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Retention of Amino Acids in the Carcass of Fish Protein Fed Rats

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Retention of Individual amino acids in the carcass of albino rats fed on fish proteins as the sole source of dietary proteins for a 3 weeks period has been studied at various levels of protein in the diet. Upto 20% protein in the diet, net synthesis of atleast some amino acids were noticed, but above 20% protein, fish proteins could meet the requirements of all amino acids of the rat. Above this level, the protein was not utilised for body protein synthesis by the rat. The protein efficiency ratio showed maximum value at 10% protein in the diet.

The influence of the nature and level of dietary proteins on the utilisation and retention of individual amino acids has been studied by various workers (Pellet & Kaba 1972; Bunce & King 1969a, b; Aguitar & Harper, 1972). Vegetable proteins from different sources and milk and egg proteins have all been used in these studies. But, such studies using fish protein have not been reported. Studies on carcass amino acids have been mainly aimed at computing the amino acid requirements during different stages of growth (Herger & Frydrych, 1985; Allison, 1964; Yoshida & Ashida, 1969). Some studies reported the effects of feeding amino acid mixtures of varying composition on the final carcass amino acid composition of rats (Yamashita & Ashida, 1969). Bunce & King (1969a) observed that the dietary level of protein is also an important factor in determining the carcass retention efficiency of amino acids.

This paper reports the results of a study on the effect of feeding varying levels of fish proteins, on the carcass amino acid composition of albino rats. A low amino acid diet containing essential (2.7%) and non-essential (2.3%) synthetic amino acids was taken as the reference diet.

Materials and Methods

Forty nine three weeks old male rats (Wistar strain) bred in the Institute's animal house were divided into seven groups. The mean initial weight of the rats was 30 g. The rats were housed individually in metabolism cages kept in a room maintained at 20±2°C with adequate light and air. Urine was collected in bottles containing toluene and dilute hydrochloric acid to prevent bacterial growth and loss of ammoniacal nitrogen.

Rats of one group were sacrificed on the initial day itself to serve as the control group. The other groups of rats were fed with diets containing varying levels of fish proteins and one group was fed with the low amino acid diet containing a mixture of 2.7% essential and 2.3% non-essential synthetic amino acids (Yamashita & Ashida 1969). The basal diet contained 45% starch, 32% glucose, 5% refined ground nut oil, 4% salt mixture (U.S.P.), 1% vitamin mixture (Chapman *et al.*, 1959) and 3% cellulose. The protein content was adjusted to 5, 10, 15, 20 and 25%. Fish powder was prepared by drying fresh Rohu (*Labeo rohita*) fish muscle in a tunnel dryer at 40°C and defatting the dried and powdered fish muscle using petroleum ether. Protein content of the defatted and solvent free powder was 88%.

All the rats were fed on the respective diets *ad libitum*. Residual feed, faeces and urine were collected quantitatively in each case. At the end of the three weeks of feeding, the rats were starved overnight and sacrificed.

To hydrolyse carcasses, faeces and urine, the samples were refluxed with 6N HCl (100 volumes/g dry matter) under N_2 for 24 h in a 2 l round bottomed flask. After hydrolysis, volume of non-lipid aqueous layer was noted. Feed was hydrolysed with 6N HCl at 110°C for 24 h in sealed evacuated tubes. Amino acids in the hydrolysates were determined using a Technicon NC 2 P amino acid analyser. Total nitrogen in carcass hydrolysate (aqueous portion only), feeds, faeces and urine were determined by the microkjeldahl method (AOAC, 1975). Tryptophan in the fish powder was determined as per Sastry & Tammuru (1985). Mean values for each group were taken from the average of duplicate determinations for each rat. Subtraction of the zero day values for amino acids from these values gave the carcass retention of individual amino acids. Net intake of individual amino acids was computed in each case from the amino acid composition of feed and the total quantity of feed intake. From these values, net catabolism/synthesis in the case of each amino acid was calculated.

