ELSEVIER

Comparative Biochemistry and Physiology Part A 137 (2004) 75–85

CBP

www.elsevier.com/locate/cbpa

Protein growth rate in rainbow trout (*Oncorhynchus mykiss*) is negatively correlated to liver 20S proteasome activity

A. Dobly, S.A.M. Martin*, S.C. Blaney, D.F. Houlihan

School of Biological Sciences, University of Aberdeen, Tillydrone Avenue, Aberdeen AB24 2TZ, UK

Received 22 May 2003; received in revised form 25 August 2003; accepted 4 September 2003

Abstract

The efficiency with which fish and other animals add and maintain body proteins is a balance between synthesis of proteins and their degradation. In fish that have similar food consumption and protein synthesis rates, a greater ratio of synthesis to degradation would be expected to produce more efficient conversion of food into growth. In addition, we hypothesised that high activities of the proteasome, a major pathway of protein degradation, would be negatively correlated with growth rate. In order to test this hypothesis we maintained rainbow trout for 62 days, during which repeat measurements of food consumption and growth were made. We selected fish for high and low growth efficiencies. Protein degradation was estimated from the difference between protein synthesis (determined by ^{15}N flux) and protein growth. We found that protein synthesis rates were significantly higher in the low growth efficiency group, as were estimated protein degradation rates. In another group of fish that also did not differ in food consumption, the activity of the proteasome in the liver, but not in the muscle, was negatively correlated with growth rates. These two experiments showed that high proteasome activity is linked to decreased growth efficiency.

Keywords: Efficiency of protein conversion; Growth; *Oncorhynchus mykiss*; Proteasome; Protein synthesis; Protein degradation; Protein turnover; Rainbow trout

1. Introduction

In salmonid fish, individuals exhibit large differences in food consumption (Jobling et al., 1989; McCarthy et al., 1993). This is partly due to the hierarchy that develops in a group of fish; when the animals are fed in a group, dominant individuals gain a higher share of the food provided than subordinate animals (McCarthy et al., 1992). Food consumption stimulates the synthesis of new proteins and also to a lesser extent, protein degradation (Houlihan et al., 1988). However, fish with similar food consumption and similar protein synthesis rates, may exhibit different efficiencies with which they deposit synthesised protein as growth (Carter et al., 1993; McCarthy et al., 1994). Hence, as protein growth results from the difference between protein synthesis and protein degradation, it has been hypothesised that protein degradation rates play a key role in regulating protein growth (Houlihan et al., 1995; Carter and Houlihan, 2001). These analyses of protein metabolism may be important for aquaculture as they reveal the mechanisms that determined the efficiency with which food is converted into growth.

Usually an indirect approach has been taken to estimate protein degradation rates in whole animals

*Corresponding author. Tel.: +44-1224-27-28-67; fax: +44-1224-27-28-36.

E-mail address: sam.martin@abdn.ac.uk (S.A.M. Martin).

1095-6433/04/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpb.2003.09.002

Table 1
Protein metabolism

	High efficiency fish	Low efficiency fish	*P*
Weight (g)	305±21	256±16	NS
Long-term food intake (g per fish per day)	4.6±0.1	3.3±0.7	NS
Long-term growth efficiency ($k_g/k_c \times 100$)	45.6±4.0	31.0±2.7	≤0.05
k_g (% of protein in fish per day)	1.61±0.14	1.00±0.03	≤0.05
k_c (% of protein in fish per day)	4.31±0.37	3.53±0.48	NS
k_s (% of protein in fish per day)	4.06±0.38	7.14±1.05	≤0.05
k_d (% of protein in fish per day)	2.45±0.60	6.14±1.00	≤0.05
Growth efficiency ($k_g/k_c \times 100$)	38.1±6.4	29.0±2.4	≤0.05
Deposition efficiency ($k_g/k_s \times 100$)	41.2±8.7	14.4±1.5	≤0.05

Long-term food intake and growth efficiency are averages of four X-ray measurements between days 23 and 55. Protein intake is on the day of the ^{15}N experiment (day 55); k_c is protein intake, k_s is protein synthesis, k_d is protein degradation and k_g is protein growth. Deposition efficiency is the efficiency of deposition of synthesised proteins. Data are shown as mean and S.E.M. We used one-tailed Mann–Whitney test with $n=6$.

from the difference between protein synthesis and growth (Carter and Houlihan, 2001). This indirect method has been required due to the difficulties in measuring protein degradation rates directly, as this process is carried out by a variety of enzyme pathways (Attaix et al., 1999) and it is not established which ones predominate in fish (Carter and Houlihan, 2001).

Protein degradation is a highly regulated and controlled process (Hershko and Ciechanover 1998). Eukaryotic cells contain two major systems for protein degradation (Attaix et al., 1999). Firstly, the lysosomal system, a membrane bound vacuole that contains acidic proteases, such as capthepsins and other hydrolases, and secondly the ubiquitin-proteasome mediated proteolysis pathway (Cuervo et al., 1995; Tanaka and Chiba, 1998; Pillay et al., 2002).

In rainbow trout the activity of the proteasome in the liver and muscle was found to decrease following two weeks starvation (Martin et al., 2002) most likely as a result of decreased protein turnover and decreased whole animal metabolism. This is in contrast to the case in mammals where proteasome activity increases dramatically following even short periods of starvation (Wing et al., 1995). In trout the activity of lysosomal enzymes, in particular cathepsin D, increase during both starvation (Krupnova et al., 1985; Martin et al., 2001) and following exposure to pollutants (Kaivarainen et al., 1998).

In this paper we have investigated the links between protein metabolism in terms of protein consumption, protein synthesis and growth efficiency. We determined the individual variation in food consumption through X-rays of fish that have eaten food containing known amount of radio-opaque beads (McCarthy et al., 1993). Protein synthesis rates were determined with a ^{15}N technique (Carter et al., 1994). In order to better understand the link between protein anabolism and catabolism, we analysed two ratios: growth compared to synthesis (the efficiency of deposition of synthesised protein) and degradation compared to synthesis. The activity of 20S proteasome was also measured in selected fish to determine if there was any relationship between the activity of proteasome and growth rates.

