, aged 21 days and averaging 85 g in weight, were obtained from the Department of Nutrition and Home Science, University of Nigeria, Nsukka. The animals were randomly assigned to seven dietary groups (six rats per group). Group 1 received a casein-based reference diet, while the remaining groups were fed test diets. Each rat was housed individually in a metabolic wire mesh-bottomed cage to prevent coprophagy. The room was maintained at $28 \pm 2^\circ\text{C}$ with a relative humidity of $50 \pm 10\%$ (Khachatryan et al. 2021; Tesalonika et al. 2025). Rats were fed 20 g of their respective diets daily and given water ad libitum for 21 days (Roschke et al. 2024). This included a 7-day adaptation period followed by a 14-day collection (balance) period (Lærke et al. 2025). Daily monitoring was conducted to record food intake, body weight, and assess biochemical indices. Digestibility, nitrogen intake, nitrogen balance, fecal nitrogen, and urinary nitrogen were also measured to evaluate the protein quality of the diets (Lærke et al. 2025). All animal handling procedures were conducted in accordance with institutional ethical guidelines and relevant international standards (Kumar et al. 2021; Chung et al. 2022).

Nutritional Composition of Formulated Diets.

The nutritional composition of the formulated diets was analyzed. Proximate composition was evaluated using standard AOAC methods: moisture by method 930.15, ash by method 942.05, and crude protein by method 984.13 (AOAC 2023). The digestible carbohydrate content was calculated by subtracting the sum of protein, fat, fiber, moisture, and ash percentages from 100%. Additionally, the micronutrient composition which included Vitamin B1, B2 and B3 as well as iron and zinc values of the diets was determined.

Statistical analysis of data. Analysis of Variance (ANOVA) was carried out on the data by using Statistical Package for Social Sciences (SPSS) Version 17.0 for Windows, SPSS Inc. Illinois, USA). Experiments were performed in duplicates. Mean separation was carried out using the Least Significant difference (LSD). Statistical significance was accepted at a 0.05 level of probability ($P \leq 0.05$).

Results and Discussion

Nutritional composition of formulated diets. The nutritional composition of formulated diets is significantly influenced by the ratios of *Hildegardia barteri*, malted maize, and blanched plantain flours. Increasing the proportion of *Hildegardia barteri* flour notably enhances protein, fiber, iron, and zinc

content in the final product (Anyadioha and Attaugwu 2024). For example, cookies with higher *Hildegardia barteri* content reached protein levels above 10%, and also showed increased crude fiber and micronutrient content. Balanced blends (e.g., 33.3% each) provided moderate to high fiber and optimal sensory acceptability (Anyadioha and Attaugwu 2024). The finding aligns with the studies by (Otoo et al. 2024) who reported that the addition of Bambara groundnut and plantain to maize-based products increased protein, β -carotene, and iron content without compromising sensory quality. Similarly, Terefe et al. (2021) observed that fermentation of maize flour with *Lactobacillus*

plantarum improved protein content and digestibility while reducing antinutritional factors. This is consistent with the report of (Akinyede et al. 2023) which showed that composite flours containing plantain, soya cake, and wheat bran exhibited higher protein and fiber content, supporting their potential use in functional foods. The findings suggest that these diets are nutritionally adequate for the albino rats, supporting normal growth and overall health. Thus, the variation in ingredients, particularly the level of *Hildegardia barteri*, is key to optimizing the nutritional benefits of the cookie.

