Journal of Food and Nutrition Research, 2023, Vol. 11, No. 11, 691-699
Available online at http://pubs.sciepub.com/jfnr/11/11/5
Published by Science and Education Publishing
DOI:10.12691/jfnr-11-11-5

Effects of Incorporating *Protomacronema* sp. Insect Meal Into the Standard Diet on Dietary, Biochemical and Physiological Parameters in Rats

Itoua Onianguet Assoba Cassiel[1,4], Elenga Michel[1,2,3,4,*], Wossolo Stephane[5], Mabossy-Mobouna Germain[1,4], Mananga Vital[1,4]

[1]Laboratoire de Nutrition et d'Alimentation Humaines BP: 69, Faculté des Sciences et Techniques, Université Marien NGOUABI, Brazzaville, Congo
[2]Laboratoire de Technologie Agro-alimentaire: Cité Scientifique ex ORSTOM, Brazzaville, Congo.
[3]Institut National de Recherche en Sciences de l'Ingénieur, Innovation et Technologie (INRSIIT): Cité Scientifique ex ORSTOM, Brazzaville, Congo
[4]Département des Formations Doctorales, Faculté des Sciences et Techniques, Université Marien NGOUABI, Congo
[5]Laboratory of Pharmacodynamics and Experimental Physiopathology (L2PE)
*Corresponding author: elengamichel@yahoo.fr

Received October 15, 2023; Revised November 17, 2023; Accepted November 24, 2023

Abstract The aim of this study was to evaluate the effect of incorporating *Protomacronema* sp flour into the standard diet on nutritional and physiological parameters, based on an animal experiment in young growing rats. The animals were weighed in batches according to diet at the start of the experiment, then at two-day intervals for 16 days. The parameters determined were weight evolution, total dry matter ingested (TDMI), weight gain, feed efficiency ratio (FE), feed conversion ratio (FCR), organ weight after dissection and biochemical parameters. The results show that rats fed insect meal show greater growth than rats fed the control diet. The 15% percentage favored growth more, with a weight gain (78.33 ±11.84 g) than that of rats fed the 5, 10 and 20% flour. The highest level of consumption was observed in rats fed 15% Protomacronema sp flour (41.43 ± 3.11). The incorporation of Protomacronema sp flour led to an improvement in the CEA values of the treated diets (0.04 ± 0.05 to 0.06 ± 0.05) compared to that of the control diet (0.03 ± 0.03). Kidney, lung and heart weights were virtually identical between treated and control diets. In contrast, statistical analysis of the data showed a significant difference ($p< 0.05$) between spleen and lung weights. Total protein levels increased in treated animals by 5% (5.07±1.77), 10% (5.26±1.90), 20% (5.77±2.22) and 15% (7.01±3.22) compared with the control (4.83±1.63). HDL levels increased in treated rats, with values of 43.08±23.73; 43.78±24.12; 46.67±25.82 and 54.44±30.45 respectively for the 5%, 10%, 15% and 20% batches, compared with the control (32.13±17.44). Plasma calcium levels increased non-significantly in rats treated with insect meal at 5% (5.17 ±1.84), 10% (7.37 ± 3.27), 15% (8.13 ± 3.76) and 20% (8.46 ± 3.74) versus control (4.43 ±1.42). In addition, plasma iron levels rose in dishes treated at 5% (0.75±0.72), 15% (1.71± 0.52) and 20% (2.63 ± 0.52). These results seem appropriate in the fight against malnutrition in the context of available food resources.

Keywords: *Protomacronema* sp, *diet, formulation, nutritional and physiological parameters, Rat*

Cite This Article: Itoua Onianguet Assoba Cassiel, Elenga Michel, Wossolo Stephane, Mabossy-Mobouna Germain, and Mananga Vital, "Effects of Incorporating Protomacronema sp Insect Meal Into the Standard Diet on Dietary, Biochemical and Physiological Parameters in Rats." Journal of Food and Nutrition Research, vol. 11, no. 11 (2023): 691-699. doi: 10.12691/jfnr-11-11-5.

1. Introduction

Edible insects generally have a high nutritional value in protein, lipids, mineral elements as well as vitamins [1,2]. They have a good nutritional value and are generally excellent sources of protein with an average content of 60 g/100g dry matter [3]. This content is higher than that of certain foods of animal (fish) and vegetable (bean) origin [4,5]. In most insects, this protein content is coupled with a richness in essential amino acids whose content is higher than that of the FAO reference protein [6,7]. They are therefore an alternative source of protein to exploit for human food or animal. Insects are also rich in lipids and have the particularity of being rich in essential fatty acids, mainly polyunsaturated fatty acids of the n-3 series, playing an important role in the prevention of metabolic diseases [8,9]. Insect consumption can contribute to a healthy diet and be used for preventive nutrition or in times of shortage, as they are rich in energy and good-quality protein. The majority of edible insects are good sources of minerals such as copper, iron, magnesium, manganese, phosphorus, selenium and zinc [7]. But they

possess insufficient amounts of calcium and potassium [3,10]. Insects generally contain more iron, zinc and calcium than beef, pork and chicken [7]. The vitamin and mineral contents of edible arthropods, vary greatly between species and orders. However, they can be controlled by their diet [7]. Most insects are a good source of riboflavin (vitamin B2), pantothenic acid (vitamin B 5), biotin (vitamin B8), cobalamin (vitamin B12) and some insect species are also rich in vitamin E and choline [2]. According to the United Nations (UN), global demographics are increasing exponentially. It could reach nearly 10 billion people on Earth by 2050 [11]. To feed this population, we need to find new means of production. Some authors propose using insects as ingredients in the manufacture of certain food products. For example, insect meal can be used to make cookies and sauces [12,13]. Their proteins can be used in the preparation of certain food emulsions, gels and foams. These properties can help control the texture of formulated foods by forming gels and stabilizing foams and emulsions. Despite Congo's enormous food resources, malnutrition is still a major problem among children [14]. The consumption of insects would be an alternative to solve this problem. Because of the consumption of Protomacronema sp by the Congolese population, it is necessary to know their contribution to the well-being of this population. The aim of this study is therefore to assess the effect of incorporating insect meal on nutritional and physiological parameters, based on an experiment with young growing rats.