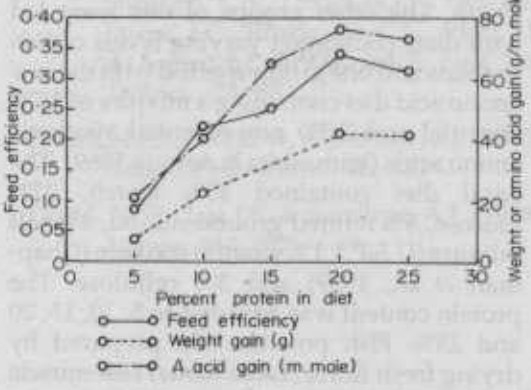

Fig. 1. Feed efficiency, weight gain and amino acid gain in rats fed with different levels of fish protein in their diet.

Results and Discussion

The fish protein used in this study (muscle powder from rohu) had a more or less balanced amino acid composition, as can be seen from the amino acid score for the essential amino acids against the FAO/WHO pattern (Table 1). Fig. 1 gives the total amino acid gain, feed efficiency (Weight gain as g/g feed consumed) and average net weight gain per rat in 3 weeks, as a function of the percentage of protein in the diet.

All the three parameters recorded a gradual increase with increasing protein content in the diet. When protein content was increased beyond 20%, all the three parameters showed a decreasing trend. While in the case of feed efficiency and amino acid gain this decrease was noticable, the weight gain curve almost levelled off beyond 20% protein in the diet. The synthetic amino acid diet was comparable to the 5% protein diet in its amino acid content. Taking the synthetic amino acid diet as the baseline, the percentage deviation in the feed efficiency, amino acid gain, weight gain and the protein efficiency ratio (PER) were plotted for the different diets (Fig. 2).

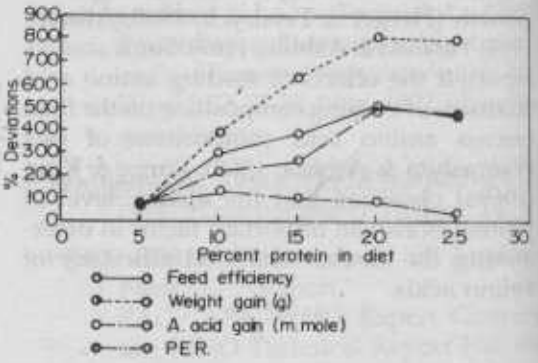

Fig. 2. Deviations of protein quality parameters from the low amino acid diet in rats fed different levels of fish protein.

PER values were generally low in these experiments which was unexpected in view of the good diet consumption and digestibility (84-96%). Though weight gain was good in all cases, it was achieved by the con-

Table 1. *Amino acid composition of the Rohu fish protein and its score against FAO/WHO reference protein*

Amino acid composition of Rohu powder g/16 g N				Amino acid score of Rohu protein against FAO/WHO reference protein	
Asp	9.50	Thr	4.5		
Ser	3.20	Val	5.03	Thr	112.5
Glu	16.05	Met	2.6	Val	99.5
Pro	2.70	Ile	4.4	Met+Cys	131.0
Gly	4.70	Leu	7.92	Ile	110.0
Ala	5.76	Phe	3.32	Leu	113.0
Cys	2.24	Trp	1.4	Phe+Tyr	103.67
Tyr	2.90	Lys	3.64	Lys	187.0
Arg	5.8	His	10.32	Trp	140.0

Table 2. *Nitrogen balance in rats fed diets at different levels in g N/rat/3 weeks*

Dietary protein	5%	10%	15%	20%	25%	Synthetic amino acids
Carcass gain	0.543	0.998	1.595	1.679	1.678	0.281
Faecal N	0.097	0.213	0.259	0.32	0.382	0.07
Urinary N	0.13	0.407	0.464	0.845	1.667	0.14
Total recovered	0.77	1.618	2.318	2.844	3.73	0.491
Total consumed	0.696	1.49	2.12	3.06	4.4	0.638
Recovery, %	110	108.6	109	93	85	77

sumption of correspondingly high amounts of protein resulting in low PER values. Under these conditions, it can be concluded that a considerable part of the amino acids absorbed is utilized for purposes other than protein synthesis. At low protein levels, increase in the fat gain also contributes to the weight gain observed. Since the protein content in diet increased, the weight gain was mainly due to the increased body protein. The PER values recorded a positive deviation from the baseline value for the low amino acid diet (Fig. 2) till the protein content reached 10%. But with further increase this deviation was reduced and the values

