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RESEARCH ARTICLE

COMPARATIVE STUDY OF THE PROXIMATE COMPOSITION OF *PYXICEPHALUS ADSPERSUS* AND *OREOCHROMIS NILOTICUS* FROM NIGERIAN WETLAND

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ABSTRACT

A study was conducted to evaluate the carbohydrate, lipid, ash, protein, moisture and fiber contents of samples of African bullfrog (Pyxicephalus adspersus) and tilapia (Oreochromis niloticus) collected from ARAC, Aluu, Port Harcourt, Nigeria from Novenber 2013 – February 2014. Slight variations in chemical compositions of both species, some of which were statistically significant, were observed in the study. The range of mean values of carbohydrate (0.54 ± 0.01 – 1.29 ± 0.10%), lipid (0.99 ± 0.33 – 4.54 ± 2.13%), ash (4.02 ± 0.51 – 7.20 ± 1.67%), protein (6.41 ± 0.46 – 13.53 ± 0.74%), moisture (71.86 ± 0.51 – 78.95 ± 3.44%) and fiber (2.31 ± 0.54 – 14.67 ± 2.31%) recorded were consistent with values reported in previous studies. P. adspersus and O. niloticus both represent an attractive and important source of animal protein, providing approximately some 10 – 13% of animal protein for human consumption. Animals with lipid content < 5% are classified to have low-oil content. Lipid contents of P. adspersus and O. niloticus recorded in this study were all below 5%, and therefore falls within the low-oil category that is nutritionally fit for human consumption.

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INTRODUCTION

All through the globe, geometric rise in human populations has been observed to impose varying degrees of threats to the availability and distribution of food. This problem is more pronounced in the third world countries in which exponential rise in human populations have come with corresponding increase in the quest for alternative sources of animal protein in order to meet the increasing need for protein. Fish is a major source of animal protein across the world and the demand for it has risen over the years. Edible frogs have been found to be proteinous and represent an alternative source of animal protein particularly in areas where fish and other protein sources are either in short supply or relatively more expensive. Therefore, providing adequate supply of protein to meet the world's teeming populations of humans is probably one of the greatest challenges facing the human race today. It is currently estimated that more than 925 million people across the globe are chronically malnourished and are targets of kwashiorkor occasioned by insufficient protein supply (FAO, 2011). Edible frogs are eaten in many parts of the world. Recently there has been an increasing trend in the exportation of frogs from developing to developed countries. Frog leg is a popular delicacy in Europe (Ashton *et al*., 1988).

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These edible frogs resemble chicken in terms of taste. Apart from having high protein content, studies have shown they also have characteristically low fats and calorie content. In many countries, including Nigeria, frogs are collected on a local scale as an essential source of animal protein (Angulo, 2008; Mohneke *et al*., 2009). Within the past decade, frogs have become international trade items involving more than 30 countries, accounting for approximately USD 48.7 million in 1998 (Teixeira *et al*., 2001). On the other hand, tilapia is one of the most commercially important aquaculture species. Despite the high dependence on fish as a source of animal protein, fish consumption in Sub-Saharan Africa is the world's lowest. The African continent is projected to be in need of additional 1.6 million tons by the year 2015 just to maintain current consumption.

Chemical composition of animals varies greatly from one species to another and even among the individuals within the same species (Alkobaby *et al*., 2008). Some studies on proximate composition of tilapia (Bombata-Fashina *et al*., 2013) and edible frogs (Cagiltay *et al*., 2011; Cagiltay *et al*., 2014; Ozugul *et al*., 2008) have been conducted around the globe in the last two decades. In Nigeria, edible frogs are important delicacies and feature prominently in the diets of many local communities. Unfortunately, very limited studies have been conducted to evaluate the chemical composition of edible frogs in Nigeria.

This study, therefore, was designed to investigate carbohydrate, lipid, ash, protein, moisture and fiber contents of African bullfrog (*Pyxicephalus adspersus*) and tilapia *(Oreochromis niloticus)* collected from the African Regional Aquaculture Center (ARAC) in Aluu, Port Harcourt, Rivers State, Nigeria.

