Fish Physiology and Biochemistry **25:** 335–345, 2001.
© 2003 *Kluwer Academic Publishers. Printed in the Netherlands.*

Digestive protease activities and free amino acids in white muscle as indicators for feed conversion efficiency and growth rate in Atlantic salmon (*Salmo salar* L.)

J. Sunde, G.L. Taranger and K. Rungruangsak-Torrissen*
*Matre Aquaculture Research Station, Department of Aquaculture, Institute of Marine Research, N-5984 Matredal, Norway; *Author for correspondence (Phone: +47 56367539; Fax: +47 56367585; E-mail: Krisnart@imr.no)*

Accepted: January 13, 2003

Key words: chymotrypsin, feed utilization, growth, light, plasma free amino acids, RNA concentration, RNA/protein ratio, triploid, trypsin, white muscle free amino acids

Abstract

The aim of the present experiment was to screen several biochemical indices in fish and their interrelations in order to select variables for future studies of growth rate and feed conversion. Several parameters [trypsin activity, chymotrypsin activity, free amino acids (FAA) in plasma and white muscle, and RNA and RNA/protein ratio in the white muscle] were measured together with specific growth rate (SGR), feed intake and feed conversion efficiency (FCE) in four groups of diploid or triploid Atlantic salmon (*Salmo salar* L.) reared under different light regimes. SGR was measured on individually tagged fish, whereas feed intake and feed conversion was estimated on tank basis. A principal component analysis (PCA) explained 80.6% of the variance in the data, using all measured parameters, regardless of ploidy and light regime. Muscle free hydroxyproline showed the highest correlation, alone explaining 55% of SGR variability. The SGR also significantly correlated with trypsin activity ($r = 0.34$), the activity ratio of trypsin to chymotrypsin (T/C) ($r = 0.39$), plasma essential FAA (EAA) ($r = 0.39$), plasma total FAA (TFAA) ($r = 0.37$), the ratio of essential to non-essential FAA (EAA/NEAA) in the white muscle ($r = -0.45$), muscle RNA ($r = -0.45$) and RNA/protein ratio ($r = -0.41$). Tank FCE correlated positively ($r = 0.97$) with SGR, T/C ratio and muscle free hydroxyproline, and negatively ($r = -0.90$) with muscle EAA/NEAA. The groups reared under continuous light (LL) regime showed significantly higher SGR than simulated natural photoperiod (SNP) groups, and with an apparently higher FCE. A higher growth rate was associated with either a higher consumption rate and/or a higher feed utilization. A negative correlation between muscle RNA concentration and SGR may indicate that increased growth rate under LL regime was not caused by an increased protein deposition rate.

Introduction

Biochemical correlates of growth rate and feed conversion efficiency in fish are in demand for measuring and predicting growth in both wild and cultured populations (Houlihan et al. 1993). A number of biochemical parameters have been found to correlate with growth rates and/or nutritional status in several fish species, such as cytochrome c oxidase activity (e.g., Foster et al. 1993a; Nathanailides and Stickland 1996; Pelletier et al. 1993), white muscle RNA concentration and RNA/protein ratio (e.g., Foster et al. 1993a; 1993b; Houlihan et al. 1993). Rate and level of nutrient influx are determined by different factors including composition and digestibility of the dietary protein, nutritional status of the fish, feeding regime, as well as individual variation in feed utilization (Rungruangsak Torrissen and Male 2000). The rate of digestion of dietary protein in the intestinal system limits the uptake of nutrients to the blood stream and can potentially limit the growth of the whole organism, as has been suggested in the case of Atlantic cod, *Gadus morhua*

(Lemieux et al. 1999), and Atlantic salmon (Rungruangsak Torrissen and Male 2000). Trypsin is a key digestive enzyme and the predominant protease in the pyloric caeca. It plays a key role in the digestion process by activating several other proteases, including chymotrypsin. The secretion rate of trypsin and chymotrypsin is related to feed intake and filling of the stomach (Einarsson et al. 1996) and the activity ratio of these two enzymes has been suggested as an indicator of the nutritional status of the fish by Rungruangsak Torrissen and Male (2000). Several trypsin genes have been reported and characterized in Atlantic salmon (Male et al. 1995). Individual variation in trypsin isozyme patterns has been characterized using isoelectric focusing techniques, and expression patterns have been found to have a significant effect on growth (Torrissen 1987, 1991) and feed utilization (Torrissen and Shearer 1992; Rungruangsak-Torrissen et al. 1998). Trypsin isozyme patterns affect digestion of dietary protein in Atlantic salmon, shown as a relation between trypsin activity and free amino acid (FAA) absorption to plasma and transport to white muscle after starving and re-feeding (Torrissen et al. 1994). Trypsin expression varies according to temperature at start-feeding in Atlantic salmon (Rungruangsak-Torrissen et al. 1998; Rungruangsak Torrissen and Male 2000) as well as according to feed quality in several fish species (Cahu et al. 1998; Nolting et al. 1999; Lazo et al. 2000), and has been proposed as an indicator of salmon growth rates in the wild (Rungruangsak-Torrissen and Stensholt 2001) and as a means of standardizing *in vitro* digestion assays for estimating feed quality (Rungruangsak-Torrissen et al. 2002). The white muscle amino acid pool functions as a reservoir for metabolism and protein growth (Carter et al. 1995) and is also involved in regulation of these processes (Millward 1989). The ratio between essential and non-essential FAA in muscle has been found to change according to different trypsin expressions (Torrissen et al. 1994). Elevated levels of the free amino acid hydroxyproline in the white muscle has been suggested to indicate increased catabolism of collagen in remodeling connective tissue for muscle growth (Torrissen et al. 1994; Rungruangsak Torrissen and Male 2000). In order to establish the reliability and suitability of parameters for estimating growth rates, groups that showed a wide range of growth rates had to be used in the experimental design. For this purpose, light regimes and ploidy were applied as treatments. Continuous light (LL) increases growth rate in Atlantic salmon in seawater (Kråkenes et al. 1991; Hansen et al. 1992; Endal et al. 2000). The use of LL regime for accelerated production is today widespread in commercial fish farming. Triploid, 'sterile' salmon were introduced in commercial farming in the mid-70s to prevent interbreeding of escapees and wild salmon (e.g., Johnstone and Stet 1995), and triploid growth performance has been reported to differ from that of diploids in some cases (e.g., Galbreath and Thorgaard, 1995; McCarthy et al. 1996; O'Flynn et al. 1997).