2. Materials and methods

2.1. Animals

Immature female rainbow trout were maintained in three circular 250 l freshwater tanks of 39, 38 and 38 fish, respectively. The tanks were outdoors with a natural photoperiod varying from 7:45L/16:15D to 12:30L/11:30D in 62 days (from 23/1/2002 until 25/3/2002). Water temperature was regulated at 14.0 °C (±0.2 °C) with pH 7.60 (±0.05) and 90% (±1%) of oxygen saturation. The fish were individually marked on the ventral surface, which enabled recognition of individuals. The initial mean fish wet weight was 34.4 g (±1.0, $n=115$) with no statistical difference between the tanks (1-Way ANOVA, $F_{2,115}=0.57$, $P=0.57$).

2.2. Feeding

Between 09.00 and 10.00 h each day of the feeding trial, the fish were hand fed a commercial

diet ad libitum (3.0 mm, Royale Optima 30, Trouw Ltd, 47% of protein; fed until at least three pellets were left uneaten on the floor of the tank). Feeding did not exceed 2.7% body weight on any one day. Total daily food consumption was monitored for each of the 62 days in each tank. Individual food consumption was recorded five times during the course of the experiment by X-radiography using a Todd Research 80/20 portable X-ray unit as described by Talbot and Higgins (1983), Jørgensen and Jobling (1989) and McCarthy et al. (1993). On days 17, 23, 31, 41 and 55, the fish were fed a diet labelled with 1% radio-opaque ballotini glass beads (400–600 μm, Jencons Scientific Ltd, Leighton Buzzard, UK; 66 beads/g of dry food). As described below, on day 55, the food also contained ^{15}N-labelled proteins. All the food (for X-rays or not) was air-dried until it reached a constant weight. One hour after the end of each feeding session, before weighing and X-rays, fish were anaesthetised with benzocaine (Sigma, 20 mg/l). The individual consumption was expressed as the mean of the X-ray sessions in grams of dry food eaten per gram of wet weight of fish per day.

In order to determine if the X-ray method could be independently verified, the total amount of food consumed by fish in a tank determined by radiography was compared with the amount of food consumed by the whole group from the ad libitum feeding procedure. This accountability of feeding with X-ray is expressed in percent and defined as:

$$\text{Accountability for whole tank} = (\text{radiographic estimate/food consumed from hand-feeding}) \times 100$$

Food consumption of individual animals was calculated from the four X-rays and the accountability was determined from a mean of four X-rays.

Out of the original 115, 86 fish were used for the final analysis. Fish were only included in the analysis if they consumed food on three or more X-ray occasions and if they remained healthy.

2.3. Growth

On every X-ray session, the length and the weight of the fish were measured. The weight of the meal estimated from radiography for that individual fish on any particular day was subtracted from the wet weight. The specific growth rate (SGR, % of body weight increase per day, Ricker, 1979), was calculated from the equation:

$$\text{SGR} = ((\ln \text{W2} - \ln \text{W1})/t) \times 100$$

where W2 is the final weight, W1 is the initial weight and t is time between W1 and W2 in days. We calculated the SGR from growth between the second (day 23) and last X-ray (day 55). The individual protein intake on a given day (k_c, % of protein content per day) is defined as:

$$k_c = (\text{gram of dry food eaten} \times \text{gram of protein per gram of dry food/protein content of fish}) \times 100$$

with gram of protein per gram of dry food being determined as 0.47. An average protein content of the fish of 16.8% was used; it is based on McCarthy et al. (1994) and Carter et al. (1994).

Protein growth efficiency (GE, %) was calculated as:

$$\text{GE} = (k_g/k_c) \times 100$$

with k_g being protein growth calculated as SGR but with protein estimated content and not wet weight (between days 23 and 55) and k_c being the protein intake expressed as a percentage of the protein content of the fish; k_c was estimated as a mean of the four X-rays carried out between days 23 and 55.

The coefficient of variation of food consumption (CV, %) was defined as:

$$\text{CV} = \text{S.D.(food intake)/mean(food intake)} \times 100$$

where S.D.(food intake) is the standard deviation of food intake and the mean food intake is in percent of body weight (McCarthy et al., 1992).

The hierarchy in terms of consumption of food for an individual fish feeding in the group was expressed in percent on a given day as (McCarthy et al. 1999):

$$\text{Food hierarchy} = (F_i/F_a) \times 100$$

with F_i being the grams of food consumed by a given individual and F_a being the average food available to a fish in its tank calculated from the ad libitum feed delivered and the number of fish in the tank. When this index is less than 100 an

individual was eating less than the average amount of food available to each individuals in the group. The index is independent of the number of fish in a tank. It was determined for the four last X-rays. This allowed a comparison between the feeding hierarchy in the three tanks.

We used data from the fourth X-ray in order to select fish for the protein synthesis experiment (see below). All fish were killed on day 62, 24 h following the meal. Their livers were frozen immediately in liquid nitrogen for the proteasome assay.

