

Assessment of Quality and Safety of Winged Termites (*Macrotermes bellicosus*) Enriched Locally Formulated Complementary Foods

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Abstract

Addition of edible insects to local staples used as complementary foods can improve their nutrient content. Nutritional quality and safety of *Macrotermes bellicosus* enriched boiled rice (BR) and yam (BY) complementary foods (CFs) was assessed using rats. *Macrotermes bellicosus* (MB) were collected, dried, and refrigerated at -4°C. Ground MB was added to BR and BY in ratios 10.0%, 15.0%, 20.0% (w/w) to give BR₁, BY₁; BR₂, BY₂, and BR₃, BY₃ respectively. Nutrient content of MB, BY, BR and MB-enriched CFs were determined by AOAC methods. Nutrient bioavailability and safety of BR₃ and BY₃ were assessed using rats fed ad libitum for 28 days. Serum trace minerals in the CFs, control and basal diets and histopathological effects of CFs on rats' organs were determined. Data were analysed using ANOVA at p<0.05. Dried MB contained 31.8g protein, 16.4g fat, 3.8g ash, 227.5mg calcium, 2.1mg iron, 15.0mg zinc, 330.4µg retinol equivalent (RE), and 529.0kcal energy/100g sample. The BR and BY contained 3.7-5.9g protein, 70.0-120mg calcium, 4.2-5.6mg iron, 1.2-1.5mg zinc and 380- 386kcal/100g compared with 7.9-15.3g protein, 242.2-264mg calcium, 2.4-4.4mg iron, 15.1-19.8mg zinc and 357-372kcal/100g enriched CFs (p<0.05). Rats Serum trace minerals ranged between 3.4- 4.3mg zinc, 23.4-27.9mg calcium, 30.6-37.0mg iron; and 52.5-56.9µg RE, compared with control (3.2, 22.2, 34.1, 48.2) and basal (2.2, 21.1, 24.0 mg, 32.3 µg) diets respectively (p<0.05). No pathological lesions were observed in internal organs of rats on CF diets. Adding *Macrotermes bellicosus* to local complementary foods is safe and improved their nutritional quality, hence its use is recommended among mothers.

Keywords: *Macrotermes bellicosus*, complementary foods, nutritional quality, trace minerals

1. Introduction

Infancy is a time of rapid physical growth as well as physiological, immunological and mental development. Malnutrition from inadequate complementary feeding is a serious problem in many low income countries where complementary foods consist of starch-based cereals or gruel that may provide sufficient energy but inadequate protein and micronutrients (Dewey, 2003). Micronutrient deficiencies, especially zinc deficiency, are associated with stunting of growth and other serious health consequences including anaemia and a greater susceptibility to infection (Bhutta et al.2008).

Access to energy and nutrient-dense foods during the complementary feeding period, along with appropriate feeding practices and continued breastfeeding is needed to ensure long-term optimal growth and development (Lutter et al., 2003). Although the causes of malnutrition are many and diverse, inadequate intake of foods and essential nutrients has been reported to be a major contributory factor to under-five malnutrition (Sarika et al., 2017; Kikafunda et al., 2003). Besides increased risk of mortality, under nutrition in early life has irreversible effects and serious health consequences such as impaired cognitive function which can result in national economic and productivity loss (Sarika et al., 2017). Studies have shown that at 6 months or less, only 17 percent of mothers in Nigeria are exclusively breastfeeding their infants, and by 6 and 9 months, 63 percent of Nigerian mothers have introduced complementary foods while continuing breastfeeding (NDHS, 2013). Therefore, complementary foods of adequate nutrient densities are needed for optimal growth and development of infants after 6 months of age.

The first Nigerian Nutrition Network meeting of 2002 also identified poor feeding practices and shortfall in food intake as the important factors responsible for malnutrition and illness among children in Nigeria. The recipes of the traditional complementary foods show that most of them are cereal based except for the egg custard, plantain pudding and yam pottage. More than half of the selected complementary foods were based on the maize gruel (pap) which is the most popular and often the first food introduced to infants by mothers in Nigeria. This is supported by the report by Ibeanu & Okeke (2001) that pap is the most commonly used complementary food in Nigeria. Yeung (1998) also reported that cereals are usually the first solid foods given to infants because they are readily available and culturally acceptable. These cereals usually form the basis for most of the traditional complementary foods (Onofiok & Nnanyelugo, 1998). After the successful introduction of cereal gruel from six months, as from eight months and beyond, the introduction of semi-solid to solid complementary foods become imperative to be able to meet energy needs of the infants and young children.

Staple foods in the family menu such as yam, rice, garri, and cocoyam are given to the child (Onofiok & Nnanyelugo, 1998). In Africa, traditional complementary foods are based on starchy cereals such as maize, sorghum, finger millet and rice, and non-cereals such as cassava, round potato, sweet potato, yams and plantains. These foods have been widely associated with nutrient deficiencies among pre-school age children (Walker, 1990). Yam (*Dioscorea* spp.) and rice (*Oryza sativa*) are major staple foods in Nigeria. Yams are rich in phenyl alanine and threonine but limiting in valine and tryptophan (Ogunlade et al. 2011), whilst rice is rich in carbohydrate but deficient in lysine, thus making their protein quality poorer than that of animals.

There is therefore need for strategic use of inexpensive high protein sources that complement the protein quality of these staple foods in order to enhance their nutritional value. Traditional complementary foods could be improved upon by combining locally available foods that complement each other in such a way that new pattern of protein quality created by this combination is similar to that recommended for infants (Okafor et al., 2008). Insects constitute quality food and feed, have high feed conversion ratios, and emit low level of greenhouse gases (van Huis, 2013). An upsurge of interest in the use of insects as sustainable diets is being encouraged as many of them are nutritionally, economically and ecologically important.

Africa insects are rich in protein and usually processed to tasty food products which are used as flavour intensifiers in soups and stews, and also add protein to protein-poor diets. Dried caterpillar has been used with cereal to prepare infant and young child complementary foods in

Democratic Republic of Congo (Bauserman et al., 2015) and found acceptable to mothers and children. Dried insects may be crushed or pulverized, and raw or boiled insects ground or mashed to become masses of protein and lipids that can be mixed with other foodstuffs such as grain, ground meat and mashed potatoes to make a variety of dishes (Mitsuhashi, 2010). The objective of this study, which is a follow up on the previous one on the use of *M. bellicosus* in enriching gruels from maize and sorghum, was to assess nutritional quality and safety of *Macrotermes bellicosus*-enriched locally formulated complementary foods using animal model.

