Nutritional Qualities of the Amino Acid profile of the Yolk and Albumen of Chicken (Hen) Egg

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The study reported was on the amino acid composition of the yolk and albumen of the egg of chicken hen which was determined on a dry weight basis. The total essential amino acid ranged from (g/100 g crude protein, cp): 48.5-45.5 or from 49.7-48.1 % respectively of the total amino acid. The total amino acid range was 97.7-94.6 g/100 g cp with the yolk predominating in the results. The amino acid scores showed lysine ranged from 1.32-1.26 (on provisional essential amino acid scoring pattern) and 1.26-1.19 (on suggested requirement of the essential amino acid of a pre-school child). The predicted protein efficiency ratio was 2.71-2.69, the essential amino acid index range was 1.46-1.39 and the calculated isoelectric point range was 5.69-5.48. The correlation coefficient (r_{xy}) was positive and significant at $r_{=0.01}$ for the amino acids, amino acid scores and the isoelectric point in the two samples.

Key words: Chicken hen, Yolk, Albumen, Amino acid.

Indigenous chickens are widely distributed in the rural areas of tropical and sub-tropical countries where they are kept by the majority of the rural poor. Indigenous chickens in Africa are in general hardy, adaptive to rural environments, survive on little or no inputs and adjust to fluctuations in feed availability. Chickens largely dominate flock composition and make up about 98 %[1] of the total poultry numbers (chickens, ducks and turkeys)kept in Africa.

Indigenous chicken constitutes 80 % of the 120 million poultry type raised in the rural areas in Nigeria[2]. They are self-reliant and hardy birds with the capacity to withstand harsh weather conditions and adaptation to adverse environment. They are known to possess qualities such as the ability to hatch on their own, brood and scavenge for major parts of their food and possess appreciated immunity from endemic diseases. Their products are preferred by the majority of Nigerians because of the pigmentation, taste, leanness and suitability for special dishes[3]. Their outputs (egg and meat) are readily available to villagers and people in urban and semi urban areas thus serving as a good source of protein in their diet, in the same vein, they serve as good source of income.

Though poultry breeding in Nigeria started in 1985 at the National Animal Production Research Institute, Zaria[4], reports have it that research on the local chicken had started earlier with comprehensive information about the local fowl. The local chicken of Nigeria is small in size and grows slowly. There have been reports on the characterisation of the local chicken in Nigeria and its potential for egg and meat production[4, 5].

There are various ecotypes of the local chicken in the different agro ecological zones in

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Nigeria as reported by different authors. Most of the classification by the different agro ecological zones considered mainly the normal feathered indigenous chicken because they are the most prominent whereas the naked neck and frizzled feathered are rare and almost becoming endangered and the gene pool they represent may be lost if not characterised and conserved. For instance, Olori[6] noted two ecotype characterised as forest and savannah or Yoruba and Fulani ecotypes, respectively. Recent works revealed that the different ecotypes can be grouped into two major categories on the basis of body size and body weight as heavy ecotype and light ecotype[7]. The heavy ecotype (also referred to as Fulani ecotype) is found in the dry savannahs (Guinea and Sahel savannah), Montane regions and cattle Kraals of the North and weigh about 0.9-2.5 kg at maturity. The light ecotype are those chicken types from Swamp, Rainforest and Derived Savannah agro ecological zones whose mature body weight ranges between 0.68-1.5 kg.

Olawunmi *et al.*[8] found that the Fulani ecotype chicken was bigger in size than the Yoruba ecotype chicken 1.7 ± 0.4 and 0.79±0.21 kg for Fulani and Yoruba ecotypes respectively. Indigenous male chicken was also bigger in size than their female counterparts 1.5±0.6 kg versus 1.29±0.04 kg, respectively[9].

Scanty reports abound in literature on the meat quality characteristics of the Nigerian indigenous chickens. Indigenous chicken meat and egg are preferred by majority of the rural dwellers mainly because of their toughness, pigmentation, taste, leanness and suitability for special dishes[10, 11]. Meat and eggs from indigenous chicken are also of moderate prices compared to products from commercial birds [10, 12]. The Nigerian indigenous chickens have the capability of being developed into meat-type and egg-type birds [13].

