Effect of fish feed processing conditions on digestive protease activities, free amino acid pools, feed conversion efficiency and growth in Atlantic salmon (*Salmo salar* L.)

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Abstract

The responses of the digestive proteases trypsin and chymotrypsin and protein metabolism to differences in feed protein quality were investigated in Atlantic salmon (*Salmo salar* L.). Two sets of experimental feeds were produced. Each set of high and low quality feeds was provided to either 150 g or 2 kg salmon. Protein in the high quality feeds had significantly higher percentages of free (reactive) sulphydryl (SH) groups than the corresponding feeds based on low quality meals. After 90 days feeding, groups given high and low quality feeds did not differ in their specific growth rates (SGR) in either experiment. However, feed conversion efficiency (FCE) was significantly different between the high and low quality feed groups in 2 kg salmon, where the difference between the high and low feed protein qualities was larger, 10% versus 4% SH/[SH + (S–S)] in 150 g salmon. Higher FCE was preceded by significantly higher trypsin and chymotrypsin specific activities on day 60. SGR, in general, changed after the first month and was stable during the last 2 months in both experiments. Concurrently, both trypsin (T) and chymotrypsin (C) decreased with an increased activity ratio of trypsin to chymotrypsin (T/C ratio), and resulting in significantly lower T/C ratio on day 90 in salmon feeding on high quality feeds in both sizes of fish. Differences in FCE were associated with significant differences in levels of total free amino acids (TFAA) in the plasma and the white muscle, as well as in the ratio of essential to non-essential free amino acids (EAA/NEAA ratio), free hydroxyproline, and RNA in the white muscle. Interestingly, after 3 days starvation (day 93), 5–7 h postprandial EAA/NEAA ratio in the plasma was significantly lower in the high quality diet groups in both experiments. Trypsin specific activity inversely correlated with muscle TFAA levels in 2 kg salmon, concurrent with higher muscle levels of RNA, lower free hydroxyproline and higher FCE in fish fed higher quality diets.

KEY WORDS: Atlantic salmon, chymotrypsin, disulphide bond, fish feed, fish meal, free amino acids, plasma, RNA, sulphydryl group, trypsin, white muscle

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Introduction

In modern aquaculture, fish meal constitutes the major protein source in fish feeds for a range of carnivorous and herbivorous species, and is the single largest cost component. A continuing worldwide increase in aquaculture has created a high demand for fish meals of high quality for production of aquaculture feeds. Until alternative protein sources become viable substitutes, protein raw material for fish feed production will remain a limited resource. It is therefore of both commercial and environmental interest that the harvested raw material is optimally processed to ensure a product of the highest possible nutritional quality. Both freshness of the raw materials and industrial processing conditions affect the nutritional value of fish meal (Pike *et al*. 1990) and high processing temperature of fish raw materials and meals has been shown to have a negative influence on the availability of amino acids *in vivo* (Cowey *et al*. 1972), and on protein digestibility both *in vitro* (Boonvisut & Whitaker 1976;

Rungruangsak-Torrissen *et al.* 2002) and *in vivo* (Opstvedt *et al.* 1984, 1987, 2003; Pike *et al.* 1990). At present several alternatives exist for evaluation of fish meal quality, including chemical methods, *in vitro* enzyme digestion and *in situ* digestibility in terrestrial and aquatic species (reviewed by Anderson *et al.* 1993).

The decrease in protein digestibility during processing of fish raw materials has been associated with several biochemical reactions, including heat-induced formation of amino acid D-enantiomers (Friedman *et al.* 1981; Luzzana *et al.* 1996, 1999) as well as oxidation of free sulphydryl (SH) groups and formation of disulphide (S–S) bonds (Boonvisut & Whitaker 1976; Opstvedt *et al.* 1984; Friedman 1994). With a new method developed for determining free SH groups directly in fish feed, Rungruangsak-Torrissen *et al.* (2002) demonstrated an adverse effect of increased S–S bond formation on the *in vitro* protein digestibility of fish meals and feeds processed under different conditions. As a result of S-S bond formation, alterations in the three-dimensional structure of the proteins occur and it has been speculated that this can reduce the availability of peptide bonds for enzyme digestion (Friedman *et al.* 1982).

Study of digestive enzymes is an essential step towards understanding the mechanism of digestion and how the organism adapts to changes in the nutritional environment. In vertebrates, adaptation of digestive enzymes to food constituents is commonly observed and trypsin, a digestive serine protease, plays a major regulatory part through its activation of a number of pancreatic proteases. In aquatic species, trypsin is often present in several isoforms, as in Atlantic salmon, *Salmo salar* L. (Torrissen 1987). Several trypsin genes have been identified in fish, for instance in Atlantic salmon (Male *et al.* 1995) and Japanese anchovy, *Engraulis japonicus* (Ahsan *et al.* 2001). In Atlantic salmon a wide range of factors has been found to affect trypsin specific activity and isoform expression (reviewed in Rungruangsak-Torrissen & Male 2000). Differences in trypsin phenotype, as determined by isoelectric focusing of caecal protein extracts, have been associated with different start-feeding temperatures (Rungruangsak-Torrissen *et al.* 1998), fish size (Torrissen 1987, 1991; Rungruangsak-Torrissen *et al.* 1998) and rates of absorption and transport of free amino acids (FAA) (Torrissen *et al.* 1994, 1995; Rungruangsak-Torrissen & Sundby 2000). Trypsin (T) activates chymotrypsin (C), and the ratio of these enzyme activities (the T/C ratio) has been related to differences in growth performance (Rungruangsak-Torrissen 2001; Sunde *et al.* 2001; Rungruangsak-Torrissen *et al.* 2002). Whole-body growth rates in fish are correlated with protein synthesis rates in the muscle (Fauconneau *et al.* 1990), and have been found to correlate with the ratio of RNA to protein, referred to as the capacity for protein synthesis, in long-term growth studies (Houlihan *et al.* 1989; Sugden & Fuller 1991). In addition, growth rate in Atlantic salmon reared under both natural and constant photoperiods was positively correlated with the FAA pools in the plasma and free hydroxyproline in the white muscle, and negatively with the ratio of essential to non-essential FAA in the white muscle as well as with white muscle concentrations of RNA and the RNA/protein ratio (Sunde *et al.* 2001). In order to test the biological quality of the experimental feeds in the current experiments, these biochemical parameters were chosen for analysis. Protease specific activities of trypsin and chymotrypsin as well as their activity ratio (T/C) were used as measures of the digestive response to different feed qualities. At the same time, nutrient influx of FAA to the plasma and the white muscle, the muscle levels of RNA and free hydroxyproline and the RNA/protein ratio were studied as indicators for protein metabolism and muscle protein synthesis rates. The degree of disulphide bond (S–S) formation, expressed as percentage of free (reactive) SH groups of total content of protein sulphur, %SH/[SH + (S–S)], was used as a feed protein quality parameter.

Materials and methods

Fish feed production

Experiment 1 Fish meals and experimental feeds were produced by the Norwegian Institute of Fisheries and Aquaculture, Department SSF, Fyllingsdalen, Norway. Norwegian whole spring spawning herring (*Clupeus harengus* L.) was cooked at low (<70 °C) or high temperature (>90 °C). The fraction cooked at low temperature was dried in an air drier with outlet meal temperature 70 °C, while the high temperature cooked fraction was dried in an indirect steam drier with outlet meal temperature 100 °C. This produced one high quality (FM1) and one low quality (FM2) fish meal, respectively. Pellets (3.5 mm Ø) were extruded under three different extrusion conditions (ET1, ET2 and ET3), designated 'gentle', 'medium' and 'tough', respectively. Production processes of the fish meals and fish feeds are described in Opstvedt *et al.* (2003). A total of six diets (FM1ET1, FM1ET2, FM1ET3, FM2ET1, FM2ET2 and FM2ET3) were prepared in a 2×3 factorial design. The diet formulations and contents of free (reactive) SH groups and S–S bonds, expressed as %SH/[SH + (S–S)], are given in Table 1. No specific pigment source was added.

Table 1 Composition of the experimental feed series. Data from Rungruangsak-Torrissen *et al.* (2002)

	FM1ET	FM2ET	FM3ET	FM4ET
Feed composition (g kg^{-1})				
Herring meal	651	651	656	656
Fish oil	208	205	200	200
Wheat flour	127	130	130	130
Vitamin C[1]	1	1	1	1
Vitamin/mineral premix[1]	3	3	3	3
Analytical values				
Moisture (g kg^{-1})	54	47	65	72
Protein (g kg^{-1})	507	512	389	393
Lipid (g kg^{-1})	255	257	276	274
Ash (g kg^{-1})	102	98	79	85
Nitrogen-free extract (g kg^{-1})[2]	82	86	191	176
Free sulphydryl groups [%SH/(SH + (S–S))][3]	12.8	8.7	20.5	10.1

[1] Provided per kg of feed: 250 mg vitamin C (Rovimix Saty C 35%), 3000 IU vitamin A acetate, 1600 IU vitamin D_3, 160 IU α-tocopheryl acetate, 12 mg vitamin K_3, 12 mg thiamine-HCl, 24 mg riboflavin, 12 mg pyridoxine-HCl, 120 mg niacin, 6 mg folate, 0.024 mg vitamin B_{12}, 0.6 mg biotin, 48 mg Ca-pantothenate, 3 mg Cu as $CuSO_4 \cdot 5H_2O$, 2.4 mg I as $Ca(IO_3)_2$, 24 mg Fe as $FeSO_4 \cdot 7H_2O$, 21 mg Mn as MnO_2, 30 mg Zn as ZnO and 0.1 mg Se as Na_2SeO_3.
[2] Calculated by difference.
[3] Disulphide bonds (S–S) calculated from difference in total reduced sulphur (S) and reactive sulphydryl groups (SH). See Rungruangsak-Torrissen *et al.* (2002) for details.

