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Nutrient bioavailability and growth performance of Wistar rats fed with graded levels of *Termitomyces robustus* substituted diets

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ABSTRACT: This study evaluated the nutrient bioavailability of Termitomyces robustus (TR) mushrooms and its potential to support growth in weanling Wistar rats. This is to promote the consumption of indigenous, nutrient-dense foods as viable high quality protein alternative for the vulnerable population**.** Fresh TR samples were sorted, oven-dried at 60 °C, and stored for feeding trials. Thirty weanling male Wistar rats (21–24 days old) were purchased and acclimatised for seven days and randomly assigned to five dietary groups (n = 6): basal, 5%, 10%, 15% T. robustus protein inclusion, and 10% casein (control). Rats were fed 10g of feed daily for 14 days, recording their daily feed intake and body weight weekly. Conversion Index (CI), Growth Rate (GR), and Efficiency of Conversion of Ingested (ECI) feeds were calculated, and rats were euthanised on the 14th day for haematological and biochemical analyses. Rats' means weight change across diet groups were basal (-21.8g), casein (+7.3g), 5% (-9.0g), 10% (+2.0g), and 15% (+9.3g) TR inclusion, respectively. Highest ECI and GR were observed in 15% TR (+99.47, +0.008) and casein (+100.27, +0.006) groups. Rats on 15% TR protein diet achieved the highest weight gain (+9.3g) compared to those on a casein diet (+7.3g), while rats on a basal diet recorded significant weight loss (-21.8 g). Red blood cells were elevated while white blood cell counts were positively modulated in 15% TR-fed group. Nutrients from Termitomyces robustus are bioavailable and support optimal growth in rats. These findings indicate that T. robustus can be incorporated into human dietary interventions without adverse health effects.

Keywords: Growth performance, malnutrition, nutrient bioavailability, protein, *Termitomyces robustus*.

INTRODUCTION

Human growth, development, and overall health cannot be separated from nutrient bioavailability from dietary sources (Krebs, 2001). Metabolic functions, immune responses, and tissue repair demand adequate nutrition with protein of high biological value (Munteanu and Schwartz, 2022). Protein deficiency in pregnancy and early life continues to be a significant public health problem with consequences including growth impairment, cognitive impairment, compromised immunity, and increased susceptibility to infections (UNICEF, 2023; Endrinikapoulos, 2023).

Access to good protein and micronutrient rich foods has been limited in sub-Saharan Africa by food insecurity and financial constraints, thereby exacerbating malnutrition as well as other nutrition-related morbidity (Fanzo, 2012). Vulnerable children and pregnant women are most affected by the impact on national economic productivity

as well as the national health system. In spite of the fact that traditional animal proteins like meat, eggs, and milk are extremely rich sources of essential amino acids, their accessibility to vulnerable populations is usually limited due to poverty and other economic barriers (Kamenju, 2022; Fanzo, 2012). This has prompted a growing interest in alternative, local, and sustainable protein sources with comparable nutritional quality. To address such diet-related issues, more importance is now being given to interventions aiming to improve food quality, nutrient bioavailability, and cultural food resources rich in essential nutrients (Ruel, 2018).

One of such undervalued resources is the *Termitomyces robustus* mushroom, a wild edible fungus commonly consumed as a staple in some West African countries. Traditionally picked from plantation and forest termite hillocks, *T. robustus,* which is also known in Nigeria as 'Olu ewe' or 'Ogogo' among the Yorubas, 'Ero mkpu' among the Igbos and 'Oru Ogbagbajele among the Igala tribes, is valued for its aroma, flavour, and perceived medicinal properties (Ijeh *et al*., 2016; Ugbogu *et al.,* 2020; Nhi *et al.,* 2022; Soumitra *et al.,* 2023). The nutrient bioavailability, serum mineral contribution, and its overall contribution to cellular growth seem to be underexplored. Mushrooms have been previously reported as possible protein alternatives with excellent amino acid profiles and bioactive compounds (AL-Hussainy and AL-Fadhly, 2019; Eke-Ejiofor and Pollyn, 2020; Ayimbila and Keawsompong, 2023). Other authors also have it documented that mushrooms are a rich source of vitamins and minerals that enhance overall well-being (Alofe *et al.,* 1996; Ijioma *et al.,* 2015; Ijeh *et al.,* 2016; Adebiyi *et al.,* 2016; Ache *et al.,* 2021). Nevertheless, data on their growth promotion and health-improvement capacity compared to conventional protein sources like casein are limited.

