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Biochemical and Haematologic Effects of Intake of *Macrotermes nigeriensis* Fortified Functional Diet

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Abstract: Twenty-four male albino rats, 4 weeks old were randomly distributed into 4 groups (A-D) and fed growers' mash as the control diet and 25, 50 and 75% oven-dried, ground *Macrotermes nigeriensis* fortified diets respectively for a total period of 28 days. The effects of the diets on hepatic, renal and haematologic function indices of albino rats were studied. The animals fed the fortified diets had non-significantly ($p>0.05$) lesser body weight gains than the control animals. The fortified diets elicited dose-dependent increases in the levels of blood total protein, albumin, packed cell volume, hemoglobin and red blood cell counts as well as on the calculated red cell indices. The fortified diets did not significantly ($p>0.05$) alter the activities of plasma alamine and aspartate aminotransferases as well as the concentrations of total bilirubin, urea and creatinine. The results indicate that the fortified diets do not have detrimental hepatic, renal or haematologic effects but rather may be recommended for fortification of human and animal food, especially in weaning diets of growing children and nursing mothers to combat food insecurity and malnutrition.

Key words: Functional food, termite diet, biochemical indices

INTRODUCTION

Entomophagy has gained prominence in recent years due, more to the increasing cases of drought and poor economic conditions and less to the reported high nutrient content of insects (Allotey and Mpuchane, 2003). Many families, especially in less developed countries incorporate insects into their diets to supplement the low protein content of their staple carbohydrate diet (Ekpo *et al*., 2009).

A number of insects or their products are used as food in some parts of the world, to a large extent eaten by women and children (Ene, 1963). Insects have served traditionally, economically and nutritionally as an important item in the history of human nutrition especially among the people of Africa, Asia and Latin America (Bodenheimer, 1951; Menzel and D'Aluision, 1998) reported that there are about 2000 edible insects around the world. The commonly consumed insect groups ranged from winged reproductive termites, beetles, caterpillars and grasshoppers. Ordinarily, insects are not consumed as sole meals during or outside food shortage periods but are served as an important part of human diet when seasonally available. Interestingly, the seasonal availability of some of these insects occur during early farming period when food stores are very low and many of such stores may have also been used as seedlings for the new farming season. Insects have been a staple of almost every indigenous culture (Ramos-Elorduy, 1998), not only because of their delicious flavour but because they provide high protein nutrients than soybean or fish and have been found to be concentrated sources of calcium, magnesium, vitamins (Igwe *et al*., 2011a) and several other nutrients (Menzel and D'Aluision, 1998).

Many insects consumed in Nigeria such as termites, grasshoppers, caterpillars, palm tree larvae, locust, dung larvae, crickets and composite beetle larvae have been reported to contain high quality fat, proteins, vitamins and minerals (Banjo *et al*., 2006; Igwe *et al*., 2011a). This is consonance with the findings that insects have high unsaturated fat content including the essential fatty acids; linoleic and/or linolenic acids (De Foliart, 1991), with termites ranking very high in fat content among many insects (Phelps *et al*., 1975).

Macrotermes nigeriensis is a eusocial insect of the Isoptera order and Termitidae family. The commonly available and consumed among them are they reproductive individuals, which are also called alates. They are locally known as Aku, Chinge and Olu-ogan or Esusu by the Ibo, Hausa and Yoruba speaking tribes of Nigeria, respectively. The winged reproductive adult termites are available during the onset of rainy season,

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during which they fly off in large numbers from their nest for their 'nuptial flight'. The 'nuptial flight' ends with a pair of male and female alates isolating themselves from others. Then, their wings fall off and they retire to a suitable spot where they establish a new colony. Here, they become the potential king and queen of the new colony (Allotey and Mpuchane, 2003).