Table 3. Nutritional composition of formulated diets

Sample	Protein, %	Ash, %	Moisture, %	Fiber, %	Fat, %	CHO, %
Diet 1	10.00±94 ^b	2.170±04 ^b	5.6±0.06 ^{ab}	0.66±0.01 ^{ab}	3.03±0.01 ^b	9.0±0.01 ^b
Diet 2	9.48±0.02 ^c	1.99±0.01 ^a	5.76±0.06 ^{ab}	0.70±0.01 ^b	0.97±0.04 ^c	87.14±0.02 ^a
Diet 3	10.31±0.44 ^b	2.55±0.02 ^{ab}	8.74±0.03 ^a	0.66±0.01 ^b	12.44±0.01 ^{ab}	79.96±0.00 ^a
Diet 4	5.76±0.02 ^{ab}	2.95±0.02	4.7±0.81 ^{abc}	0.68±0.01 ^b	13.88±0.09 ^{ab}	81.96±0.67 ^a
Diet 5	11.22±0.02 ^a	2.28±0.4 ^a	7.12±0.96 ^a	1.82±0.21 ^a	14.97±0.03 ^a	65.36±4.3 ^{ab}
Diet 6	8.25±0.07 ^{bc}	2.00±0.01 ^a	8.42±0.12 ^a	1.99±0.01 ^a	14.92±0.04 ^a	68.40±0.02 ^{ab}
Diet 7	0.3±0.01 ^a	0.09±0.01 ^a	9.5±0.01 ^a	0.9±0.01 ^b	0.05±0.01 ^b	91.3±0.01 ^b

All data presented are means ± standard deviation (n=7) of duplicate determinations.

Mean values with different superscripts in the same column are significantly different by ANOVA and post hoc LSD analysis ($p \leq 0.05$).

Abbreviation: CHO = Carbohydrate.

Key:

Diet 1 = Casein - based diet-(positive control),

Diet 2= skim milk-based diet,

Diet 3 = Diet from H_{33.3}M_{33.3}P_{33.3}, (33.3: 33.3:33.3 ratios of *Hildegardia barteri*- Malted Maize-Blanched Plantain),

Diet 4 = Diet from H_{16.7}M_{16.7}P_{66.7} (16.7: 16.7:66.7: ratios of *Hildegardia barteri*- Malted Maize-Blanched Plantain),

Diet 5 = Diet from H_{66.7}M_{16.7}P_{16.7} = (66.7: 16.7:16.7: ratios of *Hildegardia barteri*- Malted Maize-Blanched plantain),

Diet 6 = Diet from H_{16.7}M_{66.7}P_{16.7} (16.7: 66.7:16.7: ratios of *Hildegardia barteri*- Malted Maize-Blanched Plantain),

Diet 7 = Nitrogen free diet (NFD)

Table 4. Micronutrient composition of formulated diets

Sample	Thiamin, mg.100 g ⁻¹	Riboflavin, mg.100 g ⁻¹	Niacin, mg.100 g ⁻¹	Iron, mg.100 g ⁻¹	Zinc, mg.100 g ⁻¹
Diet 1	NA	NA	NA	NA	NA
Diet 2	0.12±0.00 ^a	3.34±0.03 ^a	2.04±0.04 ^a	0.33±0.00 ^c	NA
Diet 3	0.04±0.00 ^b	1.06±0.03 ^b	0.86±0.03 ^b	0.62±0.01 ^b	0.12±0.00 ^b
Diet 4	0.08±0.00 ^{ab}	0.81±0.02 ^c	0.78±0.01 ^{bc}	1.10±0.00 ^{ab}	NA
Diet 5	0.04±0.00 ^b	1.34±0.02 ^{ab}	0.63±0.03 ^c	0.54±0.02 ^{bc}	0.26±0.00 ^a
Diet 6	0.06±0.00 ^{bc}	0.96±0.01 ^{bc}	1.00±0.87 ^{ab}	1.2±0.04 ^a	0.11±0.00 ^{ab}
Diet 7	NA	NA	NA	NA	NA

All data presented are means ± standard deviation (n=7) of duplicate determinations. Mean values with different superscripts in the same column are significantly different by ANOVA and post hoc LSD analysis (p≤0.05).

Protein quality evaluation of formulated diets.

The total food intake, protein consumed, nitrogen intake, fecal nitrogen, digested nitrogen, retained nitrogen, urinary nitrogen, nitrogen balance, biological value, net protein utilization, nitrogen-free diet, and true digestibility of the treatments were presented in Table 5.