2.4. Protein metabolism

After the fourth X-ray measurement, 18 trout (chosen from the three tanks) were selected: nine with high efficiency and nine with low efficiency. The following criteria were used in this selection: (1) all 18 fish had similar food consumption values on day 41 (low efficiency fish: $3.09 \pm 0.41\%$ per day, high efficiency fish: $2.57 \pm 0.33\%$ per day, Mann–Whitney U test, $n = 18$, $P > 0.05$); (2) all 18 fish were in similar hierarchical positions on day 41 (food hierarchy, high efficiency: 140.6 ± 27.1, low efficiency: 92.6 ± 11.3, Mann–Whitney U test, $n = 18$, $P > 0.05$); and (3) nine animals with the highest and nine animals with the lowest growth efficiency on day 41 (see definition above, high efficiency: 61.6 ± 5.7, low efficiency: 35.3 ± 4.0). All fish remained as before in their original tank until the fifth X-ray session (on day 55), which followed a meal containing 1% glass beads and 0.5% *Spirulina* sp. proteins containing ^{15}N stable isotope as the labelled protein tracer (Martek Biosciences, Columbia, MD, USA). After the X-ray, the selected trout were placed in groups of three with similar total weight in six 20 l-tanks (three tanks with high growth efficiency fish and three with low efficiency fish). The water flow was stopped and an air-stone provided aeration to each tank for 6 h or 12 h periods. At 6, 12, 24, 36 and 48 h post-feeding, 50-ml and 4-l samples were collected from each tank. The water volume was reduced to approximately 5 l and fresh water flowed through for 10 min to ensure the complete change of water. The volume was readjusted to 20 l. A 50-ml sample of inflowing water was taken at each sampling time to determine ammonia, urea and ^{15}N backgrounds. All water samples were acidified with 2 M HCl to bring the pH below 2 to prevent ammonia loss (Wilkie and Wood, 1991). The samples were maintained at 4 °C until further analysis. The ammonia concentrations at the end of 6 h or 12 h periods did not reach toxic levels, as defined by Wicks and Randall (2002).

Ammonia and urea concentrations were determined in the 50-ml samples by colorimetric assays (Le Corre and Treguer, 1976; Rahmatullah and Boyde, 1980, respectively). Recovery of ^{15}N was carried out by distillation of ammonia in the 4-l sample into 1-M boric acid described by Fraser et al. (1998). The ammonium borate was then freeze-dried (Edwards Super Modulyo Freeze Drier, Edwards, Crawley, UK). Duplicate 25-mg samples of dried ammonium borate were packed in tin capsules for analysis of the enrichment of the samples with ^{15}N compared to natural ^{15}N background on a ANC Robopreprep-CN linked to a tracer mass isotope ratio mass spectrometer. These results were used to calculate the whole animal rates of protein synthesis from the end point stochastic model of Waterlow et al. (1978) as applied to fish by Carter et al. (1994) and Fraser et al. (1998). The proportion of protein mass synthesised per day was determined (k_s, % per day). Protein growth (k_g) was calculated as mentioned above. Whole animal protein degradation rates (k_d per day) were calculated as the difference between k_s and k_g. Efficiency of deposition of synthesised proteins were calculated as $(k_g/k_s) \times 100$ and called deposition efficiency.

2.5. Assay of proteasome activity in-vitro

Twenty fish (from the three tanks) were selected using the following criteria: (1) trout with data available for the five X-ray sessions, (2) animals from a wide range of growth efficiencies (range 38.6%–66.0%), (3) animals with a similar protein consumption calculated from days 16 to 55 ($3.26 \pm 0.14\%$ per day). The animals were killed by approved method, tissues were immediately frozen in liquid nitrogen and stored at -80 °C. Frozen tissue samples were homogenised in lysis buffer (50 mM Tris pH 8.0, 0.1 mM EDTA, 1.0 mM 2β-mercaptoethanol) on ice using a Dounce homogeniser (200 mg tissue to 1 ml buffer) followed by centrifugation at $20\,000 \times g$ for 1 h at 4 °C and the supernatnant retained. The concentration of soluble proteins were determined by Bradford protein assay (Bradford, 1976) using BSA for the standard curve. For the 20S proteosome peptidase activity, the proteasome-specific fluorogenic

substrate LLVT-AMC (Alexis Corporation, Nottingham, UK) was dissolved in DMSO. The final concentration of DMSO in the reaction did not exceed 4%. LLVT-AMC was incubated with tissue cytosolic proteins and SDS, as described by Shibatani and Ward (1995). Briefly, 50 μg protein was incubated with 40 μM fluorogenic substrate in 100 μl 100 mM Tris pH 8.0/0.0475% SDS. The reaction was allowed to proceed for 30 min at 15 °C and stopped by the addition of 0.3 ml 1% SDS and 1 ml 0.1 M sodium borate (pH 9.1). For control reactions protein and all reagents were stopped immediately so as to determine background fluorescence. The release of the fluorogenic reagent AMC (7-amido-4-methylcoumarin) was determined with a fluorimeter with excitation 370 nm and emission 430 nm. Enzyme activity is presented as pmol AMC h^{-1} 50 μg $protein^{-1}$. A standard curve was constructed for each assay with AMC ranging from 0 to 100 pM. Proteasome-specific inhibitor (MG115 and ZLLnV, Sigma C6706) was added to reactions to a final concentration of 50 μM to confirm that LLVT-AMC degradation was proteasome-specific (Tsubuki et al., 1993; Lee and Goldberg, 1998). The 20S proteasome activity was expressed as pmol AMC released min^{-1} 50 μg $protein^{-1}$.

2.6. Statistical analysis

Statistics were carried out using SPSS software (version 11, SPSS UK Ltd, Chertsey, UK). As no distribution was assumed to be normal, we used only non-parametric tests. We used 1-way analysis of variance and Mann–Whitney U test to determine differences between independent variables. Wilcoxon signed rank test was used for dependent variable. The correlations were identified by Spearman method. All tests were two-tailed with a level of significance of 0.05, except the comparison of high and low efficiency fish, which was one-tailed as we anticipated the direction of differences. Unless otherwise indicated mean values ± S.E.M are presented.

