DIGESTIVE EFFICIENCY, GROWTH AND QUALITIES OF MUSCLE AND OOCYTE IN ATLANTIC SALMON (SALMO SALAR L.) FED ON DIETS WITH KRILL MEAL AS AN ALTERNATIVE PROTEIN SOURCE

KRISNA RUNGRUANGSAK-TORRISSEN1

Institute of Marine Research–Matre
N-5984 Matredal, Norway

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ABSTRACT

Atlantic krill meal and Antarctic krill meal were tested to replace fish meal in Atlantic salmon diets. Different techniques were applied to precisely evaluate dietary quality and quality of fish growth performance. Inverse relationship between trypsin specific activity (T) and ratio of trypsin to chymotrypsin (T/C ratio) as a result of increased chymotrypsin specific activity (C) in the pyloric ceca indicated fish growth status as a reduction in growth rate. These protease values in the feces could also indicate fish digestive efficiency, but could not predict fish growth status. There were relationships among feed conversion efficiency (FCE), in vitro digestibility, and pyloric cecal T/C ratio, with inverse levels of krill meal in the diets. Krill meal seemed to increase muscle protein concentration through increasing protein retention, as capacity for protein synthesis was reduced. Dietary quality tests by in vitro digestibility were corresponded with growth studies, and indicated a possibility of inclusion of krill meal at 50–60% replacements, and larger fish were more sensitive to dietary quality than smaller ones. At 80–100% replacements, in vitro digestibility and FCE reduced, and oocyte quality changed through increased trypsin-like specific activity probably because of less or abnormal oocyte development.

PRACTICAL APPLICATIONS

The work illustrates differences in digestive efficiency and the quality of growth performance (protein growth efficiency and capacity for protein synthesis in muscle and oocytes) in fish feeding on diets with different levels of krill meal as fish meal replacer. The advantage of using various methods simultaneously for evaluating digestive efficiency and the quality of fish

1 Corresponding author. TEL: +47-56-36-7539; FAX: +47-56-36-7585; EMAIL: Krisnart@imr.no
growth performance is to precisely provide reasonable information for some important biological differences between fish groups that could not be explained by using growth parameter alone, especially when the numbers of samples are not high. The methods are very practical for studying food quality, food utilization and growth quality of fish in different environmental conditions and with different behaviors in aquaculture as well as in natural ecosystem where food consumption rate cannot be measured. The *in vitro* digestibility method, using fish crude enzyme extracts and standardized by trypsin activity, could also compare the digestive ability between different species and provide information on food quality in fish growth trials that may not be necessary to be performed.

**INTRODUCTION**

The requirement of protein source for the replacement of fish meal in aquaculture is high. Many studies have been conducted on the use of soybean protein as a fish meal replacer, and today, its use may have an impact on gene modified (GM) material in animal feeds as almost all soybean meals produced are from GM soybeans. In Norway, the amount of acceptable GM materials in animal feeds without notifying is ≤2%. Although vegetable proteins can save fish farming, salmon fed with marine proteins are still preferable. Recently, Krogdahl *et al.* (2003) expressed a caution in the use of extracted soybean meal in salmon feeds even at low levels as it affected pathohistological changes in the intestine as well as the pancreatic response of trypsin activity detected in feces. There are many other protein sources, and the most suitable ones are marine proteins found in the sea. Krill, the tiny shrimplike zooplankton abundant in the oceans, could become a good source of protein meal for the fish feed industry. Krill meal-supplemented diets have been reported to stimulate olfactory and gustatory responses in sea bream (Shimizu *et al.* 1990), improve larval performance in shrimp (Koshio *et al.* 1992) and improve feeding behaviors in different species (Kubitza and Lovshin 1997; Kuzmin *et al.* 1999; Campbell and Phelps 2002). Supplementation of krill meal in broodstock diets improved reproductive performance (Verakunpiriya *et al.* 1996; Izquierdo *et al.* 2001). Moreover, 30% krill meal supplemented diet with fluoride concentration of 35.8 mg% did not lead to fluoride accumulation in the tissue of Atlantic salmon after 12 weeks of feeding (Julshamn *et al.* 2004). Around 200 million tons of krill and other harvestable large zooplankton have been estimated in Norwegian waters alone (Hjellestad 2001). Before the harvest of krill and catch preserving becomes feasible, it is of importance to know the feasible limit of using krill as a fish meal replacer in aquaculture feeds.
The aim of the work was to evaluate the use of krill meals from different sources at varied levels in diets for Atlantic salmon (*Salmo salar* L.) of different sizes. Various methods, previously used in different studies, were employed simultaneously for the precise evaluation of the dietary quality and quality of fish growth performance in the current experiments. Feed *in vitro* digestibility has been shown to correlate with the chemical qualities of dietary proteins (levels of reactive sulfhydryl group, disulfide bond, and D-aspartic acid), the feed conversion efficiency (FCE) of salmon (Rungruangsak-Torrissen *et al.* 2002) and the fish growth capacity in rainbow trout (Rungruangsak-Torrissen *et al.* 2006a). Fish digestive efficiency and growth potential have been evaluated by studying trypsin specific activity and the activity ratio of trypsin to chymotrypsin (T/C ratio) (Rungruangsak-Torrissen and Sundby 2000; Sunde *et al.* 2001, 2004; Rungruangsak-Torrissen *et al.* 2006a, 2006c; Rungruangsak-Torrissen and Fosseidengen 2007). Muscle growth and muscle quality has been studied by measuring RNA and protein concentrations (Sunde *et al.* 2001, 2004; Rungruangsak-Torrissen and Fosseidengen 2007), and oocyte quality by the analyses of trypsin-like specific activity as well as the RNA and protein concentrations (Rungruangsak-Torrissen *et al.* 2006b; Rungruangsak-Torrissen and Fosseidengen 2007).