There are no reports on the chemical composition of the yolk and albumen of the chicken hen eggs. There is also a debate on whether to discard the yolk in the consumption of egg to reduce its suspected promotion of coronary heart diseases. This study wants to report on the amino acid profiles of the yolk and albumen of chicken hen cooked eggs to expose their relative contributions to the egg protein as a complete food.

MATERIALAND METHODS

Preparation of samples

The matured and fertilized eggs of chicken hen were purchased from Ado-Ekiti, Nigeria, market. The eggs were cooked in the laboratory, shells removed, yolk and albumen separated and also oven dried separately. The dried samples were pulverised, sieved and kept in freezer in McCartney bottles pending analysis.

Crude Protein Determination and Fat Extraction

The micro-Kjeldahl method[14] was followed to determine the fat-free crude protein. The fat was extracted with a chloroform/methanol (2:1 v/v) mixture using Soxhlet extraction apparatus[15].

Amino acid analysis

Details of the procedure had been given earlier[16]. To determine the amino acids, about 30 mg of defatted egg sample was weighed into glass ampoule, 7 ml of 6 M HCI added and oxygen expelled by passing nitrogen into sample. The glass ampoule was sealed with a flame and heated at 105±5°C for 22 h. The ampoule was cooled, opened and the contents filtered to remove the humins, and the filtrate was evaporated to dryness at 40 °Cunder vacuum. The residue was dissolved with 5 ml acetate buffer (pH 2.0) and stored in the freezer. The period of analysis was 76 min, with gas flow rate of 0.50 ml/min at 60 °C and the reproducibility was ±3 %. The amino acid values were the average of two determinations. Tryptophan was not determined due to cost of this specific analysis.The method of amino acid analysis was by ion-exchange chromatography (IEC)[17] using the Technicon Sequential Multisample (TSM) Amino Acid Analyser (Technicon Instruments Corporation, New York).

Estimation of isoelectric point (pI)

The theoretical estimation of isoelectric point (pI) can be carried out by the equation of the form[18];

$$IPm = \sum_{i=1}^{n} IPiXi$$

where IPm is the isoelectric point of the mixture of amino acids, IPi is the isoelectric pointof the ith amino acid in the mixture and Xi is the mass ormole fraction of the ith acid in the mixture.

Estimation of predicted protein efficiency ratio (P-PER)

The predicted protein efficiency ratio (P-PER) was estimated using one of the equations developed by Alsmeyer*et al.*,[19] that is:

P-PER = -0.468 + 0.454 (Leu)-0.105 (Tyr)

Estimation of dietary protein quality

The amino acid scores were calculated using two different methods:

(i) Calculating the amino acid score using the following formula:

Amino acid score = Amount of amino acid per test protein [mg/g]/Amount of amino acid per protein in reference pattern [mg/g]

(ii) Calculations based on the pre-school child (2-5 years) suggested requirements[20].

Estimation of essential amino acid index (EAAI)

The essential amino acid index was calculated by using the ratio of test protein to the reference protein for each eight essential amino acids plus histidine according to Steinke *et al.*[21]:

$$\text{Essential amino acid index} = \sqrt[9]{\frac{\text{mg lysine in 1 g test protein}}{\text{mg lysine in 1 g reference protein}} \times}$$

Leucine/Isoleucine ratio

The leucine/isoleucine ratios, their differences and their percentage differences were also calculated.

Statistical analysis

The statistical analysis carried out included the determination carried out included the determination of the grand mean, standard deviation(SD) and the coefficients of variation percent (CV %). Other calculations made were the simple linear correlation (r_{xy}), coefficient of determination (r_{xy}^2), coefficient of alienation (or index of lack of relationship) (C_A) 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[22].

RESULTS AND DISCUSSION

The amino acid results of the samples are shown under various headings in terms of various designations.

