

# Effects of Boiled Fish (*Silurus glanis*, *Heterotis niloticus*, *Cyprinus carpio* and *Oreochromis niloticus*) Ingestion on the Growth of Young Male and Female Wistar Albino Rats

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## Abstract

This work was focused on the determination of the *in vivo* nutritional value of some boiled freshwater fishes. Four freshwater fish species, collected in Batie (Division of the Western region of Cameroon) were boiled according to the method used in our households and then used for the composition of rats' feed. Five iso-protein diets were prepared and used for the evaluation of growth parameters of young male and female Wistar albino rats. The effects of these diets on growth performance, as well as on feed intake and digestibility were determined. The serum lipid profile and some biochemical parameters were also obtained from these rats. The results showed that, feed intake of the experimental diets were similar to those of the positive control rats. The biometry of the bodies revealed a normal evolution except for those of the negative control diet which presented renal hypertrophies. Protein Efficiency Ratio (PER) of rats that ate feed based on *Heterotis niloticus* was the best. The serum total cholesterol rates, HDL-Cholesterol, LDL-Cholesterol and (LDL VLDL)-Cholesterol revealed some significant differences, in rats fed with diets based on *Silurus glanis* and *Cyprinus carpio* whereas the others remained close to the values obtained with the positive control especially in females. These results make it possible to conclude that freshwater fish collected in the locality of Batie are of good nutritional quality.

**Keywords:** Fresh water fish; Chemical composition; Nutritional value; Feed formulation; Growth parameters; Serum lipid profile

## Introduction

In developing countries such as Cameroon, Protein-energy malnutrition (PEM) remains a major deficiency disease among children [1,2]. The efforts in the improvement of health and the nutritional status of children required new supplements. These supplements will be prepared from the locally available resources [3]. These foods are available anywhere, but their use can be limited by some anti-nutritional factors such as proteases inhibitors which can reduce the bioavailability of nutrients [4].

The amount and nature of dietary protein affects the levels of plasma cholesterol. Likewise, apart from protein under-nutrition, deficiencies of iron and Vitamin A [5]. Generally, protein malnutrition disorders include growth failure, oedema, fatty liver and hampered immune defense in human and animals [6]. Thus, the need for alternative protein sources has recently gained focus.

All these factors encourage research of new supplements among which freshwater fish, still not consumed by some group of people. The cooking treatments applied in our households depend on eating habits, traditions and ethnic groups. However, the purpose is the same: they are intended to be eaten. These animal proteins sources as all others would be essential to the growth.

Considering the benefits of fish and its place in Cameroonian food habits, its nutritional value, and on our previous work which proved that culinary treatments increased the protein and energy contents in fish, the *in vivo* study of the bioavailability of these fishes is very important in order to determine the effect of boiling on the digestibility of lipids and on growth parameters.

## Material and Methods

### Sampling preparation

Fish of varying weights from 350 to 420 g were collected in the locality of Batie (Division of the Western region of Cameroon). Four species of freshwater fish are produced here in abundance: *Silurus glanis* commonly called "bapche" by farmers, *Cyprinus carpio*, *Oreochromis niloticus* and *Heterotis niloticus* commonly called "kanga". These fishes were transferred alive to the laboratory. Upon arrival at the laboratory, they were killed and allowed to go into rigor. These fishes were then washed with tap water so as to remove adhering blood and excessive mucus. The fishes were then placed in ice-cold water (hypothermia) for five minutes prior to eviscerating and beheading. Subsequently, the fish samples were divided into six groups. After cleaning, each of the fish sample groups was separately boiled into water for 10 min. The different fish samples obtained were dried separately and used for the proximate chemical analysis and formulation of the experimental diets.

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## Proximate chemical composition of fish

The proximate composition analysis of homogenized samples of boiled and raw fish fillets were done in triplicate. The dried samples were reduced to powder using a kitchen blender and stored in a dessicator for analysis. Moisture, ash, protein and lipid contents were determined in each fish species according to methods described by the Association of Official Analytical Chemists [7].

## Animal used

48 wistar albino rats (*Rattus norvegicus*) were obtained from the animal house of the Department of Physiology in the Faculty of science of the University of Dschang. On arrival, the rats were transferred, allowed to acclimatize and maintained on the standard normal diet with water ad libitum, as proposed by Telefo [8] Table 1 in the animal house of the Biochemistry Department under normal room temperature. This research has been approved by the Biochemistry department ethics committee of university of Dschang. Experiment protocols used in this study strictly conformed to the internationally accepted standard ethical guidelines for laboratory animal use and care by as described in the European community guidelines EEC Directive 86/ 609/EEC, of 24<sup>th</sup> November 1986 (EEC, 1986) [9-11].

## Experimental design, feed components and formulation

At 6 weeks of age, rats were divided in six (6) groups and experimental diets were formulated according to the rat requirements described by [10] as presented in Table 2. 24 females and 24 male's rats were used for this experiment.

Composition	Quantity g/kg	Quantity (%)	Feed Value (Kcal) of diet
Maize	678	67.8	2513.184
soya flour	200	20	906
Fish meal	100	10	44.45
Flour of bone	10	0.1	/
Soya oil	1	0.01	/
Kitchen salt	1	0.01	/
Vitamin complex	1	0.01	/

Table 1: Composition of rats' diets during adaptation period.

Composition ( in g)	(-VE) CD	(+VE) CD	E DS	E DH	E DC	E DO
Starch	739.6	606.28	588.23	602.09	590.49	588.32
Cellulose	55.2	55.2	55.2	55.2	55.2	55.2
Protein	/	133.33	151.37	137.51	148.65	151.24
Sucrose	92	92	92	92	92	92
Soya Bean Oil	83	83	83	83	83	83
Vitamin mix	4.2	4.2	4.2	4.2	4.2	4.2
Minerals Complex	16	16	16	16	16	16
Salt	10	10	10	10	10	10
Total (g)	1000	1000	1000	1000	1000	1000
Energy (kcal)	3994.4	3940.12	3931.43	3942.36	3934.25	3912.83

(-VE) CD: Experimental diets without any protein sources; (+VE) CD: Experimental diets having as protein sources albumin; EDS: Experimental diets having as protein sources *Silurus glanis*; EDH: Experimental diets having as protein sources *Heterotis niloticus*; EDC: Experimental diets having as protein sources *Cyprinus carpio*; EDO: Experimental diets having as protein sources coming from *Oreochromis niloticus*;

Table 2: Feed Components and Formulation according to Adrian et al.

## Animal care and sample collection

Rats were individually caged in elevated stainless-steel wire-mesh cages except during the acclimatization period and had free access to food, water and toilets. The temperature was maintained a (22 ± 2) °C. At the end of the experiment, the sacrifice was done with the help of chloroform vapor, their blood collected by cardiac puncture and stored in test tubes. Serum was isolated after centrifugation.

