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Fasting and refeeding cause rapid changes in intestinal tissue mass and digestive enzyme capacities of Atlantic salmon (*Salmo salar* L.)

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Abstract

Fasting and refeeding effects on gastrointestinal morphology and digestive enzyme activities of Atlantic salmon, held in tanks of seawater at 9°C and 31‰ salinity, were addressed in two trials. Trial 1: Fish (mean body mass 1190 g) were fasted for 40 days and intestines sampled at day 0, 2, 4, 11, 19 and 40. Trial 2: Fish (1334 g), fasted for 50 days, were refed and sampled at day 0, 3 and 7. Mass, length, protein, and maltase, lactase, and leucine aminopeptidase (LAP) activities were analyzed for stomach (ST), pyloric caeca (PC), proximal (PI), mid (MI), and distal intestine (DI). PC contributed 50% of gastrointestinal mass and 75% of enzyme capacity. Fasting decreased mass and enzyme capacities by 20–50% within two days, and 40–75% after 40 days. In PC, specific brush border membrane (BBM) maltase activity decreased whereas BBM LAP increased during fasting. Upon refeeding, enzyme capacities were mostly regenerated after one week. The results suggest that refeeding should start slowly with about 25% of estimated feed requirement during the first 3 days, but may then be stepped up rapidly. Investigations of digestive processes of fed fish should only be performed when intestines are feed-filled to avoid bias due to effects of fasting.

Keywords: Starvation; Protein; Brush border membranes; Leucine aminopeptidase; Maltase; Lactase

1. Introduction

Fasting is a situation experienced by many fish species in the wild and seems to be well tolerated by many fish species (Larsson and Lewander, 1973; McLeese and Moon, 1989; Navarro and Gutiérrez, 1995; Olivereau and Olivereau, 1997; Bélanger et al., 2002). The practice of food deprivation in situations of overproduction in the aquaculture industry has therefore been less controversial than it would have been in production of terrestrial animals. Questions regarding the ethical perspectives have arisen, along with more practical questions regarding the best strategy for refeeding. The literature supplies limited and often only circumstantial evidence regarding effects of fasting on digestive capacity. Alkaline phosphatase, localized in the microvilli of the intestinal epithelium, decreased gradually in fasting carp (*Cyprinus carpio*), and after 13 months of fasting the enzyme was no longer histochemically detectable in the tissue (Gas and Noailliac-Depeyre, 1976). Mommsen et al. (2003) observed a very different effect of short-term fasting, with increases in metabolic enzyme activities in the mucosa of the stomach and along the intestinal tract of Nile tilapia (*Oreochromis niloticus*). Long term fasting in Atlantic cod (*Gadus morhua*), however, caused a decrease in metabolic enzyme activities in pyloric caeca and intestine, as well as trypsin activity in pyloric caeca homogenate, which were all largely restored upon refeeding (Bélanger et al., 2002). In Atlantic salmon, information regarding fasting responses in macronutrient digestive capability of the intestinal mucosa is preliminary (Krogdahl et al., 1999) and studies on effects of refeeding are non-existent.

The intestine of the Atlantic salmon may be divided in four, easily distinguishable sections: stomach (ST), proximal intestine (PI) with the pyloric caeca (PC), mid intestine (MI)—starting at the distal-most caecum—and the distal

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intestine (DI)—distinguishing itself by an increased diameter and pronounced circular striation of the mucosa. The relative importance of the different sections for nutrient digestion (Krogdahl et al., 1999) and absorption (Buddington and Diamond, 1987; Krogdahl et al., 1999; Bakke-McKellep et al., 2000; Nordrum et al., 2000a,b) in salmonids has been addressed to some extent in earlier studies, suggesting that the PI is responsible for 1/3 to 2/3 of total amino acid, carbohydrate, and lipid digestion and absorption. Employment of the everted sleeve method has shown highest transporter activities in the PI and PC (Buddington and Diamond, 1987; Bakke-McKellep et al., 2000; Nordrum et al., 2000a).

The current study was conducted to examine the effects of fasting and refeeding on length, mass, protein concentration, and digestive enzyme activity of the different regions of the gastrointestinal tract of Atlantic salmon. Using different biochemical methods, digestive enzymes located in the brush border of the intestinal mucosa were studied, including the disaccharidases maltase and lactase, and the peptidase leucine aminopeptidase (LAP).

2. Materials and methods

Two experiments were conducted, a fasting trial (Trial 1) and a refeeding trial (Trial 2). Both were conducted at AKVAFORSK's research station at Sunndalsøra, Norway. Atlantic salmon (*Salmo salar*) of the Sunndalsøra breed, raised at the research station, were used. The fish had not been previously used in experiments, had been fed according to estimated growth rates from start feeding (Austreng et al., 1987), and held indoors under continuous light. The two trials were conducted in the spring of two consecutive years with fish from two different populations, both starting when the fish had been fed for 25 months.

All fish were kept in tanks with surface area of 1 m^2 and with a water column height of 40 cm. Each tank was supplied with seawater at a constant temperature of 9 °C. Salinity was 31‰. Water was renewed at a rate of 8 L min^{-1}. Water velocity of the tank was not recorded. Oxygen level in the water averaged 10 g L^{-1}, measured in the outlet water of each tank twice during the trial. Sampling took place at the same hour of the day, 1.5 h after feeding on days of feeding.

In Trial 1, 20 fish were allocated to each of six tanks, one tank for each of six sampling times during the fasting period of 40 days. Average body mass was 1190 g (N=120, SD=117 g). In Trial 2, 20 fish were allocated to each of three tanks, one tank for each of three sampling days. Average body mass was 1334 g (N=60, SD=185 g). In Trial 2, fish were fasted for 50 days before the refeeding period of 7 days. In both trials the fish were fed a commercial diet produced by BioMar AS for three weeks prior to fasting. The same type of diet was used in the refeeding period. The diet was based on fishmeal (Norse LT) and capelin oil, and contained 45% crude protein and 32% lipid according to the declaration. Diet was supplied in excess of expected growth according to growth tables (Austreng et al., 1987). Feed intake was recorded during the refeeding period (Helland et al., 1996). Briefly, waste pellets were collected from the water outlet and removed every day. Total dry matter loss was estimated and corrected according to dry matter loss in a sample of the diet kept under similar conditions of running water for the same amount of time. Automatic feeders were used to feed the fish at intervals of 15 min, 24 h a day. Five fish were sampled on day 0, 2, 4, 11, 19 and 40 days of the fasting period in Trial 1. Day 0 was the last day of feeding and sampled fish represents fish in fed state. In Trial 2, six fish were sampled on day 50, 53, and 57, i.e. on day 0, 3, and 7 of the refeeding period. Fish were sampled on day 0 before feeding was started. Our choice of seven days for the refeeding period was based on preliminary results from Trial 1 indicating that the gut responses would take place within a few days.

