Correlations of Essential Amino Acid Patterns between the Dietary Protein and the Blood, Hepatopancreas, or Skeletal Muscle in Carp

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To study the relevance of the dietary essential amino acid (EAA) pattern and the free EAA pattern in fish body, free amino acid (FAA) compositions in the whole blood, plasma, erythrocytes, hepatopancreas, and skeletal muscle of carp *Cyprinus carpio* were determined at 1, 2, 4, 6, 8, 12, 24, and 72 h after feeding with a casein-gelatin diet. Correlation coefficient between the dietary EAA pattern and that of free EAA in the whole blood, plasma, or erythrocytes became rapidly higher after food intake, reached to the highest levels within 4 h, and fell gradually to non-significant levels by 24 h. The highest correlation coefficients between the whole blood, plasma and erythrocytes, and the diet were 0.8961, 0.8273 and 0.7382, respectively. Similar trend was also found in the hepatopancreas whose highest correlation coefficients observed at 4 h was 0.9142, while the skeletal muscle did not show any significant correlations to the diets during the experiment. Thus, EAA pattern of the diet was best reflected in free EAA pattern of the whole blood, plasma, and hepatopancreas at 4 h after food intake.

Recently, Murai *et al.*¹⁾ claimed that essential amino acid (EAA) index based on essential amino acid (A/E) ratio of a diet is an excellent indicator to evaluate dietary protein quality for carp *Cyprinus carpio*. However, in certain cases the dietary EAA data alone may be insufficient for evaluation of the protein quality, because of the difference in availability of the ingredients used. From this stand point, not only chemical analysis but also biological and biochemical assessments may be needed. So far, effects of dietary amino acids on free amino acid (FAA) profile in the various tissues have been intensively studied in many fishes,²⁻²⁴⁾ and assessments of dietary protein quality have been attempted based on these results.²⁻⁵⁾ Sequential changes in FAA pattern reflecting the dietary composition after food intake have been also revealed in the body of various fishes.^{3,8-9,14,16,18-20,24)} Thus, FAA pattern in the body also may be strongly affected by the time after feeding. Therefore, post-feeding change in the FAA pattern should be sufficiently studied to apply the FAA profile for assessments of the protein quality of a particular diet. The post-feeding changes of FAA levels in the whole blood, plasma, erythrocytes, hepatopancreas, and skeletal muscle were examined in order to study relationship between the dietary EAA pattern and those of free EAA in these tissues.

Methods

Composition of the experimental diet used was exactly the same as that employed in the previous experiment²⁴⁾ and has been reported in detail by Murai *et al.*¹⁾ Amino acid composition of the diet is shown in Table 1. Also, carp with mean weight of about 60 g were reared under the previously reported manner.²⁴⁾ Fish having been starved for 48 h were taken for zero time samples, and the remaining fish were immediately fed the diet at a level of 3% of body weight. At 1, 2, 4, 6, 8, 12, 24, and 72 h after feeding, blood samples were collected from the caudal peduncle after anesthetizing with 0.01% solution of MS-222. Then, the fish were sacrificed for sampling of the hepatopancreas and dorsal skeletal muscle without dark muscle. All samplings were made in duplication. Sample preparation and FAA determination of the whole blood, plasma, and erythrocytes were performed as has been reported before.^{24,25)} FAA extraction from the hepatopancreas and skeletal muscle was carried out according to a slightly modified method of Eckhart and Kemmerer.²⁶⁾ Each tissue was thoroughly minced and lyophilized. To a portion of the dried tissue, weighing 40 to 50 mg, 4 ml of 15% solution of sulfosalicylic acid was added. The mixture was mechanically shaken for 30 min at room tem-

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perature, stood at 4°C overnight, and shaken again. The suspension was centrifuged at 9,000 rpm for 15 min, and the supernatant obtained was analyzed for FAA. FAA were determined using an automatic amino acid analyzer (Hitachi Model 835).

Results

Blood

Postprandial levels of FAA in the whole blood, plasma and erythrocytes of carp are listed in Table 1, 2 and 3, respectively. In the previous report,²⁴⁾ FAA contents in the plasma and erythrocytes were expressed as levels per one liter of the whole blood to examine distribution of FAA in the blood, while the present values were shown as levels per one liter of plasma and erythrocytes, respectively. Irrespective to the representation

used, the nature of postprandial changes in FAA levels of the blood samples agreed well with the previous results²⁴⁾ which were already discussed in detail. Correlation coefficients (*r*) between the dietary EAA and free EAA in the whole blood increased from 0.5701 of the zero time to the highest value of 0.8961 at 4 h after food intake (Table 6). Then, it decreased to non significant level at 8 h, became significant again at 12 h, and returned to the initial level by 24 h. Similar trends were also observed in the plasma and erythrocytes. However, these values were slightly lower than those in the whole blood. The highest correlation coefficients of the plasma and erythrocytes were 0.8273 and 0.7382 at 4 h after food intake, respectively (Table 6).

