Mal J Nutr 22(1): 153 - 161, 2016

Assessment of Fatty Acid Profile, Protein, and Micronutrient Bioavailability of Winged Termites (*Marcrotermes bellicosus*) Using Albino Rats

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ABSTRACT

Introduction: The need for alternative protein and essential micronutrients sources for adequate complementary foods is urgent. Dried *Marcrotermes bellicosus* was reported to be a good source of dietary protein, fat, and micronutrients. This study investigated the fatty acid profile, protein, and essential micronutrient bioavailability in *M. bellicosus* using albino rats. **Methods:** *M. bellicosus* was collected around the Alegongo area, Akobo, Ibadan, Nigeria during their swarming flights, roasted at 105 °C for fifteen min, dewinged, and winnowed. The roasted sample was analysed for proximate, minerals, and antinutrients using standard methods of the AOAC International. Fatty acid profile was determined using a gas-liquid chromatographic method, whilst protein and essential minerals bioavailability were determined using weanling albino rats. **Results:** Roasted *M. bellicosus* contained 31.8 g protein, 16.4 g fat, 1.3 g ash, 46.5 g carbohydrates, 361.13 mg potassium, 227.50 mg calcium, 361.30 mg phosphorus, 15.03 mg zinc, 52.30% linolenic acid, 24.91% linoleic acid, 5.97% oleic acid and yielded 460.8 kcal gross energy/100g sample. The mean weight gain in the experimental diet group (+23.17±6.71) was significantly higher than that of the control diet group (+16.83±6.71) and the basal diet group (-19.50±9.03). The basal diet group had the least value for all serum micronutrient levels whilst the experimental diet group had the highest. **Conclusion:** *M. bellicosus* protein supported rat growth at a 15% inclusion level. The calcium, iron, zinc and vitamin A in *M. bellicosus* were bioavailable in rats. *M. Bellicosus* could be a potential novel food for humans.

Key words: Albino rates, fatty acid profile, *Marcrotermes bellicosus*, micronutrient bioavailability, protein quality

INTRODUCTION

Edible insects constitute high quality food for humans, livestock, poultry, and fish hence they are a serious alternative to conventionally produced animal-based protein sources, either for direct human consumption, or indirectly as feedstock (*Food-info.net*, 1999-2009) Termites are the most widely consumed insects in many regions of Nigeria (Fasoranti & Ajiboye, 1993; Banjo, Lawal & Songonuga, 2006; Agbidye, Ofuya & Akindele, 2009).

Winged termites are highly attracted to light, and are collected for consumption as they emerge in swarms during the rainy season (*Food-info.net*, 1999-2009). Once roasted, the wings are removed from the insects either by sifting or by rubbing them between the palms. The finished product is either eaten or sold in markets in western and eastern Nigeria as a snack (Banjo *et al.*, 2006). Dried *Marcrotermes bellicosus* is a good source of dietary protein, fat, and micronutrients (Banjo *et al.*, 2006; Ekpo, Onigbinde & Asia, 2009; Adeyeye, 2011; Adepoju & Omotayo, 2014). The fat content of the insect may contain mono and polyunsaturated fatty acids (Ekpo *et*

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Figure 1. Winged termites (*M. bellicosus)*

Figure 2. Roasted *M. bellicosus*

al., 2009) which benefit adults' health and are important for the growth, and cognitive and eye development of young children (Insel, Turner & Ross, 2007).

Roasted *M. bellicosus* can be a good source of dietary protein, calcium, phosphorus, zinc, copper and haem iron (Adepoju & Omotayo, 2014). Calcium and phosphorus are required for strong bones and teeth by all ages, while zinc and copper are required for proper enzymatic activities in the body, and zinc is also required for growth, cell replication, fertility and reproduction, and hormonal activities among others (Insel *et al.*, 2007; Rolfes, Pinna & Whitney, 2009). Hence the insect can be a good source of these minerals in complementary foods for infants. Information is available on the nutrient potential and antinutrient composition of *M. bellicosus* in the literature (Banjo *et al.*, 2006; Ekpo *et al.*, 2009; Adeyeye, 2011; Adepoju & Omotayo, 2014), but little is known about the bioavailability of micronutrients from the insect. This study aimed to determine the fatty acid profile and bioavailability of protein and micronutrients from *M. bellicosus* as a means of combating protein and micronutrient malnutrition among infants and young children.