2.1. Recordings and samples

Sampled fish were sacrificed by a blow to the head, and body mass and length measured. On the first sampling day of the fasting trial, only fish that had feed in both stomach and intestine were sampled. Likewise, on day 53 and 57 (day 3 and 7 of in the refeeding period), only fish that had feed in the stomach and intestine were sampled. The digestive tract and associated organs and tissues were removed from the carcass immediately after the fish were killed. The intestines were freed from the other organs and all visible fat removed. The pyloric caeca (PC) were individually freed from the proximal intestine (PI; section between the stomach's pyloric sphincter and the distal most caecum), counted and total length measured. The remainder of the gastro-intestinal tract was divided in four sections: stomach (ST), proximal intestine (PI), mid intestine (MI: section between the distal most caecum and the increase in diameter indicating start of the distal intestine) and distal intestine (DI: section between the distal end of the MI and anus). All sections, except the PC, were opened longitudinally, rinsed in ice-cold saline and gently blotted dry. Lengths of each section were recorded immediately. The sections were transferred to tared test tubes, frozen in liquid nitrogen and stored at −80 °C. All samples were weighed in the frozen state upon arrival at the laboratory.

2.2. Analysis of mucosal enzyme activities

Activities of the brush border enzymes leucine aminopeptidase (LAP), maltase and lactase were examined in homogenates of the intestinal tissues. In Trial 1, specific enzyme activities in the brush border membrane (BBM),

prepared according to the method developed by Storelli et al. (1986), were analyzed as well. Homogenates of the intestinal sections were prepared using an Ultra Turrax with sonication at 0 °C after thawing the tissue in ice-cold 2 mM Tris/50mM mannitol (1:20 w/v), pH 7.1, containing phenyl-methyl-sulphonyl fluoride as serine protease inhibitor. Aliquots of homogenates were frozen in liquid nitrogen and stored at −80 °C. Leucine aminopeptidase activity was measured colorimetrically with a kit (Sigma procedure no. 251) using L-leucyl-β-naphthylamide as the substrate. Maltase and lactase activities were analyzed according to the methods described by Dahlquist (1970) using reagent grade maltose and lactose, respectively, as substrates. Incubations were performed at 37 °C. Enzyme activities are expressed as molar substrate hydrolysis per hour for LAP and molar hydrolysis of substrate hydrolyzed per minute for maltase and lactase. Protein concentration of the homogenates was estimated using both BioRad Protein Assay (BioRad Laboratories, Munich, Germany) and the Kjeldahl nitrogen assay (protein = N × 6.25) (Association of Official Chemists, 1990). Both methods were needed as the results were expected to be of interest for both biochemists and nutritionists. BioRad or similar methods are used for biochemical parameters whereas Kjeldahl is standard in nutritional research. The two methods were expected to give different results, as their principles of analysis are different.

The enzyme activities measured in the intestinal tissue homogenates, which were made from the total tissue of the indicated intestinal section, are given both as specific activities in homogenates and total tissue activity calculated as follows:

$$\text{Specific activity} = \frac{\text{Enzyme activity(U/mL)}}{\text{protein(mg/mL)}}$$

$$\text{Total activity} = \text{Enzyme activity(U/mL)} \times \text{volume(mL)/body mass(kg)}.$$

Specific enzyme activities give an indication of the proportion of enzyme relative to total tissue $\text{protein}_{\text{BioRad}}$. Total activity was considered to give an estimate of enzyme capacity in the given intestinal section.

2.3. Statistical analysis

Analyses of variance and Duncan's multiple range test were used in the evaluation of the results with sampling day as class variables. For the relevant variables, the analysis was run for each intestinal section separately due to significant differences in variances. Transformation of the results to obtain similar variances was not performed as the regions responded differently both to fasting and refeeding and, hence, required separate evaluation. A regression analysis was considered inappropriate, as the models for the effects of fasting and refeeding were not known, nor were the variances of the variables. Evaluation of the results must therefore be based on visual observations of the trends in the variables along the time axis as illustrated in figures in combinations with estimates of standard errors and significances within sampling times and sections. As the two trials were conducted on fish from different populations, in the spring of two consecutive years, some variation in absolute values was expected. Correlation analysis was carried out to compare the results of Kjeldahl and BioRad methods for protein analysis. The software package SAS (Release 8.02 TS Level 02MO) was used in the evaluation. Differences between means were considered statistically significant when $p < 0.05$.

3. Results

3.1. Fish in the fed state

Pyloric caeca number averaged 52 (SD = 5) in the sampled fish. The ratio of intestinal length to body length, including all the PC, was 5.5 (SD = 0.33). In the fed state, Day 0 of Trial 1, the PC region was the dominating structure of the gastrointestinal tract regarding length, mass (Table 1; Fig. 1), and total maltase and LAP activity (Table 2; Fig. 1). The other intestinal regions were comparatively short and had higher mass per unit length (Table 1). LAP and maltase activities were below detection limit in the stomach tissue. LAP activities were at least three times higher than maltase activities throughout the intestine (Tables 2–4). The capacity of peptide and maltose hydrolysis appeared as high in the DI (Table 4) as in MI (Table 3) and PI (data not shown). Tissue protein content of the different intestinal regions, analyzed as Kjeldahl nitrogen × 6.25, did not differ significantly between the intestinal sections, and averaged 15.2% for fish in the fed state (Tables 2–4). Protein measured as Kjeldahl nitrogen × 6.25 showed low correlation with protein measured with BioRad kit:

$$\text{Protein}_{\text{BioRad}} = 0.2464\ \text{Protein}_{\text{N}\times 6.25} + 2.5395;$$

with $R^2 = 0.1682$. Within the range observed in the present study, BioRad gave lower estimates of protein concentration than the Kjeldahl method. In the present work $\text{Protein}_{\text{N}\times 6.25}$ was used for estimation of nutritionally relevant parameters, whereas $\text{Protein}_{\text{BioRad}}$ is used for estimation of specific enzyme activities as previously used by our lab when reporting enzyme activity (Krogdahl et al., 1999, 2003).

3.2. Effects of fasting and refeeding

Body mass, averaging 1190 g (SD = 120), and lengths, averaging 44.5 cm (SD = 1.8), were not affected significantly either by fasting or refeeding. However, the detection limit for changes was about 10% of both total body mass and body length.

