Influence of feeding on protein metabolism in atlantic salmon (Salmo salar)

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Influence of Feeding on Protein Metabolism in Atlantic Salmon (*Salmo salar*)

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ABSTRACT

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Two experiments were conducted to study the post-prandial changes in protein synthesis rate of different tissues in juvenile Atlantic salmon after an i.v. injection of a large dose (1.5 μmole/g) of L-[2,3-^{3}H]-leucine. In the first experiment, the changes in protein synthesis rate of the muscle and scales were followed over a period of 18 h after a meal, in salmon (mean body weight 43.4 g) starved for 1 week. The rate of protein synthesis in muscle increased until 18 h after feeding. No significant changes were observed in the protein synthesis rate of the scales. In the second experiment, the salmon (mean body weight 36.8 g) were regularly fed on a commercial diet. Immediate analysis of protein metabolism (2 h after the morning meal) as compared with analysis after an overnight fast showed no significant effect of feeding on the protein synthesis rate in liver, gill, digestive tract and muscle.

INTRODUCTION

Protein growth is under the control of two processes: protein synthesis and protein deposition (Fauconneau, 1985; Houlihan et al., 1986). It has been suggested that the utilization of amino acids for protein synthesis is much more dependent on dietary supply in fish than in mammals (Cowey and Luquet, 1983). An explanation can be found in the stimulatory effect of feeding on protein metabolism in fish as proposed by Jobling (1981). But such an effect has never been studied, and in rainbow trout, for instance, protein metabolism has been analysed either a few hours after feeding (2 to 6 h) (Fauconneau and Arnal, 1985; Fauconneau et al., 1989) or 12 and 24 h after the last meal (Smith, 1981; Loughna and Goldspink, 1984; Houlihan et al., 1986).

The aim of that work was to investigate the post-prandial changes of protein synthesis in some tissues of fish. The tissues studied were involved either in post-prandial metabolism of amino acids (liver, digestive tract) or in growth

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(muscle and scales). Protein synthesis was measured after injection of a large dose of L-[2,3-^{3}H]-leucine which was used to stabilize the specific activities of free leucine in the different pools (Garlick et al., 1980).

This experiment, carried out with Atlantic salmon (*Salmo salar*) parr, was part of a work on changes in protein synthesis during smoltification.

MATERIAL AND METHODS

Two experiments were conducted on 1-year Atlantic salmon which originated from the River Nivelle (S.W. France) and were reared for 8 months in an inland experimental hatchery (I.N.R.A., Donzacq, France) at a constant temperature of 17°C. In the first experiment 30 fish (mean body weight 43.4 g) were starved for 1 week. On the day of the experiment, 12 fish were selected according to their body weight and they were fed a single meal of a commercial diet (0.84 g/fish). At times 0, 3, 9 and 18 h after feeding, three fish received a single injection of L-[2,3-^{3}H]-leucine (1.5 μmole/g, 0.4 μCi/g) in the caudal vein. They were killed 30 min after the injection. The blood was collected by puncture in the caudal vein. A sample of 1 g of scale was collected by scraping off the skin and the mucus was removed from that sample by drying scales on absorbant paper. Muscle samples were collected from the epaxial musculature. All of the brain was also removed. The samples of tissues were weighed and frozen in liquid nitrogen, then stored at −20°C until analysis. The brain of three other control starved fish was also collected.

In the second experiment a batch of 500 Atlantic salmon was regularly fed two meals per day. In that group, 24 fish were selected (mean body weight 38.4 g). Twelve fish were studied after an overnight fast (18 h after the last meal) while twelve others were studied 2 h after the morning meal. They received a single injection of L-[2,3-^{3}H]-leucine (1.5 μmole/g, 0.4 μCi/g) in the caudal vein. Then they were killed 10 and 30 min after the injection. The blood was withdrawn, centrifuged and the plasma collected. The following tissues were removed, rinsed when necessary, weighed and frozen in liquid nitrogen: liver, digestive tract, kidney, gill, whole skeletal muscle and remains.

Tissue treatment and analysis

Samples of tissues were treated to extract free amino acids and protein (Fauconneau and Arnal, 1985). Protein was measured according to Lowry et al. (1951). Pooled samples of protein extract of each tissue were hydrolysed (HCl 6 *N*, 125°C, 24 h). Amino acids were measured in amino acid extract and hydrolysate after ortho-phtal-aldehyde (OPA) derivatization and HPLC separation (Vista 5000, Varian, France) on a C18 column (MicroPack, Varian, France), by fluorimetry (Hogan et al., 1982). Effluents from the spectrometer were collected and fractionated. The radioactivity corresponding to leucine

was measured by liquid scintillation counting (βMatic II, Kontron, France) to calculate the specific activity of leucine. The free amino acid content of the brain was also measured using the same procedure.

