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Amino Acid Profiles of the Flesh of the Heterosexual Pairs of *Neopetrolisthes maculatus*

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Abstract. This paper reports on amino acid profiles of the flesh of heterosexuals of porcellanids collected from the Atlantic Ocean at Orimedu beach in Ibeju-Lekki, Lagos, Nigeria. Results showed that high values of amino acids were observed in the heterosexual flesh of *Neopetrolisthes maculatus* (g $100g^{-1}$ protein): 17.7 – 17.8 (Glu), 9.90 – 10.0 (Asp), 8.70 – 9.07 (Arg), 7.23 – 7.94 (Leu) and 5.81 – 6.06 (Gly). Total essential amino acid values ranged from 45.2 to 46.2 g 100^{-1}g. Predicted protein efficiency ratio was 3.82 – 4.14, the range of essential amino acid index was 86.9 – 89.9, the biological values ranged from 83.0 to 86.3. The Lys/Trp was 3.31- 4.27. Serine was limiting amino acid (0.513 – 0.516) in the egg score comparison; under the essential amino acids scores, Lys (0.840) was limiting in female but Val (0.823) was limiting in the male; Lys (0.796 – 0.905) was limiting in both samples in the pre-school child amino acid requirements. It was observed that out of the twenty parameters determined, male flesh was more concentrated in 60% values than the female flesh and 40% better in female than male. Correlation coefficient result showed that significant differences existed in the amino acids composition at $r_{= 0.01}$ of the *N. maculatus* samples.

Introduction

Porcellanidae family is a group of crab-shaped anomuran crustaceans that belong to the superfamily Galatheoidea together with three other families Galatheidae, Munididae and Munidopsidae [1]. They are commonly found in rocky and coral reefs of temperate and tropical coasts.

The World Register of Marine Species (WoRMS) has given the taxonomic details of *Neopetrolisthes maculatus* (H. Milne Edwards, 1837) [2].Classification: Biota > Animalia (Kingdom) > Arthropoda (Phylum) > Crustacea (Subphylum) > Multicrustacea (Superclass) > Malacostraca (Class) > Eumalacostraca (Subclass) > Eucarida (Superorder) > Decapoda (Order) > Pleocyemata (Suborder) > Anomura (Infraorder) > Galatheoidea (Superfamily) > Porcellanidae (Family) > *Neopetrolisthes* (Genus) > *Neopetrolisthes maculatus* (Species).

Parent: *Neopetrolisthes* Miyake, 1937

Original name: *Porcellana maculata* H. Milne Edwards, 1837

Synonymised names [3]: *Neopetrolisthes ohshimai* Miyake, 1937 (Synonym)

Petrolisthes ohshimai (Miyake, 1937) (junior synonym)

Porcellana maculata H. Milne Edwards, 1837

In distribution, porcellanids are widely distributed in the Indo-West Pacific. They are found from east coast of Africa to Christmas Island and Western Australia, Bismarck Archipelago, Queensland, Moluccas, Palau, Taiwan, Southern Japan (Ryukyu Islands), New Caledonia, Marshall and Fiji Islands [4]. They live in shallow subtidal water; coral and rocky reefs, being associated with large sea anemones (*Cryptodendrum, Entacmaea, Gyrostoma, Heteractis* and *Stichodactyla*), typically found in a heterosexual pair [5].

Neopetrolisthes maculatus is a spotted crab. There are two different colour forms, although the ground colour of the bodies of both forms is white. In one form, carapace and chelipeds are white, with an uneven pattern of irregular sizes of red blotches; ambulatory legs also white, with some small red spots on meri of first pair (second pereopod). In the other form, the carapace and chelipeds have a uniform pattern of numerous small, reddish purple spots; meri of ambulatory legs also with numerous small, reddish purple spots [6].