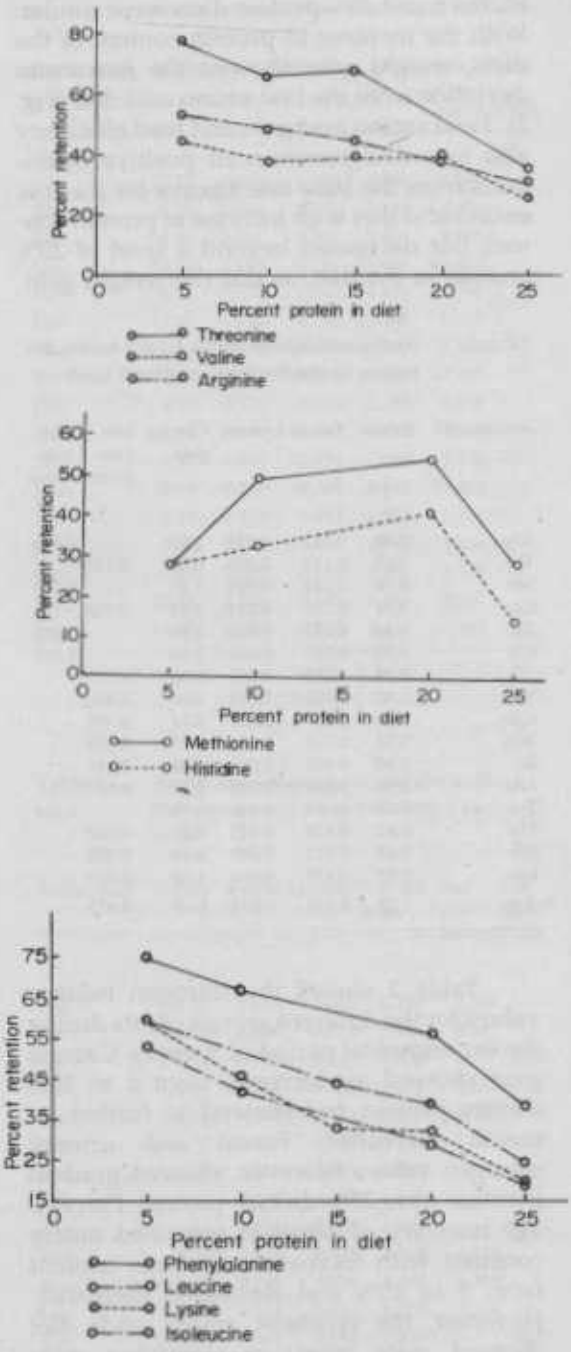

Fig.3a,b&c The retention of individual essential amino acids in the carcass of rats fed with fish protein at different levels.

for the 5 and 25% protein diets were similar. With the increase in protein content of the diets, weight gain showed the maximum deviation from the low amino acid diet (Fig. 2). Total amino acid gain and feed efficiency also recorded pronounced positive deviations from the base line figures for the low amino acid diet with increase in protein content, but decreased beyond a level of 20% protein in the diet, as did the weight gain.

Table 3. *Amino acid balance in rats fed diet having 5% protein in mmoles amino acid/rat/3 weeks*

Amino acid	Intake	Faecal	Urinary	Carcass gain	Net catabolism	Net synthesis
Asp	2.48	0.212	0.026	2.32	-	0.078
Thr	1.09	0.119	0.009	0.84	0.122	-
Ser	0.76	0.144	0.009	1.43	-	0.793
Glu	3.91	0.239	0.033	2.92	0.718	-
Pro	1.11	0.172	0.018	2.13	-	1.210
Gly	1.93	0.243	0.049	2.80	-	1.162
Ala	2.09	0.219	0.011	1.86	-	-
Val	1.80	0.152	0.018	0.80	0.830	-
Cys	0.56	0.014	0.001	0.21	0.335	-
Met	0.54	0.076	0.009	0.15	0.305	-
Ile	1.10	0.103	0.006	0.58	0.411	-
Leu	2.00	0.236	0.025	1.18	0.559	-
Tyr	0.58	0.098	0.066	0.68	-	0.264
Phe	0.82	0.128	0.015	0.61	0.067	-
His	0.65	0.112	0.003	0.18	0.355	-
Lys	2.32	0.139	0.016	1.38	0.785	-
Arg	1.28	0.116	0.011	0.68	0.473	-