Hepatopancreas

Postprandial change of FAA contents in the

Table 1. Postprandial levels of free amino acids in the whole blood of carp ($\mu\text{mol/l}$ of whole blood)

Amino acids	Hours after feeding									Diet ^{*1}
	Start	1	2	4	6	8	12	24	72	
Taurine	7682	7235	7191	7427	7021	7459	7274	7082	7280	
Aspartic acid	75	86	79	44	42	60	34	49	63	163
Threonine	223	368	544	615	718	673	414	415	740	96
Serine	190	238	366	626	801	921	527	403	358	160
Glutamic acid	119	137	163	154	179	229	158	197	232	400
Glutamine	600	562	778	1200	879	796	743	598	704	
Glycine	710	547	451	693	1076	1561	1002	908	849	365
Alanine	611	518	718	1094	1472	1250	697	770	1065	173
Valine	229	313	455	608	531	427	463	277	392	139
Cystine	6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8	3	
Methionine	46	104	175	210	131	72	80	69	60	63 ^{*2}
Isoleucine	108	146	238	361	321	218	224	113	230	97
Leucine	225	525	738	732	445	308	339	213	447	182
Tyrosine	52	274	443	333	311	243	141	157	92	
Phenylalanine	51	296	492	448	297	208	144	138	132	159 ^{*3}
Ammonia	369	474	843	1603	1182	1044	1071	1138	1186	
Tryptophan	20	64	65	39	20	19	25	33	21	13
Lysine	296	504	755	816	637	557	496	533	928	134
Histidine	191	284	398	341	234	263	220	259	348	48
Arginine	84	324	382	317	238	226	202	200	342	93
Hydroxyproline	346	415	255	319	459	956	936	672	484	80
Proline	3163	2868	1676	2664	3996	6538	4842	5032	4779	336
ΣEAA	1531	3201	4686	4818	3881	3214	2748	2415	3638	
ΣNAA	5812	5371	4486	6795	8905	12311	8937	8628	8553	
ΣNRS	15394	16281	17206	20643	20988	24028	20030	19263	20753	

All values represent the mean of two samples.

*1 Amino acid contents of the experimental diet having the mixture of casein-gelatin (5 : 2, w/w) as a protein source are expressed as mmol/100 g of dry diet.

*2 Total amount of methionine and cystine.

*3 Total amount of phenylalanine and tyrosine.

EAA: essential amino acid. NAA: nonessential amino acid. NRS: ninhydrin reacting substance. n.d.: not detected.

Table 2. Postprandial levels of free amino acids in the plasma of carp ($\mu\text{mol/l}$ of plasma)

Amino acids	Hours after feeding								
	Start	1	2	4	6	8	12	24	72
Taurine	505	502	601	613	638	766	574	496	796
Aspartic acid	29	8	10	249	9	8	84	51	13
Threonine	149	293	511	662	797	732	412	287	657
Serine	105	201	366	651	868	978	505	254	288
Glutamic acid	n.d.	17	86	n.d.	82	109	24	45	73
Glutamine	383	493	807	1321	928	850	764	411	606
Glycine	422	370	292	586	1171	1727	1117	636	720
Alanine	406	407	664	1142	1630	1261	580	515	868
Valine	240	356	522	724	613	495	506	230	431
Cystine	n.d.	n.d.	5	4	6	10	6	6	11
Methionine	45	152	258	241	159	97	86	65	81
Isoleucine	111	178	290	431	363	244	252	100	258
Leucine	238	665	891	822	472	334	375	191	496
Tyrosine	43	259	420	303	298	238	137	134	103
Phenylalanine	60	291	472	416	285	203	136	117	130
Ammonia	322	406	777	938	547	413	273	310	445
Tryptophan	12	63	54	32	16	20	20	23	23
Lysine	404	737	1127	1163	870	761	608	538	1230
Histidine	211	338	484	384	232	295	234	264	388
Arginine	107	528	591	439	309	316	238	184	434
Hydroxyproline	320	365	217	300	526	1277	1253	723	505
Proline	3318	2413	1143	2466	4335	7873	5815	5098	4951
ΣEAA	1619	3861	5623	5618	4419	3745	3009	2139	4241
ΣNAA	4954	4283	3574	6466	9541	14074	10059	7683	8010
ΣNRS	7400	9051	10574	13635	15145	18998	13915	10627	13492

See Table 1.