Crab is consumed by many individuals as it is often recommended for pregnant women. Works already reported in literature on crabs included the determination of the composition of nutritionally valuable parts of male and female common West African fresh water crab, *Sudananautes africanus africanus* [7]; relationship in the amino acid of the whole body, flesh and exoskeleton of *S. africanus africanus* [8]; proximate and mineral composition of whole body, flesh and exoskeleton of male and female *S. africanus africanus* [9]; proximate and mineral compositions of common crab species (*Calllnectes pallidus and Cardisoma armatum*) of Badagry creek, Nigeria [10]; composition and distribution of the European green crab in Prince Edward Island, Canada [11] and the comparative study of the lipid and fatty acid composition of two shell fish: lagoon and fresh water crabs [12]. There is paucity of information on the amino acid composition of *Neopetrolisthes maculatus.* The study reported in this article is an attempt to assess the amino acid quality composition of the endoskeleton of *N. maculatus* with the hope that this will contribute information to food composition tables. The colour pattern of the samples is of large and uneven blotches resembling the Pacific Ocean *N. maculatus* population [13].

Materials and methods

Collection of samples: Samples were collected from trawler catches from the Atlantic Ocean at Orimedu beach in Ibeju-Lekki area of Lagos State, Nigeria. The experiment took place between November 2014 and June 2015. The crabs were washed with distilled water to remove adhering contaminant and transported in ice crushed containers to the laboratory for identification and preservation prior to analysis. The crabs were identified in the Department of Forestry, Wildlife and Fisheries Management of Ekiti State University, Ado – Ekiti, wrapped in aluminium foil and frozen at - 4^{o}C for 2 – 3 days before analysis.

Sample treatment: More than ten matured crabs were caught with the net but three samples were used in this study. The three whole crabs were separated fresh, two were males and only one was female. Whilst the internal organs were discarded, the other separated parts were dried in the oven at 105^{o}C. For the purposes of analysis, the separated parts were the carapace and cheliped exoskeleton (to constitute the exoskeleton) and the muscle from the thoracic sterna and cheliped (to constitute flesh). The flesh from each sex was separately blended.

Extraction and analysis: Extraction and the instrumentation analysis were carried out by following AOAC method [14] and Danka *et al*. [15].

The dried pulverized sample was made to be free of water by ensuring constant weight for a period of time in the laboratory. The sample of 10.0g was weighed into the 250ml conical flask capacity. The sample was defatted by extracting the fat content of the sample with 30ml of petroleum spirit three times with Soxhlet extractor that was equipped with timble. The sample was hydrolyzed three times for complete hydrolysis to be achieved for the totality of amino acids recovery.

The pulverized and defatted sample was soaked with 30ml of 1M potassium hydroxide solution and was incubated for 48 hours at 110^{o}C in hermetically closed borosilicate glass container. After the alkaline hydrolysis, the hydrolysate was neutralized to get pH in the range of 2.5 – 5.0. The solution was purified by cation – exchange solid-phase extraction. The amino acids in purified solutions were derivatised with ethylchloroformate by the established mechanism:

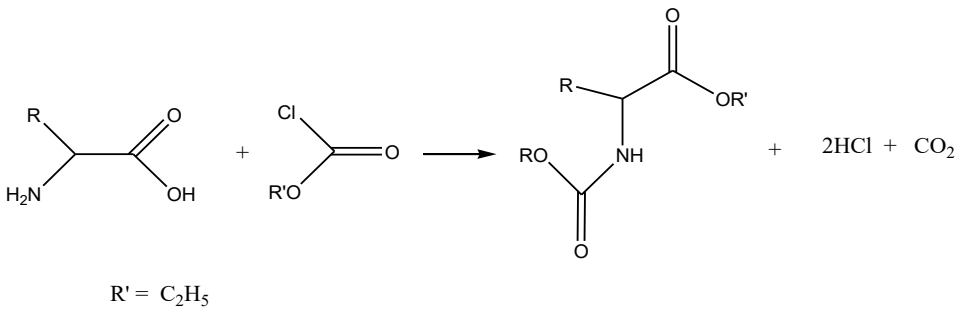

Figure 1. Derivatization process of amino acid

The derivatising reagent was removed by scavenge with nitrogen. The derivatized amino acid was made up to 1ml in a vial for gas chromatography analysis. The gas chromatographic conditions for the amino acids analysis were as follows: GC : HP6890 powered with HP Chemstation rev. A09.01 [1206] software; injection temperature: split injection; split ratio: 20:1; carrier gas: hydrogen; flow rate: 1.0ml/min; inlet temperature: 250^{o}C; column type: EZ; column dimensions: 10m x 0.2mm x 0.25µm; oven programme: initial @ 110^{o}C, first ramp @ 27^{o}C/min to 320^{o}C, second, constant for 5 mins at 320^{o}C; detector: PFPD; detector temperature: 320^{o}C; hydrogen pressure: 20 psi; compressed air: 35 psi.