Sampling

Fresh specimens of edible frogs (*Pyxicephalus adspersus*) and tilapia (*Oreochromis niloticus*) were collected from African Regional Aquaculture Center (ARAC) in Aluu Port Harcourt, Rivers State with the aid of fishing nets. The nets were thrown in a targeted area where frogs have been seen and after a few minutes the net was dragged with the animals caught in it. Samples of *O. niloticus* were collected from earthen ponds in a similar manner. The animals were transferred to plastic tanks containing water and later transported to the laboratory proximate composition analyses.

Laboratory Methods

Animals were immobilized by a blow on the head and thereafter cleaned with water and white handkerchief. In the laboratory, proximate analyses were carried out on replicate samples (n = 2) of *P. adspersus* and *O. niloticus* respectively over a period of four (4) months from November 2013-February 2014, in order to determine carbohydrate, lipid, ash, protein, moisture, and fiber content of the animals.

Carbohydrate Content

Carbohydrate was determined using Cleg Anthrone Method (AOAC, 1994) and carbohydrate as glucose calculated using the formula below:

$$\%\,CHO\,as\,glucose = \frac{25\,x\,absorbance\,of\,sample}{absorbance\,of\,standard\,glucose}$$

Lipid Content

Lipid was determined by the Soxhlet Extraction Method. Lipid was extracted with a non-polar solvent, ethyl ether using the modified Bligh and Dyer procedure (AOAC, 1994), in which the solvent was first evaporated and the extracted materials weighed from which % lipid content was calculated as shown below:

$$\%lipid = (weight of\ extract / weight of\ sample)\,x100$$

Ash Content

The ash content was determined by loss on ignition. Samples were ignited at a temperature of about 630°C for 3 h in an electric muffle furnace until samples were completely free of carbon particles (residue becomes white). After ignition, % ash content was calculated as follows:

$$\%ash = (weight of\ ash / weight of\ sample)\,x100$$

Protein Content

Protein was determined by the kjeldahl method. In this method, organic nitrogen is converted to $(NH_4)_2SO_4$ by digestion with conc. H_2SO_4 in a Kjeldahl flask. The digest was then diluted, made alkaline with 40 ml of 40% NaOH and thereafter distilled. The liberated NH_3 which was collected in a solution of H_3BO_3 was then determined titrimetrically, and the percentage of protein in sample calculated as follows:

$$\%\,protein = (c-b)\,x14\,x\,d\,x\,6.25 / a\,x1000\,x100$$

where a = sample weight (g), b = vol. of NaOH required for back titration & neutralization of 25 ml 0.1 N H_2SO_4 (for sample), c = vol. of NaOH required for back titration & neutralization of 25 ml 0.1 N H_2SO_4 (for blank), d = normality of NaOH, 6.25 = conversion factor of N to protein & 14 = atomic weight of N.

Moisture Content

Moisture was determined by drying the sample to a constant weight in a hotbox oven using the oven-dry method (AOAC, 1994), and percentage moisture content calculated as shown below:

$$\%\,moisture = (weight\ loss / original\ weight\ of\ sample)\,x100$$

Data Analysis

Analysis of variance (ANOVA) was carried out to evaluate significant differences in mean proximate compositions (carbohydrate, lipid, ash, protein, moisture and fiber content) of samples of *P. adspersus* and *O. niloticus* at the 95% significance level using the Data Analysis ToolPak of the Microsoft Excel. Samples were collected in two replicates (male and female, n = 2) for each species. Where ANOVA indicated a significant difference, pairwise comparisons were conducted using the Tukey test (Fowler & Cohen, 1990) to determine pairs of significant means. A trellis was constructed for pairwise comparison of all sample means. The *T* test statistic was computed to provide a standard against which the mean differences in the trellis were tested as shown below:

$$T = (q)\,x\sqrt{\frac{within\,variance}{n}}$$

where $n = 2$ (no of sampling units in each sample). The within variance was obtained from each ANOVA summary table, while q was extrapolated from the Tukey table for the appropriate number of means (a) and degrees of freedom (v) at $p = 0.05$ level of significance.

