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The Influence of Salinity Acclimation on Free Amino Acids and Enzyme Activities in the Intestinal Mucosa of Rainbow Trout, *Oncorhynchus mykiss* (Walbaum)

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ABSTRACT. Postprandial changes of Arg, Leu, Val, Ala, Asp, Glu, Gly, Pro and Tau as well as activities of three enzymes of the transdeamination system in the midgut mucosa and, for comparison, in the liver of freshwater and seawater acclimated *Oncorhynchus mykiss* were studied. In the mucosa a postprandial increase of Arg, Leu, Val, Ala, Asp, Glu, Gly and Pro occurred. In contrast, only the postprandial Arg level increased strongly in the liver. Levels of Leu, Val, Ala, Asp, Glu, Gly, Pro and Tau remained stable. Concentrations of Ala, Asp, Glu and Pro are higher in the liver than the mucosa. Tau is the most important osmotic effector in both organs, but its concentration is much lower in the liver. Its postprandial concentrations remained stable in both tissues but were significantly higher in seawater trout. The trend of a stronger postprandial rise of Arg, Leu, Val, Ala, Asp, Glu, Gly and Pro levels in seawater trout than in freshwater trout was shown. In mucosa tissue aspartate aminotransferase activities were higher in seawater trout. Ratios of aspartate aminotransferase, alanine aminotransferase and glutamate dehydrogenase are similar to those of the gills. Copyright © 1996 Elsevier Science Inc. COMP BIOCHEM PHYSIOL 116A;2:149–155, 1997.

INTRODUCTION

Apart from their participation in protein turnover, free amino acids perform two major functions in carnivorous teleosts. Firstly, they are central in energy metabolism as shown by Phillips (29), who observed that in natural trout food, proteins contribute about 70% to the energy supply. Secondly, FAA serve as important intracellular osmotic effectors. The total pool of FAA in the fish is divided into smaller pools, each having a different composition, specific to the particular organs (14,44). All are nevertheless linked via the blood system. FAA arise from the breakdown of nutritional and body proteins as well as by *de novo* synthesis. FAA released by digestion of nutritional protein in the intestinal lumen enter the blood by trans-epithelial transport. The principal resorption site of FAA in teleosts is the mid-intestine (7,20,39). Postprandial FAA concentrations in the blood (27,33) and in other organs (4,19,41); and recently, in the caeca (4) have been investigated. However, little is known about the midgut mucosa or its role in impor-

tant biochemical events such as ammoniogenesis and oxidation of FAA.

FAA were found to be important intracellular osmotic effectors in rainbow trout and other fish (13,16). This is of particular importance for anadromous fish like salmonids, which face large changes in salinity while migrating. As salinity influences conditions in the gut lumen (37) and FAA transport powering Na^+/K^+ ATPase (6,40) it may well affect postprandial FAA concentrations too.

The aim of this investigation was to examine the behaviour of postprandial FAA concentrations in midgut mucosa tissue and to determine the influence of different salinities on the AA metabolism of this tissue. To this end, the following were determined from mucosa tissue of both FW and SW acclimated trout: 1. Postprandial concentrations of three essential and six non-essential FAA; and 2. Maximum activities of the key enzyme of AA metabolism, GDH and two other important enzymes of the transdeamination system, AlaAT and AspAT.

As liver tissue is the main site of AA metabolism, it was included in all experiments for comparative purposes.

MATERIALS AND METHODS
Animals and Experimentation

Rainbow trout, *Oncorhynchus mykiss* (Walbaum), were obtained from a trout farm of the Versuchsanlage Born,

TABLE 1. Composition of diet

Crude protein	48.0%
Crude fibre	1.5%
Crude lipid	13.0%
Crude ash	13.5%
Mineral and vitamin mix	
Dry weight	91.1%
Ash/dry weight	16.1%
Nitrogen/dry weight	8.6%

Amino acids	(μmol/g dry weight)
Essential	
Leu	298.4
Lys	247.3
Val	221.2
Arg	199.0
Thr	180.3
Ile	159.2
His	147.9
Phe	118.6
Met	92.1
Non-essential	
Gly	659.7
Glu/Gln	509.5
Ala	422.3
Asp/Asn	371.8
Pro	297.5
Ser	233.7
Tyr	70.6
Cys	19.4
Tau	6.2

Landesforschungsanstalt für Landwirtschaft und Fischerei (Mecklenburg/Vorpommern, Germany). The fish were slowly acclimated (about 3 weeks) to the specific experimental conditions and were kept under these conditions for an additional 4 weeks.

