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Protein digestion and amino acid absorption along the intestine of the common carp (*Cyprinus carpio* L.), a stomachless fish : an *in vivo* study

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Summary. Protein hydrolysis to peptides and free amino acids and the apparent absorption of amino acids (AAaa) were evaluated in different segments of carp intestine. The AAaa analysed using Cr_2O_3 as a marker indicated that 73.2 % of the amino acids were absorbed in the first 20 % of the intestinal tract and 5.3 and 21.5, respectively, in the following segments (20 % of gut length). Except for methionine and histidine, essential free amino acid concentration decreased significantly along the intestine. Of the non-essential amino acids, glutamate and aspartate concentrations increased in the hind gut. The absolute amount of the peptide amino acid fraction decreased towards the middle intestine but, expressed as a proportion of the total amino acid content, it changed little along the intestine : 49 and 54 % in the anterior and posterior intestine, respectively. The molar concentration of the peptide amino acid fraction was much higher in carp intestine (543.9 mM) than in rainbow trout (147.3 mM) or human (143.9 mM) intestine.

Introduction.

There is a relative paucity of data on the fate of ingested protein in the fish intestine. Lied and Solbakken (1984) investigated digestion and absorption of protein in the cod digestive tract by determining the distribution of polypeptides of different molecular weights in the gut contents. Hydrolysis in the stomach of cod fed minced fish fillet led to a release of over 75 % of the peptides having molecular weights of between 300 and 4 000. However, autohydrolysis was not taken into account in that study. In rainbow trout fed a casein-gelatin diet, peptide amino acids in the intestinal content of the pyloric caeca region. This decreased in (Dabrowski *et al.*, 1986). Peptide amino acids constituted 80.3-89.0 % of the total amino acids in the intestinal content of the pyloric caeca region. This decreased in the mid-intestine and increased again in the rectum.

Studies on the localization of protein digestion and absorption in stomachless fish (Stroband and van der Veen 1981 ; Dabrowski, 1983a) have not analysed hydrolysis in various parts of the intestine. Other works on the regional differentiation of the intestinal epithelium in cyprinid fish distinguished a proximal seg-

ment (60 % of gut length) where lipids were absorbed, a middle segment (25 % of gut length) absorbing protein macromolecules, and the hind gut which seemed to have water and ion-regulation capacities (Noaillac-Depeyre and Gas, 1973 ; Stroband, 1977 ; Stroband and van der Veen, 1981). A considerable part of dietary amino acids is absorbed in the first 20 % of the intestine of grass carp (Stroband and van der Veen, 1981), roach (Hofer, 1982) and common carp (Dabrowski, 1983a). These works, however, did not study the extent of proteolysis and the disappearance of postprandial products after digestion.

In vivo studies have shown that the level of free amino acids differ markedly in the digestive tract contents of fish and mammals (Dabrowski, 1983b), and it was concluded that fish might have a higher absorption rate than endotherms due to passive transport. This divergence might originate from differences in amino acid concentrations in the gut content and in tissues (intestine and blood). Recently, this idea was challenged by an *in vitro* approach (Ferraris and Ahearn, 1984). Although each of these studies extended the knowledge available at the time, new data on both mammals (Chung *et al.*, 1979) and fish (Dabrowski *et al.*, 1986) have been published which demand a re-examination and verification of this point.

Material and methods.

Common carp with an average individual weight of 200 g were maintained at a water temperature of 20 °C and fed an experimental diet for 3 weeks prior to experimentation. The composition of the diet is given in table 1. The fish were fed 3 times a day prior to the experiment and were sacrificed 2.5 h after the morning meal. The intestine was dissected into five equal parts (Dabrowski, 1983a) and their contents were squeezed into jars maintained in liquid nitrogen. Samples from the different intestinal segments of 10 fish were pooled. The samples were freeze-dried and the dry matter determined from the difference.

TABLE 1

Diet composition used in the present trial.

Ingredients	(%)
Fish meal	35
Wheat meal	30
Meat and bone meal	10
Yeast	10
Freeze-dried krill	4
Vitamin mixture (*)	2
Mineral mixture (*)	2
Fish oil	3
Soya oil	3
Chromic oxide	1

(*) For details see Dabrowski and Dabrowska (1981).