After sacrificing the rats, the liver, heart, spleen, lungs and kidneys were removed, washed in a solution of NaCl (0.9%), on the ice wrung and weighed respectively. With the help of a mortar, 2 g of liver cuts were ground in 10 ml of NaCl 0.9%. The ground liver was centrifuged for 10 min at 3000 G and the supernatant was recovered in sterile test tubes and preserved in a freezer at -20°C.

## Chemicals

Kits for cholesterol, Triacylglycerol, HDL-Cholesterol and transaminase assays were purchased from Boehringer- Mannheim (Meylan, France) (Chod-PAP and GPOPAP methods) and from Teco diagnoses, Anhein (the USA). LDL-Cholesterol was calculated using the methods of Friedewald and Fredrickson, whereas serum and hepatic proteins were determined using Biuret's method.

## Mortality and toxicity

The rats were observed for mortality and gross signs of toxicity twice daily (morning 7 am and afternoon 7 pm). Rats' detailed physical examinations on each part were recorded prior to the study and weekly during the study period. Observations including general condition, skin, eyes, nose, oral cavity, abdomen, external genitalia and breathing evaluation recorded. Body weight and feed intake were measured every day during the experimental period. Weight and feed intake were measured from the 1<sup>st</sup> to the 12<sup>th</sup> day at the same time until sacrifice.

## Statistical analysis

The results were represented as means ± standard deviation of six determinations. The statistical analysis was made using the Graphpad Instat software program. The means were compared at p<0.05 using Dunett's test for the evaluation of the *in vivo* analysis.

## Results and Discussion

### Proximate chemical composition of freshwater fish

The chemical composition of fish is presented in Tables 3 and 4. It appears that these fishes are composed mostly of water like most living things. The protein content of *Heterotis niloticus*, *Cyprinus carpio*, *Silurus glanis*, *Oreochromis niloticus* was respectively 80.00 ± 0.07, 79.95 ± 0.10, 80.90 ± 0.27, 79.00 ± 0.57 before boiling. The results showed that these fish are sources of fat with 6.11, 4.20, 8.05 and 4.57% for *C. gariepinus*; *H. niloticus*, *C. carpio* and *O. niloticus*, respectively. Suriah et al. classified fish species according to their lipid content as: thin fish (<5%), semi-fat fish (5 to 10%) and fat fish (>10%). Based on this classification, we concluded that *C. gariepinus* and *C. carpio* are semi-fat fish whereas *O. niloticus* and *H. niloticus* are thin fish. The lipid content reported during this study was in the interval obtained by [12-15] on fish muscles (0.2 to 25% of lipid). After boiling, the proximate chemical composition of fish has change significantly (P<0.05). They reveal significant reductions (P<0.05) in the protein content between the values of the control and the cooked fish value. A decrease in the protein content of about 21.90% in *C. gariepinus*, of 16.99% in *H. niloticus*, of 20.66% in *C. carpio* and of 19.05% in *O. niloticus* was observed. The significant reduction (P<0.05) of lipid content could be explained by the exchange occurring during cooking. Indeed, during boiling, water permeates fish, and the compounds they contain which

can be largely soluble materials (amino acids, monosaccharides) are extracted. At the boiling point (100°C), the mixture is degassed and helps in reducing lipids oxidation and proteins' reactions, hence higher protein content compared to other culinary treatments. In addition, the hydrated environment would favour Maillard reaction and, therefore reduce the flavours. These results corroborate those obtained by [16-20] where the hydrated medium led to protein loss because of the Maillard reaction during the heat treatment (Table 5).

### Effects of ingestion of boiled fish flour on Wistar albino rats

#### Effects on their growth parameters

##### Feed intake:

At the beginning of the experiment (6 weeks), male rat weight was around 175 g and female rat weight were around 160 g. The value of feed intake in the distinct groups during the 2 weeks experimental period is presented in Table 6. It reveals that no significant difference is observed when compared to the control, feed intake of male rats increases significantly (P<0.05) compared to the control groups during the experiment.

In general, it comes out from our experiments that males consume more feed than females. The mean males' feed intake is approximately 22 g per day. This value is higher than that observed in females (19.05

Fish species	Treatment	W (%WM)	Protein (%DM)	Lipid (%DM)	Ash (%DM)	C (%DM)	E (Kcal) /100 g DM
<i>Heterotis niloticus</i>	Raw fish	80.00 ± 0.07 <sup>a</sup>	87.61 ± 0.30 <sup>a</sup>	4.20 ± 0.14 <sup>a</sup>	7.57 ± 0.11 <sup>a</sup>	0.62 ± 0.22	390.72 ± 10.44 <sup>a</sup>
	Boiled fish	76.03 ± 0.04 <sup>a</sup>	72.72 ± 1.01 <sup>b</sup>	1.58 ± 0.09 <sup>b</sup>	8.99 ± 0.12 <sup>a</sup>	/	375.14 ± 17.11 <sup>a</sup>
<i>Cyprinus carpio</i>	Raw fish	79.95 ± 0.10 <sup>a</sup>	84.79 ± 1.10 <sup>a</sup>	8.05 ± 0.20 <sup>a</sup>	7.23 ± 0.22 <sup>a</sup>	0.21 ± 0.06	412.45 ± 10.21 <sup>a</sup>
	Boiled fish	77.51 ± 0.06 <sup>b</sup>	67.27 ± 0.40 <sup>b</sup>	6.89 ± 0.26 <sup>b</sup>	4.19 ± 0.21 <sup>b</sup>	/	417.69 ± 13.77 <sup>a</sup>
<i>Silurus glanis</i>	Raw fish	80.90 ± 0.27 <sup>a</sup>	84.59 ± 0.40 <sup>a</sup>	6.11 ± 0.10 <sup>a</sup>	8.24 ± 0.14 <sup>a</sup>	1.06 ± 0.10	397.59 ± 12.54 <sup>a</sup>
	Boiled fish	77.26 ± 0.12 <sup>b</sup>	66.06 ± 0.45 <sup>b</sup>	04.73 ± 0.21 <sup>b</sup>	9.65 ± 0.09 <sup>b</sup>	/	385.05 ± 15.22 <sup>a</sup>
<i>Oreochromis niloticus</i>	Raw fish	79.00 ± 0.57 <sup>a</sup>	84.69 ± 0.70 <sup>a</sup>	5.57 ± 0.07 <sup>a</sup>	8.02 ± 0.09 <sup>a</sup>	0.72 ± 0.22 <sup>a</sup>	390.77 ± 21.11 <sup>a</sup>
	Boiled fish	77.43 ± 0.75 <sup>a</sup>	66.12 ± 0.14	3.32 ± 0.14	11.13 ± 0.19	/	372.08 ± 18.21 <sup>a</sup>

The results presented are the means of three values followed by their standard deviation.

