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Determination of Protein Quality of Oven Dried Clam *(Mecerneria m.)*, Whelk *(Thias c.)*, Oyster (*Crossostrea g.)* and Periwinkle *(Tympanotonus f.)* Meat Using Rat Bioassay

Ebasi Okoya Johnnie*, Achinewhu, Simeon Chituru, Kiin Kabari David Barine, Eke-Ejiofor Joy

Department of Food Science and Technology, Faculty of Agriculture, River State University Port Harcourt, Nigeria
*Corresponding author: ebasiokoya12@gmail.com

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Abstract Meat samples of four species of shell fish; Clam (Mercenaria mercenaria), Whelk (Thias coronate), Oyster (Crassostrea gasar) and Periwinkle (Tympanotonus fuscatus) were processed using oven drying and their nutritional content determined by proximate analysis. The protein content was then subjected to quality analysis using rat's bioassay to determine PER and in vivo APD values. Nutritionally the four shell fish meat samples considered in this study shows that whelk contains the highest levels of moisture with 13.96±0.01, Oyster 8.99±0.00, clam 8.98±0.01 and least is periwinkle with moisture content of 6.50±0.14. The fat content of the shell fish meat samples considered shows that oyster contains the highest fat content of 10.60±0.00, cam 10.07±0.03, whelk 9.20±0.00 and the least is periwinkle with 4.76±0.01. The ash content was highest in whelk with 5.62±0.00, clam 5.07±0.03, oyster 4.23±0.00 and the least is periwinkle with 3.38±0.01. The fibre content was highest in whelk with 10.45±0.01, clam 10.10±0.00, oyster5.33±0.04 and the least is periwinkle with 3.56±0.01. Periwinkle has the highest protein content of 70.42±0.03, oyster 64.70±0.00, whelk 47.30±0.03 and the least is clam with 46.90±0.00. Protein Efficiency Ratio (PER) and in vivo Apparent Protein Digestibility (APD) were used to determine the protein quality in the meat samples using casein as the reference protein. The PER results indicates that clam, whelk, oyster, periwinkle and casein have PER values of 1.76±0.02, 1.34±0.01, 1.83±0.00, 1.46±0.00, and 2.28±0.01 respectively. The PER values however significantly differ from each other at ($P<0.05$). The percentage in vivo APD values of 79±0.71, 81±0.63, 86±0.71, 90±0.00 for clam, whelk, oyster, periwinkle and casein respectively were obtained. Results indicates that meat samples considered in the study contains high quality proteins that can support lively wood like other conventional protein sources such as cow meat, fish, egg and milk.

Keywords: *protein quality, protein efficiency ratio, in vivo apparent protein digestibility, bioassay and proximate analysis*

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1. Introduction

Protein is a major nutrient required by the body for healthy growth and development. Protein malnutrition in humans may lead to several disease conditions such as high infant mortality rate, low resistance to disease, poor growth and development, mental retardation and kwashiorkor [1]. Many under develop and developing countries like Nigeria are having serious challenges in meeting up the daily protein intake requirements of their teaming population. Hence the ranking of Nigeria as one of the poorest nations in the world with high infant mortality rate of 119.9 per 1000 live births [2]. Nigeria also over took India in the 2018 rankings as the country with the highest poverty rate in the world and ranked 93 in the global hunger index [3]. These reports are a reflection of the fact that many families in Nigeria can hardly meet their daily protein requirement as many families feed on carbohydrate base diets and could not afford conventional proteins to meet their daily protein requirements due to the high cost of conventional proteins, therefore the need for harnessing other available protein sources in the country as alternatives to the conventional proteins which are presently very expensive. This, if achieved will enable the country to address its protein malnutrition challenges presently plaguing the nation. These alternative sources of protein are in two categories depending on their sources, plant and animal sources. Much work in form of research has been done in the area of plant protein sources, these include in the area of soy bean protein [4] and fluted

pumpkin seed were protein contents as high as 26.6% has been reported [5].

