



## Nutritional and Serum Biochemistry of the Edible Frog *Hoplobatrachus occipitalis* in Rivers State, Nigeria

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### Authors' contributions

This work was carried out in collaboration among all authors. Author SOO designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the experimental processes. Author GCA Identified the species of the amphibian and author CCA managed and proof read the literature searches. All authors read and approved the final manuscript.

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### ABSTRACT

This paper investigated the proximate, minerals and serum biochemistry in *Hoplobatrachus occipitalis*. The proximate and selected minerals of edible frog *Hoplobatrachus occipitalis* were determined using standard analytical methods. The result showed that crude protein was 16.91% carbohydrate was 1.76%, crude fibre 2.85%, The fat was 4.96% ash content was 1.84% and moisture was 71.67%. The selected mineral constituent recorded showed that sodium > iron > calcium > potassium > manganese. The nutritive serum biochemistry was determined in male and female species, the results revealed that both sexes have high nutritional profile suitable for human consumption; nevertheless, the female species have higher nutritional values than the males.

**Keywords:** *Hoplobatrachus occipitalis*; proximate analysis; serum biochemistry.

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## 1. INTRODUCTION

Meat, in general, is highly nutritional as it contains high-class nutritional entities such as protein, carbohydrates, fats and oil, in proportions essential for growth and development. The use of mammalian and the avian meat is on the increase. Its' increasing demand has caused an inflation in the price rate and the ability to purchase these products is now a major problem in most developing countries including Nigeria leading to mal – undernutrition. Thus, there is the need to provide an alternative meat source [1].

Due to the incumbent increase on the verge to provide an alternate good source of meat, as red meat gotten from the mammalian species (such as Cow) has been found to pose serious health risks and are also very expensive to purchase, most researchers have shifted their investigations into other forms of meat such as lower vertebrates (including amphibians) [2].

The nutritional composite of the anuran species has been of great focus as investigations have been carried in most advanced countries which spans from Europe [3], Netherland, Italy, Vietnam and others [4,5].

Also, local ponds and aquacultures are been constructed where these species are reared and sold for commercial purposes in countries such as Malaysia, Taiwan, Indonesia, Brazil, Mexico, USA, France, Canada, Belgium and others [6,7]. On a global scale the international trade from the frog farm/culture frog has proven to increase the socio-economic status of the countries, generating great income [8,9].

*Hoplobatrachus occipitalis* is commonly referred to as jumping chicken in Nigeria as the taste is similar to that of chicken [5]. It is a species of economic importance as it is commercially sold for food. Studies have shown that frog meat is a high-class delicacy in several civilized continents such as Europe [3]. Frog limbs that have high levels of protein are also sold in classy restaurants and local markets in Nigeria [10]. The frog is also often used as experimental models [11].

This study will, therefore, provide information on the comprehensive profile of the species thereby educating the scientific community on the nutritional benefits and sustainable dietary intake of *H. occipitalis*.

## 2. MATERIALS AND METHODS

### 2.1 Study Area and Experimental Animals

The Specimen of study (*Hoplobatrachus occipitalis*) were collected from the wild in Omoku, Ogba-Egbema-Ndoni Local Government Area, Rivers State.

### 2.2 Proximate Analysis

#### 2.2.1 Sample preparation

Each fresh sample was cleaned and cut open and the stomach content was completely removed before the proximate composition was done. The analysis were carried out in Food science laboratory, Rivers state university.

#### 2.2.2 Moisture content

Two grams (2 g) of the sample was weighed and placed into clean metallic moisture can of known weight. Samples were weighed with a weighing balance (model no AC 223). The weighted sample was allowed to dry for a period of 1hr at 130°C in a preheated oven (model No. DHG 9140A). The sample was removed with the aid of forceps and transferred to the desiccator where it was allowed to cool for 15 – 20 minutes and weighted to a constant weight (Ozogul et al., 2008).

#### Calculation:

Sample weight = wt of can+ wt of the sample before drying – wt of an empty can

Moisture loss= wt of can + sample before drying – wt of can + sample after drying

% moisture content = moisture /sample weight \*100/1

#### 2.2.3 Ash content

1 gram of sample was weighted from the dried samples; this was placed in a muffle furnace (Model No SXL) and then allowed to ash for a period of 3hrs at 550°C and then placed in a desiccator where it was allowed to cool for 30 minutes and the weighted. The percentage of residue weight was expressed as ash content.

#### Calculation:

Weight of sample = (weight of + sample – weight of empty crucible)

Weight of ash= (weight of crucible +ash - weight of empty crucible)

% ASH = weight of Ash/sample weight \* 100/1

#### 2.2.4 Protein determination

Firstly, 0.5 g of the weighted sample was placed into the Kjeldahl digestion flask and 0.3 g of copper sulphate ( $\text{CuSO}_4$ ). 3 g of sodium sulphate ( $\text{NaSO}_4$ ), serving as a catalyst, was added into the Kjeldahl flask containing the sample, then 12 ml conc.  $\text{H}_2\text{SO}_4$  was introduced and mounted into kdn -04 c digest furnace. This was allowed to digest for 1hr at  $420^\circ\text{C}$  (formation of clear solution).