The present study describes the relationship of digestive protease activities of trypsin and chymotrypsin, and FAA concentrations in plasma and white muscle tissue, with specific growth rate, feed intake and feed conversion efficiency. For relating changes in these parameters to changes in muscle growth, RNA concentration and capacity for protein synthesis (RNA/protein ratio) in the white muscle were measured (e.g., Carter et al. 1993; Suresh and Sheehan 1998). This paper is the first to measure and compare all selected parameters concurrently, using individually labeled fish.

Materials and methods

Fish

Two diploid and two triploid groups of Atlantic salmon post-smolts were grown indoors in circular 20 m^3 ($\varnothing = 5$ m) seawater tanks (about 650 fish/tank) at Matre Aquaculture Research Station, Western Norway (61°N) for 75 days during February–April. Triploidization was carried out using the protocol of Johnstone and Stet (1995). Due to limited facility, each ploidy was reared without replicates under identical simulated natural photoperiod (SNP) for six months, before two sub-groups within each ploidy were separated in different light groups at the start of the experiment, with either SNP or LL applied. Artificial illumination was provided by one asymmetric metal halogen lamp in each tank (EUROFLOODS, Siemens AS, Norway. Bulbs: Osram HQI-TS 150W/NDL), giving an illuminance of 105 ± 7 lux at 1 m below the water surface. Light spectra showed two peaks with maxima around 535 and 592 nm. Light regimes did not change during the experimental period. The water temperature was on average 8.7 ± 0.1 °C (mean ± s.e.m.) with an oxygen content of 6.80 ± 0.04 mg l^{-1} (dissolved oxygen saturation 67.8 ± 0.5%).

Specific growth rates of a random selection of 25 salmon per tank were followed throughout the experi-

ment. Initial weight of these fish in February was 870 ± 23 g. The fish were individually tagged near the dorsal fin using Floy anchor tags (Floy Tag & Manufacturing Inc., Seattle, USA) and fed 6 mm pelleted feed (Royal AB Redline, T. Skretting AS, Norway) in 10–15% excess of apparent satiation at a feeding rate of 2 h day^{-1} divided between two meals, five days a week. Dead fish weight was registered five days a week and biomass continuously estimated. Biomass was kept at an optimal level by removing a random sample of 50 fish from each tank every month. Surplus feed was collected daily for calculation of tank consumption rates.

Sampling and data analysis

Fork lengths and live body weights were measured on individually tagged fish at the beginning and at the end of the experiment. Condition factor was calculated as $K = 100 \times (w \times l^{-3})$, where w = weight in grams and l = fork length in cm (Brown 1946). The daily specific growth rate (SGR) of each fish was calculated according to Houde and Schekter (1981) as:

$$\text{SGR } [\% \text{ day}^{-1}] = (e^{g} - 1) \times 100, \quad (1)$$

where $g = (\ln W_t - \ln W_0)/(t - t_0)$, W_t = weight at day t, W_0 = weight at day t_0.

Daily feed consumption rate [% day^{-1}] was calculated as kg measured dry feed consumed × kg estimated biomass^{-1} × 100. Feed conversion efficiency (FCE) was estimated for each group as total kg estimated biomass increase (including fish taken out) during the trial divided by kg measured dry feed eaten in the same period.

Sampling of blood and tissue was performed at the end of the experiment. Fish were sedated with metomidate hydrochloride (Wildlife Laboratories, CO, USA), according to Olsen et al. (1995), and a 1–2 ml blood sample was drawn from the caudal vein via the lateral line into a heparinized syringe. Plasma was separated by centrifugation at $1,000 \times g$ for 15 min at 4 °C and kept frozen at −80 °C. The fish were killed by a blow to the head, and samples of epaxial white muscle and pyloric caeca removed. The visceral fat surrounding the pyloric caeca was discarded and the caeca immediately frozen at −80 °C.

Trypsin and chymotrypsin activity assays

Preparation of samples and trypsin activity measurements are based on Torrissen et al. (1994). Assaying chymotrypsin activity was modified from Rungruangsak-Torrissen and Sundby (2000). Initial reaction rates of trypsin and chymotrypsin activity were used for calculations and measured spectrophotometrically as the increase in absorbance at 410 nm after 1000 μl substrate solution was added to 10 μl enzyme extract. Absorbance values were logged after the addition of substrate and the activity calculated from the values at 30 and 60 seconds. The trypsin-specific substrate solution consisted of 1.25 mM benzoyl-L-arginin-*p*-nitroanilide in 0.2 M Tris-HCl buffer with 5% (v/v) dimethylformamide and the chymotrypsin-specific substrate solution was 0.1 mM *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide in 0.2 M Tris-HCl buffer with 5% (v/v) dimethylformamide. Enzyme activity was calculated in relation to protein concentration in the extract determined as described by Lowry et al. (1951).

Plasma and white muscle free amino acids analysis

Free amino acid composition in plasma was analysed according to the method developed by Rungruangsak-Torrissen and Sundby (2000). Free amino acid composition in white muscle was assayed by extracting 100 mg on ice with 800 μl 5% trichloroacetic acid and 200 μl α-aminobutyric acid as internal standard giving a final concentration of 100 nmol ml^{-1}. The supernatant was deproteinized with Millipore Ultrafree-MC 10000 NMWL Filter Unit (Millipore Corp., Bedford, MA, USA) at $5,000 \times g$ for 30 min at 4 °C. Amino acids and other amino-containing compounds in the plasma and muscle acid supernatants were derivatized with Waters AccQ·Tag reagent, according to Waters (1993) and analyzed with the buffer gradient system described by Rungruangsak-Torrissen and Sundby (2000) using the Alliance HPLC system (Waters 2690 Separations module), Waters 996 Photodiode Array Detector (λ = 254 nm) and Waters 474 Scanning Fluorescence detector (λ_{em} = 395 nm). Separation was performed using a Nova-Pak C18 column (Waters Corp., Milford, MA, USA) with dimensions 60 Å, 4 μm, 3.9 × 300 mm. Tryptophan derivative was not reliably detected on either of the detectors.

