

## BMR Biotechnology

### Research Article

# Proximate, Minerals and Amino Acids Composition of *Acanthurus monroviae* and *Lutjanus goreensis* Fish Muscle

Emmanuel Ilesanmi Adeyeye,

Department of Chemistry (Analytical Unit), Ekiti State University, P.M.B. 5363, Ado –Ekiti, Nigeria

Correspondence should be addressed to Emmanuel Ilesanmi Adeyeye.

Received 20 May 2014; Accepted 23 May 2014; Published 23 May 2014

Copyright: © 2014 Emmanuel Ilesanmi Adeyeye et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

### Abstract

The muscle of two lagoon fishes known as *Acanthurus monroviae* and *Lutjanus goreensis* was analysed for proximate, minerals and amino acids compositions. The proximate values were high in crude protein (65.4-68.5 g/100 g) and energy (1433-1436 kJ/100 g) but low in most other proximate parameters with calculated fatty acid ranging between 3.61 and 4.38 g/100 g. The proportion of total energy due to protein was highest with a range of 77.4-81.2 % and the utilizable energy due to protein was between 46.4 and 48.7 %. The two most concentrated minerals were K (392-419 mg/100 g) and P (219-269 mg/100 g) whereas each of the trace minerals was < 1.0 mg/100 g. For other mineral quality parameters, [K/(Ca+Mg)] was high at value range of 16.4-18.3. The two most abundant essential amino acids were Lys (8.88-9.57 g/100 g) and Leu (8.58-9.31 g/100 g) whereas the two most concentrated non-essential amino acids were Glu (13.4-14.7 g/100 g) and Asp (8.85-10.4 g/100 g). Total essential amino acid (with His) ranged from 47.4-48.5 g/100 g with corresponding percentage of 51.5-50.0. On other amino acid quality parameters: P-PER<sub>1</sub>, range was 3.09-3.41; EAAI was 1.39-1.44 and BV was 81.6-85.0. For amino acid scores, Ser was limiting in both samples in comparison with whole hen's egg; under provisional EAA scoring pattern, Val was limiting in the two samples; under EAA of preschool child requirement, no amino acid was limiting since each score was > 1.0. In all the parameters determined (proximate, energy, minerals, amino acids, pI, scores), significant differences occurred between the two fish samples since  $r_c > r_t$  at  $\alpha = 0.01$  in each case. In most of the determinations made, the corresponding concentrations were more in *A. monroviae* than in *L. goreensis*.

**Keywords:** *Acanthurus monroviae*, *Lutjanus goreensis*, Muscle, Chemical Compositions.

## Introduction

Animal protein intake by Nigerians has been very low due to a decrease in animal production per capita, and the rising growth in the human population. The price of fish has stabilized in recent times, thus fish have become the major source of animal protein [1].

Petrides [2] had indicated that fish and meat from wild animals are the chief sources of animal protein in the diets of the rural communities especially in the Southern States of Nigeria. The FAO calculation for apparent annual per capita consumption of fish and shellfish for human food, by region and country (2001-2008) put the expected estimate for 2008 as 26.6 kg or 58.8 pounds in Nigeria [1]. Hence work on the determination of the chemical composition of fishes should be an important part of aquaculture research.

The processor, the nutritionist, the cook and the consumer all have a direct interest in the composition of fish. The processor needs to know the nature of the raw material before he can apply correctly the techniques of chilling, freezing, smoking or canning. The nutritionist wants to know what contribution fish can make to the diet and to health, and the cook must know for example whether a fish is normally lean or fatty in order to; prepare it for table. The consumer is interested not only in whether a particular fish tastes good, which is a matter of opinion, but also in whether it is nutritious [3].

While the consumer is interested mainly in the edible part of the fish, that is the flesh or muscle, the fish meal manufacturer is concerned with the composition of the whole fish, and the processor of fish oils wants to know what is in the liver. Measurement of constituents of fish products is sometimes necessary to meet specifications or to comply with regulations. Fish is one of the most valuable sources of high grade protein available to man in this hungry world, and a knowledge of its

composition is essential if the fullest use is to be made of it [3].

The taxonomic hierarchy of the fishes are given below. *Acanthurus monroviae*: Kingdom (Animalia-Animal, animaux, animals); Subkingdom (Bilateria); Infrakingdom (Deuterostomia); Phylum (Chordata-cordés, cordado, chordates); Subphylum (Vertebrata-vertebrado, vertébrés, vertebrates); Infraclass (Gnathostomata); Superclass (Osteichthyes-bony fishes, poissons osseux, osteiceto, peixe ósseo); Class (Actinopterygii – ray-finned fishes, spiny rayed fishes, poisson épineux, poissons à nageoires rayonnées); Subclass (Neopterygii-neopterygians); Infraclass (Teleostei); Superorder (Acanthopterygii); Order (Perciformes-perch-like fishes); Suborder (Acanthuroidei-surgeon fishes); Family (Acanthuridae-surgeonfishes, tangs, cirujanos, poissons-chirurgiens); Genus (*Acanthurus* Forsskål, 1775-lancetfishes, surgeonfishes, tangs, doctorfishes, common surgeonfishes); Species (*Acanthurus monroviae* Steindachner, 1876 - surgeonfish) [4]. *Lutjanus goreensis*: *Lutjanus goreensis* (Valenciennes, 1830) synonymus with *Lutjanus guineensis* Bleeker (1863). FAO Names: En-Gorean snapper; Fr-Vivaneau de Gore; Sp-Pargo de Gorea. It's diagnostic features: Body relatively deep. Head pointed, dorsal profile of forehead steep; preorbital bone broad; maxilla extending to about mid-eye level; vomerine tooth patch triangular, with a pronounced medial posterior extension; gill rakers on lower limb of first arch (including rudiments) 13, total rakers on first arch about 19. Dorsal fin with 10 spines and 14 soft rays; anal fin with 3 spines and 8 soft rays; pectoral fins of adults not reaching level of anus, with 17 rays; caudal fin slightly emarginate. Scale rows on back parallel to lateral line. Scale rows on cheek 5 or 6. Presence of narrow blue band or row of broken spots below eye. Small specimens from shallow water mainly brownish. It's environments could be marine; freshwater; brackish; reef-associated; depth range 0-50 m; tropical; 34 °N-17°S, 27°W-14°E. Size could be

maximum length: 80.0 cm TL male/unsexed; common length: 50.0 cm TL male/unsexed. The fish occurs in Eastern Atlantic: mainly between Senegal and the Republic of Congo; also from Cape Verde. Adults occur on rocky bottoms and in the vicinity of coral reefs. Young are frequently encountered in coastal waters, particularly estuaries and sometimes in rivers. They feed mainly on fishes and bottom-dwelling invertebrates [5].