METHODS

Sample of *M. bellicosus* (Figure 1) was collected around the Alegongo area, Akobo, Ibadan, Nigeria during their swarming flights. The sample was roasted at 105^{0}C for 15 min and allowed to cool down to room temperature. The cooled sample was de-winged by rubbing between the palms and then winnowed to remove the wings. The de-winged sample (Figure 2) was put in a polythene bag and kept in a freezer at -4^{0}C until needed for analysis.

Proximate composition

Moisture content of the samples was determined by air oven at 105^{0}C (Plus 11 Sanyo Gallenkamp PLC UK) for 4 h. The crude protein of the samples was determined using the micro-Kjeldahl method (Method No. 978.04, AOAC International, 2005). Crude lipid was determined by the Soxhlet extraction method (Method No. 930.09, AOAC International, 2005) and expressed as g/100g of sample. Ash content was determined by weighing 5 g of sample in triplicate and heating it in a muffle furnace (Gallenkamp, size 3) at 550^{0}C for 4 h (Method No. 930.05, AOAC International, 2005). The total carbohydrate content was obtained by difference. Gross energy of the samples was determined using a ballistic bomb calorimeter (Cal 2k – Eco, TUV Rheinland Quality Services (Pty) Ltd, South Africa).

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 the readings were taken on a Jenway digital flame photometer/ spectronic20 (AOAC International, 2005: 975.11). Phosphorus was determined by the vanado-molybdate colorimetric method (AOAC International, 2005: 975.16). Calcium, magnesium, iron, zinc, manganese, copper, and selenium were determined spectrophotometrically using a Buck 200 atomic absorption spectrophotometer (Buck Scientific, Nor-walk) and compared with the absorption standards of these minerals (AOAC International, 2005: 975.23).

Antinutrient analysis

Oxalate was determined by extraction of the samples with water for about 3 h and standard solutions of oxalic acid were prepared and read on a spectrophotometer (Spectronic20) at 420 nm. The absorbance of the samples was also read and the amount of oxalate estimated. Phytate was determined by titration with a ferric chloride solution (Sudarmadji & Markakis, 1977). Meanwhile, trypsin inhibitory activity was determined on casein and the absorbance was compared 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 5 h, measuring their absorbance and comparing the absorbance of the sample extracts with the absorbance of standard solutions of tannic acid at 500 nm on a 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). Nitrates and nitrites were determined by the modified method of Mottran *et al.* (1977) by using ethylene diamine tetraacetic acid (EDTA) and sulphanilamide/N-1-methylethylenediamine dihydrochloride solutions respectively, and the absorbance was measured at 538 nm. All determinations were carried out in triplicate.

Protein bioavailability determination

Three iso-caloric diets, of which two sets were iso-nitrogenous, comprising a basal diet (0% protein), an experimental diet (15% protein), and a lactalbumin diet (control) (15% protein) were prepared as shown in Table 1. Eighteen 24-day old weanling Wistar albino rats were purchased from the Physiology Department, University of Ibadan, and were housed individually in metabolic cages at the animal house of the Department of Animal Science, University of Ibadan, Ibadan, Nigeria. The rats were fed *ad libitum* with commercial rat pellets,

Table 1. Rats group diet composition (g/1000 g diet)

Feed component	*Basal*	*Experimental*	*Control*
Starch	820.0	391.4	605.7
Cellulose	50.0	50.0	50.0
Vegetable oil	80.0	80.0	80.0
Vitamin mix	10.0	10.0	10.0
Mineral mix	40.0	40.0	40.0
M. bellicosus	-	428.6	-
Lactalbumin	-	-	214.3
Total	1000	1000	1000
Gross energy (kcal/g)	2.89	3.11	3.02
Nitrogen (%)	0.12	2.62	2.51

Table 2. Proximate composition and gross energy of roasted *M. bellicosus*

Components	*(g/100 g)*
Moisture	4.0 ± 0.15
Crude protein	31.8 ± 0.10
Crude lipid	16.4 ± 0.03
Ash	1.3 ± 0.03
*Total carbohydrates	46.5 ± 0.10
Gross energy (kcal)	460.8 ± 0.00

* Found by difference

and clean tap water for seven days, weighed and then randomly distributed to three diet groups (*i.e.*, basal, experimental, and control) of six rats each based on their weight. An amount of 10 g of prepared diet was supplied to each rat on a daily basis and water was changed every other day for 14 days. The left-over of the diets were collected and weighed on a daily basis. The rats were weighed on a weekly basis throughout the duration of the experiment Ayatse, Eka & Ifon, 1985; Itam, Eka & Ifon, 1986.