2. Materials and Methods

Materials Food material

The food material used consisted of Protomacronemas sp, an insect consumed in Congo.

Animal material

The animal material consisted of wistar rats (males and females) weighing between 100 and 130g, bred at the animal house of the Faculty of Science and Technology of the Université Marien Ngouabi under standard conditions (25 ± 5°C), where they were fed and given water regularly.

Methods Sourcing of food material

The insects used were purchased from the Total market, (Bacongo) Brazzaville. Fresh insects were weighed and oven-dried at 70°C until the mass was stabilized. The dry samples obtained were ground using a wooden mortar to obtain insect flour. The various feed ingredients and their proportions used in the preparation of the control and treated feeds are listed in Table 1.

Animal batches and experimentation

Fifteen (15) rats of wistar strain were used at a rate of 3 rats per diet on the basis of their weight in cages arranged in a room set up under ambient conditions (25°C). Animal experimentation was carried out according to the model [15]. It took place over a period of sixteen (16) days, including a two-day adaptation period during which the animals were fed standard feed. There were two phases: a growth phase lasting 14 days, and a nitrogen balance phase during the last 5 days of the growth phase.

Conduct of experiment and measurements

Food was distributed once a day (mornings) in freeze-dried form. The animals were weighed at the start of the experiment and then at two-day intervals. The last weighing took place at the end of the experiment. Water was served ad libitum and renewed. Growth was determined by the difference between initial and final weights. The difference between the quantities of feed served and the remainder, as well as losses, referred to dry matter, were used to determine the quantity consumed.

Expression des paramètres d'étude de la valeur nutritionnelle des régimes alimentaires [16,17]

Weight gain (GP)

Weight gain, expressed in g, represents the difference between the final weight and the initial weight of the animals. To obtain weight gain in g/d, the calculated value in g is divided by the duration of the experiment in days.

GP (g) = Final weight - initial weight

$$\mathrm{GP}\ (\mathrm{g/j}) = \frac{\text{Poids final} - \text{poids initial}}{\text{number of days}}$$

Total dry matter ingested (TDMI)

The quantity of TDSM (g) represents the total quantity of feed ingested in the form of dry matter by the animal over the duration of the experiment.

Its expression in g/d is obtained by dividing the quantity of MSTI (g) by the duration of the experiment in days.

MSTI (g): sum of the quantities of dry matter (from the feed) ingested during the experimental period.

MSTI (g/d): sum of dry matter (feed) ingested during the experimental period / number of days.

$$\mathrm{MSTI}(\mathrm{g/j}) = \frac{\text{sum of dry matter ingested during the experiment}}{\text{number of days}}$$

Feed Efficiency Coefficient (FEC)

The CEA is calculated as the ratio between weight gain (g) and the quantity of MSTI (g). It reflects the efficiency with which the feed is assimilated.

$$\mathrm{FEC} = \frac{\text{Weight gain}(\mathrm{g})}{\text{Total dry matter ingested}(\mathrm{g})}$$

Feed conversion ratio

The feed conversion ratio indicates the quantity of feed consumed to produce 1 kg of meat mass. It is determined by the following formula.

$$IC = \frac{\text{Quantity of feed in kg of dry matter}(\mathrm{DM})}{\text{Final live weight} - \text{Initial live weight}}$$

Dissection and blood sampling

Rats were fasted for 12 hours. The rats were anesthetized with ether, and blood was collected from the retrobulbar sinus using a sterile pasteur pipette, then divided into a dry tube and another containing the anticoagulant ethylene diamine tetra acetic acid (EDTA). The samples were then taken to the biomedical laboratory for biochemical analysis. After 14 days of treatment, the animals were sacrificed by decapitation in a room annexed to the animal house, and the following elements were removed and weighed: kidneys, liver, lungs, heart and spleen.

Table 1. Food ingredients used in the preparation of the different diets

Ingredients	Control diet (g)	Treaty 1 diet (5% insect or caterpillar meal)	Treaty 2 Diet (10% insect or caterpillar meal)	Treaty 3 diet (15% insect or caterpillar meal)	Treaty 4 Diet (20% insect or caterpillar meal)
Corn flour (g)	100	95	90	85	80
Wheat flour (g)	100	95	90	85	80
Soy flour (g)	100	95	90	85	80
Milk (g)	80	76	72	68	64
Peanut (g)	30	28,5	27	25,5	24
Spinach (g)	20	19	18	17	16
Bone meal (g)	4	3,8	3,6	3,4	3,2
Insect meal (g)	0	21,7	43,4	65,1	86,8
Table salt (g)	0,4	0,4	0,4	0,4	0,4
Tap water (ml)	150	150	150	150	150

Biochemical parameters

Total cholesterol assay

✽ Principle

The principle is based on an enzymatic colorimetric method. Blood total cholesterol occurs in two forms, free and esterified. Under the action of cholesterol esterase, cholesterol esters are split into free cholesterol and fatty acids. In a subsequent reaction, catalyzed by cholesterol oxidase, free cholesterol is converted to cholest-4-ene-3-one in the presence of oxygen, with the formation of hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed reacts with phenol and 4-aminophenazone to form the red-coloured derivative quinoneimine [18].

$$\text{Cholesterol esters} + \text{H2O} \xrightarrow{\text{Cholesterol esterase}} \text{HDL} + \text{RCOOH cholesterol}$$

$$\text{Cholesterol} + \text{O}_2 \xrightarrow{\text{Cholesterol oxidase}} \text{cholest} - 4 - \text{én} - 3 - \text{one} + \text{H}_2\text{O}_2$$

$$2\text{H}_2\text{O}_2 + 4 - \text{amino} - \text{phenazone} + \text{phénol phénol} \xrightarrow{\text{Peroxydase}} \text{Colorant} + 4\text{H}_2\text{O}$$

✽ Experimental protocol

In a reaction medium consisting of 47 µL of working reagent diluted in 93 µL of distilled water distilled water, 2µL of product to be assayed (standard and sample) were added. After shaking and automatic incubation at 16-25°C for 5 minutes, the measured optical density optical density measured, the absorbance (Abs) of the test material is obtained, compared with the standard using an integrated spectrophotometer at a wavelength of λ= 505 nm. The total cholesterol concentration is calculated as follows:

$$\text{Total cholesterol}(\text{mmol/L}) = \frac{\text{DO sample}}{\text{Standard DO}} x \text{Standard concentration}$$

Triglyceride assay

✿ Principle

The principle is based on the work of [19] and calls for the rapid and complete hydrolysis of triglycerides into glycerol and fatty acids, using a microorganism lipoproteinelipase; the glycerol formed is then converted into glycerol-3-phosphate, then oxidized to dihydroxyacetone-phosphate with the formation of hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed reacts with 4-aminophenazone and 4-chlorophenol to form a red-colored derivative. The intensity of the red color developed is directly proportional to the triglyceride concentration and is measured photometrically.