Protein synthetic rates (ks in % per day) were calculated from the specific activity of leucine in the free pool of the tissue (Sa) and in the protein (Sb) according to the following equation (Attaix et al., 1987):

$$Sb = ks \cdot Sa \cdot t + Cte$$

where t is the time after the injection and Cte the constant term of the regression. In the gill, liver and digestive tract only preliminary results for free leucine specific activity were available and protein synthesis rates were calculated from plasma specific activity. The significance of the differences between the slopes was tested using a covariance analysis (Snedecor and Cochran, 1971).

RESULTS AND DISCUSSION

Effects of injection of a large dose of leucine (1.5 µmole/g)

After administration of the large dose, the amount of leucine in the plasma was eight to ten times higher and in the muscle two times higher than the normal level (Fig. 1). This has already been observed in fish (Pocrnjic et al., 1983). No differences were observed in fed and fasted fish. In the brain of starved or refed fish which received a large dose of leucine, the level of leucine was respectively 50 and 200% higher than in the control starved fish (Table 1). In treated fish the level of all the other amino acids of the brain was 50% higher than that of the control. This was observed for taurine, GABA and for

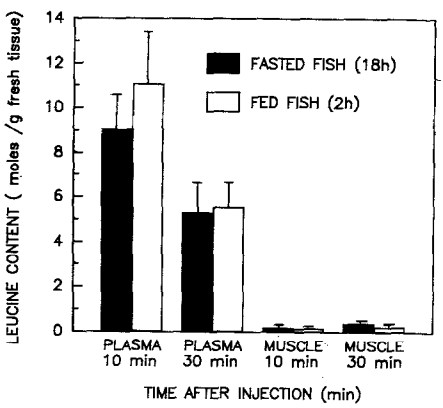

Fig. 1. Free leucine content in plasma and muscle of Atlantic salmon (*Salmo salar*) after injection of a large dose of leucine (1.5 µmole/g BW) in the caudal vein.

TABLE 1

Free amino acids in the brain of Atlantic salmon starved or refed with a single meal (in μmole/g). Fish received a single i.v. injection of a large dose of ^{3}H-L-leucine (1.5 mmole/kg)

	Control starved	Injected with ^{3}H-leucine	
		Starved	Refed
Total amino acids (without taurine)[a]	42.6	64.7	67.9
Taurine	20.7	32.8	35.4
Gamma-amino-butyric acid	5.6	7.8	7.7
Total essential amino acids	13.9	22.6	25.6
Leucine	1.07	1.54	2.92

[a]OPA-reactive compounds.
Measurements were made on pooled sample of three brains in each group.

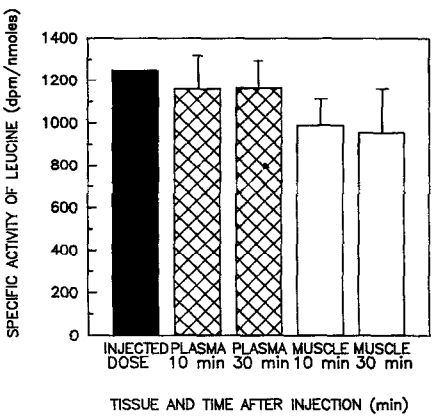

Fig. 2. Specific radioactivity of free leucine in plasma and muscle of Atlantic salmon (*Salmo salar*) after injection of a large dose of labelled (^{3}H) leucine (1.5 μmole/g BW) in the caudal vein.

total essential amino acids. Few studies have reported the levels of amino acids in the brains of fish (Fontaine and Marchelidon, 1971) and some studies have shown an increase in brain amino acids in the post-prandial phase (Dabrowski, 1982). The consequence of an increase in brain free amino acids on metabolism is not known.