**MATERIALS AND METHODS**

**Krill Diets**

Krill meals, produced from different krills (omnivorous zooplanktons), were used as fish meal replacers for the production of different experimental diets for Atlantic salmon. Atlantic krill (*Meganyctiphanes norvegica*) was used in Experiment 1, and Antarctic krill (*Euphausia superba*) was used in Experiment 2. Krill meals and experimental krill diets were produced at the Norwegian Institute of Fisheries and Aquaculture Research (Department SSF, Fyllingsdalen, Norway). Two sets of experimental diets (three diets for Experiment 1 and six diets for Experiment 2) were prepared. Preliminary tests of the dietary qualities for use in both experiments were performed by using the *in vitro* digestibility method modified from Rungruangsak-Torrissen *et al.* (2002) and Bassompierre *et al.* (1998).

**Experiment 1: Effects of Atlantic Krill Meal**

Three different krill diets (K0, K25, K50) were produced by replacing fish meal with Atlantic krill meal at 0, 25 and 50%, respectively. The experimental diets (3.5-mm pellet) contained 45% protein and 28% lipid, and the
compositions of the experimental diets are shown in Table 1. The feeding trial was carried out at the Institute of Marine Research, Matre, Norway. Atlantic salmon (approximately 100 g) were reared in freshwater in 2-m³ tank (100 fish per tank). In order to start with the fish at a low metabolic rate that would consume the new diet immediately, the fish were starved for 2 days before feeding the experimental diets. The experiment was performed for 126 days from September 2001 to January 2002 at a temperature range of 4.4–12.0°C (mean temperature 7.4°C). The fish were fed ad libitum in order to avoid restricted growth, and the diets were distributed using automatic rotating feeders, 7 h/day, from 7:00 a.m. to 2:00 p.m. The experiment was run in duplicate. Individual fish weight was measured on days 0, 75 and 110, whereas 10 and 15 individuals per tank were sampled and weighed on days 90 and 126, respectively. Individual specific growth rate (SGR) was calculated according to Houde and Schekter (1981):

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

### Table 1. Compositions of the Experimental Diets in Experiment 1

<table>
<thead>
<tr>
<th>Feed composition (%)</th>
<th>K0 (No krill meal)</th>
<th>K25 (25% krill meal)</th>
<th>K50 (50% krill meal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal 620</td>
<td>58.9</td>
<td>45.5</td>
<td>32.0</td>
</tr>
<tr>
<td>Krill meal</td>
<td>–</td>
<td>16.0</td>
<td>32.0</td>
</tr>
<tr>
<td>NorSeaOil</td>
<td>20.4</td>
<td>19.3</td>
<td>18.3</td>
</tr>
<tr>
<td>Soyalectin</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Suprex</td>
<td>18.4</td>
<td>16.9</td>
<td>15.4</td>
</tr>
<tr>
<td>Inositol</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
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<tr>
<td>Betafin</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
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<tr>
<td>Vitamin mix*</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Mineral mix*</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Vitamin C (25%)</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Carophyll pink</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
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</tbody>
</table>

Analytical values (%)

<table>
<thead>
<tr>
<th>Moisture</th>
<th>Protein</th>
<th>Lipid</th>
<th>Carbohydrate†</th>
<th>Ash</th>
<th>Gross energy (MJ/kg)</th>
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<tbody>
<tr>
<td>4.8</td>
<td>45.4</td>
<td>28.1</td>
<td>14.9</td>
<td>6.8</td>
<td>24.5</td>
</tr>
<tr>
<td>5.0</td>
<td>45.5</td>
<td>28.0</td>
<td>14.2</td>
<td>7.3</td>
<td>24.2</td>
</tr>
<tr>
<td>5.2</td>
<td>45.3</td>
<td>28.0</td>
<td>13.6</td>
<td>7.9</td>
<td>24.0</td>
</tr>
</tbody>
</table>

* Provide per kilogram of feed: 250-mg vitamin C, 3,000-IU vitamin A, 1,600-IU vitamin D, 160-IU vitamin E, 12-mg vitamin K, 12-mg thiamine, 24-mg riboflavin, 12-mg pyridoxine, 120-mg niacin, 6-mg folate, 0.024-mg vitamin B12, 0.6-mg biotin, 48-mg Ca-pantothenate, 3-mg Cu, 2.4-mg I, 24-mg Fe, 21-mg Mn, 30-mg Zn and 0.1-mg Se.

† Calculated by difference.
where \( g = \frac{(\ln W_t - \ln W_0)}{(t - t_0)} \), \( W_t = \) Weight at day \( t \), \( W_0 = \) Weight at day \( t_0 \).

Pyloric ceca samples were collected from individual salmon, 10 fish per tank on day 90 and 15 fish per tank on day 126, and were kept at \(-80\)°C for later determination of digestive efficiency. The fish were not provided the diets on the sampling days. The protease specific activities of trypsin (T) and chymotrypsin (C) as well as the protease activity T/C ratio were measured as indicators for fish digestive efficiency, as described in Sunde et al. (2001) and Rungruangsak-Torrissen et al. (2002). The epaxial white muscle was also sampled from individual salmon at the end of the experiment on day 126 (15 fish per tank), and kept at \(-80\)°C. The concentrations of RNA, protein and protein synthesis capacity as RNA/protein ratio (Carter et al. 1993) were used as indicators for muscle growth and muscle quality, as described in Sunde et al. (2001, 2004).