Protein synthesis capacity assay

The reagent TRIzol® (Life Sciences, NY, USA), a one-phase solution of phenol and guanidine isothiocyanate, was used for a one-step extraction of RNA and protein from white muscle for measuring RNA concentration and protein synthesis capacity (ratio of RNA/protein). White muscle samples of

100 mg were added with 1 ml of TRIzol® and treated with a Kontes micro-ultrasonic cell disrupter (Kimble/Kontes, Vineland, NJ, USA). The mixture was left standing for 10 min at room temperature. After adding 0.2 ml chloroform, the solution separated into an upper aqueous phase containing RNA, and a lower organic phase containing the soluble protein fraction. After centrifugation at $5,000 \times g$, 10 min, 200 μl of each phase were carefully transferred to separate tubes, and precipitated by addition of 1.5 ml of isopropanol and centrifugation at $5,000 \times g$, 10 min. Pellets were washed twice in 0.5 ml 96% ethanol and centrifugated at $5,000 \times g$, 10 min before carefully dried at 55 °C for 20 min. The RNA pellets were dissolved in 1 ml 0.1 M sodium acetate (pH 5) by heating for 10 min at 55 °C. The protein pellets were added 1 ml 1% SDS and dissolved by heating for 100 min at 55 °C. Concentrations were later determined by wavelength measurement after dilution 1:10 (RNA) and 1:50 (protein) in their respective solvents. RNA concentration was measured at 260 nm (Ashford and Pain 1986) and calculated using the extinction coefficient $E_{260} = 40$ μg RNA ml^{-1}. After TRIzol® extraction, recovery of RNA in concentrations of 5–20 μg ml^{-1} was estimated to 68.0 ± 0.7% (s.e.m.), compared to standards of yeast RNA dissolved in 0.1 M sodium acetate buffer (pH 5). Protein concentration was measured at 280 nm using a standard curve with BSA (bovine serum albumin) concentrations extracted with TRIzol® for calibration: $E_{280} = 2.1$ mg protein ml^{-1}. Recovery of protein within the concentration range of 40–100 μg ml^{-1} after TRIzol® extraction was estimated to 69.0 ± 1.0% (s.e.m.), compared to standards of BSA dissolved in 1% SDS.

Statistical analyses

Data were analyzed using SYSTAT 10 software (SPSS Inc., Chicago, IL, USA). All data analyzed exhibited normal distribution and homogeneity of variances according to criteria of Zar (1996). All results are given as mean values ± s.e.m. First, a two-way analysis of variance (ANOVA) was applied to evaluate the effects of light and ploidy. Tukey *post hoc* tests were applied in case of significance. Secondly, a principal component analysis (PCA) of the measured variables was attempted to investigate their relationship. PCA converts complex information in multivariate space to a lower number of independent latent variables (principal components). A component loading table show the covariances of the original variables and each principal component.

Results

Growth characteristics and feed consumption rates

Due to loss of Floy tags, only 78 individuals remained for sampling at the end of the experiment (Table 1). Initial weights did not show a significant correlation with subsequent growth rates ($r = 0.19$, $P = 0.09$). Less than 2% of the fish were sexually mature at the time of sampling. Condition factor (K) at the start of the experiment differed between the experimental groups, both according to ploidy (2N: 1.16 ± 0.01; 3N: 1.08 ± 0.01) and light regime (SNP: 1.16 ± 0.02; LL: 1.09 ± 0.01). At the end of the experiment, the average condition factor had increased (*t*-test, $P < 0.001$), and the triploid groups still exhibited the slimmer profile (2N: 1.22 ± 0.01; 3N: 1.14 ± 0.01). Specific growth rate was higher in the groups reared under LL light regime (SNP: 0.136 ± 0.01; LL: 0.253 ± 0.01 [% day^{-1}]). The LL groups also showed a tendency of higher consumption rates than the SNP groups (SNP: 0.418; LL: 0.513 [% body weight]). The 3N–LL group had the highest daily feed intake of all groups.

The lowest FCE was seen in the 3N–SNP group (0.295), followed by the 2N-SNP group (0.351). The two LL groups showed the highest FCE estimates, with the 3N-LL group (0.504) similar to the 2N–LL group (0.480).

Enzyme activities

The mean values of the measured biochemical parameters are summarized in Table 2. Component loadings for the four extracted factors using data from all individuals ($n = 78$) are shown in Table 3, and correlation coefficients in Table 4. Of the two measured protease activities, only trypsin activity correlated with SGR (PC1 in Table 3; Table 4) and differed between the groups (see Table 2), with a higher activity under LL light regime (NL: 163.1 ± 14.7; LL: 222.5 ± 10.2 [μmol *p*-nitroanaline h^{-1} mg protein^{-1}]). Trypsin and chymotrypsin activities were highly correlated (PC3 in Table 3; Table 4), and T/C ratios were influenced by both trypsin and chymotrypsin activity (Table 4). The T/C ratios showed a similar correlation with SGR as trypsin activity, with higher values under LL light

Table 1. Number of sampled fish (total $n = 78$), initial and final weight, condition factor (K), specific tank consumption rate (SCR), individual specific growth rate (SGR) and feed conversion efficiency (FCE) for all groups (2N: diploid, 3N: triploid, SNP: simulated natural photoperiod, LL: 24 h light). Numbers are given as mean ± s.e.m. Significances (P< 0.05) are indicated in bold and significant differences between groups by different superscripts