✽ Experimental protocol

To a volume of 2µL of product to be assayed (standard or sample), is added a reaction medium 120 µL of working reagent diluted in 28 µL of distilled water. After stirring and automatic incubation at room temperature for 2 minutes, the absorbance (Abs) of the sample was measured with a spectrophotometer at a wavelength of 505 nm, in comparison with the standard. Concentration is determined as follows:

$$\textbf{Triglycerides}(\textbf{mmol/L}) = \frac{\textbf{DOsample}}{\textbf{StandardDO}} x$$

Standard concentration

HDL Cholesterol assay

✿ Principle

The principle is based on the direct method for the determination of HDL cholesterol, which relies on The use of dextran sulfate and polyethylene glycol (PEG)-modified enzymes. In the presence of magnesium ion, dextran sulfate selectively forms water-soluble complexes with the various lipoprotein fractions LDL, VLDL and chylomicrons. These complexes are resistant to PEG-modified enzymes. HDL cholesterol concentration is then determined enzymatically using PEG-modified cholesterol esterase and cholesterol oxidase [20].Under the action of cholesterol esterase, cholesterol esters are split into free cholesterol and fatty acids. In a second reaction catalyzed by PEG-modified cholesterol oxidase, cholesterol is converted, in the presence of oxygen, to Δ4-cholestenone with the formation of hydrogen peroxide. Finally, in the presence of peroxidases, the hydrogen peroxide formed reacts with 4-amino-antipyrine and sodium N-(2-hydroxy-

3-sulfopropyl)-3,5-dimethoxyaniline (HSDA) to form a blue-violet derivative.

$$\text{Cholesterol} - \text{HDL esters} + \text{H2O} \xrightarrow{(\text{PEG})-\text{Cholesterase}} \text{Cholesterol HDL} + \text{RCOOH}$$

$$\text{Cholestérol} - \text{HDL} + O_2 \xrightarrow{(\text{PEG})-\text{Cholesterol oxydase}} \Delta^4 - \text{cholestenone} + H_2O_2$$

$$2H_2O_2 + 4-\text{amino}-\text{antipyrine} + \text{HSDA} + H^+ + H_2O \xrightarrow{\text{Peroxydase}} \text{Blue color derivative} + 5\text{H2O}$$

✽ Experimental protocol

To a reaction medium consisting of 150µL of reagent R18 and 50µL of reagent R19, 2.5µL of product to be assayed (standard and sample) were added. After shaking and automatic incubation at 16-25°C for 5 minutes, the optical density was reading of the optical density at the same wavelength of 600 nm gives the absorbance of the product to be assayed in comparison with the standard. HDL concentration is calculated as follows:

$$\text{HDL} - \text{C}(\text{mmol/L}) = \frac{\text{DO sample}}{\text{Standard DO}} x\text{Standard concentration}$$

Glucose determination

✽ Principle

Glucose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide (H2O2). The hydrogen peroxide formed is detected by action with phenolaminophenazon Mix then incubate for 5 min in a water bath and absorbance is measured at 500 nm.

✿ Experimental protocol

In a series of three tubes (blank, calibrator and sample) containing one milliliter of the glucose enzyme solution glucose enzyme solution were added 10 µL of distilled water, 10 µL of calibrator and 10 µL of test serum. The mixture was incubated at 37°C for 5 min. Optical density is measured at 500 nm against the enzymatic solution blank. Glucose concentration is determined using the formula below.

$$\text{Glucose}(\text{g/L}) = \frac{\text{DO sample}}{\text{Standard DO}} x\text{Standard concentration}$$

Total protein assay

✽ Principle

Proteins present in the sample react in an acid medium with pyrogallol red and molybdate (working reagent), forming a colored complex. Color intensity is proportional to the concentration of proteins present in the sample tested.

✿ Experimental protocol

To 1 mL reaction medium containing sodium chloride (0.15 mol/L), sodium hydroxide (0.75 mol/L), sodium potassium tartrate (6 mmol/L), potassium iodide (6 mmol/L) and cupric sulfate (6 mmol/L), is added 0.02 mL serum. After automatic shaking and incubation at room temperature for 10 min, absorbance is read on a spectrophotometer at 550 nm. A protein solution of known titre is used as a standard solution.

Total protein (g/dL) = (Sample OD / Standard OD) × Standard concentration

Creatinine determination

✿ Principle

Creatinine assay is a colorimetric kinetic test based on the [21] method. In an alkaline environment, creatinine forms an orange-yellow complex with picrate. The rate of color formation is proportional to the concentration of creatinine in the sample.

$$\text{Creatinine} + \text{picric acid} \xrightarrow[\text{Alkaline pH}]{} \text{orange} - \text{yellow complex}$$

✿ Experimental protocol

In a series of three tubes (standard, blank and test), each containing 500 µL of reaction medium 100 µL of creatinine standard, 100 µL of distilled water and 100 µL of sample were added. The tubes were shaken, and after 30 seconds a first absorbance A1 of each tube was read at 505 nm against the blank containing the reaction medium. Ninety (90) seconds after the first reading, a second absorbance A2 is read at 505 nm on the spectrophotometer. Creatinine concentration is calculated as follows:

$$\text{Creatinine}(\text{mg/L}) = \frac{\text{DO sample}}{\text{Standard DO}} x\text{Standard concentration}$$

Urea determination

✽ Principle

The technique used for urea determination is the enzymatic method using urease according to the following reaction:

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} (NH_4^+)_2 + CO_2$$

$$NH_4^+ + \text{ateSalycil} + \text{NaClO} \rightarrow \text{Indophenol}$$

Ammonium ions can react with salicylate and sodium di-hypochlorite to give a green-coloured complex. Color intensity is proportional to the concentration of urea present in the sample [22].