Some calculations from analytical results:

(i) Estimation of isoelectric point (pI): The estimation of the isoelectric point (pI) for a mixture of amino acids can be carried out by the equation of the form [16]:

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

where IP_m is the isoelectric point of the mixture of amino acids, IP_i is the isoelectric point of the ith amino acid in the mixture and X_i is the mass or mole fraction of the ith amino acid in the mixture.

(ii) Estimation of predicted protein efficiency ratio (P-PER): Computation of protein efficiency ratio (C-PER or P-PER) was done using the equations suggested by Alsmeyer *et al*. [17]:

$$P - PER_1 = - 0.468 + 0.454 \text{ (Leu)} – 0.105 \text{ (Tyr)} \quad (2)$$

$$P - PER_2 = - 0.684 + 0.456 \text{ (Leu)} – 0.047 \text{ (Pro)} \quad (3)$$

(iii) Leucine / isoleucine ratio: The leucine/isoleucine ratios, their differences and their percentage differences were calculated.

(iv) Estimation of essential amino acid index (EAAI): The method of EAAI calculation due to Oser [18] using the egg protein amino acids as the standard.

(v) Estimation of biological value (BV): Computation of biological value (BV) was calculated following the equation of Oser [18]:

$$\text{Biological value} = 1.09(\text{EAAI}) – 11.73 \quad (4)$$

(vi) Computation of Lys/Trp and Met/Trp

The ratios of Lys/Trp (L/T) and Met/Trp (M/T) were computed.

(vii) Computation of amino acid scores: The amino acid scores were computed using three different procedures:

- Scores based on amino acid values compared with whole hen's egg amino acid profile [19].
- Scores based on essential amino acid scoring pattern [20].
- Scores based on essential amino acid suggested pattern of requirements for pre-school children [21].

Statistical evaluation

Data results in Table 1 were subjected to statistical analysis of correlation coefficient (r_{xy}), regression coefficient (R_{xy}), coefficient of alienation (C_A), index of forecasting efficiency (IFE), coefficient of determination or variance (r_{xy}^2). Other calculations were grand mean, standard deviation (SD) and coefficient of variation (CV %). The r_{xy} was converted to critical Table value to see if significant difference existed among the two heterosexual sample results at $r_{=0.01}$ [22].

Results and Discussion

The amino acids composition of the heterosexual flesh of *Neopetrolisthes maculatus* (dry weight) in g $100g^{-1}$ protein (cp) can be seen in Table 1. The highest concentrated amino acid was glutamic acid (Glu), an acidic amino acid in both samples with value range of 17.7 – 17.8 and followed by another acidic amino acid, Asp with values of 10.0 – 9.90. The highest concentrated essential amino acids were Arg with values of 8.70 – 9.07 and Leu with values of 7.23 – 7.94. The total amino acids had a range of 96.6 – 97.1 which was also a reflection of the samples protein of corresponding 40.2 – 43.7 g $100g^{-1}$. The coefficient of variation percent (CV%) for all the parameters determined were all generally low with values of 0.124 (observed for Glu) - 32.0 (observed for His). This showed the very closeness of the values obtained for each parameter in the samples. The observation made in the present report for Glu and Asp also corroborated with the observation in the flesh of female West African fresh water crab (*Sudananautes africanus africanus)* with Glu (130.2 mg g^{-1} crude protein) > Asp (72.5 mg g^{-1} cp) but in reverse for Arg and Leu as shown here, Arg (58.9 mg g^{-1} cp) < Leu (66.0 mg g^{-1} cp) [8].

The concentration differences in the amino acid profiles of the two samples could also be seen in Table 1, column 7 and their corresponding differences depicted in column 8. Twenty parameters were considered in Table 1. Out of these 20 parameters, 12 parameters or 12/20 (60%) were positive towards the female flesh or to say that 60% of the parameters were more concentrated in the female than the male. It also showed that eight parameters or 8/20 (40.0%) were more positive towards the male flesh.