	2N-SNP	2N-LL	3N-SNP	3N-LL	*P* (light)	*P* (ploidy)	*P* (interaction)
No. of fish, *n*	20	20	16	22	–	–	–
Weight, initial [g]	874.8 ± 36.2	786.7 ± 44.3	841.3 ± 40.2	962.9 ± 52.8	0.683	0.123	**0.024**
Weight, final [g]	1157.0 ± 60.7^{a}	1199.8 ± 79.3^{a}	1047.8 ± 68.7^{a}	1520.9 ± 78.1^{b}	**0.001**	0.168	**0.005**
K, initial [g cm^{-3}]	1.19 ± 0.02^{a}	1.12 ± 0.01^{b}	1.11 ± 0.02^{b}	1.06 ± 0.01^{c}	**<0.001**	**<0.001**	0.511
K, final [g cm^{-3}]	1.22 ± 0.02^{a}	1.22 ± 0.02^{a}	1.11 ± 0.02^{b}	1.17 ± 0.01^{a}	0.08	**<0.001**	0.132
SCR [% day^{-1}]†	0.439 ± 0.023	0.494 ± 0.026	0.397 ± 0.020	0.532 ± 0.032	–	–	–
SGR [% day^{-1}]	0.157 ± 0.010^{a}	0.237 ± 0.012^{b}	0.117 ± 0.018^{c}	0.268 ± 0.011^{b}	**<0.001**	0.646	**0.008**
FCE†	0.351	0.480	0.295	0.504	–	–	–

† Average of 75 days on tank basis

regime. This resulted in different T/C ratios between the groups (Table 2).

Plasma and muscle free amino acids

The plasma EAA concentrations in the 3N-SNP fish were significantly lower than in the other groups (Table 2). The plasma total FAA was strongly influenced by plasma EAA concentrations (PC4 in Table 3; Table 4). However, plasma EAA/NEAA ratios were similar (0.15 ± 0.03) in all four groups (Table 2). Plasma EAA and TFAA values correlated with SGR, trypsin activity and T/C ratio (Table 4).

While muscle TFAA levels were similar in all but one group (2N-LL), the muscle EAA/NEAA ratios were significantly lower in the LL groups (1.75 ± 0.08) than in the SNP groups (2.71 ± 0.09), independent of changes in muscle TFAA levels (Table 2). The LL groups also showed higher muscle free hydroxyproline (1.78 ± 0.06 nmol mg^{-1}) than the SNP groups (0.70 ± 0.07 nmol mg^{-1}).

Similarly to the plasma values, muscle EAA concentrations strongly influenced muscle TFAA (PC2 in Table 3; Table 4). However, the variation in muscle EAA and TFAA levels were not associated with SGR variation (PC2 in Table 3; Table 4). Muscle EAA/NEAA ratio value, on the other hand, showed a strong negative correlation ($r = -0.45$, P< 0.01) with SGR (Table 4). Of all the measured parameters, muscle free hydroxyproline concentrations showed the highest correlation with SGR (PC1 in Table 3; Table 4).

Muscle RNA and protein

Muscle RNA concentrations were significantly lower in the LL groups (3.44 ± 0.07 μg mg^{-1}) than in the SNP groups (4.49 ± 0.12 μg mg^{-1}), as the RNA levels showed an inverse correlation with SGR (PC1 in Table 3; Table 4). Meanwhile, protein synthesis capacity (RNA/protein ratio) was similar, with the exception of the 3N–SNP group, where a low muscle protein concentration (0.09 ± 0.01 mg mg^{-1}) resulted in a significantly higher RNA/protein ratio (58.7 ± 4.7 μg mg^{-1}). Protein synthesis capacity correlated with SGR (Table 4: $r = -0.41$, P< 0.001).

Discussion

Variations in growth rate, feed intake and feed conversion efficiency were found between the four experimental groups, as intended in the experimental setup. Although our results are presented and discussed with ploidy and light regime in mind, we refrain from making statements about the effect of any of these factors, due to our experimental design lacking replicate groups.

Derived parameters calculated from other data (T/C, plasma EAA/NEAA, muscle EAA/NEAA and RNA/protein) were left out of the principal component analysis. Using this approach, the PCA was able to explain 80.6% of the variance in the data. For conclusive results regarding each specific parameter, a full factorial design using a reduced number of variables would be preferable. It should also be noted that although growth rates were within the normal range

Table 2. Trypsin and chymotrypsin activity, activity ratio of trypsin to chymotrypsin (T/C), essential free amino acids (EAA) and total free amino acids (TFAA) and ratio of essential to non-essential free amino acids (EAA/NEAA) in plasma and white muscle, muscle free hydroxyproline (Hyp), muscle RNA concentration, muscle protein and muscle protein synthesis capacity (RNA/protein ratio) in the four treatment groups, all given as mean ± s.e.m. (n = 78). Significances ($P < 0.05$) are indicated in bold and differences between groups by different superscripts