✽ Experimental protocol

To a series of three tubes (standard, blank, test) each containing 1000 µL of reaction medium, were added 100 µL of urea standard, 100 µL of distilled water and 100 µL of sample. After automatic shaking for 1 min and incubation at room temperature (25°C), the optical densities of each tube were read with a spectrophotometer

at 340 nm against the blank containing the reaction medium, to determine the urea concentration. The concentration of urea is determined according to the following formula:

$$\text{Urea}\left(\text{mg}/\text{dL}\right)=\frac{\text{DO sample}}{\text{Standard DO}}x\text{Standard concentration}$$

Calcium ion assay

✽ Principle

Calcium in the presence of Arsenazo III [1,8-dihydroxy-3,6-disulpho-2,7-naphthalene-bis(azo)-dibenzenearsonic acid] at neutral pH, produces a blue colored complex, whose color intensity is proportional to the concentration of calcium present in the sample measured at 620 nm [22].

✿ Experimental protocol

To determine the concentration of calcium in urine samples, 1.0 mL of the working solution was mixed with 20 µL of the urine sample in a cuvette. The mixture was then incubated for 3 minutes at 21°C and then the absorbance of each tube was determined by spectrophotometer at 620 nm and the concentration determined as follows:

$$\text{Calcium}\left(\text{mg}/\text{dL}\right)=\frac{\text{DO sample}}{\text{Standard DO}}x\text{Standard concentration}$$

Iron determination

Serum iron was measured by FerroZine colorimetry. At acid pH (citrate pH < 2.0), iron is detached from transferrin. Lipemic samples are clarified by detergent. Ascorbate reduces Fe3+ ions to Fe2+ ions, which then form a colored complex with FerroZine. The intensity of the color developed is directly proportional to the iron concentration and is measured photometrically at 570 nm against a calibration curve entered and stored during instrument calibration.

3. Results

Weight development

The weight development of the rats as a function of time is shown in figure 1. The results show that rats fed the insect meal showed greater growth than rats fed the control food. The 15% percentage favored growth more than that of rats fed the 5, 10 and 20% flour, which showed no significant difference. However, statistical analysis showed a highly significant difference (***P<0.001) with the 5% percentage from D0 to D6.

Total dry matter intake (TDMI), weight gain (g), weight gain (g/d) and feed efficiency coefficient for the different diets.

Concerning total dry matter intake, Table 2 shows that rats fed the control diet had a lower consumption level (30 ± 4.69 g/d) than those fed the test diet. The highest level of consumption was observed in rats fed the insect meal at 15% (41.43± 3.11), followed by 5% (40.12 ± 7.14), then 20% (38.76 ± 5.6) and 10% (35.88 ± 6.93). Overall, the difference was significant (**P<0.01).

In terms of weight gain (g), animals fed the 15% diet showed the best weight gain (78.33 ±11.84g), followed by 20% (67 ± 4.36g), then 5% (65 ± 9.54g), then 10% with a gain of 44.33±31g. The control batch showed a lower weight gain (29 ± 10.53g). Animals fed the test diets (5%, 10%, 15% and 20% %) showed a significant difference (**P<0.01).

With regard to weight gain (g/d) on the different diets, animals fed the 15% diet had a higher weight gain (5.59 ± 0.85 g/d) than those fed the 20%, 5% and 10% diets, as well as the control, with values of 4.78 ± 0.31; 4.64 ± 0.68; 3.16 ± 2.21 and 2.07 ± 0.75 respectively. The difference between treated and control is significant (**P<0.01).

Table 2. Total dry matter intake (TDMI), weight gain (g), weight gain (g/d) and feed efficiency ratio for different diets

Diets	Total dry matter ingested (g/d)	Weight gain (g)	Weight gain (g/d)
Indicator	30± 4.69 **	29 ± 10.53 **	2.07 ± 0.75 **
F. insect (5%)	40.12 ± 7.14 **	65 ± 9.54 **	4.64 ± 0.68 **
F. insect (10%)	35.88 ± 6.93 **	44.33±31 **	3,16 ± 2.21 **
F. insect (15%)	41.43± 3.11 **	78.33 ±11.84 **	5.59 ± 0.85 **
F. insect (20%)	38.76 ± 5.6 **	67 ± 4.36 **	4.78 ± 0.31 **

*: P < 0.05 ; ** : P< 0.01

Feeding efficiency coefficient and consumption index of the different diets.

The results obtained (Table 3) show that the 15% diet had a CEA (0.06 ± 0.05) higher than those of rats fed 20% (0.05± 0.11), 5% (0.04± 0.05) and 10% (0.04 ± 0.03). The lowest CEA values were obtained with animals fed the control diet (0.03± 0.03). The incorporation of insect meal led to an improvement in the CEA values of treated diets (0.04 ± 0.05 to 0.06 ± 0.05) compared to that of the control diet (0.03 ± 0.03). However, the differences between the CEA values of the treated and control diets were not significant ($p \leq 0.05$).

The consumption indices (CI) recorded for the five (5) diets (control, 5%, 10%, 15% and 20%) ranged from 2.52 ± 8.87 to 8.43± 5.87. The highest index was recorded for the 10%-treated diet (8.43± 5.87), followed by the control (6 ± 18.77) and 5% (3.98 ± 8.94). However, the lowest indices were recorded at 15% (2.52 ± 8.87) and 20% (3.54 ± 3.42). The 15% percentage shows the best consumption index (2.52 ± 8.87). The difference is not significant at the 5% level (P > 0.05).