Experiment 2 Experimental feeds were produced by ASA srl–Agridea, Cologna Veneta, VR, Italy. Two commercially produced fish meals (Norsildmel A/L, Fyllingsdalen, Norway), were classified as high quality FM3 (Norse LT94®) and low quality FM4 (NorSeaMink®) fish meal. Pellets (3.5 mm Ø) were extruded under three different extrusion conditions (ET1, ET2 and ET3), designated 'gentle', 'medium' and 'tough', respectively. Production processes of the fish feeds are described in Opstvedt *et al.* (2003). A total of six diets (FM3ET1, FM3ET2, FM3ET3, FM4ET1, FM4ET2 and FM4ET3) were prepared in a 2 × 3 factorial design. The diet formulations as well as feed contents of free SH groups, %SH/[SH + (S–S)], are given in Table 1. No specific pigment source was added.

Fish and sampling

Experiment 1 The experiment was conducted at the Institute of Marine Research – Matre, Western Norway (61° N), using Atlantic salmon postsmolts from a mixed Norwegian broodstock (Norsk Lakseavl, Kyrksæterøra, Norway). Six dietary fish groups (FM1ET and FM2ET series) were fed in triplicate (110 fish per tank), in 18 covered circular 3 m Ø tanks with external standpipes. Natural light fluorescent tubes simulating natural photoperiods provided light. Aerated seawater with mean temperature 10.8 ± 0.2 °C and salinity 30–33 (on the Practical Salinity Scale 1978) was supplied throughout the experimental period. Mean initial weight was 128.9 ± 17.3 g. The fish had been given 3 mm feed (Nutra Svev, Skretting, Stavanger, Norway) prior to the experiment.

During the experiment, rations were adjusted daily *ad libitum* to about 1% of body weight and were distributed using automatic rotating feeders, 6.5 h day^{-1}, from 08:00 to 14:30 hours. Daily consumption rate of each tank was recorded in the afternoon by increasing water inflow to the tanks and collecting unconsumed feed in the outlet using special grated filters fitted to the standpipes. Mortality was recorded and dead fish removed daily for estimation of tank biomass and calculation of feed conversion efficiency (FCE) as kg weight gained per kg dry feed consumed.

Fifty individuals in each tank were Floy tagged (Floy Tag & Manufacturing Inc., Seattle, WA, USA) to measure individual growth rates. Weights and fork lengths were recorded for all individuals in each tank on days 0, 30, 60 and 90 after sedation with metomidate hydrochloride, according to Olsen *et al.* (1995). The daily specific growth rate (SGR) was calculated according to Houde & Schekter (1981) as:

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

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

Blood, epaxial white muscle and pyloric caeca were sampled from 25 fish per tank at the end of the experiment (day

90). Pyloric caeca were in addition collected from three fish per tank at the start (day 0) and at day 60. After 90 days, the fish were starved for 3 days and the following day (day 93) manually fed in excess for 30 min. Blood was then sampled from three fish per tank at 5–7 h after re-feeding (based on Torrissen *et al*. 1994). Fish fillets were collected on day 0 (one pooled sample containing one fish from each tank) and day 90 (pooled samples with 10 fish from each tank).

Epaxial white muscle and plasma prepared from the blood samples were frozen at −80 °C for later determination of FAA concentrations. RNA and protein concentrations were measured in the epaxial white muscle. Pyloric caeca samples were frozen at −80 °C for later determination of trypsin and chymotrypsin specific activities. Fish fillets were frozen at −20 °C for composition analysis according to Norwegian Standard NS 9401/9402.

Experiment 2 The experiment was performed in $5 \times 5 \times 5$ m^3 sea cages with Atlantic salmon at an initial weight of 1982 ± 42 g. Six dietary fish groups (FM3ET and FM4ET series) were fed in duplicate, altogether 12 sea cages with 150 fish per cage. Water temperature was 13.4 ± 0.1 °C and salinity 30.6 ± 0.3 (at 5 m depth) during the experimental period. The fish were starved for 2 weeks before the start of the experiment, and manually fed at a ration of about 1% of body weight, in total 30 min day^{-1} between 08:00 and 16:00 hours. Waste of feed was carefully avoided by distributing the feed in small portions to ensure that all feed was consumed. Mortality was recorded daily and dead fish removed within 1–2 days for estimation of cage biomass and calculation of FCE.

Fifty individuals in each cage were Floy tagged to measure individual growth rates. Weights and fork lengths were recorded for all individuals in each cage on days 0, 30, 60 and 90 after sedation with metomidate hydrochloride, according to Olsen *et al*. (1995). The daily SGR was calculated according to Houde & Schekter (1981) as in Experiment 1. Blood, epaxial white muscle and pyloric caeca were sampled from 15 fish per cage at the end of the experiment on day 90. Additional pyloric caeca samples were collected from three fish per cage on days 0 and 60. After 90 days, the fish were starved for the next 3 days, and the following morning (day 93), they were manually fed in excess for 30 min. Blood was then sampled from three fish per cage at 5–7 h after re-feeding. Fish fillets were collected on day 0 (one pooled sample containing one fish from each cage) and day 90 (pooled samples with 10 fish from each cage).

Epaxial white muscle, plasma, pyloric caeca samples and fish fillets were kept frozen at −80 °C until analysis as described in Experiment 1.

Trypsin and chymotrypsin specific activity assays Preparations of samples and trypsin specific activity measurements were based on Torrissen *et al*. (1994). Assaying chymotrypsin specific activity was modified from Rungruangsak-Torrissen & Sundby (2000), as described by Sunde *et al*. (2001). Briefly, initial reaction rates of trypsin and chymotrypsin activity were measured spectrophotometrically as the increase in absorbance at 410 nm of *p*-nitroaniline produced during the first minute reaction after 1000 μL substrate solution was added to 10 μL enzyme extract. Trypsin activity was determined at 50 °C with a specific substrate solution of 1.25 mM benzoyl-L-arginine-*p*-nitroanilide (BAPNA) [dissolved in 5% (v/v) dimethylformamide and diluted to final volume with 0.2 M Tris–HCl buffer, pH 8.4]. Chymotrypsin activity was determined at 40 °C with a specific substrate solution of 0.1 mM *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide [dissolved in 5% dimethylformamide (v/v) and diluted to final volume with 0.2 M Tris–HCl buffer, pH 8.4]. The concentration of *p*-nitroaniline produced was compared with a standard curve. Enzyme specific activity was calculated in relation to protein concentration in the extract determined as described by Lowry *et al*. (1951), and expressed as μmol *p*-nitroaniline produced h^{-1} mg^{-1} protein.

Plasma and white muscle free amino acids analysis The concentrations of FAA in the plasma were analysed according to the method developed by Rungruangsak-Torrissen & Sundby (2000), and those in the white muscle extract analysed according to Sunde *et al*. (2001), using α-aminobutyric acid as internal standard. Filtrates of plasma and muscle acid supernatant were derivatized with Waters AccQ•Tag reagent, according to Waters (1993) and FAA separated using the buffer gradient described in Rungruangsak-Torrissen & Sundby (2000). Analysis was performed using the Alliance HPLC system, consisting of Waters 2690 Separations Module, Waters 996 Photodiode Array Detector ($\lambda = 254$ nm), Waters 474 Scanning Fluorescence Detector ($\lambda_{ex} = 250$ nm; $\lambda_{em} = 395$ nm) and Nova-Pak C_{18} column (60 Å, 4 μm, 3.9×300 mm). Tryptophan was unable to be detected in any samples.

Protein synthesis capacity assay The method used for determination of protein synthesis capacity was described in Sunde *et al*. (2001). TRIzol® (Life Technologies, Inc., Grand Island, 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 samples of approximately 100 mg. Concentration of RNA was measured at 260 nm (Ashford & Pain 1986) and calculated using the

extinction coefficient $E_{260} = 40$ μg RNA mL^{-1}. Protein concentration was measured at 280 nm and calculated using the extinction coefficient $E_{280} = 2.1$ mg mL^{-1}.

Statistical analysis Data were analysed using the Statistical Analysis System v. 8.02 for Windows® (SAS Institute Inc., Cary, NC, USA). A main factorial model (the *glm* procedure) was employed to evaluate the effects of fish meal type and extrusion conditions, and a Student's *t*-test (the *t*-test procedure) for comparison of data between sampling dates. Where significant differences (at 95% significance level) between the groups were found, a *post hoc F*-test (the *lsmeans* procedure with the *pdiff* option) was applied. Correlation coefficients (r) and probability values (P) were calculated using Pearson product–moment correlations (the *corr* procedure). All results are given as mean ± standard error of mean (SEM).

Results

Fish feed quality

The quality of the extruded fish feeds, quantified as % free (reactive) SH groups [%SH/(SH + (S–S))], was unaffected by pellet extrusion conditions. Feed data were therefore averaged by fish meal type in Table 1. The feeds of presumed high quality (FM1ET and FM3ET) had higher percentages of free SH groups ($F = 38.5$, $P < 0.0001$) than their corresponding low quality feeds (FM2ET and FM4ET). Between the FM1ET and FM2ET diets the mean difference in free SH groups was 4%, whereas there was a 10% difference between the FM3ET and FM4ET diets.

Growth, feed intake and feed conversion

Due to a problem with the water supply to one of the tanks only duplicate tanks given the FM2ET1 diet remained at the end of Experiment 1. Mean weight, SGR and FCE values for all diet groups on monthly and overall basis are given in Tables 2 & 3. Regardless of diet groups, feed intake increased during Experiment 1, while it decreased after the first month and remained low in Experiment 2. In Experiment 1, growth rates increased after the first month ($P < 0.001$), and were subsequently stable until the end of the experiment, regardless of diet groups (Table 2). However, FCE decreased during the experiment ($P < 0.001$). Mean weights were lower for the fish fed high quality diets FM1 at every sampling point ($P < 0.001$), but there were no significant differences in SGR between FM1ET and FM2ET groups. However, there were tendencies of both higher SGR (1.05 ± 0.04 versus 1.01 ± 0.02% day^{-1}) and higher FCE (1.46 ± 0.02 versus 1.44 ± 0.03) in the FM1ET groups. In Experiment 2, SGRs decreased and FCE increased after the first month ($P < 0.001$), and were stable thereafter, regardless of diet groups (Table 3). Although the fish fed on high quality FM3ET diets had higher weights than the low quality FM4ET diet groups at all sampling dates ($P < 0.0001$), group SGRs were not different. However, when using individual values from the tagged fish only, the effect of fish meal on SGR became significant ($F = 17.8$, $P < 0.0001$).