2. Materials and Methods

2.1 Sample Collection and Preparation

Macrotermes bellicosus (MB) were collected during their swarming in Oyo and Ekiti States, Nigeria, dried for 10 minutes over a gas cooker using a frying pan, cooled, dewinged and refrigerated at -4°C. About 1.5 kg each of White yam (*Dioscorea rotundata*) and Aroso rice (*Oryza sativa*) were purchased from Bodija market in Ibadan. Bodija market is one of the major markets in Nigeria where agricultural produce from different parts of the country and imported food items are being sold, hence food items purchased from this market are representative of such commodities. Sample of raw Aroso rice was cleansed of extraneous matter such as stones and chaff by hand picking. The rice was boiled for about 30 minutes at 100°C to a pastry form and mashed. The yam tubers were washed, peeled, sliced and boiled for about 30 minutes at 100°C to dryness with calculated amount of distilled water, and mashed. The two mashed samples (rice and yam) were oven-dried for 18 hours at 60°C. The dried rice and yam samples were separately milled into flour with Malex blender (DMN/DIC/PMT/02- 03/206, Kanchan International Limited), packed in cellophane nylon and kept air tight in the laboratory of Department of Human Nutrition, University of Ibadan, till when needed. Dried MB was also milled using the blender (Bauserman et al., 2015).

Various samples of basal complementary foods were prepared as follows:

Sample BR = 100 g Boiled rice, Sample BR₁=90 g Boiled rice + 10 g *M. bellicosus*

Sample BR₂= 85 g Boiled rice + 15 g *M. bellicosus* Sample BR₃ = 80 g Boiled rice + 20 g *M. bellicosus*, Sample BY = 100 g, Boiled yam, Sample BY₁=90 g Boiled yam + 10 g *M. bellicosus*

Sample BY₂ = 85 g Boiled yam + 15 g *M. bellicosus*, Sample BY₃ = 80 g Boiled yam + 20 g *M. bellicosus*

2.2 Proximate Composition Analysis

Moisture content of the samples was determined by air oven at 105°C (Plus 11 Sanyo Gallenkamp PLC UK) for 4 hours. Crude protein of the samples was determined using micro-Kjeldahl method (Method No 978.04, AOAC, 2005). Crude lipid was determined by Soxhlet extraction method (Method No 930.09, AOAC, 2005) and the crude lipid estimated as g/100g of sample. The ash content was determined by weighing 5g of sample in triplicate and heated in a muffle furnace (Gallenkamp, size 3) at 550°C for 4 h (Method No 930.05, AOAC, 2005). The total carbohydrate content was obtained by difference. Gross energy of the samples was determined using ballistic bomb calorimeter (Manufacturer: Cal 2k – Eco, TUV Rheinland Quality Services (Pty) Ltd, South Africa).

2.3 Mineral Analysis

Potassium and sodium content of the samples were determined by digesting the ash of the samples with perchloric acid and nitric acid, and then taking the readings on Jenway digital flame photometer/spectronic20 (AOAC, 2005: (975.11)). Phosphorus was determined by Vanado- molybdate colorimetric method (AOAC, 2005: (975.16)). Calcium, magnesium, iron, zinc, manganese, and copper were determined spectrophotometrically using Buck 200 atomic absorption spectrophotometer (Buck Scientific, Norwalk) and compared with absorption of standards of these minerals (AOAC, 2005: (975.23)).

2.4 Vitamin Analysis

Vitamin A Determination Vitamin A was determined through ultraviolet absorption measurement at 328 nm after extraction with chloroform. Calibration curve of vitamin A acetate was made and sample vitamin A concentration estimated as microgram (µg) of vitamin A acetate.

2.4.2 Thiamine (Vitamin B₁) Determination Thiamine content of the sample was determined by weighing 1g of it into 100ml volumetric flask and adding 50ml of 0.1M

H₂SO₄ and boiled in a boiling water bath with frequent shaking for 30 minutes. 5ml of 2.5M sodium acetate solution was added and flask set in cold water to cool contents below 50°C. The flask was stoppered and kept at 45- 50°C for 2 hours and thereafter made up to 100ml mark. The mixture was filtered through a No. 42 Whatman filter paper, discarding the first 10ml. 10ml was pipetted from remaining filtrate into a 50ml volumetric flask and 5ml of acid potassium chloride solution was added with thorough shaking. Standard thiamine solutions were prepared and treated same way. The absorbance of the sample as well as that of the standards was read on a fluorescent UV Spectrophotometer (Cecil A20 Model) at a wavelength of 285nm.

2.4.1 Riboflavin (Vitamin B₂) Determination

Riboflavin of the samples was determined by weighing 1g of each sample into a 250ml volumetric flask, 5ml of 1M HCl was added, followed by the addition of 5ml of dichloroethene. The mixture was shaken and 90ml of de-ionized water was added. The whole mixture was thoroughly shaken and was heated on a steam bath for 30 minutes to extract all the riboflavin. The mixture was then cooled and made up to volume with de-ionized water. It was then filtered, discarding the first 20ml of the aliquot. 2ml of the filtrate obtained was pipetted into another 250ml volumetric flask and made up to mark with de-ionized water. Sample was read on the fluorescent spectrophotometer at a wavelength of 460nm. Standard solutions of riboflavin were prepared and readings taken at 460nm, and the sample riboflavin obtained through calculation.

2.4.2 Niacin (Vitamin B₃) Determination

About 5g of sample was extracted with 100ml of distilled water and 5ml of this solution was drawn into 100ml volumetric flask and make up to mark with distilled water. Standard solutions of niacin were prepared and absorbance of sample and standard solutions were measured at a wavelength of 385nm on a spectrophotometer and niacin concentration of the sample estimated.

2.4.3 Pyridoxine (Vitamin B₆) Determination

The vitamin B₆ content of the sample was determined by extracting 1g of sample with 0.5g of ammonium chloride, 45 ml of chloroform and 5 ml of absolute ethanol. The mixture was thoroughly mixed in a separating funnel by shaking for 30mins, and 5ml of distilled water added. The chloroform layer containing the pyridoxine was filtered into a 100ml volumetric flask and made up to mark with chloroform. 0-10ppm of vitamin B₆ standard solutions were prepared and treated in a similar way as sample, and their absorbance measured on Cecil 505E spectrophotometer at 415nm. The amount of vitamin B₆ in the sample was then calculated.