Table 3. Postprandial levels of free amino acids in the erythrocytes of carp ($\mu\text{mol/l}$ of erythrocytes)*

Amino acids	Hours after feeding								
	Start	1	2	4	6	8	12	24	72
Taurine	19273	17549	17615	18542	19298	19357	19860	20192	21435
Aspartic acid	151	205	190	n.c.	106	152	36	76	172
Threonine	342	481	596	541	567	569	419	670	919
Serine	327	296	368	588	674	820	568	693	506
Glutamic acid	311	322	285	406	367	442	410	496	569
Glutamine	948	667	736	1006	788	701	700	960	909
Glycine	1175	819	704	871	887	1266	784	1433	1121
Alanine	940	687	804	1021	1174	1231	916	1264	1477
Valine	211	248	351	421	376	306	382	377	303
Cystine	15	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	14	n.c.
Methionine	48	30	45	159	77	26	68	77	14
Isoleucine	103	97	156	248	240	172	174	137	170
Leucine	205	311	499	584	396	264	271	254	341
Tyrosine	67	296	481	382	337	253	150	200	70
Phenylalanine	37	303	524	499	321	217	160	178	133
Ammonia	444	579	947	2688	2406	2167	2569	2789	2791
Tryptophan	35	67	84	51	27	17	34	51	18
Lysine	121	146	170	253	190	194	285	529	307
Histidine	159	201	262	270	240	206	193	245	258
Arginine	46	14	53	120	102	67	139	227	152
Hydroxyproline	385	491	316	350	329	387	339	583	447
Proline	2914	3538	2520	2996	3349	4163	3022	4921	4546
ΣEAA	1389	2192	3220	3528	2873	2289	2238	2959	2684
ΣNAA	6999	6820	5732	7239	7568	9009	6740	10395	9574
ΣNRS	28106	27139	27515	31997	32144	32819	31443	36334	36484

See Table 1.

* Free amino acid contents in the erythrocytes were calculated from the whole blood and plasma contents with correction by hematocrit values, so called "calculated erythrocytes contents".

Table 4. Postprandial levels of free amino acids in the hepatopancreas of carp (mmol/kg of dried tissue)

Amino acids	Hours after feeding								
	Start	1	2	4	6	8	12	24	72
Taurine	41.37	39.39	39.70	35.76	39.13	35.62	32.94	33.63	44.41
Aspartic acid	1.18	0.43	0.72	1.94	2.49	0.85	0.88	1.48	1.97
Threonine	1.86	1.84	1.93	2.82	2.82	2.92	2.27	2.39	2.61
Serine	2.13	1.70	2.06	3.89	4.07	3.79	3.50	2.59	2.02
Glutamic acid	12.42	4.94	4.75	12.38	18.64	15.36	13.10	13.71	12.32
Glutamine	5.42	2.73	1.88	4.18	4.68	4.39	5.87	4.40	4.50
Glycine	7.26	4.40	3.45	4.95	7.18	6.65	5.30	5.43	5.60
Alanine	14.24	6.88	7.52	14.98	20.23	15.42	11.86	13.75	12.97
Valine	1.51	2.22	2.45	3.37	2.30	1.87	2.32	1.34	1.70
Cystine	0.10	0.11	0.10	0.11	0.07	0.07	0.09	0.06	0.08
Methionine	0.63	0.80	0.75	1.11	0.59	0.55	0.87	0.55	0.43
Isoleucine	0.81	1.29	1.54	2.30	1.44	1.04	1.36	0.70	1.02
Leucine	1.75	3.68	3.80	4.28	2.28	1.95	2.89	1.57	2.11
Tyrosine	0.91	1.97	2.26	2.00	1.56	1.30	1.33	1.03	0.80
Phenylalanine	1.02	1.31	1.58	1.66	0.98	0.91	1.34	0.86	0.83
Ammonia	15.58	16.64	16.67	16.98	19.04	18.06	15.28	15.62	20.56
Tryptophan	0.11	0.30	0.21	0.22	0.12	0.13	0.17	0.12	0.14
Lysine	2.64	3.23	3.11	4.67	3.49	3.44	3.90	3.06	4.60
Histidine	1.74	2.42	3.10	2.04	1.26	1.45	1.55	1.50	1.64
Arginine	1.27	2.47	2.22	2.79	1.65	1.79	2.29	1.40	2.06
Hydroxyproline	1.24	1.32	0.52	0.89	1.41	2.42	4.15	1.93	1.85
Proline	19.06	19.36	8.21	9.83	16.41	22.79	24.86	20.99	22.24
ΣEAA	14.33	21.65	23.05	27.36	18.57	17.41	20.38	14.58	18.01
ΣNAA	62.94	41.76	29.12	53.04	75.10	71.68	69.52	64.29	63.47
ΣNRS	134.22	119.45	108.54	133.13	151.85	142.77	138.12	128.12	146.45

See Table 1.