In Table 2 were reported the summary of parameters of essential, non-essential, acidic, neutral, sulphur, aromatic, etc. amino acid contents (g $100g^{-1}$ cp) of the samples. The total amino acid of 96.6 – 97.1 g $100g^{-1}$ was higher than the value 777.0 mg g^{-1} cp in the *S. africanus africanus* female flesh [8]. The essential amino acid range was 45.2 – 46.2 g $100g^{-1}$ with a CV% of 1.42. The total sulphur amino acids (TSAA) of the samples was 4.04 – 4.85 g $100g^{-1}$ which were highly comparable to the value of 58 mg g^{-1} cp recommended for infants [21]. The aromatic amino acid range suggested for ideal protein (68 – 118 mg g^{-1} cp) [21] was highly comparable with present values in the total essential aromatic amino acid (7.72 – 9.67 g $100g^{-1}$). It means the *N. maculatus* flesh could be used to supplement cereal flours. The percentage ratio of essential amino acids to the total amino acids in the samples ranged between 46.8 – 47.5 %; values that were above the 39 % considered adequate for ideal protein food for infants, 26 % for children and 11 % for adults [21]. The EAA/TAA in egg is 50 % [23].

The predicted protein efficiency ratio (P-PER_1) was 3.39 – 3.69 and (P-PER_2) was 3.82 – 4.14. The *in vivo* P-PER is of the order of 2.2 [24]. In the flesh of female *S. africanus africanus* P-PER was reported as 3.1 [8] and in *Callinectes latimanus* (a lagoon crab), P-PER_1 was 1.21 and P-PER_2 was 1.39 [25]. These literature values showed that *N. masculatus* would be more physiologically utilized protein than the quoted references, particularly the female flesh of *N. masculatus*.

Table 1. Amino acid profiles (g $100g^{-1}$ protein) of the female and male flesh of *Neopetrolisthes maculatus*

Amino acid	Female flesh	Male flesh	Mean	SD[#]	CV%[†]	Value difference	% difference
Glycine (Gly)	6.06	5.81	5.94	0.175	2.95	+0.248	+4.09
Alanine (Ala)	5.11	5.08	5.09	0.019	0.367	+0.026	+0.518
Serine (Ser)	4.07	4.05	4.06	0.015	0.358	+0.021	+0.505
Proline (Pro)	3.42	3.36	3.39	0.040	1.17	+0.056	+1.64
Valine (Val)*	4.30	4.11	4.21	0.134	3.19	+0.190	+4.41
Threonine (Thr)*	3.88	3.51	3.69	0.262	7.11	+0.371	+9.57
Isoleucine (Ile)*	4.95	4.44	4.70	0.358	7.62	+0.506	+10.2
Leucine (Leu)*	7.94	7.23	7.59	0.503	6.63	+0.712	+8.96
Aspartic acid (Asp)	10.0	9.90	9.96	0.092	0.919	+0.129	+1.29
Lysine (Lys)*	4.62	5.25	4.93	0.445	9.01	-0.629	-13.6
Methionine (Met)*	2.75	3.24	3.00	0.347	11.6	-0.491	-17.9
Glutamic acid (Glu)*	17.7	17.8	17.7	0.022	0.124	-0.031	-0.176
Phenylalanine (Phe)*	4.26	5.16	4.71	0.638	13.6	-0.903	-21.2
Histidine (His)*	2.07	3.28	2.67	0.856	32.0	-1.21	-58.6
Arginine (Arg)*	9.07	8.70	8.89	0.261	2.94	+0.369	+4.07
Tyrosine (Tyr)	3.62	3.38	3.50	0.170	4.84	+0.240	+6.62
Tryptophan (Trp)*	1.39	1.23	1.31	0.118	8.99	+0.167	+12.0
Cystine (Cys)	1.29	1.61	1.45	0.224	15.4	-0.316	-24.5
Total amino acid	96.6	97.1	96.8	0.386	0.398	-0.545	-0.565
Protein	40.2	43.7	41.9	2.47	5.91	-3.50	-8.72

* Essential amino acid; [#]SD = standard deviation; [†]CV% = coefficient of variation; + = female flesh value > male flesh value;
- = female flesh value < male flesh value. All determinations were in duplicate and on dry weight.