W: Moisture content;

C: Carbohydrates content;

E: Energy value;

Kcal: Kilo calorie;

WM: Wet Matter;

DM: Dry Matter.

The crude energy of the modes was calculated while referring to the combustion values of the different nutrients: 4 kcal for 1g of protein, 4 kcal for 1 g of carbohydrate and 9 kcal for 1 g of lipid, n=3.

Table 3: Effects of boiling on the proximate chemical composition of four fish species.

Fish species	Minerals content (mg/1000 g DM)					
	K	Na	Ca	Mg	P	Fe
<i>Heterotis niloticus</i>	Raw fish	80.00 ± 0.07 <sup>a</sup>	87.61 ± 0.30 <sup>a</sup>	4.20 ± 0.14 <sup>a</sup>	7.57 ± 0.11 <sup>a</sup>	0.62 ± 0.22 <sup>a</sup>
	Boiled fish	76.03 ± 0.04 <sup>b</sup>	72.72 ± 1.01 <sup>b</sup>	1.58 ± 0.09 <sup>b</sup>	8.99 ± 0.12 <sup>a</sup>	17.71 ± 0.09 <sup>b</sup>
<i>Cyprinus carpio</i>	Raw fish	79.95 ± 0.10 <sup>a</sup>	84.79 ± 1.10 <sup>a</sup>	8.05 ± 0.20 <sup>a</sup>	7.23 ± 0.22 <sup>a</sup>	0.21 ± 0.06 <sup>a</sup>
	Boiled fish	77.51 ± 0.06 <sup>a</sup>	67.27 ± 0.40 <sup>b</sup>	6.89 ± 0.20 <sup>b</sup>	4.19 ± 0.21 <sup>b</sup>	21.65 ± 0.22 <sup>b</sup>
<i>Silurus glanis</i>	Raw fish	80.90 ± 0.27 <sup>a</sup>	84.59 ± 0.40 <sup>a</sup>	6.11 ± 0.10 <sup>a</sup>	8.24 ± 0.14 <sup>a</sup>	1.06 ± 0.10 <sup>a</sup>
	Boiled fish	77.26 ± 0.12 <sup>b</sup>	66.06 ± 0.45 <sup>b</sup>	04.73 ± 0.21 <sup>b</sup>	9.65 ± 0.09 <sup>a</sup>	19.56 ± 0.07 <sup>b</sup>
<i>Oreochromis niloticus</i>	Raw fish	79.00 ± 0.57 <sup>a</sup>	81.69 ± 0.70 <sup>a</sup>	4.57 ± 0.07 <sup>a</sup>	8.02 ± 0.09 <sup>a</sup>	5.72 ± 0.22 <sup>a</sup>
	Boiled fish	77.43 ± 0.75 <sup>a</sup>	66.12 ± 0.14 <sup>b</sup>	3.32 ± 0.14 <sup>a</sup>	11.13 ± 0.19 <sup>b</sup>	19.43 ± 0.33 <sup>b</sup>

The results presented are the averages of three values followed by their standard deviation.

I: Iron;

Ca: Calcium;

P: Phosphorus;

Mg: Magnesium;

K: Potassium;

Na: Sodium;

DM: Dry Matter.

Table 4: Effects of boiling on the mineral composition of four fish species

Introduced total protein (P.T.I.)	P.T.I.(g) = M.S.I X % Protein of the mode
Weight gain	Final weight - initial weight
Ponderal growth (%) =	$(p_i - P_o) \times 100/P_o$
Introduced total protein (P.T.I.)	M.S.I X % Protein of the diet
Feed efficient of effectiveness (C.E.A.)	C.E.A.= G.P. (G)/M.S.I.(g)
Protein efficiency of effectiveness (C.E.P.)	C.E.P.= G.P. (g)/P.T.I. (g)
Net protein utilization (N.P.U.)	P.N.U.= I - (F - Fpp) - (U - Upp)/I

F: Protein excreted by deposit of a subject other than that subjected to the mode without protein,  
**Fpp**: Protein excreted by deposit of a subject subjected to the mode without protein,  
**I**: Introduced protein,  
**U**: Protein excreted by the urines of a subject other than that subjected to the mode without protein,  
**Upp**: Protein excreted by the urines of a subject subjected to the mode without protein, pi: initial weight,  
**Po**: Final weight.(M.S.I.) is Total quantity of dry matter (of feed) introduced during the period of experimentation PER (Protein Efficiency Ratio) = (Weight gain by the test animal over total protein consumption) - NPR (Net Protein Ratio) = (weight gain by the test animal + weight loss by nitrogen free diet animals over total protein intake)  
 TD (True Digestibility) = Dietary Nitrogen - (Faecal Nitrogen- Faecal Nitrogen of test animal) over dietary nitrogen of test animal

Table 5: Summary table of formulas of some growth parameters.

Diets	Sex	Feed intake (g)
Negative control	Males 547.50 ± 20.30 <sup>a</sup>	1146.31 ± 36.00 <sup>a</sup>
	Females 578.50 ± 36.40 <sup>a</sup>	
Positive control	Males 664.50 ± 9.54 <sup>b</sup>	1294.00 ± 24.49 <sup>a</sup>
	Females 629.50 ± 19.30 <sup>a</sup>	
<i>Silurus glanis</i>	Males 741.50 ± 7.02 <sup>c</sup>	1366.52 ± 87.30 <sup>a</sup>
	Females 618.00 ± 31.57 <sup>a</sup>	
<i>Heterotis niloticus</i>	Males 774.50 ± 36.23 <sup>d</sup>	1439.73 ± 102.80 <sup>a</sup>
	Females 629.00 ± 13.30 <sup>a</sup>	
<i>Cyprinus carpio</i>	Males 709.50 ± 23.57 <sup>b</sup>	1367.57 ± 53.00 <sup>a</sup>
	Females 634.50 ± 20.79 <sup>a</sup>	
<i>Oreochromis niloticus</i>	Males 712.00 ± 21.58 <sup>b</sup>	1385.58 ± 42.43 <sup>c</sup>
	Females 652.00 ± 22.09 <sup>a</sup>	

Values carrying letters are significantly different with (P<0.05). The values presented are of the means and their standard deviation of a total of 8 animals (n=8).

Table 6: Effects of experimental diets on feed intake.

g). Feed intake of males in the control group is lower than the value obtained with experimental diets made of boiled fish flours. This may be due to its flavouring properties (taste, flavour). However, in the negative control group (diets which do not contain proteins), we observed a reduced feed intake. This testifies the importance and the implication of proteins in the feed palatability. This could be due to the effect of fish protein in the appetite stimulation. In females, there is no significant difference between the feed intake of the positive control diet and that of the diets made up of fish. The irregular curve of feed intake in males and female's rats could be justified by the phenomenon of appetite regulation, which tends to adjust the feed intake in order to maintain a balance between the caloric intake and the energy required for their immediate needs as well as for their growth [21]. These results agree with those obtained by [22-25].