Despite the high nutritional value of fish, there is a lack of information on the nutritional and protein quality particularly for the fishes under discussion. The major aim of this paper was to report on the investigation of the proximate, minerals and amino acids compositions of *A. monroviae* and *L. goreensis*. This type of work would improve information on the two fishes. In Nigeria, the fishes are given various local names. For *A. monroviae*, the names are: Kalabari (*Orope*), Abaji (*Atabila*), Ijaw (*Abade-Okowbo*); in *L. goreensis*, the names are: Yoruba (*Igbakere*), Kalabari (*Agbara*), Ijaw (*Ako*) [6].

## Materials and Methods

### Sampling and sample treatment

Wet samples of the fishes were collected from fish trawlers from the Lagos lagoon, the samples were brought to the laboratory under ice cover. The samples were identified in the Department of Zoology of the Ekiti State University, Ado-Ekiti. The samples were opened up in the laboratory; oven-dried till constant weight; fins, bones and viscera were removed and further oven-dried at 55°C until constant weight. The cooled dried samples were ground using mortar and pestle into fine powder. Two fish samples (of each type) were used for this exercise and the ground portions were kept in plastic containers in the laboratory freezer pending analysis.

### Proximate and mineral determination

The proximate composition was determined by the methods of AOAC [7]. Carbohydrate and organic matter were determined by difference. Minerals were determined from the ash of the proximate results. Energy was calculated using Atwater factors.

### Determination of amino acids

#### *Defatting*

A quantity of 2.0 g of each sample was weighed into the extraction thimble and the fat extracted with chloroform/methanol mixture using a Soxhlet apparatus [7]. The extraction lasted for 5-6 h.

#### *Hydrolysis of samples*

A quantity of 30 mg of the defatted sample was weighed into glass ampoules. Seven milliliters of 6 M HCl were added and oxygen expelled by passing nitrogen gas into the samples. The glass ampoules were sealed with a Bunsen flame and put into an oven at 105±5°C for 22 h. The ampoule was allowed to cool, the content was filtered to remove the humins. The filtrate was then evaporated to dryness at 40 °C under vacuum in a rotary evaporator.

Each residue was dissolved with 5 ml acetate buffer (pH 2.0) and stored in a plastic specimen bottle, and kept in the deep freezer.

#### *Samples analyses*

Amino acid analysis was by ion exchange chromatography (IEC) [8] using the Technicon Sequential Multisample (TSM) Amino Acid Analyzer (Technicon Instruments Corporation, New York). The period of analysis was 76 min for each sample. The gas flow rate was 0.50 ml/min at 60 °C with reproducibility consistent within ±3%. The net height of each peak produced by the chart recorder of the TSM (each representing an amino acid) was measured and calculated. The amino acid values reported were the averages of two determinations. Norleucine was the internal standard.

**Determination of nutritional parameters**

Nutritional parameters were determined on the basis of the amino acid profiles. Protein efficiency ratio (PER) was estimated according to the regression equations developed by Alsmeyer et al. [9] as given below:

$$PER_1 = -0.684 + 0.456 (\text{Leu}) - 0.047 (\text{Pro})$$

$$PER_2 = -0.468 + 0.454 (\text{Leu}) - 0.105 (\text{Tyr})$$

The amino acid scores were calculated using three different procedures:

- (i) Scores based on amino acid values compared with whole hen’s egg amino acid profile [10];
- (ii) Scores based on essential amino acid scoring pattern [11];
- (iii) Scores based on essential amino acid suggested pattern of requirements for preschool child [12].

The essential amino acid index (EAAI) was determined using the method of Steinke et al. [13] using the following formula:

$$\text{Essential amino acid index} = 9 \frac{\text{mg lysine in 1 g test protein}}{\text{mg lysine in 1 g reference protein}} \times \text{etc. for all 8 essential amino acids} + \text{His}$$

Computation of biological value (BV) was calculated following the equation of Oser [14] as follows:

$$BV = 49.09 + 10.53 (\text{PER})$$

where PER = protein efficiency ratio.

The leucine/isoleucine ratios, their differences and their percentage differences were also calculated. The theoretical estimation of isoelectric point (pI) was determined using the equation of Olaofe and Akintayo [15] and information provided by Finar [16]:

$$IP_m = \sum_{i=1}^n IP_i X_i$$

where IP<sub>m</sub> = the isoelectric point of i<sup>th</sup> amino acid in the mixture; X<sub>i</sub> = the mass or mole fraction of the i<sup>th</sup> amino acid in the mixture. Determination of the total essential amino acid (TEAA) to the total amino acid (TAA), i.e. (TEAA/TAA); total sulphur amino acid (TSAA); percentage cystine in TSAA (% Cys/TSAA); total aromatic amino acid (TAraA), etc.

**Statistical analysis**

The statistical analysis carried out included the determination of the grand mean, standard deviation (SD) and the coefficient of variation percent (CV %). Other calculations made were the simple linear correlation coefficient (r<sub>xy</sub>), coefficient of determination or variance (r<sub>xy</sub><sup>2</sup>), regression coefficient (R<sub>xy</sub>) coefficient of alienation (or index of lack of relationship) (C<sub>A</sub>) and index of forecasting efficiency (IFE) and subjected to Table standards to test for significance difference, the level of probability was set at r = 0.01 at n-2 degrees of freedom (df) [17].

**Results**

The proximate composition of the fish samples can be seen in Table 1. All values in the proximate composition were close within the two fish samples with low coefficient of variation (CV %) values that ranged from 0.12 to 13.6. Results with low values were

in crude fat, crude fibre, carbohydrate, fatty acid, ash and moisture. On the other hand, high values were recorded in crude protein, organic matter and energy. Values of crude fat, moisture, carbohydrate, organic matter, fatty acid and energy were correspondingly higher in *A. monroviae* than *L. goreensis* whereas crude protein, ash and crude fibre values were higher in *L. goreensis*.

In Table 2, we have values of energy contributed by protein, fat and carbohydrate to the total metabolisable energy. The value differences were low between the two samples with *A. monroviae* being higher in energy contribution from fat and carbohydrate whereas *L. goreensis* was higher in utilizable energy due to protein (UEDP).