Table 2. Concentrations of essential, aromatic, non-essential, neutral, etc. amino acid (g $100g^{-1}$ protein) of the female and male *N. maculatus* flesh

Amino acid	Female flesh	Male flesh	Mean	SD	CV%
Total amino acid (TAA)	96.6	97.1	96.8	0.386	0.398
Total non-essential amino acid (TNEAA)	51.3	50.9	51.1	0.264	0.515
% TNEAA	53.2	52.5	52.8	0.483	0.914
Total essential amino acid (TEAA) – with His	45.2	46.2	45.7	0.649	1.42
– no His	43.2	42.9	43.0	0.207	0.480
% TEAA – with His	46.8	47.5	47.2	0.483	1.02
– no His	44.7	44.2	44.4	0.339	0.878
Total essential aliphatic amino acid (TEAIAA)	28.4	26.7	27.5	1.19	4.32
% TEAIAA	29.4	27.5	28.4	1.34	4.72
Total essential aromatic amino acid (TEArAA)	7.72	9.67	8.69	1.38	15.8
% TEArAA	8.00	9.95	8.97	1.39	15.4
Total neutral amino acid (TNAA)	51.6	51.0	51.3	0.466	0.908
% TNAA	53.5	52.5	53.0	0.692	1.31
Total sulphur amino acid (TSAA)	4.04	4.85	4.44	0.571	12.8
% TSAA	4.18	4.99	4.59	0.571	12.5
%Cys in TSAA	31.9	33.1	32.5	0.852	2.62
Leu/Ile ratio	1.60	1.63	1.62	0.016	0.984
(Leu – Ile) difference	2.99	2.79	2.89	0.146	5.04
% (Leu – Ile)/ TAA	3.10	2.87	2.98	0.162	5.44
% (Leu – Ile)/ Leu	4.04	4.85	4.44	0.571	12.8
P-PER_1*	3.69	3.39	3.54	0.211	5.94
P-PER_2*	4.14	3.82	3.98	0.228	5.71
pI[#]	5.37	5.44	5.41	0.049	0.907
EAAI[†]	89.9	86.9	88.4	2.12	2.40
Biological value (BV)	86.3	83.0	84.7	2.31	2.73
Lys/Trp or L/T	3.31	4.27	3.79	0.680	17.9
Met/Trp or M/T	1.97	2.64	2.31	0.472	20.5

* Predicted protein efficiency ratio; [#]pI = isoelectric point; [†]EAAI = essential amino acid index

In general, it has been found that the better the protein, the lower the level in the diet required to produce the highest protein efficiency ratio. This is a clear reflection of the importance of the proper nutritive balance of all the amino acids to produce optimum metabolic efficiency. The Leu/Ile ratio ranged from 1.60 – 1.63. In the flesh of female *S. africanus africanus,* Leu/Ile was 1.60 [8]. The value of 1.60 – 1.63 showed that we might not experience concentration antagonism in the samples when consumed as protein source in food; this is because 2.36 is the most ideal Leu/Ile [26]. It has been suggested that an amino acid imbalance from excess Leu might be a factor in the development of pallegra [27]. A high Leu imbalance in the diet impairs the metabolism of Trp and niacin, and is responsible for the niacin deficiency in sorghum eaters [28]. Experiments in dogs showed that animals fed sorghum proteins with less than 11g 100 g^{-1} protein Leu did not suffer from nicotinic acid deficiency [29]. The present Leu values were 7.23 – 7.94 g $100g^{-1}$ protein, and therefore considered safe and could be beneficially exploited to prevent pellagra in endemic areas [30]. The percentage Cys/TSAA values were 31.9 – 33.1%. The present Cys/TSAA values were highly comparable with literature values of animal protein acids: [8] 27.3 – 32.8 % in *S. africanus africanus;* 36.3 % in *Macrotermes bellicosus*; 25.6 % in *Zonocerus variegatus;* 35.5 % in *A. marginata,* 38.8 % in *A. archatina* and 21.0 % in *Limicolaria* sp. (the last three were land snails found in Nigeria). The percentage Cys in TSAA in the diet of the rat, chick and pig is 50 % [26] but the value is unknown in man [21]. However, vegetable protein (e.g. coconut endosperm) has a percentage Cys/TSAA of 62.8 % [31]. The presence of cystine and cysteine in the diet would reduce the needs for Met and since almost all the sulphur in the diet is derived from these three amino acids the sulphur content is sometimes used as an approximate assessment of the adequacy of protein [32].