Food intake and palatability. Food intake in albino rats varied significantly ($p < 0.05$) across experimental diets, driven by factors of protein quality, fiber content, and palatability (Alahmar et al. 2024). Consistent with a compensatory feeding response to protein deficiency, the nitrogen-free diet elicited the highest recorded intake (14.14 g). The casein-based diet (13.56 g) supported the highest intake among protein-containing diets, affirming its role as a high-quality protein benchmark. These patterns are strongly supported by recent findings that rats fed a protein-free diet exhibit hyperphagia, while those fed high-quality casein maintain stable, high intake levels, directly linking protein quality to feed consumption regulation (de Almeida Moreira et al. 2024). In contrast, test diets incorporating *Hildegardia barteri* flour resulted in significantly ($p < 0.05$) lower intakes 8.77–10.33 g). The lowest intake, observed in Diet 5, was likely due to its high fiber content (3.7%), as recent evidence confirms that specific dietary fibers can reduce palatability

and increase satiety, thereby suppressing voluntary intake (Alahmari et al. 2024). The overall intake pattern underscores that dietary fiber characteristics are a critical determinant of food consumption, modulating texture, gut passage rate, and metabolic satiety signals (Suresh et al. 2024).

Nitrogen intake, digestibility, and retention.

Nitrogen intake and retention are highest in animal-based protein diets (Diet 1 and Diet 2), reflecting their superior amino acid profiles and digestibility (Musigwa et al. 2024). The test diets, especially those with incomplete amino acid profiles or higher, showed lower nitrogen digestibility and retention (Musigwa et al. 2024). However, the complementation from the composite flour blends (*Hildegardia barteri*-maize-plantain flour) - blending plant proteins or supplementing with limiting amino acids improved nitrogen utilization and biological value (Pinckaers et al. 2021).

Biological value and net protein utilization.

Biological value (BV) and net protein utilization (NPU) are highest in animal-based diets (casein, whey), but certain plant-based blends can approach these values if amino acid profiles are balanced (Calvez et al. 2024). Recent research confirms that combining plant proteins or fortifying with essential amino acids enhances protein quality and supports growth and tissue repair (Pinckaers et al. 2021).

Nitrogen intake and retention. Nitrogen intake (NI) from cookies made with composite flours of *Hildegardia barteri*, maize, and plantain varied significantly across diets ($p < 0.05$), indicating differences in protein availability and utilization. Casein and skim milk diets consistently demonstrated higher nitrogen intake, retention, and digestibility, reflecting their complete amino acid profiles and high bioavailability (Guillin et al. 2021). Test diets, while providing moderate nitrogen intake (Diets 3 and 5 (0.16–0.18 g) and retention, showed lower values than animal-based controls, likely due to the presence of anti-nutritional factors and less optimal amino acid balance. However, when plant proteins are carefully blended or supplemented, their net protein utilization and biological value can approach those of animal proteins (Calvez et al. 2024).

Fecal nitrogen and protein digestibility. Formulated test diets with higher fiber or anti-nutritional factors (e.g., Diet 5) showed the highest FN, indicating lower protein digestibility. This is consistent with findings by (Sobotka and Dražbo 2025) who reported that low-protein diets increased fecal nitrogen and reduced protein digestibility. In contrast, test diets with balanced amino acid profiles and lower fiber (Diets 3 and 4) had FN values similar to casein and skim milk, reflecting better protein absorption and utilization. Casein and skim milk diets consistently demonstrated moderate FN, supporting their high digestibility (Sobotka and Dražbo 2025).

Digested and urinary nitrogen. Casein and skim milk diets yielded the highest Digested Nitrogen, confirming superior digestibility of animal proteins. Among test diets, those with optimized blends (e.g., lower *Hildegardia barteri* content) also achieved high digested nitrogen, indicating effective protein utilization. Urinary Nitrogen values were low and stable across all groups, suggesting efficient nitrogen retention, especially in diets with balanced protein sources. This aligns with findings in pigs and rats where optimized plant-based or mixed diets improved nitrogen retention and reduced urinary nitrogen excretion (Panaite et al. 2025).

