# Original Research Article

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- DETERMINATION OF THE NUTRITIVE AND ANTI-NUTRITIVE VALUES OF
- 3 Pelophylax esculentus (EDIBLE FROG) FOUND IN HANYAN GWARI, MINNA NIGER
- 4 STATE, NIGERIA

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- **Author's contributions**
- 7 This work is carried out in collaboration between all authors. Author JTM designed the study,
- 8 wrote the protocol, wrote the first draft of the manuscript and he carried out pretreatment of the
- 9 sample. Author MMN managed the literature researches, analyses of the amino acid profile.
- Authors SSM and EYS managed the experimental process. Author UB and YA identified the
- species of insect, carried out mineral and statistical analysis.

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**ABSTRACT** 

- The proximate, selected minerals, amino acid profile, functional properties and anti-nutrient 13 composition of edible frog (Pelophylax esculentus) were determined using standard analytical 14 methods of analysis. The crude protein was 31.17±1.36%, carbohydrate was found to be 15 29.02±1.16% while the crude fibre was 11.71±0.22%. The crude fat was 16.22±0.16%, ash 16 17 content was 8.93±1.33% and moisture was 3.49±0.56%. The abundance of mineral elements 18 found in the meat of P. esculentus was found to be in the order: sodium > phosphorus > 19 potassium > calcium > zinc > magnesium > copper > iron > manganese. The calorific value was 506.17 kcal/100g while the animal was also found to have reasonable amounts of essential amino 20 21 acids: tryptophan (0.39), lysine (7.62), arginine (6.13), histidine (2.13), threosine (3.94), valine 22 (4.82), methionine (2.89), leucine (7.22), isoleucine (3.83) and phyylalanine (4.14). Based on its 23 anti-nutritional contents of P. esculentus meat could be considered as a good source of animal 24 protein for man and his animals. From the result obtained *P.esculentus* could be a good low cost and easy source of animal protein, good of calcium, reasonable amount of potassium as well as 25
  - **Keywords**: edible frog, functional properties, proximate analysis, amino acid profile

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sodium.

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### INTRODUCTION

Meat is important to human beings and could be obtained from various sources. It is very good source of nutrients and vitamins to the body. Due to its high cost and some health problems associated with red meat, research is now focused on other alternatives that would help to take care of this health challenges and which would be cheaper and safer for consumption especially the aquatic animals [1]. Since meats contain essential classes of food such as, carbohydrate, proteins, fat, vitamins and minerals, they provide the nutritional requirement of man in the appropriate quantities [2]. The provision of these nutritional entities becomes a major problem in

- 37 most developing countries such as Nigeria leading to under- or malnutrition. In a view to reduce
- 38 such menace in Nigeria some lesser known animals which can serve as food are study for their
- nutritive and non-nutritive values for human consumption. One class of such known animals that
- 40 could be considered for this purpose is the amphibian [3].
- 41 Pelophylax esculentus (edible frog), formally known as Rana esculentus is considered to be of
- 42 good nutritional value [4]. It is a widespread natural hybrid that is produced as an offspring of
- 43 the parent species *P. lessonae* and *P. ridibundus* [5]. This frog is the fertile hybrid of the Pool
- 44 Frog (Pelophylax lessonae) and the Marsh Frog (Pelophylax ridibundus). It belongs to the
- 45 kingdom: animalia, phylum: chordate, class: amphibian, order: anuran, family: ranida, genus:
- pelophylax and species: p.lessonae + p.ridibundu [5]. The aim of this study is to determine the
- 47 proximate, minerals, functional properties, anti-nutritional factors and amino acid profile of
- 48 Pelophylax esculentus in order to establish the safety or otherwise of the consumption of this
- 49 amphibian by humans.

#### 50 **3.0 MATERIAL AND METHODS**

- The sample (*pelophylax escuslentus*) used in the course of this work were obtained on 24<sup>th</sup> May,
- 52 2013 from Hanya Gwari bosso around F. U. T environment in Minna, Niger State.

# 53 **3.4 Sample preparation and treatment**

- The samples were cut opened (flesh, skin and bones) and dried in an air oven at 60°C for 10 hours for
- proper removal of moisture. The fleshy parts of the samples were scrapped using a clean laboratory stainless
- steel knife, the small pieces were dry milled, kept in air tight polythene bag and stored in a dessicator
- 57 (with dessicant) prior to further analysis.

### **3.5 METHODS**

## 3.5.1 Proximate Analysis

- 60 The standard analytical procedures for food analysis were adopted for the determination of
- 61 moisture content, crude protein, crude fibre, percentage lipids, carbohydrate, acid insoluble ash
- and caloric value.

### **Moisture Content**

- 2.00 g of the sample was put into the crucible, dried in an oven at 105°C overnight. The dried
- samples were cooled in a dessicator for 30 minutes and weighted to a constant weight. The
- 66 percentage loss in weight was expressed as percentage moisture content on dry weight basis
- 67 [6]. This was repeated three times.