Table 1 shows the amino acid (AA) of the samples. Glu and Asp were the most concentrated AA in both the yolk and the albumen with respective values (g/100 g crude protein, cp) of : 10.5-9.94 (Asp) and 14.5-14.1 (Glu). A look at Table 1 will show that AA of the yolk was slightly more concentrated (on pair wise comparisons) than the corresponding AA in the albumen in eleven or 64.7 % parameters; of the nine essential AA determined seven of them or 77.8 % were more concentrated in the yolk than the albumen on pair wise comparisons. The most concentrated essential AA (EAA) in the samples was Leu (7.85 g/100 g cp) in the yolk and 7.93 g/100 g cp in the albumen. The coefficient of variation percent (CV %) ranged between 0.72-12.7 in the AA, with Leu having the least CV % and Met the highest CV %.

The FAO/WHO/UNU[20] EAA standards for pre-school children (2-5 years) were (g/100 g protein): Leu (6.6), Phe + Tyr (6.3), Thr (3.4), Try (1.1), Val (3.5), Ile (2.8), Lys (5.8), Met + Cys (2.5), His (1.9) and total (33.9 with His) and 32.0 (no His). Based on this information, both samples would provide (individually) more than enough of the EAA for the pre-school children. Histidine is a semi-essential AA particularly useful for histamine present in small quantities in cells. Arginine is also good for children and it is high in the samples. Isoleucine is an EAA for both old and young. Methionine is needed for the synthesis of choline which in turn forms lecithin and other phospholipids in the body. When the diet is low in protein, for instance in alcoholism and kwashiorkor, insufficient choline may be formed; this may cause accumulation of fat in the liver[23]. Phenylalanine is the precursor of some hormones and the pigment melanin in hair, eyes and tanned skin. The entire above mentioned AA were high in both samples with the yolk values predominating in each case.

The result in Table 2 presents parameters on the quality of the protein of the samples. The EAA ranged from 48.5-45.5 g/100 g cp with a variation of 4.50 %. The total sulphur AA (TSAA) of the samples was 4.23 g/100 g cp (yolk) and 3.69 g/100 g cp (albumen). The values of 4.23-3.69 g/ 100 gcp are close to the value of 5.8 g/100 g cp recommended for infants[20]. The aromatic AA (ArAA) range suggested for infant protein (6.8-11.8 g/100 gcp) [20] is very favourably comparable with the present report of 12.2-12.2 g/100 g. The percentage ratio of EAA to the total AA (TAA) in the samples ranged between 49.7 % and 48.1 %. These values are well above the 39 % considered adequate for ideal protein food for infants, 26 % for children and 11 % for adults[20].

The percentage of total neutral AA (TNAA) ranged from 55.8 down to 57.1 g/100 g, indicating that these formed the bulk of the AA; total acidic AA (TAAA) ranged from 25.7-25.5 which is far lower than % TNAA, whilst the percentage range in total basic AA (TBAA) was 18.5 (yolk) and 17.5 (albumen) which made them the third largest group among the parameters. The predicted protein efficiency ratio (P-PER) was 2.71 (yolk) and 2.69 (albumen) meaning that the yolk may be more easily bioavailable than the albumen by as much as 0.74 %. The Leu/Ile ratio was low in both samples with values of 1.60 (yolk) and 1.88 (albumen) with a CV % of 11.4, hence no concentration antagonism might be experienced in the chicken (hen) egg yolk and albumen when used as the only protein source in food. The essential amino acid index (EAAI) ranged from 1.46-1.39. EAAI is useful as a rapid tool to evaluate food formulations for protein quality, although it does not account for differences in protein quality due to various processing methods or certain chemical reactions[24]. In the results of the isoelectric point (pI), there was a shift from 5.69 (yolk)down to 5.48 (albumen). This type of shift was also observed in the brain (4.64) down to 4.32 (eyes) of guinea fowl[25]. The calculation of pIfrom the AA would assist in the production of the protein isolate of an organic product. From literature on whole hen egg analysis[26]: EAA is 49.5 (no Try), EAAI is

Table 1. Amino acid composition (g/100 g crude protein) of yolk and albumen of chicken (hen) egg (dry weight)