Table 1
Results of morphometric analysis of the various regions of the gastrointestinal tract

	Unit[#]	Trial 1: Days of fasting[##]						Significance	Trial 2: Days of refeeding[###]			Significance
		0	2	4	11	19	40	ANOVA	0	3	7	ANOVA
Stomach												
Mass	g kg^{-1}	4.7^{a}	4.1^{b}	4.3^{b}	4.3^{a,b}	4.4^{a,b}	4.2^{b}	0.0343	4.1	4.4	4.7	0.3953
Length	cm kg^{-1}	12.0^{a}	11.6^{a,b}	10.9^{a,b}	11.0^{a,b}	11.9^{a}	10.4^{b}	0.0386	9.7	10.6	10.1	0.1588
Pyloric caeca												
Mass	g kg^{-1}	13.0^{a}	9.5^{b}	10.0^{b}	8.3^{c}	7.6^{c,d}	6.6^{d}	<0.0001	7.6^{b}	12.3^{a}	11.9^{a}	0.0002
Length	cm kg^{-1}	183^{a}	164^{a,b}	168^{a,b}	158^{b}	156^{b}	145^{b}	0.0260	176	206	193	0.2178
Proximal intestine												
Mass	g kg^{-1}	2.2^{a}	2.1^{a}	2.1^{a}	1.8^{b}	1.5^{b}	1.6^{b}	<0.0001	2.6	2.2	2.0	0.7528
Length	cm kg^{-1}	6.3	6.2	6.8	6.1	6.1	6.2	0.4618	5.6	5.4	6.0	0.4512
Mid intestine												
Mass	g kg^{-1}	2.1^{a}	1.7^{a,b}	1.7^{a,b}	1.6^{a,b}	1.6^{b}	1.3^{b}	0.0183	1.1^{b}	1.4^{a}	1.6^{a}	0.0025
Length	cm kg^{-1}	10.8	11.1	10.4	9.7	11.0	9.1	0.3369	9.4	9.4	8.0	0.2592
Distal intestine												
Mass	g kg^{-1}	3.8^{a}	2.9^{b,c}	3.2^{b}	2.7^{c,d}	2.5^{c,d}	2.1^{d}	<0.0001	1.7^{c}	2.0^{b}	2.5^{a}	0.0005
Length	cm kg^{-1}	7.0	6.7	5.9	6.2	6.6	6.8	0.8575	4.2	5.1	5.6	0.1517

Results with the same letter within each row and trial are not significantly different.
[#]kg=kg body mass; [##]Day 0=fed state; [###]Day 0=fasted state.

During the refeeding, average feed intake on day 1 to 7 was: 9.7, 6.3, 2.0, 2.9, 3.7, 4.8 and 6.0 g kg^{-1} fish, respectively. Expected feed intake for the fish size and water temperature in question under normal conditions was 8 g per day (Austreng et al., 1987). In other words, after a consumption above expected intake the first day, the fish reduced intake the next two days before a gradual increase took place reaching 75% of expected intake at day 7.

Results from both trials regarding morphometric measurements of the gastrointestinal tract are given in Table 1, whereas protein and enzyme activities for PC, MI, and DI are given in Tables 2–4, respectively. To clarify the trends in development of the variables with fasting and refeeding, some results from both trials regarding PC and DI, expressed as percentages of the values observed at Day 0 of Trial 1 (fed state), are presented in Figs. 2–4. Data are not shown for the PI due to high variation in this section, resulting in few parameters reaching statistical significance ($p<0.05$). The reason for the high variation was most likely of technical nature resulting from the dissection of the attached PC. The results from the PI, however, showed similar trends following fasting and refeeding observed in the other intestinal regions. Generally, total PI enzyme activities were <10% of those reported for PC.

Results of analysis of lactase activity, only measured in Trial 1 (Tables 2–4, Fig. 3), showed great variation in all tissues expect the DI. The employed experimental procedure did not appear to be suitable for study of lactase activity in the more proximal tissues. Therefore, only lactase results regarding the DI will be discussed herein.

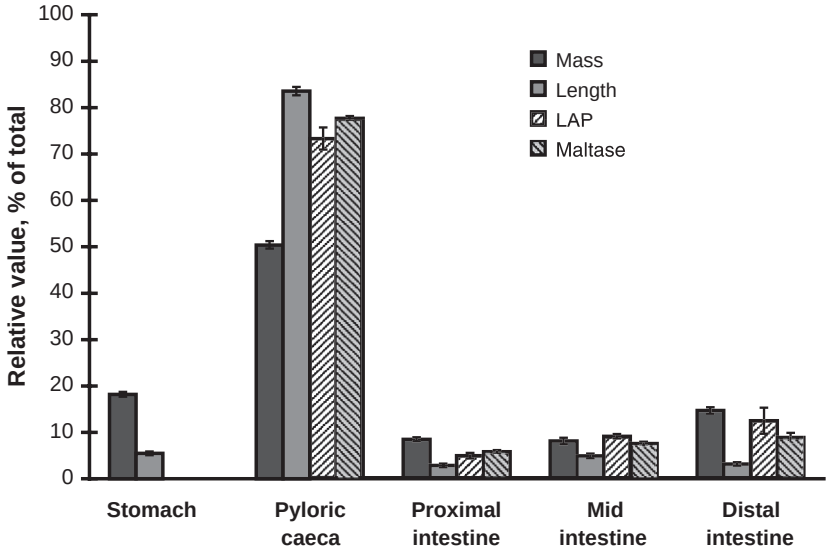

Fig. 1. Relative contribution of the gastrointestinal regions regarding mass, length, and total capacity of leucine aminopeptidase (LAP) and maltase in Atlantic salmon in the fed state, i.e. at Day 0 of Trial 1. Unit: % of total intestine. Bars indicate ±SEM.

Table 2
Effect of fasting and refeeding on protein$_{N \times 6.25}$ and enzyme activities in the pyloric caeca (PC)