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### 69 Ash Content

- 70 2.00g of the ground sample was placed in a crucible and ashed in a muffle furnace at 600°C for 3
- 71 hours. The hot crucibles were cooled in a dessicator and weighted. The percentage residual
- weight was expressed as ash content [6].

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# **Crude Lipid Content**

- 75 2.00g of the sample was used for determining crude lipid by extracting lipid from it for 5 hours
- with petroleum ether in a soxhlet extractor [6].

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#### **Protein Determination**

- 79 Total protein was determined by the Kjedahl method. 0.5 g of the sample was weighed into a
- 80 filter paper and put into a Kjedahl flask, 8-10 cm3 of concentrated H<sub>2</sub>SO<sub>4</sub> were added and then
- 81 digested in a fume cupboard until the solution becomes colourless. Distillation was carried out
- 82 with about 10 cm<sup>3</sup> of 40% of NaOH. The condenser tip was dipped into a conical flash
- containing 5 cm<sup>3</sup> of 4% boric acid in a mixed indicator till the boric acid solution turned green.
- Titration was done in the receiver flask with 0.01 M HCl until the solution turned red [6].

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### **86 Crude Fibre Content**

- 87 2.00 g of each sample were used for estimating crude fibre by acid and alkaline digestion
- methods with 20% H<sub>2</sub>SO<sub>4</sub> and 20% NaOH solution [6].

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# Carbohydrate Determination

- 91 The carbohydrate content was calculated using following:
- available carbohydrate (%), = 100 [protein (%) + Moisture (%) + Ash (%) + Fibre (%) + Crude
- 93 Fat (%)].

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# Caloric Value

- The caloric value was calculated in Kilojoules per 100 g (kcal/100g) by multiplying the crude
- 97 fat, protein and carbohydrate values by Atwater factors of 37, 17 and 17 respectively.

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### Minerals analysis

Sodium and potassium were determined using Gallenkamp Flame analyzer, while calcium, magnesium, iron, manganese, zinc and copper were determined using Buch Model 205 Atomic Absorption Spectrophotometer. Phosphorus level was determined using the phosphovanado molybdate colorimetric techniques on JENWAY 6100 Spectrophotometer [7].

### **Amino acid contents**

50 g of ground sample was defatted with chloroform and methanol mixture in a ratio 1:1, then, 30 g of the defatted sample was put into a glass ampoule, 7 ml of 6 M HCl was added and oxygen expelled by passing nitrogen into the ampoule was put in the oven at 105°C for 22 h, allowed cool and filtered. The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residue was dissolved with 5ml acetate buffer (pH 2.0) and loaded into the amino acid composition and the seed samples were determined by ion exchange chromatography (IEC) method using the Technicon Sequential Multi-sample Amino acid Analyzer (Technicon Instruments Corporation, New York) [8].

### **Functional Properties**

- The standard analytical procedures for food analysis were used for the determination of bulk density, gelation capacity, water/oil absorption capacity, wettability, gelatinization temperature, viscosity and pH determination was carried out using the method of AOAC [6] while foam capacity and stability was determine using the method as described by Abbey and Ibeh [9]. The emulsification capacity was also determined by the method of Padmashree *et al.*, [10].
- 121 Anti-nutritional Properties

- Oxalate, alkaloids and flavonoids contents were determined using the method of Day and
- 124 Underwood, [7]. Saponin and tannins content was determined by the method described by
- Wheeler and Ferrel, [8].

### **Statistical Analysis**

All experiments were performed in triplicate. The results obtained were subjected to statistical analysis using mean standard deviation and analysis of variance (ANOVA)

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## 4.0 RESULTS AND DISCUSSION

Table 1: The selected mineral contents (g/100g) of the edible frog  $(Pelophylax\ esculentus)$ 

esculentus)		
Parameter	Content	
Iron	35.93±0.67	
Zinc	219.45±0.71	
Copper	54.55±0.86	
Sodium	2,550.00±2.17	
Calcium	477.50±0.36	
Potassium	679.00±1.01	
Phosphorus	$1,220.54 \pm 1.57$	
Manganese	$2.75 \pm 0.35$	
Magnesium	87.56±0.04	

Values are means of triplicate determination  $\pm$  standard deviation

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Table 2: Some anti-nutritional factors (mg/100 g) of the edible frog (*Pelophylax esculentus*)

Anti-nutritional factors	Content	
Saponin	1.75±0.35	
Tannin	5.37±0.53	
Flavonoid	1.75±0.35	
Alkaloid	$2.80 \pm 0.00$	
Oxalate	$2.78 \pm 0.00$	

Values are means of triplicate determination  $\pm$  standard deviation

 Table 3: Functional properties of the edible frog (Pelophylax esculentus)

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-	Parameter	Content
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Bulk density (g/cm <sup>3</sup> )	0.60±0.01
Oil absorption capacity (%)	2.01±0.23
Water absorption capacity (%)	4.55±0.11
Foaming stability (cm <sup>3</sup> )	56.70±0.00
Emulsification capacity (%)	50.08±1.96
Gelation capacity (%)	2.00±0.41
Gelatinization temperature( <sup>0</sup> c)	69.00±0.71
Wettability (s)	60.04±0.66
Viscosity (s)	23.27±1.66
pH	8.60±0.00