During the flight which occurs mainly at early hours of the morning, the alates are harvested by placing a bowl of water under sources of illumination to which they are attracted. The alates trapped in the water are washed, salted to taste and fried for consumption or sale in local markets (Mbah and Elekima, 2007). However, some consumers prefer them raw. The fried termite delicacy has a nutty flavor when cooked. Oil is not usually needed during frying since its body is naturally rich in oil. It is nutritious and a good source of protein for man and animal. Solomon *et al.* (2007) reported that when de-winged reproductive termite (*Macrotermes nigeriensis*)-soybean (*Glyxine Max*) meals blend was used as base diet of *Heterobranches bidorsalis* fingerlings as sole dietary protein source, there were marked increases in body protein and lipid levels, as inclusion level of *Macrotermes nigeriensis* increased. This, they concluded was because the insect is naturally very rich in oil. They further observed that winged reproductive termite has a higher value of lysine, methionine, histidine, etc., than soybean meal (Solomon *et al.*, 2007).

Given the reported rich nutritional content and the increasing consumption of this termite as a delicacy (Igwe *et al.*, 2011a), the present study is aimed at determination of the effects of its intake on plasma markers of liver and kidney functions and hematologic indices of albino rats.

MATERIALS AND METHODS

Processing of *Macrotermes nigeriensis* diet: The alates of *M. nigeriensis* were harvested in the early morning hours of July 2012, from residential buildings in and around Federal University of Technology, Owerri, Nigeria. They were washed, oven-dried at 40°C to a constant weight and ground into coarse pellets of similar sizes with the control diet. And then used with the growers' mash for compounding of the various *M. nigeriensis* based diets. The growers' mash (Grand Cereals and Oil Mills, Nigeria) which are in coarse pellet forms with complete nutrient composition as recommended by the manufacturers and commonly used for feeding of young chicks was used as the control diet.

Animals: Twenty-four male albino rats of mean weight 45.80±6.6 g and about 4 weeks old obtained locally from Nsukka, Enugu State, Nigeria were housed in metal cages. They were fed growers' mash (Grand Cereals and Oil Mills, Nigeria) and supplied water *ad libitum* for two weeks to acclimatize them to laboratory conditions. They were later distributed randomly into 4 groups (A-D) of 6 rats each and housed in 4 different metal cages. The control animals (group A) were fed the growers' mash, while groups B-D were fed 25, 50 and 75% of *Macrotermes nigeriensis* supplemented diets, respectively.

Specimen collection: The experimental feeding period lasted for a total of 28 days. During the period, the rats' body weight changes were measured and recorded every 4 days. At the end of the 14 and 28 days of feeding, 3 randomly selected animals from each of the groups were fasted overnight, anaesthetized with chloroform and 6 mL of blood collected from each animal by cardiac puncture. This was carefully divided into two portions by dispensing into two types of anticoagulant (lithium heparin and EDTA) sample bottles. The lithium heparin anticoagulated sample was mixed, centrifuged and the plasma separated. The plasma obtained was used for the liver and kidney function indices tests. The EDTA anticoagulated sample was carefully mixed and used for the determination of the hematologic indices.

Liver function indices determination: Alanine aminotransferase (ALT) and aspartate amniotransferase (AST) activities as well as total bilirubin, total protein and albumin concentrations were determined as described by Tietz (1987), using kits supplied by Randox Industries Ltd. (UK).

Kidney function indices determination: Plasma urea and creatinine were determined by urease enzymatic and Jaffe's reaction methods as described by Tietz (1987).

Haematologic Indices determination: The haematological indices involving Packed Cell Volume (PCV), haemoglobin (Hb) concentration, White Blood Cell (WBC) count, platelet counts and the red blood cell count and its indices including Mean Corpuscular VOLUME (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were determined using an automated haematology analyzer machine (Mindray BC 2300, USA). It performs blood cell count by Direct Current detection method, haemoglobin analysis by non-cyanide haemoglobin analysis method and calculates the red blood cell constants automatically from red blood cell count, Hb and PCV.

Statistical Analysis: Data generated were presented as Mean±SD deviations and as simple percentages. They were analyzed by the use of one-way ANOVA and Tukey Posthoc test, with the aid of a computer-based statistical package (Graphpad Prism 5.3). Inferences were made at 95% confidence level.