The rats were sacrificed on the twenty-first day of the experiment using anaesthesia, and their blood samples collected for nutrient bioavailability analysis. Serum retinol was determined using the method of the Association of Official Analytical Chemists (AOAC) (1990), whilst serum ferritin and zinc were determined using AOAC International (2006) methods 983.24 and 991.11.

Measurement of nutritional indices

The consumption index (CI), growth rate (GR), and efficiency of conversion of ingested food to body tissue (ECI) were calculated using the equations described by Waldbauer (1968) as follows:

$$CI = \frac{C}{TA}; \quad GR = \frac{G}{TA}; \quad ECI = \frac{G}{C} \times 100$$

where
C = fresh weight of feed consumed
T = duration of feeding period
A = mean fresh weight of the rat during the feeding period
G = fresh weight gain of the rat

Statistical analyses

Analysis of variance (ANOVA) and least significant difference (LSD) statistics were carried out on the results obtained, and the level of significance was set at $p<0.05$.

RESULTS

The results of proximate composition of roasted *M. bellicosus* are shown in Table 2. The roasted sample contained 4.0 g moisture, 16.4 g crude lipid, 1.3 g ash, 31.8 g crude protein, 46.5 g total carbohydrates, and 460.8 kcal of gross energy/100 g roasted sample. The mineral composition of *M. bellicosus* is shown in Table 3. *M. bellicosus* was rich in potassium, calcium, and phosphorus. Sodium, magnesium, and zinc levels were moderate. Iron, manganese, and copper levels were low.

The fatty acid profile of *M. bellicosus* showed that it was very rich in linolenic acid, high in linoleic and palmitic acids, but low in oleic acid, arachidonic acid, and saturated fatty acids (arachidic and myristic acids) as shown in Table 4. *M. bellicosus* did not contain any phytates, tannins, saponins, or nitrates, but contained 0.01 mg oxalate, 0.02 TIU/mg trypsin inhibitor and 0.01 mg nitrite/100 g sample as shown in Table 5.

The mean rat feed intake of prepared diets is shown in Table 6. The mean rat feed intakes of the experimental and control groups were significantly higher than that of basal diet ($p<0.05$). A significant difference also existed between the rat feed intake of the experimental and control diet intakes with the intake of the experimental diet being slightly higher than that of the

Table 3. Mineral composition of roasted *M. bellicosus* (mg/100 g)

Potassium	361.13 ± 0.31
Sodium	98.40 ± 0.20
Calcium	227.50 ± 0.20
Magnesium	24.33 ± 0.15
Phosphorus	361.30 ± 0.20
Iron	2.07 ± 0.25
Zinc	15.03 ± 0.31
Manganese	2.35 ± 0.25
Copper	5.07 ± 0.54

Table 4. Fatty acid profile of *M. bellicosus* (%)

Palmitic acid	11.81
Oleic acid	5.97
Linoleic acid	24.91
Linolenic acid	52.30
Arachidic acid	0.02
Arachidonic acid	0.39
Erucic acid	1.56
Behenic acid	0.42
Myristic acid	0.22

Table 5. Antinutrient composition of roasted *M. bellicosus*

Components	*(mg/100 g)*
Phytate	0.00 ± 0.00
Oxalate	0.01 ± 0.00
Tannin	0.00 ± 0.00
Saponin	0.00 ± 0.00
Trypsin Inhibitor (TIU/mg)	0.02 ± 0.00
NO3-	0.00 ± 0.00
NO2-	0.01 ± 0.00

control group. The rat feed intakes were significantly different with the basal group having consumed slightly above half of the daily supply of 10 g each, whilst the experimental group (insect-source protein diet) recorded the highest mean feed intake, which was slightly higher than that of the control group (casein-source protein diet).