	Units[#]	Trial 1: Days of fasting[##]						Significance	Trial 2: Days of refeeding[###]			Significance
		0	2	4	11	19	40	ANOVA	0	3	7	ANOVA
Protein	%	16.4	13.8	14.6	14.5	14.0	15.4	0.7112	12.1^{b}	12.4^{b}	14.5^{a}	0.0290
Total protein	g kg^{-1}	2.1^{a}	$1.3^{b,c}$	1.5^{b}	$1.2^{b,c}$	1.1^{c}	1.0^{c}	0.0019	0.9^{b}	1.5^{a}	1.7^{a}	<0.0001
Total enzyme capacity												
LAP[####]	mmol h^{-1} kg^{-1}	8.8^{a}	$6.0^{b,c}$	6.2^{b}	$4.4^{c,d}$	4.0^{d}	2.3^{e}	<0.0001	1.6^{b}	3.0^{b}	7.7^{a}	<0.0001
Maltase	μmol min^{-1} kg^{-1}	48^{a}	26^{b}	$25^{b,c}$	$16^{b,c,d}$	$14^{c,d}$	11^{d}	<0.0001	10^{b}	34^{a}	34^{a}	<0.0001
Lactase	μmol min^{-1} kg^{-1}	0.2	0.3	0.4	0.3	0.6	0.4	0.2620	–	–	–	–
Specific enzyme activity												
LAP	μmol h^{-1} mg^{-1}	8^{a}	8^{a}	6^{b}	6^{b}	5^{b}	4^{b}	0.0004	4.5	5.1	6.0	0.4562
Maltase	nmol min^{-1} mg^{-1}	42^{a}	36^{a}	22^{b}	20^{b}	18^{b}	21^{b}	0.0024	22^{b}	48^{a}	24^{b}	0.0041
Lactase	nmol min^{-1} mg^{-1}	0.2^{c}	0.5^{b}	$0.3^{b,c}$	0.4^{b}	0.8^{a}	0.7^{a}	0.0002	–	–	–	–
BBM[#####] enzyme activity												
LAP	μmol h^{-1} mg^{-1}	74^{b}	98^{a}	98^{a}	91^{ab}	101^{a}	$91^{a,b}$	0.0778	–	–	–	–
Maltase	nmol min^{-1} mg^{-1}	380^{a}	196^{b}	191^{b}	155^{b}	302^{a}	367^{a}	<0.0001	–	–	–	–
Lactase	nmol min^{-1} mg^{-1}	0.9	0.9	0.8	1.0	1.0	0.9	0.8101	–	–	–	–
Enzyme purification[######]												
LAP		10^{d}	$13^{c,d}$	$17^{b,c}$	$17^{b,c}$	$19^{a,b}$	24^{a}	0.0002	–	–	–	–
Maltase		9^{b}	6^{c}	9^{b}	$8^{c,b}$	17^{a}	17^{a}	<0.0001	–	–	–	–
Lactase		5^{a}	2^{b}	3^{b}	3^{b}	1^{b}	1^{b}	0.0051	–	–	–	–

See Materials and methods for calculations, definitions, and unit explanations of total and specific enzyme activities.
* Results with the same letter within row and trial are not significantly different.
[#]kg=kg body weight; mg=mg protein$_{BioRad}$.
[##]Day 0=fed state.
[###]Day 0=fasted state.
[####]LAP=leucine aminopeptidase.
[#####]BBM=brush border membrane.
[######]Specific activity of BBM/specific activity of tissue.

3.2.1. Tissue mass

Onset of fasting initiated a rapid decrease in tissue mass of the gastrointestinal sections (Table 1, Fig. 2). All sections seemed to respond similarly. However, the PC showed the most pronounced effects with a 25% reduction in mass of during the first two days. After the initial two days of fasting, the decrease in intestinal tissue mass slowed down, but continued throughout the fasting period. 40 days of fasting caused reductions of 45–50%. The mass of the ST, however, did not change after the initial drop. Upon refeeding, mass of the intestinal tissues increased steeply and the PC regained most of its mass, relative to the value observed in the fed

Table 3
Effect of fasting and refeeding on protein$_{N \times 6.25}$ and enzyme activities in the mid intestine (MI)

	Units[#]	Trial 1: Days of fasting[##]						Significance	Trial 2: Days of refeeding[###]			Significance
		0	2	4	11	19	40	ANOVA	0	3	7	ANOVA
Protein	%	15.5	11.4	11.2	12.7	8.3	13.7	0.5906	10.7^{b}	11.0^{b}	14.9^{a}	0.0004
Total protein	g kg^{-1}	0.33^{a}	$0.19^{b,c}$	$0.19^{b,c}$	0.20^{b}	0.14^{c}	$0.17^{b,c}$	0.0700	0.12^{b}	0.16^{b}	0.24^{a}	<0.0001
Total enzyme capacity												
LAP[####]	mmol h^{-1} kg^{-1}	1.1^{a}	$0.8^{a,b}$	1.0^{a}	$0.7^{b,c}$	$0.6^{b,c}$	0.5^{c}	0.0002	1.1	1.6	1.4	0.9574
Maltase	μmol min^{-1} kg^{-1}	4.7^{a}	$3.0^{b,c}$	$3.6^{a,b}$	$2.3^{b,c}$	2.1^{c}	1.5^{c}	0.0004	0.5^{b}	0.8^{a}	0.6^{b}	0.0077
Lactase	μmol min^{-1} kg^{-1}	0.08^{b}	$0.10^{a,b}$	0.16^{a}	$0.10^{a,b}$	0.07^{b}	0.07^{b}	0.0333	–	–	–	–
Specific enzyme activity												
LAP	μmol h^{-1} mg^{-1}	7.0^{a}	7.0^{a}	$5.8^{a,b}$	$5.9^{a,b}$	$6.1^{a,b}$	5.5^{b}	0.0004	5.0	4.2	3.5	0.1887
Maltase	nmol min^{-1} mg^{-1}	32^{a}	$25^{a,b}$	20^{b}	19^{b}	21^{b}	19^{b}	0.0194	$18^{a,b}$	22^{a}	12^{b}	0.0160
Lactase	nmol min^{-1} mg^{-1}	0.5	0.9	0.9	0.8	0.8	0.9	0.1457	–	–	–	–
BBM[#####] enzyme activity												
LAP	μmol h^{-1} mg^{-1}	65^{a}	$44^{a,b}$	$49^{a,b}$	39^{b}	$47^{a,b}$	35^{b}	0.0652	–	–	–	–
Maltase	nmol min^{-1} mg^{-1}	310^{a}	65^{b}	79^{b}	66^{b}	167^{b}	164^{b}	0.0014	–	–	–	–
Lactase	nmol min^{-1} mg^{-1}	1.0	0.7	1.1	0.8	1.0	1.1	0.2776	–	–	–	–
Enzyme purification[######]												
LAP		10	6	9	7	8	5	0.2074	–	–	–	–
Maltase		9^{a}	3^{b}	4^{b}	4^{b}	8^{a}	9^{a}	0.0002	–	–	–	–
Lactase		2^{a}	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	0.0804	–	–	–	–

See Table 2 for the significance of symbols in this table.