Understanding the nutritional impact of *T. robustus* in experimental diets is essential to ascertaining its use as an alternative source of protein in human nutrition. The objective of this study was to determine the nutrient bioavailability and the effect of T. robustus in weanling Wistar rats and the scientific rationale of its inclusion into human diets.

MATERIALS AND METHODS

Preparation of the sample

Freshly harvested Termitomyces robustus mushrooms were obtained from Eporo farm settlement, Emure Ekiti, Emure Local Government Area (LGA), Ekiti State, Nigeria (7.4317° N, 5.4621° E). The mushrooms were separated, oven-dried at 60 °C for 48 hours, and stored in an airtight container until the feeding trial. Based on earlier findings of 35g/100g crude protein content (Olaoye and Adepoju, in press), the dried sample was incorporated into formulated rat feed at protein inclusion levels of 5%, 10%, and 15% (Table 1).

Determination of *T. robustus* nutrient bioavailability

Nutrient Bioavailability of *Termitomyces robustus* was determined as described by Adepoju and Ajayi (2017). Thirty 21–24-day-old male weanling Wistar rats were purchased from the Department of Veterinary Physiology and Biochemistry, University of Ibadan, Oyo State, Nigeria. The rats were weighed and kept individually in metabolic cages for seven days to acclimatise to laboratory conditions with a 12-hour light/dark cycle. They were provided ad libitum access to rat pellets and water.

After acclimatisation, the rats were weighed and randomly assigned to five dietary groups of six rats/group with a weight variation of not more than ±2.2 g. All the diets were iso-caloric, while two diets (Experimental 2 and Casein) were iso-nitrogenous (Table 1). Each rat was fed 10 g of the formulated diets for 14-days. Leftover feeds were collected, weighed, and recorded daily, while each rat was weighed weekly. On the final day of the experiment, the rats were weighed before being sacrificed using anaesthesia. Blood samples were collected at the end of the study for packed call volume (PCV), haemoglobin (Hb), red blood cells (RBC), and white blood cells (WBC) determination according to Baker and Silverton (1985); and biochemical (total protein, albumin, liver enzymes, kidney function markers) analyses as described by Lowry *et al.* 1951; Swedko, *et al.,* 2003; Asad *et al.,* 2020.

Statistical analysis

Data were analysed using SPSS version 29, and the differences in means across groups were estimated using one-way analysis of variance (ANOVA) at $p<0.05$.

Ethical consideration

This study (Protocol No: UI-ACUREC/071-0723/24) was conducted in strict compliance with ethical guidelines and regulations set by the University of Ibadan ACUREC, prioritising animal welfare and minimising potential risks or distress.

RESULTS

Weight changes and growth performance

In Table 2, the rats' feed intake varied across the groups, with the highest intake observed in the 15% *T. robustus* group and the lowest being the basal diet group. The

Table 1. Formulated rats' diet composition (g/1000g).

Feed component	Rats Groups				
	A	B	C	D	E
Starch	820	677	534	391	712
Cellulose	50	50	50	50	50
Vegetable oil	80	80	80	80	80
Minerals	40	40	40	40	40
Vitamins	10	10	10	10	10
T. robustus powder	-	143	286	429	-
Casein	-	-	-	-	108
Total	1000	1000	1000	1000	1000

Group A (Basal diet) = No protein, Group B = 5% T. robustus protein inclusion, Group C = 10% T. robustus protein inclusion, Group D = 15% T. robustus protein inclusion, E (Control) = 10% casein protein inclusion. CI - consumption index, GR - growth rate, and ECI - efficiency of conversion of ingested food. CI= C/TA; GR = G/TA; ECI = (G/C) x100 where C is fresh weight of feed consumed, T is the feeding duration; A is the mean weight of the rat during the feeding period and G is the fresh weight gain of the rat.