Significant differences existed between the feed CI, GR, and ECI for all the rat groups ($p<0.05$). The basal group had the least CI, GR, and ECI; the experimental diet was highest in ECI, whilst the control diet was the highest in feed CI and GR. Rat weight change could be attributed to feed intake and protein content of the diet with the basal group losing weight whilst the experimental and control groups gained weight.

The serum micronutrient levels of the rats fed the basal diet were significantly lower in value than that of the other groups whilst the rats fed the experimental diet had the highest value as shown in Table 6. All the micronutrient values of the rats fed the

Table 6. Mean feed intake, weight gain/loss and serum micronutrient bioavailability of rats fed basal, control and experimental diets

Parameters	*Basal*	*Experimental*	*Control*
Feed intake (g)	5.99 ±0.40	9.50 ± 0.24	9.01 ± 1.13
Weight change (g)	-19.50 ±9.03	+23.17 ± 6.71	+16.83 ± 6.71
Iron (mg)	22.38 ±2.78a	37.15 ± 2.68b	32.95 ± 4.00c
Zinc (mg)	2.14 ±0.70a	3.92 ± 0.46b	2.78 ± 0.29c
Calcium (mg)	18.72 ±1.62a	26.83 ± 1.04b	23.23 ± 1.95c
Vitamin A (μg/)	31.28 ±1.71a	55.70 ± 2.66b	48.98 ± 1.35c
CI	8.60a	11.48b	14.98c
GR	3.64a	6.41b	8.03c
ECI	5.64a	7.47b	7.14b

Means with different superscripts in a row are significantly different ($p<0.05$).
CI = consumption index, GR = growth rate, ECI = efficiency of conversion of ingested feed.

experimental diet were significantly higher than that of the control group ($p<0.05$).

DISCUSSION

The low moisture level (4.0 g/100 g) for roasted *M. bellicosus* obtained in this study is in agreement with the value reported by Adepoju & Omotayo (2014) but lower than value reported by Ekpo *et al.* (2009). The difference in value compared with that of Ekpo *et al.* (2009) could be due to the extent of roasting of *M. bellicosus* as well as differences in the geographic locations from where the samples were obtained. The low moisture content of the roasted *M. bellicosus* is an indication that it can be kept for a period of time with or without refrigeration before it goes bad. Its low moisture content also implied high dry matter content.

The value of crude protein (31.8 g/100 g) of *M. bellicosus* in this study was slightly lower than the one reported in the literature (Ekpo *et al.*, 2009; Adepoju & Omotayo, 2014). The observed variation in nutrient content might be due to the geographic location and method of sample preparation. *M. bellicosus* is high in animal protein, and can be a good source of the protein if it is bioavailable.

The lipid value (16.4 g/100 g) obtained in this study is significantly lower than that reported by Ekpo *et al.* (2009) and Adepoju & Omotayo (2014). The difference in lipid values might also be attributed to variation in method of sample preparation, as well as geographic location. The moderate lipid content of *M. bellicosus* may reduce the rate of its susceptibility to spoilage through rancidity, and promote its shelf life. Its lipid value is suggestive of the fact that it can be a source of fat soluble vitamins. The lipid content of *M. bellicosus* was comparatively higher than that of other edible insects (Adepoju & Daboh, 2013).

The ash value (1.3 g/100 g) of *M. bellicosus* was relatively high, and this is indicative of it being a good source of minerals. The value of ash in this study was very similar to the value reported by Adepoju & Omotayo (2014). *M. bellicosus* was surprisingly high in total carbohydrate content (46.5 g/100 g), and the value obtained was significantly higher than the value reported by Ekpo *et al.* (2009) and Adepoju & Omotayo (2014). The observed variation in nutrient content of *M. bellicosus* in this study compared to reported values in the literature is believed to have arisen from seasonal variation and soil conditions/composition of areas the samples were obtained.

The gross energy content (460.8 kcal/100 g) of *M. bellicosus* was high. This is believed to be a direct result of its high protein, fat and total carbohydrate values. However, this value is significantly lower than the reported value of gross energy by Adepoju & Omotayo (2014). This could be due to higher crude lipid value of their sample.