Amino acid	Yolk	Albumen	Mean	SD	CV %
Lys[a]	7.28	6.91	7.10	0.26	3.69
His[a]	3.25	3.00	3.13	0.18	5.66
Arg[a]	7.55	6.62	7.09	0.66	9.28
Asp	10.5	9.94	10.2	0.42	4.08
Thr[a]	4.20	4.45	4.33	0.18	4.09
Ser	3.65	4.35	4.00	0.49	12.4
Glu	14.5	14.1	14.3	0.28	1.92
Pro	4.86	5.08	4.97	0.16	3.13
Gly	5.52	4.89	5.21	0.45	8.56
Ala	5.00	5.20	5.10	0.14	2.77
Met[a]	2.86	2.39	2.63	0.33	12.7
Cys	1.37	1.30	1.34	0.05	3.71
Val[a]	5.38	4.95	5.17	0.30	5.89
Ile[a]	4.91	4.21	4.56	0.49	10.9
Leu[a]	7.85	7.93	7.89	0.06	0.72
Phe[a]	5.22	5.05	5.14	0.12	2.34
Tyr	3.69	4.17	3.93	0.34	8.64
Try[a]	-	-	-	-	-
Protein (fat free)	75.3	75.6	75.5	0.21	0.28

[a]Essential amino acid; - not determined; mean value is grand mean from the mean of the amino acids.

1.37 (no Try), P-PER is 2.88, TAAA is 22.7 g/100 g, TBAA is 14.7 g/100 g, Leu/Ile is 1.48, TArAA is 11.5 (no Try), TNAA is 60. 7 g/100 g and pI is 5.64 (no Try). From an unpublished work on the amino acid profile for the yolk and albumen of guinea fowl (*Numidameleagris*) egg, the following information were obtained: P-PER was 3.74 (yolk) and 3.17 (albumen), Leu/Ile ratio was 1.60 (yolk) and 1.54 (albumen), EAAI range was 1.44-1.38, pI was 5.69 (yolk) down to 5.48 (albumen) (Adeyeye, E.I., 2011 article sent for publication).

Table 2. EAA, non-EAA, acidic, neutral, sulphur and aromatic acid contents (g/100 gcrude protein) of yolk and albumen of chicken (hen) egg (dry weight)

Amino acid	Yolk	Albumen	Mean	SD	CV %
Total amino acid (TAA)	97.7	94.6	96.1	2.17	2.26
Total non-essential amino acid (TNEAA)	49.2	49.1	49.1	0.06	0.12
Total EAA-with His	48.5	45.5	47.0	2.11	4.50
-no His	45.2	42.5	43.9	1.92	4.38
% TNEAA	50.3	51.9	51.1	1.13	2.21
% Total EAA -with His	49.7	48.1	48.9	1.13	2.31
-no His	47.9	46.4	47.2	1.06	2.25
Total neutral amino acid (TNAA)	54.5	54.0	54.2	0.38	0.70
% TNAA	55.8	57.1	56.5	0.92	1.63
Total acidic amino acid (TAAA)	25.1	24.1	24.6	0.69	2.82
% TAAA	25.7	25.5	25.6	0.14	0.55
Total basic amino acid (TBAA)	18.1	16.5	17.3	1.10	6.33
% TBAA	18.5	17.5	18.0	0.71	3.93
Total sulphur amino acid (TSAA)	4.23	3.69	3.96	0.38	9.64
% TSAA	4.33	3.90	4.12	0.30	7.39
% Cys in TSAA	32.4	35.2	33.8	1.98	5.86
Total aromatic amino acid (TArAA)	12.2	12.2	12.2	0.04	0.35
% TArAA	12.5	12.9	12.7	0.28	2.23
P-PER[a]	2.71	2.69	2.70	0.01	0.52
Leu/Ile ratio	1.60	1.88	1.74	0.20	11.4
Leu-Ile (difference)	2.94	3.72	3.33	0.55	16.6
% Leu-Ile (difference)	37.5	46.9	42.2	6.65	15.8
EAAI[b]	1.46	1.39	1.43	0.05	3.47
Isoelectric point (pI)	5.69	5.48	5.59	0.15	2.66

[a]Predicted protein efficiency ratio; [b]Essential amino acid index.