In this study however attention is giving to nonconventional protein from animal sources mainly marine shell fish such as clam (*Mercenaria mercenaria),* whelk (*Thias coronata),* oyster (*Crassostrea gasar)* and periwinkle (*Tympanotonus fuscatus*) which are abundant in the Niger Delta region of South- South Nigeria. Shell fish in general have been reported as rich sources of protein and other nutrients such as minerals [6]. However the quality of these proteins are yet to be tested an evaluated so as to be recommended as good alternative sources of protein to supplement or substitute the highly expensive conventional protein sources. Shell fish meat samples were obtained from per boiling the shell fish in hot water for few minutes and the meat removed with sterilized needles or knives and the meat oven dried in an electric oven model BHG-9140A at 60^{oc} for 24 hrs and the meat sample used in the analysis. In the protein quality analysis Protein Efficiency Ratio (PER) Assay and In vivo Apparent Protein Digestibility (APD) methods were used to determine the shell fish meat protein qualities. Protein Efficiency Ratio PER Assay [7] is easy to conduct and had been used extensively. The PER is the widely used standard by the U.S food industry to evaluate protein quality in foods. It is also used in the calculation of the recommended daily allowance for protein and calculation of food tables in the U.S.A [8]. In using PER to determine protein quality, diets of 10% protein are formulated from the test protein and used to feed young rats and their growth and feed consumption rates monitored. It has been observed that rats fed with poor protein gains little weight and eats little protein, while rats fed with good quality protein gain more weight and eat more protein [9].

2. Materials and Method

All the four shell fish samples were bought from market women in Ogbia market capital of Ogbia Local Government Area of Bayelsa State South -South Nigeria. The shell fish are then properly washed and parboiled for a few minutes and the meat removed with sterilized needles or knives and packed into iced cool plastic buckets with cover and transported to the Food Science and Technology laboratory were they are kept in deep freezer and samples taken from the bulk for further processing by oven drying. Shell fish meat samples of clam, whelk, oyster, and periwinkle were collected from the bulk samples and placed in aluminium containers and dried in oven at 60^{oc} for 24hrs. The meat samples are then taken for proximate analysis. Each shell fish meat sample was subjected to proximate analysis to determine their nutritive value.

The moisture, protein, ash, fibre, crude fat and carbohydrate content were determine using the [7] method.

The percentage moisture content was calculated using the formula

$$\%\ moisture\ content = \frac{W1 - W2\ x100}{W1}$$

Where W1= weight of fresh sample
W2 = weight of dried sample

The nitrogen content was determined using the micro-kjeldahl method.

Percentage crude protein was determine using the formula

$$\%\ Nitrogen = \frac{titre - blank\ x\ N\ of\ acid\ x1.4}{Wt\ of\ sample}$$

$$\%\ crude\ protein = N\ x\ 6.25$$

Where 6.25 is the conversion factor of protein.

$$Percent\ crude\ ash = \frac{weight\ of\ ash\ x\ 100}{Weight\ of\ sample}.$$

$$Percentage\ crude\ fat = \frac{weight\ of\ fat\ x\ 100}{Weight\ of\ sample}$$

Using the soxhlet method

$$Percentage\ crude\ fibre = \frac{weight\ of\ residue}{weight\ of\ sample}.Prot.\ ash - blank\ x\ 100$$

Percentage carbohydrate was calculated by difference

$$\%\ CHO = 100 - \begin{pmatrix} \%\ moisture + \%\ protein \\ +\%ash + \%\ fibre + \%\ fat \end{pmatrix}.$$

2.1. Rat Diet Formulation

The formulation of diet was done using the procedures outlined by [7] for PER with casein as reference protein. Each diet was formulated to give 10% protein content using their protein content from the proximate analysis as recommended for PER. Shell fish meat samples serving as the only source of protein in the diet. Other ingredients include maize flour, bone meal, rice bran, wheat bran, vitamin mix, salt, fat, and vitamin c. after the diet have been prepared proximate analysis is done for each diet.

2.2. Feed Composition Using Periwinkle Meat as the Sole Source of Protein in 1kg of Feed

Maize flour	560g
Periwinkle protein	142
Wheat bran	80
Rice bran	80
Bone meal	100
Fat (butter)	50
Salt	0.5
L/stone	0.4
Vit C	0.4
Premix	0.25
Total 1013.55	

Calculation of ingredients in the diet was based on the proximate analysis of the test protein. Each type of diet formulated with clam, whelk, oyster, periwinkle and casein based protein sources is then fed to 5 young rats kept in separates cages. The young rats used in this experiments were obtained from a known private breeder based in Nsuka South- East Nigeria. The casein used as the standard protein was obtained from an authorized

dealer in Port Harcourt capital of River State South- South Nigeria.