3. Results

3.1. Growth and food consumption

We observed no statistical difference between the three tanks in growth rate, mean food consumption determined by visual observation or mean food hierarchy, so data for all tanks were pooled (1-Way ANOVA, growth rate: $F_{2,84}=1.33$, $P=0.27$; mean food: $F_{2,85}=1.14$, $P=0.32$; food hierarchy: $F_{2,88}=0.009$, $P=0.99$). The mean specific growth rate after 62 days was 1.29% per day ± 0.05 ($n=86$, day 23 to 55). Individual SGRs varied from 0.15 to 2.87% per day. Mean food consumption determined by visual observation was not different from consumption of the tank determined by the X-ray method (0.79 ± 0.04 vs. 0.90 ± 0.14 g per day per fish, Mann–Whitney U test, $n=29$ and 4, $P>0.05$)

The mean food consumption determined from the X-rays in percent of wet body weight was 1.21% per day ± 0.04 ($n=86$, minimum: 0.49% per day, maximum: 2.28% per day). The corresponding k_c was 3.39% per day ± 0.11(minimum: 1.37% per day, maximum: 6.39% per day); this was used to calculate growth efficiency. The mean accountability of feeding with X-ray, similar between the three tanks, was 95.3% ± 0.5 of the food seen to be eaten by the fish in the tanks. The coefficient of variation of food consumption (CV) from the X-ray determinations of individual food consumption in three tanks was 42.5% ± 2.1 ($n=86$, minimum: 10.5, maximum: 99.0).

Protein growth efficiency (ratio between protein growth and protein consumption) was highly variable between individuals: the mean ratio was 38.3% ± 1.04 ($n=86$, minimum: 10.8, maximum: 66.0). The 20 fish selected for assays for 20S proteasome activity had a wide range of protein growth efficiencies (from 38.6% to 66.0%) with a very similar k_c for the 10 most efficient fish and the 10 less efficient fish (high efficiency: 3.25% per day ± 0.22, low efficiency: 3.27% per day ± 0.19). The specific growth rate was 1.77% per day ± 0.18 for the high efficient fish and 1.36% per day ± 0.08 for the low efficient fish. At the end of the experiment (day 62), these 20 fish had a weight of 91.2 g ± 6.7.

3.2. Protein metabolism

After a meal containing ^{15}N-labelled proteins, protein synthesis was determined over 48 h for isolated groups of low and high efficiency fish. The average weight of fish groups and the mean food consumption were not significantly different between low and high efficiency fish (Table 1). Nitrogen excretion peaked 24 h following the meal in all tanks and had returned to basal levels of

Table 2
Spearman's rho and *P* from correlations between the main physiological variables

		Specific growth rate	Protein intake (k_c)	Growth efficiency	Proteasome in liver	Proteasome in muscle
Final body weight	rho	0.105	0.208	−0.448	−0.328	−0.272
	P	0.658	0.379	0.048*	0.158	0.245
Specific growth rate	rho		0.501	0.397	−0.507	0.060
	P		0.024*	0.083	0.023*	0.802
Protein intake (k_c)	rho			0.172	−0.476	−0.266
	P			0.469	0.034*	0.257
Growth efficiency	rho				0.184	0.102
	P				0.438	0.668
Proteasome in liver	rho					0.302
	P					0.196

The first line of each variable gives the correlation coefficient, the second line gives the significance. All weights are in g, protein intake is in % of body weight. Proteasome in liver and in muscle are the 20S proteasome activity in these tissues (see Section 2 for the details of calculations). Correlations are 2-tailed Spearman's rho ($N=20$), $*<0.05$.

nitrogen excretion by 48 h following the meal (6.8 ± 1.4 μg N h^{-1} g^{-1}). Percent of total nitrogen excreted as urea was found to be $6.6\%\pm3.0$ for the high efficiency fish and $10.9\%\pm2.0$ for the low efficiency fish (not significantly different: one-tailed Mann–Whitney U test, $n=6$, $P>0.05$). The cumulative excretion of ^{15}N had reached a plateau by 48 h following the meal indicating that the amino acid free pool was depleted of the label.

The values for protein metabolism are shown in Table 1. As fish were chosen with similar protein intakes and different growth efficiencies, the low efficiency fish as expected had a lower growth rate than the high efficiency fish. The synthesis rates and the protein degradation were higher in low efficiency fish than in high efficiency fish (see Table 1). The efficiency of deposition of synthesised protein (k_g/k_s) was higher in high efficiency fish (Table 1). The ratio between protein degradation and synthesis (k_d/k_s) was negatively correlated with the specific growth rate (Spearman's rho, $n=6$, $r=-0.829$, $P=0.042$). The low efficiency fish had a higher mean k_d/k_s ratio than the high efficiency fish (low efficiency fish: 88.7 ± 1.1, high efficiency fish: 67.8 ± 6.8, 1-tailed Mann–Whitney test, $n=6$, $P\leq0.05$).

3.3. Proteasome activity and growth efficiency

A second experiment tested with linear correlations the presence of significant associations between the main physiological variables already obtained (SGR, k_c, GE) and proteasome activity. The 20S proteasome activity was significantly greater in the liver than in the muscle tissue ($n=20$, 42.0 ± 2.77 vs. 24.2 ± 3.75 pmol AMC h^{-1} 50 μg $protein^{-1}$, Wilcoxon Test, $P\leq0.001$). Table 2 shows that there were no correlations between the proteasome activity in the muscle and all the physiological variables tested. Liver proteasome activity was negatively correlated to k_c and to SGR (Fig. 1). SGR was positively correlated to k_c (Fig. 2). Growth efficiency was negatively correlated to final weight.

4. Discussion

In this study, we observed a relatively low mean coefficient of variation in food consumption (42%, range: 10–99%). These values are in agreement with other studies where variation was between 26 and 62% (McCarthy et al., 1992) and 1–62% (Jobling and Baardvik, 1994). It is known that low rations increase the variation (McCarthy et al., 1992; Carter et al., 1996). As in this study the fish were hand-fed until all fish has cessed feeding, the food was probably difficult to defend (Koebele, 1985) and most fish could eat as much as they wanted. This method of feeding is, therefore, probably the reason for the relatively low individual variability (McCarthy et al., 1999).