RESULTS

The means and standard deviations of carbohydrate content of *Pyxicephalus adspersus* and *Oreochromis niloticus* are as presented in Table 1. Mean carbohydrate content generally ranged from a minimum in *P. adspersus* (0.54 ± 0.01%) to a maximum in *O. niloticus* (1.29 ± 0.10%). Carbohydrate was not recorded in tissue samples of animals during the months of November and December. However, in January comparatively higher values were observed in both species, with a peak value (1.29 ± 0.10%) in *O. niloticus*. The ANOVA on carbohydrate content was significant between species ($F_{1,8} = 34.82$, $p < 0.05$) and periods ($F_{3,8} = 99.44$, $p < 0.05$) (Table 2). ANOVA also showed a significant interaction between species and periods. Significantly higher values of carbohydrate content were recorded in *O. niloticus* in January and February compared to those of *P. adspersus* in the preceding months (Tukey, $p < 0.05$).

Table 1. Carbohydrate Content ($\bar{x} \pm SD, n=2$) of *Pyxicephalus adspersus* and *Oreochromis niloticus* from ARAC, Aluu Port Harcourt (Nov 2013-Feb 2014)

Species	Carbohydrate Content (%)			
	Nov 2013	Dec 2013	Jan 2014	Feb 2014
Pyxicephalus adspersus	0.00 ± 0.00	0.00 ± 0.00	0.64 ± 0.26	0.54 ± 0.01
Oreochrmis niloticus	0.00 ± 0.00	0.00 ± 0.00	1.29 ± 0.10	1.12 ± 0.09

Table 2. ANOVA on Carbohydrate Content (%) of *Pyxicephalus adspersus* and *Oreochromis niloticus* from ARAC, Aluu Port Harcourt (Nov 2013-Feb 2014)

Source of Variation	SS	df	MS	F	P-value
Species	0.38	1	0.38	34.82	< 0.05*
Periods	3.24	3	1.08	99.44	< 0.05*
Interaction	0.38	3	0.13	11.70	< 0.05*
Within	0.09	8	0.01		
Total	4.09	15			

* Significant at $p < 0.05$

Table 3. Lipid Content ($\bar{x} \pm SD, n=2$) of Pyxicephalus adspersus and Oreochromis niloticus from ARAC, Aluu Port Harcourt (Nov 2013-Feb 2014)

Species	Lipid Content (%)			
	Nov 2013	Dec 2013	Jan 2014	Feb 2014
Pyxicephalus adspersus	1.66 ± 0.76	2.73 ± 0.67	3.69 ± 0.93	2.88 ± 0.17
Oreochrmis niloticus	4.33 ± 1.28	4.54 ± 2.13	2.33 ± 0.54	0.99 ± 0.33

Table 4. ANOVA on Lipid Content (%) of *Pyxicephalus adspersus* and *Oreochromis niloticus* from ARAC, Aluu Port Harcourt (Nov 2013-Feb 2014)

Source of Variation	SS	df	MS	F	P-value
Species	0.37	1	0.37	0.35	> 0.05
Periods	5.94	3	1.98	1.86	> 0.05
Interaction	15.44	3	5.15	4.84	< 0.05*
Within	8.50	8	1.06		
Total	30.25	15			

* Significant at $p < 0.05$

Table 5. Ash Content ($\bar{x} \pm SD, n=2$) of *Pyxicephalus adspersus* and *Oreochromis niloticus* from ARAC, Aluu Port Harcourt (Nov 2013-Feb 2014)

Species	Ash Content (%)			
	Nov 2013	Dec 2013	Jan 2014	Feb 2014
Pyxicephalus adspersus	4.02 ± 0.51	5.76 ± 0.66	5.96 ± 0.77	5.98 ± 0.61
Oreochrmis niloticus	6.51 ± 1.02	7.20 ± 1.67	6.39 ± 0.33	6.17 ± 0.28

Table 6. ANOVA on Ash Content (%) of Pyxicephalus adspersus and Oreochromis niloticus from ARAC, Aluu Port Harcourt (Nov 2013-Feb 2014)