2.4.4 Cyanocobalamin (Vitamin B₁₂) Determination

Cyanocobalamin content of the sample was determined by extracting 1g of sample with distilled water with shaking for 45min followed by filtering the mixture. The first 20 ml of the filtrate was rejected, and another 20mls filtrate collected. To the collected filtrate, 5mls of 1% Sodium dithionite solution was added. Standard cyanocobalamin solutions (0-10 µg/ml) were prepared and absorbance of sample as well as standard was read on spectronic21D spectrophotometer at 445nm. The amount of sample cyanocobalamin was then estimated through calculation.

2.4.5 Ascorbic Acid Determination

Ascorbic acid in the sample was determined by titrating its aqueous extract with solution of 2, 6-dichlorophenol-indophenol dye to a faint pink end point.

2.5 Antinutrient Analysis

Oxalate was determined by extraction of the samples with water for about three hours and standard solutions of oxalic acid prepared and read on spectrophotometer (Spectronic20) at 420 nm. The absorbance of the samples was also read and amount of oxalate estimated. Phytate was determined by titration with ferric chloride solution (Sudarmadji & Markakis, 1977); while trypsin inhibitory activity was determined on casein and comparing the absorbance with that of trypsin standard solutions read at 280 nm (Makkar & Becker, 1996). The tannin content was determined by extracting the samples with a mixture of acetone and acetic acid for five hours, measuring their absorbance and comparing the absorbance of the sample extracts with the absorbance of standard solutions of tannic acid at 500 nm on spectronic20 (Griffiths & Jones 1977). Saponin was also determined by comparing the absorbance of the sample extracts with that of the standard at 380 nm (Makkar & Becker, 1996). All determinations were carried out in triplicate.

2.6 Micronutrient Bioavailability and Histopathological Study

2.6.1 Rat Feeding Trial

Four iso-caloric diets, of which three sets were iso-nitrogenous, comprising a basal diet, two experimental diets (yam and rice at 20% MB inclusion level) and casein diet (control) were prepared as shown in Table 1. Twenty four weanling albino rats of Wister strain brand of not more than 24-day old were purchased from Physiology Department, University of Ibadan, and were housed individually in metabolic cages at the Department of Animal Science, University of Ibadan. The animal house was maintained at a 12 h light–dark cycle, constant temperature of $20 \pm 3^\circ\text{C}$ and relative humidity of $65 \pm 15\%$. The rats were allowed to acclimatise for one week, fed ad libitum with commercial rat pellets and clean tap water. At the end of seven days, they were weighed and then randomly distributed to four diet groups of six rats per group based on their weight. An amount of 10 g of prepared diets was supplied to each rat on daily basis and water was changed every other day for 21 days. The left-over of the diets were collected and weighed on daily basis. The rats were weighed on a weekly basis throughout the duration of the experiment (Ayatse et al., 1985).

Table 1. Composition of formulated rats diets (g)

Nutrient	Basal diet	Yam diet	Rice diet	Control diet
Starch	1033.2	499.9	506.8	778.2
Cellulose	63	63	63	63
Vegetable oil	100.8	100.8	100.8	100.8
Minerals	50.4	50.4	50.4	50.4
Vitamins	12.6	12.6	12.6	12.6
BY ₃	-	533.3	-	-
BR ₃	-	-	526.4	-
Casein	-	-	-	280.0
TOTAL	1260	1260	1260	1260

2.6.2 Serum Analysis for Zinc, Calcium and Ferritin

Ocular bleeding method of collecting blood from the orbital sinus was employed (Janet Hoff, 2000). On the 28th day of feeding trial, blood sample of each rat was taken. The rats were anesthetized using chloroform and blood collected in EDTA bottles containing anticoagulant, and digested a clear colourless solution with concentrated HNO₃, H₂O₂ deionized water. The concentration of calcium, iron and zinc were read on a Buck 211 VGP atomic absorption spectrophotometer using their hollow cathode lamp at their respective wavelength (AOAC, 2006).

2.6.3 Determination of Serum Retinol

A 0.5 ml homogenized blood sample was refluxed with methanol and potassium hydroxide solution in a water bath at 100°C for 30 minutes, cooled in ice and kept in the dark for 1 hour. Standard solution of the mixture was prepared by adding methanol:H₂O (3:1) mixture and made up to the mark in a 250 ml volumetric flask and kept in the dark overnight. The absorbance of resulting solution as well as standards were read on a cecil 2483 spectrophotometer at a wavelength of 430nm and vitamin A concentration as retinol equivalent was calculated.

2.6.4 Histological Study of Rat Organs

Histopathological study was carried out on the kidney, liver and spleen of the rats by preparing slides of the rat organs and examining them under the microscope. Photomicrographs were taken at x40, x100 and x400 magnifications.

2.7 Statistical analysis

Data was analysed using SPSS (Statistical Package for Social Science) version 15.0. The data obtained were subjected to analysis of variance (ANOVA), Fisher's Least Significance Difference and Duncan multiple range tests at $p < 0.05$

3. Results

3.1 Proximate Composition of *M. bellicosus*, Boiled Yam and Rice and Complementary Foods

The result of proximate composition of roasted *M. bellicosus*, boiled yam and rice is as shown in Table 2(A). Roasted *M. bellicosus* has low moisture content while the crude protein, fat, carbohydrate and gross energy content were very high. The moisture, crude protein and crude fat content of both boiled yam and rice were low, while the ash and total carbohydrates content were moderately high. The moisture and crude protein values of boiled yam were significantly higher than boiled rice ($p > 0.05$). There was no significant difference in crude fat and ash values of the samples. Boiled rice was significantly higher in total carbohydrate and gross energy ($p < 0.05$) than boiled yam. In Table 2(B), moisture content of the formulated complementary foods reduced significantly, it continue to reduce with increase in quantity of MB inclusion ($p < 0.05$). Significant differences also exist between the enriched complementary foods, the level of reduction in moisture content increasing with increase in inclusion level ($p < 0.05$). There were significant increase in values of crude protein, fat, ash, gross energy and decrease in total carbohydrate of the enriched rice and yam complementary foods ($p < 0.05$).

3.2 Mineral Composition of *M. bellicosus*, Boiled Rice and Yam and Formulated Complementary Foods

M. bellicosus is a rich source of potassium, calcium, phosphorus, zinc, and copper, moderate in sodium, iron and manganese, but low in magnesium (Table 3(A)). Boiled rice and yam are good sources of magnesium, phosphorus and calcium, moderate in potassium, sodium, iron and zinc respectively. Boiled yam was significantly higher in all the minerals compared with boiled rice ($p < 0.05$).