Table 3. The feed efficiency coefficient and consumption index for the different diets

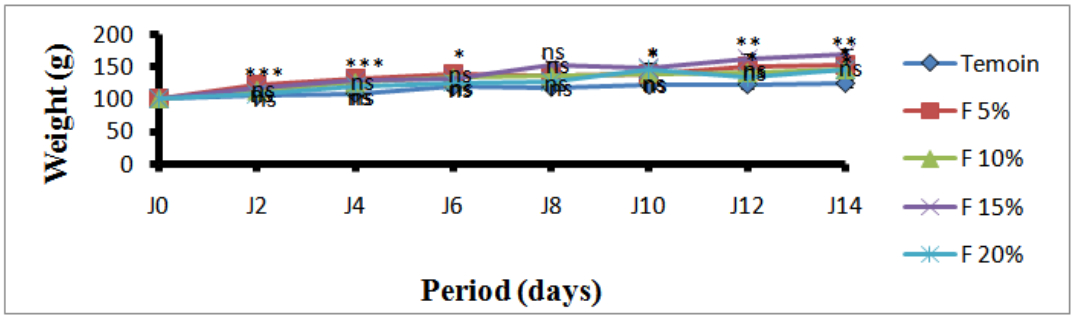
Diets	CEA	IC
Indicator	0.03± 0.03 ns	6 ± 18.77 ns
F. insect (5%)	0.04± 0.05 ns	3.98 ± 8.94 ns
F. insect (10%)	0.04 ± 0.03 ns	8.43± 5.87 ns
F. insect (15%)	0.06 ± 0.05 ns	2.52 ± 8.87 ns
F. insect (20%)	0.05± 0.11 ns	3.54 ± 3.42 ns

F. flour; ns: non-significant difference

Food consumption

The results obtained showed no disturbance in the general behavior of the animals for the dishes tested after 14 days of observation. On the other hand, there was a slight increase in food consumption (Figure 2) of the treated dishes compared with the controls from day 2 to

day 14. This increase in feed intake was reflected in a weight gain (figure 1) in the animals. These variations were significant from day 2 to day 14 of experimentation (P<0.05).



ns : non-significant difference ;* : P<0.05 ; ** : P<0.01 ; *** : P<0.001

Figure 1. Weight evolution as a function of time

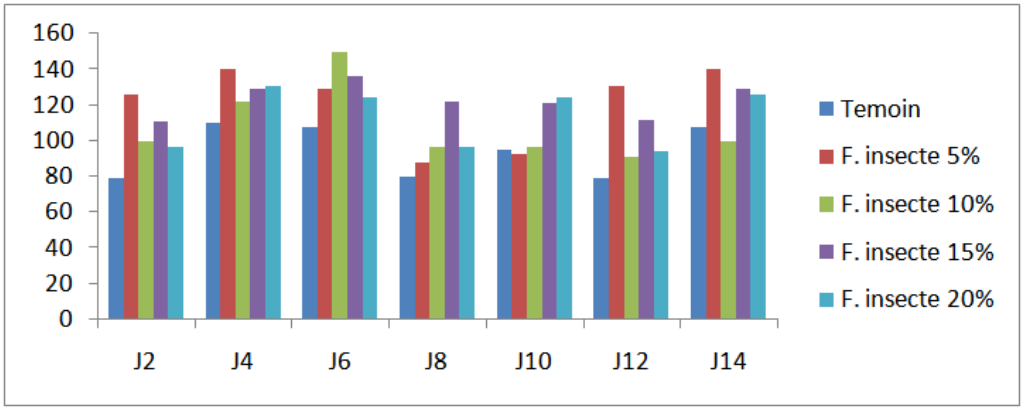


Figure 2. Rat food consumption as a function of time

Table 4. Different organs after dissection in rats

Organs	Rat groups				
	Indicator	Insect f . 5%	Insect f. 10%	Insect f. 15%	Insect f. 20%
Left kidney	0.44 ± 0.90	0.48 ± 0.88 ns	0.45 ± 0.89 ns	0.51 ± 0.86 ns	0.57 ± 0.83 ns
Right kidney	0.62 ± 0.81	0.48 ± 0.88 ns	0.53 ± 0.85 ns	0.53 ± 0.85 ns	0.58 ± 0.82 ns
Liver	4.76 ± 1.59	6.36 ± 2.53 ns	5.08 ± 1.84 ns	5.54 ± 2.17 ns	5.96 ± 2.35 ns
Lung	0.86 ± 0.66	0.98 ± 0.59 ns	0.93 ± 0.62 ns	1.07 ± 0.53 *	1.05 ± 0.55 ns
Heart	0.67 ± 0.77	0.67 ± 0.77 ns	0.73 ± 0.73 ns	0.74 ± 0.73 ns	0.67 ± 0.77 ns
Spleen	0.29 ± 0.99	0.57 ± 0.83 *	0.27 ± 0.99 *	0.43 ± 0.91 ns	0.51 ± 0.86 ns

ns: non-significant difference;*: P<0.05

Table 5. Lipid biomarkers for different groups of rats

	Rat groups				
	Indicator	**Insect f. 5%**	**Insect f. 10%**	**Insect f. 15%**	**Insect f. 20%**
CT	25.43 ± 13.57	31.27 ± 17.21 ns	31.28 ± 16.94 ns	33.12 ± 18.35 ns	34.38 ± 18.69 ns
TG	33.39 ± 18.17	29.51 ± 15.89 *	25.64 ± 13.69 ns	42.19 ± 27.39 ns	22.38 ± 11.84 *
HDL	32.13 ± 17.44	43.08 ± 23.73 *	43.78 ± 24.12 ns	46.67 ± 25.82 ns	54.44 ± 30.45 *

TC: Total cholesterol; TG: Triglycerides; HDL: High-density lipoprotein. ns: non-significant difference;* : P<0.05

Organ weight after dissection

Table 4 shows the weights of the various organs treated with the experimental diet.It can be seen that the weights of kidneys, lungs and heart are more or less the same between treated dishes and controls. On the other hand, statistical analysis of the data showed a significant difference (p< 0.05) between the weights of the spleen and lungs.

Biochemical parameters

The results obtained in Table 5 show a disturbance of the lipid profile characterized by a non-significant increase in plasma total cholesterol (TC) levels. However, there was a significant decrease (*p<0.05) in triglycerides (TG) in rats fed 5% (29.51 ± 15.89) and 20% (22.38 ± 11.84) insect meal, a non-significant decrease in TG in rats fed 10% (25.64 ± 13.69) and a non-significant increase in TG in rats fed 15% insect meal.