The mineral composition of the samples is depicted in Table 3. The trace elements were widely distributed with the CV % range of 24.7 to 113 whereas the major elements had lower CV % range of 2.77 to 19.7. Similar values of Co were observed for the samples. The trace elements were of very low value with each being < 1.0 mg/100 g. Thus, the two samples were not sources of appreciable levels of trace elements. High values of P (219-269 mg/100 g) and K (392-419 mg/100 g) were recorded for the fish samples and also observed were appreciable values in Ca, Mg and Na. In other calculated mineral quality parameters, [K/(Ca+Mg)] value was high at 16.4-18.3 whereas each of these were < 1.0: Na/K, Ca/P and Ca/Mg.

The amino acid profiles of the fish samples are depicted in Table 4. The variation of each amino acid in the fishes was with the CV % range of 0.49 to 30.3. In both samples, Glu was the most concentrated amino acid (AA) with values of 13.4-14.7 g/100 g crude protein (cp). Most of the amino acid values in the *A. monroviae* were correspondingly higher than in the *L. goreensis*.

The various quality parameters of the fish samples are shown in Table 5. Again the CV % values were generally low at 0.37-10.4, these values were lower than CV % observed in Table 4. The values of the major AA groups and their corresponding percentages as well as P-PER (1 and 2), EAAI, BV, pl and Leu-Ile were shown in the Table 5.

The differences between the AA corresponding values in the samples can be seen in Table 6. Whilst *A. monroviae* had fourteen positive differences, *L. goreensis* had four positive differences. The percentage differences were also shown. The highest percentage difference came from Phe as follows: [*A. monroviae* (3.92)-(6.06) *L. goreensis* = - 2.14 or 54.6 %].

The amino acid scores (AAS) of the fish samples in comparison with the whole hen's egg are in Table 7. The variation (CV %) values were generally low (0.98-30.3) although equal values of AAS were observed in Val, hence no CV % value for Val. Similar AA was the limiting AA in the samples, Ser, but their AAS were not similar; the value was 0.52 in *A. monroviae* and 0.45 in *L. goreensis*. Essential amino acid scores based on the provisional EAA scoring pattern are shown in Table 8. The CV % ranged from 0.56 to 13.5. Val was limiting in both samples with very close values: *A. monroviae* (0.87) and *L. goreensis* (0.88) and CV % of 0.81. The AAS of the samples based on the EAA of the preschool child requirement are shown in Table 9. No limiting amino acid (LAA) was recorded in any of the fish samples because each score was > 1.1 with Met + Cys even recording AAS of 2.00 in *A. monroviae*.

The statistical summary of the data in Tables 1-5 and 7-9 is shown in Table 10. Correlation coefficients for all the parameters in the various Tables depicted were significantly different at  $n=2$  and  $r = 0.01$ . The  $r_{xy}$  ranged between 0.8480-0.9999. The Table 11 explained the summary of the amino acids profiles into Factors A and B means. It is a quick summary of the EAA and NEAA terminating at the same target.

**Table 1. Proximate composition of the muscle of *Acanthurus monroviae* and *Lutjanus goreensis* fish (g/100 g dry weight)**

Component	<i>A. monroviae</i>	<i>L. goreensis</i>	Mean	SD	CV %
Crude fat	6.25	5.15	5.70	0.78	13.6
Crude protein	65.4	68.5	66.9	2.16	3.22
Total ash	8.58	9.10	8.84	0.37	4.16
Crude fibre	0.80	0.90	0.85	0.07	8.32
Moisture	13.2	11.5	12.3	1.22	9.88
Carbohydrate	5.80	4.95	5.38	0.60	11.2
Organic matter	91.4	90.9	91.2	0.37	0.40
Fatty acid*	4.38	3.61	3.99	0.54	13.6
Energy (kJ/100 g)	1436	1433	1435	1.73	0.12

\*Crude fat x 0.70. SD = standard deviation. CV % = coefficient of variation.

**Table 2. Energy values as contributed by protein, fat and carbohydrate in the fish samples**

Parameter	<i>A. monroviae</i>	<i>L. goreensis</i>	Mean	SD	CV %
Total energy	1436	1433	1435	1.73	0.12
PEP %	77.4	81.2	79.3	2.69	3.39
PEF %	16.1	13.3	14.7	1.98	13.5
PEC %	6.46	5.53	6.00	0.66	11.0
UEDP %	46.4	48.7	47.6	1.61	3.39

PEP = proportion of total energy due to protein.

PEF = proportion of total energy due to fat.

PEC = proportion of total energy due to carbohydrate.

UEDP = utilizable energy due to protein.

**Table 3. Mineral composition of the muscle of *A. monroviae* and *L. goreensis* fish (mg/100 g dry weight)**

Component	<i>A. monroviae</i>	<i>L. goreensis</i>	Mean	SD	CV %
Fe	0.38	0.68	0.53	0.22	41.1
Cu	0.005	0.001	0.003	0.003	94.3
Co	0.001	0.001	0.001	-	-
Mn	0.009	0.001	0.005	0.006	113
Zn	0.62	0.43	0.52	0.13	24.7
Pb	0.0003	0.0005	0.0004	0.0001	35.4
Ca	15.6	15.0	15.3	0.42	2.77
Mg	27.2	36.0	31.6	6.22	19.7
K	392	419	406	19.1	4.71
Na	51.0	63.8	57.4	9.05	15.8
P	269	219	244	35.6	14.5
Se	0.012	0.048	0.030	0.025	84.9
[K/(Ca + Mg)]*	18.3	16.4	17.4	1.34	7.74
Na/K	0.130	0.152	0.141	0.016	11.0
Ca/P	0.058	0.068	0.063	0.007	11.2
Ca/Mg	0.57	0.42	0.50	0.11	22.4

\*Milliequivalent.