**Experiment 2: Effects of Antarctic Krill Meal**

Six different krill diets (K00, K20, K40, K60, K80, K100) were produced by replacing the fish meal with Antarctic krill meal at 0, 20, 40, 60, 80 and 100%, respectively. The experimental diets (4-mm pellet) contained 45% protein and 28% lipid, and were prepared similarly to those in Experiment 1. Atlantic salmon (approximately 1 kg) were reared in seawater of salinity 30 (on the Practical Salinity Scale 1978) in a 2-m³ tank (20 fish per tank). The fish were also starved for 2 days, to induce a low metabolic rate, before feeding the experimental diets for 168 days from November 2002 to April 2003 with a temperature range of 7.2–11.9°C (mean temperature 9.90 ± 0.15°C). The experiment was also run in duplicate. In order to avoid restricted growth, daily ration was adjusted *ad libitum* to more than 1% of body weight and distributed using automatic rotating feeders, 7 h/day, from 7:00 a.m. to 2:00 p.m. Daily consumption rate of each tank was recorded in the afternoon after 2:30 p.m. by increasing water inflow to the tanks and collecting unconsumed feed in the outlet. On days 76 and 146, calculations of FCE were performed for each tank as kilogram weight gained per kilogram dry feed consumed. Fish weight was measured individually on days 0, 76, 146 and 168. The fish were not provided the diets on the sampling days. The pyloric ceca samples were collected on days 76 and 168 (5–10 individuals per tank), and the epaxial white muscle was sampled at the end of the experiment on day 168. All samples were kept frozen at \(-80\)°C for later determinations of digestive efficiency, muscle growth and muscle quality as described in Experiment 1. Individual SGR was calculated as in Experiment 1. At the end of the experiment, oocytes were also dissected from the female fish and kept frozen at \(-80\)°C for later determinations of trypsin-like specific activity, and RNA and protein concentrations as suggested
Determination of *In Vitro* Digestibility

Two sets of dialyzed crude enzyme extracts for *in vitro* digestibility tests were prepared from the pyloric ceca of Atlantic salmon that had never been fed with the experimental diets. Seven samples from 100-g salmon for Experiment 1 and four samples from 1-kg salmon for Experiment 2 were collected. After removing the fat by dissection, pyloric ceca samples were homogenized (1:3 w/v) on ice in 50-mM Tris buffer pH 8.2 containing 200-mM NaCl. The homogenate was centrifuged at 15,000 \( \times g \) for 60 min at 4°C, and the supernatant was dialyzed overnight at 4°C against a 10-mM phosphate buffer pH 8.2 using Pierce Slide-A-Lyzer Dialysis Cassette (Pierce Chemical Company, Rockford, IL). The dialyzed crude enzyme extracts obtained were concentrated using Pierce Slide-A-Lyzer Concentrating Solution (Pierce Chemical Company) and kept frozen at \(-80^\circ\text{C}\) until final use. *In vitro* digestibility was performed as described in Rungruangsak-Torrissen *et al.* (2002), modified by using a 50-mM phosphate buffer pH 8.2 to increase buffering capacity. The *in vitro* digestion mixture containing a 20-mg diet (in 40 mL of 50-mM phosphate buffer pH 8.2 and 0.2 mL of 0.5% chloramphenicol as bacteriostatic agent) was digested with 0.5 mL of enzyme extract. After exactly 24 h of digestion at 15°C, the pH of the digested mixtures was reduced from 8.2 to 7.9–8.1. The liberated reactive amino groups of peptides was determined by the trinitrobenzene sulfonic acid (TNBS) method, in the reaction mixture containing 0.2 mL of digested mixture, 2 mL of 50-mM phosphate buffer pH 8.2 and 1 mL of 0.1% TNBS in 50-mM phosphate buffer pH 8.2, modified from Ihekoronye (1986), Bassompierre *et al.* (1998) and Rungruangsak-Torrissen *et al.* (2002).

Digestion was performed in triplicate for each diet and each dialyzed enzyme extract, 21 assays (7 enzyme extracts \( \times 3 \) replicates) for each diet using the enzyme extracts from 100-g salmon, and 12 assays (4 enzyme extracts \( \times 3 \) replicates) for each diet using the enzyme extracts from 1-kg salmon. A commercial feed containing 46% protein and 22% lipid (Bio-Optimal SVEV, BioMar AS, Trondheim, Norway) used before the start of the experiments was also tested as another control *in vitro* digestibility value aside from the experimental control diets, K0 and K00. The *in vitro* digestibility value was expressed as nmol or \( \mu \text{mol} \) DL-alanine equivalent liberated reactive amino group of cleaved peptides per milligram feed sample after standardizing trypsin activity in the dialyzed crude enzyme extracts to 500-\( \mu \text{mol} \) p-nitroaniline produced/h/mL. Trypsin activity of each dialyzed crude enzyme extract was determined by measuring the rate of p-nitroaniline produced during the first minute of reaction as described in Rungruangsak-Torrissen *et al.* (2002).
Determinations of Trypsin and Chymotrypsin Specific Activities

Pyloric ceca and feces sampled from individual salmon were analyzed for trypsin and chymotrypsin specific activities. Oocytes sampled from maturing females were analyzed for trypsin-like specific activity. Protease activities of trypsin/trypsin-like and chymotrypsin were determined using specific enzyme substrates as described in Sunde et al. (2001) and Rungruangaksak-Torrissen et al. (2002). Briefly, crude enzyme extract was obtained after homogenizing pyloric ceca sample with 1-mM HCl (1:5 w/v) and centrifuging at 15,000 × g at 4°C for 60 min, and kept at −80°C for final use (Rungruangak and Utne 1981). The oocyte sample was extracted with 0.2-M Tris buffer pH 8.4. The enzymatic activities were determined spectrophotometrically at 410 nm by measuring the initial reaction rates of p-nitroaniline production within 60 s, using the Pharmacia Biotech Ultraspec 4000 with Peltier heated cell holder (Pharmacia Biotech, Uppsala, Sweden), with 1.25-mM benzoyl-L-arginine-p-nitroanilide as trypsin or trypsin-like substrate and 0.1-mM N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide as chymotrypsin substrate. Each substrate was dissolved in dimethylformamide (5% final concentration) before making up to final volume with 0.2-M Tris buffer pH 8.4. The enzyme activities were assayed at around their optimal temperatures (Fig. 1A, adapted from Rungruangaksak-Torrissen and Male 2000) in enzyme reaction mixture containing 10 μL of crude enzyme extract, and 1,000 μL of preheated specific substrate at 50°C for trypsin or trypsin-like and 40°C for chymotrypsin. It is important to note on the thermostability study of the enzymes in the absence of substrates that 40% of trypsin activity remained after a 30-min incubation at 50°C, while chymotrypsin activity remained intact after a 30-min incubation at 40°C (Fig. 1B, Sunde 2006). The use of the initial reaction rate is practical as it is not necessary to dilute the enzyme extracts and the enzymes are not denatured at these temperatures (especially in the presence of substrates) within a short period of 60 s (see Fig. 1). The protein concentration in the crude enzyme extract was determined using Bio-Rad DC (Detergent Compatible) Protein Assay (Bio-Rad Laboratories, Hercules, CA). The enzyme specific activities were expressed as μmol p-nitroaniline produced/h/mg protein.