The difference observed in male and female rats could be explained by the expression of leptine which could be higher in male rats. This peptide hormone is also produced, in smaller quantity, by the stomach, skeletal muscles and the placenta [24]. Studies made by [24] revealed that the secretion of leptine is in addition inhibited by testosterone whereas it is increased by female hormones. These results are in agreement with those of [25].

### Effects on the rat's growth

Figure 1 illustrates the effect of experimental diets on rat growth. The five protein diets present an ascending growth while the negative control diet of rats decreases significantly (P<0.05). The weight loss in this group is normal since the animal of this group were nourished with diets deprived of proteins; this would explain the inability to ensure growth.

Differences observed in male and female rats would be due to secretions of hormone controlling satiety. According to Beaufriere, proteins count for approximately 15% of our muscle mass. A lack of protein logically causes a loss of the muscular mass because of its incapacity of synthesis, and thus a stop of growth. These results corroborate those of Poitier de Courcy, who found out that rat need proteins for their maintenance, and possibly for growth or lactation [26].

### Effects on the relative weights of some vital organs.

Table 7 shows the various values of the relative weights of organs. In protein diets, it reveals significant increases (p<0.05) in the relative weights of kidneys, rate, liver, heart of females nourished with diet containing *Cyprinus carpio*. We can also observe a significant decrease in weight in the negative control group of the kidneys, liver and heart. A significant increase of the kidney's weight (p<0.05) of the negative control group is observed compared to positive control.

The liver plays a very significant role in metabolic processes. The atrophy of the liver of animals nourished with the negative control diet group can be due to a decrease of its activity while the weight of liver of the other animal nourished with the protein diet could suggest a normal metabolism of amino acids in the body. According to increase of the liver's weight translates its hyper activity. The weight of the liver of male rats fed with the experimental diet is closed to that of the positive control; this can be due to a normal metabolism of freshwater fish amino acids.

### Effects of the experimental diet on PER

The results reveal that the PER and Net Protein Ratio (NPR) of



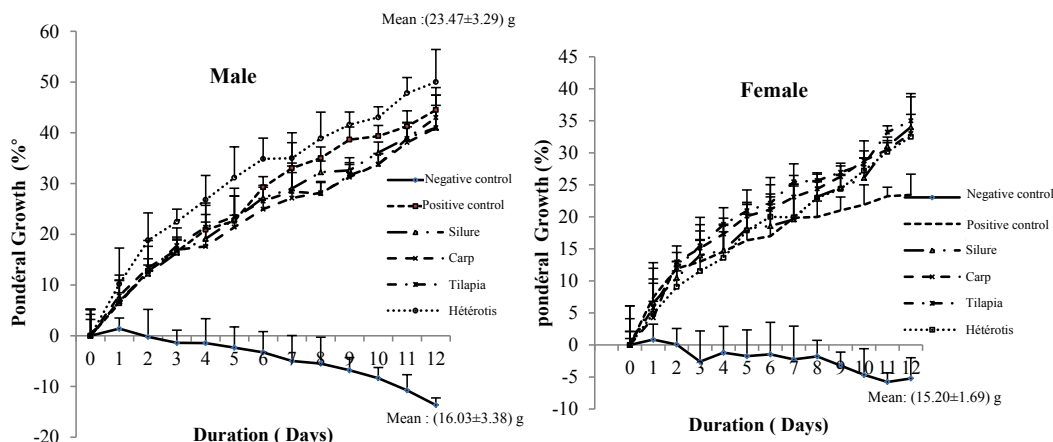


Figure 1: Effects of experimental diets on growth of the young male (A) and female rats (B).

Experimental Diets	Sex	Kidney (%)	Spleen (%)	Liver (%)	Heart (%)	Ileum (%)
Negative control	Male	0.72 ± 0.01 <sup>b</sup>	0.24 ± 0.01 <sup>a</sup>	5.71 ± 0.35 <sup>b</sup>	0.44 ± 0.02 <sup>b</sup>	3.49 ± 0.25 <sup>a</sup>
	Female	0.89 ± 0.03 <sup>a</sup>	0.40 ± 0.09 <sup>a</sup>	5.18 ± 0.07 <sup>a</sup>	0.50 ± 0.04 <sup>b</sup>	2.81 ± 0.40 <sup>a</sup>
Positive control	Male	1.03 ± 0.00 <sup>a</sup>	0.35 ± 0.02 <sup>a</sup>	4.90 ± 0.32 <sup>a</sup>	0.49 ± 0.01 <sup>a</sup>	3.12 ± 0.87 <sup>a</sup>
	Female	0.88 ± 0.03 <sup>a</sup>	0.36 ± 0.00 <sup>a</sup>	5.50 ± 0.42 <sup>a</sup>	0.44 ± 0.03 <sup>a</sup>	2.77 ± 0.33 <sup>a</sup>
<i>Silurus glanis</i>	Male	0.80 ± 0.05 <sup>a</sup>	0.43 ± 0.00 <sup>a</sup>	4.18 ± 0.35 <sup>a</sup>	0.42 ± 0.00 <sup>c</sup>	1.95 ± 0.27 <sup>b</sup>
	Female	0.92 ± 0.02 <sup>a</sup>	0.40 ± 0.05 <sup>a</sup>	4.74 ± 0.04 <sup>b</sup>	0.43 ± 0.01 <sup>a</sup>	2.41 ± 0.29 <sup>a</sup>
<i>Heterotis niloticus</i>	Male	0.89 ± 0.00 <sup>a</sup>	0.35 ± 0.04 <sup>a</sup>	5.13 ± 0.23 <sup>a</sup>	0.45 ± 0.02 <sup>a</sup>	2.64 ± 0.11 <sup>a</sup>
	Female	0.93 ± 0.02 <sup>a</sup>	0.41 ± 0.03 <sup>a</sup>	4.82 ± 0.11 <sup>b</sup>	0.41 ± 0.01 <sup>a</sup>	2.27 ± 0.19 <sup>a</sup>
<i>Cyprinus carpio</i>	Male	0.91 ± 0.02 <sup>a</sup>	0.46 ± 0.08 <sup>a</sup>	4.73 ± 0.15 <sup>a</sup>	0.41 ± 0.03 <sup>c</sup>	2.44 ± 0.09 <sup>a</sup>
	Female	0.97 ± 0.06 <sup>b</sup>	0.47 ± 0.01 <sup>b</sup>	4.55 ± 0.11 <sup>c</sup>	0.39 ± 0.03 <sup>b</sup>	2.29 ± 0.07 <sup>a</sup>
<i>Oreochromis niloticus</i>	Male	0.84 ± 0.02 <sup>a</sup>	0.50 ± 0.15 <sup>b</sup>	5.05 ± 0.71 <sup>a</sup>	0.39 ± 0.02 <sup>c</sup>	2.52 ± 0.31 <sup>a</sup>
	Female	0.83 ± 0.01 <sup>a</sup>	0.44 ± 0.04 <sup>a</sup>	5.14 ± 0.63 <sup>a</sup>	0.32 ± 0.00 <sup>c</sup>	2.35 ± 0.18 <sup>a</sup>

Values carrying letters are significantly different with (P<0.05). The values presented are of the means and their standard deviation of total of 8 animals by n=8 group.