Chromic oxide was measured according to the method of Bolin *et al.* (1952). Dry samples were transferred to 5 % sulfosalicyclic acid at the ratio of 1 : 10, homogenized and centrifuged. Part of the supernatant was diluted with citrate buffer, pH 2.2, and used directly for free amino acid analysis ; another part (1-2 ml) was hydrolysed in 25 ml of 6 N HCl in vacumized tubes at 100 °C for 24 h. The total amino acid contents were analysed in lyophilized samples using the same procedure as for the peptide plus free amino acids. Free amino acids were subtracted from the supernatant hydrolysates (peptide + free). The amino acids were determined using an amino acid analyser Jeol JLC-6AH (Japan) in standard conditions. All samples were analysed three times ; the standard error is given throughout.

Apparent absorbability of amino acids (AAaa) was calculated as follows :

$$AAaa = 100/100 \frac{\%\ Cr\ in\ feed}{\%\ Cr\ in\ gut\ content} \times \frac{\%\ amino\ acid\ in\ gut\ content}{\%\ amino\ acid\ in\ feed}.$$

The molar concentration of amino acids in the gut content was calculated taking into account the water content in the digesta.

Results.

The amount of essential amino acids in the experimental diet is shown in table 2. Amino acid requirements determined with a diet containing a free amino acid mixture (Nose, 1979) are questionable since fish growth is less than with a protein diet. However, this preliminary evaluation of the dietary protein source used in the present trial suggests that, except for threonine, most of the essential

TABLE 2

Amino acid composition of experimental diet and amino acid requirements (Nose, 1979) *of common carp.*

Amino acids	Diet		Requirements
	(% dry matter)	(% protein)	(% protein)
Phe	1.86	3.6	3.4
Tyr	1.75	3.4	2.6
Leu	3.66	7.3	3.3
Ile	1.98	3.9	2.5
Met	1.09	2.1	2.1
Val	2.76	5.6	3.6
Ala	2.63	5.2	—
Gly	3.72	7.4	—
Pro	6.36	12.9	—
Glu	9.55	19.3	—
Ser	2.21	4.5	—
Thr	1.76	3.4	3.9
Asp	3.17	6.3	—
Arg	2.28	4.5	4.3
His	1.16	2.2	2.1
Lys	3.40	6.8	5.7

amino acids exceeded the recommended level. In diets based on animal protein, the tryptophan level is satisfactory, especially if we consider that the required level is lower (Dabrowski, 1981) than indicated by Nose (1979).

From different parts of the intestine, we chose several essential amino acids showing the characteristic pattern of amino acid groups (fig. 1). In all cases, the lowest levels of total amino acids were found in the third intestinal segment. The increasing level of amino acids present in non-hydrolysed protein in the hind gut suggests diminished hydrolysis of the first portion of the consumed meal. With the exception of Met, His, Tyr and Phe, the other essential amino acids decreased significantly in the hind gut (see table 3 also).

