

Review Article

Consumption of Macrotermes Subhyalinus: Effects on the Physiological Parameters of Wistar Strain Rats

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Abstract

Protein deficiency is a public health problem in developing countries. The search for available and cheaper animal proteins is recommended as a solution. The objective of this study is to promote winged *Macrotermes subhyalinus* termites as a source of protein to contribute to the resolution of protein-energy malnutrition. The overall composition of these termites was first determined, followed by an animal experiment conducted on 12 growing Wistar rats, with soy as a control feed. Two isoenergetic and isoproteic diets were formulated (a control diet containing fish meal (RFP), and a diet containing dried termite flour (RFT)). The experiment evaluated growth parameters, blood biochemistry, and organ biometrics of the animals. The results for the overall composition showed $97 \pm 0.35\%$ dry matter, $30.187 \pm 0.6\%$ protein, $56.89 \pm 2.1\%$ fat, $9.92 \pm 1.38\%$ of total carbohydrates with an energy content of 672.44 ± 0.15 Kcal/100 g of dry matter. Regarding biochemical blood parameters and organ biometrics, the comparative analysis revealed similar values in rats subjected to both diets, except for total cholesterol and HDL cholesterol levels. These termites are likely to provide essential nutrients to humans in their diets.

Keywords

Termites, Protein, Nutrient and Diet

1. Introduction

Malnutrition affects individuals at all stages of life, from conception to old age, including childhood, adolescence, and adulthood. It can be due to a deficiency in macronutrients (carbohydrates, fats, or proteins) or micronutrients [1]. Malnutrition due to deficits in proteins, energy, or micronutrients continues to be a public health problem in developing countries. The grains and tubers available to a large portion of the population are relatively low in protein [2]. In Côte d'Ivoire, the main sources of protein in the diets of the population are

animal-based proteins, particularly fish and beef. These products, often imported from outside the country for consumption, are commonly subject to price inflation. The inaccessibility of these foods. Thus, the use of insects such as termites and caterpillars presents itself as an alternative and complementary source to meet the protein nutritional needs of populations. Insects are nutritious, rich in proteins, fats, and minerals [3]. The nutritional quality of insects and the possibility of producing them at a low cost could be a solu-

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Received: 28 July 2025; Accepted: 8 August 2025; Published: 3 September 2025



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tion to address many protein deficiency problems [4]. Termites are consumed raw or fried, usually as part of a complete meal or accompanied by tapioca, bread, or roasted corn. *Macrotermes subhyalinus*, simply called "Termite," is an important part of the food culture in Côte d'Ivoire. The consumption of *Macrotermes subhyalinus* could be greatly improved if it were introduced into human diets using drying methods and modern conservation. The general objective of this work is the food valorization of insects, specifically those of *Macrotermes subhyalinus* in Côte d'Ivoire.

2. Material and Methods

2.1. Material

2.1.1. Animal Material

The animal material was made up of adult winged edible termites of the species *Macrotermes subhyalinus* (Figure 1). These *Macrotermes subhyalinus* termites were raised in their natural environment and were captured during the swarming period on the campus of Felix Houphouët Boigny University in Cocody (Abidjan, Ivory Coast). This material also consisted of 12 young Wistar strain rats aged 40 to 53 days, weighing on average 54 to 55 g. These rats were raised in the VIVARIUM of the Ecole Normale Supérieure (E.N.S) of Abidjan, Ivory Coast (Figure 1 and Figure 2).