Table 3. Amino acid scores of the chicken (hen) egg yolk and albumen based on the provisional essential amino acid scoring pattern

Amino acid	Yolk	Albumen	Mean	SD	CV %
Lys	1.32	1.26	1.29	0.04	3.29
Thr	1.05	1.11	1.08	0.04	3.93
Met + Cys	1.21	1.05	1.13	0.11	10.0
Val	1.08	0.99	1.04	0.06	6.15
Ile	1.23	1.05	1.14	0.13	11.2
Leu	1.12	1.13	1.13	0.01	0.63
Phe + Tyr	1.49	1.54	1.52	0.04	2.33
Try	-	-	-	-	-
Total	1.22	1.18	1.20	0.03	2.36

-not determined.

The % Cys in TSAA was low with a range value of 32.4 – 35.2 like most animal AA values[28]. Cys can spare with Met in improving protein quality and has positive effects on mineral absorption, particularly zinc[29].

The result in Table 3 shows the essential AA scores (EAAS) based on the provisional amino acid scoring pattern[30]. EAAS less than 1.0 in the albumen was Val (0.99) thereby serving as the limiting AA (LAA) in the corresponding sample. Normally the EAA most often acting in a limiting capacity are Lys, Met + Cys, Thr and Try in that order. Try was not determined, Val would then be limiting in the albumen. To make correction for the LAA in the sample if it serves as the sole source of protein food therefore, it would be 100/99 x protein of albumen or 1.01 x protein of albumen. The result in Table 4 shows the EAAS based on suggested requirement of the EAA of a pre-school child[20]. All the EAAS were greater than 1.00. Whilst Ile had the highest score in Table 4 (1.75, yolk; but His, 1.58 in albumen), Phe + Tyr the highest score (1.49-1.54) in Table 3.

The following values would show the position of the quality of the chicken (hen) egg yolk and albumen protein: the EAA requirements across board are (values with His) (g/100 g protein): infant (46.0), pre-school (2-5 years), (33.9)school

Table 4. Amino acid scores of the chicken (hen) egg yolk and albumen based on the suggested requirement of the essential amino acid of a pre-school child

Amino acid	Yolk	Albumen	Mean	SD	CV %
Lys	1.26	1.19	1.23	0.05	4.04
His	1.71	1.58	1.65	0.09	5.59
Thr	1.24	1.31	1.28	0.05	3.88
Val	1.69	1.48	1.59	0.15	9.37
Met + Cys	1.54	1.41	1.48	0.09	6.23
Ile	1.75	1.50	1.63	0.18	10.9
Leu	1.19	1.20	1.20	0.01	0.59
Phe +Tyr	1.41	1.46	1.44	0.04	2.46
Try	-	-	-	-	-
Total	1.40	1.35	1.38	0.04	2.57

Table 5. Summary of the amino acid profiles into factors A and B

	Samples (Factor A)		
	Yolk	Albumen	Factor B means
Amino acid composition (Factor B)			
Total essential amino acid	48.5	45.5	47.0
Total non-essential amino acid	49.2	49.1	49.1
Factor A means	48.8	47.3	48.1

Table 6. Summary of the statistical analysis of the data in Tables 1, 2, 3 and 4

From Table	r_{xy}	r_{xy}^2	R_{xy}	C_A %	IFE %	Remark
1	0.9903	0.98	0.12	13.9	86.1	*
2 (pI only)	0.9889	0.98	2.45	14.9	85.1	*
3	0.8567	0.73	-0.11	51.6	48.4	*
4	0.8999	0.81	0.56	43.7	56.3	*

*Result significant at $r_{=0.01}$ at n-2 degrees of freedom.

child (10-12 years) (24.1) and adult (12.7) and without His: infant (43.4), pre-school (32.0), school child (22.2) and adult (11.1)[20]; from the present results based on these standards, we have: 48.5 g cp (with His) and 45.2 (no His) in yolk; 45.5 g cp (with His) and 42.5 (no His) in albumen; Try was not determined. The yolk would satisfy the requirements of all age groups but slightly less for the albumen.