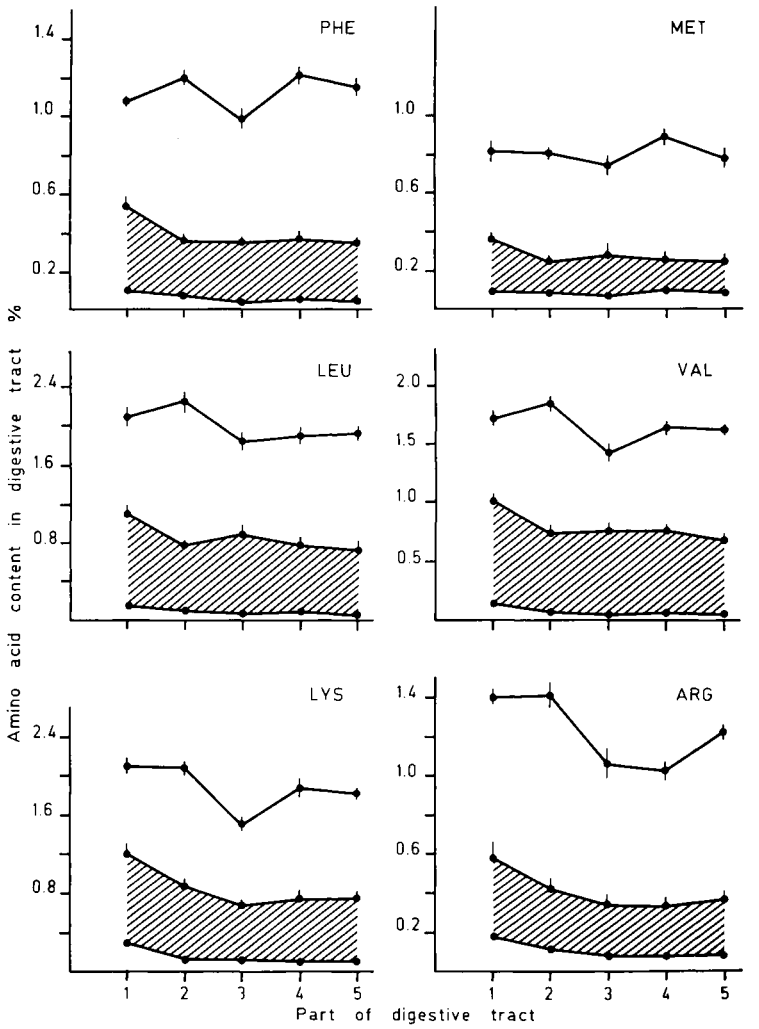

FIG. 1. — *Amino acid content in digestive tract of carp.* Upper curve : total a.a. content ; dashed area : peptide a.a. content ; bottom curve : free a.a. content. All values are expressed in percentage of intestinal content dry matter. Vertical bar indicates S.E.M. for analysis.

TABLE 3

Amino acid content in digestive tract of common carp (% of dry matter).

Amino acids	Part of digestive tract 1			2			3			4			5			S.E. (%)		
	Free	Peptide	Total	Free	Peptide	Total	Free	Peptide	Total	Free	Peptide	Total	Free	Peptide	Total	Free	Peptide	Total
Tyr	0.0764	0.297	0.81	0.0520	0.184	0.94	0.0309	0.200	0.68	0.0412	0.173	0.97	0.0262	0.206	0.73	7.4	1.0	6.8
Ile	0.1464	0.732	1.34	0.0783	0.502	1.40	0.0688	0.491	1.20	0.0762	0.504	1.26	0.0674	0.452	1.28	2.9	2.1	1.6
Ala	0.0871	1.715	2.17	0.0716	1.283	2.26	0.0326	1.260	1.87	0.0382	1.285	2.06	0.0312	1.134	1.99	4.8	1.1	5.1
Gly	0.0657	3.109	3.38	0.0762	2.376	3.30	0.0317	2.316	2.91	0.0432	2.376	3.22	0.0368	2.104	2.80	7.0	2.2	4.6
Pro	0.0411	2.365	2.44	0.0242	2.456	3.32	0.0704	1.864	2.20	0.0351	2.315	3.67	0.0313	2.011	2.56	4.0	2.6	4.3
Glu	0.2576	4.639	5.16	0.1227	3.600	5.61	0.0849	3.423	4.73	0.1188	3.633	5.41	0.1655	3.097	4.70	3.2	1.9	2.9
Ser	0.0100	1.001	1.48	0.0500	0.794	1.60	0.0150	0.753	1.28	0.0200	0.825	1.72	0.0400	0.817	1.40	4.9	0.4	7.2
Thr	0.0194	1.193	1.66	0.0700	0.946	1.73	0.0284	0.958	1.49	0.0336	0.986	1.49	0.0672	0.812	1.58	6.9	1.0	4.5
Asp	0.1309	3.144	3.73	0.0900	2.489	4.05	0.0336	2.490	3.58	0.0581	2.773	3.85	0.0790	2.134	3.53	8.0	1.6	6.9
His	0.0205	0.557	0.92	0.0198	0.404	0.95	0.0304	0.267	0.59	0.0301	0.439	0.94	0.0209	0.380	0.78	5.7	7.6	11.7
Total (*)	2.0436	23.621	32.30	1.2229	18.471	34.76	0.8827	17.320	28.12	0.9696	18.548	33.14	0.9679	16.268	30.10	—	—	—

(*) Including those amino acids presentend in figure 1.