Table 4
Effect of fasting and refeeding on protein$_{N \times 6.25}$ and enzyme activities in the distal intestine (DI)

	Units[#]	Trial 1: Days of fasting[##]						Significance	Trial 2: Days of refeeding[###]			Significance
		0	2	4	11	19	40	ANOVA	0	3	7	ANOVA
Protein	%	13.2	12.5	11.6	10.9	11.6	12.2	0.8691	12.1^{b}	12.3^{b}	14.3^{a}	0.0091
Total protein	g kg^{-1}	0.51^{a}	0.36^{b}	0.37^{b}	$0.29^{b,c}$	$0.28^{b,c}$	0.26^{c}	0.0011	0.20^{b}	0.25^{b}	0.35^{a}	<0.0001
Total enzyme capacity												
LAP[####]	mmol h^{-1} kg^{-1}	1.5^{a}	$1.2^{a,b}$	$1.3^{a,b}$	$0.8^{b,c}$	$0.7^{b,c}$	0.5^{c}	0.0165	0.6^{b}	0.7^{b}	2.0^{a}	<0.0001
Maltase	μmol min^{-1} kg^{-1}	5.5^{a}	3.3^{b}	3.3^{b}	1.7^{c}	2.0^{c}	1.3^{c}	<0.0001	1.3^{b}	2.8^{b}	6.7^{a}	0.0006
Lactase	μmol min^{-1} kg^{-1}	0.7^{a}	$0.4^{a,b}$	0.3^{b}	0.2^{b}	0.1^{b}	0.2^{b}	0.0331	–	–	–	–
Specific enzyme activity												
LAP	μmol h^{-1} mg^{-1}	7.6^{a}	5.8^{b}	$4.8^{b,c}$	$4.0^{b,c}$	$4.1^{b,c}$	3.3^{c}	<0.0001	5.5^{b}	5.0^{b}	11.3^{a}	0.0001
Maltase	nmol min^{-1} mg^{-1}	22^{a}	$16^{a,b}$	$12^{b,c}$	9^{c}	$11^{b,c}$	8^{c}	0.0005	11^{b}	18^{b}	31^{a}	0.0003
Lactase	nmol min^{-1} mg^{-1}	2.8^{a}	$1.8^{a,b}$	0.9^{b}	1.1^{b}	0.8^{b}	1.2^{b}	0.0720	–	–	–	–
BBM[#####] enzyme activity												
LAP	μmol h^{-1} mg^{-1}	56^{a}	$46^{a,b}$	$44^{a,b,c}$	$31^{c,d}$	$33^{b,c,d}$	29^{d}	0.0011	–	–	–	–
Maltase	nmol min^{-1} mg^{-1}	179^{a}	$68^{c,d}$	$75^{c,d}$	43^{d}	$121^{b,c}$	$129^{a,b}$	<0.0001	–	–	–	–
Lactase	nmol min^{-1} mg^{-1}	4.7^{a}	1.1^{b}	1.1^{b}	0.8^{b}	0.9^{b}	0.6^{b}	<0.0001	–	–	–	–
Enzyme purification[######]												
LAP		8	8	9	8	8	8	0.6610	–	–	–	–
Maltase		9^{bc}	4^{d}	6^{cd}	5^{d}	12^{b}	15^{a}	<0.0001	–	–	–	–
Lactase		2^{a}	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	0.0169	–	–	–	–

See Table 2 for the significance of symbols in this table.

state (Day 0 of Trial 1), within the three first days (Table 1; Fig. 2). The other tissue masses developed more slowly.

3.2.2. Tissue length

The section lengths (Table 1) were less affected by fasting than mass, and only the stomach and PC of the fish showed significant changes. PC length decreased 10% the first two days and 21% during the whole fasting period. Stomach length decreased more slowly, showing a 13% decrease during the course of the fasting period. During refeeding, the tissue lengths increased in most tissues, but the changes were highly variable and did not reach significance (Table 1).

3.2.3. Intestinal protein

The results from both trials are given for each section in Tables 2–4 and Fig. 2 (PC and DI only). As the variance of protein concentration for the intestinal sections were quite similar, a statistical evaluation was performed on the total data set, as well as for the individual sections. The protein concentrations averaged over the whole experimental period for PC: 14.6%^{a}, PI: 13.0%^{b}, MI: 11.9%^{b}, and DI: 11.9%^{b} (numbers with different letters are significantly different). Fasting affected protein concentration significantly ($p<0.0001$): the average of all sections on Day 0 was 15.2%^{a}, Day 2: 12.2%^{b,c}, Day 4: 12.4%^{b,c}, Day 11: 13.1%^{b}, Day 19: 11.2%^{c}, and Day 40: 13.9%^{a,b}. Adding up the absolute amounts of protein in all the intestinal sections showed a decrease from 3.3 to 1.7 g kg^{-1} fish during the fasting period and 75% of the reduction took place the first two days. Upon refeeding, total protein increased to 70–80%, depending on intestinal region, relative to the fed state after 7 days (Table 2–4, Fig. 2). No clear trend was apparent in PI.

3.2.4. Enzyme activities

Rapid decreases in total activity (relative to body weight) of the investigated enzymes were observed during the initial days of fasting (Tables 2–4; Figs. 2–4). The decrease in total enzyme capacity of PC during the two first days was close to 40% for both maltase and LAP, after which the decline was slower but continued throughout the fasting period, reaching a 70–80% total reduction. Upon refeeding, immediate and rapid increases in total enzyme activities took place. Maltase activity in PC seemed to level off following 3 days, reaching 70% of the fed state values in 7 days of refeeding. For LAP, however, activity in PC seemed still to be increasing at day 7 when it had attained nearly 90% of the fed state value. In DI the changes in enzyme activities upon refeeding seemed initially slower than in the more proximal PC, and had not reached stable levels within the observation period. The levels were however, for maltase 120% and for LAP over 130% of fed state values.

Specific activity (relative to tissue protein) of maltase decreased rapidly during the first four days of fasting in all intestinal sections (Tables 2–4). Thereafter the levels remained relatively constant. Upon refeeding, the specific maltase activity in PC and MI showed a transient peak at 3 days, decreasing again over the next few days to levels similar to the fasted state (Tables 2–3). Specific maltase activity in DI (Table 4) showed a different pattern during the refeeding period with a steady increasing trend in activity to a value about 40% above the value observed in the fed state.