Determinations of RNA and Protein Concentrations

RNA and protein concentrations were determined in the white muscle and oocyte samples. TRIzol reagent (Life Technologies, New York, NY) was used for a single step separation of RNA and protein. The method was modified from Sunde et al. (2001). Briefly, 1.0-mL TRIzol was added to a known amount of frozen tissue sample (50–60 mg). The mixture was sonicated and incubated for at least 10 min at room temperature, and RNA and protein were separated by the addition of 0.2-mL chloroform. RNA measurement was performed from...
200 μL of the upper aqueous phase (60% of total volume), while protein determination from 50 μL of the lower organic phase (40% of total volume). DNA at the interphase of the phenol phase will not contaminate protein if the transfer of lower organic phase is carefully performed. The material in each phase was precipitated separately by 1.0-mL isopropanol. The RNA precipitate was washed once, while the protein precipitate was washed twice, with 1.0-mL ethanol for each wash. The procedure was performed at room temperature, and the mixtures were left standing for 10–20 min at each step before centrifuging at 9,000 ¥ g at 4°C for 15–20 min. The precipitate was carefully dried on a hot plate at 55°C for about 20 min. The clean RNA precipitate was then dissolved in 1.0 mL of 0.1-M sodium acetate pH 5 for at least 10 min at 55°C, diluted to 1:10 in the same buffer. The concentration of RNA was measured at 260 nm (Ashford and Pain 1986). The clean protein precipitate was dissolved in 1.0 mL of 1% sodium dodecyl sulfate (SDS) for at least 100 min (mixed every 20 min) at 55°C, diluted to 1:3 with 1% SDS solution and the absorbance measured at 280 nm. RNA concentration was calculated using the extinction coefficient \( E_{260} = 40 \mu g \text{ RNA/mL} \), and protein concentration using the extinction coefficient \( E_{280} = 2.1 \text{ mg protein/mL} \) as reported by Sunde et al. (2001).

**FIG. 1. OPTIMAL TEMPERATURES AND TEMPERATURE STABILITY OF TRYPSIN AND CHYMOTRYPSIN ACTIVITIES IN SALMON PYLORIC CECA**

(A) The enzyme activities of trypsin (●) and chymotrypsin (○) were measured at 410 nm (A410) as a function of assayed temperatures, the enzyme activities were stopped after a 10-min reaction by 5% trichloroacetic acid (adapted from Rungruangsa-Torrissen and Male 2000). (B) Thermostability of trypsin (– – – – – – ●) and chymotrypsin (●—●—●) after 30-min preincubation at different temperatures before measuring the remained enzyme activities using the initial reaction rate (A410/min) at optimal temperature of each enzyme (Sunde 2006).
Statistical Analyses

The values are given as mean ± standard error of mean (SEM) throughout. Linear regression, analysis of variance and t-test at 95% significance level were performed. Differences in elevation and regression coefficient between two regressions were tested using the statistical software SAS System 6.12 for Windows, general linear model (GLM) procedure for unbalanced data with the SOLUTION option (SAS Institute Inc., Cary, NC).

RESULTS

Experiment 1: Effects of Atlantic Krill Meal

In Vitro Digestibility. In vitro digestibility values of the experimental diets (K0, K25, K50) and the commercial feed are shown in Fig. 2A, using the crude enzyme extracts from both 100-g and 1-kg salmon. At the same trypsin activity, the crude enzyme extracts from the 100-g salmon showed a significantly higher digestion than the extracts from the 1-kg salmon (P < 0.001). The control K0-diet showed a better quality than the commercial feed as the resulting in vitro digestibility from both 100-g and 1-kg salmon enzyme extracts were significantly higher (P < 0.004). Increasing in krill meal levels seemed to reduce the in vitro digestibility value of the diets. Although there were no significant differences in the in vitro digestibility among the experimental diets using the 100-g salmon enzyme extract (P > 0.05), a significant difference between the diets with (K25, K50) and without (K0) krill meal was observed using the 1-kg salmon enzyme extract (P < 0.001).

Fish Growth. Fish weight, cumulative SGR and periodic SGR are shown in Fig. 3A–C. At the end of the experiment on day 126, the average weight (Fig. 3A) and cumulative SGR (Fig. 3B) of the Atlantic salmon fed with diets containing krill meal (K25 and K50) were significantly lower than the control groups fed with diets without krill meal (K0) (P < 0.001, see also Table 2). In studying the periodic SGR, a decrease in SGR during days 75–90, followed by an increase in SGR during days 90–110 to a similar level as during days 0–75, were observed in all tanks, except for one tank of the fish fed with the K50-diet where a stable SGR was observed until day 110, followed by a decrease in SGR during days 110–126 as in the other tanks provided with diets containing krill meal (Fig. 3C). Differences in SGR among fish groups were observed during days 110–126 when an adverse effect of krill meal started to be observed (Fig. 3C).

Protease Expressions of Trypsin and Chymotrypsin. High significant correlations (P < 0.0001) between specific activities of trypsin and chymo-
trypsin were observed on both day 90 and day 126, with significant different regression coefficients ($P < 0.003$) between the two regressions (Fig. 4A). Although the higher activated chymotrypsin specific activities on day 90 (based on the same trypsin specific activities that activated chymotrypsin specific activities) was not statistically different ($P_{\text{elevation}} > 0.05$, Fig. 4A), the T/C ratios were significantly lower ($P_{\text{elevation}} < 0.004$, Fig. 5A), compared with the values on day 126. Regression coefficients, of trypsin specific activity and T/C ratio, between days 90 and 126 were not different (Fig. 5A). Significantly inverse correlations between trypsin specific activity and T/C ratio value on days 90 and 126 were observed (Fig. 5A). The T/C ratio ($y$) had slight corre-

![Graph A](image1)

![Graph B](image2)

