

## EVALUATION OF PROTEINS OF SOME MARINE EDIBLE FISH FOUND AROUND KARACHI COAST

(Miss) MASARRAT RIAZ, (Miss) RIAZ FATIMA and A.H. KHAN

*PCSIR Laboratories, Karachi 39*

S. MAQSOOD ALI and (Miss) RAZIA ISHAQUE

*PCSIR Laboratories, Lahore 16*

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**Abstract.** Proteins extracted from fourteen different marine edible fish found around Karachi coast were analysed for amino acid contents and net protein utilisation (NPU) value by rat assay. When compared with FAO reference protein, the fish proteins were found to contain adequate amounts of essential amino acids except for slight deficiencies for tryptophan, sulphur, amino acids and lysine. NPU standardised values ranged from 86-100% which corroborated the results of chemical analysis. It was observed that NPU (st) values compared within  $\pm 10\%$  (except for dawan and khokhar) with the protein scores calculated by using FAO 1957 Amino Acid Pattern. Protein scores calculated by FAO 1965 Pattern are consistently lower than those calculated by 1957 Pattern of NPU (st) values.

Marine edible fish have been found to be a rich source of vitamin A, protein, oil and unsaturated fatty acids. Their consumption is very high in this country, as they form a major constituent in the diet of the local population.

In a previous communication,<sup>1</sup> the annual catch, consumption and distribution of vitamin A of fourteen species of fish found around the Karachi coast has been reported. Later on, a number of unsaturated fatty acids in their body oils were reported.<sup>2</sup>

The present studies have been undertaken for determining the amino acid contents of proteins from the same fourteen varieties of edible fish. Their protein value, on the basis of protein scores, calculated from amino acid make-up and from NPU value (by rat assay) was also evaluated. A comparative study of their nutritive assessment has also been undertaken.

### Material and Methods

**Extraction of Protein.** The fourteen species of fish were procured from the fish harbour in the early morning in fresh condition. After removal of the viscera, the entire edible part (free from skin and bones) was coarsely minced, mixed well, and extracted by the following method.

The protein of the flesh was separated from nitrogenous extractives by macerating the mince with 95%, 80% and finally 60% hot alcohol. The precipitated protein was collected by filtration, air-dried and then refluxed for 4-6 hr with petroleum ether (b.p. 60-80°C). The solvent was removed and the fish meal was dried in air. It was then mixed with acetone for 24 hr to remove water from it. Acetone was decanted off and the meal was placed in a blowing oven at 45-50°C for 24 hr. The dried material obtained was powdered and sieved through 60 mesh.

**Analysis.** The proteins so obtained were analysed for moisture content according to A.O.A.C. method<sup>3</sup> and for nitrogen by standard Kjeldahl technique and

converted to protein by multiplying with 6.25. The results were expressed on dry weight basis.

Amino acid composition was determined by hydrolysing protein (50 mg), with 6N HCl in a sealed-tube and keeping it at 110°C for 24 hr in an oven. After complete removal of the acid, the hydrolysate was used for estimation. Tryptophan was determined by the method of Spies and Chambers<sup>4</sup> by alkaline hydrolysis. Methionine was determined according to the method of Sullivan-McCarthy.<sup>5</sup>

Net protein utilisation at 10% protein level<sup>10</sup> was determined according to the method of Miller and Bender<sup>6</sup> using male albino rats weighting 30-40 g. Calories were determined by means of a ballistic bomb calorimeter according to the method of Miller and Payne.<sup>7</sup> NPU<sup>10</sup> values were converted to NPU(st) or protein score according to the formula.<sup>8</sup>

$$\text{NPU (st)} = \frac{\text{NPU} \times 54}{54 - \text{Protein cal\%}} - 8$$

**Protein Score (Chemical Score).** Protein score is a measure of protein quality and is determined by comparing with the amino acid content of a test protein with that of a selected standard such as FAO provisional amino acid pattern of 1957.<sup>9</sup> The amino acid showing the lowest percentage is called limiting amino acid and the percentage is the protein score. In 1965, the FAO Committee of Protein Requirements<sup>10</sup> changed its ideal amino acid pattern in favour of egg protein. Since a good deal of data on protein scores of various proteins reported in the literature is based on FAO 1957 recommendations, we considered it worthwhile to calculate protein scores by using both FAO 1957<sup>9</sup> and 1965<sup>10</sup> ideal amino acid patterns (Table 3).