Diet type did not affect group SGR or feed intake in either experiment. Likewise, FCE was generally unaffected by fish meal quality. However, total FCE did differ ($F = 5.84$, $P = 0.05$) between the high quality FM3ET groups (0.93 ± 0.03) and the low quality FM4ET groups (0.83 ± 0.04), and showed a strong correlation with group SGRs ($r = 0.821$, $n = 12$, $P < 0.002$).

Fillet composition was analysed in Experiment 2 (Table 4). The composition of the pooled fillet samples from each cage at the end of the trial (day 90) was unaffected by diet type. There was, however, a tendency of higher lipid content (130 ± 2 g kg^{-1}) in fish fed the low quality FM4ET feeds versus the high quality FM3ET feeds (122 ± 4 g kg^{-1}).

Digestive enzyme specific activities

Results from the enzyme specific activity measurements are summarized in Tables 5 & 6. In general, fish meal type had a significant influence on enzyme specific activities, whereas the effect of extrusion conditions did not show a consistent pattern. In neither experiment did enzyme activities show any correlation with feed intakes ($P > 0.05$). When SGR was stable during the last 2 months in both experiments (Tables 2 & 3), both trypsin and chymotrypsin specific activities decreased together with increased T/C ratios. On day 90, the high quality feed groups (FM1ET and FM3ET) showed higher trypsin (albeit insignificant) and chymotrypsin ($P < 0.002$) specific activities, and consequently higher total enzyme specific activities and lower T/C ratios ($P < 0.01$) in fish fed higher quality feeds, than the corresponding low quality feed groups (FM2ET and FM4ET). These differences were significant already on day 60 in Experiment 2 (Table 6), preceding the differences in FCE seen at the end of the experiment on day 90 (Table 3). In Experiment 1, differences in chymotrypsin specific activity and T/C ratio became significant only at the end of the experiment (day 90), and no FCE differences due to feed type were seen within the experimental period (Table 2). However, none of the enzyme

Table 2 Initial weight, final weight, individual feed intake, specific growth rate (SGR) and feed conversion efficiency (FCE) development in all diet groups, and averaged by fish meal type, in Experiment 1. Values of *F* and associated probabilities (*P*) are given for a main factorial model (the *glm* procedure) considering the effects of fish meal quality (FM) and extrusion conditions (ET) during processing. Bold type indicates significant effect. Different superscripts identify significant differences ($P \leq 0.05$) between groups after *post hoc* testing (the *pdiff* option). All numbers are given as mean ± SEM

							Mean		FM		ET		FM × ET	
Growth parameter	FM1ET1	FM1ET2[1]	FM1ET3	FM2ET1	FM2ET2	FM2ET3	FM1ET	FM2ET	*F*	*P*	*F*	*P*	*F*	*P*
Initial weight (g)	130 ± 2	110 ± 3	129 ± 2	134 ± 2	132 ± 2	130 ± 2	125 ± 1	132 ± 1	–	–	–	–	–	–
Final weight (g)	346 ± 6^{a}	306 ± 5^{b}	342 ± 6^{a}	352 ± 8^{a}	332 ± 5^{a}	343 ± 5^{a}	330 ± 3	340 ± 3	5.82	**0.02**	16.9	**<0.001**	3.06	**0.05**
Feed intake (g)														
Days 0–30	24 ± 1	21 ± 1	23 ± 2	25 ± 1	24 ± 1	25 ± 1	23 ± 1	25 ± 1	2.99	0.11	0.97	0.41	0.42	0.67
Days 30–60	58 ± 7	50 ± 2	55 ± 5	59 ± 9	51 ± 2	55 ± 3	54 ± 3	55 ± 2	0.05	0.83	1.36	0.30	0.01	0.99
Days 60–90	71 ± 6	62 ± 1	68 ± 7	75 ± 16	64 ± 3	69 ± 7	67 ± 3	68 ± 4	0.15	0.70	1.16	0.35	0.02	0.98
Total feed intake	163 ± 16	140 ± 1	153 ± 15	171 ± 31	148 ± 5	155 ± 12	152 ± 7	155 ± 8	0.19	0.67	1.55	0.26	0.03	0.97
SGR (% day^{-1})														
Days 0–30	0.80 ± 0.02	0.92 ± 0.05	0.81 ± 0.08	0.76 ± 0.06	0.87 ± 0.09	0.86 ± 0.03	0.84 ± 0.03	0.84 ± 0.04	0.05	0.82	1.71	0.23	0.34	0.72
Days 30–60	1.23 ± 0.16	1.11 ± 0.15	1.17 ± 0.16	1.19 ± 0.22	0.99 ± 0.13	1.13 ± 0.07	1.17 ± 0.08	1.09 ± 0.07	0.29	0.60	0.60	0.57	0.04	0.96
Days 60–90	1.09 ± 0.06	1.10 ± 0.06	1.12 ± 0.04	1.06 ± 0.01	1.07 ± 0.03	1.08 ± 0.05	1.10 ± 0.03	1.07 ± 0.02	0.86	0.37	0.16	0.86	0.01	0.99
Total SGR	1.06 ± 0.08	1.05 ± 0.08	1.04 ± 0.08	1.01 ± 0.06	0.98 ± 0.03	1.03 ± 0.05	1.05 ± 0.04	1.01 ± 0.02	0.53	0.48	0.09	0.91	0.08	0.92
FCE (weight gain per feed consumed)														
Days 0–30	1.40 ± 0.04	1.64 ± 0.18	1.51 ± 0.21	1.38 ± 0.15	1.59 ± 0.24	1.50 ± 0.07	1.52 ± 0.09	1.50 ± 0.09	0.04	0.85	0.83	0.46	0.01	0.99
Days 30–60	1.53 ± 0.09	1.38 ± 0.07	1.50 ± 0.10	1.48 ± 0.08	1.37 ± 0.11	1.48 ± 0.04	1.47 ± 0.05	1.44 ± 0.05	0.13	0.73	1.46	0.27	0.02	0.98
Days 60–90	1.38 ± 0.08	1.39 ± 0.05	1.45 ± 0.03	1.32 ± 0.12	1.43 ± 0.05	1.39 ± 0.04	1.41 ± 0.03	1.39 ± 0.04	0.26	0.62	0.67	0.53	0.48	0.63
Total FCE	1.44 ± 0.03	1.47 ± 0.02	1.49 ± 0.06	1.40 ± 0.06	1.46 ± 0.06	1.46 ± 0.02	1.46 ± 0.02	1.44 ± 0.03	0.50	0.49	0.88	0.44	0.07	0.93

[1] Two replicate groups only.

Table 3 Initial weight, final weight, individual feed intake, specific growth rate (SGR) and feed conversion efficiency (FCE) development in all diet groups, and averaged by fish meal type, in Experiment 2. Values of *F* and associated probabilities (*P*) are given for a main factorial model (the *glm* procedure) considering the effects of fish meal quality (FM) and extrusion conditions (ET) during processing. Bold type indicates significant effect. Different superscripts identify significant differences ($P \leq 0.05$) between groups after *post hoc* testing (the *pdiff* option). All numbers are given as mean ± SEM

							Mean		FM		ET		FM × ET	
Growth parameter	FM3ET1	FM3ET2	FM3ET3	FM4ET1	FM4ET2	FM4ET3	FM3ET	FM4ET	*F*	*P*	*F*	*P*	*F*	*P*
Initial weight (g)	2018 ± 40	2004 ± 41	1984 ± 38	1886 ± 36	1986 ± 41	2013 ± 39	2002 ± 23	1958 ± 22	–	–	–	–	–	–
Final weight (g)	3156 ± 47[a]	3193 ± 51[a]	2998 ± 52[b]	2741 ± 47[c]	2977 ± 50[b]	2960 ± 53[b]	3115 ± 29	2867 ± 29	36.84	<**0.001**	5.07	**0.006**	6.05	**0.002**
Feed intake (g)														
Days 0–30	626 ± 4	655 ± 1	613 ± 29	632 ± 80	599 ± 51	564 ± 95	631 ± 11	598 ± 37	0.52	0.50	0.33	0.73	0.18	0.84
Days 30–60	202 ± 1	197 ± 4	196 ± 8	194 ± 29	181 ± 8	174 ± 29	198 ± 3	183 ± 11	1.11	0.33	0.28	0.76	0.07	0.93
Days 60–90	324 ± 9	321 ± 5	328 ± 2	303 ± 34	301 ± 17	281 ± 37	324 ± 3	295 ± 14	2.63	0.16	0.09	0.92	0.25	0.79
Total feed intake	1185 ± 3	1200 ± 11	1215 ± 1	1171 ± 160	1128 ± 82	1051 ± 174	1200 ± 6	1117 ± 68	1.00	0.36	0.10	0.90	0.27	0.77
SGR (% day^{-1})														
Days 0–30	0.65 ± 0.12	0.74 ± 0.08	0.63 ± 0.01	0.52 ± 0.12	0.63 ± 0.02	0.53 ± 0.04	0.67 ± 0.04	0.56 ± 0.04	2.87	0.14	1.12	0.39	0.02	0.98
Days 30–60	0.36 ± 0.06	0.34 ± 0.02	0.33 ± 0.01	0.35 ± 0.06	0.22 ± 0.03	0.31 ± 0.03	0.34 ± 0.02	0.30 ± 0.03	2.07	0.20	1.96	0.22	1.32	0.34
Days 60–90	0.39 ± 0.11	0.35 ± 0.00	0.31 ± 0.04	0.29 ± 0.01	0.39 ± 0.05	0.35 ± 0.07	0.35 ± 0.03	0.34 ± 0.03	0.01	0.91	0.29	0.76	0.97	0.43
Total SGR	0.49 ± 0.06	0.50 ± 0.03	0.45 ± 0.04	0.40 ± 0.07	0.44 ± 0.03	0.41 ± 0.05	0.48 ± 0.02	0.42 ± 0.02	2.89	0.14	0.43	0.67	0.16	0.86
FCE (weight gain per feed consumed)														
Days 0–30	0.88 ± 0.08	0.97 ± 0.04	0.90 ± 0.13	0.63 ± 0.12	0.88 ± 0.04	0.78 ± 0.02	0.92 ± 0.04	0.76 ± 0.06	5.44	0.06	2.17	0.20	0.56	0.60
Days 30–60	1.31 ± 0.29	1.28 ± 0.14	1.08 ± 0.17	1.18 ± 0.13	0.85 ± 0.11	1.25 ± 0.07	1.22 ± 0.10	1.09 ± 0.09	0.99	0.36	0.58	0.59	1.67	0.27
Days 60–90	0.99 ± 0.21	0.94 ± 0.00	0.77 ± 0.08	0.72 ± 0.02	1.03 ± 0.09	1.00 ± 0.18	0.90 ± 0.07	0.92 ± 0.08	0.01	0.91	0.63	0.56	2.13	0.20
Total FCE	0.96 ± 0.06	0.99 ± 0.01[a]	0.84 ± 0.00	0.72 ± 0.08[b]	0.88 ± 0.02	0.89 ± 0.06	0.93 ± 0.03	0.83 ± 0.04	5.84	**0.05**	1.97	0.22	4.38	0.07