**FIG. 2. IN VITRO DIGESTIBILITY VALUES OF THE DIFFERENT EXPERIMENTAL DIETS AND THE COMMERCIAL FEED**

The crude enzyme extracts were from 100-g and 1-kg salmon, and standardized to trypsin activity level of 500-$\mu$mol $p$-nitroaniline/h/mL. The average *in vitro* digestibility value ($\mu$mol DL-alanine equivalent/mg feed) is indicated in each bar. The bars with different superscripts are significantly different ($P < 0.05$).
lations, but highly significant, with fish weight \(y = 0.0015x + 0.38, r^2 = 0.095, n = 90, P < 0.004\) and cumulative SGR \(y = 0.53x + 0.37, r^2 = 0.122, n = 90, P < 0.001\) at the end of the experiment on day 126, but not on day 90. Trypsin specific activity \(P < 0.001\) and T/C ratio \(P < 0.02\) increased from day 90 to day 126, regardless of diet type (Table 2). Although a relatively lower T/C ratio in association with increasing krill meal in the diets was observed on day 90, there were no differences in these enzyme values among the different fish groups even when growths were different among them on day 126 (Table 2).

**Muscle Growth and Muscle Quality.** There were significant differences \(P < 0.05\) in the levels of RNA and RNA/protein ratio in the white muscle between fish groups in association with fish growth with the ranking of K0 > K25 > K50 (Table 2). No correlation between the concentrations of RNA and protein was observed in the white muscle (Fig. 6A), and protein synthesis capacity (RNA/protein ratio) was significantly correlated with the concentration of RNA (Fig. 6B). Muscle protein concentration was statistically similar among different fish groups, but it seemed to increase with increasing krill meal

**FIG. 3. GROWTH MEASUREMENTS OF ATLANTIC SALMON IN EXPERIMENT 1 AND EXPERIMENT 2**

Weight and specific growth rate (SGR), both cumulatively and periodically, were measured during the experimental periods. Water temperature ranges during each period were indicated in C and F.

The bars with asterisk (*) or different superscripts are significantly different \(P < 0.05\).
levels in the diet (Table 2). Muscle protein concentration was significantly decreased from \(183.8 \pm 3.04\) mg/g at the start to \(169.6 \pm 1.28\) mg/g after 126 days of feeding the experimental diets, regardless of the diet type \((P < 0.01)\).

**Experiment 2: Effects of Antarctic Krill Meal**

**In Vitro Digestibility.** In vitro digestibility values of the commercial feed and the control K00-diet were similar (Fig. 2). The in vitro digestibility values decreased as the krill meal levels increased in the diets (Fig. 2B). At the krill meal levels of 40–100% fish meal replacement, significant decreases in the in vitro digestibility values were observed \((P < 0.03)\) compared to the control K00-diet (Fig. 2B). Replacements up to 60% krill meal in the experimental diets showed similar in vitro digestibility values to the commercial feed, while replacements of 80–100% had an adverse effect on digestibility as the in vitro digestibility values were significantly lower \((P < 0.04)\) (Fig. 2). Interestingly,
FIG. 4. RELATIONSHIPS BETWEEN SPECIFIC ACTIVITIES OF TRYPSIN AND CHYMOTRYPSIN IN THE PYLORIC Ceca AND IN THE FECES

The protease specific activities were expressed as micromole p-nitroaniline produced per hour per milligram protein. The full line (●—●) indicates the day at the end of the experiment (day 126 or 168), and the broken line (◇——◇) indicates the day during experimental period (day 90 or 76).
FIG. 5. RELATIONSHIPS BETWEEN TRYPSIN SPECIFIC ACTIVITY AND THE PROTEASE ACTIVITY RATIO OF TRYPSIN TO CHYMOTRYPSIN (T/C RATIO) IN THE PYLORIC CECAL AND IN THE FECES