### Discussion

**Amino Acid Make-Up.** The FPC are generally rich in essential amino acids and are comparable to

TABLE 1. AMINO ACID CONTENT OF PAKISTANI FISHES, COMPARED WITH MILK, BEEF AND FAO 1957 PROVISIONAL AMINO ACID REFERENCE PATTERN.

Amino acids	Category A				Category B							Category C			Range	Average	whole* cows milk	Beef	FAO†
	White pomfret	Black pomfret	Rawas	Surmal	Aal	Boi	Dawan	Dhother	Hira	Palla	Sua	Khagga	Mushka	Khokhar					
Alanine	6.394	6.215	6.693	6.776	6.474	6.884	6.259	6.327	6.934	6.461	6.793	6.600	5.934	6.190	5.93-6.93	6.495	3.50	5.77	—
Arginine	6.210	7.604	8.897	7.284	1.828	5.693	5.010	6.486	6.900	5.243	7.281	3.177	3.867	1.548	1.55-8.90	5.502	3.72	6.45	—
Aspartic	10.350	10.249	11.195	11.313	12.029	11.312	10.359	10.887	11.189	10.567	11.854	10.165	10.045	9.863	9.86-12.3	10.812	7.44	9.33	—
Methionine	4.013	2.802	4.825	3.751	3.841	2.831	3.014	3.021	4.174	2.900	3.130	3.744	2.980	3.990	2.80-4.8	3.501	2.49	2.48	—
Cystine	1.380	1.186	1.137	1.403	1.414	1.658	1.375	1.356	1.242	1.508	1.376	1.289	1.254	1.368	1.19-1.66	1.353	0.91	1.26	—
Total sulphur -amino acid	5.393	3.988	5.962	5.154	5.255	4.489	4.389	4.377	5.416	4.408	4.506	5.033	4.234	5.358	4.23-5.9	4.854	3.41	3.74	4.3
Glutamic acid	6.019	14.384	17.585	15.633	14.869	18.283	16.996	17.191	17.399	17.195	17.238	19.621	17.797	18.446	14.38-19.626	17.046	15.00	15.14	—
Glycine	4.749	4.553	4.651	5.099	4.749	4.948	4.543	4.936	4.979	4.837	4.628	4.622	4.000	4.879	4.00-4.95	4.720	2.02	6.19	—
Histidine	2.495	2.791	2.459	3.521	3.139	2.584	2.316	2.587	2.725	2.540	2.985	0.920	1.376	2.830	0.92-3.52	2.519	2.60	3.47	—
Isoleucine	5.324	5.457	5.950	6.437	5.658	6.219	5.680	5.700	6.175	5.858	6.515	6.132	4.950	5.380	4.95-6.18	5.816	6.51	4.42	4.3
Lencine	8.544	9.299	9.488	10.236	10.315	9.918	9.051	9.222	10.154	9.396	10.112	9.721	8.155	8.226	8.16-10.32	9.416	10.02	8.19	4.9
Lysine	9.085	9.944	9.520	12.910	4.611	9.231	8.435	10.328	10.959	8.723	11.510	4.333	5.517	5.999	4.33-10.96	8.650	7.93	8.74	4.3
Phenylalanine	3.979	4.109	4.709	5.130	5.209	4.999	4.677	5.073	4.715	4.779	5.250	4.896	4.400	4.518	3.98-5.25	4.746	5.94	4.11	2.9
Tyrosine	3.829	2.745	3.642	3.400	3.461	3.740	3.481	3.363	2.645	3.538	3.984	4.131	3.820	3.925	2.64-4.13	3.550	5.20	3.40	2.9
Total aromatic amino acid	7.808	6.854	8.351	8.530	8.670	8.739	8.158	8.436	7.360	8.317	9.234	9.027	8.420	8.443	7.32-9.23	8.310	11.14	7.50	—
Proline	4.174	4.034	4.373	4.948	4.508	4.374	4.020	4.411	4.910	5.150	4.628	3.415	3.318	3.674	3.32-5.15	4.281	11.34	4.93	—
Serine	4.255	4.553	4.396	5.069	4.588	4.543	4.019	3.796	5.175	4.210	3.973	3.743	3.732	3.830	3.73-5.18	4.277	6.02	4.19	—
Threonine	5.370	5.796	6.043	5.820	5.025	6.373	5.643	4.753	6.290	5.759	7.126	7.331	4.758	4.887	4.78-7.33	5.783	4.70	4.42	2.9
Tryptophan	1.449	1.310	1.600	1.573	1.299	1.355	1.243	1.573	1.242	1.397	1.332	1.584	1.542	1.594	1.24-1.60	1.435	1.44	1.17	1.4
Valine	5.520	6.181	6.403	6.727	6.773	6.858	6.191	5.928	6.704	6.405	6.737	6.941	5.978	6.006	5.52-6.86	6.382	7.01	5.55	—
Protein (% × 6.25)	86.8	87.97	86.0	82.63	86.5	82.09	90.80	87.22	86.41	86.0	89.78	87.50	90.04	87.63					