For both experiments, we chose fish that had high or low efficiency. To avoid effects of protein consumption on protein synthesis, fish were chosen with no significant difference in food consumption as increased protein consumption results in

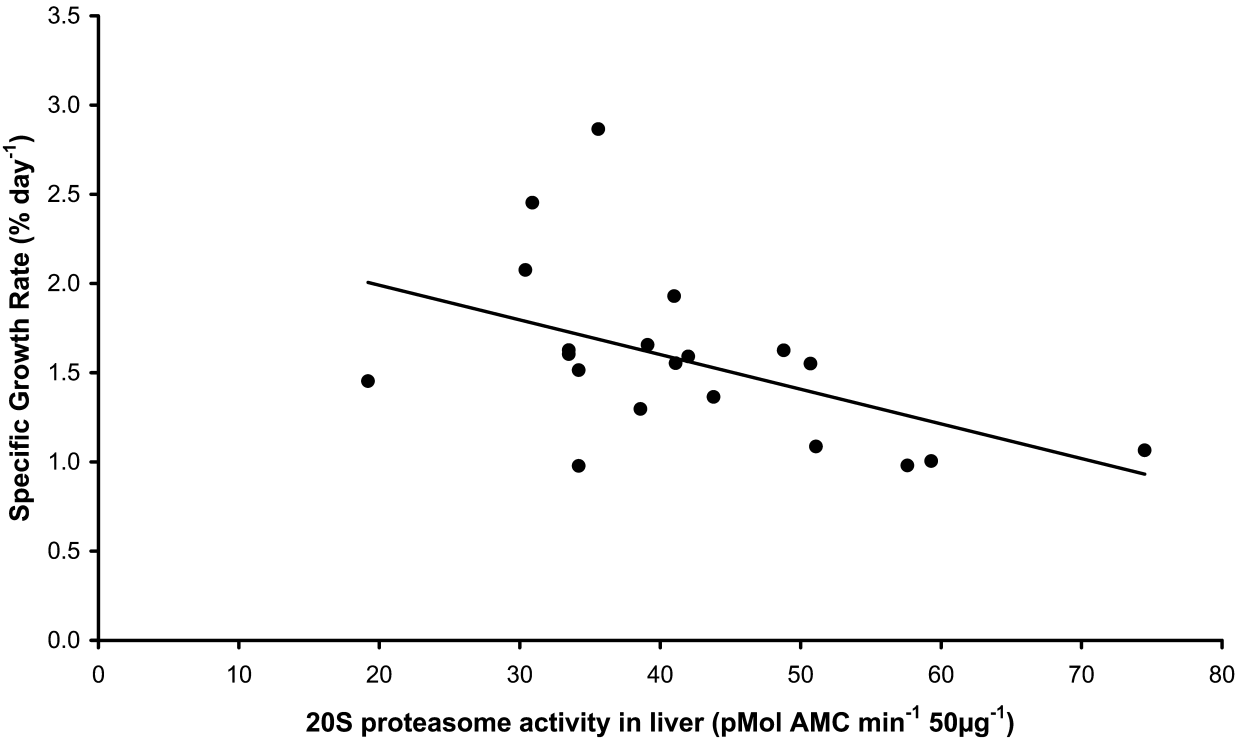

Fig. 1. Correlation between specific growth rate and 20S proteasome activity in liver expressed in pmol AMC released per minute per 50 μg protein ($N=20$, $y=-12.6x+61.6$, $r=0.507$, $P=0.023$).

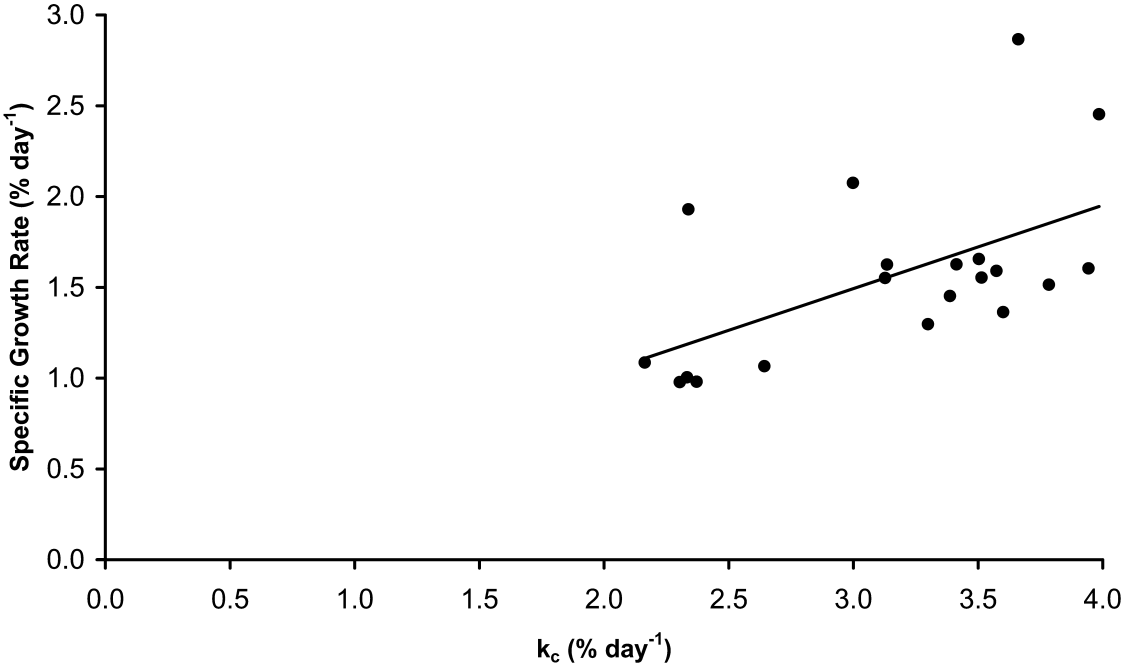

Fig. 2. Correlation between specific growth rate and protein consumption of 20 fish; both measures are expressed as percent of body weight per day ($y=0.46x+0.12$, $r=0.524$, $P=0.018$).

increased protein synthesis in both fish (Houlihan et al., 1989; Carter et al., 1998) and mammals (reviewed in Houlihan et al., 1995). This increase in protein synthesis may be brought about by a result of amino acid and hormonal stimulation (Sugden and Fuller, 1991). The relationship between protein degradation and consumption is less clear, with some studies indicating degradation is stimulated by increased consumption (Houlihan et al., 1989; Millward, 1989) whereas other studies show degradation rates are not linked to ration (McCarthy et al., 1994).