Table 7: Effects of the experimental diets on the relative weights of some vital organs.

*Heterotis niloticus* are better than those obtained with other freshwater fishes. Indeed, there is no significant difference between them and the control. On the other hand, we note a significant difference between the positive control and the other diets. In spite of this difference, these parameters are close to each other 1.92; 1.78 and 1.69 respectively for *Silurus glanis*, *Cyprinus carpio* and *Oreochromis niloticus*. These values are higher than those found by Solomon et al. for *Zonocerus variegatus*; through which we can suggest that the quality of their proteins are close and consequently, this feed would be able to promote growth and contains proteins of good quality. In fact, Adrian et al. showed that a protein that produces a weight gain of 25 g in 4 weeks when it is provided to rats as the only source of protein is generally regarded as a good quality protein source. The positive values of PER obtained with test diets (ranging from 1.52 to 2.82) mean that the protein they contain are either of intermediate quality or very good quality. Indeed, the quality of a protein is bad if the PER is lower than 1.5; intermediary if it's between 1.5 and 2; and good or very good when this value is higher than 2 (Friedman, 1996). These values are higher than those obtained (PER ranging between 0.9 and 2.1) on leguminous plants and 3 on egg [15-19].

## Effects on the digestibility

### Effects on the UDCr

Figure 2 reveals that the UDCr of male rats of the positive control (94.68 ± 0.18) is significantly different (P<0.05) from that of the diet containing *Heterotis niloticus* (92.67 ± 1.02), but remains comparable

with that of the groups containing *Silurus glanis*, *Cyprinus carpio* and *Oreochromis niloticus*. It also arises that the CUDr of female control groups (95.8 ± 0.35) is significantly different from that of the groups containing *Heterotis niloticus* (92.77 ± 1.11) and *Cyprinus carpio* (93.22 ± 0.40), but remains comparable (P<0.05) to the diets containing *Silurus glanis* and *Oreochromis niloticus*.

## Effects on the bioavailability parameters

### Effects on the net protein ratio (NPR)

This parameter presented in Figure 3, determines the proportion of nitrogen retained by young [12]. The NPR or percentage of subsequent body nitrogen, digested and absorbed does not present a significant difference compared to the control. That would explain the fact that protein's nitrogen of fish meals would return fully in the tissue. The protein's nitrogen of these fish meals could correspond to a biologically effective form since it would have crossed the stereo chemical tests of specificity of the varied enzymatic systems of digestion and cellular metabolism. This nitrogen would be accumulated consequently more in the tissues and functional systems.

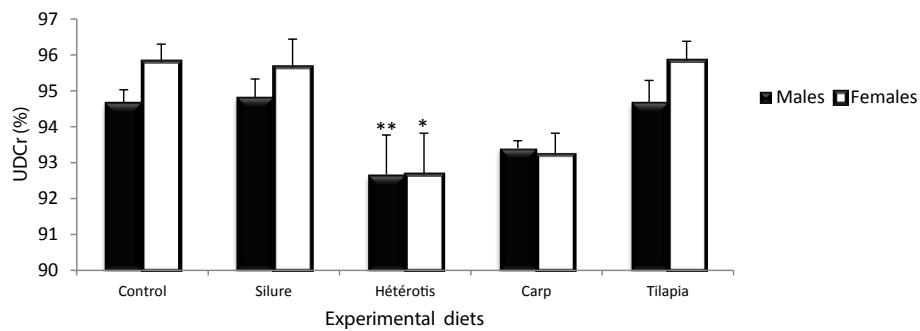
### Effects of experimental diet on serum lipid level

#### Protein content of organs (expressed in mg/g of body) and of the serum

Figure 4a presents the protein content of liver (a) and serum (b) of

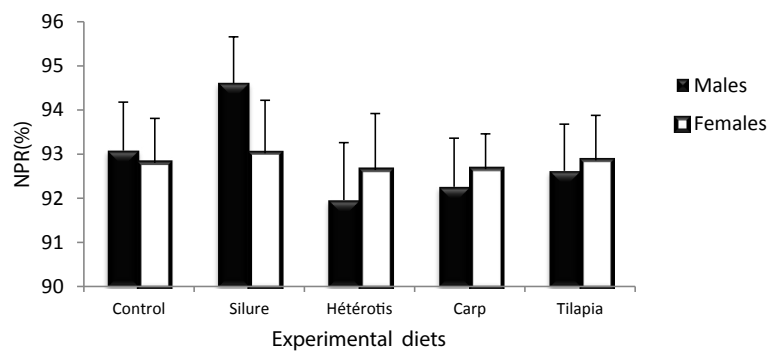
test animals. The negative control group presented a significant weight loss; the positive control group does not present any difference with the diets containing protein. The same report is made with female rats. The liver is an organ of synthesis and storage of certain nutrients. The higher amount of proteins in males would be under the influence of hormonal factors. Moreover, this could also be explained by the anabolizing role of androgens (male hormones).

Figure 4b illustrates the animal serum proteins contents of the experimental diets. A very significant difference ( $p < 0.01$ ) is observed between the control group and the other female groups. However, in males, the difference is very significant with diets composed of *Cyprinus carpio*, *Heterotis niloticus*, and *Oreochromis niloticus*. In both cases, no difference is observed between the reference groups and the negative control groups.