Table 2. Rats' feed intake, weight changes and growth performance (g).

Parameter	Group A	Group B	Group C	Group D	Group E
Mean feed intake	5.15±0.56	7.23±0.41	9.13±0.41	9.38±0.25	7.31±0.13
Final weight	54.17± 5.42^{a}	65.67±7.21^{b}	78.33±8.82^{c}	84.17±5.34^{c}	82.00±7.38^{c}
Initial weight	76.00±7.46^{a}	74.67±8.59^{a}	76.33±7.53^{a}	74.83±8.84^{a}	74.67±9.22^{a}
Weight change	-21.83±2.64^{a}	-9.00±10.43^{b}	2.00±9.49^{c}	9.33±10.54^{d}	7.33±10.11^{e}

The values are means of six rats. Values along the same rows with similar superscripts do not differ significantly. **Keys:** Group A = Basal diet, Group B = 5% T. robustus inclusion, Group C = 10% T. robustus inclusion, Group D = 15% T. robustus inclusion, Group E = 10% Casein diet (control).

Table 3. Rats' consumption index, growth rate and efficiency of conversion of ingested food.

Parameter	Group A	Group B	Group C	Group D	Group E
Consumption index	0.0068	0.0078	0.0080	0.0083	0.0079
Growth Rate	-0.029	-0.0097	0.002	0.0079	0.006
ECI	-423.88	-124.48	21.91	99.47	100.27

The values represent the mean of six rats. **Key:** Group A = Basal diet (Negative control); Group B = 5% T. robustus inclusion; Group C = 10% T. robustus inclusion; Group D = 15% T. robustus inclusion; Group E = 10% Casein diet (control).

formulated diets influenced weight gain in the experimental rats, the 15% *T. robustus*-supplemented diet (Group D) recording the highest mean weight gain compared to the casein diet and other levels of inclusion of the mushroom ($p<0.05$). The 10% *T. robustus* group (Group C) exhibited marginal weight gain, while the 5% inclusion level group (Group B) and basal diet group (Group A) recorded weight losses.

Table 3 reveals that the consumption index (CI) of the diets followed the same trend, with the 15% *T. robustus* and casein groups exhibiting the highest CI values. Growth rate and efficiency of conversion of ingested food (ECI) were highest in the 15% *T. robustus* and casein-fed groups, while the lowest values were recorded in the 5% *T. robustus* and basal diet groups.

Haematological parameters

Packed Cell Volume (PCV) and haemoglobin (Hb) levels of rats showed a gradual increase from Group A to Group D, suggesting a potential enhancement of erythropoietic activity with increasing levels of *T. robustus* inclusion. Conversely, red blood cell (RBC) counts remained relatively consistent across all groups, ranging from 7.14 to 7.51 × 10^{6}/mm³. Despite these observed trends, the differences in PCV, Hb, and RBC values were not significant ($p> 0.05$) across the treatment groups (Table 4). Notably, the highest PCV and Hb values were observed in the group receiving 15% *T. robustus* (Group D), which also recorded the peak RBC count of 7.51 × 10^{6}/mm³. White blood cell (WBC) counts had no significant variation across

Tables 4. Haematological parameter of rats on nutrient bioavailability.