Specific LAP activity in PC and MI did not change significantly the first two days of the fasting period (Tables 2–3), whereas in the DI a significant 25% reduction was observed (Table 4). Between day 2 and 4, the activity decreased 25% in the PC and 17% in the MI and DI. Thereafter, the decreases progressed more slowly with total

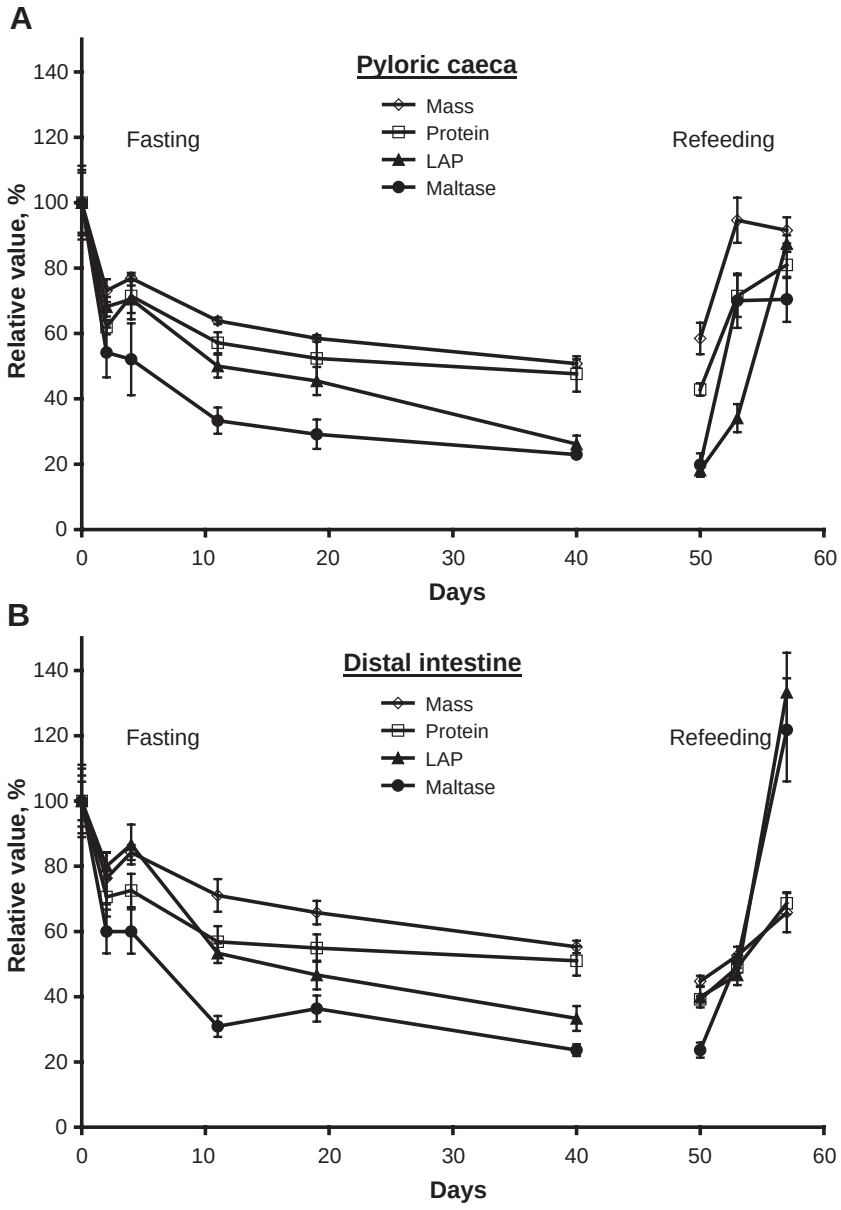

Fig. 2. Relative mass, protein content, and total leucine aminopeptidase (LAP) and maltase capacity of pyloric caeca (A) and distal intestine (B) as a function of time during fasting and refeeding. Unit: % of section value in the fed state at Day 0. Bars indicate ±SEM.

declines of between 20% and 55% depending on region. In the refeeding period, specific LAP activity did not change markedly during the first two days in any tissue. Thereafter, the activity increased rapidly in the DI, in which the activity at 7 days of refeeding was more than twice the activity at 0 and 3 days and about 50% higher than the values observed in the fed fish. A comparison of effects of fasting and refeeding (Tables 2–4) on specific activity of LAP and maltase strongly indicates that the LAP activity reacted more slowly to fasting and refeeding than maltase.

In the DI, total and specific lactase decreased rapidly the first four days of fasting and seemed to stabilize near the value observed at day 4 (Table 4; Fig. 3). In the more proximal regions, lactase activities tended to increase during the fasting period (Tables 2–3).

3.2.5. Specific activities of brush border membranes (Trial 1 only)

In isolated brush border membranes (BBM), specific LAP activity showed different developments in the various intestinal sections (Tables 2–4; Fig. 4A). In PC the activity increased rapidly the first two days of fasting and remained elevated. A similar trend was observed for PI, although the effect was not significant (data not shown). The more distal regions showed decreasing activities until day 11 at which the activities seemed to stabilize. Specific BBM maltase activity decreased rapidly the first two days in the range of 50% to 80% in the various tissues (Tables 2–4; Fig. 4B). The activities were fairly stable between 2 and 11 days of the fasting period after which they increased towards the values observed in fed individuals. Specific lactase activity

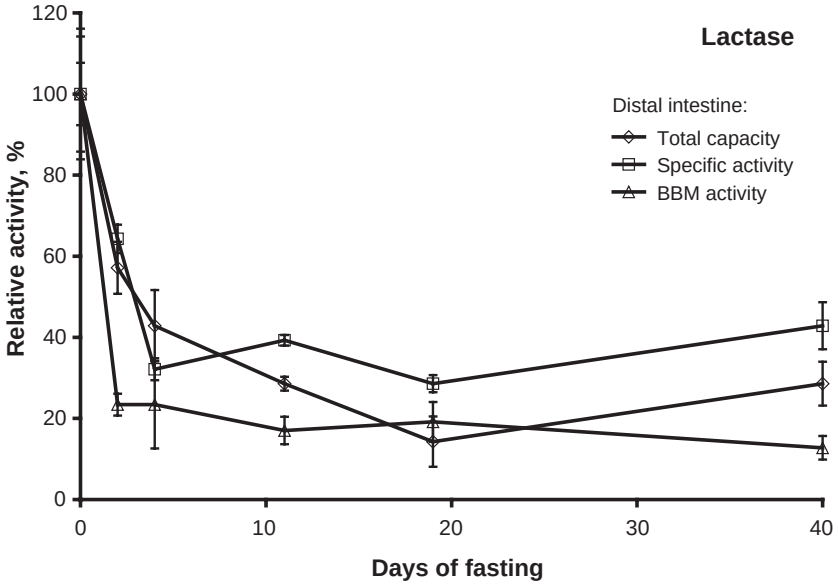

Fig. 3. Relative total capacity, specific activity, and brush border membrane (BBM) activity of lactase in the distal intestine as a function of time during fasting. Unit: % of section value in the fed state at Day 0. Bars indicate ±SEM.

in BBM from distal intestinal tissue decreased about 80% during the first two days of fasting (Table 4; Fig 3). Thereafter specific lactase activity did not change markedly. In more proximal tissues, no significant changes were observed (Tables 2–3).