During the protein synthesis experiment, the profile of ammonia excretion showed a peak between 12 and 24 h following the meal, this is later than that reported previously (6–12 h, Gélineau et al., 1998). This may be a reflection of temperature (14 °C in this experiment, 16 °C in Gélineau et al. (1998)). The proportion of digested nitrogen excreted as urea (7% and 11%, respectively, for high and low efficiency fish) is within the normal range for rainbow trout (between 4% and 13%, Dosdat et al., 1996; Médale et al., 1998). The rates of protein synthesis were within the range of published whole animal synthesis rates for salmonid fish (Houlihan et al., 1986; McMillan and Houlihan, 1992; McCarthy et al., 1994).

Protein synthesis rates were significantly greater in the low efficiency groups of fish (Table 1). This result is in contrast to that reported by McCarthy et al. (1994) where a radioactive tracer method was used to measure protein synthesis 24 h after a meal. In that report, rainbow trout with different growth efficiencies had similar protein synthesis rates and hence protein degradation was suggested as being the major controlling factor in growth efficiency. As the method used here integrates protein synthesis over 48 h following a meal, greater reliance can be placed on the results compared with the short-term radioactive method. Using this approach protein synthesis is measured over a short time (1–2 h) and thus the variations in synthesis rates over a full day following feeding are not accounted for. The end product measurement of protein synthesis used here also integrates temporal fluctuations and measurements are taken without disturbing the fish (reviewed by Carter and Houlihan, 2001).

Here we find degradation rates higher in the low efficiency group (6.4% per day) compared to the high efficiency group (2.8% per day, Table 1). Degradation rates published for trout are highly variable; Peragòn et al. (1999) report degradation rates between 3.5% per day for fed fish whilst this increases to 11% per day for trout that have been starved for 70 days. With the flooding dose method, McCarthy et al. (1994) report lower degradation rates in high efficiency fish than in low efficiency fish (1.6 and 2.4% per day).

Protein growth efficiencies for rainbow trout have been reported to be 35–45% (Owen et al., 1999) and 14.4–34.6% (McCarthy et al., 1994), our results for high and low efficient fish are within this range (45.6% and 31.0%, respectively, see Table 1). This study also showed that fish with high protein consumption show a higher growth rate.

In all animals in this study the proteasome activity was greater in liver than in muscle as had been previously described for fish (Martin et al., 2002) and for mammals (Selman et al., 2002). This is likely to reflect the high metabolic activity of the liver. No relationship was found between 20S proteasome activity and growth efficiency in the muscle, however, a significant negative correlation was found between liver 20S activity and growth rate, demonstrating that fast growing animals had lower activity of 20S proteasome in the liver (Table 2). This is of great interest as it could indicate that fast growing animals show lower overall protein turnover in liver. These results parallel the higher deposition efficiency (k_g/k_s) in high efficiency fish. Our assays were performed on tissues extracted 24 h following a meal, which could reflect basal rates of protein degradation. It is possible that the 20S activity varies with time following a meal similar to the synthesis rates. Protein synthesis in both rainbow trout and cod are know to increase following a meal, with maximum rates being observed before 6 h in both species (McMillan and Houlihan, 1989, 1992; Lyndon et al., 1992). A significant negative correlation was also found between 20S proteasome and food consumption. This is unexpected and may be due to temporal changes in proteasome activity 24 h after the meal. Further studies will be required to fully elucidate this.

Both protein synthesis and protein degradation are energy requiring processes, with an estimated 20–40% of oxygen consumption being required for protein synthesis (Houlihan et al., 1988; Houlihan, 1991) and up to 15% of ingested energy may be required for protein degradation (Siems et al., 1984). Hence, efficient fish are using less

energy for the continual turnover of proteins. In chickens selected for high growth efficiency, protein breakdown is significantly reduced in fast growing chickens compared to slower growing chickens (Maruyama et al., 1978; Muramatsu et al., 1987; Flannery and Beynon, 1991). Recent studies on ubiquitin proteasome mediated proteolysis in chickens showed that ubiquitin conjugation was not related to differences in growth rates (Harper et al., 1999). Evidently, caution needs to be taken when synthesis rates and derived degradation rates are measured over two days and the activity of proteasome is taken at a fixed time 24 h following the meal. Turnover of cellular proteins can be extremely rapid. Up to 30% or more of newly synthesised proteins can be degraded almost immediately following ubiquitination (Yewdell et al., 2002). They suggest these proteins are in some way defective and cannot reach their intended conformation or cellular location in a time frame deemed appropriate by the cells.

Efficiency of protein deposition is a key goal in aquaculture and in the underlining regulation of growth in other cultivated animals. In this paper we have demonstrated that fish that are poor in terms of growth efficiency also have higher rates of protein turnover, this high rate of turnover is energy demanding and means poor utilisation of nutrients. In parallel we have shown that the 20S proteasome activity is decreased in livers of fish that have high specific growth rates, which indicates reduced protein degradation by the ubiquitin proteasome pathway.

Acknowledgments

We thank Rosie Morland for her technical help. This work was funded by EU grants PROGRESS (QLRT-2000-00994) and PEPPA (Q5RS-2000-30068).

References

Attaix, D., Combaret, L., Taillandier, D., 1999. Mechanisms and regulation in protein degradation. In: Lobley, G.E. (Ed.), Protein metabolism an nutrition, Proceedings VIII. Purdue University Press, 51–67.

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Carter, C.G., Houlihan, D.F., Buchanan, B., Mitchell, A.I., 1993. Protein-nitrogen flux and protein growth efficiency of individual Atlantic Salmon (*Salmo salar* L.). Fish Physiol. Biochem. 12, 305–315.