The essential amino acid index (EAAI) of 86.9 – 89.9 and their corresponding biological values (BV) of 83.0 – 86.3 depicted the high quality of the protein of *N. maculatus*. In comparison, some literature values of EAAI and BV are as follows [18]: milk, cow (whole, nonfat, evaporated or dry), EAAI (88) and BV (84, predicted; 90, observed); human, EAAI (87) and BV (83); eggs, chicken (whole, raw or dried), EAAI (100), BV (97, predicted; 96, observed); whites (raw or dried), EAAI (95), BV (92, predicted; 93, observed); yolks (raw or dried), EAAI (93), BV (89, predicted); shellfish (shrimp, including prawns, raw or canned), EAAI (67), BV (61, predicted). These literature results show the quality position of *N. maculatus* flesh under discussion. EAAI is useful as a rapid tool in the evaluation of food formulation for protein quality. The isoelectric point, pI, was 5.37 – 5.44 showing the samples to be in the acidic medium of the pH range. The pI calculation from amino acids would assist in the quick production of certain isolate of organic product without evaluating the protein solubility to get to the pI.

In protein requirements of infants, a growth pattern of amino acid requirements was obtained by assigning value of unity to the Trp need [33]. Similar calculation of the amino acid content of mammalian tissues showed that there exist good agreement of growth needs and tissue amino acid patterns. This agreement is good for the Lys/Trp (L/T) and Met/Trp (M/T) ratios of muscle proteins which constitute approximately 75% of the infant body proteins. The present results had L/T values of 3.31 – 4.27 and M/T of 1.97 – 2.64.

Mammalian tissue patterns have the following values: L/T: muscle (6.3), viscera (5.3), plasma proteins (6.2). M/T: muscle (2.5), viscera (2.0), plasma proteins (1.1) [34]. The available evidence indicates that the utilsation of dietary proteins increases as their Lys and Trp content approaches that of muscle tissues. In the present results the flesh L/T tried to meet the muscle standard whilst M/T value of 1.97 was lower than the muscle value of 2.5 but the value of 2.64 was greater than 2.5.

The summary of the statistical analysis of the data from Table 1 is shown in Table 3. The r_{xy} value was positively high (0.9920) and showed the samples to be significantly different at $r_{=0.01.}$ The r_{xy}^2 was also high. The R_{xy} (regression) was positive at 0.2257 meaning that for every one unit (g $100g^{-1}$ protein) increase in the amino acid of the female flesh of *N. maculatus*, there was a corresponding increase of 0.2257 in the flesh of male amino acid of *N. maculatus.* The values of coefficient of alienation (C_A) and index of forecasting efficiency (IFE) parameters always affect

each other simultaneously; this is because C_A + IFE = 1.00 or C_A + IFE = 100%. The C_A was low at 12.6% but correspondingly high value of IFE (87.4%). The IFE is an indication that the relationship between the two flesh samples *N. maculatus* could easily be predicted because error of prediction was just 12.6% which was relatively low. The IFE is a measure of the reduction in the error of prediction of relationship between two related samples. The mean of female flesh was 5.36 ± 3.90 g $100g^{-1}$ with CV% of 72.8 and male flesh was 5.39 ± 3.79 g $100g^{-1}$ and CV% of 70.3.

Table 3. Statistical analysis of the data from Table 1 pertaining to amino acid profiles of female and male flesh of *Neopetrolisthes maculatus*

Statistics	Female flesh		Male flesh
Total amino acid value	96.6		97.1
Mean	5.36		5.39
SD	3.90		3.79
CV%	72.8		70.3
Correlation coefficient (r_{xy})		0.9920	
Variance (r_{xy}^2)		0.9841	
Regression coefficient (R_{xy})		0.2257	
Coefficient of alienation (C_A)		0.1262 (12.6%)	
Index of forecasting efficiency (IFE)		0.8738 (87.4%)	
Remark*		Results significantly different	

*Results significantly different at $n - 2$ and $r_{=0.01}$ (critical value = 0.590). (NOTE: $n - 2 = 18 - 2 = 16$.)