The protease specific activities were expressed as micromole p-nitroaniline produced per hour per milligram protein. The full line (●—●) indicates the day at the end of the experiment (day 126 or 168), and the broken line (◇---◇) indicates the day during experimental period (day 90 or 76).
the in vitro digestibility values seemed to be correlated with the final fish weight (Fig. 7A) and the pyloric cecal T/C ratio values on day 76 (Fig. 7B), and highly correlated with the total FCE (Fig. 7B). The in vitro digestibility value was not correlated with SGR (Fig. 7B).
Fish Growth and Feed Conversion Efficiency. The fish fed diets containing 20% krill meal (K20) showed a higher growth rate, as fish in this group were larger than the other groups with relatively high cumulative SGR (Fig. 3D,E). This seemed to be due to a higher consumption rate in this group as their total average FCE was slightly lower than the control group (Fig. 7B and Table 3), although the values were not significantly different.
### TABLE 3.
**BIOLOGICAL PARAMETERS OF ATLANTIC SALMON IN EXPERIMENT 2**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>K00 (No krill meal)</th>
<th>K20 (20% krill meal)</th>
<th>K40 (40% krill meal)</th>
<th>K60 (60% krill meal)</th>
<th>K80 (80% krill meal)</th>
<th>K100 (100% krill meal)</th>
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<tr>
<td><strong>Initial weight (g)</strong></td>
<td>553 ± 18</td>
<td>559 ± 13</td>
<td>561 ± 15</td>
<td>519 ± 19</td>
<td>499 ± 16</td>
<td>541 ± 138</td>
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<tr>
<td>In vitro digestibility</td>
<td>899 ± 19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>884 ± 18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>772 ± 8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>749 ± 41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>707 ± 21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>615 ± 25&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Day 76</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Weight (g)</td>
<td>966 ± 32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1132 ± 35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1066 ± 40&lt;sup&gt;c&lt;/sup&gt;</td>
<td>996 ± 36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>975 ± 50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>926 ± 27&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SGR days 0–76 (%/day)</td>
<td>0.74 ± 0.05&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.93 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.84 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.86 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.87 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.71 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>FCE (weight gain per feed consumed)</td>
<td>1.14 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.20 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.14 ± 0.14</td>
<td>0.97 ± 0.17</td>
<td>0.84 ± 0.17</td>
<td>0.87 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Pyloric cecal values</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trypsin (T)</td>
<td>14.5 ± 1.5</td>
<td>11.9 ± 1.7</td>
<td>12.3 ± 2.8</td>
<td>15.5 ± 2.1</td>
<td>13.8 ± 1.0</td>
<td>13.1 ± 0.9</td>
</tr>
<tr>
<td>Chymotrypsin (C)</td>
<td>24.9 ± 4.0</td>
<td>20.3 ± 3.7</td>
<td>20.1 ± 6.3</td>
<td>28.2 ± 5.4</td>
<td>24.1 ± 1.6</td>
<td>26.9 ± 4.0</td>
</tr>
<tr>
<td>T/C ratio</td>
<td>0.62 ± 0.03</td>
<td>0.64 ± 0.05</td>
<td>0.63 ± 0.04</td>
<td>0.62 ± 0.07</td>
<td>0.58 ± 0.03</td>
<td>0.55 ± 0.06</td>
</tr>
<tr>
<td><strong>Fecal values</strong></td>
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<td></td>
</tr>
<tr>
<td>Trypsin-like (T)</td>
<td>162.5 ± 10.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.0 ± 7.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92.5 ± 15.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>101.0 ± 21.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.5 ± 15.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.3 ± 13.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chymotrypsin-like (C)</td>
<td>273.7 ± 20.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>177.8 ± 32.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>154.0 ± 19.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>179.3 ± 29.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>156.2 ± 25.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>119.7 ± 22.6&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>T/C ratio</td>
<td>0.64 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.50 ± 0.05</td>
<td>0.58 ± 0.08</td>
<td>0.54 ± 0.06</td>
<td>0.56 ± 0.05</td>
<td>0.49 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Day 168</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>1827 ± 98</td>
<td>1946 ± 80</td>
<td>1904 ± 105</td>
<td>1827 ± 97</td>
<td>1744 ± 98</td>
<td>1661 ± 75&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>SGR days 0–168 (%/day)</td>
<td>0.71 ± 0.03</td>
<td>0.74 ± 0.03</td>
<td>0.71 ± 0.03</td>
<td>0.75 ± 0.02</td>
<td>0.73 ± 0.03</td>
<td>0.66 ± 0.03</td>
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<tr>
<td>FCE (weight gain per feed consumed)</td>
<td>1.10 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.04 ± 0.10</td>
<td>1.00 ± 0.10</td>
<td>0.91 ± 0.08</td>
<td>0.88 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.83 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td><strong>Pyloric cecal values</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Trypsin (T)</td>
<td>11.8 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.7 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.1 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.5 ± 0.7</td>
<td>10.0 ± 0.9</td>
<td>8.7 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chymotrypsin (C)</td>
<td>23.9 ± 3.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.9 ± 1.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.2 ± 2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.0 ± 2.4</td>
<td>18.2 ± 2.0</td>
<td>16.6 ± 1.5</td>
</tr>
<tr>
<td>T/C ratio</td>
<td>0.53 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.66 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.58 ± 0.03</td>
<td>0.59 ± 0.07</td>
<td>0.58 ± 0.03</td>
<td>0.55 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Parameters</td>
<td>K00 (No krill meal)</td>
<td>K20 (20% krill meal)</td>
<td>K40 (40% krill meal)</td>
<td>K60 (60% krill meal)</td>
<td>K80 (80% krill meal)</td>
<td>K100 (100% krill meal)</td>
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<tr>
<td>Fecal values</td>
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<td></td>
</tr>
<tr>
<td>Trypsin-like (T)</td>
<td>53.3 ± 6.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.1 ± 5.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.8 ± 4.2&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>41.3 ± 5.6&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>37.2 ± 5.9&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>23.7 ± 5.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chymotrypsin-like (C)</td>
<td>104.9 ± 11.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>128.6 ± 10.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.3 ± 11.9&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>97.9 ± 11.5&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>75.3 ± 9.0&lt;sup&gt;ad&lt;/sup&gt;</td>
<td>57.2 ± 11.5&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>T/C ratio</td>
<td>0.51 ± 0.05&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.54 ± 0.03&lt;sup&gt;ad&lt;/sup&gt;</td>
<td>0.37 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.41 ± 0.03&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.48 ± 0.03&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>0.41 ± 0.02&lt;sup&gt;bde&lt;/sup&gt;</td>
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<tr>
<td>Oocyte values</td>
<td></td>
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<tr>
<td>Trypsin-like</td>
<td>0.44 ± 0.04</td>
<td>0.38 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.36 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.37 ± 0.09</td>
<td>0.54 ± 0.06</td>
<td>0.63 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>RNA (µg/g oocyte)</td>
<td>4895 ± 322</td>
<td>4694 ± 127</td>
<td>4293 ± 223</td>
<td>4637 ± 268</td>
<td>5041 ± 220</td>
<td>4753 ± 266</td>
</tr>
<tr>
<td>Protein (mg/g oocyte)</td>
<td>107.1 ± 3.8</td>
<td>102.3 ± 2.6</td>
<td>98.1 ± 3.8</td>
<td>118.9 ± 10.1</td>
<td>106.7 ± 3.0</td>
<td>101.7 ± 2.7</td>
</tr>
<tr>
<td>RNA/Protein ratio (µg/mg)</td>
<td>46.4 ± 4.0</td>
<td>46.3 ± 1.8</td>
<td>44.1 ± 3.4</td>
<td>41.9 ± 4.6</td>
<td>47.7 ± 2.4</td>
<td>46.7 ± 2.3</td>
</tr>
<tr>
<td>White muscle values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNA (µg/g muscle)</td>
<td>2684 ± 64a</td>
<td>2538 ± 52</td>
<td>2536 ± 46</td>
<td>2442 ± 37b</td>
<td>2500 ± 69</td>
<td>2590 ± 67</td>
</tr>
<tr>
<td>Protein (mg/g muscle)</td>
<td>245.3 ± 11.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>260.6 ± 8.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>309.7 ± 7.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>279.3 ± 6.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>283.2 ± 12.8&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>260.1 ± 11.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>RNA/Protein ratio (µg/mg)</td>
<td>11.2 ± 0.4a</td>
<td>9.9 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.3 ± 0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.8 ± 0.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.1 ± 0.4&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>10.4 ± 0.7&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