In the first experiment (for FAA determination) it was necessary to keep the animals separately to avoid hierarchy effects during feeding and to ensure that trout would feed the whole portion without any delay. The animals were kept in containers with a closed water circulation at temperatures between 18 and 21°C. Acclimation salinity was either 0 or 32‰ and the pH in the freshwater and seawater tanks was 7.1 and 7.9, respectively. The fish were fed daily on a ration of artificial pellet food (for composition see Table 1), equivalent to 1% of the individual body mass. Average mass was 168 ± 33 g (n = 48) measured after the acclimation period. Pellets were obtained from the Kraftfutterwerk Beeskow e.G. (Germany). In the first experiment animals were trained to feed from hand. Fish were starved for 24 hr prior to experimentation. The times of killing (see Table 2) were chosen after a pilot experiment, which showed that 12 hours after feeding the FAA concentrations remain at the level attained 6 hours after feeding.

In a second experiment (for enzyme assays) animals were kept as described above, except that they were fed *ad libitum*. Body mass in this experiment was 144 ± 26 g (n = 15) measured after killing.

The day/night cycle in the two experiments was natural (March–May/June–August, respectively). Animals were killed by a sharp blow to the head and tissue samples dissected out immediately. The midgut (see Fig. 1) was taken out first and cut open longitudinally. The gut content present was removed and the tissue rinsed with ice-cold 0.9% NaCl using a soft paintbrush. Samples were then dried using a dry paintbrush. The gut "mucosa" was separated from the gut musculature by means of a scalpel, wrapped in aluminium foil, and immediately frozen in liquid N_2. All the tissue was subsequently stored at −70°C for later analysis. Subsequently, the whole liver was rinsed with 0.9% NaCl, dried with a cotton cloth and frozen (as described above). The whole procedure was performed on ice and did not take longer than 3 min for the two tissue samples.

FAA Analysis by HPLC

Treatment of tissue and analysis of FAA by HPLC was carried out according to Schiedek and Schöttler (32) except that the column was a LiChroCART 125-4 column (Merck) with a guard column consisting of the same material. Mixtures (1 and 2 mM) of the FAA measured (Arg, Leu, Val, Ala, Asp, Glu, Gly, Pro, Tau) were used as standards.

Enzyme Assays

Tissue samples were disrupted in ice-cold bi-distilled H_2O by means of an Ultraturrax (Janke and Kunkel) for 30 sec at maximum speed, followed by sonification for a further 30 sec with an HD 60 (Bandelin). The homogenates were centrifuged at 20,000 g (4°C) and the supernatants obtained were used for assays (within 1 hour). Enzyme assays were carried out as follows: GDH (E.C. 1.4.1.2.) in the direction of glutamate formation according to Schmidt (34), AlaAT (E.C.2.6.1.2.) in the direction of pyruvate formation according to Hørder and Rej (12), AspAT (E.C.2.6.1.1.) in the direction of oxaloacetate synthesis according to Rej and Hørder (30), with the exception that pyridoxal phosphate was omitted for AlaAT and AspAT. Changes in absorbance were detected by means of a DU 64 spectrophotometer (Beckman) at 340 nm after 5 min of pre-incubation at 25°C. All reactions were initiated by adding α-ketoglutarate. It was omitted for control.

DNA Determination

Extraction was carried out using the Schmidt-Thannhauser method modified according to Munro and Fleck (24). DNA was derivatized with indol according to Ceriotti (5) and absorbance measured at 490 nm by means of a Spekol 11 (VEB Carl Zeiss Jena). DNA from herring sperm (Sigma) was used as a standard. The DNA concentration of the same tissue sample as the enzyme assay but from a separate homogenate, was determined.

TABLE 2. Postprandial Changes of FAA Concentrations in the Mucosa of Fresh- and Seawater Acclimated Trout