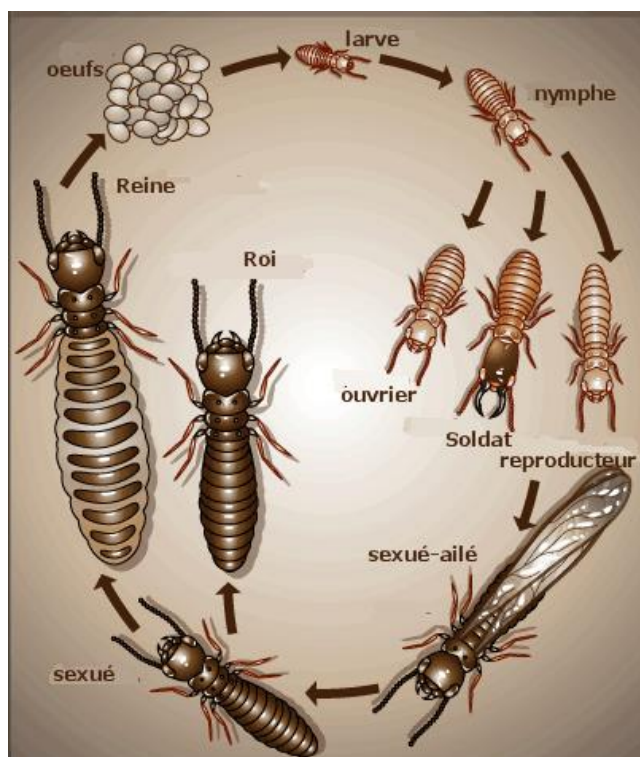


Figure 1. Reproduction cycle of termites.



Figure 2. Rat (*Rattus Norvegicus*) de souche Wistar.

2.1.2. Food Material

Various ingredients were used in the preparation of the different diets for the rats. Corn starch "ma žana" purchased from supermarkets in Abidjan and sugar were the main sources of carbohydrates. Sunflower oil (Lesieur) was used in the formulation of diets for its lipid and essential fatty acid intake. Dried herring fish, bought at the Adjamé market in Abidjan, was used as a reference protein source. Vitamin and mineral supplements were used to balance the diets (total amino acids).

2.2. Methods

2.2.1. Preparation of *Macrotermes Subhyalinus*

After their arrival at the Laboratory, the termites were rinsed three times in tap water and then placed on aluminum foil in an oven at 62 °C, to avoid denaturing the proteins, for 72 hours to dry. After drying, they were sorted and cleaned of all kinds of debris, then ground using a NASCO brand blender (BL1008AK-CB) to obtain the meal. The flour was stored in a jar and kept in the refrigerator at 27 °C until the experiment began.

2.2.2. Determination of the Chemical Composition of Termites

The determination of moisture and dry matter, protein content, fat content, ash content, and sesame seeds was carried out according to the methods described by AOAC [5]. The total carbohydrate content was calculated by difference. The energy value was calculated according to method the FAO [6].

2.2.3. Animal Experimentation

Animal experimentation was conducted according to the method of [7]. It includes two phases: a growth experiment that lasted 12 days and nitrogen balance assessments that covered the last five days. The experimental period was preceded by three (03) days of adaptation during which the rats were fed with the standard food provided by the FACI company.

2.2.4. Animals and Housing

This experience was conducted with 12 animals due to six (06) rats per diet, individually housed based on their more or

less homogeneous weights in metabolism cages during the experimental period. Located at the Vivarium of the Ecole Normale Supérieure of Abidjan (ENS), these cages were arranged in a room adapted to ambient temperature conditions (26 °C) and relative humidity (estimated hygrometry between 70% and 80%) and illuminated $\pm$ 12 hours/day. The rats were raised in metal cages, lined with a bedding made of wood shavings. Additionally, their cages were equipped with feeders and water bottles to facilitate free access to water and food.

2.2.5. Dietary Regimes

Two iso-caloric (4000 kcal/kg of dry matter) and iso-protein (10%, or 100 g/kg of dry matter) diets were prepared:

1. A control diet containing fish meal (RFP)
2. A diet containing dried termite meal (RFT)

Table 1. Composition of the different dietary regimes.

Ingredients	RFP (g)	RFT (g)
Fish meal	16,315	-
Termite meal	-	33,167
Minerals	0,1	0,1
Vitamin	0,1	0,1
sugar	5	5
Maize starch	78,56	61,733
Sunflower oil	0,025	0
Protein	10	10
Grass energy (kcal/kg M. S)	4000	4000

The grass energy of diets is calculated by referring to the combustion values of the different nutrients based on 4 kcal for 1 g of protein, 4 kcal for 1 g of carbohydrate, and 9 kcal for 1 g of lipid. RFP: fish meal diet, RFT: termite meal diet,

2.2.6. Feeding and Growth Measurement

The diets were provided ad libitum once a day in the form of a puree for 12 days to avoid food waste. Every noon, the leftover food from all the rats is collected and weighed, which allows us to determine the fresh weight ingested by the animal. After weighing, the food was placed in the oven at 60 °C for 24 hours. As for water, it was provided freely and changed every three (3) days. The animals were weighed at the start of the experiment and then at three-day intervals. The last weighing took place at the end of the experiment.