In vitro digestibility (nmol DL-alanine equivalent/mg feed), weight, specific growth rate (SGR), feed conversion efficiency (FCE), protease specific activities (µmol p-nitroaniline produced/h/mg protein), and the concentrations of RNA, protein, and RNA/protein ratio in the white muscle and in the oocytes, after 76 and 168 days of feeding on different experimental krill meal diets. Within the same row, the values with different superscripts are significantly different ($P \leq 0.05$).
Fish weight declined when the level of krill meal increased in the diets, and the average weights between the Atlantic salmon consumed diets containing 20% (K20) and 100% (K100) krill meal were significantly different ($P < 0.02$) on day 76 until the end of the experiment on day 168 (Fig. 3D). Cumulative SGRs were different among fish groups on day 76, after that, they were similar with a lower SGR in K100 groups (Fig. 3E). By studying periodic SGR, a decrease in SGR during days 76–146 was observed in all fish groups feeding on krill meal (K20–K100) while the control fish (K00) had an increased growth rate, followed by a decrease in SGR during days 146–168 in all groups (Fig. 3F).

Significant differences in FCE between the K00 and the K100 groups were observed on days 0–76 ($P < 0.03$), and the differences were observed during the entire experimental period (Table 3). Due to ad lib feeding, FCE did not correlate to SGR. On the other hand, total FCE was highly correlated with the in vitro digestibility values (Fig. 7B). Similar correlation between in vitro digestibility ($x$) and FCE ($y$) was also observed on day 76 ($y = 0.0012x + 0.06$, $r^2 = 0.76$, $n = 6$, $P < 0.03$). Interestingly, the correlation ($y = 0.20x + 0.40$, $r^2 = 0.75$, $n = 6$, $P < 0.03$) between the FCE ($x$) and the T/C ratio in the pyloric ceca ($y$) was observed only on day 76 when differences in SGR were observed (Table 3). The higher the krill meal concentration in the diets, the lower the FCE, the pyloric cecal T/C ratio and the in vitro digestibility value.

**Protease Expressions of Trypsin and Chymotrypsin.** Specific activities of trypsin and chymotrypsin were correlated both in the pyloric ceca (Fig. 4B) and in the feces (Fig. 4C). Only the T/C ratio values in the pyloric ceca on day 76 were correlated to the FCE values when growth differences among the groups were seen (see earlier result). Similar to Experiment 1, when growth differences among the groups were observed on day 76 (Fig. 3D,E) trypsin in the pyloric ceca activated somewhat lower chymotrypsin specific activity (albeit insignificant), compared to the values on day 168, but the regression coefficients were similar between the two regressions (Fig. 4B). On the other hand, the regressions of trypsin specific activities and T/C ratios in the pyloric ceca were significantly different between day 76 and day 168 in both elevations ($P < 0.001$) and regression coefficients ($P < 0.03$) (Fig. 5B). Similar to Experiment 1, significantly inverse correlations between trypsin specific activity and T/C ratio value on days 76 and 168 were observed (Fig. 5B). There were no correlations between T/C ratio with either fish weight or cumulative SGR at any time on individual basis, except for between the pyloric cecal T/C ratio ($y$) and fish weight ($x$) on the group basis on day 76 ($y = 0.0004x + 0.26$, $r^2 = 0.77$, $n = 6$, $P < 0.03$). Both trypsin and chymotrypsin specific activities in the pyloric ceca were higher in the control (K00) than in the other groups on day 168 (Table 3). Similar to final fish weights, the pyloric cecal T/C ratio values
were highest in the K20 groups, and on day 168 they were significantly higher \((P \leq 0.05)\) than the K00 and K100 groups (Table 3).

Protease specific activities were lower in the pyloric ceca than in the feces because of very low protein levels in the feces, and on day 168, the T/C ratios were significantly higher \((P < 0.005)\) in the pyloric ceca \((0.58 \pm 0.002)\) than in the feces \((0.45 \pm 0.001)\), regardless of the diet type (Table 3). By comparing the values in the pyloric ceca, a significant decline in the T/C ratio values in the feces was observed in the fish fed on krill meal diets. On day 168, the decline was significantly higher \((P < 0.02)\) in the fish fed with krill meal diets \((19.83 \pm 0.39 \% \ decline \ on \ average)\), compared to the control groups (K00) whose T/C ratio values in the feces \((0.51 \pm 0.05)\) and in the pyloric ceca \((0.53 \pm 0.03)\) were still similar (Table 3). Between the values in the pyloric ceca \((x)\) and the feces \((y)\), trypsin specific activity was slightly correlated on day 168 \((y = 2.06x + 23.83, r^2 = 0.06, n = 89, P < 0.03)\), while T/C ratio value was correlated on day 76 \((y = 0.37x + 0.32, r^2 = 0.12, n = 49, P < 0.02)\). Chymotrypsin specific activity was not correlated between the values in the pyloric ceca and the feces at any time.

**Muscle Growth and Muscle Quality.** There were significant differences \((P < 0.01)\) in the levels of RNA \((2684 \pm 64 \ versus \ 2519 \pm 25 \mu g/g)\), protein \((245 \pm 11 \ versus \ 278 \pm 5 \ mg/g)\), and RNA/protein ratio \((11.2 \pm 0.4 \ versus \ 9.3 \pm 0.2 \mu g/mg)\) in the white muscle between the control groups (K00) and the groups fed with krill meal diets (K20–K100) (Table 3). No correlation was observed between the concentrations of RNA and protein in the white muscle (Fig. 6A), and the protein synthesis capacity (RNA/protein ratio) was significantly correlated with the concentration of RNA (Fig. 6B). Similar to Experiment 1, RNA concentration and RNA/protein ratio in the white muscle were higher with lower protein concentration in the control K00 groups (Table 3). In contrast to Experiment 1, no associations were observed between muscle RNA level or RNA/protein ratio with fish growth.