Table 5. Postprandial levels of free amino acids in the dorsal skeletal muscle of carp (mmol/kg of dried tissue)

Amino acids	Hours after feeding								
	Start	1	2	4	6	8	12	24	72
Taurine	57.85	71.61	61.28	70.56	68.98	60.24	63.04	66.77	68.43
Aspartic acid	0.16	0.09	n.d.	0.55	0.29	0.20	0.41	0.32	0.38
Threonine	3.23	3.48	4.32	3.90	4.06	3.79	3.58	3.55	4.62
Serine	3.12	1.77	3.21	4.16	5.02	5.10	5.09	4.34	3.36
Glutamic acid	2.44	2.62	3.22	4.13	2.88	3.12	3.71	2.78	3.03
Glutamine	6.37	4.06	3.83	5.75	3.74	2.89	5.52	3.35	3.65
Glycine	33.51	25.04	27.79	29.22	32.21	29.80	31.82	24.74	23.14
Alanine	13.37	9.72	12.77	12.97	11.82	11.70	10.17	10.98	12.05
Valine	1.31	1.56	1.83	2.44	2.50	2.19	2.27	1.37	2.00
Methionine	0.38	0.47	0.77	1.09	0.93	0.64	0.58	0.48	0.59
Isoleucine	0.64	0.61	0.85	1.27	1.40	1.11	1.12	0.58	1.29
Leucine	1.13	1.55	2.50	2.82	1.99	1.40	1.48	0.93	2.23
Tyrosine	0.37	0.59	1.41	1.57	1.63	1.25	0.83	0.80	0.67
Phenylalanine	0.37	0.45	1.33	1.51	1.33	0.92	0.62	0.49	0.57
Ammonia	28.63	27.37	30.26	30.45	31.44	29.50	27.85	26.10	30.33
Tryptophan	n.d.	0.16	0.24	0.22	n.d.	n.d.	n.d.	n.d.	n.d.
Lysine	27.23	23.92	23.03	23.03	23.48	26.00	27.45	22.78	27.25
Histidine	70.98	71.69	77.09	73.75	71.02	85.38	72.92	72.12	78.44
Arginine	2.77	2.19	3.98	4.86	5.48	4.69	3.86	3.20	4.59
Hdroxyproline	6.15	6.54	4.23	5.49	6.25	6.89	7.42	5.98	4.19
Asparagine	3.06	n.d.	0.58	3.57	1.93	2.22	2.34	1.97	1.80
Proline	22.32	24.73	15.99	22.51	25.55	29.91	25.22	21.67	17.59
ΣEAA	108.41	106.66	117.36	116.91	116.33	128.83	110.05	110.78	125.49
ΣNAA	90.50	74.57	71.62	88.35	89.69	91.83	91.71	76.13	69.19
ΣNRS	285.39	280.20	280.52	306.28	306.44	310.39	292.65	279.78	293.44

See Table 1.

Table 6. Correlation coefficients between the dietary EAA pattern and free EAA pattern in the whole blood, plasma, erythrocytes, hepatopancreas, or dorsal skeletal muscle of carp

Hours after feeding	Correlation coefficients ($\times 10$)				
	Whole blood	Plasma	Erythrocytes	Hepatopancreas	Skeletal muscle
Start	5.701	5.455	3.715	4.573	-2.710
1	8.374* ³	7.749* ¹	5.493	8.298* ²	-2.812
2	8.395* ³	8.049* ²	6.025	7.853* ¹	-2.819
4	8.961* ³	8.273* ²	7.382* ¹	9.142* ⁴	-2.724
6	7.362* ¹	6.937* ¹	6.514	7.793* ¹	-2.634
8	5.853	5.538	5.377	6.502	-2.831
12	7.175* ¹	6.970* ¹	6.803* ¹	8.106* ²	-2.853
24	4.944	4.666	4.737	5.651	-2.724
78	4.495	4.424	2.917	5.278	-2.604

*¹ $p < 0.05$ *² $p < 0.01$ *³ $p < 0.005$ *⁴ $p < 0.001$

hepatopancreas was principally similar to those found in the blood as shown in Table 4. Almost all of free EAA in the hepatopancreas rose rapidly and attained their highest levels within 4 h. Whereas, levels of most of free nonessential amino acids (NAA) decreased shortly after food intake, and attained the highest values at 6 or 8 h. In all tissues examined, the highest correlation coefficient (0.9142) was found between the diet and hepatopancreas at 4 h after food intake. Post-feeding change in correlation coefficients was also almost similar to those in the blood.