Table 4 presents the total amino acid scores based on whole hen's egg amino acid profile. The following amino acids were more concentrated in both flesh samples than the whole hen's egg values as shown by their scores: Gly (1.94 – 2.02), Glu (1.48 – 1.48) and Arg (1.43 – 1.49); however, these amino acids were more concentrated in the male flesh than the whole hen's egg: Met (1.01), Phe (1.01) and His (1.37). The limiting amino acid in both samples was Ser (0.516, female; 0.513, male). Therefore, in order to fulfil the day's needs for all the amino acids in *N. maculatus* samples, 100/51.6 or 1.94 times as much female flesh protein, or 100/51.3 or 1.95 times as much male flesh protein, would have to be eaten when they are the sole protein source in the diet. In Table 5 is the essential amino acid scores of *N. maculatus* based on FAO/WHO [20] standards. The following scores were greater than 1.0: Ile (1.11 – 1.24), Leu (1.03 – 1.13), Met + Cys (1.15 – 1.39), Phe + Tyr (1.31 – 1.42), Trp (1.23 – 1.39) and total (1.08 – 1.09). Whilst Lys was limiting with a value score of 0.804 in the female flesh, Val was limiting with a score of 0.823 in the male flesh. Whilst the correction factor was 100/84.0 or 1.19 in the female flesh, it was 100/82.3 or 1.22 in the male flesh sample protein. For essential amino acid scores of *N. maculatus* samples based on requirements of pre-school child (2-5 years), the scores are in Table 6. All the scores were greater than 1.00 except for Lys in both samples. This meant that the first limiting amino acid, Lys, was limiting in both samples with values of 0.796 (female) and 0.905 (male). Therefore, the correction to get all the requirements in full would be female 100/79.6 or 1.26 times female protein and 100/90.5 or 1.10 times male protein for male sample.

Table 4. Amino acid scores of *Neopetrolisthes masculatus* based on whole hen's egg amino acid

Amino acid	Female flesh	Male flesh	Mean	SD	CV%
Gly	2.02	1.94	1.98	0.058	2.95
Ala	0.946	0.941	0.943	0.003	0.367
Ser	0.516	0.513	0.514	0.002	0.365
Pro	0.899	0.884	0.892	0.010	1.17
Val	0.574	0.549	0.561	0.018	3.19
Thr	0.760	0.687	0.724	0.051	7.11
Ile	0.882	0.793	0.838	0.063	7.51
Leu	0.957	0.871	0.914	0.061	6.63
Asp	0.937	0.925	0.931	0.009	0.919
Lys	0.745	0.846	0.796	0.072	9.01
Met	0.859	1.01	0.936	0.109	11.6
Glu	1.48	1.48	1.48	0.002	0.124
Phe	0.835	1.01	0.923	0.125	13.6
His	0.861	1.37	1.11	0.357	32.0
Arg	1.49	1.43	1.46	0.043	2.94
Tyr	0.906	0.846	0.876	0.042	4.84
Trp	0.775	0.682	0.729	0.066	8.99
Cys	0.717	0.892	0.804	0.124	15.4
Total	0.966	0.972	0.969	0.004	0.398

Table 5. Essential amino acid scores of *Neopetrolisthes masculatus* based on FAO/WHO (1973) [20] standards

Amino acid	Female flesh	Male flesh	Mean	SD	CV%
Val	0.861	0.823	0.842	0.027	3.19
Thr	0.969	0.876	0.923	0.066	7.11
Ile	1.24	1.11	1.17	0.089	7.62
Leu	1.13	1.03	1.08	0.072	6.63
Lys	0.840	0.954	0.897	0.081	9.01
Met + Cys	1.15	1.39	1.27	0.163	12.8
Phe + Tyr	1.31	1.42	1.37	0.078	5.71
Trp	1.39	1.23	1.31	0.118	8.99
Total	1.08	1.09	1.09	0.003	0.278

Table 6. Essential amino acid scores of the *Neopetrolisthes masculatus* samples based on requirements of pre-school child (2 – 5 years)