**Table 4. The amino acids composition (g/100 g crude protein) of the fish samples (dry weight)**

Amino acid	<i>A. monroviae</i>	<i>L. goreensis</i>	Mean	SD	CV %
Lys	9.57	8.88	9.23	0.49	5.29
His	3.01	2.58	2.80	0.30	10.9
Arg	6.01	6.33	6.17	0.23	3.67
Asp	10.4	8.85	9.63	1.10	11.4
Thr	4.36	3.96	4.16	0.28	6.80
Ser	4.12	3.53	3.83	0.42	10.9
Glu	14.7	13.4	14.1	0.92	6.54
Pro	3.16	2.93	3.05	0.16	5.34
Gly	4.39	3.61	4.00	0.55	13.8
Ala	5.76	6.08	5.92	0.23	3.82
Cys	1.62	1.39	1.51	0.16	10.8
Val	4.35	4.38	4.37	0.02	0.49
Met	3.38	3.29	3.34	0.06	1.91
Ile	4.07	4.04	4.06	0.02	0.52
Leu	9.31	8.58	8.95	0.52	5.77
Tyr	3.46	2.89	3.18	0.40	12.7
Phe	3.92	6.06	4.99	1.51	30.3
Trp	1.48	1.39	1.44	0.06	4.43



**Table 5. Essential, non-essential, acidic, sulphur, aromatic, other amino acid quality parameters, etc. (g/100 g crude protein) of the fish samples**

Amino acid	<i>A. monroviae</i>	<i>L. goreensis</i>	Mean	SD	CV %
Total amino acid (TAA)	97.1	92.2	94.6	3.46	3.66
Total essential amino acid (TEAA)	45.5	44.9	45.2	0.47	1.03
% TEAA	46.9	48.7	47.8	1.25	2.62
Total essential amino acid with His	48.5	47.4	48.0	0.77	1.61
% TEAA (with His)	50.0	51.5	50.7	1.05	2.06
Total non-essential amino acid (TNEAA)	51.6	47.3	49.4	3.00	6.07
% TNEAA	53.1	51.3	52.2	1.27	2.44
Total aromatic amino acid (TArAA)	8.86	10.3	9.58	1.02	10.6
% TArAA	9.12	11.2	10.2	1.47	14.5
Total acidic amino acid (TAAA)	25.1	22.3	23.7	1.98	8.35
% TAAA	25.9	24.1	25.0	1.27	5.09
Total neutral amino acid (TNAA)	51.9	50.7	51.3	0.85	1.65
% TNAA	53.5	55.1	54.3	1.13	2.08
Total sulphur amino acid (TSAA)	5.0	4.68	4.84	0.23	4.68
% TSAA	5.15	5.08	5.12	0.05	0.97
% Cys/SAA	32.4	29.7	31.1	1.91	6.15
Total basic amino acid (TBAA)	18.6	17.8	18.2	0.57	3.11
% TBAA	19.2	19.3	19.3	0.07	0.37
P-PER <sub>1</sub>	3.41	3.09	3.25	0.23	6.96
P-PER <sub>2</sub>	3.40	3.12	3.26	0.20	6.07

EAAI	1.44	1.39	1.42	0.04	2.50
BV <sub>1</sub>	85.0	81.6	83.3	2.40	2.89
BV <sub>2</sub>	84.9	81.9	83.4	2.12	2.54
Leu/Ile ratio	2.29	2.12	2.21	0.12	5.45
Leu-Ile	5.25	4.53	4.89	0.51	10.4
% Leu-Ile	56.3	52.9	54.6	2.40	4.40
% Leu-Ile /TAA	5.41	4.91	5.16	0.35	6.85
pI	5.66	5.44	5.55	0.16	2.80

---

P-PER = predicted protein efficiency ratio.

EAAI = essential amino acid index.

BV = predicted biological value.

pI = predicted isoelectric point.

**Table 6. Differences (g/100 g crude protein) between the amino acids composition of *A. monroviae* and *L. goreensis* (dry weight)**

Amino acid	<i>A. monroviae</i>	<i>L. goreensis</i>	Difference *
Lys	9.57	8.88	+0.69 (7.21 %)
His	3.01	2.58	+0.43 (14.3 %)
Arg	6.01	6.33	-0.32 (5.32 %)
Asp	10.4	8.85	+1.55 (14.9 %)
Thr	4.36	3.96	+0.40 (9.17 %)
Ser	4.12	3.53	+0.59 (14.3 %)
Glu	14.7	13.4	+1.30 (8.84 %)
Pro	3.16	2.93	+0.23 (7.28 %)
Gly	4.39	3.61	+0.78 (17.8 %)
Ala	5.76	6.08	-0.32 (5.56 %)
Cys	1.62	1.39	+0.23 (14.2 %)
Val	4.35	4.38	-0.03 (0.69 %)
Met	3.38	3.29	+0.09 (2.66 %)
Ile	4.07	4.04	+0.03 (0.74 %)
Leu	9.31	8.58	+0.73 (7.84 %)
Tyr	3.46	2.89	+0.57 (16.5 %)
Phe	3.92	6.06	-2.14 (54.6 %)
Trp	1.48	1.39	+0.09 (6.08 %)

\*Positive values meant amino acid value in *A. monroviae* is higher than in *L. goreensis* and vice versa for negative sign; same sign runs before and within the brackets.

**Table 7. Amino acid scores of the fish samples based on whole hen's egg**

Amino acid	<i>A. monroviae</i>	<i>L. goreensis</i>	Mean	SD	CV %
Lys	1.54	1.43	1.49	0.08	5.24
His	1.25	1.08	1.17	0.12	10.3
Arg	0.99	1.04	1.02	0.04	3.48
Asp	0.97	0.83	0.90	0.10	11.0
Thr	0.85	0.78	0.82	0.05	6.07
Ser	0.52	0.45	0.49	0.05	10.2
Glu	1.23	1.12	1.18	0.08	6.62
Pro	0.83	0.77	0.80	0.04	5.30
Gly	1.46	1.20	1.33	0.18	13.8
Ala	1.07	1.13	1.10	0.04	3.86
Cys	0.90	0.77	0.84	0.09	11.0
Val	0.58	0.58	0.58	-	-
Met	1.06	1.03	1.05	0.02	2.03
Ile	0.73	0.72	0.73	0.01	0.98
Leu	1.12	1.03	1.08	0.06	5.92
Tyr	0.87	0.72	0.80	0.11	13.3
Phe	0.77	1.19	0.98	0.30	30.3
Trp	0.82	0.77	0.80	0.04	4.45
Total	0.97	0.92	0.95	0.04	3.74

**Table 8. Amino acid scores of the fish samples based on provisional essential amino acid scoring pattern**

Amino acid	<i>A. monroviae</i>	<i>L. goreensis</i>	Mean	SD	CV %
Lys	1.74	1.61	1.68	0.09	5.49
Thr	1.09	0.99	1.04	0.07	6.80
Met + Cys	1.43	1.34	1.39	0.06	4.59
Val	0.87	0.88	0.88	0.01	0.81
Ile	1.02	1.01	1.02	0.01	0.70
Leu	1.33	1.23	1.28	0.07	5.52
Phe + Tyr	1.23	1.49	1.36	0.18	13.5
Trp	1.48	1.39	1.44	0.06	4.43
Total	1.26	1.25	1.26	0.01	0.56