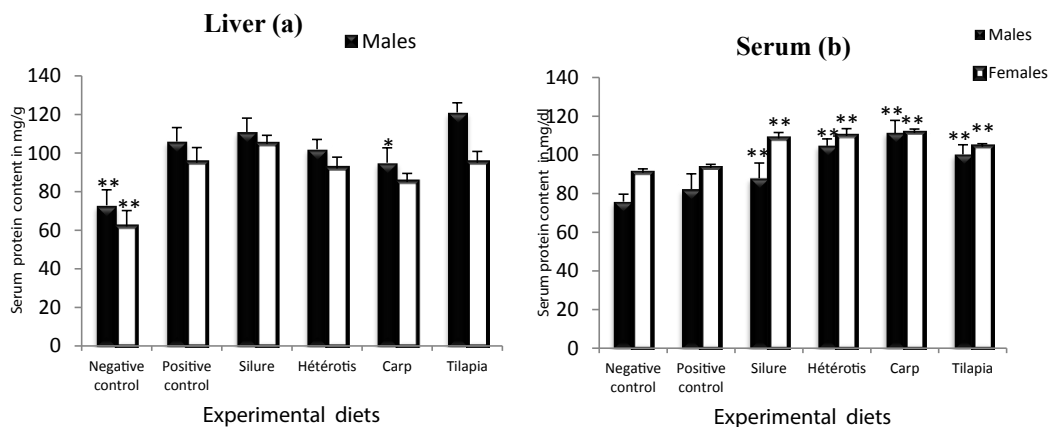
Value presenting a significant difference; \* ( $p < 0.05$ ); \*\* very significant ( $P < 0.01$ ); \*\*\* highly significant ( $P < 0.001$ ) compared to that of the positive control.

Figure 2: Effects of experimental diets on CUDr.



Value presenting a significant difference: \* ( $p < 0.05$ ); \*\* very significant ( $P < 0.01$ ); \*\*\* highly significant ( $P < 0.001$ ) compared to that of the positive control.

Figure 3: Effects of the various diets on the NPR of male and female's rats.



Value presenting a significant difference \* ( $p < 0.05$ ), \*\* very significant ( $P < 0.01$ ), \*\*\* highly significant ( $P < 0.001$ ) compared to that of the positive control.

Figure 4: (a, b) Effects of experimental diets on protein content of liver and serum.

Figure 5 Females contain more proteins circulating in blood. The rise of the serum rate in females nourished with fish could be due to the implication of fish components in the synthesis of protein molecules which play the role of antibodies, enzymes and hormones. This role of hormone would be developed more especially as males have very high rate of androgens which would stimulate the synthesis of proteins at the level of liver in particular.

### Protein content of the various body organs

It comes out from Table 7 that, the quantities of proteins in various organs of the animal in protein diets are comparable to those of the control (P<0.05) in males, as well as in females. However, a significant difference is observed in the negative control group diets. This difference would suggest the implication of proteins in the metabolic processes. This table also reveals a rise in the quantities of proteins in male bodies compared to female bodies.

The significant increase (P<0.05) in protein content of the heart in the experimental groups compared to the negative control group would represent an accumulation of proteins at this level of the body. This result could imply a biological effectiveness of the protein's shape brought by the diets, more especially as there are no significant differences with the control.

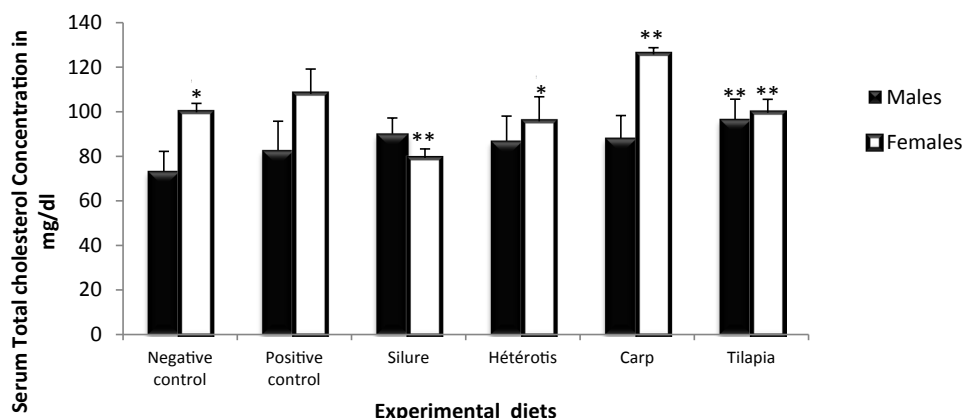
The significant increase in renal proteins of animal protein diets compared to those of the negative control group (P<0.05) could only illustrate the biological effectiveness of proteins of these freshwater fishes. However, this increase can be explained by an abnormal metabolism due to the presence of water-soluble molecules in the blood.

Table 8 The significant increase (P<0.05) in protein content of the spleen of females (P<0.05) compared to the spleen of males, would illustrate the varied levels of performance in the diet of the spleen whose principal role is the production of white blood cells and the blood purification.

According to Holst and Williamson, the various functions of the intestinal mucus membrane would be accelerated or slowed down according to the nutrients contribution. The brush border enzymes of the small intestine are sensitive at the level of protein contribution [16].

### Effects of the diets on the Total-cholesterol concentration in male and female rats

Figure 6 illustrates the serum HDL-Cholesterol in male and female rats. It comes out that a significant decrease is observed between rats of the negative control group compared to the positive control. Diets composed of *Silurus glanis* and *Cyprinus carpio*, present significant



Value presenting a significant difference: \* (p<0.05); \*\* very significant (P<0.01); \*\*\* highly significant (P<0.001) compared to that of the positive control.

Figure 5: Effects of experimental diets on Total cholesterol concentration of male and female's rats.

Experimental group	Sex	Kidneys	Spleen	Heart	Ileum
Negative control	Male	72.42 ± 4.54 <sup>b</sup>	72.78 ± 5.01 <sup>b</sup>	88.90 ± 3.13 <sup>b</sup>	8.67 ± 0.52 <sup>b</sup>
	Female	41.90 ± 3.03 <sup>b</sup>	136.97 ± 4.09 <sup>a</sup>	85.04 ± 7.20 <sup>b</sup>	8.81 ± 0.40 <sup>b</sup>
Positive control	Male	95.76 ± 7.09 <sup>a</sup>	107.71 ± 8.02 <sup>a</sup>	109.42 ± 4.73 <sup>a</sup>	10.95 ± 0.87 <sup>a</sup>
	Female	78.09 ± 5.12 <sup>a</sup>	130.79 ± 9.21 <sup>a</sup>	102.00 ± 4.03 <sup>a</sup>	9.57 ± 0.33 <sup>a</sup>
<i>Silurus glanis</i>	Male	101.71 ± 9.05 <sup>a</sup>	97.71 ± 7.57 <sup>a</sup>	95.33 ± 2.21 <sup>b</sup>	9.25 ± 0.87 <sup>c</sup>
	Female	77.42 ± 2.51 <sup>a</sup>	106.91 ± 3.05 <sup>b</sup>	88.83 ± 7.01 <sup>c</sup>	7.63 ± 0.29 <sup>b</sup>
<i>Heterotis niloticus</i>	Males	95.90 ± 3.45 <sup>a</sup>	114.27 ± 9.54 <sup>a</sup>	108.62 ± 5.02 <sup>a</sup>	7.66 ± 0.56 <sup>b</sup>
	Female	70.09 ± 4.82 <sup>a</sup>	124.40 ± 8.01 <sup>a</sup>	103.03 ± 6.47 <sup>a</sup>	6.80 ± 0.19 <sup>b</sup>
<i>Cyprinus carpio</i>	Male	90.97 ± 7.78 <sup>a</sup>	103.83 ± 7.01 <sup>a</sup>	97.82 ± 6.22 <sup>b</sup>	8.24 ± 0.88 <sup>b</sup>
	Female	73.45 ± 1.58 <sup>a</sup>	150.51 ± 9.33 <sup>a</sup>	88.24 ± 4.52 <sup>c</sup>	7.62 ± 0.07 <sup>b</sup>
<i>Oreochromis niloticus</i>	Male	103.33 ± 7.02 <sup>a</sup>	121.00 ± 7.15 <sup>a</sup>	97.04 ± 1.32 <sup>b</sup>	8.73 ± 0.54 <sup>b</sup>
	Female	69.80 ± 4.25 <sup>a</sup>	149.12 ± 8.04 <sup>a</sup>	101.80 ± 6.52 <sup>a</sup>	8.44 ± 0.07 <sup>b</sup>