2.2.7. Blood Sampling and Measurement of Blood Biochemical Parameters

(i). Blood Collection

At the end of the animal experiment, the 12 rats were anesthetized with chloroform and then sacrificed. Blood was collected in dry tubes immersed in an ice bath. These samples were transferred to the University Hospital of Cocody, Abidjan, Ivory Coast, where they underwent centrifugation at 3000 RPM for three (3) minutes in a CWS 4236 centrifuge (Italy) to collect the serum. The serum was then collected in Eppendorf tubes and stored in a cold room at -20 °C before analysis. The measurement was performed at the Laboratory of the University Hospital Centre (CHU) of Cocody, Abidjan, Ivory Coast.

(ii). Organ Removal and Determination of the Relative Weight of Organs

After scarification of the rats, organs such as the spleen, liver, kidneys, abdominal fat and heart of each rat were quickly removed, dehumidified, and then weighed. These organs were weighed and their masses reduced to the percentage of body mass. These organs after weighing were kept in boxes containing formalin diluted to 10th.

(iii). Statistical Analysis of the Data

The data, expressed as mean $\pm$ standard deviation, were analyzed using analysis of variance (ANOVA) with software, GraphPad Prism 8.4.3 (686). The statistical analysis of the differences between means was carried out using the Newman-Keuls test. Differences were considered significant if $p > 0.05$. Graphs and histograms were created using GraphPad Prism 8.4.3 (686).

(iv). Ethical Consideration

Rats were treated according to good laboratory practices 15. The experimental protocols were conducted by the protocols for the protection of experimental animals of the European Council on Legislation 2012/70716.

3. Results and Discussion

3.1. Results

3.1.1. Overall Composition of Macrotermes Subhyalinus

After a biochemical analysis of the dried *Macrotermes subhyalinus* flour, for a quantity of 100 g, it is composed of a moisture content of 2.8 ± 0.35 (g/100gDM) and 97 ± 0.35 (g/100gDM) dry matter (Table 3). This same quantity of dry matter consists of 30.187 ± 0.6 (g/100gDM) of protein, 56.89 ± 2.1 (g/100gDM) of lipid. (Fat), 2.66 ± 0.12 (g/100gDM) of Ash, 9.92 ± 1.38 (g/100gDM) of Total Carbohydrates.

3.1.2. Growth Parameters

(i). Total Protein Intake (TPI)

The total protein intake of rats on a fish diet is 0.80 ± 0.04 g/day while that of rats fed with a termite diet is 0.28 ± 0.03 g/day (Table 4). The statistical analysis of total protein ingested per day by the experimental rats reveals that those on the termite diet are significantly lower ($p < 0.05$) than those on the fish control diet.

(ii). Body Weight Gain

The body weight gain of rats that consumed the fish meal diet (RFP) (3.11 ± 0.20) is significantly higher than that of rats on the termite meal diet (RFT) (-0.63 ± 0.06) ($P \leq 0.05$).

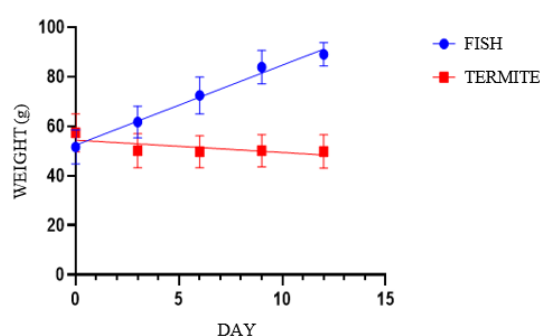


Figure 3. Body weight gain of rats subjected to the two diets.

Table 2. Overall composition of the termite *Macrotermes subhyalinus*.

Parameter	content
Moisture (g/100gFM)	2.8 ± 0.35
Dry matter (g/100gFM)	97 ± 0.35
Protein (g/100gDM)	30.187 ± 0.6
Lipid (g/100gDM)	56.89 ± 2.1
Ash (g/100gDM)	2.66 ± 0.12
Total carbohydrates (g/100gDM)	9.92 ± 1.38
Energy (Kcal / 100gDM)	672.44 ± 0.15

Each value is the mean $\pm$ standard deviation of 3 repetitions FM: Fresh Material DM: Dry Material

Table 3. Consumption parameters.