**Table 9. Amino acid scores of the fish samples based on the essential amino acids of the pre-school child requirement**

Amino acid	<i>A. monroviae</i>	<i>L. goreensis</i>	Mean	SD	CV %
Ile	1.45	1.44	1.45	0.01	0.49
Leu	1.41	1.30	1.36	0.08	5.74
Lys	1.65	1.53	1.59	0.08	5.34
Met + Cys	2.00	1.87	1.94	0.09	4.75
Phe + Tyr	1.17	1.42	1.30	0.18	13.7
Thr	1.28	1.16	1.22	0.08	6.96
Trp	1.35	1.26	1.31	0.06	4.88
Val	1.24	1.25	1.25	0.01	0.57
His	1.58	1.36	1.47	0.16	10.6
Total	1.43	1.40	1.42	0.02	1.50

**Table 10. Statistical summary of the data in Tables 1 and 2, 3, 4, 5 (pI only), 7, 8 and 9**

Table	Parameter	$r_{xy}$	$r_{xy}^2$	$R_{xy}$	$C_A$	IFE	df(n-2)	$r = 0.01$	Remark
1 and 2	Proximate + Energy	0.9999	0.9999	0.1057	0.005	0.995	11	0.684	Significant
3	Minerals	0.9909	0.9820	0.2184	0.134	0.866	14	0.623	Significant
4	Amino acids	0.9034	0.8161	-0.0437	0.429	0.571	17	0.575	Significant
5	pI (only)	0.9786	0.9577	0.2439	0.206	0.794	16	0.590	Significant
7	Scores (chicken egg)	0.8583	0.7366	0.1571	0.513	0.487	17	0.575	Significant
8	Scores (PEAA)	0.8919	0.7954	0.1874	0.452	0.548	7	0.798	Significant
9	Scores (PSEAAR)	0.8480	0.7192	0.4137	0.530	0.470	8	0.765	Significant

$r_{xy}$  = correlation coefficient;  $r_{xy}^2$  = coefficient of determination;  $R_{xy}$  = regression coefficient;  $C_A$  = coefficient of alienation; IFE = index of forecasting efficiency; df = degree of freedom;  $r = 0.01$  = confidence level; PEAA = provisional essential amino acid; PSEAAR = preschool essential amino acid requirement.

**Table 11. Summary of amino acids profiles into factors A and B means**

Amino acid composition	Factor A means		Factor B means
	Samples		
	<i>A. monroviae</i>	<i>L. goreensis</i>	
Total EAA	45.5	44.9	45.2
Total NEAA	51.6	47.3	49.4
Factor A means	48.6	46.1	47.3

## Discussion

The organic matter (OM) as shown in the proximate composition (90.9-91.4 g/100 g) were close to the value of 91.07 g/100 g in trunk fish, but higher than the values reported for four fresh water fishes of *Mormyrops delicious* (86.4 g/100 g), *Bagrus bayad* (75.0 g/100 g), *Synodontis budgetti* (84.0 g/100 g) and *Hemichronis fasciatus* (76.0 g/100 g) [18]. The protein content was lower than 72.89 g/100 g reported for trunk fish as well as another report on trunk fish (74.5 g/100 g) [19]. However the protein of *L. goreensis* was 4.53 % higher than the value in *A. monroviae*. The crude fat (and calculated fatty acid) levels had the highest (although low) disparity between the samples (CV % =13.6). Low level of fat showed the samples to be likely white fish. The total ash was moderate in each sample at 8.58 -9.10 g/100 g with that of *L. goreensis* being slightly higher. With higher ash content, *L. goreensis* would be expected to have higher overall concentration of minerals than in the *A. monroviae*. The energy levels of 1.43-1.44 MJ were close to 1.33-1.37 MJ in turkey-hen muscle and skin respectively [20], close to 1.3-1.6 MJ from cereals [12]

showing the samples muscle to be a reasonable source of energy. The daily energy requirement for an adult is between 10455-12548 kJ depending on his physiological state whilst that of infants is 3095 kJ [21, 22]. This implies that whilst an adult man would require between 728-874 g *A. monroviae* or 730-876 g *L. goreensis* to meet his minimum requirement, infants would require 216 g of each sample. The utilizable energy due to protein (UEDP %) for the fish samples (assuming 60 % utilisation) was 46.4 (*A. monroviae*) and 48.7 (*L. goreensis*). Both values were far greater than the recommended safe level of 8 % for an adult man who requires about 55 g protein per day with 60 % utilization. This shows that the protein concentration in the fishes in terms of energy would be more than enough to prevent protein energy malnutrition in children and adults fed solely on the fish samples as main sources of protein. The PEF % (Table 2) was lower than recommended level of 30 % [23] and 35 % [24] for total fat energy intake, this is useful for people wishing to adopt the guidelines for a healthy diet.



All the minerals determined were detected in both samples. Despite the fact that the total ash in *L. goreensis* was slightly higher than that of *A. monroviae* (by a value of 6.06 %), the mineral composition took a reverse position as *A. monroviae* was 0.26 % higher than in the *L. goreensis*. Virtually all the micro elements were almost negligible as their values ranged between 0.005-0.68 mg/100 g. The most widely varied mineral was Mn with CV % of 113. The values of Ca, Mg, K, Na and P ranged from moderate to high and with much less CV % values. Whilst K was high (392-419 mg/100 g), Na was moderate (51.0-63.8 mg/100 g). Both of them are required to maintain osmotic balance of the body fluid, the pH of the body, regulation of muscles and nerve irritability, control of glucose absorption and enhancement of normal retention of protein during growth [25]. Fe requirement by humans is 10-15 mg for children, 18 mg for women and 12 mg for men [26]; Fe in the present report was < 1.0 mg in each sample. The P levels of 219-269 mg/100 g in this report were much below the recommended daily allowance (RDA) level of 800 mg; the samples would contribute about 27.4-33.6 % of this value in the diet. Also, the Ca levels of 15.0 -15.6 mg/100 g were much lower than the RDA level of 800 mg. If Ca is adequately present in the diet, Fe is utilized to better advantage. This is an instance of 'sparing action' [26]. The Zn levels (0.43-0.62 mg/100 g) were lower than the Zn allowance of about 15-20 mg/day [26]. The K/Na ranged from 6.57-7.69 whereas the Na/K ratios ranged from 0.130-0.152. The Na/K ratios were good as they were lower than the 0.60 requirement to avoid high blood pressure [27]. The ratios in K/Na showed that more Na would be required to balance up to 1:1. K/Na enhances the salt balance of the body fluid [27]. The Ca/P weight ratio with values of 0.058 and 0.068 in the samples respectively were both lower than the recommended value of 1.0 [25]. The Ca/P ratio was reported to have some effects on Ca in the blood of many animals [25] and also in the absorption of phosphorus. Food is considered 'good' if the Ca/P ratio is above 1.0 and 'poor' if the ratio is less than 0.5 [28]. The Ca/Mg weight ratios in the samples fell within the range of 0.42-0.57. These values were far below the recommended value of 1.0 [25]. It means more Ca would have to be supplied from

other sources when the samples serve as sources of food in the diet. The milliequivalent ratios of  $[K/(Ca + Mg)]$  were all much greater than 2.2 with values of 16.4-18.3. The likely result of this is that consumers may suffer from hypomagnesamia in man [25].