Peptide amino acids in the first segment constituted a large fraction (74 %) of the total amino acids ; this rate decreased towards the rectum, but was still 53 %. The total content of free amino acids in the digesta decreased towards the mid-intestine and increased slightly again in the last 20 % of the intestinal tract (table 3). However, the individual amino acids in the digesta along the carp intestine showed specific levels which were evident when expressed in molar concentrations (fig. 2). Except for Met and His (fig. 2), the other essential amino acids decreased in the gut content. A significant drop occurred in levels of Lys, Arg (fig. 2) and Thr (from 4.28 to 1.81 mM).

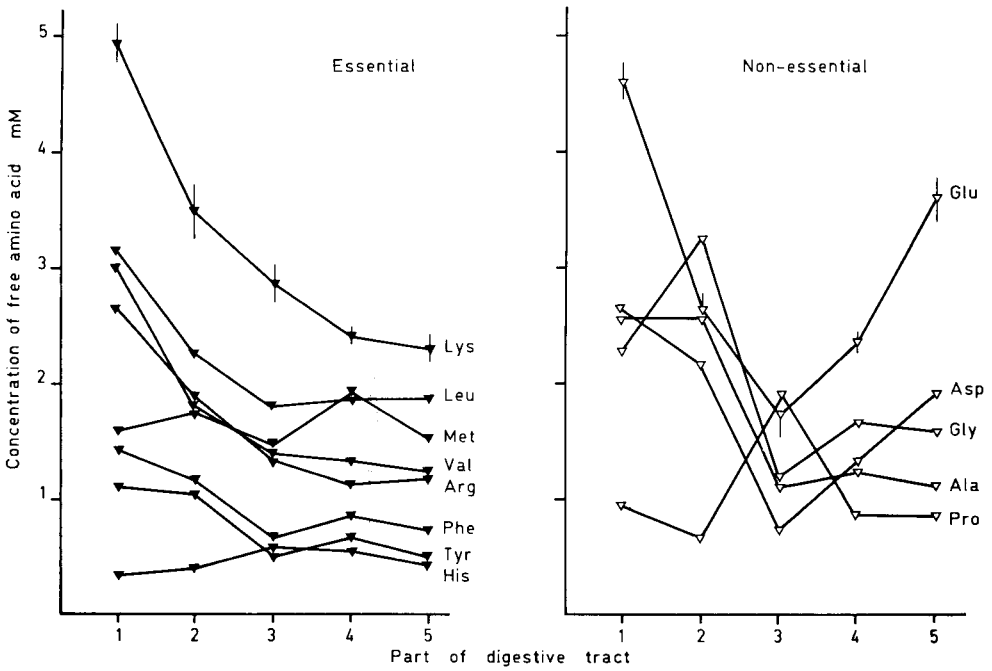

FIG. 2. — *Molar concentration of free amino acids in digestive tract content.* Vertical bars indicate S.E.M. for analysis. For clarity of presentation in some amino acids S.E.M. were omitted.

Total molar concentrations of free amino acids decreased from 41 to 20 mM between the first and third segments (table 4) and increased slightly in the hind gut (21.3 to 23.5 mM).

The patterns of non-essential amino acids were different from those of the essential amino acids (fig. 2). Glu and Asp concentrations, highest in the first part of the intestine, decreased most in the third segment, but showed a significant increase again in the hind gut. Proline, which constituted a high proportion of the peptide fraction (table 3), appeared in low concentrations as a free form, except in the third intestinal segment.