Carter, C.G., Owen, S.F., He, Z.-Y., Watt, P.W., Scrimgeour, C., Houlihan, D.F., et al., 1994. Determination of protein synthesis in rainbow trout, *Onchorhynchus mykiss*, using a stable isotope. J. Exp. Biol. 189, 279–284.

Carter, C.G., Purser, G.J., Houlihan, D.F., Thomas, P., 1996. The effect of decreased ration on feeding hierarchies in groups of greenback flounder (*Rhombosolea tapirina*: Teleosti). J. Mar. Biol. Assoc. 76, 505–516.

Carter, C.G., Houlihan, D.F., Owen, S.F., 1998. Protein synthesis, nitrogen excretion and long-term growth of juvenile Pleuronectes flesus. J. Fish Biol. 53, 272–284.

Carter, C.G., Houlihan, D.F., 2001. Protein synthesis. In: Walsh, P.J., Mommsen, (Eds.), Nitrogen Excretion, Vol. 20. Academic Press, New York, pp. 31–75 Fish Physiol.

Cuervo, A.M., Knecht, E., Terlecky, S.R., Dice, J.F., 1995. Activation of a selective pathway of lysosomal proteolysis in rat liver by prolonged starvation. Am. J. Physiol. 269C, 1200–1208.

Dosdat, A., Servais, F., Métailler, R., Huelvan, C., Desbruyères, E., 1996. Comparison of nitrogenous losses in five teleost fish species. Aquaculture 141, 107–127.

Flannery, A.V., Beynon, R.J., 1991. Genetic differences in turnover of glycogen phosphorylase in broiler and layer chickens. Biomed. Biochim. Acta 50, 361–363.

Fraser, K.P.P., Lyndon, A.R., Houlihan, D.F., 1998. Protein synthesis and growth in juvenile Atlantic halibut, *Hippoglossus hippoglossus* (L.): application of 15N stable isotope tracer. Aquac. Res. 29, 289–298.

Gélineau, A., Médale, F., Boujard, T., 1998. Effect of feeding time on postprandial nitrogen excretion and energy expenditure in rainbow trout. J. Fish Biol. 52, 655–664.

Harper, J.M.M., Mee, M.P., Arnold, J.E., Boorman, K.N., Mayer, R.J., Buttery, P.J., 1999. Ibiquitin gene expression and ubiquitin conjugation in chicken muscle do not reflect diiferences in growth rate between broiler and layer birds. J. Anim. Sci. 77, 1702–1709.

Hershko, A., Ciechanover, A., 1998. The ubiquitin system. Annu. Rev. Biochem. 67, 425–479.

Houlihan, D.F., McMillan, D.N., Laurent, P., 1986. Growth rates, protein synthesis and protein degradation in rainbow trout: effects of body size. Physiol. Zool. 59, 482–493.

Houlihan, D.F., Hall, S.J., Gray, C., Noble, B.S., 1988. Growth rates and protein turnover in Atlantic cod, *Gadus morhua*. Can. J. Fish. Aquat. Sci. 45, 951–964.

Houlihan, D.F., Hall, S.J., Gray, C., 1989. Effects of ration on protein turnover in cod. Aquaculture 79, 103–110.

Houlihan, D.F., 1991. Protein turnover in ectoderms and its relationship to energetics. Adv. Comp. Environ. Physiol. 7, 1–44.

Houlihan, D.F., Carter, C.G., McCarthy, I.D., 1995. Protein turnover in animals. In: Wright, P.J., Walsh, P.A. (Eds.), Nitrogen Metabolism and Excretion. CRC Press, Boca Raton, pp. 1–32.

Jobling, M., Baardvik, B.M., Jørgensen, E.H., 1989. Investigations of food-growth relationships of Artic charr, *Salvinus alpinus* L., using radiography. Aquaculture 81, 367–372.

Jobling, M., Baardvik, B.M., 1994. The influence of environmental manipulations on inter-and intra-individual variation

in food acquisition and growth performance of Arctic charr, *Salvelinus alpinus*. J. Fish Biol. 44, 1069–1087.

Jørgensen, E.H., Jobling, M., 1989. Patterns of food intake in Artic charr, *Salvenus alpinus*, monitored by radiography. Aquaculture 81, 155–160.

Kaivarainen, E.I., Nemova, N.N., Krupnova, M.Y., Bondareva, L.A., 1998. The effect of toxic factors on intracellular proteinase activity in freshwater fish. Acta Vet. Brno 67, 309–316.

Koebele, B.P., 1985. Growth and the size hierarchy effect: an experimental assessment of three proposed mechanisms; activity differences, disproportional food acquisition, physiological stress. Environ. Biol. Fish 12, 181–188.

Krupnova, M.Y., Vysotskaya, R.U., Ruokolainen, T.P., 1985. Variation in lysosome enzyme-activity in trout during fasting. Ukr. Biokhim. Zh. 57, 62–65.

Le Corre, P., Treguer, P., 1976. Contribution à l'étude de la matière organique dissoute et des sels nutritifs dans l'eau de mer. Caractéristiques chimiques du Golfe de Gascogne et des upwellings côtiers de l'Afrique du Nord-Ouest, Ph.D. Thesis UBO Brest, p. 490.

Lee, D.H., Goldberg, A.L., 1998. Proteasome inhibitors: valuable new tools for cell biologists. Trends Cell Biol. 8, 397–403.

Lyndon, A.R., Houlihan, D.F., Hall, S.J., 1992. The effects of short term fasting and a single meal on protein synthesis and oxygen consumption in cod. J. Comp. Physiol. B 162, 209–215.

Martin, S.A.M., Cash, P., Blaney, S., Houlihan, D.F., 2001. Proteome analysis of rainbow trout (*Onchorhynchus mykiss*) liver proteins during short term starvation. Fish Physiol. Biochem. 24, 259–270.

Martin, S.A.M., Blaney, S., Bowman, A., Houlihan, D.F., 2002. Ubiquitin-proteasome-dependent proteolysis in rainbow trout (*Onchorhynchus mykiss*): effect of food deprivation. Eur. J. Physiol. 445, 257–266.