Parameter	Group A	Group B	Group C	Group D	Group E
PCV (%)	44.17±3.43[a]	44.83±1.6[a]	45.83±4.40[b]	46.33±6.02[b]	45.67±3.98[b]
HB (g/dl)	14.08±1.1[a]	14.38±0.93[a]	14.72±1.46[b]	14.87±1.95[b]	14.7±1.13[b]
RBC (million/mm^3)	7.14±0.51[a]	7.26±0.25[a]	7.5±0.93[a]	7.51±1.26[a]	7.40 ±0.75[a]
MCV (µm3) fL	61.83±1.20[a]	61.85±2.78[a]	61.30±2.11[a]	62.14±4.31[a]	61.90±1.97[a]
MCHC (%)	31.89±0.25[a]	32.06±1.03[a]	32.10±0.32[a]	32.08±0.13[a]	32.22±0.62[a]
MCH (pg)	19.72±0.43[a]	19.83±1.33[a]	19.67±0.67[a]	19.93±1.33[a]	19.95±0.89[a]
WBC ($x10^9$/L)	5.24±0.65[a]	5.03±0.90[ab]	6.68±2.29[c]	5.89±0.63[b]	5.48±0.42[d]
Platelets (10^9/L)	123.33±18.11[a]	139.33±32.16[b]	115.67±16.27[c]	117.67±6.12[b]	122.00±9.23[d]
% Lymphocyte	70.83±3.54[a]	74.83±1.94[a]	73.17±3.19[a]	73.67±5.01[a]	73.13±1.47[a]
% Neutrophil	25.67±3.14[a]	22.17±2.29[a]	23.00±3.1[a]	23.17±4.17[a]	22.33±2.25[a]
% Monocyte	1.33±0.82[a]	2±0.63[a]	2.33±0.82[a]	1.83±0.75[a]	2 ±0.89[a]
% Eosinophil	2.17±0.98[a]	1±0.63[a]	1.5±1.5[a]	1.33±0.81[a]	2.5±0.83[a]

The values represent the mean of 6 rats. Values along the same row with similar superscripts do not differ significantly. PCV = packed cell volume, HB = haemoglobin, RBC = red blood cell MCV = mean corpuscular volume, MCHC = mean corpuscular haemoglobin concentration, MCH = mean corpuscular haemoglobin.

Table 5. Effect of treatments on liver profile and kidney function indices of rats.

Parameters	Group A	Group B	Group C	Group D	Group E
Protein (g/dl)	6.52±0.38[a]	6.57±0.7[a]	6.62±0.82[a]	6.65±0.31[a]	6.72±0.36[a]
Albumin (g/dl)	2.62±0.19[a]	2.72±0.41[a]	2.67±0.44[a]	2.80±0.22[a]	2.83±0.12[a]
Globulin (g/dl)	3.90±0.21[a]	3.85±0.31[a]	3.95±0.39[a]	3.85 ±0.14[a]	3.88±0.31[a]
A:G	0.67±0.03[a]	0.71±0.08[a]	0.67±0.06[a]	0.73±0.05[a]	0.73±0.06[a]
AST (µ/L)	36.83±1.83[a]	37.67±0.08[a]	37.50±2.59[a]	37.17±2.64[a]	38.00±2.37[a]
ALT (µ/L)	26.5±1.38[a]	28.00±.61[a]	27.50±2.17[a]	27.67±2.34[a]	28.50±1.05[a]
ALP (µ/L)	83.0±5.29[a]	86.00±16.3[a]	84.67±14.00[a]	82±13.27[a]	92.7±11.5[a]
BUN (mg/dl)	15.03±0.92[a]	14.80±0.97[a]	15.38±1.24[a]	15.28±0.75[a]	14.90±0.70[a]
Creatinine (mg/dl)	0.68±0.04[a]	0.70±0.09[a]	0.72±0.08[a]	0.72±0.04[a]	0.73±0.05[a]
T. Bilirubin (mg/dl)	0.22±0.08[a]	0.22±0.08[a]	0.20±0.09[a]	0.22±0.04[a]	0.23±0.05[a]

Values are mean of six rats. Values along the same row with similar superscripts do not differ significantly. AST- Aspartate aminotransferase, ALP- Alkaline phosphatase, ALT- Alanine aminotransferase.

groups. Similarly, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) did not differ significantly across groups.

Biochemical parameters

The biochemical indices assessing liver and kidney function revealed stable trends across all experimental groups. Serum total protein levels increased progressively from Group A to Group E with increasing *T. robustus* inclusion, and the albumin levels followed a similar trend. Conversely, globulin levels remained relatively stable, fluctuating marginally between 3.85 and 3.95 g/dL across the groups (Table 5).

