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Full Length Research Paper

Nutrient composition of *Rana galamensis*

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The proximate composition, amino acid, fatty acid and mineral profiles of Rana galamensis were investigated. The results of the proximate analysis revealed that R. galamensis has high level of protein (53.74 ± 0.89) with considerable amount of lipid (9.52 ± 0.31) and very low fibre content. The amino acid profile showed that it contains seventeen amino acids with nine indispensable amino acids being higher than those recommended by WHO/FAO reference; the ether extract from R. galamensis contains four common fatty acids, lauric acid, palmitic acid, stearic acid and linoleic acid. The result also revealed that R. galamensis is a good source of dietary minerals especially selenium, calcium and phosphorous. R. galamensis is therefore considered to be a rich source of essential nutrients that would be useful for both animal and human.

Keywords: Rana galamensis; proximate analysis; amino acid profile; fatty acid profile; mineral profile.

INTRODUCTION

Of all the food nutrients, requirements for protein are very crucial without which life would be impossible (Brain and Allan, 1977a). But most people in less developed countries, to which Nigeria belongs, are facing inadequate intake of protein called protein–energy–malnutrition (PEM), common in growing children (Franco *et al*., 1999). This is the most important nutritional problem in the world (Murray *et al*., 2000). Due to the economic, agriculture and social deterioration in Nigeria and most African countries, most people cannot afford the exorbitant prices of egg, milk, meat and fish (Oloyede and Fowomola, 2003). Thus, it is important for the nutritionist to search for alternative (new) sources of high protein quality. One of the most readily and cheapest (than fish and other animal's) sources of protein is *Rana galamensis.*

Rana galamensis, commonly called galam white-lipped frog belongs to the family Ranidae, which has widest distribution of any frog family, and the class amphibian. It is a strongly aquatic species, where it lives in and around permanent lakes, rivers, ponds and swamps. *R. galamensis* is abundant throughout most of the continents except Antarctica. In Africa they are found in savannah region of West Africa, South Africa and East Africa (Rodel *et al.,* 2004). In Nigeria, they are found in many states such as Lagos, Ogun, Oyo, Kwara, Osun, Ondo, Ekiti, Kaduna and Benin City (Walker, 1967). The fresh *R. galamensis* could be salted and sundried to make them into stock for transportation into various parts of the country. Consumption of *R. galamensis* in various parts of Nigeria and other West Africa countries had been reported (Mohneke *et al*., 2010), however, information on its nutritive value is still very scanty in literature. Therefore, the objective of this study was to determine the nutrient compositions of *R. galamensis.*

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MATERIALS AND METHODS

Twenty-five dried, adult *Rana galamensis* were obtained from 'Oja Tuntun' market, in Ilorin, the North Central part of Nigeria. The samples were milled, using the local grinding machine and used for analysis.

Proximate Analysis

The crude protein, lipids, fibre, and ash were determined in triplicates using the procedures of AOAC (1990), while the carbohydrate content was determined by difference method (calculated by subtracting the sum of crude protein, crude fat, ash and crude fibre from total dry matter content). The nitrogen concentration of the milled sample was determined by the micro-kjeldahl method and multiplied by 6.25 to estimate the crude protein content. The lipid content was determined by the usual procedure of continuously extracting the fat content of a sample using

Table 1: Proximate analysis of *Rana galamensis* in g /100g

Parameters	*Rana galamensis*
Ash	6.10 ± 1.08
Lipid content	9.52 ± 0.31
Fibre	1.60 ± 0.30
Protein	53.74 ± 0.89
Carbohydrate (by differences)	29.04 ± 0.01

Each value is a mean of three determinations ± SEM

Table 2: Amino acid profile of *Rana galamensis* (g / 100g protein)

Amino acids	*Rana galamensis*	FAO/WHO (1990)
Lysine	6.93± 0.02	5.80
Histidine	3.13± 0.01	2.5
Arginine	6.55± 0.02	5.2
Asparatic acid	9.66±0.21	7.7
Threonine	4.33±0.05	3.40
Serine	5.15±0.03	7.7
Glutamic acid	13.24 ±0.25	14.7
Proline	5.10 ±0.03	10.7
Glycine	5.01±0.02	2.2
Alanine	6.10±0.11	6.1
Cystine	1.06±0.01	3.0
Valine	4.82±0.04	5.00
Methionine	3.00±0.34	2.50
Isoleucine	4.00±0.02	2.80
Leucine	7.05±0.02	6.60
Tyrosine	4.02±0.01	1.10
Phenylalanine	5.02±0.02	6.30