Nitrogen balance and growth implications. Casein diets produced the highest NB, but all protein-containing test diets supported positive NB and weight gain, indicating adequate protein quality

for growth and tissue repair. Nitrogen-free diets, while resulting in the highest food intake, led to poor nitrogen retention and growth, underscoring the necessity of dietary protein (Schipper et al. 2024). These results reinforce that well formulated plant-based diets can approach the protein quality of animal-based diets if amino acid balance and digestibility are optimized. Efficient protein utilization in formulated diets is achievable with careful ingredient selection and processing, supporting sustainable alternatives to animal proteins. However, high fiber or anti-nutritional factors can impair digestibility, emphasizing the need for balanced formulations. Biological value (BV) varied significantly across diets, indicating differences in nitrogen utilization. Diet 4 (84.3%) recorded the highest BV, comparable to casein (84.0%) and skim milk (83.7%), while Diet 3 (81.1%) also showed strong amino acid balance. Except for Diet 5, all diets exceeded the 70% BV threshold for quality protein. Similar findings were reported by (Akinyemi et al. 2022) where extruded millet–soybean–carrot blends achieved BV comparable to casein, and by (Salles et al. 2021) and (Guillin et al. 2021) who found pea protein to match casein in digestibility and nitrogen utilization. Biological value (BV) varied significantly across diets, indicating differences in nitrogen utilization. Diet 4 (84.3%) recorded the highest BV, comparable to casein (84.0%) and skim milk (83.7%), while Diet 3 (81.1%) also showed strong amino acid balance. Except for Diet 5, all diets exceeded the 70% BV threshold for quality protein. Similar findings were reported by (Akinyemi et al. 2022), where extruded millet–soybean–carrot blends achieved BV comparable to casein, and by (Salles et al. 2021) and (Guillin et al. 2021) who found pea protein to match casein in digestibility and nitrogen utilization. Overall, the results underscore that well-formulated cereal–legume blends can provide protein quality comparable to animal sources. Net protein utilization (NPU) of the experimental diets ranged from 50% to 77.8%, with Diet 2 recording the highest value and Diet 5 the lowest, showing significant differences ($p < 0.05$). The low NPU of Diet 5 was attributed to reduced food intake and higher urinary nitrogen loss, resulting in poor nitrogen retention. This aligns with (Musigwa et al. 2024) who reported a strong correlation between urinary nitrogen excretion and NPU in rats. NPU, which reflects protein

digestibility and biological value, varied with protein source and amino acid composition. Supporting studies by (Salles et al. 2021) and (Olanrewaju et al. 2024) showed that both plant- and animal-based proteins can achieve NPU values of 70–77% when amino acid profiles and digestibility are optimized. Similarly, (Berghaus et al. 2023) linked nitrogen utilization efficiency to protein synthesis rates and urinary nitrogen excretion in pigs. These findings emphasize the need to optimize protein source, amino acid balance, and intake to enhance NPU, promote efficient nitrogen retention, and reduce nitrogen waste with potential environmental benefits. Rats in groups 1, 2, and 3 showed significant ($p < 0.05$) weight gain, comparable to those fed the casein-based diet (14.8 g), indicating good protein quality and nutrient utilization. Variations among groups were attributed to differences in food intake and diet composition. Similar finding by (Jeong et al. 2022) showed that milk- and casein-based diets promote higher weight gain and food acceptance than lower-quality protein sources. (Nychyk et al. 2021) also confirmed that protein quality, quantity, and dietary fat jointly influence body weight and tissue growth. Reduced fecal and urinary nitrogen losses likely supported higher weight gain, emphasizing that protein quality and digestibility are major determinants of growth performance in rats. True digestibility (TD) values were generally high (92.5–95.0%) across most diets, indicating efficient protein digestion, with casein-based diets showing consistently superior TD due to their balanced amino acid profile (Jeong et al. 2021). Diet 5 showed a lower TD (83.6%), likely influenced by anti-nutritional factors or suboptimal amino acid composition (Anyiam et al. 2025). These results agree with (Salles et al. 2021) who reported similarly high digestibility for animal and refined plant proteins like pea protein, while less refined

plant sources exhibited reduced digestibility. Overall, TD reflects the proportion of dietary protein absorbed and utilized for metabolism. The Feeding Efficiency Ratio (FER) ranged from 5.65 to 8.79, indicating significant variation in diet quality and protein utilization. Diet 1 (8.79), followed by Diets 2 (8.54) and 3 (8.49), showed superior feed conversion efficiency, reflecting balanced amino acids and (Sawant et al. 2021) high the results were similar to the findings of who reported that probiotics enhanced PER and GFCE in *Oreochromis niloticus* and (Miller et al. 2025) confirmed that trypsin inhibitors and excess fiber reduce feed efficiency. Overall, FER trends mirrored protein quality and digestibility, with higher FERs linked to better growth and vitality.