Values carrying different letters are significantly different with (P<0.05). The values presented are of the means and their standard deviation for a total of 8 animals by n=8 group.

Table 8: Effects of the experimental diets on the proteins content of the rat organs expressed in mg/g of body.

differences ( $P < 0.01$ ) compared to the positive control. Female rats' diets containing *Silurus glanis* and *Heterotis niloticus* present a significant decrease compared to the control. This increase of serum HDL-Cholesterol of the rats nourished with experimental diets opposed to a reduction in the serum LDL-cholesterol in rats of the negative control group, would be due to the inhibition of the regulation of cholesterol synthesis enzyme in the liver (beta-hydroxy-beta-methyl glutaryl-ScoA reductase), and the inhibition of LDL-Cholesterol membrane receptor by proteins.

### Effects of the experimental diets on HDL-Cholesterol concentration of male and female rats

Figure 6 illustrates the HDL serum cholesterol concentration in male and female rats. We observe a significant decrease between males and females of the negative control group compared to the positive control. Diets containing *Silurus glanis*, and *Cyprinus carpio*, present a significant difference ( $P < 0.01$ ) compared to the positive control. Female rats' diets containing *Silurus glanis* and *Heterotis niloticus* present a significant decrease compared to the positive control.

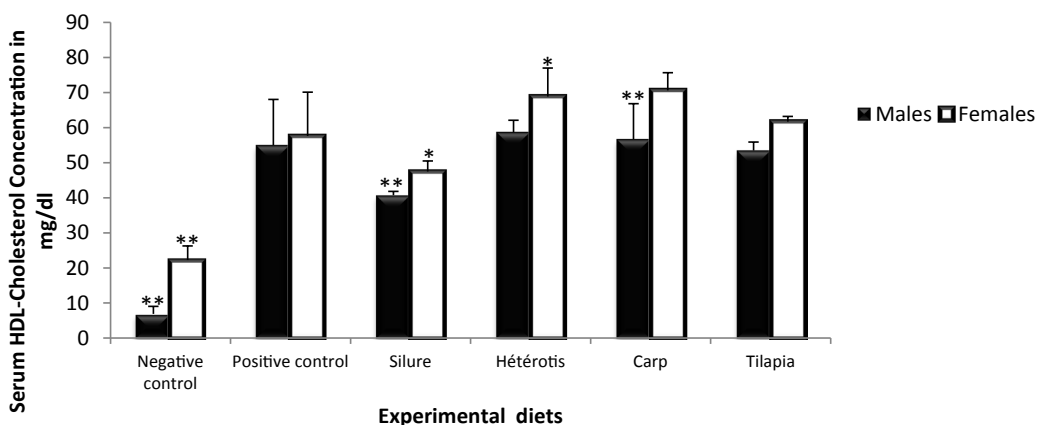
The higher rate of HDL-Cholesterol obtained with rats nourished

with diets is opposed to the reduction of LDL-cholesterol in the serum of the negative control group. This would be explained by the inhibition of the regulator enzyme included in cholesterol synthesis in the liver (beta-hydroxy-beta-methyl glutaryl-ScoA reductase); this could also be due to the inhibition of LDL-Cholesterol membrane receptors. The consumption of proteins would increase blood cholesterol level in two ways: by supporting the synthesis of cholesterol in the liver, and by inhibiting the specific low density (LDL) lipoproteins receptor of liver cells. Similar results were found by Luchini who revealed the increase in the serum cholesterol level.

In addition, the higher rate of serum HDL-Cholesterol in females would be explained by hormones' implication. Indeed, oestrogens stimulate the distribution of the lubricating mass [20].

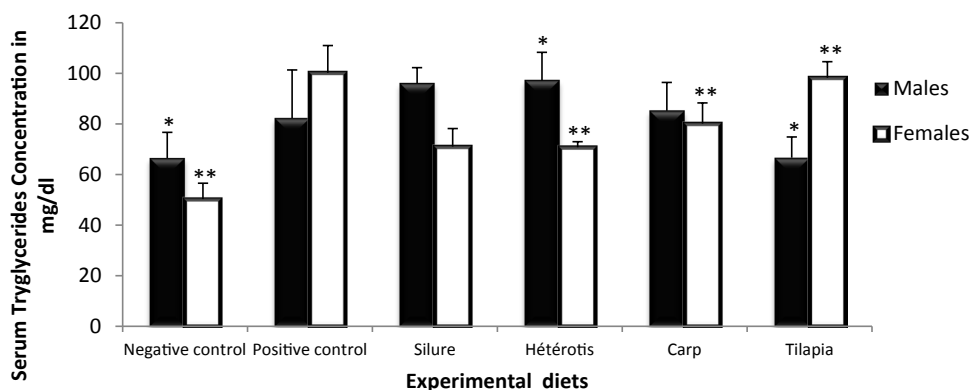
### Effects of diets on the serum Triacylglycerol concentration of male and female rats

Figure 7 presents the effect of experimental diets on serum Triacylglycerols concentration. We observe a significant difference ( $P < 0.05$ ) between the male control diets and the negative control diets made of *Heterotis niloticus* and *Oreochromis niloticus*. However, a significant difference is observed between the control group and other



Value presenting a significant difference: \* ( $p < 0.05$ ); \*\* very significant ( $p < 0.01$ ); \*\*\* highly significant ( $p < 0.001$ ) compared to that of the positive control.

Figure 6: Effects of experimental diets on the HDL-Cholesterol concentration of males and female's rats.



Value presenting a significant difference: \* ( $P < 0.05$ ); \*\* very significant ( $P < 0.01$ ); \*\*\* highly significant ( $P < 0.001$ ) compared to that of the positive control.