Each value in column 2 is a mean of three determinations ± SEM

Table 3: Fatty acids composition of the oil of *Rana galamensis*

Fatty acids	Composition (%)
Lauric acid (12:0)	45
Palmitic acid (16:0)	1.23
Stearic acid (18:0)	0.100
Linoleic acid (18:2)	0.00114

petroleum ether (40 – 60 ^{o}C) as a solvent in a soxhlet extractor, which is based on the principle that non-polar components of a sample are easily extracted into organic solvent-ether. Crude fibre was determined by defatting a known weight of the sample with petroleum ether. The defatted sample was boiled under reflux with H_2SO_4, filtered and washed with boiling water till the filtrates were no longer acidic. The residue was boiled with NaOH, filtered and washed with boiling water till the filtrates were no longer alkaline. The residue was dried in the oven at 100 ^{o}C, cooled in a desiccator and weighed. This was then incinerated in a muffle furnace at about 600 ^{o}C, cooled in a dessiccator and weighed. The ash content was estimated by heating a known weight of the sample inside a pre-weighted porcelain crucible in a muffle furnace at 600 ^{o}C

Amino acid determination

The amino acids profile of the sample was determined using the method described by Spackman *et al.,* (1958). The samples were dried to a constant weight, defatted (so as to remove non-polar component of the sample), hydrolysed (by 7ml of 6N HCl), evaporated using a rotatory evaporator and between five to ten microlitre of the hydrolysate was loaded into the Technicon Sequential Muti- sample Acid Analyzer (TSM). The TSM analyzer was designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of an analysis lasted for 76 minutes. The chromatogram obtained showed amino acids peaks corresponding to the magnitudes of their concentrations. The net height of each peak produced by the chart–recorder of TSM (each representing an amino acid) was measured. The half–height of the peak on the chart and width of the peak on the half–height were measured and recorded. Approximate area of each peak was then obtained by multiplying the height with the width at half- height. Finally, the amount of each amino acid present in the sample was calculated in g/ 100g protein.

Fatty acid determination

This was carried out using AKTA high performance liquid chromatography due to its high sensitivity and resolution. HPLC makes use of high - pressure pumps that speed the movement of the protein molecules down the column, as well as higher–quality chromatographic materials that can withstand the crushing force of the pressurized flow, by reducing the transit time on the column. AKTA HPLC (made in Europe) with a mobile phase consisting of acetonitrile to acetone (51:49) was used for this analysis. Concentrated fatty acid methyl esters was injected into the column (ODS 2 G8) at injection point. Fatty acid detection was carried out by using retention minute to detect the fatty acid profile in the sample (AOAC, 1990).

Mineral determination

The determination of the levels of inorganic minerals of *R. galamensis* was carried out using the Perchloric acid digestion (wet oxidation) procedure. The minerals were brought into solution by wet digestion using conc. HNO_3, H_2SO_4 and perchloric acid in the ratio (4.1.1) (Harris, 1979). P and Fe were determined using the colorimetry method while determination of the Zn, Ca, Mn and Mg were carried out using the atomic absorption spectrophotometer (Perkin – Elmer Model 290B) (Gomori, 1942; Piper, 1944; Perkin-Elmer Corp., 1968; AOAC, 1990).

Results and Discussion

The proximate compositions of *R. galamensis* are shown in Table 1. *R. galamensis* contains a high protein and understandably, low fibre content. The protein content of *R. galamensis* (53.73g/100g) is higher than those reported in moon fish (39.3g/100g) and cat fish (43.70g/100g) (Abdullahi, 1999; Abdullahi *et al*., 2001), Haddock (17g/100g), Sardine (20g/100g), Mackerel (12g/100g) and Oyster (11g/100g) (Brain and Allan, 1977b; Pearson, 1981), and beef (18g/100g), lamb (16g/100g) and pork (10g/100g) (Bhulyan *et al*., 1993). Therefore *R. galamensis* can serve as a better source of protein especially, with its high levels of indispensable (essential) amino acids (Table 2). The carbohydrate

Table 4: Mineral content of *Rana galamensis* (mg / 100g)

Minerals	*Rana galamensis*	FAO/WHO (1974)
Fe	59.00 ± 0.09	6 – 15/day
Mg	429.00 ± 0.02	460/day
Se	8065.36± 0.52 (ppm)	1000 – 4000µg/day
Cu	1.60 ± 0.01	0.2 – 1.3/day
Co	7.00 ± 0.001	10 – 15/day
P	14.86 ± 0.13	20 – 23.8/day
Ca	2105.00 ± 0.40	1000 – 1400/day
Zn	Trace	3 – 5/day

Each value is a mean of three determinations ± SEM

content of the *R. galamensis* was higher than those reported in moon and cat fishes (Abdullahi, 1999; Abdullahi *et al.,* 2001). The carbohydrate content, if digestible could serve as a source of energy. The relatively low lipid content (9.52 ± 0.31 %) of *R. galamensis* (Table 1), compared to that of moon fish (27.7 ± 1.06g/100g) and cat fish (32.30 ±1.32g/100g) (Abdullahi, 1999; Abdullahi *et al*., 2001), would probably make it a useful ingredient in poultry feeds than the fish meals. This is because very high fat content in feed ingredients would cause difficulty in mixing the feed and could even predispose the feed to oxidative rancidity (Ewing, 1951; Atteh, 2002). The roughage (fibre) quantity of *R. galamensis* may due to its tough skin. This plays an important role in preventing colon cancer and constipation (Ahmed, 1995; Bingham *et al*., 2003; Park *et al*., 2005).