The value of sodium (98.40 mg/100 g), calcium (227.50 mg/100 g), magnesium (24.33 mg/100 g), and phosphorus (361.30 mg/100 g) were in close agreement with that of Adepoju & Omotayo (2014). However, the value for potassium (361.13 mg/100 g) and zinc (15.03 m/100 g) were significantly higher in this study compared to that reported by Adepoju & Omotayo (2014). The high value of the essential minerals such as potassium, calcium, phosphorus, zinc, and moderate value of iron (2.07 mg/100 g) qualify *M. bellicosus* as good source of these minerals for locally formulated complementary foods.

The value of linoleic acid (24.91%) in this study was close to the value reported by Ekpo *et al.* (2009), but the palmitic acid, oleic acid, and arachidonic acid components were significantly lower (p<0.05), whilst linolenic acid in this study was significantly higher (p<0.05). *M. bellicosus* is rich in polyunsaturated fatty acids of omega 6 and omega 3, which are essential for the growth of infants and young children (Insel *et al.*, 2007; Rolfe *et al.*, 2009). It also contained a reasonable level of mono unsaturated fatty acid of omega 9, which is also important nutritionally. The high level of unsaturated fatty acid coupled with a low level of saturated fatty acid content makes *M. bellicosus'* fat content a desirable, health promoting one.

The value of all the antinutrients studied was very low (lower than 0.10 mg/100 g of sample). This finding supports the findings of Adepoju & Omotayo (2014) who report that *M. bellicosus* is very low in antinutrients and toxicants making it very safe for consumption.

The pronounced weight loss of rats fed the basal diet confirmed the absence of protein in their diet, whilst the significant weight gain in the experimental and control groups indicated that those rats' diets contained protein of high biological value. Protein of high biological value, which is usually of animal origin, is required for proper growth and development of infants and young children (Roth & Townsend, 2003).

The least feed consumption index(CI), growth rate (GR) and efficiency of conversion of ingested feed (ECI) was experienced by the basal group rats and could be due to a lack of protein. Protein-rich diets are found to be more appealing and palatable (Roth & Townsend, 2003). However, the experimental and control diets were relished by the rats in these groups, and their consumption indices were significantly higher than that of basal diet group (p<0.05), confirming the fact that protein-containing foods are attractive and palatable. The higher CI and GR values of the control diet did not translate to significantly different ECI between the experimental and control groups (p<0.05), but rather, the higher ECI for experimental group led to significantly higher weight gain in experimental group compared with control group.

The comparatively higher mean weight gain in the experimental rat group above that of control group is an indication that *M. bellicosus* protein is of high biological value and is bioavailable for the rats' growth. The level of protein in *M. bellicosus* coupled with its high gross energy qualify it as source of essential integral part of complementary foods, hence, its inclusion in locally formulated complementary foods will ameliorate the effect of protein-energy malnutrition among this vulnerable group.

The mean serum calcium, iron, zinc, and vitamin A content of rats fed the experimental diet were significantly higher than that of both the basal and control groups (p<0.05), indicating the contribution of *M. bellicosus* to these micronutrients. The contribution of *M. bellicosus* to rat serum level of these micronutrients was an indication that these micronutrients were bioavailable in rats, and by extrapolation, will be bioavailable in humans at the 15% inclusion level (Sanchez-Muniz *et al.*, 1998).

CONCLUSION

Roasted *M. bellicosus* is high in protein of high biological value, healthy dietary fat, gross energy, and essential minerals such as iron and zinc, calcium, phosphorus, copper, and vitamin A. It is very low in antinutritional factors and its macro and micronutrients were bioavailable for rat utilisation, supporting rat growth at 15% level of inclusion with highly comparable mean weight gain which was higher than that of the control protein (casein). Incorporation of this nutritious insect in locally formulated complementary foods and consumption by adults can serve as a means of reducing the prevalence of infant and young child morbidity and mortality rate, adult micronutrient malnutrition, and reduce protein energy malnutrition, which is rampant especially in the rural communities of Nigeria.

ACKNOWLEDGEMENT

The author wishes to acknowledge the financial support from the authority of the University of Ibadan Research Grant to carry out this research work.

Conflict of Interest

The author had no conflict of interest in carrying out and reporting this work.

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