Table 2 shows the nitrogen balance values for the different groups of rats during the experimental period of 3 weeks. Carcass gain showed an increase, from 5 to 20% dietary protein, but showed no further increase thereafter. Faecal and urinary nitrogen values however, showed gradual increase upto 25% dietary protein. Percentage recovery of nitrogen remained nearly constant with increase in protein content from 5 to 15% and decreased thereafter. However, the synthetic amino acids diet showed poor retention signifying poor utilisation of the amino acids. Eventhough the synthetic amino acid and the 5% fish protein diets were consumed at the same level and were similar in their amino acid composition, the latter was utilised by the rats more efficiently (Table 2).

Figures 3a, b and c present the data on the percentage retention efficiency (carcass gain as % of intake) of the essential amino acids at each protein level. The essential amino acids showed three distinctly different patterns of behaviour in this respect. Threonine, arginine and valine showed almost the same retention efficiency from 5 to 20% and decreased thereafter (Fig. 3a). Methionine and Histidine showed a very low efficiency at 5% level, but increased gradually at higher levels of protein and decreased beyond 20% protein level (Fig. 3b). This was possibly because these two amino acids could not be utilised fully when the other amino acids were supplied at a low level. Methionine, besides its use in the synthesis of body protein, is a major donor of methyl groups for synthesis of various other essential compounds in the body. Increased percentage of retention may be due to its reduced availability as a result of its easy oxidation (Njaa, 1962). Phenylalanine, leucine, isoleucine and lysine showed a gradual decrease in retention efficiency from

Table 4. *Amino acid balance in rats fed diets having 10% protein in mmoles amino acid/rat/3 weeks*

Amino acid	Intake	Faecal	Urinary	Carcass gain	Net catabolism	Net synthesis
Asp	5.3	0.607	0.066	5.330	-	0.703
Thr	2.33	0.273	0.023	1.535	0.499	-
Ser	1.625	0.404	0.039	2.390	-	1.208
Glu	8.36	0.709	0.096	4.795	2.760	-
Pro	2.38	0.652	0.032	3.075	-	1.379
Gly	4.14	0.608	0.081	8.360	-	4.910
Ala	4.46	0.620	0.078	4.145	-	0.385
Val	3.85	0.530	0.060	1.320	1.940	-
Cys	1.18	0.093	0.004	0.040	1.043	-
Met	1.15	0.212	0.014	0.565	0.359	-
Ile	2.36	0.283	0.029	0.990	1.058	-
Leu	4.28	0.531	0.069	1.850	1.830	-
Tyr	1.25	0.172	0.033	0.940	0.105	-
Phe	1.75	0.239	0.030	1.170	0.311	-
His	1.38	0.054	0.007	0.450	0.869	-
Lys	4.97	0.403	0.019	2.260	2.290	-
Arg	2.75	0.212	0.032	0.340	1.170	-

Table 5. *Amino acid balance in rats fed diets of 15% protein in mmoles amino acid/rat/3 weeks*

Amino acid	Intake	Faecal	Urinary	Carcass gain	Net catabolism	Net synthesis
Asp	8.31	0.574	0.034	5.130	2.560	-
Thr	3.65	0.244	0.160	2.465	0.781	-
Ser	2.55	0.279	0.174	4.375	-	2.278
Glu	13.09	0.533	0.690	6.145	5.720	-
Pro	3.73	0.217	0.146	3.945	-	0.578
Gly	6.48	0.459	0.573	7.450	-	2.002
Ala	6.99	0.483	0.628	3.200	2.680	-
Val	6.03	0.340	0.014	2.345	3.330	-
Cys	1.86	0.062	0.027	0.190	1.581	-
Met	1.81	0.133	0.248	0.785	0.639	-
Ile	3.70	0.180	0.118	0.950	2.452	-
Leu	6.70	0.311	0.222	2.990	3.172	-
Tyr	1.96	0.234	0.107	1.240	0.379	-
Phe	2.75	0.143	0.087	0.665	1.850	-
His	2.18	0.039	0.060	0.560	0.521	-
Lys	7.79	0.220	0.073	2.570	4.922	-
Arg	4.30	0.240	0.057	1.910	2.093	-