RESULTS AND DISCUSSION

The changes in body weight of the rats within the experimental period are shown in Figure 1. The control animals (group A) showed a non-significant (p>0.05) weight gain (149.30±16.29 g) than the animals fed the *M. nigeriensis* fortified diets; group B (148.60±18.56 g), group C (140.62±17.78 g) and group D (120.29±19.36 g). The results show that the body weight gain of the animals fed the respective *M. nigeriensis* fortified diets were not significantly (p>0.05) different from the body weight gains in the age-matched control animals. This is in corroboration with our earlier observations that although the termite diet is rich in lipids and fatty acids (Igwe *et al*., 2011a), the consumer might not be prone to excessive and abnormal weight gain, obesity and its related dyslipidaemia (Igwe *et al*., 2011b). Interestingly, the least overall weight gains were observed among the animals fed the 75 and 50% termite fortified diets. This indicates a possible role of the intake of this delicacy in control of food items. A practice common among the indigenous consumers of this delicacy in Nigeria (Igwe *et al*., 2011b) overweight. It also buttresses the advantages accruable from intake of *M. nigeriensis* in combination with other and a fact of functional food research and application (Okoye, 2011).

Table 1 shows the effect of 14 and 28 days feeding of *M. nigeriensis* fortified diets on liver function indices of the albino rats. It shows that the fortified diets in comparison with the control diet increased, although non-significantly (p>0.05), the total protein and albumin concentrations of the animals after 14 days of feeding. However, a more pronounced increase in plasma total protein concentration was observed in the animals fed the termite fortified diet for up to 28 days, especially and significantly (p<0.05) among the animals fed the 25% fortified diet. This may be attributed to the high crude protein and essential amino acids contents of edible termites (Igwe *et al*., 2011a; Adeyeye, 2005; Mbah and Elekima, 2007). However, these observed effects of the 25% fortified diet in comparison with higher doses of the termite diet calls for further research to elucidate any advantage of intake this delicacy at lower amounts. The results also showed that feeding of the animals with the

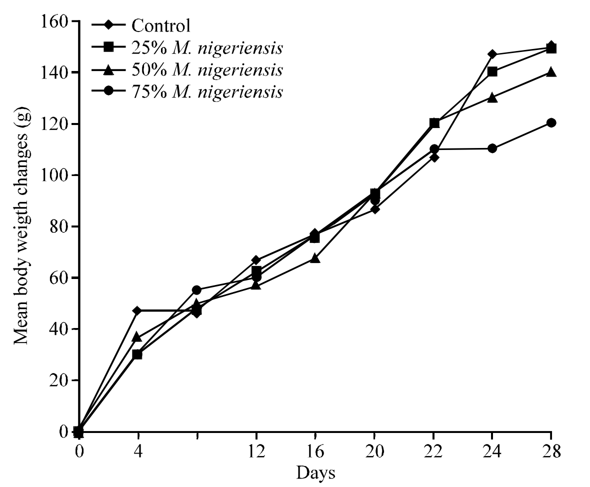

Fig. 1: Mean body weight change (g) of the albino rats fed control diet and *Macrotermes nigeriensis* (MN) fortified diets for 28 days

Table 1: Liver function indices of albino rats fed *Macrotermes nigeriensis* fortified diets

Indices	Group A (Control)	Group B (25% MN)	Group C (50% MN)	Group D (75% MN)
After 14 days feeding				
Total Protein (g L^{-1})	59.37±1.38	65.85±2.84	59.30±7.57	60.70± 5.02
Albumin (g L^{-1})	38.30±5.26	46.45±5.39	45.981±3.26	40.73±1.13
ALT (IU L^{-1})	25.33±1.86	30.00±1.42	27.25±3.08	30.00±2.68
AST (IU L^{-1})	99.33±4.50	101.25±4.30	111.50±12.69	113.25±10.24
Bilirubin (μmol L^{-1})	3.03±1.06	3.90±1.46	3.531.38	3.18±1.74
After 28 days feeding				
Total protein (g L^{-1})	62.10±1.65	75.48±2.14*	61.96±2.23	69.75±7.51*
Albumin (g L^{-1})	42.1±5.38	44.74±5.47	43.18±5.23	42.55±7.80
ALT (IU L^{-1})	23.33±4.51	23.75±3.36	35.50±9.20	35.33±5.41
AST (IU L^{-1})	101.33±4.50	109.75±9.37	111.57±14.56	112.67±12.60
Bilirubin (μmol L^{-1})	3.33±1.30	3.80±1.18	3.63±1.35	3.53±1.82