The second phase which is the distillation proceeded and also the final stage of titration which resulted in a pink coloured solution was seen. [12].

#### 2.2.5 Carbohydrate determination

The carbohydrate content was determined using the mathematical equation:

Available carbohydrate (%) =  $100 - [\text{Protein} (\%) + \text{Moisture} (\%) + \text{Ash} (\%) + \text{Fibre} (\%) + \text{Crude fat} (\%)]$

#### 2.2.6 Fat/lipid determination

Two gram (2 g) of the sample was used for fat determination; the 2 g weighted sample in a thimble was transferred into a Soxhlet extractor, 150 ml of hexane was added and placed on the extraction unit which extracted for 3 hours. The thimble was taken away and the solvent covered. Finally, the extraction flask was placed in an oven and allowed to evaporate resident solvents at  $105^\circ\text{C}$  for 30 minutes and placed in a desiccator to cool down. The duplicate sample was used to achieve duplicate values and the mean was calculated.

#### Calculation:

Weight of fat = [weight (wt) of flask + fat] – [weight (wt) of empty flask]

% fat = weight of fat /sample weight \*100/1

#### 2.2.7 Crude fiber determination

A total of 0.5 g of moisture-free samples was extracted for 3hours with petroleum ether using Soxhlet apparatus. The fat-free sample was put

in a 100 ml beaker where 25 ml of 1.2% of sulphuric acid was introduced; and covered with watch glass.

The content was gently heated on a Gehardt hot plate for approximately 5 minutes and later filtered under vacuum through a Buchner funnel fixed with filter paper and washed with boiling water until the washings were no longer acidic to the litmus. The residue was then washed again into the beaker with 1.25% NaOH and covered with the glass which was allowed to boil for 5 minutes.

#### Calculation:

Crude fibre (%) = weight of fibre/weight of sample \*100/1

#### 2.3 Mineral Determination

Sodium and potassium were determined using the Gallenkamp Flame analyzer, while calcium, iron, manganese, were determined using Buch Model 205 atomic absorption spectrophotometer [13].

#### 2.4 Serum Biochemical Examination

An automated biochemical analyzer was used to determine the nutritional composite of the species (male and female), the total cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein, albumin and total protein were analysed [14].

#### 2.5 Statistical Analysis

The Serum biochemical data resulting from this study were expressed as means and standard deviation (SD) via Microsoft excel software for windows. The results were compared between sexes. Significant differences were determined using an independent sample t-test model. Results were considered significant at  $P=0.05$ . Proximate Analyses were performed in replicates, results were expressed in means.

### 3. RESULTS AND DISCUSSION

#### 3.1 Proximate Results

The Proximate composition (Table 1) shows 71.669% Moisture content, 4.961% Ash content, 1.843% Fat, 16.911% Crude protein, 2.852% Crude fibre and 1.7643% Carbohydrate Content as the mean value of replicate samples of Edible Frog (*Hoplobatrachus occipitalis*).

**Table 1. Showing the proximate composition (%) of the meat of edible frog (*Hoplobatrachus occipitalis*) meat**

Parameter	Percentage
Moisture content	71.67
Ash content	4.96
Fat	1.84
Crude protein	16.91
Crude fibre	2.85
Carbohydrate	1.76

Values of triplicates where expressed in mean

The fat content in *Hoplobatrachus occipitalis* was low compared to other meat sources as reported for *Oreochromis niloticus* [15], edible *Pelophylax esculentus* [16]. Increased level of fat promotes or facilitates an increase in the serum cholesterol level, whereby exposing one to the risk and high susceptibility to coronary heart diseases, hypertension, diabetes and breast cancer [17,18]. The fat percentage value obtained was similar to the report on *Rana esculenta* [19,20]. Protein is an essential component of the human diet needed for the repair of dead tissues and the supply of the adequate amount of required amino acid [21]. The protein percentage value was observably high in *H. occipitalis* though this value is lower than the 22.80% crude protein value of chicken [22]. This was, however, a fairly rich alternate source of meat. The moisture content percentage was the highest recorded this is similar to the report of (Onadeko et al., 2011)

Crude fibre aids digestions. The crude fibre was 2.8% although this is higher than the percentages recorded in fat and carbohydrate it cannot be regarded as a rich source of fibre as it is deficient or insufficient in ranges of 19-25%, 21-30% and 29% required by infant, adult pregnant and breastfeeding mothers, respectively [17,18].

### 3.2 Mineral Constituents

The mineral content as seen in (Table 2) the Manganese content recorded was 0.49mg/100g followed by Potassium with 1.60mg/100g,

Calcium at 3.35mg/100g, Iron was 3.44mg/100g and Sodium having the highest value 18.52 mg/100g.