Liver enzyme activities (AST, ALT, and ALP) did not follow any dose-dependent trend, indicating hepatic enzyme stability irrespective of *T robustus*' inclusion level (Table 5). Blood urea nitrogen (BUN) and creatinine varied only slightly among the groups. Total bilirubin concentrations were uniform across groups, indicating no evident hepatic or renal stress.

Effect of treatments on serum mineral composition of weanling Wistar rats

The serum sodium, potassium, chloride, and calcium concentrations remained within normal physiological limits across all treatment groups, with no apparent pattern of increase or decline (Table 6). The 15% *T. robustus* inclusion was associated with the highest concentrations of zinc and iron, representing a nutritional advantage at 15% inclusion level. While zinc values did not differ significantly, the increase in serum iron concentration in

Table 6. Effect of treatments on serum mineral composition of rats.

Parameter	Group A	Group B	Group C	Group D	Group E
Sodium (mEq/L)	143.83±4.58[a]	142.67±2.73[a]	143.67±5.12[a]	143.17±4.53[a]	145.5±4.14[a]
Potassium(mEq/L)	5.08±0.35[a]	5.12±0.78[a]	5.12±0.82[a]	5.45±0.84[a]	5.45±0.52[a]
Chloride (mEq/L)	92.5±7.89[a]	91.00±10.47[a]	90.67±11.15[a]	93.5±4.85[a]	90.00±6.93[a]
Calcium (mg/dl)	9.38±0.70[a]	9.50±0.43[a]	9.48±0.74[a]	9.63±0.74[a]	9.62±0.47[a]
Zinc (µg/dl)	10.85±1.31[a]	10.65±1.81[a]	10.90±0.63[a]	12.43±1.61[b]	10.98±1.15[a]
Iron (µmol/L)	16.24±0.85[a]	18.57±0.62[b]	18.90±0.93[b]	19.15±0.34[b]	18.53±0.35[b]

Values represent the mean of 6 rats. Values along the same rows with similar superscripts do not differ significantly.

Groups B to E compared to Group A was significant ($p<0.05$), suggesting improved mineral bioavailability with *T. robustus* supplementation, especially for iron.

DISCUSSION

Growth performance and nutrient utilization

The feed intake among the T. robustus-fed rat groups varied and increased progressively as the level of inclusion increased, with the 15% T. robustus inclusion group being the highest. This finding is in consonance with the earlier findings of Ukoima *et al*. (2009) and Ayimbila and Keawsompong (2023) on the digestibility of edible mushrooms and their high palatability, which must have enhanced their intake in the experimental animals. This was also corroborated by the findings of Poddar *et al*. (2012), who documented that edible mushrooms are highly palatable, low in energy density and rich in nutrients.

Rats in the experimental groups B, C, and D recorded higher weight gain with increased feed consumption, indicating that efficient utilisation of the ingested nutrients is essential for optimal growth performance. The highest weight loss was seen in the basal diet-fed group with negative growth, while rats on the 5% mushroom inclusion diet experienced less weight loss when compared to basal diet-fed rats. The weight loss in both groups could be attributed to the lack of protein (basal diet) and the low protein inclusion level (5% inclusion) in the diets. Pinckaers *et al*. (2021) reported that inclusion of no less than 10% protein of high biological value is necessary for optimal growth in growing rats.

Feed conversion efficiency was substantially improved in groups with higher levels of T. robustus inclusion, which reflects its role in improved nutrient bioavailability and metabolic utilisation. This is in line with Ogbe (2009), where mushroom-based diets significantly improved the feed conversion efficiency of chicken feed. It therefore suggests that the improved performance of rat groups fed on T. robustus is likely attributable to the increased feed intake and the observed improved nutrient conversion efficiency in the *T. robustus* fed groups. Evidenced by its ability to enhance growth rate, conversion index, and feed conversion efficiency, which are key indices of growth performance and feed utilisation, T. robustus can be compared favourably with conventional protein sources like casein and soy protein.

The observed weight loss in the group fed with 5% *T. robustus* diet inclusion suggests that a lower inclusion level may not be adequate to optimise growth performance or support the formulation of protein-rich diets for experimental animals, and possible use in applied human nutrition.