	Initial composition	Composition at day 90					
		FM3ET1	FM3ET2	FM3ET3	FM4ET1	FM4ET2	FM4ET3
Water (g kg^{-1})	696	678 ± 13	676 ± 5	671 ± 5	666 ± 1	664 ± 4	666 ± 6
Protein (g kg^{-1})	198	200 ± 1	202 ± 1	202 ± 0	201 ± 0	203 ± 2	203 ± 0
Lipid (g kg^{-1})	105	121 ± 13	119 ± 8	126 ± 7	133 ± 0	129 ± 4	129 ± 4
Protein/lipid ratio	19	17 ± 2	17 ± 1	16 ± 1	15 ± 0	16 ± 1	16 ± 0
Ash (g kg^{-1})	13	12 ± 0	13 ± 0	12 ± 0	13 ± 0	13 ± 0	12 ± 0

Table 4 Initial ($n = 1$) and final ($n = 12$) fillet contents of water, protein, lipid and ash in Experiment 2 (mean ± SEM)

parameters were correlated with individual SGRs or FCE (on group basis) in either experiment, although there were tendencies in Experiment 1 that SGR and trypsin specific activities ($r = 0.438$, $n = 17$, $P = 0.08$) as well as FCE and T/C ratios ($r = 0.444$, $n = 17$, $P = 0.08$) were correlated on group basis.

Plasma free amino acids

The plasma samples (Tables 7 & 8) were taken from fish during normal routine feeding (day 90) and also after a short starvation period with subsequent re-feeding (day 93). In Experiment 1, where growth rates and FCE were unaffected by diet type, total FAA (TFAA) levels in the plasma on day 90 were likewise not different between groups (Table 7). In Experiment 2, where FCE was different due to fish meal type (Table 3), elevated essential FAA (EAA) ($F = 7.54$, $P < 0.007$), non-essential FAA (NEAA) ($F = 9.52$, $P < 0.002$), and consequently TFAA ($F = 10.8$, $P < 0.001$) plasma levels were observed in the high quality FM3ET groups (Table 8). In this case FCE, but not SGR, correlated with plasma TFAA ($r = 0.574$, $n = 12$, $P = 0.05$) and showed a close to significant correlation with plasma EAA ($r = 0.552$, $n = 12$, $P = 0.06$) on group basis.

After starving and re-feeding (day 93), fish in both experiments (Tables 7 & 8) showed lower plasma EAA/NEAA ratios in the high quality fish meal groups (FM1ET < FM2ET; FM3ET < FM4ET). These EAA/NEAA ratios showed a close to significant negative correlation with FCE in Experiment 1 ($r = -0.769$, $n = 17$, $P < 0.08$), but not in Experiment 2.

White muscle free amino acids and protein synthesis capacity

White muscle FAA concentrations and protein metabolism parameters on day 90 are shown in Tables 9 & 10. In both experiments the same pattern was seen; muscle TFAA concentrations were higher ($P < 0.03$) in the low quality fish meal groups (FM1ET < FM2ET; FM3ET < FM4ET) due to elevated NEAA levels. The muscle EAA/NEAA ratio was thus higher ($P < 0.05$) in the high quality fish meal groups (FM1ET > FM2ET; FM3ET > FM4ET). Interestingly, muscle TFAA correlated with trypsin specific activity in Experiment 2 on group basis ($r = -0.635$, $n = 12$, $P < 0.04$). However, this was not seen in Experiment 1 and neither did individual data show this correlation. Free hydroxyproline in the white muscle was lower ($P < 0.03$) in the high quality fish meal groups in Experiment 2 (FM3ET < FM4ET), but not different between groups in Experiment 1.

A significant effect of extrusion conditions was found on protein synthesis capacity (RNA/protein ratio) in the white muscle in both experiments (Tables 9 & 10), whereas protein concentration was unaffected by feed type. In Experiment 2, where a difference in FCE was observed, fish meal quality affected RNA concentration in the white muscle (FM3ET > FM4ET, $P < 0.005$). A similar tendency was seen in Experiment 1, but insignificant.

Discussion

Fish feed quality

Other characteristics of the feeds used in this study were reported in Rungruangsak-Torrissen *et al.* (2002). Both the extent of D-aspartic acid racemization (see Luzzana *et al.* 1996, 1999 for details), as well as SH-group oxidation with concurrent S–S bond formation (Opstvedt *et al.* 1984) are related to the thermal history of the fish raw material during processing, and increases with increasing drying time or processing temperatures (Rungruangsak-Torrissen *et al.* 2002). Both chemical reactions were concomitant with a decrease in protein digestibility both in mink as well as *in vitro* using fish crude enzyme extract (Rungruangsak-Torrissen *et al.* 2002).

Growth, feed intake and feed conversion efficiency

Under conditions approaching those of commercial aquaculture production, the extent of difference in protein qualities between the high and low quality feeds within each experiment had little or no effect on growth and feed

Table 5 Protease specific activities (μmol *p*-nitroaniline h^{-1} mg^{-1} protein) of trypsin (T), chymotrypsin (C), and total protease specific activity as well as the ratio of trypsin to chymotrypsin (T/C ratio) on days 0, 60 and 90 for all diet groups, and averaged by fish meal type, in Experiment 1. Values of *F* and associated probabilities (*P*) are given for a main factorial model (the *glm* procedure) considering the effects of fish meal quality (FM) and extrusion conditions (ET) during processing. Bold type indicate significant effects. Different superscripts identify significant differences ($P \leq 0.05$) between groups after *post hoc* testing (the *pdiff* option). All numbers are given as mean ± SEM

Enzyme parameter	FM1ET1	FM1ET2	FM1ET3	FM2ET1	FM2ET2	FM2ET3	Mean		FM		ET		FM × ET	
							FM1ET	FM2ET	*F*	*P*	*F*	*P*	*F*	*P*
Trypsin														
Day 0	151 ± 18	202 ± 23	169 ± 26	184 ± 39	190 ± 28	145 ± 30	–	–	–	–	–	–	–	–
Day 60	270 ± 23	324 ± 29	282 ± 27	300 ± 31	226 ± 75	269 ± 34	283 ± 18	261 ± 20	0.65	0.43	0.38	0.69	3.41	**0.04**
Day 90	202 ± 8	180 ± 8	183 ± 10	172 ± 8	177 ± 7	186 ± 9	189 ± 4.8	178 ± 5	2.14	0.14	0.47	0.63	1.89	0.15
Chymotrypsin														
Day 0	508 ± 90	651 ± 57	455 ± 77	492 ± 89	636 ± 144	444 ± 70	–	–	–	–	–	–	–	–
Day 60	974 ± 140	1250 ± 90	1035 ± 27	1169 ± 163	914 ± 305	1027 ± 184	1045 ± 81	1020 ± 104	0.01	0.94	0.46	0.64	2.33	0.11
Day 90	716 ± 39^{a}	694 ± 34^{a}	563 ± 30^{b}	549 ± 43^{b}	599 ± 30^{b}	567 ± 28^{b}	658 ± 19	572 ± 20	9.97	**0.002**	3.30	**0.04**	2.91	0.06
Total														
Day 0	659 ± 108	853 ± 80	624 ± 101	676 ± 122	827 ± 171	589 ± 99	–	–	–	–	–	–	–	–
Day 60	1244 ± 153	1573 ± 116	1317 ± 154	1470 ± 189	1140 ± 380	1295 ± 215	1328 ± 94	1281 ± 122	0.04	0.84	0.46	0.63	2.69	0.08
Day 90	918 ± 46^{a}	875 ± 40^{a}	746 ± 37^{b}	721 ± 48^{b}	776 ± 36^{b}	753 ± 34^{b}	846 ± 22	750 ± 24	8.85	**0.003**	2.18	0.11	2.92	0.06
T/C ratio														
Day 0	0.30 ± 0.02	0.31 ± 0.01	0.38 ± 0.03	0.37 ± 0.04	0.31 ± 0.03	0.32 ± 0.02	–	–	–	–	–	–	–	–
Day 60	0.30 ± 0.03	0.26 ± 0.01	0.28 ± 0.02	0.27 ± 0.02	0.27 ± 0.09	0.30 ± 0.04	0.28 ± 0.02	0.28 ± 0.02	0.03	0.85	0.65	0.53	0.33	0.72
Day 90	0.32 ± 0.01^{ac}	0.30 ± 0.02^{c}	0.36 ± 0.02^{a}	0.44 ± 0.06^{b}	0.33 ± 0.02^{ac}	0.36 ± 0.02^{ac}	0.33 ± 0.01	0.38 ± 0.01	8.37	**0.004**	4.01	**0.02**	3.68	**0.03**