Table 2(A). Proximate composition of roasted *M. bellicosus*, Boiled rice and yam (g/100g dry matter)

Sample	<i>M. bellicosus</i>	Boiled rice (BR)		Boiled yam (BY)
		(Dry)	(Dry)	RV
Moisture	4.0±0.04	5.52±0.04	7.17±0.02	≤ 5
Crude Protein	31.8±0.10	3.78±0.11	5.90±0.09	≥ 15
Crude Fat	16.4±0.03	2.66±0.61	2.40±0.03	10-25
Ash	3.8±0.03	2.70±0.02	2.57±0.02	≤ 3
Total Carbohydrates	43.0±0.10	85.38±0.70	81.98±0.09	64±4
Gross Energy (kcal/)	450.7±0.00	380.50±0.00	373.12±0.00	400-425

Values are means±standard deviations of triplicate determinations. (CODEX CAC/GL08.Rev. 2013): Codex alimentarius: Guidelines on formulated complementary foods for older infants and young children RV = Recommended values (g/100g)

Table 2(B). Proximate composition of enriched complementary foods (g/100)

Sample	Moisture	C. Protein	C. Fat	Ash	T. Carbohydrates	G E
BR	5.52±0.04 ^a	3.78±0.11 ^c	2.66±0.61 ^l	2.70±0.02 ^h	85.34±0.70 ^c	380.42±0.00 ^f
BR ₁	5.40±0.02 ^c	11.57±0.11 ^z	3.57±0.03 ⁿ	3.33±0.02 ^a	76.12±0.02 ^p	382.89±0.01 ^r
BR ₂	4.58±0.02 ^b	13.78±0.10 ^y	3.86±0.03 ^c	3.69±0.02 ^s	74.09±0.10 ^q	386.22±0.01 ^c
BR ₃	4.01±0.03 ^k	15.31±0.05 ^j	4.08±0.03 ^s	4.16±0.03 ^d	72.52±0.22 ^f	388.04±0.00 ^c
BY	7.17±0.02 ^j	5.90±0.09 ^l	2.40±0.03 ^e	2.57±0.02 ^c	81.98±0.09 ^k	373.12±0.00 ^e
BY ₁	7.02±0.04 ^d	7.88±0.10 ^k	2.89±0.03 ^e	4.06±0.03 ^r	75.99±0.12 ^h	381.48±0.00 ^s
BY ₂	4.03±0.04 ^e	11.44±0.11 ^b	5.28±0.03 ^m	4.45±0.03 ^h	74.80±0.15 ⁿ	392.48±0.00 ^k
BY ₃	4.02±0.02 ^a	15.26±0.08 ^c	5.02±0.03 ^m	4.20±0.03 ^h	71.48±0.10 ^c	392.14±0.00 ^h
*RV	<5	>15	10-25	<3	64	400-425

Values are means±standard deviations of triplicate determinations. Means with different superscripts in a column are significantly different ($p<0.05$). *RV = Recommended values (g/100g) *(CODEX CAC/GL 08. Rev. 2013): Codex alimentarius: Guidelines on formulated complementary foods for older infants and young children. BR = 100g boiled rice sample; BR₁ = 90g boiled rice + 10g *M. bellicosus* sample, BR₂ = 85g boiled rice + 15g *M. bellicosus* sample; BR₃ = 80g boiled rice +20g *M. bellicosus* sample BY = 100g boiled yam sample; BY₁ = 90g boiled yam + 10g *M. bellicosus* sample; BY₂ = 85g boiled yam + 15g *M. bellicosus* sample, and BY₃ = 80g boiled yam+20g *M. bellicosus* sample

Adding *M. bellicosus* to boiled rice and yam (Tables (3B) and (3C)) brought about significant increase in minerals ($p<0.05$) in the formulated complementary foods; the values increased with increase in inclusion level of *M. bellicosus*. However, the mineral content of the formulated complementary foods were lower than the recommended values by FAO/WHO. The values of the minerals of enriched complementary foods were significantly different from one another ($p<0.05$), for boiled rice and yam, the 10% *M. bellicosus* incorporated diets having the lowest values while 20% *M. bellicosus* incorporated food samples had the highest values.

3.3 Vitamin Composition of Boiled Rice and Yam and *M. bellicosus* Enriched Complementary Foods

Vitamin composition of boiled rice, boiled yam and *M. bellicosus* enriched complementary foods is shown in Tables 4 (A) and (B) respectively. Addition of *M. bellicosus* increased significantly ($p<0.05$) the β -carotene level in boiled rice and yam. Water soluble vitamins such as thiamine, riboflavin and niacin decreased in value in BR₁ and BR₂. Vitamin B₆ level in *M. bellicosus*- enriched rice complementary foods increased at all levels of addition of the insect, while the addition does not apparently improve the vitamin C content of the complementary food. In boiled yam (Table 3A) the reverse was the case, as addition of *M. bellicosus* progressive improved vitamins at all levels of inclusion of the insect.

3.4 Antinutritional Factors of Boiled Rice, Boiled Yam and Enriched Complementary Foods

Antinutritional factors in boiled rice, boiled yam and *M. bellicosus* enriched complementary foods are shown in Tables 5(A) and (B)). Boiled rice and yam were very low in all the antinutritional factors studied. *M. bellicosus* inclusion does not bring about any observable change in the level of some antinutritional factors, while oxalates and tannins were not detectable at 15% and 20% (BR₂ and BR₃) inclusion level. Trypsin inhibitor was not detected at any level

Table 3(A). Mineral composition of *M. bellicosus*, boiled rice and yam (mg/100)

Parameter	<i>M. bellicosus</i>	Boiled rice (BR)	Boiled yam (BY)
Potassium	361.13±0.31	80.04±0.00	120.00±0.00
Sodium	98.40±0.20	30.00±0.00	90.00±0.00
Calcium	227.50±0.20	70.03±0.00	120.00±0.00
Magnesium	24.33±0.15	140.02±0.00	160.03±0.00
Phosphorus	361.30±0.20	210.01±0.00	290.01±0.00
Iron	2.07±0.25	4.20±0.04	5.60±0.03
Zinc	15.03±0.31	1.18±0.02	1.53±0.02
Manganese	2.35±0.25	0.53±0.15	0.81±0.03
Copper	5.07±0.54	0.37±0.35	0.43±0.21

Values are mean ± standard deviation of triplicate determinations.