Measured parameter	2N-SNP	2N-LL	3N-SNP	3N-LL	P (light)	P (ploidy)	P (interaction)
Trypsin activity*	209.2 ± 20.8^{a}	200.2 ± 12.5^{a}	105.6 ± 7.0^{b}	242.9 ± 14.7^{a}	**<0.001**	0.056	**<0.001**
Chymotrypsin activity*	422.2 ± 35.3	358.7 ± 31.2	277.0 ± 32.0	395.9 ± 32.8	0.501	0.148	**0.005**
T/C ratio	0.52 ± 0.04^{ab}	0.59 ± 0.03^{ac}	0.43 ± 0.03^{b}	0.65 ± 0.03^{ac}	**<0.001**	0.450	0.103
Plasma EAA [nmol ml^{-1}]	1026 ± 70^{a}	994 ± 49^{a}	686 ± 54^{b}	1093 ± 67^{a}	**0.004**	0.065	**0.001**
Plasma TFAA [nmol ml^{-1}]	7796 ± 519^{a}	8094 ± 317^{a}	5408 ± 213^{b}	7786 ± 280^{a}	**<0.001**	**<0.001**	**0.005**
Ratio of plasma EAA/NEAA	0.15 ± 0.01	0.14 ± 0.00	0.14 ± 0.01	0.16 ± 0.00	0.778	0.309	**0.02**
Muscle EAA [nmol mg^{-1}]	57.0 ± 1.8^{a}	38.0 ± 3.9^{b}	57.0 ± 4.5^{a}	59.2 ± 2.8^{a}	**0.013**	**0.002**	**0.002**
Muscle TFAA [nmol mg^{-1}]	79.5 ± 2.0^{a}	62.9 ± 4.4^{b}	76.7 ± 5.0^{a}	89.2 ± 3.6^{a}	**<0.001**	**<0.001**	**0.005**
Ratio of muscle EAA/NEAA	2.57 ± 0.11^{a}	1.49 ± 0.14^{b}	2.87 ± 0.14^{a}	1.98 ± 0.07^{c}	**<0.001**	**0.001**	0.426
Muscle free Hyp [nmol mg^{-1}]	0.75 ± 0.08^{a}	1.63 ± 0.07^{b}	0.64 ± 0.13^{a}	1.91 ± 0.10^{b}	**<0.001**	**0.247**	0.111
Muscle RNA [μg mg^{-1}]	4.32 ± 0.16^{a}	3.43 ± 0.10^{b}	4.71 ± 0.19^{c}	3.44 ± 0.11^{b}	**<0.001**	0.176	0.142
Muscle protein [mg mg^{-1}]	0.14 ± 0.01^{a}	0.12 ± 0.01^{a}	0.09 ± 0.01^{b}	0.13 ± 0.01^{a}	0.076	**0.037**	**0.008**
Protein synthesis capacity [μg mg^{-1}]	34.9 ± 3.3^{a}	31.5 ± 2.6^{a}	58.7 ± 4.7^{b}	31.2 ± 2.9^{a}	**<0.001**	**0.001**	**0.001**

* Expressed as μmol p-nitroaniline h^{-1} mg protein^{-1}.

Table 3. Component loadings of each parameter ($n = 78$) and percent variance explained using PCA with four extracted principal components (PC) and varimax rotation. Numbers in bold indicate the main components of each PC.
(SGR: specific growth rate, EAA: essential free amino acids, TFAA: total free amino acids, Hyp: hydroxyproline)

Parameter	PC1	PC2	PC3	PC4
SGR [% day^{-1}]	**0.815**	0.084	0.091	0.261
Trypsin activity*	0.263	0.034	**0.890**	0.224
Chymotrypsin activity*	−0.054	0.079	**0.961**	−0.034
Plasma EAA [nmol ml^{-1}]	0.157	−0.098	−0.006	**0.944**
Plasma TFAA [nmol ml^{-1}]	0.203	0.050	0.174	**0.909**
Muscle EAA [nmol mg^{-1}]	−0.019	**−0.979**	−0.064	0.051
Muscle TFAA [nmol mg^{-1}]	0.178	**−0.961**	−0.047	0.087
Muscle free Hyp [nmol mg^{-1}]	**0.885**	−0.164	0.108	0.106
Muscle RNA [μg mg^{-1}]	**−0.778**	0.003	−0.013	−0.063
Muscle protein [mg mg^{-1}]	0.315	0.361	0.023	0.196
Variance explained [%]†	23.2	20.7	17.7	19.0

* Expressed as μmol *p*-nitroaniline h^{-1} mg protein^{-1}.
†Percent of total variance in data set. Total explained variance 80.6% (ΣPC).

during our sampling period, they were lower than expected for Atlantic salmon of this size reared under optimum conditions at similar temperatures (Austreng et al. 1987). This could possibly be due to a limited supply of oxygen to the water during the experimental period, which for salmonids in particular can have adverse effects on growth and feed conversion probably already at levels approaching 50–70% (Jobling 1994). However, we registered no differences in oxygen concentration between the experimental tanks.

There is evidence that the activity of trypsin might limit growth rate in Atlantic cod when growth is stimulated by injection with recombinant somatotropin (Lemieux et al. 1999). Our results also showed an overall significant correlation between trypsin activity and individual growth rates ($r = 0.34$, P< 0.002), however within each group, the variation in growth rates was probably too low for this correlation to reach significance. Trypsin activity also correlated with overall plasma EAA, although the coefficient was low ($r = 0.24$, P< 0.04). Trypsin activity might be the limiting factor in supplying amino acids and peptides for growth processes (Torrissen et al. 1994). Interestingly, the group with the lowest feed intake, growth rate and trypsin activity (3N-SNP), showed a stronger correlation between trypsin activity and plasma EAA than the other groups ($r = 0.50$, P< 0.05). Chymotrypsin activity, in spite of its overall correlation with trypsin activity ($r = 0.76$, P< 0.001), did not correlate with growth rate. The trypsin-chymotrypsin correlation was consistent also within the groups, although weakest in the 3N–SNP group ($r = 0.60$, P< 0.02). Very high correlation between trypsin and chymotrypsin activities (PC3 in Table 3; Table 4) suggests that trypsin activates chymotrypsin in fish like in mammals. The activity ratio of trypsin to chymotrypsin might also indicate to what extent chymotrypsin is activated by trypsin, and this in turn may indicate growth potential of the fish, as suggested by Rungruangsak Torrissen and Male (2000). It was suggested that the higher the T/C ratio, the higher the absorption/transport rate of EAA for protein synthesis and plasma insulin level (Rungruangsak-Torrissen and Sundby 2000). This in turn stimulates amino acid uptake and protein synthesis, especially in muscle (Murat et al. 1981; Matty 1986). However, trypsin activity alone did not explain more than 11.5% of the variance in SGR (Table 4). Using the ratio of trypsin to chymotrypsin gave a slightly better correlation, but even then accounted for only 15.2% of the variability in SGR. The significant, but poor, correlation with growth rate may be due to the fact that these proteases do not directly affect growth rate, but are key factors influencing other biochemical parameters (Rungruangsak Torrissen and Male 2000). However, the T/C ratios rank the four groups correctly, according to their average growth rates and FCE (Tables 1 and 2; see Rungruangsak-Torrissen et al. 2002) and may be useful in establishing whether growth differences exist between groups of fish.