When expressed in molar concentrations, peptide amino acid levels in fish digesta were 13.5-22.0-fold higher than free ones (table 4). The respective propor-

TABLE 4

Concentration of free and peptide amino acids (mM) *in intestinal content of fishes and humans.*

Amino acids	Human jejunal content (*)		Trout mid-intestine (**)		Carp intestine content			
					1st segment		3rd segment	
	Free	Peptide	Free	Peptide	Free	Peptide	Free	Peptide
Phe	5.34	7.70	0.150	1.05	1.43	8.55	0.67	6.35
Tyr	5.56	5.66	0.200	0.72	1.10	4.27	0.51	3.35
Leu	14.20	16.08	0.532	3.01	3.17	21.95	1.80	19.99
Ile	2.00	4.28	0.366	4.19	2.93	14.65	1.54	11.36
Met	0.80	1.38	0.597	0.88	1.58	6.62	1.48	5.63
Val	6.62	13.34	0.547	5.87	3.03	23.42	1.36	19.71
Ala	5.58	16.56	0.499	11.36	2.57	50.60	1.11	42.91
Gly	0.38	9.72	1.495	34.78	2.30	108.83	1.28	93.51
Pro	0.0	17.60	0.351	18.12	0.94	67.81	1.85	49.13
Glu	4.80	18.10	0.882	22.90	4.63	83.38	1.75	70.58
Ser	4.16	5.28	0.632	12.04	2.50	25.02	0.43	21.71
Thr	3.78	11.36	0.522	7.85	4.28	26.32	0.72	24.37
Asp	2.04	15.84	0.520	15.45	2.58	61.97	0.76	56.70
Arg	1.97	0.66	0.240	2.49	2.66	8.99	1.34	6.03
His	2.12	4.98	0.245	2.16	0.35	9.51	0.59	5.22
Lys	8.91	14.06	0.587	3.58	4.95	22.00	2.88	14.63
Total	57.26	143.90	10.27	147.29	41.00	543.89	20.07	451.18

(*) Chung *et al.* (1979), (**) Dabrowski *et al.* (1985).

tion in human digesta is 3. In the fifth segment, the proportions of peptide and free amino acids did not change (447.3 and 23.5 mM, respectively).

Apparent absorption of amino acids was maximal in the third intestinal segment (fig. 3) and decreased in the hind gut. 73,2, 5.3 and 21.5 % of the total amino acids were absorbed in the first three parts of the intestine, respectively. In the fourth segment, absorption decreased by 5.8 % compared to the third segment, but this negative difference decreased to only 1.8 % in the last 20 % of the gut length. For these reasons, the AAaa in the fourth and fifth segments were omitted in figure 3. AAaa differed in the first segment (Tyr : 61 %, His : 32 %) but this was less significant in the third segment. Basic amino acids (Arg, Lys) appeared to have a common absorption pattern. Thr was less than the indicated requirement in the diet used in the present study (table 2) and was not as well absorbed as the other essential amino acids.

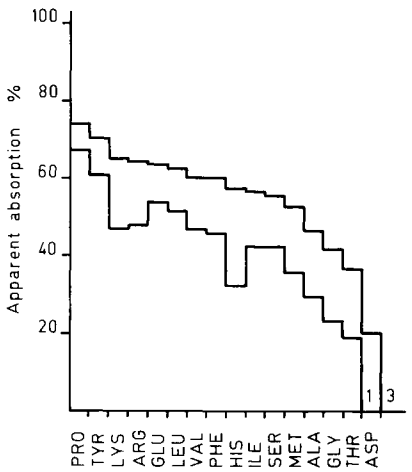

FIG. 3. — *Apparent absorbability of amino acids in the first 20 and 60 % of intestinal length.* Number 1 and 3 respectively.

Discussion.

Protein digestion. — Lied and Solbakken (1984) developed a biochemical technique based on the fractionation of the digestive tract content into protein, polypeptide and oligopeptide plus free amino acids. In the present work, sulfosalicyclic acid was used to precipitate the protein and polypeptides. According to Adibi and Mercer (1973), di-, tri- and tetra-peptides constitute a major portion of the soluble nitrogenous compounds in this preparation. Chung *et al.* (1979) confirmed that the fraction separated on Sephadex G-25 column into oligopeptides and supernatant after sulfosalicyclic acid precipitation had essentially the same -amino nitrogen concentrations. This provides a basis for directly comparing oligopeptide distribution in the digestive tract of rainbow trout (Dabrowski *et al.*, 1986), humans (Chung *et al.*, 1979) and common carp (table 4).