Diets		
Parameter	RFP	RFT
	(n=6)	(n=6)

Diets		
Parameter	RFP	RFT
PTI (g/j)	0.80 ± 0.04^b	0.28 ± 0.03^a

n: Number of rats per treatment. a, b on the same line means the means followed by different letters are significantly different ($p \leq 0.05$). RFP: fish-based diets; RFT: caterpillar-based diet; PTI: total protein ingested.

3.1.3. Blood Biochemical Parameters and Organ Biometrics

(i). Effects of Different Diets on the Average Value of Blood Biochemical Parameters

The results of serum metabolite assays show that urine, HDL, and triglycerides are not statistically different ($P > 0.05$) in all rats fed with the two diets (RFT and RFT). The levels of LDL and blood glucose are higher ($p \leq 0.05$) in rats subjected to the fish diet RFP compared to those on the RFT diet, and also higher in RFP rats for proteins and total cholesterol (g/L), with respective values of (64.92 ± 2.92 and 0.99 ± 0.01) (Table 4).

(ii). Effects of Different Diets on Organ Biometrics

The results indicate that the average heart weight of rats consuming both formulated diets RFP and RFT (0.43 ± 0.03 and 0.55 ± 0.05 respectively) show a significant difference ($p \leq 0.05$). The average liver weights of rats fed with RFP and RFT diets show no significant difference (3.73 ± 0.40 and 3.98 ± 0.50 respectively) ($p > 0.05$). The statistical analysis of the average kidney weights of rats subjected to RFP (0.69 ± 0.04) is statistically lower than those of rats subjected to RFT (0.79 ± 0.01). There is a significant difference ($p \leq 0.05$) between the average spleen weights of rats subjected to the two diets RFP and RFC (0.35 ± 0.01 and 0.27 ± 0.03 respectively), (Table 4). The weight of abdominal fat in rats consuming the RFT diet is statistically lower ($p > 0.05$) than that of rats consuming the RFP diet, with RFT and RFP having respective values of (0.18 ± 0.00 and 0.54 ± 0.03).

Table 4. Biochemical parameters of rats after consuming the two diets.

PARAMETER	RFP	RFT
	(n=6)	(n=6)
Blood glucose (g/L)	11.32 ± 0.50^a	11.36 ± 0.40^a
Triglycerides (g/L)	0.73 ± 0.03^a	0.75 ± 0.02^a
Total cholesterol (g/L)	0.38 ± 0.01^a	0.99 ± 0.01^b

PARAMETER	RFP	RFT
	(n=6)	(n=6)
LDL (g/L)	0,35 ±0,02 ^b	0,17 ±0,02 ^a
HDL (g/L)	0,11 ±0,01 ^a	0,17 ±0,00 ^b
Creatinine (mg/L)	5,5 ±0,1 ^a	6 ±0,2 ^a
Urea (g/L)	0,11 ±0,00 ^a	0,11 ±0,00 ^a
Proteins	57,83 ±1,01 ^a	64,92 ±2,92 ^b

(n = 6): number of rats per treatment. a, b on the same line, means followed by different letters are significantly different ($p \leq 0.05$). LDL: Low Density Lipoprotein. HDL: High Density Lipoprotein.

Table 5. Relative weight of the organs of rats subjected to fish and termite diets.

Diets Organs	RFP	RFT
	(n=6)	(n=6)
Liver	3,73 ±0,40 ^a	3,98 ±0,50 ^a
Kidneys	0,69 ±0,04 ^a	0,79 ±0,01 ^a
Heart	0,43 ±0,03 ^a	0,55 ±0,05 ^a
Spleen	0,35 ±0,01 ^a	0,27 ±0,03 ^a
Abdominal fats	0,54 ±0,03 ^b	0,18 ±0,00 ^a

(n = 6): number of rats per treatment a, b on the same line, means followed by different letters are significantly different ($p \leq 0.05$)