Table 4. Correlation table giving correlation coefficients (r) for all measured parameters ($n = 78$). Significant correlations ($P < 0.05$) are indicated in bold

	SGR	Trypsin	Chymotrypsin	T/C ratio	Plasma EAA	Plasma TFAA	Plasma EAA/NEAA	Muscle EAA	Muscle TFAA	Muscle EAA/NEAA	Muscle free Hyp	RNA	Protein
SGR	**1**												
Trypsin	**0.34**	**1**											
Chymotrypsin	0.05	**0.76**	**1**										
T/C ratio	**0.39**	**0.25**	**−0.38**	**1**									
Plasma EAA	**0.39**	**0.24**	−0.04	**0.43**	**1**								
Plasma TFAA	**0.37**	**0.40**	0.13	**0.37**	**0.82**	**1**							
Plasma EAA/NEAA	0.17	−0.007	−0.19	**0.25**	**0.65**	0.12	**1**						
Muscle EAA	−0.09	−0.08	−0.14	0.05	0.13	−0.04	**0.29**	**1**					
Muscle TFAA	0.06	−0.01	−0.13	0.15	0.19	0.05	**0.27**	**0.97**	**1**				
Muscle EAA/NEAA	**−0.45**	**−0.27**	−0.10	**−0.26**	−0.07	**−0.28**	**0.27**	**0.74**	**0.55**	**1**			
Muscle free Hyp	**0.74**	**0.31**	0.05	**0.35**	**0.24**	**0.32**	−0.03	0.11	**0.30**	**−0.44**	**1**		
RNA	**−0.46**	**−0.25**	0.03	**−0.37**	−0.21	**−0.25**	0.02	0.02	−0.13	**0.41**	**−0.55**	**1**	
Protein	**0.23**	0.18	0.04	0.16	0.15	0.18	0.01	−0.22	−0.13	**−0.36**	0.17	−0.16	**1**
RNA/protein	**−0.41**	**−0.30**	−0.01	**−0.37**	**−0.28**	**−0.31**	−0.07	0.13	0.02	**0.39**	**−0.36**	**0.63**	**−0.77**

Muscle EAA/NEAA ratio showed a somewhat stronger, but negative, correlation with growth rate ($r = -0.45$, P< 0.001). This is in accordance with previous studies, where low EAA/NEAA ratios were found to accompany high growth rates and high incorporation of essential free amino acids into muscle protein (Torrissen et al. 1994; Rungruangsak Torrissen and Male 2000). Muscle free hydroxyproline seems to be the most promising of all the measured parameters in estimating growth rate. Free hydroxyproline in muscle by itself accounted for about 55% (Table 4) of the variation in growth rate. Free hydroxyproline appears in muscle tissue from breakdown of collagen and could be an indicator of the catabolic activity in the muscle tissue (Torrissen et al. 1994; Rungruangsak Torrissen and Male 2000). The increase in muscle free hydroxyproline was associated with a lower RNA concentration ($r = -0.55$, P< 0.001) and a lower muscle EAA/NEAA ratio ($r = -0.44$, P< 0.001).

RNA concentrations ($r = -0.46$, $P < 0.001$) and RNA/protein ratio ($r = -0.41$, $P < 0.001$) showed negative correlations with growth rate, contrary to that reported in previous studies (e.g., Houlihan et al. 1993; Foster et al. 1993a; Suresh and Sheehan 1998). However, the use of different photoperiods might have affected different metabolic parameters in the fish. Photoperiod has been hypothesized to be the main cause of metabolic adaptations in fish associated with change of season (Endal et al. 2000). Muscle RNA concentration was higher under shorter (8L:16D, 6 °C) than longer (11L: 13D, 10 °C) photoperiod in juvenile Atlantic cod (Foster et al. 1993b), in agreement with our findings. A lower white muscle RNA concentration has also been associated with a higher protein turnover rate and higher RNA activity (Rungruangsak Torrissen and Male 2000). It is possible that extended photoperiods may affect growth in Atlantic salmon through an increased RNA activity and protein turnover rate. Higher values of white muscle free hydroxyproline in the LL groups also seem to suggest higher rates of protein turnover, compared to the SNP groups. High protein growth efficiency has been shown to associate with a low protein turnover rate (Rungruangsak Torrissen et al. 1999). The higher growth rates found in the LL groups, were therefore most likely not due to an increased protein growth efficiency. Since growth is affected by both protein and lipid deposition, and higher RNA activity is associated with lower proximate composition ratio of protein to lipid (Rungruangsak Torrissen and Male 2000), LL regime under the conditions studied might be postulated to increase fish growth through a relative increase in lipid deposition rate.

All measured parameters indicate a general low anabolic activity in the 3N–SNP group. This is supported by the feeding data (not shown), showing a tendency of a decreasing feed intake throughout the experimental period. This seemed to be a temporary phenomenon that coincided with a slightly higher mortality in this group. In the same period, an increasing trend of feed intake was observed in the 3N–LL group while both 2N groups showed a stable feed intake. Although statistical analysis could not be performed on the FCE values, the biochemical measurements seem to indicate higher feed conversion efficiency in the LL groups. Also, diploid and triploid fish may have shown different responses to light treatment regarding feed conversion efficiencies (Table 2). Interestingly, the coefficient of variance (CV) for most of the measured parameters (including SGR) decreased under LL regime, regardless of ploidy.