Haematological and biochemical indices

The haematological indices in the current study show that Termitomyces robustus supplementation at 10% and 15% levels of protein has a positive impact on the synthesis of red (erythropoiesis) and white (leukopoiesis) blood cells. The observed haematological increase in RBC and WBC in the T. robustus-supplemented group reflects the ability of the mushroom to modulate blood cell production and promote immunity responses. The 10% and 15% T. robustus protein supplemented groups were found to exert similar haematological effects to those of casein-fed groups, positioning *T. robustus* as a promising non-conventional high-quality protein alternative for the vulnerable. Erythropoiesis is an essential nutrient-regulated process involving iron, vitamin B12, and folate (Koury and Ponka, 2004). The increased RBC indices of the T. robustus groups indicate that the mushroom would have a potentiating effect on erythropoiesis, likely due to improved bioavailability of the involved cofactors. Similarly, the elevated leukocyte counts indicate their immune system-modulating role.

Similar haematological profiles were observed by authors of other mushroom-based diets. Supplementation in Nile tilapia diet with Pleurotus djamor at doses of 15–25% provided huge increases in haemoglobin, haematocrit, erythrocytes, total leukocytes, and lymphocyte ratios (Cruz-García *et al.,* 2022). Ganoderma lucidum extract (GLE) also provided significant haematological benefits to rodent models. The platelet and haemoglobin levels were raised with the introduction of an intermediate dose (150 mg/kg) of GLE, possibly due to its rich antioxidant content and bioactive substances like tannins and triterpenes (Ahmed and Aslam, 2018). The changes in the haematological parameters observed in this study corroborate the above findings with Ganoderma

lucidum and Pleurotus djamor, indicating the modulatory roles of edible mushrooms on blood cell production and immune function.

Liver and renal function indicators (ALT, AST, ALP, and total bilirubin, blood urea nitrogen and creatinine) in all the supplemented diet groups were within the normal range, a clear indication that *Termitomyces robustus* is neither hepatotoxic nor has any negative impact on kidney function. This observation is in consonance with previous study reports by Alam *et al.* (2009) and Nweze *et al.* (2022) who noted that rats' feed supplementation with Pleurotus sajor-caju, P. ostreatus, and P. florida significantly improved lipid profiles while maintaining liver enzymes and kidney function parameters in rats. This study showed the potential of T. robustus to improve haematological indices without causing liver or kidney impairment and supports its potential inclusion in nutrient-rich therapeutic diets.

Micronutrient bioavailability

This study showed that there was a significant increase in serum levels of minerals like iron, zinc, calcium, chloride and potassium in rats fed on 15% Termitomyces robustus supplemented diet compared to basal and casein control diet groups. This finding confirms the earlier research of Adepoju and Ajayi (2017) that bio-fortification with underutilised edible insects, such as *Macrotermes bellicosus*, improves the serum micronutrient concentration of consumers. This study also showed increased serum concentration of Iron and Zinc which are key micronutrients in immunological competence and haematopoietic efficiency. Iron is an important constituent of haemoglobin and myoglobin, necessary for cellular respiration and oxygen transport (Abbaspour *et al.,* 2014). Zinc serves as a cofactor for the immune function and cell health-implicated enzymes (Weyh *et al.,* 2022). The high serum Iron and Zinc concentration suggest the possible therapeutic role of T. robustus in iron-deficiency anaemia as well as zinc-associated immune complications in nutritionally vulnerable groups. The group on a basal diet (no protein) had the lowest serum mineral concentration, suggesting the essential role of dietary protein in mineral absorption and utilisation.

Conclusion and Recommendations

Termitomyces robustus at 10% and 15% protein inclusion levels supported growth and nutrient bioavailability in Wistar rats with no negative haematological and biochemical impact. Given its positive effects on growth performance and haematological indices, consumption of T. robustus should be promoted and its incorporation into functional foods as a measure towards alleviating malnutrition and enhancing dietary diversity in vulnerable populations.

CONFLICT OF INTEREST

All authors confirm that there are no conflicts of interest.

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