Table 6 Protease specific activities (μmol *p*-nitroaniline h^{-1} mg^{-1} protein) of trypsin (T), chymotrypsin (C), and total protease specific activity as well as the ratio of trypsin to chymotrypsin (T/C ratio) on days 0, 60 and 90 for all diet groups, and averaged by fish meal type, in Experiment 2. Values of *F* and associated probabilities (*P*) are given for a main factorial model (the *glm* procedure) considering the effects of fish meal used (FM) and extrusion conditions (ET) during processing. Bold type indicates significant effects. Different superscripts identify significant differences ($P \leq 0.05$) between groups after *post hoc* testing (the *pdiff* option). All numbers are given as mean ± SEM

							Mean		FM		ET		FM × ET	
Enzyme parameter	FM3ET1	FM3ET2	FM3ET3	FM4ET1	FM4ET2	FM4ET3	FM3ET	FM4ET	*F*	*P*	*F*	*P*	*F*	*P*
Trypsin														
Day 0	124 ± 61	154 ± 29	154 ± 37	74 ± 48	104 ± 74	141 ± 41	–	–	–	–	–	–	–	–
Day 60	333 ± 71	394 ± 45	382 ± 78	249 ± 64	273 ± 22	230 ± 69	370 ± 37	250 ± 30	5.49	**0.03**	0.24	0.79	0.14	0.87
Day 90	237 ± 20	214 ± 24	238 ± 19	172 ± 20	239 ± 27	210 ± 18	230 ± 12	207 ± 13	1.67	0.20	0.63	0.54	2.17	0.12
Chymotrypsin														
Day 0	279 ± 159	404 ± 9	302 ± 93	95 ± 85	200 ± 196	294 ± 15	–	–	–	–	–	–	–	–
Day 60	891 ± 253^{a}	1036 ± 201^{a}	869 ± 207^{a}	581 ± 333	646 ± 140	296 ± 92^{b}	932 ± 121	499 ± 104	6.37	**0.02**	0.86	0.43	0.21	0.81
Day 90	438 ± 46^{a}	387 ± 51^{a}	420 ± 40^{a}	248 ± 37^{b}	333 ± 44	321 ± 36	415 ± 26	301 ± 23	10.9	**0.001**	0.22	0.81	1.31	0.27
Total														
Day 0	404 ± 221	558 ± 19	456 ± 130	168 ± 132	304 ± 270	434 ± 26	–	–	–	–	–	–	–	–
Day 60	1225 ± 322	1430 ± 235^{a}	1251 ± 280^{a}	830 ± 397	920 ± 157	526 ± 160^{b}	1302 ± 154	749 ± 127	6.54	**0.02**	0.66	0.53	0.21	0.81
Day 90	674 ± 64^{a}	601 ± 72^{a}	658 ± 58^{a}	420 ± 56^{b}	572 ± 70	530 ± 53	645 ± 37	508 ± 35	7.27	<**0.01**	0.33	0.72	1.62	0.20
T/C ratio														
Day 0	0.47 ± 0.05	0.38 ± 0.08	0.52 ± 0.04	1.59 ± 0.91	4.22 ± 3.78	0.49 ± 0.17	–	–	–	–	–	–	–	–
Day 60	0.41 ± 0.04^{a}	0.42 ± 0.06^{a}	0.47 ± 0.04^{ac}	0.65 ± 0.14^{bc}	0.49 ± 0.07^{ac}	0.79 ± 0.05^{b}	0.43 ± 0.03	0.64 ± 0.05	15.7	<**0.001**	3.90	**0.03**	2.23	0.13
Day 90	0.62 ± 0.06^{a}	0.83 ± 0.17	0.63 ± 0.05^{a}	1.08 ± 0.15	0.94 ± 0.12	1.17 ± 0.32^{b}	0.69 ± 0.06	1.06 ± 0.12	7.19	<**0.001**	0.04	0.96	0.92	0.40

Table 7 Concentrations (nmol mL^{-1}) of essential (EAA), non-essential (NEAA), the ratio of essential to non-essential (EAA/NEAA), and total (TFAA) free amino acids in the plasma on day 90, and 5–7 h postprandial levels on day 93 in all groups, and averaged by fish meal type, in Experiment 1. Values of *F* and associated probabilities are given for a main factorial model (the *glm* procedure) considering the effects of fish meal used (FM) and extrusion conditions (ET) during processing. Bold type indicates significant effects. Different superscripts identify significant differences ($P \leq 0.05$) between groups after *post hoc* testing (the *pdiff* option). All numbers are given as mean ± SEM

							Mean		FM		ET		FM × ET	
Plasma parameter	FM1ET1	FM1ET2	FM1ET3	FM2ET1	FM2ET2	FM2ET3	FM1ET	FM2ET	*F*	*P*	*F*	*P*	*F*	*P*
Day 90														
EAA	1215 ± 158	1334 ± 170	1275 ± 172	1539 ± 190	1777 ± 249	1388 ± 189	1266 ± 94	1585 ± 124	2.75	0.11	0.60	0.55	0.25	0.78
NEAA	2466 ± 156	2299 ± 385	3142 ± 471	2811 ± 428	2627 ± 245	2059 ± 313	2545 ± 179	2559 ± 209	0.21	0.65	0.15	0.86	2.14	0.14
EAA/NEAA	0.52 ± 0.10	0.64 ± 0.12	0.42 ± 0.07	0.68 ± 0.15	0.74 ± 0.14	0.73 ± 0.15	0.54 ± 0.06	0.71 ± 0.08	2.37	0.14	0.33	0.72	0.22	0.80
TFAA	3680 ± 118	3632 ± 397	4417 ± 517	4350 ± 364	4404 ± 268	3447 ± 368	3812 ± 179	4143 ± 208	0.31	0.59	0.03	0.97	3.27	0.053
Day 93														
EAA	1135 ± 76	1390 ± 86	1288 ± 111	1575 ± 65	1366 ± 53	1181 ± 152	1267 ± 57	1337 ± 75	1.33	0.26	1.11	0.34	3.42	**0.04**
NEAA	2465 ± 169	2807 ± 175	2783 ± 184	2655 ± 304	2652 ± 171	2347 ± 223	2684 ± 103	2521 ± 130	0.63	0.43	0.43	0.65	1.17	0.32
EAA/NEAA	0.46 ± 0.02^{a}	0.50 ± 0.02^{a}	0.46 ± 0.02^{a}	0.62 ± 0.05^{b}	0.53 ± 0.04^{a}	0.49 ± 0.02^{a}	0.47 ± 0.01	0.53 ± 0.02	9.40	**0.004**	2.64	0.08	2.97	0.06
TFAA	3600 ± 231	4197 ± 247	4071 ± 282	4229 ± 350	4018 ± 192	3528 ± 373	3951 ± 152	3858 ± 194	0.02	0.90	0.58	0.57	1.96	0.15

Table 8 Concentrations (nmol mL^{-1}) of essential (EAA), non-essential (NEAA), the ratio of essential to non-essential (EAA/NEAA), and total (TFAA) free amino acids in the plasma on day 90, and 5–7 h postprandial levels on day 93 in all groups, and averaged by fish meal type, in Experiment 2. Values of *F* and associated probabilities are given for a main factorial model (the *glm* procedure) considering the effects of fish meal used (FM) and extrusion conditions (ET) during processing. Bold type indicates significant effects. Different superscripts identify significant differences ($P \leq 0.05$) between groups after *post hoc* testing (the *pdiff* option). All numbers are given as mean ± SEM

							Mean		FM		ET		FM × ET	
Plasma parameter	FM3ET1	FM3ET2	FM3ET3	FM4ET1	FM4ET2	FM4ET3	FM3ET	FM4ET	*F*	*P*	*F*	*P*	*F*	*P*
Day 90														
EAA	2322 ± 98^{a}	2273 ± 134^{a}	1606 ± 69^{b}	1608 ± 89^{b}	2361 ± 249^{c}	2002 ± 93.6^{ac}	2007 ± 72	1754 ± 71	7.54	**0.007**	5.04	**0.007**	26.11	<**0.001**
NEAA	4955 ± 211^{a}	4454 ± 282^{ac}	3255 ± 138^{b}	3035 ± 140^{b}	3792 ± 245^{cd}	4011 ± 189.5^{cd}	4277 ± 144	3715 ± 129	9.52	**0.002**	3.41	**0.04**	14.11	<**0.001**
EAA/NEAA	0.49 ± 0.03	0.54 ± 0.03	0.51 ± 0.02	0.58 ± 0.06	0.58 ± 0.03	0.53 ± 0.03	0.49 ± 0.02	0.49 ± 0.02	0.01	0.92	0.17	0.84	5.84	**0.004**
TFAA	7277 ± 257^{a}	6726 ± 384^{a}	4861 ± 180^{b}	4644 ± 152^{b}	6153 ± 485^{c}	6013 ± 228.4^{c}	6284 ± 198	5469 ± 185	10.82	**0.001**	4.69	**0.01**	21.12	<**0.001**
Day 93														
EAA	1294 ± 150	1120 ± 24	1335 ± 62	1410 ± 30	1317 ± 82	1466 ± 142	1256 ± 68	1398 ± 53	2.64	0.12	1.37	0.28	0.07	0.93
NEAA	5065 ± 455	5365 ± 347	5725 ± 409	4984 ± 417	4972 ± 54	4864 ± 503	5339 ± 243	4940 ± 198	1.70	0.21	0.22	0.81	0.45	0.64
EAA/NEAA	0.26 ± 0.03	0.21 ± 0.01^{a}	0.23 ± 0.01	0.29 ± 0.03^{b}	0.27 ± 0.02	0.30 ± 0.01^{b}	0.24 ± 0.02	0.29 ± 0.01	5.96	**0.024**	1.36	0.28	0.40	0.68
TFAA	6359 ± 498	6485 ± 351	7060 ± 464	6394 ± 427	6289 ± 106	6330 ± 637	6595 ± 265	6338 ± 234	0.60	0.45	0.29	0.75	0.35	0.71