The absolute quantities of the short peptide fraction in the cod stomach was not changed compared to the food (5.1 % of dry matter), but in the pyloric caeca

region, oligopeptides constituted 24.2 % of the dry matter (Lied and Solbakken, 1984). This fraction decreased to 17.6 % of dry matter content in the anterior ileum of cod. If the protein content in minced saithe fillet is assumed to be 60 % (86 % of the diet), the extent of hydrolysis can be estimated approximately. In rainbow trout fed a casein diet, the peptide fraction of the pyloric caeca region contains a maximum of 89 % of total amino acids (Dabrowski *et al.,* 1986). However, the similitude between the stomachless carp (maximal value : 75 %) and the rainbow trout with a stomach could result from the composition of the diet offered to the fish in these studies.

Current knowledge of protein digestion in fish is derived mostly from *in vitro* studies (Ferraris and Ahearn, 1984) ; therefore the role of peptidization is difficult to estimate. Dietary treatment and postprandial time are likely to affect the peptide fraction as they do the amount of free amino acids. Because the concentration of free amino acids is higher in the digesta of fish than in that of mammals or reptiles (Dabrowski, 1983b), it has been claimed that the absorption of amino acids in fish could be due to passive transport. However, amino acid absorption in stomachless fish fed a diet containing a mixture of amino acids led to loss of body weight, and Kaushik and Dabrowski (1983) postulated gill excretion of amino acids. This phenomenon was recently confirmed by Murai *et al.* (1984).

The free amino acid fraction was highest in carp intestinal content 3 h after a meal, but 6 h after feeding (Dabrowski, 1983a) it had decreased to less than the level found in the present trial 2.5 h after feeding. However, water temperature in the present study was lower than in the former study.

The most recent approach by Grabner (1985), who simulated the conditions in the carp digestive tract by *in vitro* methods, is of great value in comparing the present *in vivo* study. In his study Grabner analysed the products of protein hydrolysis and peptidization by fractionation to nitrogenous compounds of various molecular weights, and this seems to be an alternative to *in vivo* studies. It resolves several of the shortcomings of amino acid absorption study in the intestine when a single free amino acid, or a mixture of them, is exclusively used in a medium. Coulson and Hernandez (1983) concluded that when single amino acid absorption is tested *in vitro,* several transport systems can be distinguished. However, a different and more efficient transport starts to operate when the amino acids are absorbed from digested protein. Thus, the conclusion of Ferraris and Ahearn (1984) is not very relevent to *in vivo* amino acid absorption in the fish intestine. When both carp (Plakas *et al.,* 1980) and rainbow trout (Yamada *et al.,* 1981) were fed a diet containing a free amino acid mixture, the amino acids were transported quickly into the blood. The upper intestine plays a major role in amino acid absorption in both stomachless fish and those with a stomach. Adaptation to sea water tends to increase amino acid absorption in the anterior intestine of rainbow trout (Dabrowski and Leray, 1986) as compared to freshwater fish. Contrary to the large backflux or secretion of organic solutes suggested by Ferraris and Ahearn (1984), we have demonstrated increased absorption of amino acids in the mid-intestine of rainbow trout in sea water. In the trout rectum, the influx of free amino acids evidently diminishes apparent amino acid absorption. The acute change in salinity should not be overlooked in the experiments of Dabrowski *et al.*

(1986), although the digestion of dietary protein in fully adapted fish might be improved.

Stroband and Van der Veer (1981) argued against the nutritional importance of protein macromolecule pinocytosis in stomachless fish. A recent study by McLean and Ash (1985) demonstrates an increase of horseradish peroxidase in the blood circulation of rainbow trout following ingestion. This suggests that intact protein hydrolysis is not restricted to enterocytes, but that other organs, like liver, could be involved in the use of protein entering via pinocytosis. The major products of pancreatic protease digestion, *i.e.* small peptides containing 2 to 6 amino acid residues, are likely to enter the blood system intact in mammals (Sleisenger *et al.,* 1977). But this mechanism has not been studied in fish. The high concentrations of peptide amino acids in the carp gut lumen suggest the importance of this absorption process in fish.