Biochemical indices and organ weight of rats.

Table 6 shows the biochemical indices and organ weight of rats fed different diets. Biochemical indices and organ weights of rats fed the test diets showed normal serum levels of AST, ALT, and ALP, all within reference ranges, indicating no hepatic toxicity. Similar findings by (Barszcz et al. 2021) confirmed that well-formulated animal- and plant-based proteins do not elevate liver enzymes. Organ weights, including liver and kidney, were also unaffected, supporting the diets' safety (Amusa et al. 2020). The stability of these enzymes suggests that the diets were well-tolerated and did not cause hepatic or organ damage. The average organ weights of rats ranged from 0.5–0.7 g (kidney), 3.0–3.6 g (liver), and 0.46–0.60 g (heart), with no significant ($p \leq 0.05$) differences between test and control groups. This stability indicates that the diets did not adversely affect organ function or health. Consistent with (Amusa et al. 2020) properly formulated dietary protein sources do not significantly alter major organ weights, confirming the safety of the test diets.

Table 5. Protein quality evaluation of formulated diets

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
FI, g	13.56±0.00 ^b	11.64±0.04 ^c	10.33±0.80 ^d	10.00±0.30 ^d	8.77±0.04 ^e	9.98±0.02 ^d	14.14 ^a
PC, g	1.36±0.00 ^a	1.11±0.02 ^b	1.06±0.02 ^c	0.63±0.02 ^e	0.98±0.01 ^c	0.87±0.01 ^d	
NI, g	0.21±0.02 ^a	0.18±0.01 ^b	0.16±0.00 ^c	0.14±0.00 ^e	0.16±0.00 ^c	0.11±0.00 ^d	0.00
FN, g	0.02±0.00 ^b	0.02±0.00 ^b	0.008±0.00 ^c	0.004±0.00 ^c	0.03±0.00 ^a	0.01±0.00 ^b	
DN, g	0.18±0.00 ^a	0.17±0.00 ^a	0.152±0.00 ^b	0.096±0.00 ^d	0.131±0.00 ^c	0.133±0.00	
RN, g	0.16±0.00 ^a	0.14±0.00 ^a	0.12±0.00 ^a	0.10±0.00 ^b	0.08±0.00 ^b	0.08±0.00 ^a	
UN, g	0.02±0.00 ^a	0.03±0.00 ^a	0.03±0.00 ^a	0.02±0.00 ^c	0.05±0.00 ^a	0.02±0.00	0.02
NB, g	0.16±0.00 ^a	0.14±0.01 ^a	0.12±0.00 ^b	0.08±0.00 ^b	0.08±0.00 ^d	0.11±0.00 ^c	
BV, %	84.00±0.04 ^c	83.7±0.60 ^b	81.1±0.40 ^d	84.3±0.30 ^c	60.3±0.80 ^e	84.0±0.60 ^b	
NPU, %	76.0±0.30 ^a	77.8±1.10 ^b	75.0±0.60 ^b	71 ^b ±0.30	50.0±0.06 ^c	72.6±0.40 ^a	
TD, %	95.0±0.00 ^a	93±0.500 ^a	92.5±0.60 ^a	89 ^b ±0.30	83.6±0.20 ^a	93.5±0.60 ^a	
FER	8.79±0.01 ^a	8.54±0.01 ^b	8.49±0.01 ^c	7.97±0.00 ^d	5.65±0.01 ^f	6.96±0.01 ^e	

All data presented are means ± standard deviation (n=7) of duplicate determinations. Mean values with different superscripts in the same column are significantly different by ANOVA and post hoc LSD analysis ($p \leq 0.05$). Abbreviation: FI = Food Intake, PC= Protein consumed, NI = Nitrogen Intake, FN = Fecal, Nitrogen, DN = Digested Nitrogen, RN= Retained Nitrogen, UN = Urinary Nitrogen, NB = Nitrogen Balance, BV = Biological Value, NPU = Net Protein Utilization, NFD = Nitrogen Free Diet, TD = True Digestibility (%), FER= Feed efficiency ratio.