Table 9 Concentrations (nmol mg^{-1}) of essential (EAA), non-essential (NEAA), the ratio of essential to non-essential (EAA/NEAA), total (TFAA) free amino acids and free hydroxyproline, as well as RNA concentration (μg mg^{-1}), and protein synthesis capacity [RNA/protein ratio (μg mg^{-1})] in the white muscle (mean ± SEM) in all groups, and averaged by fish meal type, on day 90 of Experiment 1. Values of *F* and associated probabilities are given for a main factorial model (the *glm* procedure) considering the effects of fish meal used (FM) and extrusion conditions (ET) during processing. Bold type indicates significant effects. Different superscripts identify significant differences ($P \leq 0.05$) between groups after *post hoc* testing (the *pdiff* option). All numbers are given as mean ± SEM

							Mean		FM		ET		FM × ET	
White muscle parameter	FM1ET1	FM1ET2	FM1ET3	FM2ET1	FM2ET2	FM2ET3	FM1ET	FM2ET	*F*	*P*	*F*	*P*	*F*	*P*
EAA	2.63 ± 0.23	3.32 ± 0.39	2.73 ± 0.28	3.02 ± 0.31	2.87 ± 0.14	2.62 ± 0.24	2.90 ± 0.19	2.81 ± 0.13	0.15	0.71	1.33	0.28	1.11	0.34
NEAA	16.79 ± 0.99^{a}	20.52 ± 1.72	18.01 ± 1.09^{ac}	22.28 ± 2.87^{bc}	22.57 ± 1.86^{bc}	23.05 ± 1.80^{bd}	18.47 ± 0.83	22.68 ± 1.12	8.66	**0.007**	0.66	0.53	0.60	0.56
EAA/NEAA ratio	0.16 ± 0.01	0.17 ± 0.02^{a}	0.15 ± 0.02	0.14 ± 0.03	0.13 ± 0.02	0.11 ± 0.01^{b}	0.16 ± 0.01	0.13 ± 0.01	4.32	**0.05**	0.68	0.52	0.38	0.68
TFAA	19.42 ± 1.20^{a}	23.84 ± 1.63	20.74 ± 1.14	25.30 ± 2.81^{b}	25.45 ± 1.81^{b}	25.67 ± 1.99^{b}	21.37 ± 0.88	25.50 ± 1.14	8.11	**0.008**	0.85	0.44	0.82	0.45
Hydroxyproline	2.15 ± 0.19	2.51 ± 0.35	1.95 ± 0.34	2.35 ± 0.42	2.32 ± 0.38	2.15 ± 0.41	2.22 ± 0.17	2.26 ± 0.22	0.05	0.83	0.56	0.58	0.21	0.81
RNA	5.34 ± 0.16	5.17 ± 0.13	5.18 ± 0.14	5.25 ± 0.17	5.28 ± 0.15	4.95 ± 0.17	5.23 ± 0.08	5.15 ± 0.09	0.31	0.58	1.16	0.32	0.69	0.50
RNA/protein ratio	60.8 ± 5.9^{a}	95.6 ± 7.8^{bc}	108.4 ± 18.3^{bc}	83.2 ± 10.7^{ac}	123.8 ± 18.4^{b}	48.1 ± 4.1^{a}	88.3 ± 7.2	85.2 ± 8.3	0.10	0.75	5.23	**0.006**	8.10	**<0.001**

Table 10 Concentrations (nmol mg^{-1}) of essential (EAA), non-essential (NEAA), the ratio of essential to non-essential (EAA/NEAA), total (TFAA) free amino acids and free hydroxyproline, as well as RNA concentration (μg mg^{-1}), and protein synthesis capacity [RNA/protein ratio (μg mg^{-1})] in the white muscle (mean ± SEM) in all groups, and averaged by fish meal type, on day 90 of Experiment 2. Values of *F* and associated probabilities are given for a main factorial model (the *glm* procedure) considering the effects of fish meal used (FM) and extrusion conditions (ET) during processing. Bold type indicates significant effects. Different superscripts identify significant differences ($P \leq 0.05$) between groups after *post hoc* testing (the *pdiff* option). All numbers are given as mean ± SEM

							Mean		FM		ET		FM × ET	
White muscle parameter	FM3ET1	FM3ET2	FM3ET3	FM4ET1	FM4ET2	FM4ET3	FM3ET	FM4ET	*F*	*P*	*F*	*P*	*F*	*P*
EAA	3.51 ± 0.16	4.96 ± 0.36	5.02 ± 0.37	4.47 ± 0.30	4.51 ± 0.26	3.93 ± 0.22	4.65 ± 0.21	4.30 ± 0.15	0.58	0.45	2.80	0.06	5.19	**0.007**
NEAA	13.23 ± 0.67^{ad}	10.90 ± 0.38^{b}	9.22 ± 0.46^{b}	14.55 ± 0.80^{ac}	11.35 ± 0.67^{d}	12.47 ± 0.78^{d}	10.84 ± 0.33	12.77 ± 0.45	8.54	**0.004**	10.31	**<0.001**	2.29	0.10
EAA/NEAA ratio	0.28 ± 0.02	0.47 ± 0.03	0.58 ± 0.05	0.33 ± 0.03	0.41 ± 0.02	0.34 ± 0.03	0.46 ± 0.03	0.36 ± 0.01	8.87	**0.003**	12.00	**<0.001**	8.48	**<0.001**
TFAA	16.73 ± 0.66	15.85 ± 0.43^{ac}	14.24 ± 0.62^{a}	19.02 ± 0.91^{b}	15.86 ± 0.80^{ac}	16.39 ± 0.79^{c}	15.49 ± 0.34	17.08 ± 0.50	5.35	**0.02**	5.31	**0.006**	1.45	0.24
Hydroxyproline	1.07 ± 0.13^{a}	0.78 ± 0.06^{b}	0.68 ± 0.08^{b}	1.40 ± 0.10^{c}	0.78 ± 0.06^{b}	0.82 ± 0.07^{ab}	0.81 ± 0.05	1.00 ± 0.05	5.14	**0.02**	18.25	**<0.001**	1.86	0.16
RNA	4.81 ± 0.15^{a}	4.77 ± 0.15^{a}	4.75 ± 0.12^{a}	4.84 ± 0.12^{a}	3.81 ± 0.03^{b}	4.57 ± 0.17^{a}	4.78 ± 0.08	4.40 ± 0.11	8.04	**0.005**	5.69	**0.004**	5.23	**0.006**
RNA/protein ratio	112.1 ± 13.5	118.8 ± 26.1^{a}	138.0 ± 14.1^{a}	140.2 ± 13.7^{a}	63.7 ± 5.8^{b}	160.1 ± 26.8^{a}	123.1 ± 10.9	120.7 ± 10.9	0.01	0.91	5.16	**0.007**	3.27	**0.04**

conversion during a growth period of 3 months. However, several interesting observations were made that deserve further attention.

The different feeding methods used in Experiments 1 and 2 were reflected in the results. With *ad libitum* feeding in Experiment 1, no correlations were seen between feed intake, SGR and FCE. However, there was a tendency of slightly higher intake of the ET1-extruded feeds (Table 2). The differences in weight between the FM1ET and FM2ET groups were probably due to significantly lower start weights in the FM1ET2 group. In Experiment 2, the fish were on restricted rations for most of the experimental period (on average <1% of body weight per day) due to practical problems with hand feeding related to the small pellet size; 3.5 mm versus 12 mm recommended for fish of this size (Skretting A/S, Stavanger, Norway). Feeding data (Table 3) revealed that larger amounts of feed were distributed the first month during the 'running-in' of feeding routines, and probably in part explains both the higher growth rates and lower FCE seen in this period (from days 0 to 30). Weight differences between the FM3ET and FM4ET groups on days 30, 60, and 90 ($P < 0.0001$) were probably due to errors inherent to the lower initial weights of the FM4 groups. Collection of feed waste was not performed in Experiment 2, so the accuracy of the feed conversion estimates may be questionable. However, considering that feed intake did not differ between groups and that FCE correlated with growth rates ($r = 0.821$, $n = 12$, $P < 0.002$) by the end of the experiment (day 90), the FCE estimates seem reasonable. The significant differences in SGR seen when using the values from tagged fish only also suggest that the FCE estimates are accurate. The tagged fish had better condition and higher growth rates than the cage mean in 11 of 12 cases.

Starving the fish in Experiment 2 prior to feeding the experimental feeds may have had an adverse effect on protein and lipid contents in the fillet on day 0, as these were slightly lower than on day 90. There was a correlation between the protein-to-lipid ratio at the end of the experiment and group SGR ($r = 0.650$, $n = 12$, $P < 0.03$), suggesting that either growth rate, or a combination of feed protein quality and growth rate, affected fillet composition. There was a tendency of lower fat contents, as well as significantly higher muscle RNA concentrations in the fish fed higher digestible diets, suggesting that the balance between protein and lipid deposition was affected by feed protein quality. However, neither protein synthesis capacity (RNA/protein), muscle RNA concentrations nor muscle protein content correlated with growth rate.

Digestive enzyme activities

In the present experiment, chymotrypsin showed consistently higher specific activity than trypsin. This is in accordance with other studies where specific synthetic substrates were used (Kristinsson & Rasco 2000; Sunde *et al.* 2001), but may not be representative of the actual proteolytic activity *in situ*, where substrates are more complex. Rungruangsak-Torrissen & Male (2000) imitated protein digestion by these enzymes by using a common protein substrate (casein) and found trypsin-like activity to dominate digestion in the pyloric caeca of Atlantic salmon whereas both trypsin-like and chymotrypsin-like activities dominated digestion in the small and large intestine.