Table 6. *Amino acid balance in rats fed diet with 20% protein in mmoles amino acids/rat/3 weeks*

Amino acid	Intake	Faecal	Urinary	Carcass gain	Net catabolism	Net synthesis
Asp	14.08	0.838	0.290	6.620	6.300	-
Thr	6.19	0.480	0.130	4.860	0.720	-
Ser	4.32	0.531	0.059	4.920	-	1.190
Glu	22.16	0.659	0.167	10.450	10.880	-
Pro	6.35	0.636	0.240	5.760	-	0.286
Gly	10.98	0.545	0.426	10.620	-	0.611
Ala	11.84	1.090	0.452	4.070	6.230	-
Val	10.21	0.459	0.066	4.010	5.675	-
Cys	3.16	0.065	0.019	0.200	2.876	-
Met	3.05	0.231	0.066	1.670	1.083	-
Ile	6.26	0.301	0.137	1.800	4.022	-
Leu	11.35	0.454	0.098	4.400	6.398	-
Tyr	3.32	0.170	0.026	1.900	1.224	-
Phe	4.64	0.270	0.071	2.610	1.686	-
His	3.69	0.061	0.079	1.530	2.020	-
Lys	13.19	0.391	0.123	4.240	8.456	-
Arg	7.30	0.205	0.009	4.550	2.536	-

Table 7. *Amino acid balance in rats fed diet with 25% protein in mmoles amino acid/rat/3 weeks*

Amino acid	Intake	Faecal	Urinary	Carcass gain	Net catabolism	Net synthesis
Asp	22.17	1.500	0.540	7.590	12.540	-
Thr	9.75	0.584	0.159	3.340	5.670	-
Ser	6.81	0.561	0.181	5.460	0.608	-
Glu	34.94	1.150	0.291	11.490	22.010	-
Pro	9.96	0.571	0.367	5.630	3.390	-
Gly	17.30	1.020	0.795	15.060	0.425	-
Ala	18.66	0.978	0.420	10.960	6.300	-
Val	16.08	0.517	0.153	3.930	11.480	-
Cys	4.97	0.171	0.024	0.290	4.485	-
Met	4.81	0.211	0.060	1.400	3.140	-
Ile	9.87	0.560	0.254	1.890	7.170	-
Leu	17.88	0.783	0.169	4.460	12.468	-
Tyr	5.22	0.284	0.043	1.950	2.943	-
Phe	7.30	0.290	0.076	2.790	4.144	-
His	5.80	0.202	0.260	0.860	4.478	-
Lys	20.78	1.110	0.109	4.180	15.381	-
Arg	11.49	0.197	0.028	3.450	7.815	-

Table 8. *Amino acid balance in rats fed diet with synthetic amino acids in mmoles amino acid/rat/3 weeks*

Amino acid	Intake	Faecal	Urinary	Carcass gain	Net catabolism	Net synthesis
Asp	2.35	0.365	0.045	0.350	1.587	-
Thr	1.65	0.376	0.028	0.905	0.339	-
Ser	1.54	0.338	0.027	1.218	-	0.041
Glu	4.86	0.574	0.080	1.330	2.877	-
Pro	1.46	0.287	0.030	0.850	0.291	-
Gly	1.57	0.776	0.157	2.160	-	1.519
Ala	1.82	0.592	0.065	1.360	-	0.198
Val	2.20	0.376	0.045	0.049	1.302	-
Cys	0.36	0.151	0.008	0.270	-	0.074
Met	0.99	0.148	0.003	0.324	0.510	-
Ile	1.56	0.276	0.016	0.350	0.916	-
Leu	2.27	0.376	0.041	0.560	1.286	-
Tyr	0.66	0.061	0.041	0.590	-	0.033
Phe	1.57	0.111	0.013	0.550	0.892	-
His	0.56	0.223	0.007	0.274	0.056	-
Lys	1.69	0.371	0.044	8.720	0.554	-
Arg	0.64	0.192	0.018	0.580	-	0.150

5 to 25% protein level. When the protein content of the diet is increased, the carcass gain as a percentage of intake of most of the essential amino acids generally showed a decreasing trend (Fig. 3c). Bunce and King (1969a, b) found that most of the essential amino acids reached a maximum retention efficiency at 6% protein diet.