			Time After Feeding (hr)				
			0	0.5	2	3	6
Essential							
Arginine	FW	(4) 0.52 ± 0.08	(7) 1.24 ± 0.12**	(4) † 1.35 ± 0.21**	(5)	(5) 1.35 ± 0.09**	
	SW	(4) 0.42 ± 0.09	(5) 1.04 ± 0.09**	(5) † 2.05 ± 0.23**	(4)	(5) 1.63 ± 0.12**	
Leucine	FW	1.07 ± 0.28	1.04 ± 0.18	1.14 ± 0.04	1.69 ± 0.29	1.95 ± 0.21*	
	SW	0.85 ± 0.09	0.98 ± 0.21	1.36 ± 0.11*	2.33 ± 0.39*	2.73 ± 0.59*	
Valine	FW	0.61 ± 0.09	0.67 ± 0.03	0.61 ± 0.04	0.77 ± 0.11	1.06 ± 0.09**	
	SW	0.53 ± 0.04	0.69 ± 0.08	0.81 ± 0.12*	1.15 ± 0.19*	1.47 ± 0.30*	
Non-essential							
Alanine	FW	0.78 ± 0.07	0.82 ± 0.08	1.11 ± 0.11*	1.61 ± 0.19**	1.41 ± 0.12**	
	SW	0.63 ± 0.04	0.93 ± 0.11*	1.47 ± 0.20**	1.81 ± 0.31**	1.69 ± 0.30**	
Aspartate	FW	0.22 ± 0.03	† 0.59 ± 0.07**	0.47 ± 0.08*	0.73 ± 0.11**	0.7 ± 0.04**	
	SW	0.22 ± 0.01	† 0.27 ± 0.03	0.63 ± 0.11**	0.72 ± 0.23*	0.85 ± 0.11*	
Glutamate	FW	1.08 ± 0.12	1.21 ± 0.21	† 1.49 ± 0.09*	† 1.65 ± 0.21*	1.53 ± 0.11*	
	SW	1.55 ± 0.28	1.63 ± 0.22	† 2.06 ± 0.19	† 2.31 ± 0.20	1.93 ± 0.20	
Glycine	FW	†† 1.3 ± 0.09	1.12 ± 0.29	1.62 ± 0.18	2.04 ± 0.24*	† 1.61 ± 0.10	
	SW	†† 0.83 ± 0.11	1.17 ± 0.21	1.36 ± 0.22	2.30 ± 0.21*	† 2.63 ± 0.41*	
Proline	FW	0.47 ± 0.11	0.62 ± 0.09	† 0.70 ± 0.10	0.91 ± 0.08*	0.74 ± 0.03*	
	SW	0.32 ± 0.03	0.53 ± 0.02**	† 0.97 ± 0.09**	0.99 ± 0.18**	1.05 ± 0.21**	
Taurine	FW	†† 23.0 ± 0.5	†† 23.0 ± 0.7	23.8 ± 1.0	†† 24.3 ± 1.6	†† 21.2 ± 1.4	
	SW	†† 28.1 ± 1.5	†† 28.6 ± 1.5	30.0 ± 3.4	†† 33.1 ± 1.9	†† 30.2 ± 2.4	

Values are expressed in μmol/gfw as means ± SE with n in parentheses.
  Significance levels for comparison with value at time 0: $P < 0.05$ (*), $P < 0.01$ (**).
  Significance levels for differences between FW and SW animals: $P < 0.05$ (†), $P < 0.01$ (††).

Protein Determination

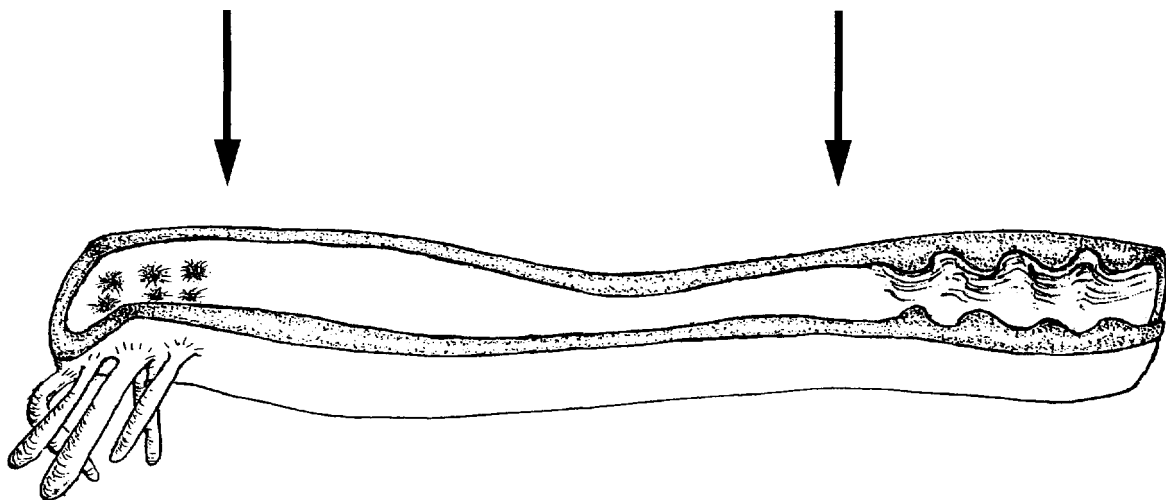Protein was measured according to Lowry et al. (18) using human serum albumin as a standard.

Statistical Analysis

Student's t-test or Welch's t-test (when F-test gave significantly different variances) were used, where appropriate.

RESULTS

Postprandial Changes of FAA

MUCOSA. Values and significance levels are reported in Table 2. An increase in postprandial concentrations of Arg, Leu, Val, Ala, Asp, Glu, Gly and Pro occurred. Only the concentration of Tau remained stable during the investigated period. After roughly 3 hours FAA levels reached a plateau. There were no major differences in FAA concen-

pylorus region **midgut** **posterior intestine**

FIG. 1. Mid-intestine of O. mykiss. Mucosa of the section between the arrows was used for experimentation.