It is known that a change in feed proteins can affect the measured activity of digestive enzymes in mammals (e.g. Lhoste *et al.* 1993, 1994), as well as in fish (e.g. Abi-Ayad & Kestemont 1994; El-Saidy *et al.* 2000). In particular, adaptation of the pancreatic enzymes to dietary proteins has been described. Changes in the activity of these enzymes have also been associated with transition between different life stages, e.g. maturation in fish (Torrissen & Torrissen 1984, 1985) and moulting in shrimp (Klein *et al.* 1996; Van Wormhoudt *et al.* 1995). Lhoste *et al.* (1994) found digestive enzyme activity in rats to be regulated on either the transcriptional or post-transcriptional level, depending on the nature of the dietary protein, and also that protein source affected chymotrypsin, and to a lesser degree trypsin, specific activities. In contrast to the current study, after 30 days of feeding, Pacific shrimp (*Penaeus vannamei*) showed higher chymotrypsin activity when protein quality decreased, in this case correlating with higher feed content of aromatic amino acids (Ezquerra *et al.* 1997). El-Saidy *et al.* (2000), however, found that slow growing channel catfish fry (*Ictalurus punctatus* R.) showed suppressed trypsin, but not chymotrypsin, activity in the viscera after 13 weeks feeding on isonitrogenous starter diets with different concentrations of fish meal sources.

In order to explain the contrasting results listed above, Rungruangsak-Torrissen (2001) proposed a model for understanding the complex interactions of digestive enzyme activity and growth, by suggesting that organisms during growth are in one of two distinct physiological states, one of continuous growth under stable conditions, and one in which growth is interrupted due to changes in environmental and/or physiological conditions, including food deprivation. According to this hypothesis, trypsin correlates with growth rate under conditions where growth is uninterrupted, whereas chymotrypsin plays a role when growth opportunity is limited, such as in periods of food

deprivation or adaptation to new feed. The ratios of trypsin to chymotrypsin activity (T/C ratio), as well as trypsin specific activities, were therefore suggested as predictors of potential growth differences. Sunde *et al.* (2001) found positive correlations between trypsin specific activities, T/C ratios and growth rates when groups of salmon were subjected to different photoperiods. In cod, *Gadus morhua*, injected with recombinant somatotropin, trypsin specific activity was similarly found to increase with SGR and FCE (Lemieux *et al.* 1999). However, in the current study where growth rates were not different between groups, neither trypsin specific activity nor T/C ratio correlated with growth rates. Due to increased chymotrypsin activities during the steady growth phase, the T/C ratios instead were lower in groups with a potential for higher growth rates, in contrast to that observed when SGRs were different between groups in Sunde *et al.* (2001).

In light of previous (Rungruangsak Torrissen & Male 2000; Sunde *et al.* 2001) and the current studies, it seems that differences in FCE can be either accompanied by (Sunde *et al.* 2001), or preceded by (present study) differences in the digestive protease activity profile (T/C ratio) and that this parameter could indicate potential differences in feed utilization and growth. One can therefore suggest a possible use of these parameters for prediction of differences in dietary protein quality or the digestive ability of the organism that may in turn lead to differences in FCE and growth rate. The decrease in trypsin and chymotrypsin specific activities and increase in T/C ratios during the last period of steady growth in the current experiments may indicate a complete adaptation of the digestive system to the experimental feeds.

Plasma and white muscle free amino acids and protein synthesis capacity

In vitro digestion of protein yielded different patterns of FAA and peptides under different assay temperatures (Kristinsson & Rasco 2000), when using enzymes from fish possessing different trypsin phenotypes (Bassompierre *et al.* 1998; Rungruangsak Torrissen & Male 2000), when using extracts from fish adapted to different feeds (Rungruangsak-Torrissen *et al.* 2002), when enzyme-to-substrate ratio was altered (Robbins 1978), and when the ratio between the digestive enzymes in the extract was different (Kristinsson & Rasco 2000). Sveier *et al.* (2001) also demonstrated by adding potato trypsin inhibitors to fish feed that a change in digestive protease activity altered digestion and absorption rate of dietary protein in salmon. Similarly, Atlantic salmon with different digestive abilities (possessing different trypsin phenotypes) had different rates and levels of absorption and transport of FAA (Torrissen *et al.* 1994), affecting the FAA profile and insulin secretion (Rungruangsak-Torrissen & Sundby 2000), as well as the capacity for protein synthesis and protein turnover rate (Rungruangsak-Torrissen *et al.* 1999). Differences in enzyme specific activities or digestive enzyme profiles may therefore result in different plasma or muscle FAA profiles, that in turn can affect protein synthesis, which is stimulated both by the amount as well as the composition of the FAA pools in the tissues (Millward & Rivers 1988). The study of dietary FAA absorption, measured as the removal of EAA from the plasma (the EAA/NEAA ratio), seems to be more sensitive when sampled after a single meal than during routine feeding, especially when SGR differences are small between fish (Torrissen *et al.* 1994; Rungruangsak-Torrissen & Sundby 2000; present experiment). Both lower (present experiment; Sunde *et al.* 2003) and higher (Torrissen *et al.* 1994; Sunde *et al.* 2003) values have however been reported for fish with higher feed utilization, possibly depending on the metabolic status of the fish. The ratio of EAA/NEAA in the plasma were found to be lower 5–7 h postfeeding (day 93) in the fish fed high quality diets (Tables 7 & 8), in accordance with a rapid reduction in plasma EAA/NEAA ratio 0–6 h postfeeding in Arctic charr with higher feed utilization, but in contrast to an increase in plasma EAA/NEAA ratio up to 12 h postfeeding in salmon with higher feed utilization (Rungruangsak Torrissen & Male 2000). In white muscle, EAA levels has been found to decrease following feeding in rainbow trout (Carter *et al.* 1995). Lower EAA/NEAA ratios in the white muscle were also reported in fish with higher SGR and FCE (Sunde *et al.* 2001). In contrast, the higher muscle EAA/NEAA ratio observed in fish with higher FCE in the current experiment might be due to the fact that their growth phase was different from those in Sunde *et al.* (2001). The range of growth rates in Sunde *et al.* (2001) was also larger than in the current study. The lower muscle free hydroxyproline concentration observed in fish given high quality diets in Experiment 2 is in contrast to Torrissen *et al.* (1994), Rungruangsak Torrissen & Male (2000) and Sunde *et al.* (2001), where higher feed utilization and growth was concomitant with higher levels of free hydroxyproline in the white muscle. Carter *et al.* (1995) observed an increase in free hydroxyproline and free proline concentrations in caecum and liver after feeding, but found white muscle free hydroxyproline to be unaffected by feeding a single meal. In the present experiment, where the fish had regular access to different types of feed, differences in both muscle free hydroxyproline and EAA pools were found.

Differences in white muscle FAA pools were also observed at 6 h postfeeding in salmon with genetic differences in feed utilization (Rungruangsak Torrissen & Male 2000). Considering the conclusions of Carter *et al*. (1995) in light of the present experiment, one can imply that like feeding has an effect on white muscle FAA pool composition, genetic variation in feed utilization (Rungruangsak-Torrissen & Male 2000), different stages of growth (Rungruangsak-Torrissen 2001; Sunde *et al*. 2001; present experiment), or different feed protein qualities (present experiment) may also give rise to differences in white muscle FAA pools. Trypsin specific activity may be involved, as it showed a negative correlation with muscle TFAA concentrations in Experiment 2 ($r = -0.635$, $n = 12$, $P < 0.04$), probably suggesting a higher FAA assimilation for muscle protein synthesis in fish with a higher digestive capacity. Concurrently, a higher level of RNA in the white muscle indicated a higher rate of protein synthesis, and thus a higher growth potential, in the high quality fish meal groups in Experiment 2 (Table 10), but were not significantly different in Experiment 1 (Table 9), where differences in FCE were insignificant. However, correlations between RNA concentrations and FCE or SGR on individual or group level were not observed in either experiment. Foster *et al*. (1993a) found RNA measurements of intestine and stomach to be more sensitive than white muscle to alterations in nutrition, and deviations from the correlation between growth rates and RNA concentration in the white muscle have been reported in several studies (e.g. Pelletier *et al*. 1995; Sunde *et al*. 2001). In addition, a changed protein synthesis rate may result from either a change in the number of ribosomes (estimated as tissue RNA concentration) or an altered synthetic activity per ribosome, the RNA activity (Houlihan 1991). For instance, Foster *et al*. (1993b) found RNA activity to be affected by temperature in juvenile cod. Protein synthesis capacity (RNA/protein), another growth rate correlate, also did not differ between diet groups in either experiment. The appearance of free hydroxyproline in muscle tissue could be an indicator of catabolic as well as anabolic activity in the muscle tissue (Torrissen *et al*. 1994; Rungruangsak Torrissen & Male 2000; Sunde *et al*. 2001), and fish fed high quality feeds could probably have a lower protein turnover rate during steady growth, as lower free hydroxyproline levels were observed in the white muscle of these fish.

Conclusion

Fish feeds differing in *in vitro* digestibility, due to different degrees of disulphide bond formation, were used in a salmon growth study to determine the effect on digestive enzyme activities, growth and feed utilization. The extent of difference between dietary qualities and duration of experiment determined the significance of differences in digestive enzyme profiles of trypsin and chymotrypsin in the diet-adapted fish. Measuring digestive enzyme activity profiles (trypsin to chymotrypsin ratio) could be an alternative way of predicting whether growth differences might be experienced between fish groups, as digestive enzyme activities were different one month prior to observed differences in feed utilization. Concurrent monitoring of plasma and muscle FAA, and RNA and protein levels in the white muscle provided more information on muscle growth and protein metabolism as well as the metabolic status of the fish.

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References

Abi-Ayad, A. & Kestemont, P. (1994) Comparison of the nutritional status of goldfish (*Carassius auratus*) larvae fed with live, mixed or dry diet. *Aquaculture*, **128,** 163–176.

Ahsan, M.N., Funabara, D. & Watabe, S. (2001) Molecular cloning and characterization of two isoforms of trypsinogen from anchovy pyloric caeca. *Mar. Biotechnol*., **3,** 80–90.