This study demonstrated how several biochemical parameters changed with growth rate and outlines their possible future use as estimators of individual and group growth rates of fish. Trypsin activity, T/C ratio, muscle free EAA/NEAA ratio and muscle free hydroxyproline levels show promise as parameters that together can account for most of the variance in measured growth rates. The four groups were ranked similarly as 3N–SNP< 2N–SNP < 2N–LL < 3N–LL with regards to SGR, FCE, trypsin activity, T/C ratio and muscle free hydroxyproline. Measuring a combination of these parameters will provide more information on how growth rate is affected under different conditions. Our results show that an increase in growth rate may not always be associated with a higher RNA concentration in the white muscle depending on the changes in body protein/lipid ratio.

Acknowledgements

The authors would like to thank the staff at Matre Aquaculture Research Station, and in particular Ph.D. student Frode Oppedal, for daily fish husbandry and assistance during sampling. The work was financed by the Norwegian Research Council (NFR) and in part carried out with financial support from the Commission of the European Communities, Agriculture and Fisheries, specific RTD program FAIR-CT96-1329, 'Effect of processing technology on the quality of aquaculture feeds' and program AIR-3-CT94-2216,

'Minimising the interaction of cultured and wild fish: A comprehensive evaluation of the use of sterile triploid Atlantic salmon'. The paper does not necessarily reflect its views and in no way anticipates the Commission's future policy in this area.

References

Ashford, A.J. and Pain, V.M. 1986. Effect of diabetes on the rates of synthesis and degradation in ribosomes in rat muscle and liver *in vivo*. J. Biol. Chem. 261: 4059–4065.

Austreng, A., Storebakken, T., Åsgård, T. 1987. Growth rate estimates for cultured Atlantic salmon and rainbow trout. Aquaculture 60: 157–160.

Brown, M.E. 1946. The growth of brown trout (*Salmo trutta* Linn.). II The growth of two-year-old trout at a constant temperature of 11.5 °C. J. Exp. Biol. 22: 130–144.

Cahu, C.L., Infante, J.L.Z., Peres, A., Quazuguel, P. and Le Gall, M.M. 1998. Algal addition in sea bass (*Dicentrarchus labrax*) larvae rearing: effect to digestive enzymes. Aquaculture 161: 479–489.

Carter, C.G., Houlihan, D.F., Buchanan, B. and 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., He, Z.-Y., Houlihan, D.F., McCarthy, I.D. and Davidson, I. 1995. Effect of feeding on the tissue free amino acid concentrations in rainbow trout (*Oncorhynchus mykiss* Walbaum). Fish Physiol. Biochem. 14: 153–164.

Einarsson, S., Spencer Davies, P. and Talbot, C. 1996. The effect of feeding on the secretion of pepsin, trypsin and chymotrypsin. Fish Physiol. Biochem. 15: 439–446.

Endal, H.P., Taranger, G.L., Stefansson, S.O. and Hansen, T. 2000. Effects of continuous additional light on growth and sexual maturity in Atlantic salmon, *Salmo salar*, reared in sea cages. Aquaculture 191: 337–349.

Foster, A.R., Houlihan, D.F. and Hall, S.J. 1993a. Effects of nutritional regime on correlates of growth-rate in juvenile Atlantic cod (*Gadus morhua*) – comparison of morphological and biochemical measurements. Can. Fish. Aquat. Sci. 50: 502–512.

Foster, A.R., Hall, S.J. and Houlihan, D.F. 1993b. The effects of seasonal acclimatization on correlates of growth rate in juvenile cod, *Gadus morhua*. J. Fish Biol. 42: 461–464.

Galbreath, P.F. and Thorgaard, G.H. 1995. Saltwater performance of all-female triploid Atlantic salmon. Aquaculture 138: 77–85.

Hansen, T., Stefansson, S.O. and Taranger, G.L. 1992. Growth and sexual maturation in Atlantic salmon, *Salmo salar* L., reared in sea cages at two different light regimes. Aquacult. Fish. Manage. 23: 275–280.

Helland, S.J., Grisdale-Helland, B., and Nerland, S. 1996. A simple method for the measurement of daily feed intake of groups of fish in tanks. Aquaculture 139: 157–163.

Houde, D.E. and Schekter, R.C. 1981. Growth rate, rations and cohort consumption of marine fish larvae in relation to prey concentration. Rapp. P.-V. Reun. Cons. Int. Explor. Mer 178: 441–453.

Houlihan, D.F., Mathers, E.M. and Foster, A. 1993. Biochemical correlates of growth rate in fish. *In*: Fish ecophysiology. pp. 45–71. Edited by J.C. Rankin and F.B. Jensen. Chapman & Hall, London.

Jobling, M. 1994. Environmental factors and growth. *In*: Fish Bioenergetics. pp. 166–168. Edited by M. Jobling. Chapman & Hall, London.

Johnstone, R., and Stet, R.J.M. 1995. The production of gynogenetic Atlantic salmon, *Salmo salar* L. Theor. Appl. Genet. 90: 819–826.

Kråkenes, R., Hansen, T., Stefansson, S.O. and Taranger, G.L. 1991. Continuous light increases growth rate of Atlantic salmon (*Salmo salar* L.) postsmolts in sea cages. Aquaculture 95: 281–287.

Lazo, J.P., Dinis, M.T., Holt, G.J., Faulk, C. and Arnold, C.R. 2000. Co-feeding microparticulate diets with algae: Toward eliminating the need of zooplankton at first feeding in larval red drum (*Sciaenops ocellatus*). Aquaculture 188: 339–351.

Lemieux, H., Blier, P.U. and Dutil, J.-D. 1999. Do digestive enzymes set a physiological limit to growth rate and food conversion efficiency in the Atlantic cod (*Gadus morhua*)? Fish Physiol. Biochem. 20: 293–303.

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

Nolting, M., Ueberschar, B. and Rosenthal, H. 1999. Trypsin activity and physiological aspects in larval rearing of European sea bass (*Dicentrarchus labrax*) using live prey and compound diets. J. Appl. Ichthyol. 15: 138–142.