Although not significant ($P > 0.05$), the specific activity of leucine in the muscle was slightly lower than that in the plasma. The leucine levels in both tissues were close to the injected dose (Fig. 2). The intake of amino acid in muscle is very low. It is considered to be a limiting factor for the application of the large dose method in fish (Loughna and Goldspink, 1985). A difference of 20% between plasma and muscle specific activity of the injected amino acid has been observed (Pocrnjic et al., 1983). In our experiment such a difference

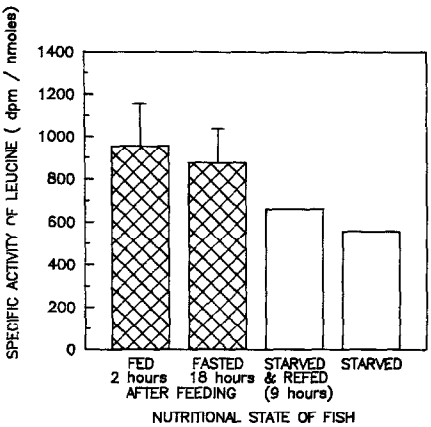

Fig. 3. Specific radioactivity of free leucine in muscle of fed (2 h and 18 h after feeding) and 1-week-starved (starved and refed) Atlantic salmon (*Salmon salar*) after injection of a large dose of labelled leucine (1.5 μmole/g BW).

was not seen. Therefore, an accurate measurement of protein synthesis rate in muscle was possible.

In fact, it was observed that the specific radioactivity of free leucine in muscle was dependent on the nutritional status of fish (Fig. 3). It was higher in fish 2 h after feeding and lower in starved fish. It is suspected that labelled leucine was diluted by leucine coming from protein breakdown (Fauconneau, 1985) in starved and overnight-fasted fish.

Effect of feeding on protein synthesis

There is a significant increase in the rate of protein synthesis of muscle in starved and refed fish (Table 2). This was observed at 9 h after the meal. Such stimulation of protein synthesis of muscle could be related mainly to an increase in protein accretion of muscle, taking into account the strong relationship between protein growth and protein synthesis in that tissue (Smith, 1981; Houlihan et al., 1986). In scales of refed fish an increase of protein synthesis rate was also observed 9 and 18 h after the meal, as compared with starved fish, but it was not significant. The rate of protein synthesis in scales measured in vitro (Smith, 1986) was ten times lower than that observed in our experiment. Large daily variations of protein synthesis rate of scales have been observed in vitro (Smith, 1986) and have been related to daily increment of protein in scale and thus to daily variation of fish growth. In fact, it seems that the growth response of scales was delayed as compared to that of muscle.

In regularly fed fish analysed either 2 h or 18 h after feeding, significant differences were not observed in the rate of protein synthesis of muscle, liver, gill and digestive tract (Table 3). The fractional protein synthesis rate seemed

TABLE 2

Fractional protein synthesis rate (%/day) in muscle and scale of salmon starved for 1 week and refed with a single meal

Hours after single meal	Muscle	Scale
0	0.18	8.89
	(0.08)	(5.00)
2	0.22	8.13
	(0.11)	(1.17)
9	0.38	9.86
	(0.13)	(3.46)
18	0.41	11.49
	(0.25)	(2.96)
Analysis of variance: significance	$P<0.05$	$P<0.10$

( ): Standard deviation.

TABLE 3

Influence of feeding on fractional protein synthesis rate in Atlantic salmon fasted (18 h after last meal) or fed (2 h after meal) in %/day (mean body weight 33.8 g, mean specific growth rate 1.06 %/day)

	Muscle	Liver	Gill	Digestive tract
Fasted fish	0.24	7.84	5.83	2.43
($n=12$)	(0.06)	(0.53)	(0.04)	(0.08)
Fed fish	0.19	8.71	5.92	3.99
($n=12$)	(0.04)	(1.30)	(0.07)	(1.15)

( ): Standard deviation.

to be higher in liver and digestive tract of recently fed fish (2 h post-prandial) as compared to that of overnight-fasted fish (18 h post-prandial). However, the differences were not significant ($P>0.05$). The rate of protein synthesis in muscle was, however, very low when compared to the specific growth rate of the group of fish (1.06%/day) during that time. If the treated fish and its muscle were supposed to grow at the same rate as that of other fish of the group, it means indirectly that protein synthesis rate of muscle expressed its minimum level at the maximum time of fasting (18 h) over a 24-h period. It means, also, that large daily variation of protein synthesis rate and of protein accretion in muscle could be observed with a maximum which could take place at another time of the day: 9 to 12 h after feeding. No information is available on daily variation of protein synthesis. Such large changes in protein synthesis in muscle have been observed during starvation (Smith, 1981) but they are not very

rapid (Loughna and Goldspink, 1984). If a daily variation of protein accretion in muscle could be confirmed, it would strengthen the idea of a close relationship between protein synthesis and amino acid supply.

CONCLUSIONS

The protein synthesis rate of different tissues is not significantly affected by immediate feeding. A stimulation of protein synthesis rate in liver and digestive tract in the post-prandial period is not excluded. Protein synthesis rate in muscle showed daily variations in relation to feeding. The maximum rate of protein deposition in muscle is supposed to take place around 9 h after feeding.

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