Source of Variation	SS	df	MS	F	P-value
Species	5.18	1	5.18	7.29	< 0.05*
Periods	3.22	3	1.07	1.51	> 0.05
Interaction	3.32	3	1.11	1.56	> 0.05
Within	5.68	8	0.71		
Total	17.39	15			

* Significant at $p < 0.05$

Mean lipid content remained fairly constant in *O. niloticus* between November and December (4.33 ± 1.28% – 4.54 ± 2.13%) but dropped abruptly in January (2.33 ± 0.54%), reaching a minimum value (0.99 ± 0.33%) in February (Table 3). Although lipid content rose to a peak value in P. adspersus in January (3.69 ± 0.93%), it fell slightly again in February (2.88 ± 0.17%). The result of ANOVA on lipid content was not found to be significant between species and periods (Table 4).

Ash content showed roughly similar trend in both *P.adspersus* and *O. niloticus*, with mean values ranging from 4.02 ± 0.51% – 5.98 ± 0.61% and 6.17 ± 0.28% – 7.20 ± 1.67% for *P.adspersus* and *O. niloticus* respectively (Table 5). The ANOVA on ash content was significant between species ($F_{1,8}$ = 7.29, $p < 0.05$) but not between periods ($F_{3,8}$ = 1.51, $p > 0.05$) (Table 6). Mean protein content of *P. adspersus* fell progressively from a maximum in November (12.04 ± 0.87%) to a minimum in February (6.41 ± 0.46%).

Table 7. Protein Content ($\bar{x} \pm SD, n=2$) of *Pyxicephalus adspersus* and *Oreochromis niloticus* from ARAC, Aluu Port Harcourt (Nov 2013-Feb 2014)

Species	Ash Content (%)			
	Nov 2013	Dec 2013	Jan 2014	Feb 2014
Pyxicephalus adspersus	12.04 ± 0.87	10.63 ± 1.48	8.58 ± 0.47	6.41 ± 0.46
Oreochrmis niloticus	9.03 ± 2.32	13.53 ± 0.74	12.72 ± 1.90	10.85 ± 1.94

Table 8. ANOVA on Protein Content (%) of *Pyxicephalus adspersus* and *Oreochromis niloticus* from ARAC, Aluu Port Harcourt (Nov 2013-Feb 2014)

Source of Variation	SS	df	MS	F	P-value
Species	18.00	1	18.00	8.64	< 0.05*
Periods	24.05	3	8.02	3.85	> 0.05
Interaction	36.37	3	12.12	5.82	< 0.05*
Within	16.67	8	2.08		
Total	95.09	15			

* Significant at $p < 0.05$

Table 9. Moisture Content ($\bar{x} \pm SD, n=2$) of Pyxicephalus adspersus and Oreochromis niloticus from ARAC, Aluu Port Harcourt (Nov 2013-Feb 2014)

Species	Ash Content (%)			
	Nov 2013	Dec 2013	Jan 2014	Feb 2014
Pyxicephalus adspersus	78.47 ± 1.65	77.80 ± 0.69	72.23 ± 2.67	71.86 ± 0.51
Oreochrmis niloticus	77.03 ± 0.66	77.75 ± 3.27	78.95 ± 3.44	78.72 ± 2.28

Table 10. ANOVA on Moisture Content (%) of *Pyxicephalus adspersus* and *Oreochromis niloticus* from ARAC, Aluu Port Harcourt (Nov 2013-Feb 2014)

Source of Variation	SS	df	MS	F	P-value
Species	36.51	1	36.51	7.54	< 0.05*
Periods	21.78	3	7.26	1.50	> 0.05
Interaction	57.73	3	19.24	3.97	> 0.05
Within	38.75	8	4.84		
Total	154.77	15			

* Significant at $p < 0.05$

Table 11. Fiber Content ($\bar{x} \pm SD, n=2$) of Pyxicephalus adspersus and Oreochromis niloticus from ARAC, Aluu Port Harcourt (Nov 2013-Feb 2014)