The amino acid profiles of *R. galamensis* are shown in Table 2. Seventeen amino acids were observed; out of which nine were indispensable with leucine having the highest concentration. The seventeen amino acids present in *R. galamensis* were also reportedly present in moon and cat fishes (Abdullahi, 1999; Abdullahi *et al.,* 2001). The indispensable amino acids (lysine, histidine, arginine, threonine, valine, methionine, isoleucine, leucine, and phenylalanine) are within FAO/ WHO 1990 reference values for daily intake. These amino acids serve as raw materials for the synthesis of many other cellular products, including hormones, enzymes and pigments. In addition, several of these amino acids are key intermediates in cellular metabolism (Murray *et al.,* 2000). The present study indicates that *R. galamensis* can serve as a source of indispensable amino acids for consumers. Also, the lower quantity of proline in *R. galamensis* would probably make it more digestible, thus releasing all the amino acids in it into the amino acid pools of the body. The absence of tryptophan as the tenth indispensable (essential) amino acid could be that it was destroyed by hydrolysis. This is because it had been reported by Wilson and Walker (2000), that hydrolysis procedure either destroys or chemically modifies the asparagines, glutamine and tryptophan residues in protein. Asparagine and glutamine are converted to their corresponding acids (aspartic and glutamic acids) while tryptophan is completely destroyed. Therefore, tryptophan of unhydrolysed *R. galamensis* should be determined spectrophotometrically.

Also, the fatty acid compositions of ether extract of *R. galamnesis* are shown in Table 3. Out of the four fatty acids detected, lauric acid has the highest concentration and traces of the essential fatty acid, linoleic acid, was equally detected. The present of four fatty acids out of twenty fatty acids common in aquatic animal could be has as a result of factors such as water temperature, time of capture, salinity and feed type (de Castro *et al.,* 2007). Lauric acid, found in high quantity in *R. galamensis*, is the main acid in coconut and palm kernel oils, and is believed to have antimicrobial properties (Hoffman *et al.,* 2001; Ouattar *et al*., 2000; Dawson *et al*., 2002). It is also found in human milk (5.8% of total fat), cow milk (2.2%), and goat milk (4.5%), this fatty acid can undergo β-oxidation to produce energy and also be stored in adipose tissues (Nelson and Cox, 2005). Palmitic acid is one of the most common saturated fatty acids found in animals and plants tissues. Palmitic acid is the first fatty acid produced during lipogenesiss (fatty acid synthesis) and from which longer fatty acids can be produced (Murray *et al.,*2000). *R. galamensis* also has stearic acid which is a very useful ingredient in dietary supplements (Wootthikanokkhan and Tunjongnawin, 2002). Linoleic acid, an unsaturated omega- 6 fatty acid, is a polyunsaturated fatty acid used in the biosynthesis of arachidonic acid (AA) and thus some prostaglandins (Nelson and Cox, 2005). It is found in the lipids of cell membranes. These four common fatty acids are also found in fishes (Ackman, 1990).

In addition, Table 4 shows the macro and micro minerals of *R. galamensis*. The levels of the minerals compared favorably with those reported for moon and cat fishes (Abdullahi, 1999; Abdullahi *et al.,* 2001). The levels of iron, selenium, copper, and calcium observed in *R. galamensis* (Table 4) are above the WHO (1974) recommended values, while the concentration of the other minerals are close to the WHO (1974) values, except zinc which was in trace amount, thus *R. galamensis* could be a good source of dietary minerals. Calcium, magnesium and phosphorous are very important minerals in bone and teeth development, iron is very crucial in haemoglobin and cytochromes, cobalt is a constituent of vitamin B12, copper plays a crucial role in iron absorption (Murray *et al*., 2000). The high quantity of selenium in *R. galamensis*, may be as a result of consumption of aquatic plants by the animal. Selenium, apart from its role in glutathione peroxidase (Rotruck, 1973; Muhammad *et al*., 2001), is very important in the treatment of cancer, cardiovascular disease, strokes and heart attacks (Stranges *et al*., 2006; Bardia *et al*., 2008).

CONCLUSION

The results of this study demonstrate the potentials of *R. galamensis* as a rich source of essential nutrients required in the diets of both man and his animals.

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