TABLE 3. Postprandial Changes of FAA Concentrations in the Liver of Fresh- and Seawater Acclimated Trout

			0		0.5		2		3		6
					Time After Feeding (hr)						
Essential											
Arginine	FW	(7)	0.44 ± 0.11	(4)	1.87 ± 0.21**	(5)	1.37 ± 0.50**	(5)		(6)	1.23 ± 0.09**
	SW	(5)	0.86 ± 0.18	(5)	1.55 ± 0.28	(4)	1.83 ± 0.22**	(5)		(5)	1.65 ± 0.20*
Leucine	FW	††	0.55 ± 0.04		0.65 ± 0.03		0.62 ± 0.11		0.93 ± 0.18*		0.96 ± 0.10**
	SW		0.81 ± 0.11		0.52 ± 0.02**		0.73 ± 0.07		1.22 ± 0.10*		1.29 ± 0.21
Valine	FW		0.45 ± 0.03		0.32 ± 0.03*		0.29 ± 0.08	†	0.24 ± 0.03**		0.53 ± 0.11
	SW		0.54 ± 0.10		0.38 ± 0.03		0.38 ± 0.03		0.52 ± 0.09		0.7 ± 0.09
Non-essential											
Alanine	FW		3.27 ± 0.22		3.42 ± 0.28		4.12 ± 0.49		4.16 ± 0.19*		2.92 ± 0.13
	SW		3.12 ± 0.71		3.34 ± 0.48		4.37 ± 0.80		5.54 ± 0.71*		3.11 ± 0.19
Aspartate	FW		0.53 ± 0.09		0.38 ± 0.08		0.48 ± 0.10		0.34 ± 0.02*		0.42 ± 0.06
	SW		0.48 ± 0.02		0.54 ± 0.07		0.72 ± 0.11*		0.48 ± 0.09		0.63 ± 0.20
Glutamate	FW		5.91 ± 0.69		5.09 ± 0.39		5.85 ± 0.32		5.78 ± 0.68		5.52 ± 0.24
	SW		6.05 ± 1.28		3.94 ± 0.30		5.11 ± 0.67		6.54 ± 0.58		4.98 ± 0.50
Glycine	FW		1.31 ± 0.12		1.27 ± 0.30		1.87 ± 0.41		2.19 ± 0.41*		1.81 ± 0.13*
	SW		1.54 ± 0.20		1.24 ± 0.27		1.48 ± 0.19		1.97 ± 0.14		2.03 ± 0.22
Proline	FW		0.74 ± 0.08		0.60 ± 0.10		0.71 ± 0.06	†	0.81 ± 0.11	†	0.73 ± 0.07
	SW		0.84 ± 0.28		0.46 ± 0.10		0.81 ± 0.06		1.15 ± 0.10		0.98 ± 0.09
Taurine	FW	†	13.6 ± 0.8		12.7 ± 0.4	††	14.1 ± 1.0	††	15.9 ± 0.8	†	15.8 ± 0.8
	SW		17.5 ± 1.4		19.5 ± 2.7		19.2 ± 0.8		21.5 ± 0.6		19.7 ± 1.2

Values are expressed in μmol/gfw as means ± SE with n in parentheses.
For significance levels see Table 2.

trations between pre-fed animals of the two experimental groups. The only exception of the FAA investigated was Tau. It had by far the highest concentration and its level was consistently higher (22–36%) in SW trout. However, the sum of the FAA measured is 26, 24 and 35% higher in SW trout than FW trout after 2, 3 and 6 hours, respectively.

LIVER. Values and significance levels are reported in Table 3. Postprandial levels of Leu, Val, Ala, Asp, Glu, Pro and Tau remained stable. Only Arg showed a strong increase. It is noteworthy that in the liver concentrations of some, FAA (Leu, Asp, Glu, Gly, Pro) dropped within the first 30 min after feeding and reached the previous level or a slightly higher one later. Tau also remained stable in liver tissue and, similar to the findings in the mucosa, the level was higher (24–54%) in SW animals. Tau concentration in the liver is only about half of that measured in the mucosa of the midgut. In general, it can be reported that FAA concentrations in the liver are more stable than in the midgut mucosa and are less influenced by food uptake.

Maximum Enzyme Activities

MUCOSA. Results and significance levels are shown in Table 4. GDH activities showed no significant differences between animals of the different salinities. Maximum activity of AspAT was significantly higher in rainbow trout kept in SW. Also AlaAT showed a greater maximum activity in SW acclimated trout, but not significant because of large individual differences. DNA concentrations were not differ-

ent in both groups either, so, cell number per g liver weight remained unchanged by salinity. Thus, measured results are comparable. A similar feature occurred in a second experiment (results not shown), except that the difference of the AlaAT activities was significant ($P < 0.05$).

LIVER. GDH activity was slightly higher (21%) in SW trout. Specific activities were similar. AspAT and AlaAT showed slightly higher values (22 and 11%, respectively) in SW animals but again there were no differences in the specific activities of these enzymes in trout of either group. This was caused by the 19% higher content of soluble protein in liver tissue of SW acclimated fish, while the DNA concentrations were similar.