The amino acid (AA) Table showed the AA compositions for each fish species. Glu had the highest concentrations among their groups and is acidic AA. Whilst Lys constituted the highest EAA in *A. monroviae*, Phe + Tyr constituted the highest EAA in *L. goreensis*. The various concentration parameters as total AA (TAA), total essential AA (TEAA), total acidic AA (TAAA), total neutral AA (TNAA), total sulphur AA (TSAA), total aromatic AA (TArAA) and their percentage levels were all depicted. Other quality parameters were included.

Glu, Asp and Phe + Tyr trends in the present study followed the trend as observed in *Gymnarchus niloticus* (trunk fish) [29]. Arginine (6.01-6.33 g/100 g cp) is essential for children and reasonable levels were present here. The lysine contents of 8.88-9.57 g/100 g cp were more than the content of the reference egg protein (63 mg/g), and they will therefore serve as good sources for fortification of cereal weaning foods. The His levels (2.58-3.01 g/100 g cp) were close to the levels in ostrich (2.03) [30], beef (3.20) [31] and chicken (3.04) [32]. Phe levels in the turkey-hen muscle (4.79 g/100 g) and skin (3.80 g/100 g cp) [20] were highly comparable with present results with values of 3.92-6.06 g/100 g cp; they are also favourably comparable with Phe levels in ostrich (4.84) [30], beef (4.48) [31] and chicken (4.48) [32].

The contents of TEAA of 47.4-48.5 g/100 g cp with tryptophan or 46.0 -47.0 g/100 g cp without Trp were close to the value for egg reference protein (56.6 g/100 g cp) [10]. The present contents of TEAA are higher than some literature values (mg/g cp); i.e., 351 (*Zonocerus variegatus*) [33]; 350 (*Macrotermes bellicosus*) [34]; 428 (*Limicolaria* sp.), 361 (*Archartina archartina*), 450 (*Archachatina marginata*) [35] and 355 (*G. niloticus*) [29]. Some literature values for freshwater fish TEAA contents were (mg/g cp): *M. deliciosus* (330), *B. bayad* (395), *S. budgetti* (389) and

*H. fasciatus* (394) [18] which were all favourably comparable to the present report. The present EAA were also better in concentration than in *Clarias anguillaris* (317 mg/g cp), *Oreochromis niloticus* (313 mg/g cp) and *Cynoglossus senegalensis* (300 mg/g cp) [36]. The contents of TSAA were lower than the 58 mg/ g cp recommended for infants [12]. The ArAA range suggested for ideal infant protein (68-118 mg/g cp) [12] has present values greater than the maximum, i.e. 11.9 – 12.9 g/100 g cp.

The ArAA are precursors of epinephrine and thyroxin [37]. The percentage ratios of TEAA to the TAA in the samples were 50.0 -51.5 % which were well above the 39 % considered to be adequate for ideal protein food for infants, 26 % for children and 11 % for adults [12]. The TEAA/TAA percentage contents were strongly comparable to that of egg (50 %) [38].

Most animal proteins are low in cystine (Cys) and hence in Cys in TSAA. For examples, (Cys/TSAA) % were 36.3 % in *M. bellicosus* [34]; 25.6 % in *Z. variegatus* [33], 35.5 % in *A. marginata*, 38.8 % in *A. archatina* and 21.0 % in *Limicolaria* sp., respectively [35] and 29.8 % in *G. niloticus* [34]. In contrast, many vegetable proteins contain substantially more Cys than Met, for example, 62.9 % in coconut endosperm [39]. Thus for animal protein diets, or mixed diets containing animal protein, cystine is unlikely to contribute up to 50 % of the TSAA [40]. Although FAO/WHO/UNU [12] did not give any indication of the proportion of TSAA which can be met by Cys in man, for rats, chicks and pigs the proportion is about 50 % [40]. Information on the agronomic advantages of increasing the concentration of sulphur-containing amino acids in staple foods show that Cys has positive effects on mineral absorption, particularly zinc [41, 42].

The protein efficiency ratio (PER) determines the effectiveness of a protein through the measurement of animal growth. The computed values in the samples were: P-PER<sub>1</sub>, (3.09-3.41) and P-PER<sub>2</sub> (3.12-3.40) which were higher than the standard value of 2.7, which is the standard value of casin protein. Any value that exceeds 2.7 is considered to be an excellent protein source [43]. These

values were better than the values in three fresh water fishes of *C. anguillaries*, *O. niloticus* and *C. senegalensis* whose P-PER ranged from 1.89-2.22 [36]. P-PER was 1.93 (muscle) and 2.27 (skin) of turkey-hen [20]. The biological value (BV) provides a measurement of how efficient the body utilizes protein consumed in the diet. The present results had BV<sub>1</sub> values of 81.6-85.0 and BV<sub>2</sub> as 81.9-84.9 in the samples. A food with a high value correlates to a high supply of the essential amino acids. Animal sources typically possess a higher biological value than vegetable sources due to the vegetable source's lack of one or more of the EAA. These literature values are favourably comparable to the samples, PER, BV: beef (2.9, 80). casein (2.5, 77); egg (3.9, 100); milk (2.5, 91). soy protein (2.2, 74); wheat gluten (0.8, 64); whey protein (3.2, 104) [44, 45]. The essential AA index (EAAI) ranged from 1.39 - 1.44. These values were better than the results obtained in the fancy meats of African giant pouch rat (*Cricetomys gambianus*) with EAAI range of 1.14-1.30 [46]. EAAI is useful as a rapid tool to evaluate food formulations for protein quality, although it does not account for difference in protein quality due to various processing methods or certain chemical reactions [47]. The EAAI of defatted soybean is 1.26 [47]. EAAI in African pouch rat brain was 1.10 and in the eyes -1.10 [48]. In the results of the isoelectric points (pI) the values ranged from 5.44 to 5.66. These values were slightly acidic. pI values in *C. gambianus* fancy meats ranged from 4.97-5.22 [46]; *C. gambianus* brain (4.28) and eyes (4.25) [48]; also in turkey meat: skin (4.41) and muscle (5.01) [20]. The calculation of pI from AA would assist in the quick production of the protein isolate of an organic product without going through the protein solubility determination to get the pI. The Leu/Ile ratio was low in the samples (2.12-2.29) with CV % of 5.45; hence no concentration antagonism might be experienced in the samples when used as protein source in food. The Leu/Ile ratio of the samples were in the ideal range suggested by FAO/WHO [40]. Deosthale et al. [49] showed that excess leucine in foods interfered with the utilization of Ile and Lys.