Table 3(B). Mineral composition of enriched rice complementary foods (mg/100g)

Parameter	BR	BR ₁	BR ₂	BR ₃	*RV
Potassium	80.04±0.00 ^a	158.70±0.46 ^b	170.07±0.51 ^c	176.63±0.51 ^d	516
Sodium	30.00±0.00 ^a	102.67±0.25 ^b	109.10±0.36 ^c	113.63±0.45 ^d	296
Calcium	70.03±0.00 ^a	242.23±0.50 ^b	252.13±0.29 ^c	258.06±0.42 ^d	500
Magnesium	140.02±0.00 ^a	24.10±0.40 ^b	25.93±0.35 ^c	27.13±0.35 ^d	60
Phosphorus	210.01±0.00 ^a	359.40±1.57 ^b	372.40±0.30 ^c	381.76±0.57 ^d	460
Iron	4.20±0.04 ^a	2.40±0.20 ^b	2.80±0.30 ^c	3.23±0.35 ^d	11.6
Zinc	1.18±0.02 ^a	2.40±0.20 ^b	2.67±0.40 ^c	2.73±0.35 ^d	8.3
Manganese	0.53±0.15 ^a	2.50±0.12 ^b	2.62±0.42 ^c	2.83±0.42 ^d	0.60**
Copper	0.37±0.35 ^a	0.49±0.60 ^b	0.57±0.35 ^c	0.65±0.40 ^d	0.34**

Values are mean ± standard deviation of triplicate determinations. Mean value with different superscripts in a row are significantly different (p<0.05). *RV = Recommended values (mg/100g) *(CODEX CAC/GL 08.Rev. 2013) Codex alimentarius: Guidelines on formulated complementary foods for older infants and young children **RDA (Sareen, Jack & James, 2009).

Table 3(C). Mineral composition of formulated yam complementary Foods (mg/100g)

Parameter	BY	BY ₁	BY ₂	BY ₃	*RV
Potassium	120.00±0.00 ^e	162.20±0.36 ^f	174.10±0.45 ^g	181.50±0.30 ^h	516
Sodium	90.00±0.00 ^e	99.70±0.50 ^f	110.63±0.42 ^g	116.00±0.36 ^h	296
Calcium	120.00±0.00 ^e	232.90±15.68 ^f	253.87±0.35 ^g	264.50±0.30 ^h	500
Magnesium	160.03±0.00 ^e	25.87 ^g ±0.25 ^f	28.17±0.32 ^g	31.47 ^j ±0.25 ^h	60
Phosphorus	290.01±0.00 ^e	359.43 ^s ±1.06 ^f	380.20±0.87 ^g	392.83±0.31 ^h	460
Iron	5.60±0.03 ^e	2.57±0.25 ^f	3.37 ^l ±0.25 ^g	4.40±0.20 ^h	11.6
Zinc	1.53±0.02 ^e	16.10±0.20 ^f	17.57±0.25 ^g	19.80±0.30 ^h	8.3
Manganese	0.81±0.03 ^e	25.03±0.31 ^f	27.17±0.35 ^g	30.00±0.36 ^h	0.60**
Copper	0.43±0.21 ^e	5.23±0.12 ^f	6.50±0.20 ^g	7.40±0.20 ^h	0.34**

Values are means ± standard deviations of triplicate determinations. Mean value with different superscripts in a row are significantly different (p<0.05) *RV=Recommended values (mg/100g) *(CODEX CAC/GL 08. Rev. 2013) Codex alimentarius: Guidelines on formulated complementary foods for older infants and young children **RDA (Sareen, Jack & James, 2009).

Table 4(A). Vitamin composition of enriched rice complementary foods (mg/100 g)

Parameter	BR ₁	BR ₂	BR ₃	*RV
Vitamin A (µg/)	50.13±0.31 ^a	58.97±0.42 ^b	61.47±0.25 ^c	400
Thiamine	0.29±0.02 ^a	0.39±0.02 ^b	0.45±0.02 ^c	0.5
Riboflavin	0.01±0.02 ^a	0.02±0.01 ^b	0.03±0.02 ^c	0.5
Niacin	0.61±0.03 ^a	0.69±0.03 ^b	0.81±0.03 ^c	6
Vitamin B ₆	1.07±0.02 ^a	1.19±0.02 ^b	1.26±0.03 ^c	0.5
Vitamin C	0.00±0.00 ^a	0.00±0.00 ^b	0.00±0.00 ^c	30

Values are means ± standard deviations of triplicate determinations. Means with different superscripts in a row are significantly different (p<0.05). *RV=Recommended values (mg/100g) *(CODEX CAC/GL 08. Rev. 2013) Codex alimentarius: Guidelines on formulated complementary foods for older infants and young children

Table 4(B). Vitamin composition of enriched yam complementary foods (mg/100 g)

	BY ₁	BY ₂	BY ₃	*RV
Vitamin A (µg/)	56.60±0.30 ^f	61.33±0.15 ^g	64.5±0.20 ^h	400
Thiamine	0.33±0.02 ^f	0.38±0.03 ^g	0.51±0.02 ^h	0.5
Riboflavin	0.11±0.02 ^f	0.14±0.02 ^g	0.17±0.02 ^h	0.5
Niacin	0.61±0.03 ^f	0.69±0.02 ^g	0.82±0.02 ^h	6
Vitamin B ₆	1.16±0.02 ^f	1.26±0.03 ^g	1.33±0.03 ^h	0.5
Vitamin C	0.44±0.02 ^f	0.37±0.03 ^g	0.25±0.04 ^h	30

Values are means ± standard deviations of triplicate determinations. Means with different superscripts in a row are significantly different ($p < 0.05$). *RV=Recommended values (mg/100g) *(CODEX CAC/GL 08. Rev. 2013) Codex alimentarius: Guidelines on formulated complementary foods for older infants and young children

Table 5. Antinutritional factors in boiled rice, yam and enriched complementary foods (mg/100 g)

Sample	Phytate	Oxalate	Tannin	Saponin	T. I (TIU/mg)
BR	0.01±0.01 ^f	0.01±0.00 ^f	0.00±0.00 ⁿ	0.04±0.00 ^f	ND
BR1	0.01±0.00 ^f	0.01±0.00 ^f	0.03±0.00 ^f	0.01±0.00 ^s	ND
BR2	0.01±0.00 ^f	0.00±0.00 ^b	0.00±0.00 ⁿ	0.01±0.00 ^s	ND
BR3	0.01±0.00 ^f	0.00±0.00 ^b	0.00±0.00 ⁿ	0.01±0.00 ^s	ND
BY	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^b	0.05±0.00 ^a	ND
BY ₁	0.01±0.00 ^b	0.01±0.00 ^b	0.00±0.00 ^b	0.01±0.00 ^b	ND
BY ₂	0.20±0.00 ^c	0.11±0.00 ^c	0.00±0.00 ^b	0.08±0.00 ^c	ND
BY ₃	0.01±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^b	0.01±0.00 ^b	ND

Mean value with different superscripts in a row are significantly different ($p < 0.05$).