DISCUSSION

MUCOSA. The intestine plays a special role in maintaining AA homeostasis. The passage of AA from the lumen across the intestinal wall into the circulation involves transcellular transport. When considering the FAA absorption process, it is necessary to distinguish between uptake by the brush-border membrane, and its basolateral release into the circulation. This study reports an increase of postprandial concentrations of the essential (Arg, Leu, Val) (43) as well as the non-essential FAA (Ala, Asp, Glu, Gly, Pro) in mucosa tissue of the mid-intestine. Similar increases of FAA levels after feeding were observed in blood plasma of rainbow trout (25,27,33) and in the caeca of rainbow

TABLE 4. Maximum Enzyme Activities in Mucosa and Liver of Fresh- and Seawater Acclimated Trout

		Mucosa		Liver	
		FW	SW	FW	SW
n		9	6	9	6
DNA	mg/gfw	2.1 ± 0.1	2.0 ± 0.1	4.5 ± 0.5	4.3 ± 0.6
Soluble Protein	mg/gfw	129.9 ± 3.5	122.3 ± 7.8	89.2 ± 6.7	106.1 ± 8.9
AspAT	U/gfw	9.1 ± 0.5 †	13.2 ± 1.3	8.3 ± 0.7	10.1 ± 1.8
AlaAT	U/gfw	3.4 ± 0.3	4.7 ± 0.9	20.8 ± 2.2	23.0 ± 4.8
GDH	U/gfw	15.6 ± 0.5	13.1 ± 1.4	57.0 ± 4.8	68.9 ± 5.7

Values are given as means ± SE.

1U = consumption of 1 μmol of the respective substrate per min.

Significance level for differences between FW and SW animals: $P < 0.01$ (†).

trout (4). In contrast, stable postprandial FAA concentrations were measured in white muscle of cod (19) and rainbow trout (4), stomach tissue of rainbow trout (4) and in rainbow trout liver (4,41). The high stable levels of Tau recorded in this experiment are in contrast to the findings of Nose (27) and Murai et al. (25), who measured strong postprandial increases of Tau in trout blood. Unfortunately, they did not mention the Tau content of the diet fed. Thus, fish meal or marine invertebrates cannot be excluded as the possible source for Tau. Feed used in this experiment had a low Tau concentration (see Table 1). The lower Tau level in the liver (about 50% of that in the mucosa) might result from the high amount of other osmoeffectors (e.g. Ala and Glu). Murai et al. (25) found a surprisingly strong increase of Pro concentration in the portal vein blood of O. mykiss in comparison with the composition of the food. This is unlikely a random observation since it was found in two independent experimental groups. This feature can be explained by a postprandial Pro synthesis from Glu in the enterocytes. However, the present results do not support this hypothesis. Since we do not know what the basolateral transport rate of Pro is, our results do not disprove it, either. The FAA concentrations measured in the midgut mucosa of FW animals occur in the following quantitative order:

Tau ≫ Gly > Glu, Leu >
Ala > Val > Arg > Pro > Asp

Quantitatively, the effect of salinity acclimation on the FAA in the mucosa is relatively small. According to investigations in Anguilla anguilla the general osmoregulation is essentially achieved in the anterior part of the gut: oesophagus, the stomach and the pyloric section of the intestine (17). Accordingly, in rainbow trout the highest activity of Na⁺/K⁺ATPase was found in the pyloric section (40).

Only Tau has obvious and significantly higher levels in mucosa of SW trout, which underlines its importance for cell volume regulation.

Essential (Arg, Leu, Val) and non-essential FAA (Ala, Glu, Gly, Pro) show a rapid postprandial increase in SW trout. Although this increase is not statistically significant

in all instances, the general trend observed is clear. One reason might be a higher activity of Na⁺/K⁺ATPase in the small intestine in SW trout (40). Reshkin et al. (31) have shown that the transapical transport is Na⁺ dependent, while active transport across the basolateral membrane occurs for only a few FAA. The Na⁺ dependent transport is driven by the basolateral located Na⁺/K⁺ATPase (14). Mucosal AspAT activity (Table 4) in rainbow trout increased by roughly the same extent as that shown by Vökler et al. (40) for Na⁺/K⁺ATPase after salinity acclimation. The AspAT might be indirectly implicated in the energization of transport in the malate-aspartate shuttle.

Considering the mucosa and liver AspAT, AlaAT and GDH activities in relation to those reported by Bittorf and Jürss (2) for kidney, gill, red muscle, white muscle and heart of FW rainbow trout, it can be seen that there is a great similarity between the proportions in mucosa to gill tissue, and liver to kidney, respectively (Table 5).

A similar situation has been noted in the case of Ictalurus punctatus (42). AspAT activity is much higher than AlaAT activity in the mucosa, and is possibly linked to ion transport in the two organs (gill and mucosa).