The problem of enteropancreatic circulation of digestive enzymes is linked to intact protein absorption. This has raised considerable discussion (Rothman and Grendell, 1984) but recent evidence argues against the biological significance of this phenomenon in mammals (Bohe *et al.,* 1984 ; Udall *et al.,* 1984). This process has not been studied experimentally in fish, though Hofer (1982) is of the opinion that the reabsorption of proteolytic enzymes plays a major role, while protease absorption efficiency is one order of magnitude higher than that of soluble proteins. Even assuming that autodigestion and inactivation of proteases are negligible in the cyprinid intestine (Hofer, 1982), there are some analytical problems concerning the enzymes firmly bound to digesta particle (Kaspar and Neumann, 1984). Although this example applies to the mammalian situation, it is necessary to be cautious when analysing fish digesta. The quantitative estimation of the absorption of intact protein and of the eventual role of digestive proteins in this absorption in the fish intestine needs further study. However, in my opinion it is less important than the role of small peptides in amino acid absorption in the fish gut.

Amino acid absorption. — AAaa in the present study was lower due to the shorter postprandial time than in the previous work where fish were sampled 3 or 6 h after feeding (Dabrowski, 1983a). In that experiment using a fish meal diet, 30 and 35 % of the amino acids were absorbed in the first and second intestinal segments, respectively (each 20 % of gut length). In grass carp weighing 100-180 g, 50 % of the amino acids were absorbed in the first 30 % of the gut (Stroband and Van der Veen, 1981).

The preferential absorption of individual amino acids in different parts of the intestine changes depending on the postprandial time. The consistent differences between common carp and grass carp was the low AAaa of Met and Thr in the former species compared to the high AAaa of Met in the latter species. The essential amino acids appeared to be preferentially absorbed in the more anterior parts of the intestine, particularly during the early phase of digestion.

In other studies, Dabrowski and Schwarz (1986) demonstrated fast potassium absorption which, in carp anterior intestine, is closely associated with amino acid absorption. Although the acid-base balance plays a large role in nutrient

absorption in fish, Murai *et al.* (1983) found that supplementing K up to the equimolar level of chloride significantly improved the growth of fish fed an amino acid diet. Dabrowski and Schwarz (1986) observed a large influx of Na in the anteror carp intestine which was maintained at a similar level of 229.2-272.0 mM in the carp lumen. It has been shown that dietary electrolytes, with no separation of the K and Na roles, contribute to histidine absorption and growth in rainbow trout (Chiu *et al.*, 1984).

In *conclusion,* the results of the present work emphaize the need to study *in vivo* absorption of amino acids and peptides in the fish gut since the *in vitro* approach lacks several basic components of the fish gut « environment ».

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Résumé. *La digestion des protéines et l'absorption des acides aminés dans l'intestin de la carpe, poisson sans estomac : une étude* in vivo.

L'hydrolyse des protéines en peptides et acides aminés libres et l'absorption apparente des acides aminés (AAaa) ont été évaluées dans plusieurs segments de l'intestin de la carpe. L'AAaa a été mesurée en utilisant Cr_2O_3 comme indicateur. On a constaté que 73,2 % des acides aminés étaient absorbés dans les premiers 20 % de la longueur de l'intestin, tandis que la participation des deux segments suivants n'était que de 5,3 et 21,5 %. La concentration en acides aminés essentiels, à l'exception de la méthionine et de l'histidine, diminuait le long de l'intestin.

Parmi les acides aminés non essentiels, la concentration des acides glutamique et aspartique augmentait au niveau de l'intestin postérieur. La quantité absolue d'acides aminés de la fraction peptidique diminuait vers l'intestin moyen, mais exprimée en proportion du contenu d'acides aminés, elle variait insensiblement le long de l'intestin : 49 et 54 % respectivement dans les parties antérieure et postérieure de l'intestin.

La concentration molaire des amino-acides de la fraction peptidique était remarquablement plus forte dans l'intestin de la carpe (543,9 mM) que dans celui de la truite arc-en-ciel (147,3 mM) ou de l'intestin humain (143,9 mM).

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