3.2. Discussion

Analysis of growth parameters and digestive balance was conducted with the aim of measuring the nutritional impact of the diet based on herring fish and termites of the species *Macrotermes subhyalinus*. Following this analysis, the overall composition of the edible termite flour of the species *Macrotermes subhyalinus* given in (Table 2), we notice that it contains a very important nutritional source. A dry matter content of (97 ± 0.35) (g/100gDM) is noted, which is lower than that found by [8] on the same species (92 ± 1.5). This difference could be explained by the roasting method employed. This dry matter content allows for good physical preservation of the termites, thus preventing their decomposition [9]. The flour of *Macrotermes subhyalinus* is an important source of protein (30.187 ± 0.6 g/100gDM) and fat (56.89 ± 2.1 g/100gDM). The fat content obtained is higher than that collected by [8] (46.3 ± 1.0 g/100g fat) on the same species. The protein content of the termites *Macrotermes subhyalinus* is similar to that of the larva of *Oryctes rhinoc-*

eros (30.15%) but higher than the protein content for *Rhynchophorus phoenicis* (22.06%) obtained by [10]. The essential function of a food protein is to meet the body's needs for nitrogen and essential amino acids. It follows from this study that the termites *Macrotermes subhyalinus* rank among the most protein-rich foods [11]. Given these results, it was therefore appropriate to assess the impact that these termites could have on the growth of Wistar rats during an animal experiment. At the end of the experiment, the animals that consumed the fish diet (RFP) experienced a weight gain, while the animals fed with the termite diet (RFT) suffered a weight loss. This growth is manifested through the daily weight gains observed in the animals. The negative effect, characterized by weight loss, was also observed by [12], who fed rats with snail meat flour, which agrees with those of [13], who also identified the same types of termites used in poultry feeding. Despite the considerable amount of the protein source and In terms of fat content in the termites *Macrotermes subhyalinus*, this did not allow the rats to gain weight; on the contrary, they lost weight during the experimentation. These poor performances were also observed in the work of [14], who fed Wistar rats with a diet based solely on snail meat as the only source of protein. The weight loss of rats subjected to the RFT could be explained, on one hand, by the amount of fat present in the termite. According to [15], dietary lipids can, in fact, have a direct impact on the use of proteins. The effect of the grillage can also have negative effects on protein digestibility. [16] explain that severe thermal treatment applied to model protein systems containing very few carbohydrates (meat or fish) leads to the formation of isopeptide covalent bonds between lysine and glutamine or asparagine residues. From a nutritional perspective, the formation of these intra- or intermolecular bonds decreases protein digestibility. However, the digestibility of RFT remains similar to that of RFP. Weight loss would therefore not be caused by poor protein digestibility. This difference could be explained by the fact that the different diets were administered without prior cooking, which likely negatively affected the organoleptic properties of the formulated foods. Beyond growth parameters, the effect of termites has also been evaluated on blood biochemical parameters and the biometrics of organs. The results of the analysis of blood biochemical parameters do not show a significant difference between the blood glucose, triglycerides, creatinine, and urea (g/l) of rats subjected to a fish diet and those of rats subjected to a termite diet. These results are inconsistent with those obtained by [17] after a 28-day treatment with cadmium on rats. On the other hand, the protein content, HDL, and total cholesterol are significantly higher in rats subjected to the termite diet. The analysis revealed that the LDL cholesterol content and blood glucose (g/L) of rats on the RFP are higher than that of rats fed with RFC. These results are similar to those obtained by (2023) [18] during studies on the consumption of sesame seeds in Wistar rats. The triglyceride levels observed are close to the reported norms. Regarding

creatinine, the low levels recorded may be due to insufficient weight gain in the rats. At the organ level, the rats from both diets had equivalent relative weights. The results obtained were consistent with those reported by [19] and [18] on the nutritional quality of sesame. Termites are a rich food source that can be incorporated into cereals and legumes to reduce protein-energy malnutrition. A significant difference in abdominal fat is observed in rats subjected to the termite diet compared to the fish diet. The low level of abdominal fat observed in rats on the termite diet would be due to low food intake combined with an energy intake deficit according to [20].

4. Conclusion

In order to effectively address the growing problem of malnutrition in the world each year, this work was undertaken to valorize winged *Macrotermes subhyalinus* termites. This valorization made it possible to determine the overall composition of the termites. Indeed, it has a very low water content of 2.8% and is very rich in fat at 56.89% and in protein at 30.187%. The consumption of termites caused weight loss in rats. However, the termite has good digestibility comparable to that of a fish diet. In terms of blood biochemical parameters, the effect of termites on urea, creatinine, and triglycerides shows it is a good alternative to common animal proteins, although it is preferable to consume them in moderation or in deodorized form due to their high fat content. To benefit from the nutritional potential of termites, more in-depth research is needed necessary.

Abbreviations

RFP Fish-based Diets
RFT Termite Meal Diet

Conflicts of Interest

The authors declare no conflicts of interest.

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