T. I = Trypsin Inhibitors; ND =Not detected at milligramme level

4.5 Bioavailability of Selected Minerals and Vitamin in Formulated Complementary Foods

The serum ferritin of rats on rice and yam groups were significantly higher than the control (Figure 1) ($p < 0.05$). Experimental animals on rice and yam groups had significantly higher serum zinc than the control and basal groups. The serum zinc values observed in the control and rice groups were not significantly different ($p > 0.05$). Serum calcium was more bioavailable in yam and rice groups compared to the control and basal groups. The serum retinol level of rats fed with the formulated complementary foods was significantly higher than those fed with control and basal diets ($p < 0.05$). Total protein values observed in rice group was significantly higher than other groups ($p < 0.05$).

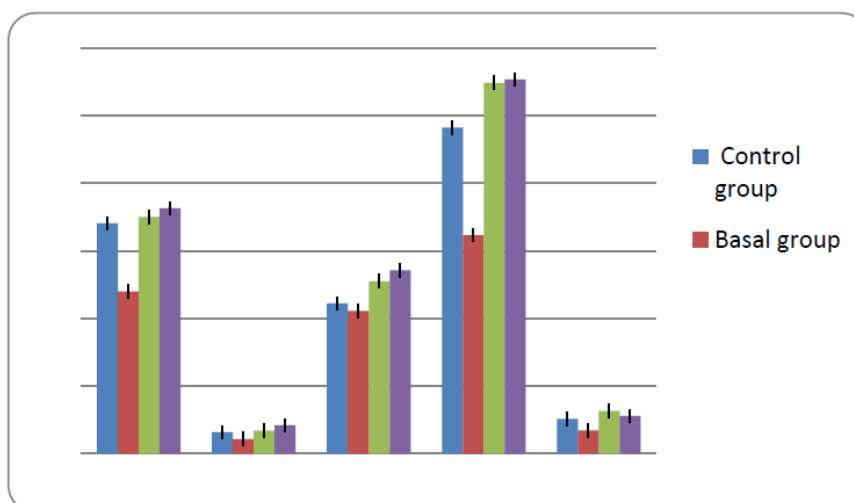


Figure 1. Mean (±SD) Bioavailability of selected minerals, vitamin and serum total protein (mg/100ml) of formulated complementary foods, basal diet and control diets ($p < 0.05$)

4.6 Histopathological Assessment of Rats' Organs

4.6.1 Rats Kidney

Histopathological examination of kidney section of rats is shown in Figure 2. Rats from control group (C) showed no histopathological changes and normal structure of renal parenchyma after the feeding trial. It also shows normal glomerular morphology (G) and tubular arrangement. Photomicrograph of kidney of rats from basal group (B) indicated some histopathological changes. These included slight degeneration with increased urinary space size. Rats belonging to rice group (R) also showed normal glomerular morphology (G) and tubular arrangement just like the rats in the control group, while the yam (Y) group was not well processed.

4.6.2 Rats Liver

Photomicrographs of liver from rats on different diets are illustrated in Figure 3. Liver from rats on control diet (Plate C) yam diet (Plate Y) displayed normal histological structure of hepatic lobules. They also showed normal morphology of the hepatocyte (H) arrangements with a well-defined central vein (CV). On the other hand, rats fed on basal diet show slightly different hepatocyte arrangement with active macrophage action, indicating slight insult on the liver; while rats fed rice diet showed great insult on the liver as there is a loss of hepatocytes around the CV area which is radiating outward.

4.6.3 Rats spleen

Figure 4 shows the effect of feeding control, basal and experimental diets on the lymphocytes and red to white pulp ratio in the spleen of rats. The photomicrography of rats' spleen fed with the control and rice diets revealed no histopathological changes, and they had normal arrangement of the lymphocytes with the branching pattern of the veins. The rats on basal diet were poorly processed and could not be interpreted. On the other hand, the spleen of rats fed yam diet showed irregular arrangement of lymphocytes.