An evaluation of the degree of purification of LAP and maltase in the tissue homogenates relative to BBM (Tables 2–4) showed similarities between the tissues in fed fish. During the course of the fasting period, however, purification of LAP in MI and DI remained fairly constant, whereas it increased in PC and PI. For maltase activity the patterns of purification were quite similar for all sections, but did not follow the pattern of LAP in any of the sections during fasting. After a decreasing trend during the first 11 days, it doubled towards the end of the fasting period. The different patterns of purification of the two enzymes activities, both considered to be BBM bound, indicate other cellular locations of the two enzymes besides the BBM. Recovery for maltase averaged 36.4% (SD=13.5), for LAP 46.7% (SD=11.2). Purification of lactase (Tables 2–4) from PC tissue was highest. For samples taken during fasting, however, purification of lactase in the BBM isolation procedure was very low for all intestinal sections. Recovery for lactase was low, on average 11.3% (SD=15.1).

4. Discussion

Fasting and refeeding affected enzyme activities, mass, and protein content of the intestinal sections in similar patterns — a rapid decrease the first two days of fasting and rapid increase when feed was made available. The developments in PC were, in general, somewhat ahead of that in the DI, a phenomenon most likely due to differences in evacuation and appearance of digesta in the these regions, and an indication that nutrients in the intestinal lumen act as signals for tissue regeneration. This has been shown in the Burmese python (*Python molurus*), a reptile that naturally undergoes periods of fasting (Secor et al., 2000, 2002). The rapid reductions in all intestinal sections in the first two days after food was withdrawn was, however, surprisingly fast since “half time” for food to pass the intestinal tract of Atlantic salmon from the last feeding is approximately 18 h (Storebakken et al., 1999). The absence of feeding seemed to cause an immediate mobilization of protein resources from the intestine, presumably for systemic use. Protein losses from the intestine during the first two days of fasting, 1.2 g/kg of fish, represents about 0.7% of total body protein, assuming a value around 18% for total body protein (Nordrum et al., 2000b). This rapid protein degradation in the intestinal tract is possibly due to rapid protein degradation (Houlihan et al., 1988) as well as decreased fractional rate of protein synthesis (McMillan and Houlihan, 1989). Previous studies indicate higher protein degradation in intestinal tissue than other tissues in well-nourished fish during early phases of fasting (Theilacker, 1978; Weatherley and Gill, 1981; Houlihan et al., 1988). As fasting continued, however, an apparent shift occurred and intestinal wasting slowed down. Protein degradation in other tissues, especially white muscle apparently increases at this time to provide amino acids for vital body functions (reviewed by Navarro and Gutierrez, 1995). The rate changes indicate that shifts from luminal signals to hormonal and other regulatory pathways take place. From reviews of studies in reptiles and mammals, it is clear that gastrointestinal hormones can act as growth hormones in intestinal tissue (Karasov and Diamond, 1983; Walsh, 1994; Secor et al., 2000). The regulatory processes involved in intestinal changes during

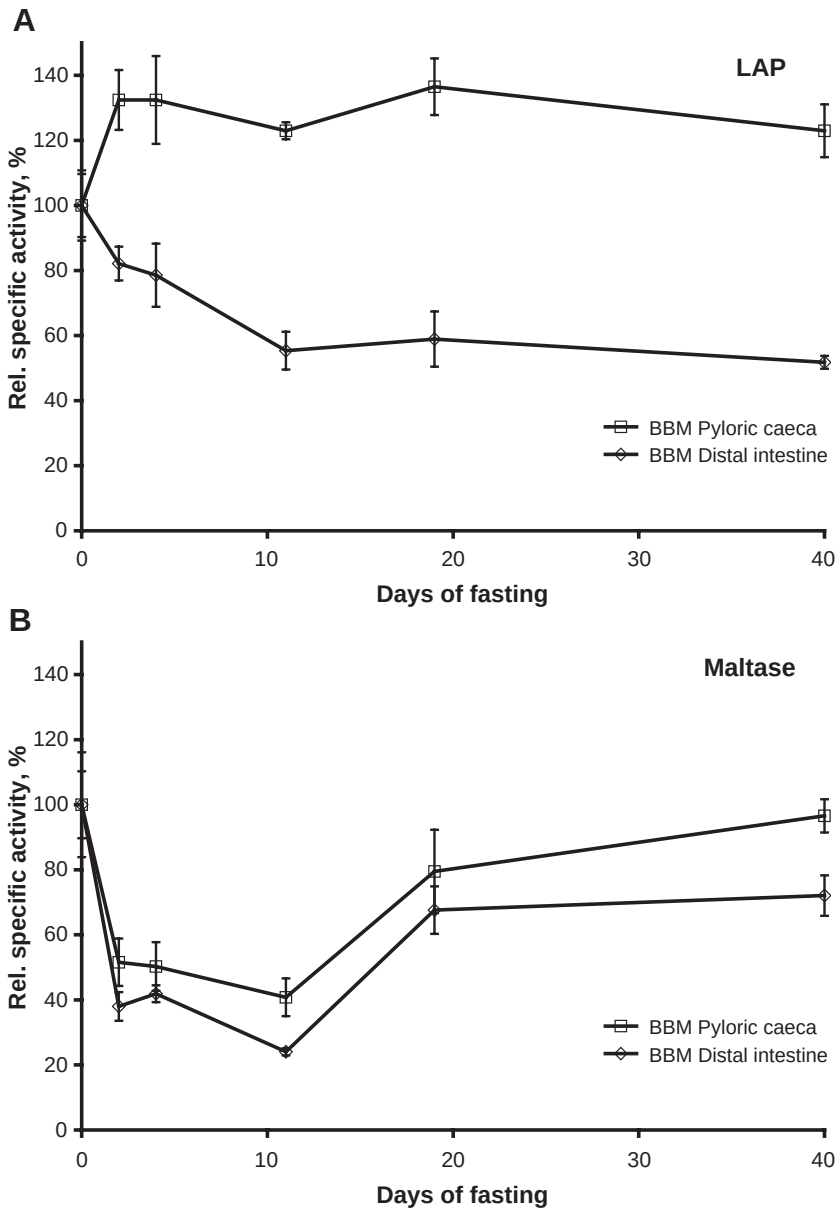

Fig. 4. Specific brush border membrane (BBM) leucine aminopeptidase (LAP; A) and maltase (B) activity of pyloric caeca and distal intestine as a function of time during fasting. Unit: % of section value in the fed state at Day 0. Bars indicate ±SEM.

fasting and refeeding are only poorly understood in other animals and barely studied in fish. We may, however, conclude that the high degree of complexity and fine-tuning involved demands precise concerted actions between all elements, and cannot be understood until knowledge of the regulation of digestive processes is elevated substantially.