Anderson, J.S., Lall, S.P., Anderson, D.M. & McNiven, M.A. (1993) Evaluation of protein quality in fish meals by chemical and biological assays. *Aquaculture*, **115,** 305–325.

Ashford, A.J. & 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.

Bassompierre, M., Ostenfeld, T.H., McLean, E. & Rungruangsak-Torrissen, K. (1998) *In vitro* protein digestion, and growth of Atlantic salmon with different trypsin isozymes. *Aquacult. Int*., **6,** 47–56.

Boonvisut, S. & Whitaker, J.R. (1976) Effect of heat, amylase and disulphide bond cleavage on the in vitro digestibility of soybean proteins. *J. Agric. Food Chem*., **24,** 1130–1135.

Carter, C.G., He, Z.-Y., Houlihan, D.F., McCarthy, I.D. & 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.

Cowey, C.B., Pope, J.A., Adron, J.W. & Blair, A. (1972) Studies on the nutrition of marine flatfish. The protein requirement of plaice (*Pleuronectes platessa*). *Br. J. Nutr*., **28,** 447–456.

El-Saidy, D.M.S.C., Dabrowski, K. & Bai, S.C. (2000) Nutritional effects of protein source in starter diets for channel catfish in suboptimal water temperature. *Aquacult. Res.*, **31,** 885–892.

Ezquerra, J.M., García-Carreño, F.L. & Haard, N.F. (1997) Effects of feed diets on digestive proteases from the hepatopancreas of white shrimp (*Penaeus vannamei*). *J. Food Biochem.*, **21,** 401–419.

Fauconneau, B., Aguirre, P. & Blanc, J.M. (1990) Protein synthesis in different tissues of mature rainbow trout (*Salmo gairdneri* R.). Influence of triploidy. *Comp. Biochem. Physiol.*, **97C,** 345–352.

Foster, A.R., Houlihan, D.F. & 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. J. Fish. Aquat. Sci.*, **50,** 502–512.

Foster, A.R., Hall, S.J. & 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.

Friedman, M. (1994) Improvements in the safety of foods by SH-containing amino acids and peptides. A review. *J. Agric. Food Chem.*, **42,** 3–20.

Friedman, M., Zahnley, J.C. & Masters, P. (1981) Relationship between in vitro digestibility of casein and its content of lysinoalanine and D-amino acids. *J. Food Sci.*, **46,** 127–131,

Friedman, M., Grosjean, O.-K. & Zahnley, J.C. (1982) Inactivation of soya bean trypsin-inhibitors by thiols. *J. Sci. Food Agric.*, **33,** 165–172.

Houde, D.E. & 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. (1991) Protein turnover in ectotherms and its relationships to energetics. In: *Advances in Comparative and Environmental Physiology*, Vol. 7 (Gilles, R. ed.), pp. 1–43. Springer-Verlag, Berlin.

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

Klein, B., LeMoullac, G., Sellos, D. & Van Wormhoudt, A. (1996) Molecular cloning and sequencing of trypsin cDNAs from *Penaeus vannamei* (Crustacea, Decapoda): use in assessing gene expression during the moult cycle. *Int. J. Biochem. Cell Biol.*, **28,** 551–563.

Kristinsson, H.G. & Rasco, B.A. (2000) Hydrolysis of salmon muscle proteins by an enzyme mixture extracted from Atlantic salmon (*Salmo salar*) pyloric caeca. *J. Food Biochem.*, **24,** 177–187.

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

Lhoste, E.F., Fiszlewicz, M., Gueugneau, A.-M. & Corring, T. (1993) Effects of dietary proteins on some pancreatic mRNAs encoding digestive enzymes in the pig. *J. Nutr. Biochem.*, **4,** 143–152.

Lhoste, E.F., Fiszlewicz, M., Gueugneau, A.-M. & Corring, T. (1994) Adaptation of exocrine pancreas to dietary proteins: effect of the nature of protein and rat strain on enzyme activities and messenger RNA levels. *J. Nutr. Biochem.*, **5,** 84–94.

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

Luzzana, U., Mentasti, T., Moretti, V.M., Albertini, A. & Valfrè, F. (1996) Aspartic acid racemization in fish meal as induced by thermal treatment. *Aquacult. Nutr.*, **2,** 95–99.

Luzzana, U., Mentasti, T., Opstvedt, J., Nygård, E., Moretti, V.M. & Valfrè, F. (1999) Racemization kinetics of aspartic acid in fish material under different conditions of moisture, pH, and oxygen pressure. *J. Agric. Food Chem.*, **47,** 2879–2884.

Male, R., Lorens, J.B., Smalås, A.O. & Torrissen, K.R. (1995) Molecular clotting and characterization of anionic and cationic variants of trypsin from Atlantic salmon. *Eur. J. Biochem.*, **232,** 677–685.

Millward, D.J. & Rivers, J.P.W. (1988) The nutritional role of indispensable amino acids and the metabolic basis for their requirements. *Eur. J. Clin. Nutr.*, **42,** 367–393.

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

Opstvedt, J., Miller, R., Hardy, R.W. & Spinelli, J. (1984) Heat-induced changes in sulphydryl groups and disulfide bonds in fish protein and their effect on protein and amino acid digestibility in rainbow trout (*Salmo gairdneri*). *J. Agric. Food Chem.*, **32,** 929–935.

Opstvedt, J., Søbstad, G. & Hansen, P. (1987) Fish protein concentrates for pre-ruminant calves: effects of processing temperatures and source of fish on protein digestibility and biological value. *Anim. Feed Sci. Technol.*, **18,** 181–196.

Opstvedt, J., Nygård, E., Samuelsen, T. A., Venturini, G., Luzzana, U. & Mundheim, H. (2003) Effect on protein digestibility of different processing conditions in the production of fish meal and fish feeds. *J. Sci. Food Agric.*, **83,** 775–782.

Pelletier, D., Blier, P.U., Lambert, Y. & Dutil, J.-D. (1995) Deviation from the general relationship between RNA concentration and growth rate in fish. *J. Fish Biol.*, **47,** 920–922.

Pike, I.H., Andorsdóttir, G. & Mundheim, H. (1990) The role of fish meals in diets for salmonids. *IAFMM Tech. Bull.*, **24,** 35 pp.

Robbins, R.C. (1978) Effect of ratio of enzymes to substrate on amino acid patterns released from proteins *in vitro*. *Int. J. Vit. Nutr. Res.*, **48,** 44–53.

Rungruangsak-Torrissen, K. (2001) Important parameters for growth study. *The 2nd International Symposium on Cultivation of Salmon II (Abstract)*, Bergen, Norway, 7–10 May, p. 14.

Rungruangsak Torrissen, K. & Male, R. (2000) Trypsin isozymes: development, digestion and structure. In: *Seafood Enzymes, Utilization and Influence on Postharvest Seafood Quality* (Haard, N.F. & Simpson, B.K. eds), pp. 215–269. Marcel Dekker, Inc., New York, USA.

Rungruangsak-Torrissen, K. & 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., Pringle, G.M., Moss, R. & Houlihan, D.F. (1998) Effects of varying rearing temperatures on expression of different trypsin isozymes, feed conversion efficiency and growth in Atlantic salmon (*Salmo salar* L.). *Fish Physiol. Biochem.*, **19,** 247–255.

Rungruangsak-Torrissen, K., Carter, C.G., Sundby, A., Berg, A. & 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., Rustad, A., Sunde, J., Eiane, S., Jensen, H., Opstvedt, J., Nygard, E., Samuelson, T., Mundheim, H., Luzzana, U. & Venturini, G. (2002) *In vitro* digestibility based on fish crude enzyme extract for prediction of feed quality in growth trials. *J. Sci. Food Agric.*, **82,** 644–654.

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

Sunde, J., Taranger, G.L. & Rungruangsak-Torrissen, K. (2001) 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. Fish Physiol. Biochem.*, **25,** 335–345.

Sunde, J., Kiessling, A., Higgs, D., Opstvedt, J., Venturini, G. & Rungruangsak-Torrissen, K. (2003) Evaluation of feed protein quality by measuring plasma free amino acids in Atlantic salmon (*Salmo salar* L.) after dorsal aorta cannulation. *Aquacult. Nutr.*, **9,** 351–360.

Sveier, H., Kvamme, B.O. & Raae, A.J. (2001) Growth and protein utilization in Atlantic salmon (*Salmo salar* L.) given a protease inhibitor in the diet. *Aquacult. Nutr.*, **7,** 255–264.

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. & Torrissen, O.J. (1984) Digestive proteases of Atlantic salmon (*Salmo salar*) from different river strains: development after hatching, rearing temperature effect and effect of sex and maturation. *Comp. Biochem. Physiol.*, **77B,** 15–20.

Torrissen, K.R. & Torrissen, O.J. (1985) Protease activities and carotenoid levels during the sexual maturation of Atlantic salmon (*Salmo salar*). *Aquaculture*, **50,** 113–122.

Torrissen K.R., Lied E. & 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.

Torrissen, K.R., Lied, E. & Espe, M. (1995) Differences in utilization of dietary proteins with varying degrees of partial pre-hydrolysis in Atlantic salmon (*Salmo salar* L.) with genetically different trypsin isozymes. In: *Biopolymers and Bioproducts: Structure, Function and Applications* (Svasti, J., Rimphanitchayakit, V., Tassanakajorn, A., Pongsawasdi, P., Sonthayanon, B., Packdibamrung, K., Soontaros, S., Limpaseni, T., Wilairat, P., Boonjawat, J. & Kamolsiripichaiporn, S. eds), pp. 432–442. Proceedings of the 11th FAOBMB Symposium, Samakkhisan (dokya) Public Company Limited, Bangkok, Thailand.

Van Wormhoudt, A., Sellos, D., Donval, A., Plaire-Goux, S. & Le Moullac, G. (1995) Chymotrypsin gene expression during the intermolt cycle in the shrimp *Penaeus vannamei* (Crustacea; Decapoda). *Experientia*, **51,** 159–163.

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