Species	Fiber Content (%)			
	Nov 2013	Dec 2013	Jan 2014	Feb 2014
Pyxicephalus adspersus	3.82 ± 1.25	2.31 ± 0.54	14.67 ± 2.31	12.34 ± 0.40
Oreochrmis niloticus	3.11 ± 1.92	4.88 ± 0.27	5.61 ± 1.55	2.39 ± 0.78

Table 12. ANOVA on Fiber Content (%) of Pyxicephalus adspersus and Oreochromis niloticus from ARAC, Aluu Port Harcourt (Nov 2013-Feb 2014)

Source of Variation	SS	df	MS	F	P-value
Species	73.53	1	73.53	41.67	< 0.05*
Periods	124.46	3	41.49	23.51	< 0.05*
Interaction	114.75	3	38.25	21.68	< 0.05*
Within	14.12	8	1.76		
Total	326.85	15			

* Significant at $p < 0.05$

On the other hand, protein content of *O. niloticus* was found to lie in the range 9.03 ± 2.32% – 13.53 ± 0.74% (Table 7). The result of ANOVA on protein content was found to be significant between species ($F_{1,8} = 8.64$, $p < 0.05$) (Table 8). Moisture content was fairly stable in *O. niloticus* with mean values ranging from 77.03 ± 0.66 – 78.95 ± 3.44%. *P. adspersus* showed slight variations in moisture content with means that fell in the interval 71.86 ± 0.51 – 78.47 ± 1.65% (Table 9).

ANOVA showed differences in means which were significant between species ($F_{1,8} = 7.54$, $p < 0.05$) (Table 10). Significantly higher mean values were recorded for *O. niloticus* in January and February compared to *P. adspersus* (Tukey, $p < 0.05$). Mean fiber content of *O. niloticus* varied minimally across sampling period and ranged from a minimum value of 2.39 ± 0.78% to a maximum of 5.61 ± 1.55%. Comparatively higher values were recorded in *P. adspersus* especially during the months of January and February in which

mean values were recorded as 14.67 ± 2.31% and 12.34 ± 0.40% respectively (Table 11). The ANOVA on fiber content showed that the observed differences were significant between species ($F_{1,8} = 41.67$, $p < 0.05$) and periods ($F_{3,8} = 23.51$, $p < 0.05$) (Table 12). ANOVA also revealed significant interaction between species and periods ($F_{3,8} = 21.68$, $p < 0.05$). Mean fiber content of both species tended to be higher in January compared to the preceding months.

DISCUSSION

Frog meat is an attractive food choice in high class restaurants. In Nigeria, it is delicacy in many rural settings especially in the southern part of the country where wetlands and floodplains provide conducive environment for breeding. Frogs are mainly collected from nature. However, with rising human activities involving various degrees of industrial operations, the population of edible frogs has declined considerably. Destruction of wetlands, unsustainable capture operations and declining water resources are some of the factors that have been implicated in the progressive decline of populations of edible frogs (Cagiltay *et al*., 2014). In Nigeria the African bullfrog, *Pyxicephalus adspersus* is widely consumed and features in the diet of many local communities. This is also true of the American bullfrog (*Rana catesbeiana*) , Indian bullfrog (*Rana tigrina*) and the green frog (*Rana esculenta*) which have been reported to be widely consumed in the Middle and Southern American as well as Asian countries (Alvarez & Real, 2006). Trade in frozen frog leg is a flourishing business in Europe, with the highest demand recorded in France. France alone imports 3000-4000 tons of frozen frog legs and 700-800 tons of live frogs annually (Cagiltay *et al*., 2014). In Europe, Turkey, Bangladesh, Albania, China, Malaysia and Indonesia have been listed as countries with ready markets for trade in edible frogs (Negroni, 1997; Neveu, 2004; Neveu, 2009). In 2008, Nile tilapia (*Oreochromis niloticus*) culture alone was ranked fifth among the most cultured species in the world with a total aquaculture production of 2.3 million. *O. niloticus* represents ≈ 84% of the total global tilapia production (FAO, 2009), with China reported to be the largest consumer and producer (≈ 46% of global production) of tilapia. China alone is estimated to have produced 1.15 million tons of tilapia in 2009. Tilapia has been rated man's most important single source of high quality protein, providing 16% of the animal protein consumed by the world's population (FAO, 1997). Is it any wonder, therefore, that numerous studies have been conducted to evaluate the nutritional potential of edible frogs and tilapia in the last two decades?