The concentration differences in the amino acids composition of the two fishes showed that *A. monroviae*

was more concentrated in 14/18 (77.8 %) amino acids whilst *L. goreensis* was more concentrated in 4/18 (22.2 %) amino acids. For the EAA, *A. monroviae* was more concentrated in 7/9 (77.8 %) amino acids whilst *L. goreensis* was more concentrated only in 2/9 (22.2 %) amino acids. These percentage differences further showed that extreme variability of composition do occur among different species of fish which accounts to some extent for the large variety of dishes that can be made from them. The composition of a particular species often appears to vary from one fishing ground to another, and from season to season, but the basic causes of change in composition are usually variation in the amount and quality of food that the fish eats and the amount of movement it makes [3].

The amino acid scores (AAS) based on whole hen's egg profile had values > 1.0 in Lys, His, Glu, Gly, Ala, Met, and Leu in *A. monroviae* and AAS > 1.0 in *L. goreensis* were Lys, His, Arg, Glu, Gly, Ala, Met, Leu and Phe. This meant that 7/18 (38.9 %) AAS were > 1.0 in *A. monroviae* and 9/18 (50.0 %) > 1.0 in *L. goreensis*. Also in the EAAS, 4/9 (44.4 %) of *A. monroviae* EAAS had values >1.0 whereas it is 5/9 (55.6 %) in *L. goreensis*. On the provisional essential amino acid scoring pattern, Val (0.87) was the limiting amino acid (LAA) in *A. monroviae* but the LAA in *L. goreensis* was also Val (0.88). The EAA often acting in a limiting capacity are Lys, Met + Cys, Thr and Trp [50]. Therefore in *L. goreensis*, Thr (0.99 or 99 %) would be the real limiting AA. In order to fulfil the daily need for all the EAA in the sample, it would require 100/99 or 1.01 times as much *L. goreensis* protein to be taken (eaten) when it is the sole protein in the diet. Under this condition, *A. monroviae* had no LAA. The scores based on the EAA based on the preschool child requirement, all the AA had scores > 1.0. This meant that each AA would supply more than the requirement of the preschool child.

The statistical summary of the data in Tables 1-5 and 7-9 showed that significant differences existed between the two samples in the parameters in each Table. The calculated  $r_{xy}$  ranged between 0.8480 to 0.9999 whilst the Table (confidence level)  $r_{xy}$  ranged from 0.575 to 0.798 at df (n-2) and  $r = 0.01$ . This trend was followed in  $r_{xy}^2$ . From Table 4,

$R_{xy}$  showed that for every unit increase in the AA of *A. monroviae* there was a corresponding decrease of 0.0437 in the *L. goreensis*. The coefficient of alienation ( $C_A$ ) values were high only from Tables 7 and 9 (0.513-0.530) but low from Tables 1-5 and 8. The index of forecasting efficiency (IFE) is an index of  $C_A$ , the higher the  $C_A$ , the lower the IFE and vice versa since  $C_A + IFE = 1.0$ . The IFE is a reduction in the error of prediction of relationship between two samples. For example from Table 1 and 2, IFE was 0.995 or 99.5 % meaning that the error of prediction was 100-99.5 or 0.5 %; this meant that the relationship between the two samples was very high and easily predictable. Where the IFE was high, it means that one sample could substitute the other in its physiological functions. The summary of the AA profiles into columns under factor B means had a value of 47.7 g/100 g and the factor A means also had 47.3 g/100 g. Both factor B individual values were close, similar observation was made for factor A individual values.

## Conclusion

Two essential amino acids Lys and Met were generally found in high concentrations in the fish proteins, in contrast to cereal proteins for example. Thus fish and cereal protein can supplement each other in the diet. Fish protein provides a good combination of amino acids that are highly suited to man's nutritional requirements and compares favourably with that provided by meat, milk and eggs. The fish samples had demonstrated these good characteristics as demonstrated in their amino acid profiles.

## References

- Adeyeye EI: *Aletes macrolepidotus*: nutritional implications of the lipid profile of its skin and muscle. Elixir Food Science 2013, 62: 17416-17424.
- Petrides GA: Advisory report on wild life and national parks in Nigeria. In: Olayide SO, Olatunbosun D, Idusoye EO, Abiagom JD (Eds.): A quantitative analysis of demands in Nigeria, 1968-1985, Lagos, Nigeria. The Federal Department of Agriculture 1972, p. 2.