Skeletal Muscle

Levels of free lysine and histidine in the skeletal muscle were surprisingly high compared with those of other free EAA detected (Table 5). Free lysine and histidine represented approximately 25 and 65% of the total free EAA content, respectively. Levels of lysine and histidine did not show any consistent changes after food intake, though postprandial contents of other free EAA became up to 1.3 to 4.4 times higher than initial values of these. This phenomenon is reflected in the postprandial correlations of free EAA in the skeletal muscle to the dietary EAA, *i.e.* the correlation coefficients between these did not become significant during the experiment (Table 6).

Discussion

Changes in post-feeding profile of tissue FAA has been extensively studied in fishes. Nose⁹⁾ found that the plasma FAA pattern shows the best correlation coefficient against the dietary amino acid pattern at 12 h after food intake in his preliminary study on rainbow trout fed a commercial diet. Kaushik and Luquet⁵⁾ also obtained a similar result in the whole blood of

rainbow trout. Whereas, Schlisio and Nicolai¹²⁾ demonstrated that there is no distinct correlation between the amino acid contents in the diet and plasma at 12 h after food intake using rainbow trout fed a synthetic diet containing pure amino acids. The reason for this discrepancy might be partly due to the difference in the experimental diet used. While in carp, Plakas *et al.*¹⁴⁾ reported that the plasma free EAA pattern at 4 h after food intake is highly and positively correlated to the EAA pattern in a casein diet used. Similarly, the plasma pattern estimated from the results of Murai *et al.*³⁾ where a casein-gelatin mixture was used as a protein source seemed to show the best correlation against the dietary pattern at 4 h. However, the most significant correlation between the values of BEAAp (balance of essential amino acids in plasma which is proposed for an assessment of the quality of dietary protein) and the growth responses was obtained at 6 h by Murai *et al.*³⁾ Unlike carp, Dabrowska⁵⁾ recently reported that BEAAp index seemed to be not suitable for rainbow trout. Thus, sequential phase of fluctuation in FAA composition and content in a tissue due to food intake might differ among the species of experimental animals used and rearing temperature. Quality and quantity of the experimental diets taken, and time after feeding can also affect the response of fish. Especially, the very last factor can drastically affect tissue FAA profile. Therefore, when a study on FAA dynamics under various nutritional and physiological conditions is conducted, nutritional history of the experimental fish should be thoroughly studied in advance.

In the skeletal muscle of carp, any significant correlation was not found against the dietary EAA pattern. Absence of distinct peak in the skeletal

muscular free EAA and NAA during the first 24 h after food intake was also observed in rainbow trout reared in freshwater by Kaushik and Luquet.⁹⁾ Thus unlike in the other tissues, FAA patterns in the skeletal muscle seem to be relatively stable and be hardly affected by the time elapsed after food intake. However, the dietary levels of certain amino acids are reflected in those of the skeletal muscle. Therefore, the muscular analysis also can be used for biochemical prediction of the EAA requirement of fishes as have been described in higher vertebrates by Pion.²⁷⁾ According to this principal, Kaushik¹³⁾ made the biochemical estimation of arginine needed for rainbow trout in different salinities and obtained the results which are well consistent with those through growth studies using diets containing graded levels of arginine.

Free EAA pattern in the whole blood at 4 h after food intake showed stronger correlation against the dietary EAA pattern than that of plasma. As have been claimed by Pion,²⁷⁾ Kaushik and Luquet,⁹⁾ and Kaushik,¹³⁾ whole blood seems to reflect the dietary amino acid composition better than plasma. Recently, the author found in the study using carp fed a casein-gelatin diet that certain EAA, especially aromatic amino acids, can distribute not only in the plasma but also in the erythrocytes after food intake and appeared to be transported partially by the erythrocytes.²⁴⁾ Therefore, plasma analysis would result in underestimation owing to amino acids present in erythrocytes. However, simple amino-graph can be obtained by the plasma analysis compared to the whole blood and hepatopancreas analyses. Also, the degree of fluctuation in the plasma FAA by food intake seems to be more distinct and wider than those in the whole blood and hepatopancreas. Thus, judging from these points, plasma analysis appears to be almost as good as whole blood and hepatopancreas analysis in evaluation of the quality of dietary protein.

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