Amino acid balance data giving net synthesis/catabolism figures, for the control and test diets are presented in Tables 3 to 8. The loss of amino acids through the faeces ranged from 2.5 to 10.6 mmoles and through the urine 0.33 to 3.9 mmoles. Considering the low intake the loss of amino acids through faeces and urine was relatively high for the synthetic amino acid diet. Maximum synthesis of amino acids was found at 10% protein level. When the supply of amino acid was increased, the net synthesis decreased and at 25% level, there was no synthesis of any of the amino acids. Incorporation of the dietary amino acids into the tissue was highest in rats on the 20% protein diet.

Generally net synthesis occured in the case of aspartic acid, serine, proline, glycine and alanine. Serine and glycine are interconvertible. Since both serine and glycine are synthesised, synthesis of serine must be by the rat liver enzyme, 3 phosphoglycerase. Aspartic acid and alanine are readily synthesised from oxaloacetic acid and pyruvic acid respectively. In the case of proline, glutamic acid or arginine can be precursors. At 5% level, it was found that proline synthesis almost equalled the amount of glutamic acid and arginine catabolised. At higher levels of protein, however, the amount of proline synthesised was much less compared to the other two amino acids catabolised. Proline and glycine synthesis continued upto 20% protein. This may be due to their content being very low in control rats because of the lower levels of collagen in the young animals. At 5% level of protein, synthesis of tyrosine was greater than the amount of phenylalanine catabolised. Tyrosine synthesis took place in the case of synthetic amino acid diet also. In this case, the amount synthesised was lesser than amount of phenylalanine catabolised. Synthesis of cystine was also lesser than the methionine catabolised.

It can be concluded that for this particular diet, the amino acids supplied by the protein above 20% level are in excess of the requirements for the synthesis of body protein. The capacity of the rat to utilise the amino acids for synthesis of body protein reached a maximum at 20% protein concentration. Elevated levels of protein are not utilised fully by the rat. At 10% level, the protein has the highest PER. Upto a level of 20% protein in diet, a net synthesis of several amino acids is observed. Above 20% protein content, the protein seems to meet all the requirements of amino acids of the rat. Consequenty, synthesis of amino acids either does not take place or is retarded to the bare minimum.

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References

Aguitar, T.S. & Harper, A.E. (1972) *J. Nutr.* **102**, 1100

Allison, J.B. (1964) in: *Mammalian Protein metabolism* Vol. 2 (Munro, H.N. and Allison J.B., Eds.), Chapt. 12, Academic Press, New York, USA

AOAC (1975) *Official Methods of Analysis* (Horowitz, W. Ed.) 12th edn., Association of Official Analytical Chemists, Washington DC, USA

Bunce, G.E. & King, K.W. (1969a) *J. Nutr.* **98**, 159

Bunce, G.E. & King, K.W. (1969b) *J. Nutr.* **98**, 168

Chapman, D.G., Castillo, R. & Campbell, J.A. (1959) *Can. J. Biochem. Physiol.* **37**, 679

Heger, J. & Frydrych, Z. (1985) *Brit. J. Nutr.* **54**, 499

Njaa, L.R. (1962) *Brit. J. Nutr.* **16**, 571

Pellet, P.L. & Kaba, H. (1972) *J. Nutr.* **102**, 61

Sastry, C.S.P. & Tammuru, K.M. (1985) *J. Food Sci. Technol.* **22**, 146

Yamashita, K. & Ashida, K. (1969) *J. Nutr.* **99**, 267

Yoshida, A. & Ashida, K. (1969) *Agr. Biol. Chem.* **33**, 43