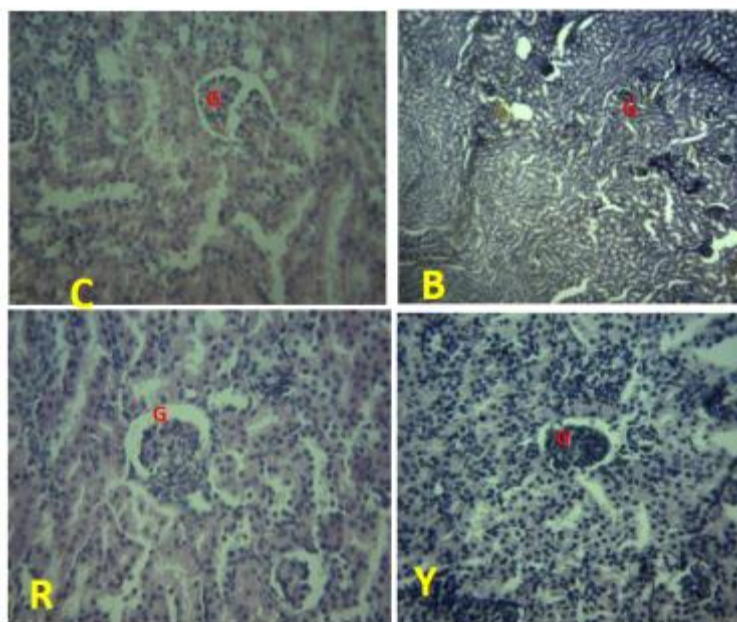


Figure 2. Histomorphology of rats kidneys

C = Control group, B = Basal group, R = Rice group, and Y = Yam group

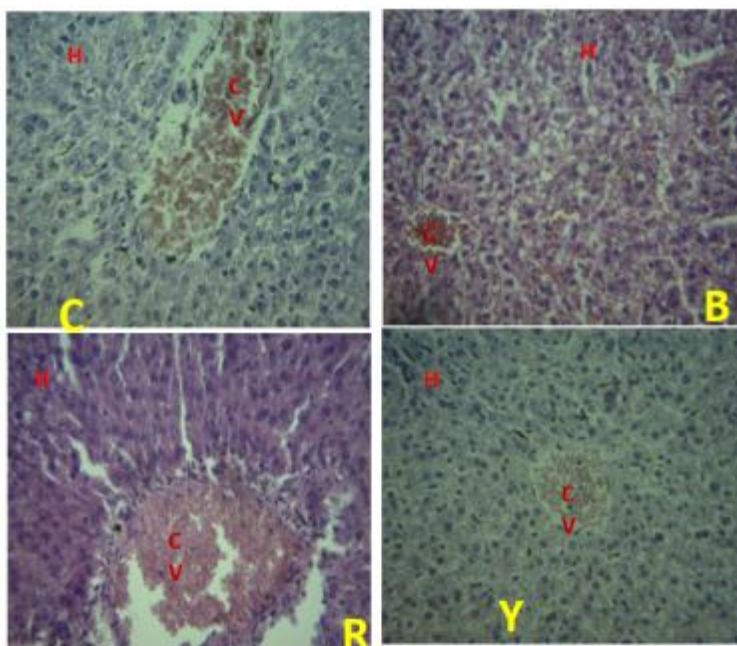


Figure 3. Histomorphology of liver tissue of rats

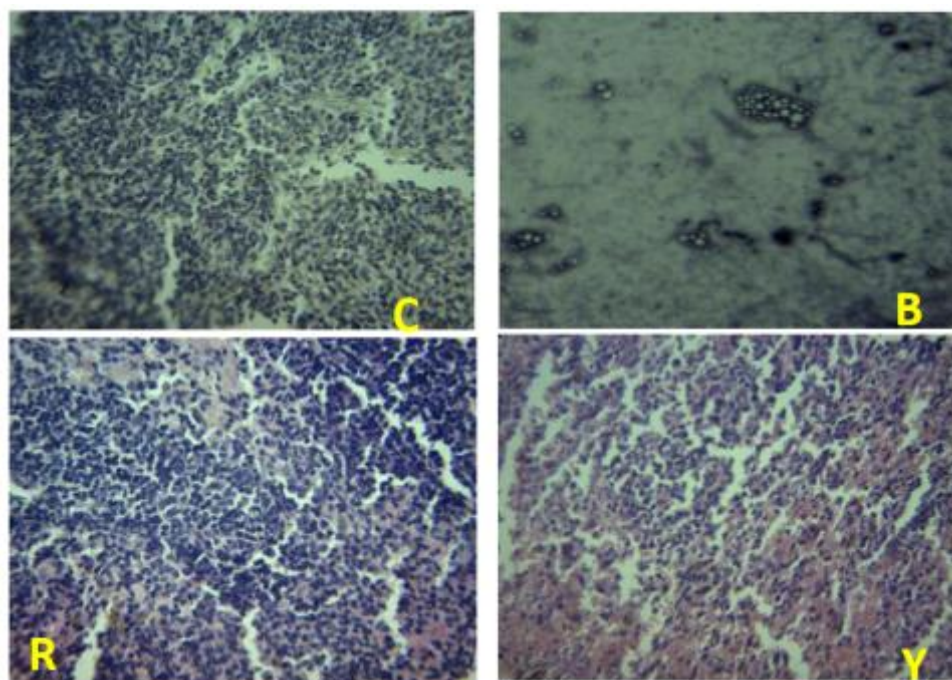


Figure 4. Histomorphology of rats spleen

5. Discussion

5.1 Proximate Composition of Roasted *M. bellicosus*, Boiled Yam, Rice and Complementary Foods

The value of moisture content for *M. bellicosus* in this study is comparable to Adepoju & Omotayo, (2014). Its low moisture content underscores its high dry matter, and consequently, high macronutrients. The value obtained for the macronutrients of the insect was related to (Adepoju, 2016), and within the range stated for termite (*Trinervitermes germinates*, Afiukwa et al., 2013). Generally, the insect is rich in crude protein, crude lipid, ash, total carbohydrates and gross energy. The high values of the macronutrients of the insect can contribute significantly to macronutrients of infant complementary foods. The value of crude protein reported in the present

study falls within the range (33.51–39.74 g/100 g) observed by Kinyuru et al, (2013) for four species of termites, and similar to dried termite (35.70 g/ 100 g) reported in the NFCT (Sehmi, 1993). Crude protein value of winged termites in this study is superior to raw red meats reported by Williams, (2007) hence, may offer an affordable source of protein for complementary foods.

The moisture content of cooked rice and yam (dry matter basis) used in preparing the rats diets was low and closer to the recommended value (≤ 5 g) (Table 1 (A)). Food commodities proposed to be used for preparation of dry complementary foods should be properly dried to reduce eventual growth of unwanted microorganisms, and only small amounts should be prepared at a time to avoid extended storage (WHO, 1998; Solomon, 2005). The low moisture content of the raw material may aid the storage quality of the formula, because low moisture content in foods will reduce microbial growth. Both cooked rice and yam were high in total carbohydrates and gross energy content. The high values of total carbohydrates and gross energy content underline why they are used as basic staples for family foods, and can serve well in meeting energy needs of old infants and young children even when fed in little quantity. The reduction in moisture content of *M. bellicosus* enriched complementary foods was similar to Adepoju & Daboh (2013) for Cirina forda powder and Adepoju & Ajayi (2016) for *M. bellicosus*–enriched maize and sorghum complementary foods. Addition of *M. bellicosus* to rice and yam resulted in significant increase in protein, fat, ash, total carbohydrate and gross energy content of enriched complementary foods. High energy value of the formulated foods is believed to be due to high lipid and carbohydrate content of the foods. FAO and WHO recommended that foods fed to infants and children should be energy-dense so as to improve total energy intake and the utilization of other nutrients (FAO/WHO, 1998). The formulated rice and yam enriched complementary foods met the recommended values for protein, ash, total carbohydrate, and can supply up to half of fat requirement (CODEX CAC/GL 08. 1991). Feeding infants and young children with the *M. bellicosus*–enriched complementary foods two times a day may provide the larger part of their recommended value of macronutrient needs on daily basis, especially at 20% level of inclusion, though our preparations are meant to serve as basic ingredients to which other sources of nutrients and dietary fibre can be added.