Table 6. Biochemical indices in IU.L⁻¹per organ weight of rats

Groups	AST, IU.L ⁻¹	ALT, IU.L ⁻¹	ALP, IU.L ⁻¹	Kidney, g	Liver, g	Heart, g	AW, g	WG, g
Group 1	22.1±0.76 ^a	28.0±6.5 ^a	25.66±1.5 ^b	0.70±0.10 ^a	3.66±0.3 ^a	0.60±0.10 ^a	108±12.7 ^a	15.0
Group 2	29.0±6.06 ^a	21.0±3.46 ^c	25.00±0.00 ^b	0.76±0.02 ^b	3.3±0.10 ^a	0.50±0.00 ^{ab}	99.3±4.0 ^b	14.7
Group 3	24.0±1.0 ^a	25.6±0.57 ^b	26.66±1.15 ^b	0.73±0.05 ^a	3.3±0.15 ^a	0.50±0.00 ^{ab}	87.75±4.8 ^b	13.0
Group 4	31.0±11.2 ^a	28.3±4.9 ^a	31.66±3.7 ^b	0.70±0.10 ^b	3.3±0.05 ^a	0.50±0.00 ^{ab}	77.1±2.1 ^b	8.00
Group 5	23.1±4.01 ^a	30.0±3.6 ^a	32.33±4.7 ^a	0.70±0.10 ^b	3.3±0.10 ^a	0.46±0.05 ^{ab}	69.5±3.1 ^b	12.0
Group 6	21.0±01 ^b	23.0±0.1 ^c	28.20±0.09 ^b	0.5±0.00 ^c	3.0±0.01 ^b	0.47±0.0 ^{ab}	56.3±0.02 ^c	6.00

All data presented are means ± standard deviation (n=7) of duplicate determinations. Mean values with different superscripts in the same column are significantly different by ANOVA and post hoc LSD analysis ($p \leq 0.05$). Key: AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase, Kidney = Organ weight in grams: Liver = Organ Weight in grams, Heart = Organ Weight in grams, AW = Average weight of rats in groups 1-6, weight gain (g per day).

Conclusions

This study demonstrates that high-fat cookies made from composite flours of maize, *Hildegardia barteri*, and plantain are safe and support healthy growth. The test diets resulted in significant weight gain, with high true protein digestibility (95%), net protein utilization (78.6%), and biological value (84%). Among the test diets, Diet 3 (H333M333P333) was the most effective, showing superior nutrient utilization that was comparable to the casein and skim milk control diets. This was reflected in the animals' higher activity and energy levels. In contrast, Diets 4, 5, and 6 led to lower nutrient absorption and reduced activity, confirming their poorer quality. Critically, all diets were found to be non-toxic, as evidenced by relative organ weights (liver 3.0-3.6 g) and key biochemical indices (AST, ALT, ALP) that all remained within normal physiological ranges. These findings confirm the potential of these composite flour cookies as a safe, nutritious food product, contributing to enhanced food security and healthier dietary options.

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Author Contribution

Conceptualization, A.J.I. and E.J.I; methodology, A.J.I and ARN; Formal analysis A.J.I and ARN, investigation A.J.I; resources A.J.I.data curation E.J.I. and N.C.C. ; writing original draft preparation A.J.I. and A.R.N; writing A.J.I, A.R.N. and N.C.C., review and editing, E.J.I; visualization, E.J.I; supervision, E.J.I; project administration E.J.I; funding acquisition A.J.I. , A.R.N and N.C.C. All authors have read and agreed to published version of the manuscript.

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Institutional Review Board Statement

The experimental protocol was designed and implemented in compliance with the ethical standards for animal research in Nigeria, guided by the principles promoted by the Nigerian Institute of Animal Science (NIAS). Approval was obtained from the Research Ethics Committee of Chukwuemeka Odumegwu Ojukwu University, Igbariam, Anambra State Nigeria, with approval No: REC/COOU/02/001 (16.5.2025) and all procedures conformed to the provisions of the Animal Diseases (Control) Act and international best practices for laboratory animal care.

Informed Consent Statement

Not applicable

Data Availability Statement

The original and relevant data in this study are contained in the article. For additional enquiries, please contact the corresponding author.

Conflicts of Interest

The authors report no conflicts of interest related to this work.

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