Interesting for explanation of the differences in postprandial FAA level increase are probably also the true conditions in the intestinal lumen. Since SW teleosts have to replace water lost due to osmosis by drinking (9,36), one could expect a higher ion content (Na⁺,Cl⁻) as well as a slightly higher pH in the lumen of such animals. Usher et al. (37) indeed found a significant higher pH in the midgut

TABLE 5. Proportions of Maximum Activities of AspAT, AlaAT and GDH in Various Tissues of Rainbow Trout

	AspAT	:	AlaAT	:	GDH
Mucosa	1.0	:	0.37	:	1.71
Gill	1.0	:	0.22	:	1.55
Liver	1.0	:	2.50	:	6.87
Kidney	1.0	:	2.09	:	4.86

Values for gill and kidney calculated from results of Bittorf and Jürss (2).

of SW acclimated Atlantic salmon. According to Shehadeh and Gordon (35) the concentration of Na⁺ decreases with increasing milieu salinity in the midgut, while it rises in the plasma. This might also be a result of the higher Na⁺/K⁺ATPase activity. In addition, Usher et al. (37) found that proteolytic enzymes in SW and FW salmon intestines have identical pH optima (~pH 9). Thus, conditions in SW trout are closer to the optimum. Although the pH of the stomach is not different, that of the intestine is more alkaline, probably due to higher bicarbonate secretion in SW acclimated salmon (37).

In a short-term experiment, Dabrowski et al. (8) always found higher FAA concentrations in the gut lumen of SW than FW trout. Thus, one reason for the higher postprandial levels of some FAA in mucosa tissue of SW rainbow trout are, very likely, higher FAA concentrations in the apical part of the enterocytes. A further reason might be a limited release into the blood.

LIVER. The liver is regarded to be the main organ of AA homeostasis (14). The results presented here show that postprandial concentrations of FAA in the liver remain relatively stable and do not follow the increase measured in the mucosa. Nor do they mirror those as reported by Nose (27) and Murai et al. (25) in the blood of trout or carp (28). Similar observations were made by Walton and Wilson (4) for trout. Only Arg showed a large increase. The drop in levels of several FAA (Leu, Val, Ala, Glu, Gly, Pro) 30 min after feeding might serve as an indication of increased protein synthesis induced by feeding and the arrival of absorbed dietary FAA (4,21).

The quantitative order of the FAA in liver tissue is:

Tau $\gg$ Glu $>$ Ala $\gg$ Gly
$>$ Pro $>$ Asp, Leu $>$ Arg, Val

The high Glu and Ala levels are similar to results of van der Boon et al. (38) for Carassius auratus and of Wilson and Poe (44) for Ictalurus punctatus. The high activities of GDH and AlaAT measured by Jürss and Nicolai (15) and in this study are corresponding with that. The high Glu concentrations are understandable considering that in the liver of the goldfish (Carassius auratus) Glu is formed rapidly from Ala, Asp, Tyr and the branched chain AA. In addition, Glu is released in larger quantities than any other FAA from nutritional (Table 1) and body proteins (10,45). Glu and Ala are known to be the favoured substrates for oxidation and gluconeogenesis in teleost liver (22,23,26). Additionally, Ala is preferred to glucose for lipogenesis in trout liver (11).

Although the portal vein directly links gut and liver, FAA seem to be taken up selectively during blood circulation. An indication for this is that postprandial FAA levels in the liver do not follow the pattern measured in the mucosa (present study) and in the blood (27). Remarkable in this context is a comparison of our results with those of

Ash et al. (1). These authors determined amino acid profiles relating to blood drawn simultaneously from dorsal aorta and hepatic portal vein and calculated the arterio-portal difference for 3-hr post-fed rainbow trout. The greatest differences were reported for Ala and Gly, which in the mentioned experiment rose strongest 3 hr after feeding in FW trout. As demonstrated by Campbell et al. (3), for Ictalurus punctatus at least for Glu, Gln and Asp transport into the hepatocytes is limited, maybe to avoid a "flooding" by FAA. On the other hand, as indicated by the high activities of GDH, AlaAT and AspAT, FAA undergo a rapid transdeamination. The influence of salinity seems to occur only to the pre-fed levels of Leu and Tau. Although concentration in the liver is lower than in mucosa tissue Tau is similarly important for osmoregulation.

In conclusion, influence of salinity acclimation is greater on the intestinal mucosa than on the liver. The increased capacity of ion transport in the mucosa cells and the changes in the luminal milieu seem to support the resorption of FAA.