Fasting fish for periods of 1–2 months had little effect on body mass and length, as observed by others (Foster and Moon, 1991; Navarro et al., 1993; Bélanger et al., 2002). Replacement of lipid with water is an explanation for the stability of fish mass during fasting. Actual energy losses during the fasting period can give an estimate of maintenance requirement for energy. Few estimates for fish have been presented in the literature and none for Atlantic salmon. Estimates for red drum, *Sciaenops ocellatus* (McGoogan and Gatlin, 1998), and yellowtail, *Seriola quinqueradiata* (Watanabe et al., 2000), indicate values in the range of 60 kJ kg^{-1} body mass per day. Using this value for our salmon, which most likely is too high because of the higher ambient water temperature red drum and yellowtail are exposed to compared to Atlantic salmon, gives an estimated requirement during the fasting period of 40 days of 2.4 MJ kg^{-1}. The estimate represents the energy content of about 80 g of adipose tissue, an amount close to "detection limit" of most fish experiments with fish of the size of the present salmon. In our experiment the least significant differences was about 100 g, 10% of body mass. A similar "detection limit" was observed in a study with brown trout (Navarro et al., 1993) in which a 50 day fasting period caused an insignificant reduction in body mass from 135 to 121 g. The energy

expenditure was, estimated as above, about 300 kJ, corresponding to 10 g of adipose tissue.

Regarding effects of fasting on intestinal morphology, the shortening of PC is comparable to results from studies of the intestines of carp (Gas and Noailliac-Depeyre, 1976) and Red Sea surgeonfish *Arcanthurus nigrofuscus* (Montgomery and Pollak, 1988). In a number of salmonid species, fasting causes alterations in the absorptive cell structure, characterized by reductions in cell height, amount of cytoplasm, RNA in cytoplasm, and a disturbance of regular arrangement of nuclei (Vasil'yeva and Korovina, 1969; Baeverfjord and Krogdahl, 1996). In the DI of fasting Atlantic salmon histological changes were perceptible at two days, prominent after 7 days, and included indentations in the simple folds and transformation of the supranuclear cytoplasm from distinctly vacuolated to finely granular (Baeverfjord and Krogdahl, 1996). Reductions in gut length, surface area, and cell height have been described for *Pomacentrus coelisetis*, a marine aquarium fish (Hall and Bellwood, 1995). A similar study with winter flounder (*Pseudopleuronectes americanus*) during natural summer feeding and winter fasting indicate that fasting may not affect the microstructure of absorptive cells equally in all species (McLeese and Moon, 1989). However, when atrophy does occur, a concomitant reduction in mass and protein in the tissue would be expected. Although apparently never directly measured in fish, the microvilli of the Burmese python shorten many-fold and cell proliferation apparently decreases during fasting (Secor et al., 2000). This was correlated to lower brush border nutrient transport. A similar link is feasible for brush border enzyme activities.

Thus, changes in total enzyme activities may be the result of a combination of changes in tissue mass, cell numbers, morphology, and enzyme synthesis in the absorptive cells. Specific enzyme activities in tissue homogenate and in isolated BBM may throw light on the events taking place in the intestinal cells during fasting. The development in specific enzyme activities in tissue homogenate largely followed the development seen in total activities for both maltase and LAP during fasting, whereas protein level in the tissues generally decreased less. Hence, it appears as if the digestive enzymes were sacrificed faster than other proteins. BBM maltase and LAP developed differently and events differed in the DI and more proximal intestinal sections. This indicates that protein degradation does not occur uniformly but rather follows different strategies. Similar findings have been reported for hepatic enzymes in fasting yellow perch, *Perca flavescens* (Foster and Moon, 1991). Metabolic enzyme activities in the gastrointestinal tract of tilapia increased following short term (5 day) fasting (Mommsen et al., 2003). Thus it is tempting to speculate that although digestive enzyme activities of the enterocytes rapidly decrease with the onset of fasting, enzymes involved in metabolic activity may remain intact and even slightly elevated, although in the long term these may also eventually dwindle (Bélanger et al., 2002).

The specific activities for maltase along the intestinal tract of fed and fasted fish in the present study were similar to our preliminary observations (Krogdahl et al., 1999). After an initial sharp decrease in the MI maltase following 5 days of fasting in the earlier study, an increase to an intermediate level was observed that did not vary significantly during the rest of the fasting period. This type of fluctuation was not observed during the first 11 days of fasting in the current study, although the total reduction in specific maltase activity following 40 days of fasting was similar to the reduction after 60 days reported in Krogdahl et al. (1999).

Despite variable feed intake, refeeding caused an increase in tissue mass, protein content, and enzyme activities of a similar rapidity as the response to fasting, possibly due to a combination of increased cellular proliferation (Secor et al., 2000) and high rates of protein synthesis and low protein degradation (Houlihan et al., 1988). In rainbow trout (*Oncorhynchus mykiss*) force-fed a meal after a 6-day fasting period, fractional rates of protein synthesis in the intestine more than doubled 3 h after feeding and were brought about by an increase in protein synthesis per unit of RNA (McMillan and Houlihan, 1989). The results indicate that the intestine of fish have resting capacity for protein synthesis that can be mobilized within hours when feed becomes available.

At the end of the refeeding period, values well above fed state values were observed for enzyme activities in DI. This may indicate that enzyme regeneration in this part of the intestine is given priority in a refeeding situation. Also, comparatively low digestibility of the feed in the more proximal regions, due to low enzyme activities, may cause the digesta that reached the distal intestine to contain higher levels of digestible nutrients, serving as additional luminal signals for regeneration. Overcompensation of various parameters upon refeeding has been observed in rainbow trout, *Salmo gairdneri* Richardson, (Weatherley and Gill, 1981) and Atlantic cod (Bélanger et al., 2002) and been related to compensatory growth.

The data confirms that the pyloric caeca are quantitatively the most important part of the gastrointestinal tract regarding nutrient digestion in salmonids (Buddington and Diamond, 1987; Krogdahl et al., 1999; Bakke-McKellep et al., 2000). Both morphometrically and enzymatically these appendages stand out. The ratio of intestinal length to body length puts the Atlantic salmon in the same group as other carnivores, such as cat and mink.

5. Conclusions

Fasting caused substantial rapid decreases in tissue mass, protein, and enzyme capacities within two days. Cell concentration and distribution of maltase, lactase, and leucine aminopeptidase seemed to be regulated separately. Upon refeeding, regeneration of both mass and enzyme activities also occurred rapidly. Taking the observed feed

intake into account, refeeding after fasting should start with less than maximal feeding rate, such as 25% of estimated feed intake for the first 3 days, which may be increased rapidly by 25% increments each subsequent day to normal levels by the end of the first week. Another important conclusion that may be drawn is that when conducting studies on intestinal parameters for fed fish, samples should be taken only from fish in the fed state with intestines filled with chyme.

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