\*M.L. Gr and B.K. Watt, *Amino Acid Content of Foods* (Home Economics Research Report No. 4, Washington, 1966). †FAO Nutritional Studies No. 16(1957 b).

those of other protein sources such as milk and beef. (Table 1). However, a few depressions from the normal values are as follows:

'Aal' and 'khokhar' are particularly low in agrinine, 1.83 g and 1.55 g/16 g N, respectively, as against 3.87-8.90 for the remaining fish. It will be noted that all the three fish of category C are generally deficient in agrinine.

Fish proteins are generally rich in lysine and, therefore, very well complement cereal proteins which are poor in this amino acid. But 'aal', 'khagga', 'mushka' and 'khokhar' appear to be poor in lysine containing almost half the normal quantity but still comparable to lysine contained in FAO 1957 Ideal Reference Protein (Table 1, last column). However, such low values are not exceptional to the above fish and lysine as low as 3.7 g/16 g N has been reported in Indian fish, *Stromateus niger*.<sup>11</sup> As against these 'surmai' and 'sua' contain 12.9 and 11.5 g lysine/16 g N which is more than double the quantity of lysine found in the above fish.

All fish proteins were found to contain appreciable quantity of sulphur-containing amino acids. Black pomfret, 'boi', 'dother', 'palla' and 'mushka' had slightly lesser contents. Sulphur-amino acids content of Pakistani fish was found to be comparable or even higher than those of Indian<sup>11</sup> and English<sup>12,13</sup> fish. These are richer in this amino acid than either milk or beef.

Histidine was found to be deficient in 'khagga' and 'mushka' (0.9 and 1.4 g/16 g N) as against normal figures of 2.46-3.52 g/16 g N in other cases. But this is not peculiar to the above-mentioned Pakistani fish. Some Indian fish, e.g. *Stromateus niger*, *Sardinella fimbriata* and *Sardinella longiceps*, were found to contain 0.7, 0.8 and 1.7 g histidine/16 g N respectively.<sup>9</sup>

*NPU and Protein Score (Chemical Score)*. The NPU (st) of fish proteins are quite high and range between 86-100 (Table 2). Fish having NPU(st) of over 95% are 'rawas' (101%) 'mushkas' (97.5%) and 'dawan' (95.9%), while lower values are given

TABLE 2. COMPARISON OF NET PROTEIN UTILIZATION (NPU) VALUES AND PROTEIN SCORES CALCULATED BY FAO 1957 AND 1965 AMINO ACID PATTERNS.