HDL levels increased in treated rats, with values of 43.08 ± 23.73; 43.78 ± 24.12; 46.67 ± 25.82 and 54.44 ± 30.45 respectively for the 5%, 10%, 15% and 20% batches, compared with the control (32.13 ± 17.44). The difference was significant for the 5% and 20% batches

(*p < 0.05), then insignificant for the 10% and 15% batches.

Table 6 shows a reduction in blood glucose levels for rats treated at 5% (0.97± 0.59), 15% (0.76 ± 0.72) and 20% (0.57 ± 0,83) compared with the control (0.98 ± 0.59). In addition, an increase in total protein levels was observed for treated animals at 5% (5.07±1.77), 10% (5.26 ± 1.90), 20% (5.77 ± 2.22) and 15% (7.01 ± 3.22) compared with the control (4.83 ± 1.63). Overall, the difference was not significant (P > 0.05).

Table 6. Carbohydrate and protein biomarkers and different groups of rats

	Rat groups				
	Indicator	**Insect f. 5%**	**Insect f. 10%**	**Insect f. 15%**	**Insect f. 20%**
Blood glucose	0.98 ±0.59	0.97±0.5 9 ns	0.98 ± 0.59 ns	0.76 ± 0.72 ns	0.57 ± 0.83 ns
Protein Total	4.83 ± 1.63	5.07 ± 1.77 ns	5.26 ± 1.90 ns	7.01± 3.22 ns	5.77± 2.22 ns

ns: non-significant difference

According to the results described in Table 7, there was a non-significant decrease (p > 0.05) in plasma creatinine and urea levels in treated rats compared with control rats. The lowest creatinine and urea levels were observed in rats fed 20% insect meal, with values of 0.51 ± 0.86 and 21.67 ± 12.44 respectively.

Table 7. Renal biomarkers in different groups of rats

	Rat groups				
	Indicator	**Insect f. 5%**	**Insect f. 10%**	**Insect f. 15%**	**Insect f. 20%**
Creatinine	0,59 ± 0,81	0,43 ± 0,91 ns	0,56 ± 0, 84 ns	0,53 ± 0,85 ns	0,51 ± 0,86 ns
Urea	42,31 ± 23,39	29,79 ±17,45 ns	28,08 ± 15,45 ns	23,18 ±12,41 ns	21,67 ±12,44 ns

ns: non-significant difference

Results on plasma calcium and iron levels are presented in Table 8. The table shows a non-significant increase in calcium (Ca2+) levels in rats treated with insect meal at 5% (5.17±1.84), 10% (7.37±3.27), 15% (8.13±3.76) and 20% (8.46±3.74) compared with the control (4.43±1.42). In addition, plasma iron levels were elevated in rats treated with 5% (0.75 ± 0.72), 15% (1.71±0.52) and 20% (2.63±0.52) iron, followed by a decrease in rats treated with 10% (0.57 ± 0.94) iron compared with control (0.66 ± 0.78) (P > 0.05).

Table 8. Blood ionograms for different groups of rats

	Rat groups				
	Indicator	**Insect f. 5%**	**Insect f. 10%**	**Insect f. 15%**	**Insect f. 20%**
Calcium	4.43 ±1.42	5.17 ± 1.84 ns	7.37 ± 3.27 ns	8.13 ± 3.76 ns	8.46 ± 3.74 ns
Iron	0.66 ± 0.78	0.75 ± 0.72 ns	0.57 ± 0.94 ns	1.71 ± 0.52 ns	2.63 ± 0.52 ns

ns : non-significant difference

4. Discussion

The incorporation of insect meal into the rat diet influenced weight development, food consumption, organ weight after dissection and biochemical parameters.

Rats fed the 15% diet showed greater weight growth and weight gain than those fed the 20%, 5% and 10% diets, as well as the control. This performance can be explained by the higher protein and major mineral content of the dishes [23]. Other authors such as [24,25] have clearly confirmed the benefits of protein administered to rats fed protein-free diets (prive protein).

The results on the total dry matter ingested of incorporated insect meal (35.88 ± 6.93 to 41.43 ± 3.11) are higher than those found by [26] on the nutritional efficacy of three Ivorian dishes (7.05 ± 1.64 to 9.22 ± 0.88). Food consumption depends on several factors, including the physiological state of the organism, as well as factors linked to food characteristics such as aroma, flavour and chemical composition [27].

The daily growth rate results obtained in the present study are higher than those of [28], who reported a rate of 0.24 g/d, and [29], who recorded 1.36 g/d in Bagré (Burkina Faso) and 0.8 to 1.27 g/d in Niger, respectively, in a floating cage rearing cycle, as well as [30], with cassava-, soy-, maize- and groundnut-based diets, respectively. In the present study, the average consumption index obtained with the test foods over the 14 days (2.52 ± 8.87 to 8.43± 5.87) is much higher than that of [29], who obtained a value of 2,7; of [31], which obtained lower consumption indices, ranging from 1.41 to 2.57 and to those obtained by [32,33,34], who obtained respectively 2,3 – 2,7; 2,3 – 2,4 and 2,5 – 2,4; for trials of animal meal in broiler rations. However, our results on the feed efficiency coefficient (0.03± 0.03 to 0.06 ± 0.05) are lower than those obtained by [26] on the nutritional efficiency of three Ivorian dishes (0.13 ± 0.00 to 0.36 ± 0.11).

The similarity obtained for kidney, lung and heart weights of the treated subjects (5, 10, 15 and 20%) compared with the control explains why insect meal had no marked adverse effects on the functioning of these organs. The increase in lung weight to 15% in the treatment may be linked to this organ's need to increase its efficiency [35]. With regard to biochemical parameters, the results show a disturbance in the lipid profile, with a decrease in HDL-cholesterol levels. These lipid changes could be explained by the onset of atherosclerosis, characterized by the abnormal accumulation of lipids and HDL-cholesterol on artery walls, leading to vessel obstruction [36].