- Murray J, Burt JR: The composition of Fish. FAO Corporate Document Repository, Advisory Note No. 38, 2001, 14 pp.
- Eschmeyer WW (Ed.): Catalogue of fishes: Special Publication of the Center for Biodiversity Research and Information. California Academy of Sciences, San Francisco, California, USA 1998, 2905pp.
- Allen GR: FAO Species Catalogue. Vol. 6. Snappers of the world. An annotated and illustrated catalogue of lutjanid species known to date. FAO Fish Synop 1985, 125(6): 208 pp.
- Federal Ministry of Science and Technology: Trawl fishes of the Nigerian Continental Shelf (No. 1). Compiled and produced by Marine Research Division, Nigerian Institute of Oceanography and Marine Research of the Federal Ministry of Science and Technology, Victoria Island, Lagos, Nigeria 1975, p. 1.
- AOAC: Official Methods of Analysis (18<sup>th</sup> Edn.). Association of Official Analytical Chemists, Washington DC, USA 2006.
- Spackman DM, Stein WH, Moore S: Automatic recording apparatus for use in the chromatography of amino acids. Analytical Chemistry 1958, 30: 1190-1206.
- Alsmeyer RH, Cunningham AE, Happich ML: Equations predict PER from amino acid analysis. Food Technology 1974, 28: 34-40.
- Paul AA, Southgate DAT: McCance and Widdowson's The Composition of Foods (4<sup>th</sup> Edn.). Her Majesty's Stationery Office, London, Britain 1978.
- FAO/WHO: Energy and protein requirements: Technical report series no. 522. Geneva, Switzerland 1973.
- FAO/WHO/UNU: Energy and protein requirements: Technical report series no. 724. Geneva, Switzerland 1985.
- Steinke FH, Precher EE, Hopkins DT: Nutritional evaluation (PER) of isolated soybean protein and combinations of food proteins. Journal of Food Science 1980, 45: 323-327.
- Oser BL: An integrated essential amino acid index for predicting the biological value of proteins. In AA Albanes (Ed.), *Protein and amino acid nutrition* (pp. 295-311). Academic Press, New York 1959.
- Olaofe O, Akintayo ET: Prediction of isoelectric points of legume and oilseed proteins from amino acid compositions. The Journal of Technoscience 2000, 4: 49-53.
- Finar LL: Organic chemistry, vol.2 (5<sup>th</sup> Edn.). ELBS and Longman Group, London, UK 1975.
- Oloyo RA: Fundamentals of research methodology for social and applied sciences. ROA Educational Press, Ilaro, Nigeria 2011.
- Abdullahi SA, Abolude DS: Investigation of protein quality of some fresh water fish species of Northern Nigeria. Academy Journal of Science and Technology 2002, 2(1):18-25.
- Adeyeye EI, Akpambang VOE, Adebomojo IA: Determination of protein, nitrite and nitrate in animal protein sources in Nigeria. Pakistan Journal of Science and Industrial Research 2003, 46(4): 270 – 276.
- Adeyeye EI, Ayejuyo OO: Proximate, amino acid and mineral composition of turkey-hen muscle and skin. Oriental Journal of Chemistry 2007, 23 (3): 879-886.
- Bingham S: Nutrition: A consumer's guide to good eating. Transworld Publishers, London 1978.
- Edem DO, Amugo CI, Eka OU: Chemical composition of yam beans (*Sphenostylis stenocarpa*). Tropical Science 1990, 30 (1): 59-63.
- National Advisory Committee on Nutrition Education (NACNE): Proposal for nutritional guidelines for healthy education in Britain. Health Education Council, London 1983.



Committee on Medical Aspects (COMA) of Food Policy, Diet and Cardiovascular Disease. HMSO, London 1984.

National Research Council (NRC): Recommended Daily Allowances (10<sup>th</sup> Edn.). National Academic Press, Washington DC, USA 1989.

Fleck H: Introduction to nutrition (3<sup>rd</sup> Edn.). Macmillan, New York, USA 1976.

Nieman DC, Butterworth DE, Nieman CN: Nutrition. Wm. C. Brown Publishers, Dubuque, USA 1992.

Shills MEG, Young VR: Modern nutrition in health and disease. Lea and Febiger, Philadelphia, USA 1988.

Adeyeye EI, Adamu AS: Chemical composition and food properties of *Gymnarchus niloticus* (Trunk fish). Biosciences Biotechnology Research Asia 2005, 3(2): 265-272.

Sales J, Hayes JP: Proximate, amino acid and mineral composition of ostrich meat. Food Chemistry 1996, 36(2): 167-170.

USDA: Composition of foods: Beef products. Agriculture handbook no. 8-13. United States Department of Agriculture, Washington DC, USA 1986.

USDA: Composition of foods: Poultry products. Agriculture handbook no. 8-5. United States Department of Agriculture, Washington DC, USA 1979.

Adeyeye EI: Amino acid composition of variegated grasshopper, *Zonocerus variegatus*. Tropical Science 2005a, 45(4): 141-143.

Adeyeye EI: The composition of winged termites, *Macrotermes bellicosus*. Journal of Chemical Society of Nigeria 2005b, 30(2): 145-149.

Adeyeye EI, Afolabi EO: Amino acid composition of three different types of land snails consumed in Nigeria. Food Chemistry 2004, 85: 535-539.

Adeyeye EI: Amino acid composition of three species of Nigerian fish: *Clarias anguillaris*, *Oreochromis niloticus* and *Cynoglossus senegalensis*. Food Chemistry 2009, 113: 43-46.

Robinson DE: Food biochemistry and nutritional value. Longman Scientific and Technical, London, UK 1987.

FAO/WHO: Protein quality evaluation. Report of Joint FAO/WHO Expert Consultation. FAO Food and Nutrition Paper 51, Rome, Italy 1990.

Adeyeye EI: The chemical composition of liquid and solid endosperm of ripe coconut. Oriental Journal of Chemistry 2004, 20(3): 471-476.

FAO/WHO: Protein quality evaluation. Report of Joint FAO/WHO Consultation Held in Bethesda, USA, 4-8 December, 1989. FAO, Rome, Italy 1991.

Mendoza C: Effect of genetically modified low phytic acid plants on mineral absorption. International Journal of Food Sciences and Nutrition 2002, 37: 759-767.

Sandstrom B, Almgren A, Kivisto B, Cederblad A: Effect of protein and protein source on zinc absorption in humans. Journal of Nutrition 1989, 199: 48-53.

Hoffman JR, Falvo MJ: Protein- which is best ? Journal of Sports Science and Medicine 2004, 3: 118-130.

United States Dairy Export Council: Reference Manual for US Whey Products (2<sup>nd</sup> Edn.) 1999.

Sarwar G: The protein digestibility-corrected amino acid score method overestimates quality of proteins containing antinutritional factors and of poorly digestible proteins supplemented with limiting amino acids in rats. Journal of Nutrition 1997, 127: 758-764.

Adeyeye EI: Amino acids profiles of the fancy meats of the African giant pouch rat (*Cricetomys gambianus*). Pakistan Journal of Science and Industrial Research, Series B: Biological Sciences 2013, 56(3): 129-136.

Nielsen SS: Introduction to the chemical analysis of foods. CBS Publishers and Distributors, New Delhi, India 2002.

Oyarekua MA, Adeyeye EI: The amino acid profile of the brain and eyes of African gaint pouch rat (*Cricetomys gambianus*). Agriculture and Biology Journal of North America 2011, 2: 368-375.

Deosthale YG, Mohan VS, Rao KV: Varietal deficiencies in protein lysine and leucine content of gram sorghum. Journal of Agricultural and Food Chemistry 1970, 18: 644-646.

Bingham S: Dictionary of nutrition. Barrie and Jenkins, London, UK 1977.