2.3. Rat Bioassay

The rat bioassay experiment was conducted for twenty eight days (28). Each test protein was fed to a set of 5 young rats of between 28 to 30 days old, kept in separate cages. In addition to the feeds water was supplied on a daily basis and weight and feed consumption recorded every two days (2). Before the commencement of the experiment the rats on arrival were fed with the original diets for about two days before the new diets were introduced. At the end of the twenty eight days the PER values were then calculated as follows:

$$PER = \frac{increase\ in\ body\ weight}{Weight\ of\ protein\ consumed}$$

In vivo Apparent Protein Digestibility (APD)

Feed consumption and faecal data were recorded daily for eight days from day (10-18) of the twenty eight days and the data used to determine the in vivo apparent protein digestibility (APD) [7] and is calculated as follows.

$$\%\ in\ vivo\ APD = \frac{N\ in\ diet - N\ in\ faces}{N\ in\ diet} x100.$$

3. Results and Discussion

3.1. Proximate Analysis

The proximate composition of the oven dried meat samples of the four selected shell fish clam, whelk, oyster, and periwinkle are presented on Table 1.

The moisture content of whelk was the highest with moisture content of 13.96 followed by oyster with a moisture content of 8.99, clam 8.98, and periwinkle is the least with values of 6.50. Crude protein values of the shell fish indicates that periwinkle has the highest amount of crude protein with a protein content of 70.42, followed by oyster with a protein content of 64.70, whelk 47.34 and clam 46.90. Fat contents of 10.60 was recorded for oyster which is the highest followed by clam 10.0, whelk 9.20, and the least is periwinkle with 4.76.

3.2. Rat Bioassay

During the bioassay all the twenty five rats used in the experiment survived and gained weight within the twenty eight days and the results are presented on Table 2. Results from the study shows that casein recorded the highest PER value of 2.28 followed by oyster with PER values of 1.83, clam 1.76, periwinkle 1.46 and whelk 1.34 been the least.

Percentage in vivo apparent protein digestibility (APD) experimental results are shown on Table 3. Results indicates that casein the reference protein has the highest % ADP of 90 and faeces dried weight of 6.46 closely followed by oyster with APD of 87 and weight of dried faeces 7.01, periwinkle with % APD of 86 with dried weight of faeces 7.32, whelk with % APD of 81, with dried weight faeces of 9.40 and the least clam with % APD of 79 and dried weight faeces of 10.45.

The results obtained in the proximate analysis indicates that the moisture content of whelk was 13.96% which is above the recommended 10% for storage. This may be due to different in the sizes of the meat samples that were oven dried. All the other samples contain moisture content lower than 10%. The protein content of periwinkle was the highest with 70.42% this result is in line with the report of [10] that reported protein content of periwinkle as 74.74 on dried matter basis. While the results of the protein content of oyster in the study is 64.70 which is also in line with the works of [6] who reported protein content of oven dried oyster as 63.03. The protein content of clam is 47.34 which is in line with the reported findings of [11] who reported a protein content of 47.38 for clam.

Table 1. Proximate composition of oven dried sample of 4 selected shell fish meat

Samples	%moisture Content	% crude protein	%crude ash	% crude fat	% crude fiber	%carbohydrate
Clam	8.98 ±0.01^{b}	46.90 ±0.00^{e}	5.07 ±0.03 ^{b}	10.07 ±0.03^{b}	10.10 ±0.00 ^{b}	18.88 ±0.04^{a}
Whelk	13.96 ±0.01^{a}	47.34 ±0.03^{d}	5.62 ±0.00 ^{a}	9.20 ±0.00^{c}	10. 45±0.01^{a}	13.43±0.06 ^{b}
Oyster	8.99 ±0.00^{b}	64.70±0.00^{c}	4.23±0.00^{c}	10.60 ±0.00^{a}	5.33 ±0.04^{c}	6.15±0.04^{d}
Periwinkle	6.50 ±0.14^{c}	70.42 ±0.03^{b}	3.38 ±0.01^{d}	4.76 ±0.01^{d}	3.56 ±0.01^{d}	11.38±0.13^{c}
Casein	4.00 ±0.00^{d}	90.64±0.00 ^{a}	0.86±0.01^{e}	1.50±0.00 ^{e}	0.30 ±0.00^{e}	2.70±0.01^{e}

Values are mean ± SD of duplicate samples

Values bearing different subscript in the same column differ significantly $p < 0.05$.