Amino acid	Female flesh	Male flesh	Mean	SD	CV%
Val	1.23	1.18	1.20	0.038	3.19
Thr	1.14	1.03	1.09	0.077	7.11
Ile	1.77	1.59	1.68	0.128	7.62
Leu	1.20	1.10	1.15	0.076	6.63
Lys	0.796	0.905	0.851	0.077	9.01
Met + Cys	1.62	1.94	1.78	0.228	12.8
Phe + Tyr	1.25	1.36	1.30	0.074	5.71
Trp	1.27	1.12	1.19	0.107	8.99
His	1.09	1.72	1.41	0.450	32.0
Total	1.21	1.25	1.23	0.028	2.31

The summary of the amino acid groups is shown in Table 7 [35]. The concentration trend of the classes could be seen to follow as shown in g $100g^{-1}$ protein: class I (26.7 – 28.4) > class IV (27.6 – 27.7) > class V (15.8 – 17.2) > class VI (11.3 – 12.6) > class II (7.56 – 7.95) > class III (4.04 – 4.85) > class VII (3.36 – 3.42). It could be observed that the percentage values were close to their individual principal values, e.g. value (percentage): class I, 26.7 – 28.4 (27.5 – 29.4); class II, 7.56 – 7.95 (7.79 – 8.23); class III, 4.04 – 4.85 (4.18 – 4.99); class IV, 27.6 – 27.7 (28.4 – 28.7), class V, 15.8 – 17.2 (16.4 – 17.7), class VI, 11.3 – 12.6 (11.7 – 13.0) and class VII, 3.36 – 3.42 (3.46 – 3.54). The CV% values were generally low at 0.251 – 12.8.

Table 7. Amino acid groups of *Neopetrolisthes masculatus* samples

	Value in g $100g^{-1}$ protein (% value)				
Class	Female flesh	Male flesh	Mean	SD	CV%
I [with aliphatic side chains (hydrogen and carbons) = Gly, Val, Leu, Ile]	28.4 (29.4%)	26.7 (27.5%)	27.5	1.19	4.32
II [with side chains containing hydroxylic (OH) groups = Ser, Thr]	7.95 (8.23%)	7.56 (7.79%)	7.76	0.277	3.57
III [with side chains containing sulphur atoms = Cys, Met]	4.04 (4.18%)	4.85 (4.99%)	4.44	0.571	12.8
IV [with side chains containing acidic groups or their amides = Asp, Glu]	27.7 (28.7%)	27.6 (28.4%)	27.7	0.070	0.251
V [with side chains containing basic groups = Arg, Lys, His]	15.8 (16.4%)	17.2 (17.7%)	16.5	1.04	6.30
VI [containing aromatic rings = His, Phe, Tyr, Trp]	11.3 (11.7%)	12.6 (13.0%)	11.9	0.856	7.16
VII [imino acids = Pro]	3.42 (3.54%)	3.36 (3.46%)	3.39	0.040	1.17

The summary of the amino acid profiles into Factors A and B is shown in Table 8. Factor A means constituted amino acids of the two samples along the vertical axis whilst Factor B constituted the amino acids values along the horizontal axis as shown in the Table; both containing the essential and non – essential amino acids. Column under Factor B means showed close values at a range of 45.7 – 51.1 g $100g^{-1}$ protein. However, the mean of Factor A means and Factor B means gave a value of 48.4 g $100g^{-1}$ protein.

Table 8. Summary of the amino acid profiles into factors A and B

	Samples (Factor A)		
Amino acid composition	Female flesh	Male flesh	Factor B means
Total essential amino acid	45.2	46.2	45.7
Total nonessential amino acid	51.3	50.9	51.1
Factor A means	48.3	48.5	48.4

Conclusions

Neopetrolisthes maculatus flesh samples were found to be good sources of high-quality protein of more than average requirements of essential amino acids, high P-PER, high EAAI, high BV and low Leu/Ile ratios. The WHO recommended Val and Ile requirements for school children aged 10 – 12 years of 33 and 30 mg amino acid kg^{-1} body weight day^{-1} [20, 21]. For example, a 30 kg child will require 990 and 900 mg of Val and Ile day^{-1} respectively. From Table 1, 100 g of female flesh protein would provide 1728 mg Val and 1987 mg Ile to 30 kg child whereas the male flesh *N. masculatus* would provide 1796 mg Val and 1939 mg Ile to 30 kg child. *N. masculatus* would therefore meet almost double the requirements of school children in Val and Ile.

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