The result in Table 5 gives a brief summary of the AA profile in the samples. Column under Factor B shows that the values were very close with a range of 47.0-49.1. However, Table 6 depicts the summary of the statistical analysis from Table 1, 2 (pI only), 3 and 4. The simple linear correlation coefficient (r_{xy}) values showed high positive and significant results for all the Tables 1-4 but r_{xy} values being highest in 1 and 2 at $r_{=0.01}$ at n-2 degrees of freedom. The regression coefficient (R_{xy}) showed that for every unit increase in the yolk AA parameter, the increase was 0.12 (Table 1), 2.45 (Table 2, pI only), - 0.11 (Table 3) and 0.56 (Table 4).

The coefficient of alienation was low in Table 1 (13.9 %), Table 2 (14.9 %), slightly high in Table 3 (51.6 %) and slightly low in Table 4 (43.7 %). The Index of forecasting efficiency (IFE) was high in Table 1 (86.1 %), Table 2 (85.1 %), slightly low in Table 3 (48.4 %) and slightly above average in Table 4 (56.3 %). Low IFE versus high C_A makes prediction of relationship difficult. The C_A produces an index of lack of relationship whilst the IFE gives the reduction in errors of prediction or relationship. The C_A and IFE values showed that a good relationship existed between the yolk and albumen AA in chicken (hen) eggs particularly with the results in Tables 1,2 and 4.

Table 7. Amino acid composition (g/100 g crudeprotein) of yolk and albumen of chicken (hen) and guinea fowl eggs (dry weight) compared

Amino	Yolk		Albumen	
acid	Chicken	Guinea fowl	Chicken	Guinea fowl
Lys	7.28	7.01	6.91	7.20
His	3.25	2.90	3.00	3.09
Arg	7.55	7.12	6.62	6.87
Asp	10.5	9.63	9.94	9.84
Thr	4.20	3.95	4.45	4.15
Ser	3.65	4.94	4.35	5.00
Glu	14.5	13.9	14.1	13.1
Pro	4.86	5.60	4.89	4.89
Gly	5.52	5.60	5.08	4.86
Ala	5.00	4.70	5.20	5.08
Met	2.86	2.73	2.39	2.49
Cys	1.37	1.30	1.30	1.24
Val	5.38	5.61	4.95	4.60
Ile	4.91	5.03	4.21	4.91
Leu	7.85	8.07	7.93	7.55
Phe	5.22	5.56	5.05	5.22
Tyr	3.69	3.69	4.17	3.53
Try	-	-	-	-
Protein (fat free)	75.3	81.1	75.6	77.1

For clear comparison between the amino acid profiles of the yolk and albumen of chicken (hen) and guinea fowl eggs, Table 7 depicts their comparison on one to one parameter bases.

CONCLUSIONS

The study showed that the amino acid in the yolk of the egg of chicken (hen) is better than its albumen in TAA, TEAA, TSAA, P-PER, Leu/Ile ratio and EAAI. Removal of yolk before consumption of the egg will therefore reduce the availability/function of the parameters enunciated. Also to be lost would be all phospholipids (including those needed for brain development), all essential fatty acids and all forms of sterols. The bird is free-range and its yolk cholesterol would not be high enough to promote incidence of coronary heart disease.