Fishes	NPU* at 10% protein level	NPU standard- dised	FAO protein scores	
			1957	1965
White pomfret	82.3	93.0	100.0	93.5(†)
Black pomfret	79.2	89.2	88.8(s)	73.1(s)
Rawas	89.0	101.0	100.0	96.7(†)
Surmal	80.5	90.8	100.0	80.6(†)
Aal	87.5	99.4	90.0(†)	66.1(†)
Boi	76.3	85.6	94.4(†)	77.7(s)
Dawan	84.0	95.9	84.6(†)	70.9(†)
Dhother	80.1	90.3	100.0	80.5(s)
Hira	81.1	91.5	86.8(†)	64.5(†)
Palla	78.0	87.7	96.9(†)	79.6(s)
Sua	78.5	88.3	92.2(†)	70.9(s)
Khagga	83.3	94.2	100.0	69.3(†)
Mushka	86.0	97.5	97.7(s)	88.8(s)
Khokhar	77.1	86.5	100.0	100.0

\*Mean NPU value of 8 rats per group.

†Limiting amino acids are shown in parenthesis.  
s, tryptophan; t, sulphur-amino acids; j, lysine.

by 'boi' (85.6%), 'khokhar' (86.5%) and 'pallas' (87.7%).

Miller and Payne<sup>13</sup> observed that NPU determined at the maintenance level or NPU(st) of a protein is equal to protein score calculated from amino acid make-up. Table 2 shows that our NPU(st) figures compare well with the protein score calculated by FAO 1957 method. Except for 'dawan' and 'khokar', these agree well within 10%. Protein scores calculated by FAO 1965 method are consistently lower than those obtained by the FAO 1957 method or NPU(st) values.

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13. Appendix A

#### Appendix A

##### AMINO ACIDS CALCULATED ON THE BASIS OF 16 g N.

Fish species	Agr.	Hist.	Isolene	Leu.	Lys.	Meth.	Ph. alan	Thr.	Tryp.	Valine
Sardines ( <i>Sardinops caerulea</i> )	5.1	4.7	4.6	7.2	8.4	2.8	3.7	4.3	1.0	5.2
Herring ( <i>Clupea hereugus</i> )	5.5	2.4	4.9	7.1	7.8	2.7	3.4	4.4	0.8	5.0
Herring Pallasi	5.9	2.0	6.4	8.0	8.2	2.2	4.5	4.5	0.7	5.1
Meckerel ( <i>Scomber scombrus</i> )	5.8	3.8	5.2	7.2	8.1	2.7	3.5	4.9	1.0	5.4
Pneumatophorusdego	5.5	5.4	5.0	7.4	8.5	2.8	3.8	4.5	1.0	5.2
Scorbermorus Maculatus	5.3	1.5	—	—	6.5	—	—	—	1.4	—
Tuna:germo ( <i>Thunnus</i> and <i>Meothunnus</i> sp)	5.3	5.7	4.7	7.2	8.3	2.8	3.5	4.5	1.0	5.1
Tuna:enthyunnus Pelanis	7.8	3.0	—	10.4	7.4	—	4.1	—	—	—
Gaduscallaras and <i>Melono grammus aeglefinus</i>	6.1	2.1	5.5	8.1	8.8	3.0	3.9	4.9	1.0	5.3
Sebastodes sp. (Redcod)	4.3	1.6	6.4	11.4	14.4	2.6	4.4	5.1	0.1	5.0
<i>S. pagus</i> sp.	5.1	2.0	—	8.8	6.2	—	4.7	—	—	—
<i>Micropogun undulatus</i>	5.8	1.4	—	—	6.1	—	—	—	1.2	—
<i>Hippoglossus hippglossis</i>	6.0	1.7	—	10.3	6.7	—	3.0	—	1.6	—
<i>Lutianus black fordii</i>	6.2	1.6	—	—	6.7	—	—	—	1.2	—
<i>Mugil</i> sp.	5.8	1.6	—	—	6.7	—	—	—	1.4	—
<i>Alosa sapidissima</i>	4.5	1.1	—	—	6.4	—	—	—	1.2	—
Salmon:Oncorhynchus pschawytscha	5.8	2.6	4.9	7.3	8.0	3.0	3.7	4.4	0.9	5.6
Salmon: keta	5.5	1.3	—	—	5.7	—	—	—	1.3	—
Salmon:Namaycush	5.7	1.4	—	—	7.1	—	—	—	1.2	—
Trout ( <i>Cynoscion regalis</i> )	5.9	1.4	—	—	6.8	—	—	—	1.0	—
<i>Cristivomer namaycush</i>	5.7	1.4	—	—	7.1	—	—	—	1.2	—