References

1. Ash, R.; McLean; Westcott, P.A.B. Arterio-portal differences and net appearance of amino acids in hepatic portal vein blood of the trout (Salmo gairdneri). In: Pauw, N.D.; Jaspers, E.; Ackefors, H.; Wilkins, N. (eds). Aquaculture—A Biotechnology in Progress. Bredene, Belgium: European Aquaculture Society; 1989:801–806.
2. McLean, E.; Bittorf, T.; Jürss, K. Die Aktivitäten der Glutamat-Dehydrogenase, Aspartat-Aminotransferase und der Alanin-Aminotransferase in verschiedenen Organen der Regenbogenforelle (Salmo gairdneri Richardson). WZ Rostock XXXII: 64–67;1983.
3. Campbell, J.W.; Aster, P.L.; Vorhaben, J.E. Mitochondrial ammoniogenesis in liver of the channel catfish Ictalurus punctatus. Am. J. Physiol. 244, (Regulatory Integrative Comp. Physiol. 13): 709–717;1983.
4. Carter, C.G.; He, Z.Y.; Houlihan, D.P.; McCarthy, I.D.; Davidson, I. Effect of feeding on the tissue free amino acid concentrations in rainbow trout (Oncorhynchus mykiss Walbaum) Fish Physiol. Biochem. 10:153–164;1995.
5. Ceriotti, G.A. Microchemical determination of desoxyribonucleic acid. J. Biol. Chem. 198:297–303;1952.
6. Colin, D.A.; Nonnotte G.; Leray, C.; Nonnotte, L. Na⁺ transport and enzyme activities in the intestine of the freshwater and seawater adapted trout (Salmo gairdneri R.). Comp. Biochem. Physiol. 81A:695–698;1985.
7. Dabrowski, K.; Dabrowska, H. Digestion of protein by rainbow trout and absorption of amino acids within alimentary tract. Comp. Biochem. Physiol. 69A:99–111;1981.
8. Dabrowski, K.; Leray, C.; Nonnotte, G.; Colin, D.A. Protein digestion and ion concentrations in rainbow trout (Salmo gairdneri R.) digestive tract in sea- and freshwater. Comp. Biochem. Physiol. 83A:27–39;1986.
9. Eddy, F.B. Osmotic and ionic regulation in captive fish with

particular reference to salmonids. Comp. Biochem. Physiol. 73B:125–142;1982.

10. Gatlin, D.M. III Whole-body amino acid composition and comparative aspects of amino acid nutrition of the goldfish, golden shiner and fathead minnow. Aquaculture 60:223–229; 1987.

11. Henderson, R.J.; Sargent, J.R. Lipid biosynthesis in rainbow trout, Salmo gairdneri, fed diets of differing lipid content. Comp. Biochem. Physiol. 69C:31–37;1981.

12. Hørder, M.; Rej, R. Alanine aminotransferase. In: Bergmeyer, O. (ed). Methods of Enzymatic Analysis, Vol. III, Chapt. 6. Weinheim, Germany: VCH Verlagsgesellschaft mbH; 1981: pp. 444–450.

13. Jürss K.; Bittorf, T.; Vökler, T.; Wacke, R. Influence of nutrition on biochemical sea water adaptation of the rainbow trout (Salmo gairdneri Richardson). Comp. Biochem. Physiol. 75B: 713–717;1983.

14. Jürss, K.; Bastrop, R. Amino acid metabolism in fish. In: Biochemistry and Molecular Biology of Fishes, Vol. 4. Amsterdam: Elsevier; 1995: pp. 159–189.

15. Jürss, K.; Nicolai, B. Biochemische Veränderungen in Leber und Muskel der Regenbogenforelle (Salmo gairdneri) bei Hunger. Zool. Jb. Physiol. 80:101–109;1976.

16. Kaushik, S.J.; Harache, Y.; Luquet, P. Variations in the total free amino acids level in rainbow trout muscle and blood during its adaptation to seawater. Ann. Hydrobiol. 8:145–151; 1977.

17. Kirsch, R.; Humbert, W.; Simonneaux, V. The gut as an osmoregulatory organ: Comparative aspects and special references to fishes. In Gilles R. and Gilles-Baillien M. (eds). Transport processes, Iono- and Osmoregulation. Berlin, Germany: Springer-Verlag. 1985; pp. 265–277.

18. Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275;1951

19. Lyndon, A.R.; Davidson, I.; Houlihan, D.F. Changes in plasma and tissue free amino acid concentrations after feeding in Atlantic cod. Fish Physiol. Biochem. 10:365–375;1993.

20. Marcotte, G.; de la Noüe, J. In vitro intestinal absorption of glycine and L-alanine by rainbow trout, Salmo gairdneri, Richardson. Comp. Biochem. Physiol. 79A:209–213;1984.

21. McMillan, D.N.; Houlihan, D.F. Protein synthesis in trout liver is stimulated by both feeding and fasting. Fish Physiol. Biochem. 10:23–34;1992.

22. Mommsen, T.P. Comparative gluconeogenesis in hepatocytes from salmonid fishes. Can. J. Zool. 64:1110–1115;1986.

23. Moon, T.W.; Walsh, P.J.; Mommsen, T.P. Fish hepatocytes: A model metabolic system. Can. J. Zool. 42(11):1772–1782; 1985.

24. Munro, H.N.; Fleck, A. The determination of nucleic acids. Methods Biochem. Anal. 14:113–176;1966.

25. Murai, T.; Ogata, H.; Hirasawa, Y.; Akiyama, T.; Nose, T. Portal absorption and hepatic uptake of amino acids in rainbow trout force-fed complete diets containing casein or crystalline amino acids. Nippon Suisan Gakkaishi 53 (10):1847–1859;1987.