**Table 2. The selected mineral contents (mg/100g) of the edible frog (*H. occipitalis*) meat**

Parameter	Content
Calcium	3.35
Iron	3.44
Potassium	1.60
Sodium	18.52
Manganese	0.49

The iron content recorded (see Table 2) was 3.44. This was the second-highest value in the selected nutrients recorded. High iron content is of great benefit to pregnant women, nursing mothers, infants and as well as elderly ones as it helps in preventing anemia and other related diseases [23]. Nevertheless, the iron in this work was considered low compared to the report on *Pelophylax esculentus* with 35.93mg/100g [16] and *Rana esculenta* [19,20].

Calcium helps in the regulation of muscle contraction and it is needed by young infants for strength and development of strong healthy bones and teeth. The calcium in this report was lower than the values obtained in literature for *Pelophylax esculentus*, quail, beef, lamb, turkey, broiler and ostrich at 477.50mg/100g, 126.55 mg/kg, 46.50 mg/kg, 19.04 mg/kg, 16.11 mg/kg, 7.83 mg/kg and 11.71 mg/kg, respectively [24]. Although *Hoplobatrachus occipitalis* is not a good source of calcium compared to the other meat source, research has proven that calcium in conjunction with manganese and protein is part of the components that facilitates bone formation [25]. Thus, *Hoplobatrachus occipitalis* will be not be regarded as a fairly good source mineral as the manganese ratio is also deficient. Thus *Hoplobatrachus occipitalis* may not a suitable source of mineral for children this can be augmented by other sources. The sodium was

**Table 3. Showing the biochemical parameters in male and female *Hoplobatrachus occipitalis***

Parameter	Unit	Male	Females
Total cholesterol	Mmol/l	2.0± 0.07	2.5±0.12
Total protein	g/l	51 ±4.42	60±6.29
Tryglycerol	Mmol/l	0.3± 0.11	0.2 ±0.12
Albumin	g/l	34±6.67	41±1.94
High density lipoprotein	Mmol/l	1. ± 0.16	1.13± 0.14
Low density lipoprotein	Mmol/l	0.92 ±0.13	1.0± 0.08

Significant (P = .05) difference according to different sex, values where expressed in mean and STD

considered high compared to other values in this report but greatly lower than the values in the literature that was recorded for *Pelophylax esculentus* with Na value of 2550mg/100g [16].

### 3.3 Serum Biochemistry Results

According to our results (Table 3) total protein concentration in the male and female species of *Hoplobatrachus occipitalis* was recorded as  $51 \pm 4.42$ g/l and  $60 \pm 6.29$ g/l, respectively. There was a significant difference between males and females at ( $P = .05$ ). In female species, the significant ( $P = .05$ ) increase in the serum total protein might be due to estrogen-induced increase of total protein, as all the blood samples were taken from matured species [26]. Also, the serum albumin concentration differs significantly ( $P = .05$ ) between males ( $34 \pm 6.67$ g/l) and female species ( $41 \pm 1.94$ g/l) in *Hoplobatrachus occipitalis*. Usually, Serum albumin increases when protein intake exceeds the amount required for growth and maintenance. Total cholesterol differed significantly ( $P = .05$ ) between males and females. The total cholesterol as recorded was higher in females ( $2.5 \pm 0.12$  mmol/l) than in males ( $2.0 \pm 0.067$  mmol/l). This was similar to the record of *L. castesbeianus* as females were higher than male thus the cholesterol level in both males and female of this study were higher than inactive and hibernating farmed bullfrog [14]. Cholesterol serves as the precursor for a variety of biologically important products, including steroid hormones (e.g. progesterone, glucocorticoids, mineralocorticoids, androgens, estrogen, etc), bile acids and Vitamin D. The Tryglycerol value in males was slightly higher than the females although there was no significant difference between both sexes. There was no significant difference in the lipoprotein (high-density lipoprotein and low-density lipoprotein) of the male and female *Hoplobatrachus occipitalis*, though the mean values obtained were increased in females than in males. Lipoproteins are large water-soluble complexes formed by a combination of lipids and protein that transport insoluble lipids through the blood between different organs and tissues, lipoprotein can be divided into the class of high-density lipoprotein and low-density lipoprotein. The serum biochemical parameters presented in this study are the first report for *H. occipitalis*, as previous scholars were concerned with the liver enzymes and biochemical alteration due to cadmium effect on the liver [27,28].

## 4. CONCLUSION

From the data gotten from this research on the proximate analysis, it could be inferred that the meat of *H. occipitalis* has low fat levels which are also seen on the serum cholesterol. This implies that *H. occipitalis* may be a good source of meat for diabetic patients compared to other meat sources such as *Oreochromis niloticus* and edible *Pelophylax esculentus*. However, the minerals were deficient making this an unsuitable meat source for infants and pregnant women although this deficiency can be provided from other sources.

The serum biochemistry showed that both male and female species have high nutritional profile suitable for human consumption; nevertheless, the female species have higher nutritional values than the males.

Thus, this represent a crucial baseline data for wild *H. occipitalis* in Nigeria and this can also be expanded upon use on nutrition as a tool in bio-monitoring of the health status of the anuran population in the future.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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