Male, R., Lorenz, J.B., Smalås, A.O. and Torrissen, K.R. 1995. Molecular cloning and characterization of anionic and cationic variants of trypsin from Atlantic salmon. Eur. J. Biochem. 232: 677–685.

Matty, A.J. 1986. Nutrition, hormones and growth. Fish Physiol. Biochem. 2: 141–150.

McCarthy, I.D., Carter, C.G., Houlihan, D.F., Johnstone, R., Mitchell, A.I. 1996. The performance of all-female diploid and triploid Atlantic salmon smolts on transfer together to sea water. J. Fish Biol. 48: 545–548.

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

Murat, J-C., Plisetskaya, E.M., Woo, N.Y.S. 1981. Endocrine control of nutrition in cyclostomes and fish. Comp. Biochem. Physiol. 68A: 149–158.

Nathanailides, C. and Stickland, N.C. 1996. Activity of cytochrome c oxidase and lactate dehydrogenase in muscle tissue of slow growing (lower modal group) and fast growing (upper modal group) Atlantic salmon. J. Fish Biol. 48: 549–551.

O'Flynn, F.M., McGeachy, S.A., Friars, G.W., Benfey, T.J. and Bailey, J.K. 1997. Comparisons of cultured triploid and diploid Atlantic salmon (*Salmo salar* L.). ICES J. Mar. Sci. 54: 1160–1165.

Olsen, Y. A., Einarsdottir, I.E. and Nilssen, K.J. 1995. Metomidate anasthesia in Atlantic salmon, *Salmo salar*, prevents cortisol increase during stress. Aquaculture 134: 155–168.

Pelletier, D., Guderley, H. and Dutil, J.D. 1993. Does the aerobic capacity of fish muscle change with growth rates. Fish Physiol. Biochem. 12: 83–93.

Rungruangsak-Torrissen, K., Carter, C.G., Sundby, A., Berg, A. and Houlihan, D.F. 1999. Maintenance ration, protein synthesis capacity, plasma insulin and growth of Atlantic salmon (*Salmo salar* L.) with genetically different trypsin isozymes. Fish Physiol. Biochem. 21: 223–233.

Rungruangsak-Torrissen, K., Lied, E. and Espe, M. 1994. Differences in digestion and absorption of dietary protein in Atlantic salmon (*Salmo salar*) with genetically different trypsin isozymes. J. Fish Biol. 45: 1087–1104.

Rungruangsak-Torrissen, K., Pringle, G.M., Moss, R. and Houlihan, D.F. 1998. Effects of varying rearing temperatures on expres-

sion of different trypsin isozymes, feed conversion efficiency and growth in Atlantic salmon (*Salmo salar* L.). Fish Physiol. Biochem. 19: 247–255.

Rungruangsak Torrissen, K. and Male, R. 2000. Trypsin isozymes: Development, digestion and structure. *In*: Seafood Enzymes, utilization and influence on post-harvest seafood quality. pp. 215–269. Edited by N.F. Haard and B.K. Simpson. Marcel Dekker, Inc., New York.

Rungruangsak-Torrissen, K. and Sundby, A. 2000. Protease activities, plasma free amino acids and insulin at different ages of Atlantic salmon (*Salmo salar* L.) with genetically different trypsin isozymes. Fish Physiol. Biochem. 22: 337–347.

Rungruangsak-Torrissen, K. and Stensholt, B.K. 2001. Spatial distribution of Atlantic salmon post-smolts: Association between genetic differences in trypsin isozymes and environmental variables. *In*: Proceedings of the International Symposium on Spatial Processes and Management of Fish Populations. pp. 415–429. Edited by G.H. Kruse, N. Bez, A. Booth, M.W. Dorn, S. Hills, R.N. Lipcius, D. Pelletier, C. Roy, S.J. Smith and D. Witherell. University of Alaska Sea Grant, AK-SG-01-02, Fairbanks.

Rungruangsak-Torrissen, K., Rustad, A., Sunde, J., Eiane, S.A., Jensen, H.B., Opstvedt, J., Nygård, E., Samuelsen, T.A., Mundheim, H., Luzzana, U. and Venturini, G. 2002. *In vitro* digestibility based on fish crude enzyme extract for prediction of feed quality in growth trial. J. Sci. Food Agric. 82: 644–654.

Suresh, A.V. and Sheehan, R.J. 1998. Biochemical and morphological correlates of growth in diploid and triploid rainbow trout. J. Fish Biol. 52: 588–599.

Torrissen, K.R. 1987. Genetic variation of trypsin-like isozymes correlated to fish size of Atlantic salmon (*Salmo salar*). Aquaculture 62: 1–10.

Torrissen, K.R. 1991. Genetic variation in growth rate of Atlantic salmon with different trypsin-like isozyme patterns. Aquaculture 93: 299–312.

Torrissen, K.R. and Shearer, K.D. 1992. Protein digestion, growth and food conversion in Atlantic salmon and Arctic charr with different trypsin-like isozyme patterns. J. Fish Biol. 41: 409–415.

Torrissen, K.R., Lied, E. and Espe, M. 1994. Differences in digestion and absorption of dietary protein in Atlantic salmon (*Salmo salar*) with genetically different trypsin isozymes. J. Fish Biol. 45: 1087–1104.

Waters 1993. Waters AccQ•Tag Chemistry Package Instruction Manual. Waters Corporation, Milford, MA, USA.

Zar, J.H. 1996. Biostatistical analysis. 3rd ed. Prentice-Hall Inc., New Jersey, USA.

ERRATA PAPER I - Sunde *et al.* (2001)

The SGR and FCE values in Table 1 should read as follows:

	2n-SNP	2n-LL	3n-SNP	3n-LL	P (light)	P (ploidy)	P (interaction)
SGR [% day^{-1}]	0,355 ± 0,024	0,546 ± 0,026	0,278 ± 0,042	0,615 ± 0,024	**<0.001**	**0.646**	**0.008**
FCE	0,809	1,105	0,700	1,156	-	-	-

All references to the original values in the text should be substituted accordingly.