The results obtained show a reduction in blood glucose levels for rats treated at 5% (0.97 ± 0.59), 15% (0.76 ± 0.72) and 20% (0.57 ± 0.83) compared with the control (0.98 ± 0.59). This hypoglycemia may have occurred as a result of liver failure [37].

Renal biomarkers (creatinine and urea) are filtered by the kidneys. Their elevated levels in the blood generally indicate renal failure followed by a decrease in glomerular filtration [38]. Creatinine is formed in the body by non-enzymatic dehydration of creatine synthesized by the liver and stored in muscle [39].Whereas urea comes from protein destruction [40]. In our experimental study, a decrease in plasma creatinine and urea concentrations was noted in treated rats. This is explained by kidney damage or dysfunction.

In addition to renal biomarkers, mineral elements play an important role in the construction of human tissues and in the regulation of vital reactions as cofactors of many

metalloenzymes [41]. In the present study, a quantitative determination of blood ionic composition such as calcium (Ca2+) and iron (Fe) was performed. The blood ionogram shows an increase in calcium and iron levels linked respectively to a parathyroid and/or bone problem and to hemolysis of red blood cells or liver cirrhosis [42].

5. Conclusion

Incorporation of Protomacronema sp meal resulted in higher growth and food consumption in rats fed treated diets compared with rats fed control diets. Treated diets incorporating Protomacronema sp meal showed higher plasma levels of total protein, calcium, iron and HDL in rats compared with rats fed control diets. These treated diets also helped to reduce blood sugar levels in the rats. These results appear to be well-suited to combating malnutrition in the context of available insect food resources.

References

[1] PAYNE, C. L. R., SCARBOROUGH, P., RAYNER, M., & NONAKA, K. (2016). A systematic review of nutrient composition data available for twelve commercially available edibleinsects, and comparison with reference values. *Trends in Food Science &Technology*, *47*, 69–77.

[2] AKHTAR, Y., & ISMAN, M. B. (2018). Insects as an alternative protein source. In Rickey Y. Yada (Ed.), *Proteins in food processing* (pp. 263–288). Elsevier.

[3] RUMPOLD, B. A., & SCHLUTER, O. K. (2013). Nutritional composition and safety aspects of edible insects. *Molecular Nutrition & Food Research*, *57*(5), 802-823.

[4] ALAMU, O., AMAO, A., NWOKEDI, C., OKE, O., & LAWA, I. (2013). Diversity and nutritionalstatus of edible insects in Nigeria : A review. International Journal of Biodiversity and Conservation, 5 (4), 215–222.

[5] BARROSO, F. G., HARO, C. DE, SANCHEZ-MUROS, M., VENEGAS, E., MARTINEZ-SANCHEZ, A., & PEREZ-BANON, C. (2014). The potential of various insect species for use as food for fish. Aquaculture, 422-423, 193–201.

[6] WOMENI, H. M., TIENCHEU, B., LINDER, M., NABAYO, M. C., TENYANG, N., TCHOUANGUEP, F. VILLENEUVE, P., FANNI, J., & PARMENTIER, M. (2012). Nutritional Value and Effect of Cooking, Drying and Storage Process on Some Functional Properties of Rhynchophorus Phoenicis. International Journal of Life Science and Pharma Research, 2 (3), 203–219.

[7] ZIELINSKA E., BARANIAK, B., KARAS, M., RYBCZYNSKA, K. & JAKUBCZYK, A. (2015). Selected species of edible insects as a source of nutrient composition. Food Research International, 77, 460–466.

[8] YANG, L. F., SIRIAMOMPUN, S., & LI, D. (2006). Polyunsaturated fatty acid content of edible insects in Thailand. Journal of Food Lipids, 13 (3), 277–285.

[9] SIHAMALA, O. BHULAIDOK, S., LI-RONG, S., & DUO, L. (2010). Lipids and Fatty Acid Composition of Dried Edible Red and Black Ants. Agricultural Sciences in China, 9 (7), 1072–1077.

[10] FINKE M. D., & OONINCX, D. (2014). Insects as food for insectivores. In J. Morales-Ramos,G. Rojas & D. I. Shapiro-Ilan (Eds.) *Mass Production of Beneficial Organisms* (pp. 583–616). Elsevie.

[11] NATIONS UNIES (2022).La population mondiale atteindra 8 milliards le 15 novembre 2022, rapport 2022. 3p.

[12] IDOLO, I. (2010). Nutritional and Quality Attributes of Wheat Buns Enriched with the Larvae of Rhynchophorus phoenicis F. Pakistan Journal of Nutrition, 9 (11), 1043–1046.

[13] OJINNAKA, M. C., UKAH, N., & OKORIE, S. U. (2015). Production and quality evaluation ofwheat cookies enriched with edible african termites. Journal of Biological Sciences and Bioconservation, 7 (1), 55–74.

[14] EDS-Congo, (2012). Enquête Démographique et de Santé au Congo (EDSC-II 2011-2012). Rapport préliminaire, 38p.

[15] AFASS, 2002 Les fibres alimentaires: définition méthodes de dosage allégations nutritionnelles. Rapport du comité des experts spéciales Nutrition humaine 62pp.

[16] A.M. ESTEVEZ, F. FIGUEROLA, M. VASQUEZ, E. CASTILLO, E. YANEZ, (1987) Supplementation of wheat flour with chickpea (Cicer arietinum) flour. II Chemical composition and biological quality of breads made with blends of the same. Arche Latinoam Nutr; 37 515-524.

[17] S. DHINGRA, S. JOOD (2002b). Effect of supplementation on physicochemical, sensory and nutritional characteristics of bread. *Nutr Health*., 16 (2002b) 313-329.

[18] ALLAIN C.C., POON L.S., Chan C.S., RICHMOND W., FU P.C., (1974). Enzymatic determinationof total serum cholesterol. Clinical chemistry, 20 (4): 470-475.

[19] BUCOLO G. & David H., (1973). Quantitative determination of serum triglycerides by the use ofenzymes. Clinical chemistry, 19 (5): 476-482.

[20] ALI K.M., WONNERTH A., HUBER K., WOJTA J. (2012). Cardiovascular disease risk reduction byraising HDL cholesterol–current therapies and future opportunities. British Journal of Pharmacology, 167 (6): 1177-1194.