4.2 Mineral Content of *M. bellicosus*, Boiled Rice, Yam and Formulated Complementary Foods

The insect is rich in potassium, calcium, phosphorus, zinc, and copper, moderate in sodium, iron and manganese, but low in magnesium (Table 2(A) when compared with the daily requirements of these micronutrients. Boiled rice and yam are high in magnesium, phosphorus and calcium; and moderate in potassium, sodium iron and zinc respectively. Boiled yam was significantly higher in all the minerals compared with boiled rice ($p < 0.05$). Addition of *M. bellicosus* to boiled rice (Table 2B) and boiled yam (Table 2C) resulted in significant increase in values of the minerals in all the formulated complementary diets ($p < 0.05$), the values increasing with increase in its inclusion level. The formulated diets at 15% and 20% inclusion levels of *M. bellicosus* almost met the recommended value for zinc, met that of manganese, while all levels of inclusion can meet recommended value for copper (FAO/WHO 1991). Intake of the formulated complementary foods twice daily can satisfy requirement for calcium and phosphorus, while daily intake of the formulated diets 2-3times at 20% inclusion of the insect will meet the requirement for iron. Zinc is an essential mineral, vital to human metabolism, growth and immune function (Aggett & Comerford, 1995). Its deficiency may significantly contribute to stunting in young children. Mild to moderate zinc deficiency may present clinically as impaired growth, which have previously been attributed to other factors (Brown et al., 1998).

Iron deficiency is the most common cause of anemia though other nutrition and non-nutrition related cause can be involved in its origin. Hallberg & Rossander (1984) reported that anemia is most prevalent in children between 6 and 24 months of age and the major causes are inadequate dietary intake of bioavailable iron, malaria and parasitic infections. Fernandez et al., (2002) reported that prevalence of anaemia is higher during infancy and early childhood than at any other time in the life cycle. The high prevalence is consistent with inadequate intake of dietary iron and low bioavailability in most complementary foods (Lutter, 2003).

4.3 Vitamin Composition of Boiled Rice, Yam and Formulated Complementary Foods

Addition of *M. bellicosus* powder improved the vitamin A content of the enriched foods (Tables 3A and 3B), the observed improvement was due to vitamin A content of the insect, which has been previously reported to be a good source of vitamin A (330.42 ± 0.12 $\mu\text{g}/100\text{g}$, Adepoju & Omotayo, 2014). Vitamin A is an antioxidant which prevents cells from damage by free radicals, essential for maintaining healthy eyes and skin, needed for normal growth and reproduction, promotion of healthy immune system and prevention of infections (Rolfe et al., 2009; Roth & Townsend, 2003). The enriched boiled rice and yam will meet recommended value for vitamin B6 (CODEX CAC/GL 08. 1991). The complementary food were low in water soluble vitamins, hence, their

alternative sources need to be included in the complete complementary foods since the preparation here are meant for basic ingredients and not complete complementary foods.

4.4 Antinutrient Composition in Boiled Food Samples and Enriched Complementary Foods

The boiled rice and yam and *Macrotermes bellicosus*-enriched samples were very low in the antinutritional factors studied. Addition of *M. bellicosus* to the samples did not increase the level of antinutrients in the formulated diets. The level of antinutrients was very low and cannot hinder nutrient bioavailability in the enriched complementary foods.

4.5 Micronutrient Bioavailability in the *Macrotermes bellicosus*-enriched Diets

The significantly higher serum levels of iron, zinc, calcium and vitamin A in rats groups fed the control and experimental diets confirmed the contribution of *M. bellicosus* to serum micronutrients in the two experimental diet groups, implying that addition of *M. bellicosus* to complementary foods at 20% inclusion level will improve the bioavailability of iron, vitamin A, and zinc which are micronutrients of public health importance. Micronutrient requirements are high during the first 2 years of life to support the rapid growth and development during this period. Iron and zinc are among micronutrients whose daily requirements' are difficult to meet with plant-based complementary foods alone, hence, the need to obtain them from animal-source foods, or be fortified in some way (Dewey 2013). Micronutrients are essential for growth, development, and prevention of illness in young children (WHO, 2009). Adequate intakes of micronutrients, such as iron, zinc, and calcium, are important for ensuring optimal health, growth, and development of infants and young children (Caballero et al., 2005; Rolfes et al., 2009)

4.6 Histopathological Assessment of Rat Internal Organs

The result obtained for histopathological effects of feeding rats with the control and experimental diets revealed that the diets had no pathological alteration on the kidney morphology of rats in their groups. The result of the basal diet showed that inadequate/lack of protein intake for a period of time can have negative effect on kidney morphology and function. The kidney is an excretory organ that removes metabolised and nonmetabolised toxic materials from the body (Robbins et al., 1985); hence this organ would be exposed to high concentrations of the noxious materials that could have caused the lesions. This result has confirmed that *M. bellicosus* was not nephrotoxic to the kidney. While the basal diet showed slightly different hepatocyte arrangement with active macrophage action indicating slight insult on the liver; the diets enriched with *M. bellicosus* at 20% inclusion level and the control had no pathological lesions, demonstrating further that *M. bellicosus* is safe for consumption. The slight insult observed in the basal group may be due to absence of any protein source in their diet. The rats' spleen fed with the control and experimental diets had no histopathological changes, and also showed normal arrangement of the lymphocytes with the branching pattern of the veins. Also, normal red to white pulp ratio was observed after the feeding trial. This further confirmed that both control and experimental diets if properly prepared will not encourage the growth of unpleasant bacteria when consumed.

5. Conclusion and Recommendation

The nutrient content of *M. bellicosus* improved the nutrient quality of rice and yam used as staple for complementary foods in Southwest Nigeria, its protein supported rats growth at the three levels of inclusion, especially at 20% inclusion level. The nutrients were bioavailable without pathological effects on kidney, liver and spleen of rats fed the diets, showing that *M. bellicosus* had no toxic effects on these organs. This discovery suggests that *M. bellicosus* may be a promising animal source of protein and essential micronutrients without any negative effects on the organs of humans. The use of this insect as a cheap source of protein, energy and essential micronutrients is therefore recommended for nursing mothers especially from rural communities as a means of reducing infants and young children malnutrition when in season.

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