Chemical composition of animals varies greatly from one species to another and even among the individuals within the same species (Alkobaby *et al*., 2008). Mean protein content recorded in this study was found to vary from 6.41 ± 0.46% - 12.04 ± 0.87% and 9.03 ± 2.32% - 13.53 ± 0.74% for of *P. adspersus* and *O. niloticus* respectively. Wide ranging variations in protein content was reported by Bombata-Fashina *et al*. (2013) in a study conducted to investigate the proximate composition of some wild tilapiine fishes in Epe Lagoon, Lagos, Nigeria. According to these workers, protein content of tilapia ranged from 18.08 – 21.80%. In a similarly related study, Cagiltay *et al*. (2014) also reported mean values of protein in wild marsh frog (*Rana ridibunda*) that fell within the interval 17.82 – 19.22%. Other related studies include the works of Ozugul *et al*. (2008) who also reported a mean value of 19.22% in studies conducted on *Rana esculenta* and Cagiltay *et al*. (2011) who recorded values within the range 16.58 ± 0.14%, 18.94 ± 0.01% and 19.37 ± 0.03% in wild frogs collected from Adana, Bursa and Trakya regions respectively in Turkey. The results of protein content of *P. adspersus* and *O. niloticus* obtained in this study were, therefore, found to be consistent with values reported in previous investigations. Lipid content of *P. adspersus* and *O. niloticus* recorded in this study ranged from 1.66 ± 0.76 – 3.69 ± 0.93% and 0.99 ± 0.33 – 4.54 ± 2.13% respectively and was found to be slightly higher than ranges of 0.40 – 0.90% and 0.59 – 0.89% which were reported for tilapia (Bombata-Fashina *et al*., 2013) and edible frogs (Cagiltay *et al*., 2014; Ozugul *et al*., 2008) respectively. According to Stansby (1982) and Ackman (1989), animals with lipid content < 5% are generally classified as lean animals. Mean values obtained in the current study were generally below 5% and fall within the range classified as low-oil by Bombata-Fashina *et al*., (2013). Ash content of 1.00 – 1.75% and 1.14 – 1.60% reported in previous studies in tilapia (Bombata-Fashina *et al*., 2013) and edible frogs (Cagiltay *et al*., 2014) respectively were found to be smaller than the range reported in *P. adspersus* (4.02 ± 0.51 – 5.98 ± 0.61%) and *O. niloticus* (6.17 ± 0.28 – 7.20 ± 1.67%) in the current study. It is well known and documented that moisture and lipid contents in fish muscles are inversely related and that their sum is ≈ 80%, with other components accounting for the remaining 20% (FAO, 1999). This finding agrees with the observations made in the current study in which sum of moisture and lipid contents were found to be over 70%, ranging from ≈74 – 81% and 80 – 82% in *P. adspersus* and *O. niloticus* respectively.

Conclusion

Although chemical composition of animals have been shown to vary greatly from one species to another and even among the individuals within the same species, the variations in protein and lipid contents observed between *P. adspersus* and *O. niloticus* are within normal ranges. This study represents at least an attempt to evaluate the nutritional potential of edible frogs (*P. adspersus*) and tilapia (*O. niloticus*) which have been found to feature prominently in the diets of many local communities in Nigeria, especially within the South-Eastern region of the country. Edible frogs have been found to be proteinous and represent an alternative source of animal protein particularly in areas where fish and other protein sources are either in short supply or relatively more expensive. Frog leg is a popular delicacy and resembles chicken in terms of taste. Apart from having high protein content, studies have shown they also have characteristically low fats and calorie content. Results of this study showed that *P. adspersus* and *O. niloticus* provide approximately some 10 – 13% of protein available for local consumption, and that their lipid contents are considerably low and healthy for human consumption.

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