[21] FABINY D.L. & ERTINGSHAUSEN G. (1971). Automated reaction-rate method for determination of serum creatinine with the CentrifiChem. Clinical chemistry, 17 (8): 696-700.

[22] BURTIS C.A., ASHWOOD E.R., BRUNS D.E., (2006). Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 4th ed. St Louis Missouri Elsevier Saunders, 549p.

[23] RAHAMAN A, ABDEL S. M, ELMAKIL H.B, HASSAN W.I, BABIKER E.E, and ElTINAY A.H (2005). Proximate composition, anti-nutritional factors and mineral content and availability of selected legumes and cereal grown in Sudan.

[24] BOUAFOU K. G. M. (2007). Bilan azoté chez le rat en croissance de la farine d'asticots sehés. Tropicultua Agri Overseas ASBL (Belgique).

[25] ZANNOU T.V.J, (2005). Stratégies d'amélioration des farines infantiles a base de manioc et de soja de haute densité énergétique par incorporation de farine de maïs germés. Thèse de doctorat 3e cycle. Université de Cocody-Abidjan, Côte d'Ivoire. 124 p.

[26] DALLY THEODOR, MEITE ALASSANE, KOUAME KOFFI G, BOUAFOU KOUAME G. M. et KATI–COULIBALI SERAPHIN (2010). Efficacité nutritionnelle de trois mets Ivoiriens: cabatoh à la sauce dah au nord; foutou igname à la sauce gouagouassou au centre; riz cuit à la sauce graine à l'ouest. Journal of Applied Biosciences 33: 2084 – 2090.

[27] J. M. BORYS (2001). Sucres et prise de poids. *Med. Nut*., 37 13-18.

[28] FANDA NGANDEU JP (2012). Effet du type d'aliment sur la croissance d'Oreochromis niloticus. Mémoire de fin de cycle, diplôme d'Ingénieur de travaux halieute, ISH Yabassi, Université de Douala, CAMEROUN.51 p.

[29] DIBALA CI, YOUGBARE MC, KONATE K, COULIBALY ND. et DICKO MH (2018). Production du tilapia du Nil (Oreochromis niloticus Linneaus, 1758) avec des aliments à base de protéines végétales. Journal of Applied Biosciences 128: 12943-12952.

[30] ELENGA MICHEL, MASSAMBA Joachim SILOU THOMAS (2012). Effet de l'incorporation de malt sur la fluidité et la densité énergétique des bouillies de maïsarachide destinées aux nourrissons et aux jeunes enfants. Journal of Applied Biosciences 55: 3995– 4005.

[31] IGA-IGA R (2008). Contribution à la mise au point d'aliments pour tilapia Oreochromis niloticus à base d'intrants locaux: cas du Gabon. Mémoire de fin d'études, diplôme de Master en Sciences Agronomiques et Agroalimentaires, IRAF Libreville, GABON 47p.

[32] OUEDRAOGO B, GNANDA IB, SANFO R, ZOUNDI SI, BAYALA B (2015). Etude comparative des performances réalisées avec l'incorporation de la farine de co-produits de volaille et la farine des asticots dans des rations de poulets de chair au Burkina faso. *Rev. Ivoir.Sci. Technol.*, 25, 148-161.

[33] DIOMANDE M, KOUSSEMON M, ALLOU KV, KAMENAN A. (2008). Effect of snail (*Achatina fulica*) meal broiler production and meat sensorial quality, Livestock Research for Rural Developpement, 20 (12) paper 2.

[34] DONGMO T, NGOU NJD, POUILLE DMJ (2000). Utilisation de quelques farines animales locales dans l'alimentation du poulet de chair, *Tropicultura*, 18 (3): 122-125. Int. J. Biol. Chem. Sci. 12 (2): 716-727.

[35] MASSE DIOMANDE, MAXWELL GRAT AVIT BEUGRE, Benjamin Kan KOUAME et LOUIS GUICHARD BOHOUA (2018). Effets de la farine de chenille (*Imbrasia oyemensis*) sur les performances de croissances et le rendement des organes de poulets de chair en Côte d'Ivoire.

[36] BOBRYSHEV YV (2006). Monocyte recruitment and foam cell formation in atherosclerosis. Micron. avr 2006; 37 (3): 208‑222.

[37] EISSA F, ZIDAN N (2010). Haematological, biochemical and histopathological alterations induced by abamectin and *Bacillus thuringiensis* in male albino rats. Acta Biologica Hungarica. mars 2010; 61 (1): 33‑44.

[38] JABALLI I, BEN SAAD H, BKHAIRIA I, KAMMOUN I, DROGUET M, MAGNE C, et al. (2017).Increasing maneb doses induces reactive oxygen species overproduction and nephrotoxicity in adult mice. Toxicology Mechanisms and Methods. 13 juin 2017; 27 (5): 382‑393.

[39] PRITCHARD JC, BUM CC, BARR ARS, WHAY HR (2009). Haematological and serum biochemical reference values for apparently healthy working horses in Pakistan. Research in Veterinary Science. déc 2009; 87 (3): 389‑395.

[40] BEN SAAD H, GARGOURI M, KALLEL F, CHAABENE R, BOUDAWARA T, JAMOUSSI K, et al. (2017) Flavonoid compounds from the red marine alga *Alsidium corallinum* protect against potassium bromate-induced nephrotoxicity in adult mice: *Alsidium corallinum* PROTECT AGAINST KBrO3- INDUCED NEPHROTOXICITY. Environmental Toxicology. mai 2017; 32 (5): 1475‑1486.

[41] YENGKHOM R, SINGH P, MUWEL N, RAJE K, HANDIQUE B,VENKATESWARAN K.(2019). Supplementation of Brown Seaweed (*Turbinaria conoids*) Powder and its Effect on Blood Metabolites and Mineral Profile in Adult Goats. Ind Jour of Anim Nutr. 2019; 36 (1): 103.

[42] SCHROPP DM, KOVACIC J (2007). Phosphorus and phosphate metabolism in veterinary patients. J Veter Emer Crit. Juin 2007; 17 (2): 127‑134.