Maruyama, K., Sunde, M.L., Swick, R.W., 1978. Growth and muscle protein turnover in the chick. Biochem. J. 176, 573–582.

McCarthy, I.D., Carter, C.G., Houlihan, D.F., 1992. The effect of feeding hierarchies on individual variability in daily feeding of rainbow trout, *Onchorhynchus mykiss* (Walbaum). J. Fish Biol. 41, 257–263.

McCarthy, I.D., Houlihan, D.F., Carter, C.G., Moutou, K., 1993. Variation in individual consumption rates of fish and its implications for the study of fish nutrition and physiology. Proc. Nutr. Soc. 52, 427–436.

McCarthy, I.D., Houlihan, D.F., Carter, C.G., 1994. Individual variation in protein turnover and growth efficiency in rainbow trout, *Onchorhynchus mykiss* (Walbaum). Proc. R. Soc. London B 257, 141–147.

McCarthy, I.D., Gair, D.J., Houlihan, D.F., 1999. Feeding rank and dominance in Tilapia rendalli under defensive and indefensive patterns of food distribution. J. Fish Biol. 55, 854–867.

McMillan, D.N., Houlihan, D.F., 1989. Short term responses of protein synthesis to refeeding in rainbow trout. Aquaculture 79, 37–46.

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

Médale, F., Boujard, T., Vallée, F., Blanc, D., Mambrini, M., Roem, A., et al., 1998. Voluntary feed intake, nitrogen and phosphorus losses in rainbow trout (*Onchorhynchus mykiss*) fed increasing dietary levels of soy protein concentrate. Aquat. Living Resour. 11, 239–246.

Millward, D.J., 1989. The nutritional regulation of muscle growth and protein turnover. Aquaculture 79, 1–28.

Muramatsu, T., Kita, K., Tasaki, I., Okumura, J., 1987. Influence of dietary protein intake on whole-body protein turnover in chicks. Br. Poult. Sci. 28, 471–482.

Owen, S.F., McCarthy, I.D., Watt, P.W., Ladero, V., Sanchez, J.A., Houlihan, D.F., et al., 1999. In vivo rates of protein synthesis in Atlantic salmon (*Salmo salar* L.) smolts measured using a single flooding dose injection of 15N-phenylalanine. Fish Physiol. Biochem. 20, 87–94.

Peragòn, J., Barroso, J.B., García-Salguero, L., Aranda, F., de la Higuera, M., Lupiáñez, J.A., 1999. Selective changes in the protein-turnover rates and nature of growth induced in trout liver by long-term starvation followed by re-feeding. Mol. Cell. Biochem. 201, 1–10.

Pillay, C.S., Elliot, E., Dennison, C., 2002. Endolysosomal proteolysis and its regulation. Biochem. J. 363, 417–429.

Rahmatullah, M., Boyde, T.R.C., 1980. Improvements in the determination of urea using diacetyl monoxime: methods with and without deproteinisation. Clin. Chem. Acta 107, 3–9.

Ricker, W.E., 1979. Growth rates and models. In: Hoar, (Ed.), Fish Physiology, Vol. VIII. Randalland Brett, Academic Press, London, pp. 677–743 Bioenergetics and Growth.

Selman, C., Grune, T., Stolzing, A., Jakstadt, M., McLaren, J.S., Speakman, J.R., 2002. The consequences of acute cold exposure on protein oxidation and proteasome activity in short-tailed voles, Microtus agrestis. Free Radic. Biol. Med. 15, 259–265.

Shibatani, T., Ward, W.F., 1995. Sodium dodecyl sulfate (SDS) activation of the 20S proteasome in rat liver. Arch. Biochem. Biophys. 321, 160–166.

Siems, W., Dubiel, W., Dumdey, R., Müller, M., Rapoport, S.M., 1984. Accounting for the ATP-consuming processes in rabbit reticulocytes. Eur. J. Biochem./FEBS 139, 101–107.

Sugden, P.H., Fuller, S.J., 1991. Regulation of protein turnover in skeletal and cardiac muscle. Biochem. J. 273, 21–37.

Talbot, C., Higgins, P.J., 1983. A radiographic method for feeding studies on fish using metallic iron powder as marker. J. Fish Biol. 23, 211–220.

Tanaka, K., Chiba, T., 1998. The proteasome: a protein-destroying machine. Genes Cells 3, 199–510.

Tsubuki, S., Kawasaki, H., Saito, Y., Miyashita, N., Inomata, M., Kawashima, S., 1993. Purification and characterization of a Z-Leu-Leu-Leu-MCA degrading protease expected to regulate neurite formation: a novel catalytic activity in proteasome. Biochem. Biophys. Res. Commun. 196, 1195–1201.

Waterlow, J.C., Garlick, P.J., Millward, D.J., 1978. Protein Turnover in Mammalian Tissues and in the Whole Body. Elsevier/North Holland Publishing, Amsterdam, pp. 804.

Wicks, B.J., Randall, D.J., 2002. The effect of feeding and fasting on ammonia toxicity in juvenile rainbow trout, *Onchorhynchus mykiss*. Aquat. Toxicol. 59, 71–82.

Wilkie, M.P., Wood, C.M., 1991. Nitrogenous waste excretion, acid–base regulation and ionoregulation in rainbouw trout (*Onchorhynchus mykiss*) exposed to extremely alkaline water. Physiol. Zool. 64, 1069–1086.

Wing, S.S., Haas, A.L., Goldberg, A.L., 1995. Increase in ubiquitin-protein conjugates concomitant with the increase in proteolysis in rat skeletal muscle during starvation and atrophy denervation. Biochem. J. 307, 639–645.

Yewdell, J.W., Schubert, U., Bennink, J.R., 2002. At the crossroads of cell biology and immunology: DriPs and other sources of peptide ligands for MHC class I molecules. J. Cell Sci. 114, 845–851.