Values are Mean±Standard deviation; * Values in comparison with the control value per row are statistically significant (p<0.05); n = 3 per feeding sub-period. MN: *Macrotermes nigeriensis*, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase

Table 2: Haematologic indices of albino rats fed *Macrotermes nigeriensis* fortified diets

Indices	Group A (Control)	Group B (25% MN)	Group C (50% MN)	Group D (75% MN)
After 14 days feeding				
PCV (%)	35.40±1.42[a]	39.05±1.50[ab]	41.10±1.96[b]	43.03±2.24[b]
Hb (g dL^{-1})	12.03±0.32[a]	12.78±0.38[ab]	13.48±0.51[b]	13.88±0.63[b]
PLT(10^3 L^{-1})	389.67±96.54	346.75± 82.42	417.00±77.68	481.25±38.3
WBC (10^3 L^{-1})	4.40±1.47	5.85±1.80	4.78±0.91	4.30±1.61
RBC (10^3 L^{-1})	6.49±0.42[a]	6.71±0.27[a]	7.11±0.28[ab]	7.61±0.24[b]
MCV (fl)	54.63±2.25	58.28±2.97	57.80± 1.12	56.50±1.68
MCH (pg)	18.60±0.70	19.05±0.41	18.98± 0.21	19.23±0.54
MCHC (g dL^{-1})	34.00±0.90	33.75±1.16	34.80± 0.36	34.28±0.72
After 28 days feeding				
PCV (%)	39.27±3.67	38.98±2.14	36.88±3.04	41.10±2.59
Hb (g dL^{-1})	12.73±1.11	13.33±0.72	12.55±0.74	12.80±1.31
PLT(10^3 L^{-1})	392.67±81.80	227.50± 51.42	281.00±56.57	303.00±33.73
WBC (10^3 L^{-1})	5.10±0.61	4.98±1.39	4.63±1.17	5.70±3.30
RBC (10^3 L^{-1})	7.07±0.44	7.19±0.45	6.69±0.63	7.11±0.58
MCV (fl)	55.50± 1.91	57.53±2.70	55.20±0.70	57.85±2.69
MCH (pg)	18.00±0.87	19.68±0.86	18.83±0.72	18.95±0.71
MCHC (g dL^{-1})	32.29±1.29	34.20±0.18	34.07± 0.87	35.10±1.80

Values are mean±standard deviation; Values with different superscripts per row are statistically significant ($p<0.05$), n = 3 rats per feeding sub-period, MN: *Macrotermes nigeriensis*, PCV: Packed cell volume Hb: haemoglobin, PLT: platelet count, WBC: White blood cell count, RBC: Red blood cell count, MCV: Mean cell volume, MCH: Mean cell haemoglobin, MCHC: Mean cell haemoglobin concentration

fortified diets for up to 14 and 28 days respectively did not significantly ($p>0.05$) alter plasma total bilirubin concentrations as well as alanine and aspartate aminotransferases (ALT and AST) activities. These liver function biomarkers are usually localized within the hepatocytes. Persistent elevation in the concentration of bilirubin and activities of ALT and AST in the blood indicate continuing destruction of liver cells (Igwe *et al*., 2011c). Thus, the results of this study indicate that *M. nigeriensis* diet fortification does not have any detrimental effect on hepatocellular function. Rather, it may enhance effective synthetic functional capacity of the liver cells as indicated by increased total protein and albumin concentrations of the animals fed the diet as well as its previously reported significant positive effect on HDL-cholesterol concentration (Igwe *et al*., 2011b).