REFERENCES

1. Gweye, EF, *J World Poultry,***19**: 12 (2003).
2. RIM (Resources Inventory and Management Ltd), *Nigeria National Livestock Survey*. Federal Department of Livestock and PestControl, Lagos, Nigeria (1992).
3. Horst, P, *Native fowl as reservoir for genomes and major genes with direct and indirect effects on production adaptability*. Proceedings of the 18th World Poultry Congress, September 4-9, pp 105-106, Nagoya, Japan (1988).
4. Nwosu, CC, *Characterization of the local chicken in Nigeria and its potential for egg and meat production*. Proceedings of the 1st National Seminar on Poultry Production, December 11-13, pp 187-210, Ahmadu Bello University, Zaria, Nigeria (1979).
5. Adebambo, OA, *Proposed national animal breeding programmes in Nigeria*. Proceedings of the Research Planning Workshop, African Animal Genetic Resources, February 19-21, pp 137-139, International Livestock Center for Africa, Addis Ababa, Ethiopia (1992).
6. Olori, VE, *An evaluation of two ecotypes of the Nigerian indigenous chicken*, M.Sc. Thesis, ObafemiAwolowo University, Ile Ife (1992).
7. Momoh, OM, Ehiobu, NG, Nwosu, CC, *Egg production of two Nigerian local chicken ecotypes under improved management*. Proceedings of 32nd Annual Conference of Nigerian Society for Animal Production, March 18-22, pp 278-281, University of Calabar, Calabar, Nigeria (2007).
8. Olawunmi, OO, Salako, AE, Afuwape, AA, *Int J Morphol*, **26**: 975 (2008).
9. Ajayi, FO, Agaviezor, BO, *Phenotypic characteristics of indigenous chicken in selected local government areas in Bayelsa State, Nigeria*. Proceedings of the 3rd Nigeria International Poultry Summit, February 22-26, pp 75-78, Abeokuta, Nigeria (2009).
10. Horst, P, *Anim Res Dev*, 33: 63 (1991).
11. Islam, MA, Nishibori, M, *World's PoultSci J*, **65**: 125 (2009).
12. Gueye, EF, *J World PoultSci*, **54**: 73 (1998).
13. Ajayi. FO, *Asian J PoultSci*, **4**: 164 (2010).
14. Pearson, D, *Chemical Analysis of Foods*, 7thedn., Churchill, London, 7-11 (1976).
15. AOAC, *Official Methods of Analysis*, 18thedn., Association of official Analytical Chemists, Washington DC (2005).
16. Adeyeye, EI, Arifalo, MKO, *Biosci, Biotech Res Asia*, **6**(2): 559(2009).
17. FAO/WHO, *Protein Quality Evaluation*, Report of Joint FAO/WHO Expert Consultation, FAO Food and Nutrition Paper 51, FAO/WHO. Rome, 4-66 (1991).
18. Olaofe, O, Akintayo, ET, *The Journal of Techno-Science*, **4**: 49(2000).
19. Alsmeyer, RH, Cunningham, AE, Happich, ML, *Food Technol*, **28**: 34 (1974).
20. FAO/WHO/UNU, *Energy and Protein Requirements*, WHO Tech Report Ser. No. 724, Geneva, 205 (1985).
21. Steinke, FH, Prescher, EE, Hopkins DT, *J. Food Sci*, **45**: 323 (1980).
22. Oloyo, RA, *Fundamentals of Research Methodology for Social and Applied Sciences*, ROA Educational Press, Ilaro, Nigeria (2001).
23. Bingham, S, *Dictionary of Nutrition*, Barrie and Jenkins Ltd., London, 76-281 (1977).
24. Nielsen, SS, *Introduction to the Chemical Analysis of Foods*, CBS Publishers and Distributors, New Delhi (2002).
25. Adeyeye, EI, Aremu MO, *TheOpen Nutraceuticals Journal*, **3**: 220 (2010).
26. Paul, AA, Southgate, DAT, *First Supplement to McCance and Widdowson's The Composition of Foods*, HMSO, London, UK, 16 (1976).
27. Adeyeye, EI, Amino acid composition of some aquaculture fauna resources in Nigeria. In: *Handbook of Nutritional Biochemistry: Genomics, Metabolomics and Food Supply*, Editors: S.Haugen, S. Meijer, pp 225-254. Nutrition and Diet Research Progress Series, Nova Science Publishers, Inc, New York (2010).
28. Adeyeye, EI, *Orient J Chem*, **20** (3): 471 (2004).
29. Mendoza, G, *Int J Food SciTechnol*, **37**: 759 (2002).
30. FAO/WHO, *Energy and Protein Requirements*, Technical Report Series No.522, WHO, Geneva, Switzerland, 1-118 (1973).