Values are means of triplicate determination  $\pm$  standard deviation

Table 4: Proximate composition (%) of the edible frog (Pelophylax esculentus)

Parameter	Percentage
Moisture content	$3.49\pm0.56$
Ash content	8.93±1.33
Crude fat	16.22±0.16
Crude fibre	11.71±0.22
Crude protein	31.17±1.36
Carbohydrate	29.02±1.16
Calorific value (kcal/100 g)	506.17

Values are means of triplicate determination  $\pm$  standard deviation

Table 5: Result of amino acids contents in edible frog (Pelophylax esculentus)

Parameter	Concentration in mg/100 g
*Lysine	7.62

*Histidine 2.13	
ΨA · · ·	
*Arginine 6.13	
Asparti acid 9.16	
*Threosine 3.94	
Serine 4.24	
Glutamic acid 13.86	
Proline 4.04	
Glycine 7.24	
Alanine 5.60	
Cysteine 0.93	
*Valine 4.82	
*Methionine 2.89	
*Isoleucine 3.83	
*Leucine 7.22	
Tyrosine 3.06	
*Phenylalanine 4.14	
*Tryptophan 0.93	
EAA (%) 47.60	
NEAA(%) 52.40  * - assential amine acid. EAA - assential amine acid. NEAA - non assential amine acid.	

<sup>\* =</sup> essential amino acid, EAA = essential amino acid, NEAA = non-essential amino acid.

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### 4.1 DISCUSSION OF RESULT

The nutritional value of a given food depends on the nutrient and anti-nutritional constituents of the food [13]. Table 1 shows that the presence of the selected mineral elements in the sample was in order: sodium > phosphorus > potassium > calcium > zinc > magnesium > copper > iron > manganese. The calculated ratio of Na/K in the body is of great importance in the control of high blood pressure. Na/K ratio of less than one is recommended, [14]. Hence *Pelophylax esculentus* meat may not be a good protein source for a diabetic patient since it had a Na/K ratio of 3.76. McDonald [15] reported that calcium in conjunction with magnesium, phosphorus, manganese,

vitamin A, C and D, chlorine and protein is involved in bone formation. From the results obtained *Pelophylax esculentus* will serve as a good source of minerals involved in bone formation since it contains large amounts of calcium and considerable amounts of magnesium but little amount of manganese. Ozkan, [16] considered a food source to be good if its Ca/P ratio is above one and poor if the ratio is less than 0.5. The Ca/P ratio of *Pelophylax esculentus* was 0.39 and based on this, the meat may have to be augumented with a higher calcium source in order to meet up the calcium requirement of the body.

Tannins and oxalate affect the bioavailability of composite nutrients, complexing with bivalent ions Ca<sup>2+</sup>, Mg<sup>2+</sup>, Fe<sup>2+</sup> and Zn<sup>2+</sup>. This makes them unavailable especially in monogastric animals [17]. From Table 2, all the anti-nutrient contents of *Pelophylax esculentus* were very low compared with the values reported for other meat sources [18].

From Table 4 it indicates that, the meat of *Pelophylax esculentus* contains lower moisture value (3.39%) which means that it might have a good shelf value [20]. The ash content of this sample was slightly high (8.71%) and this was expected because the sample was prepared by crushing both the meat and bones together. The carbohydrate value of 29.02% showed that *Pelophylax esculentus*, being an animal, is not a good source of carbohydrate. The crude fat value in the meat was much 16.22%, since crude fat is important part of diet, which decreases serum cholesterol levels risk of coronary heart disease, hypertension, diabetes and breast cancer [21]. The crude fibre contents of the meat was 11.71%, which meant that *Pelophylax esculentus* could not be a rich source of crude fibre because since this value fell short of the respective ranges of 19-25%, 21-30% and 29% required for children, adult, pregnant and lactating mothers as reported by Ishida *et al.*, [21]. The crude protein of *Pelophylax esculentus* was 31.17% which could be used to qualify it as a good source of low cost animal protein and relatively high biological value.

The result of essential and non essential amino acid profile of the *Pelophylax esculentus* was presented in Table 5. The result showed that non-essential amino acids content had higher percentage - 52.40% while essential amino acid contents amount to 47.60%. Similar amino acid compositions was recorded for *Hoplobat rachus occipitalis* reported by Onadeko *et al.*, [3]. The percentage present in both essential and non-essential amino acid were to complement each other when present in food; though they were desire in a certain quantity.

# 4.2 CONCLUSION

 From the results obtained in this study, it shows that the meat of *Pelophylax esculentus* have higher nutrient composition and calorie value. It also indicate high content of mineral elements composition given the Na/K ratio is above 1 which may not be too good for a diabetic patient. *Pelophylax esculentus* also showed higher nutritional values compared to some meat most especially in terms of crude protein, this will make them a good source of animal protein.

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