Figures 2 and 3 show the effects of 14 and 28 days feeding of *M. nigeriensis* fortified diet on plasma urea and creatinine concentrations of albino rats. They show that the diets did not significantly ($p>0.05$) affect the plasma concentrations of urea and creatinine, two commonly used biomarkers of kidney function. The marginal but non-significant ($p>0.05$) effect noted in the plasma urea concentration upon feeding with each of the fortified diets for 14 days was adequately counter balanced with continued fortified diets feeding for up to 28 days.

The effects of the 14 and 28 days feeding of the albino rats with *M. nigeriensis* fortified diets on haematologic indices are shown in Table 2. The results that the fortified diets elicited dose-dependent significant ($p<0.05$) increases in Packed Cell Volume (PCV), haemoglobin (Hb) concentration and Red Blood Cell (RBC) counts of the animals fed the diets for 14 days in comparison with the control animals. The results also show that platelet and white blood cell counts as well as the calculated red cell indices, namely Mean Cell Volume

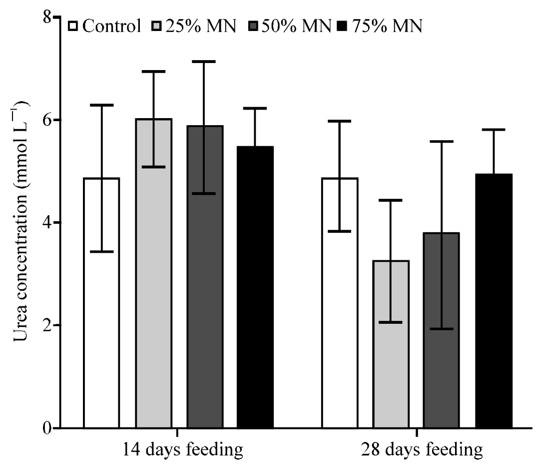

Fig. 2: Effect of 14 and 28 days of administration of *Macrotermes nigeriensis* (MN) fortified diets on plasma urea (mmol L^{-1}) concentration of albino rats

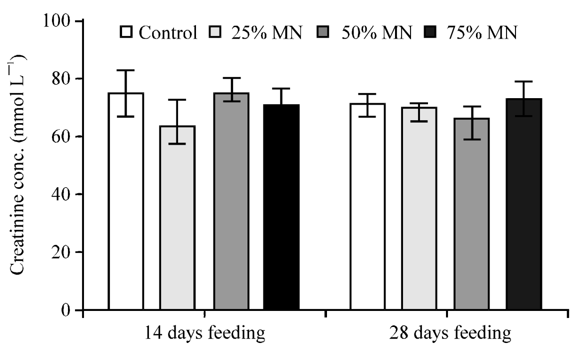

Fig. 3: Effect of 14 and 28 days of administration of *Macrotermes nigeriensis* (MN) fortified diets on plasma creatinine (mmol/l) concentration of albino rats

(MCV), Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC) were not significantly ($p>0.05$) increased by feeding with the fortified diets. However, continued feeding with the fortified diets for up to 28 days non-significantly ($p>0.05$) maintained PCV, Hb, RBC and the red cell indices at positively higher levels than their corresponding control animal values. Packed cell volume is a measure of the volume of blood consisting of solid cells, Hb is the oxygen-carrying macromolecule of the blood, while the red cell indices are used to mathematically define the concentration of Hb within the cell (Igwe *et al*., 2011a). Deducing from the above, it may be stated that the fortified diets have the potential to effectively enhance oxygen-transporting capacity of the blood. This could be attributed to the reported high iron (9.56 mg kg^{-1} dry weight) and vitamin C (17.76±1.60 mg 100 g^{-1}) contents of *M. nigeriensis* (Igwe *et al*., 2011b). Among several other functions, iron is an essential co-factor in haemoglobin, while vitamin C maintains blood vessels flexibility and improves blood circulation in the arteries.

In conclusion, intake of *M. nigeriensis* fortified diet had appreciable positive effect on blood total protein, albumin, PCV, Hb, RBC and red cell indices. Thus, it does not have detrimental effects on liver and kidney cellular functions as well as on the haematologic indices of albino rats. Considering the above points, fortification of human and animal food with *M. nigeriensis* is recommended, especially in weaning diet of growing children and nursing mothers during this period of acute food insecurity and global economic meltdown.

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