Table 2. PER values of protein used in the Diets for Rat assay

Diets	Body Weight Increase (G)	Total Amount of Feed Intake (g / rat/28days)	Percentage Protein In feeds (N x 6.25)	Protein Consumed (g /rat/28d)	PER
Clam	79.33±0.02^{c}	440.20±0.00^{c}	10.25±0.01^{e}	45.12±0.00^{e}	1.76±0.02^{c}
Whelk	63.31±0.00^{e}	420.50±0.00^{e}	11.26±0.00^{b}	47.35±0.03^{d}	1.34±0.01^{e}
Oyster	90.28±0.01^{b}	451.45±0.01^{b}	10.91±0.00 ^{d}	49.25 ±0.01^{b}	1.83±0.00^{b}
Periwinkle	70.25±0.00^{d}	431.31±0.00^{d}	11.15±0.02^{c}	48.09±0.00^{c}	1.46±0.00^{d}
Casein	120.61±0.01^{a}	456.41±0.01^{a}	11.59±0.00^{a}	52.90 ±0.00^{a}	2.28±0.01^{a}

Values are means ±SD of five replications

Mean values bearing different subscript in the column differ significantly $p < 0.05$.

Table 3. Percentage Apparent Protein Digestibility of Clam, Whelk, Oyster, Periwinkle and Casein

Protein Sources	Weight of feeds consumed (g/rat/ 8days)	% Nitrogen	Total nitrogen consumed	Dried Weight of feces	% Nitrogen	Total nitrogen in feces	% APD
Clam	88.68±0.01^{b}	1.64±0.01^{e}	1.45±0.02^{b}	10.45±0.01^{a}	2.61±0.01^{e}	0 .30 ±0.00^{a}	79 ±0.71^{e}
Whelk	78.30 ±0.00^{e}	1.80±0.00^{b}	1.41±0.00^{c}	9.40± 0.00^{b}	2.73±0.00^{c}	0.26±0.01^{b}	81±0.00^{d}
Oyster	82.44±0.01^{c}	1.75± 0.00^{d}	1.44 ±0.12^{b}	7.01± 0.00^{d}	2.70± 0.00^{d}	0.19± 0.00^{c}	87 ±0.63^{b}
Periwinkle	80.25±0.01^{d}	1.78±0.01^{c}	1.43±0.00 ^{bc}	7.32± 0.01^{c}	2. 76±0.02^{b}	0.17± 0.00^{d}	86± 0.71^{c}
Casein	97.68± 0.00^{a}	1.85±0.00^{a}	1.81± 0.00^{a}	6.46 ± 0.00^{e}	2.81±0.00 ^{a}	0.18± 0.01^{cd}	90± 0.00^{a}

The results obtained in the PER assay indicates that casein has the highest PER values of 2.28 which is in line with the PER values reported for casein by [12] which gives casein PER values of 2.5. The PER values of the shell fish are relatively high as compared to the PER values of casein and other conventional proteins such as cow meat 1.29, fish 2.20, and egg 2.28. [13].

The in vivo apparent protein digestibility (APD) is another parameter used in measuring the protein quality of the tested proteins. Casein the test protein has % APD of 90 while oyster 87, periwinkle 86, whelk 81 and clam79. The quality of protein is related to its amino acid composition, digestibility and ability to supply essential amino acid in the amounts required by the animal species consuming the protein [8], therefore higher the digestibility of the protein the better the quality. The high digestibility values recorded in the experiment for the shell fish under consideration is an indication of high quality proteins.

4. Conclusion

The diet with casein had the highest PER values indicating better quality protein content than the shell fish diets. However the shell fish diets also contain good quality protein as their PER values compares favorably with that of cow meat, fish and egg. Casein protein has the highest % APD values indicating a highly digestible protein. The % APD values for the shell fish are also relatively very high indicating good quality protein content.

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