Figure 7: Effects of the diets on the serum Triacylglycerol concentration on Males and Females rats.



groups. The increase of the Triacylglycerol level in rats nourished with diet based on fish protein would be due to the function of fatty acids which is selective, then affected by their chain length [21] poorly formulated.

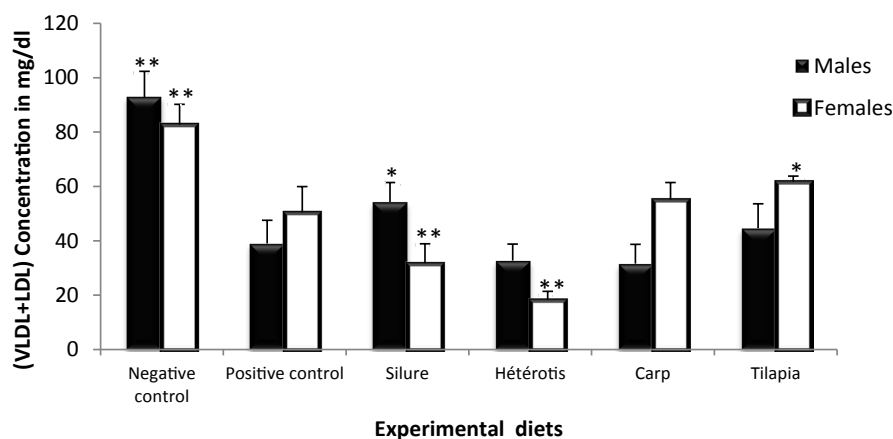
### Effects on the concentration of (VLDL+LDL) – Cholesterol

The (VLDL+LDL) – Cholesterol count for 55 to 65 % of the total lipoproteins. They contain no esterified cholesterol. Figure 8 shows this cholesterol level in male and female rats of the experimental diets. This reveals that, the negative control group present a significant increase ( $p < 0.01$ ) of the (VLDL +LDL)–Cholesterol concentration. We can also observe a significant decrease of the VLDL–Cholesterol rate in female rats nourished with diets base on fish protein. The results of our study show that the experimental fish could be without notable effects on the VLDL+LDL lipoproteins serum except in animal of group containing feed based on *Heterotis niloticus*. This would be explained by the high concentration of HDL-Cholesterol ( $68.04 \pm 1.05$  mg/dL). The studies made on the Sprague-Dawley rats nourished with a mixture of soya dried milk

and casein gave similar results for 2 weeks experimental period. Similar results to those of [20] compared with other fish protein were obtained by [22] on the albino rats and [24-29] on the *Syrian hamsters*. Our results also make it possible to suggest that freshwater fish used in the present study could also be without notable effects on the sum of hepatic VLDL+LDL lipoproteins.

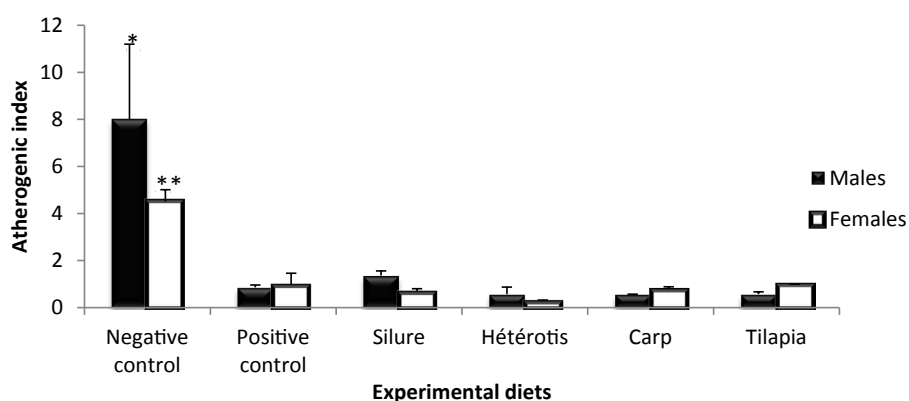
### Effects on the arteriosclerosis index

Figure 9 presents the atherogenic index of rats. We observe significant differences between controls group and the negative control nourished with a diet made without proteins ( $p < 0.05$ ). There was no significant difference observed between the atherogenic indices in experimental protein groups. This observation would translate the incapacity of fish to induce this disease. This reduction could indicate the presence of antioxidant substances and  $\omega$ -6/ $\omega$ -3 ratio which could have the capacity to remove from the blood reactive oxygen, thereby inhibiting oxidation of the  $\beta$ -lipoproteins and consequently decrease the risk of atherogenic index. These results corroborate those obtained by [21] on albino rats.



Value presenting a significant difference: \* ( $p < 0.05$ ); \*\* very significant ( $P < 0.01$ ); \*\*\* highly significant ( $P < 0.001$ ) compared to that of the positive control.

**Figure 8:** Effects of experimental diets on the concentration in of VLDL+LDL- Cholesterol serum of in male and female's rats.



Value presenting a significant difference: \* ( $p < 0.05$ ); \*\* very significant ( $P < 0.01$ ); \*\*\* highly significant ( $P < 0.001$ ) compared to that of the positive control.

**Figure 9:** Effect of experimental diets on the atherogenic index in Male and Female rats

## Effects of the treatments on some biochemical toxicity parameters related to the ingestion of freshwater fish proteins

The null death rate obtained after 12 days of experimentation is in conformity with the experimental design of [14] with reveals that, lasted maximum for an acute or minimal toxicity for a sub-acute toxicity could explain a positive effect of the cooking treatments in the reduction of the microbiological factors being able to find themselves in freshwaters and penetrate the fish, thus contributing to the improvement of food safety.

## Conclusion

The protein quality was determined by its aptitude to promote the growth. This was observed clearly with the weight gain comparable to the value obtained with rats fed with experimental diets made using reference protein. The males certainly, eat more than the females because of the hormonal factors, but that does not have a major influence on the growth and digestibility parameters. These male hormones, which stimulate appetite, have effects on certain biochemical parameters, particularly the abundance of proteins in male bodies. Treatments used in this study make it possible to obtain the Total cholesterol, HDL-Cholesterol, LDL+VLDL-Cholesterol values similar to those of rats nourished with the positive control diet. The nutritional assessments, the digestibility parameters and protein used made it possible to conclude that freshwater fishes of Cameroun can be used for food formulation.

## Competing Interests

The authors declare that they have no competing interests.

## Authors' Contribution

All the authors i.e. CTT, SHN, HMW, FT and ML designed the study. CTT carried out the research work for various biochemical analyses, growth parameters were done by CTT, HMW and SHN parameters. FT and ML drafted the manuscript. All authors read and approved the final manuscript.

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