26. Nagai, M.; Ikeda, S. Carbohydrate metabolism in fish-IV. Effect of dietary composition on metabolism of acetate-U-^{14}C and L-alanine-U-^{14}C in carp. Bull. Japan. Soc. Sci. Fish. 39: 633–643;1973.

27. Nose, T. Changes in pattern of free plasma amino acid in rainbow trout after feeding. Bull. Fresh. Fish. Res. Lab. 22:137–144;1972.

28. Ogata, H. Post-feeding changes in distribution of free amino acids and ammonia in plasma and erythrocytes of carp. Bull. Japan. Soc. Sci. Fish. 51:1705–1711;1985.

29. Phillips, A.M. Nutrition, digestion and energy utilisation. In Hoar W.S. and Randall J.D. (eds). Fish Physiology, Vol. I. New York: Acad. Press; 1969:391–432.

30. Rej, R.; Hørder, M. Aspartate aminotransferase. In Bergmeyer, U. (ed). Methods of Enzymatic Analysis III, Chapt. 6, Weinheim, Germany, VCH Verlagsgesellschaft mbH. 1987: pp. 416–424.

31. Reshkin, S.J.; Vilella, S.; Cassano, G.A.; Ahearn, G. Storelli, C. Basolateral amino acid and glucose transport by the intestine of the teleost, Anguilla anguilla. Comp. Biochem. Physiol. 91A:779–788;1988.

32. Schiedek, D.; Schöttler, U. The influence of freshwater runoff on the population density of juvenile Arenicola marina L. (Polychaeta) In Elliott, M.; Ducrotoy, J. P. (eds). Estuaries and coasts: spatial and temporal intercomparisons. Olsen & Olsen, Fredensborg, Denmark. 1991: pp 195–200.

33. Schlisio, W.; Nicolai, B. Kinetic investigations in the behaviour of free amino acids in the plasma and of two aminotransferases in the liver of rainbow trout (Salmo gairdneri Richardson) after feeding on a synthetic composition containing pure amino acids. Comp. Biochem. Physiol. 59B:373–379;1978.

34. Schmidt, E.; Glutamat-Dehydrogenase, In: Methoden der enzymatischen Analyse I, Chapt. C. Weinheim, Germany; VCH Verlagsgesellschaft mbH; 1974: pp. 689–699.

35. Shehadeh, Z.H.; Gordon, M.S. The role of the intestine in salinity adaptation of the rainbow trout, Salmo gairdneri. Comp. Biochem. Physiol. 30:397–418;1969.

36. Smith, H.W. The absorption and excretion of water and salts by marine teleosts. Am. J. Physiol. 93:480–505;1930.

37. Usher, M.L.; Talbot, C.; Eddy, F.B. Effects of transfer to seawater on digestion and gut function in Atlantic salmon smolts (Salmo salar L.). Aquaculture 90:85–96;1990.

38. van der Boon, J.; van der Thillart, G.E.E.J.M.; Addink, A.D.F. Free amino acid profiles of aerobic (red) and anaerobic (white) skeletal muscle of the cyprinid fish, Carassius auratus L. (goldfish). Comp. Biochem. Physiol. 94A:809–812;1989.

39. Vernier, J.M. Intestine ultrastructure in relation to lipid and protein absorption in teleost fish. In: Animal·Nutrition and Transport Processes. Vol. 5, Chapt. 1. Basel, Switzerland: Karger; 1990: pp. 166–175.

40. Vökler, T.; Jürss, K.; Holtz, M. Na$^+$/K$^-$-ATPase activity in different tissues of the rainbow trout, Salmo gairdneri Richardson. Zool. Jb. Physiol. 91:457–466;1987.

41. Walton, M.J.; Wilson, R.P. Postprandial changes in plasma and liver free amino acids of rainbow trout fed complete diets containing casein. Aquaculture 51:105–115;1986.

42. Wilson, R.P. Nitrogen metabolism in channel catfish, Ictalurus punctatus-I. Tissue distribution of aspartate and alanine aminotransferases and glutamic dehydrogenase. Comp. Biochem. Physiol. 46B:617–624;1973.

43. Wilson, R.P. Amino acids and proteins. In Halver, J. E. (ed). Fish Nutrition, Chapt. 3, Academic Press, London, U.K. 1989: pp. 111–151.

44. Wilson, R.P.; Poe, W.E. Nitrogen metabolism in channel catfish, Ictalurus punctatus-III. Relative pool sizes of free amino acids and related compounds in various tissues of the catfish. Comp. Biochem. Physiol. 48B:545–556;1974.

45. Wilson, R.P.; Cowey, C.B. Amino acid composition of whole body tissue of rainbow trout and Atlantic salmon. Aquaculture 48:373–376;1985.