**Oocyte Quality.** Trypsin-like specific activity was highest in the oocytes of females fed with a diet with 100% krill meal (K100), which was significantly higher \((P < 0.05)\) than those of K20 and K40 groups (Table 3). The concentrations of RNA, protein and RNA/protein ratio were not different among the oocytes from fish fed with different diets (Table 3). Interestingly, trypsin-like specific activity in the oocytes was correlated negatively with protein concentration and positively with RNA/protein ratio, regardless of the diet type (Fig. 8). As in white muscle, no correlation was observed between the concentrations of RNA and protein in the oocytes (Fig. 6A), and protein synthesis capacity (RNA/protein ratio) in the oocytes was significantly correlated with the concentration of RNA (Fig. 6B).
DISCUSSION

**In Vitro Digestibility, Growth and Feed Conversion**

In comparison to the commercial feed, replacements up to 60% krill meal in the experimental diets showed acceptable *in vitro* digestibility values while replacements of 80–100% had an adverse effect on digestibility (Fig. 2). A reduction in the *in vitro* digestibility as a result of increasing krill meal levels among K0, K25 and K50 without statistical differences (using 100-g salmon enzyme extracts) indicates that if there would be any effect on dietary quality, it would take more than 3 months to affect the FCE and SGR of the fish, as suggested by Rungruangsak-Torrissen et al. (2002). This prediction is supported by the significant differences in fish growth in Experiment 1 after 3 months of feeding on day 126 (Fig. 3). In Experiment 2, where significant differences in the *in vitro* digestibility were observed among the diets (Fig. 2B), differences in fish growth (Fig. 3D–F) and FCE (Table 3) were seen within 3 months of feeding on day 76. The current experiment supports the *in vitro* digestibility value as a reliable indicator of dietary quality for growth experiment as described by Rungruangsak-Torrissen et al. (2002) where the

![Graph showing relationships between trypsin-like specific activity, protein concentration, and RNA/protein ratio in oocytes from females in Experiment 2.](image)
value was also found to relate to chemical qualities of dietary proteins (levels of reactive sulfydryl group, disulfide bond and D-aspartic acid) that affect the nutritional value of the diet. Higher values of in vitro digestibility using 100-g salmon enzyme extracts observed in comparison to 1-kg salmon enzyme extracts and based on the same trypsin activity (Fig. 2A) indicates that small fish could utilize the diets better than large fish. This also indicates that, within the same range of difference in dietary quality, it would take longer to see the effect of the diets on growth in small fish than in large fish. Based on these findings and with regard to similar in vitro digestibility values between the control diets K0 (Fig. 2A) and K00 (Fig. 2B) using 1-kg salmon enzyme extracts, a possibility of using any krill meal in aquaculture at 50–60% of fish meal replacement in diets was observed, as their in vitro digestibility values were within a similar range and also similar to those of commercial feed. The diets K80 and K100 had significantly lower in vitro digestibility values (Fig. 2) and affected significantly lower weights and FCE of the fish (Table 3) compared to the other diets (P < 0.04 by pooled data), indicating that krill meal levels at 80–100% replacements are not suitable for fish diets. Variation in growth due to different dietary quality may not be easily seen in ad lib feeding experiment unless FCE is included in the experimental design. As a result of ad lib feeding, SGR is not correlated with FCE and the in vitro digestibility value (Fig. 7B). As FCE is highly correlated with in vitro digestibility (Rungruangsak-Torrissen et al. 2002; Fig. 7B), it would be practical, quick and reliable to determine the in vitro digestibility of the experimental diets (by using crude enzyme extract from the species of interest and standardized by trypsin activity) before starting up growth experiments that might not be necessary.

Fish growth could be affected by dietary quality as well as by frequent samplings. The SGR fluctuations in Experiment 1 (Fig. 3C) did not seem to be influenced by stress after samplings as the growth rate increased on day 110 after sampling on day 90. Changes in SGR during each period were not always related to changes in water temperature as the SGR decreased at the end of both experiments during a constant temperature of 8°C (Fig. 3C,F).

**Protease Expressions of Trypsin and Chymotrypsin**

The relationship between trypsin and chymotrypsin specific activities in the pyloric ceca was observed when growth was limited or depressed such as during starvation or food deprivation, and when growth was reduced after a rapid growth (Rungruangsak-Torrissen et al. 2006c). The relationship, on the other hand, has disappeared after feeding and with a rapid growing phase (Rungruangsak-Torrissen and Male 2000; Rungruangsak-Torrissen et al. 2006c). The relationship observed between specific activities of trypsin and
chymotrypsin in the current experiments (Fig. 4) should be because of a limitation in growth as well as a reduction in growth as seen during the last period of both experiments (Fig. 3C,F). Based on the same trypsin specific activity, higher activated chymotrypsin specific activity on day 90 in Experiment 1 (Fig. 4A) and on day 168 in Experiment 2 (Fig. 4B), although not significant, resulted in a significantly lower T/C ratio (P < 0.004, Fig. 5A,B) and corresponded with a significantly lower cumulative SGR (P < 0.0001) compared to day 126 and day 76, respectively, regardless of diet type (see Tables 2 and 3). Inverse correlations between trypsin specific activity and T/C ratio in the pyloric ceca (Fig. 5A,B) also confirmed the reduction in growth rate seen during the last periods of both experiments (Fig. 3C,F) as a result of increased chymotrypsin specific activity. If we do not know the history of the fish (i.e., wild fish), studies of the relationships between trypsin specific activity, chymotrypsin specific activity, T/C ratio and fish weight would indicate growth status of the fish—whether they are during the growing phase, steady growth phase, or a reduction in growth rate as seen in the current work (details in Rungruangsak-Torrissen et al. 2006c). The T/C ratio is significantly correlated to fish weight when there are differences in cumulative SGR (Sunde et al. 2001; Rungruangsak-Torrissen et al. 2006c; current work) and the correlation coefficient is higher on group basis (r² = 0.77 in Experiment 2) than on individual basis (r² = 0.095 in Experiment 1).

Specific activity of trypsin, but not that of chymotrypsin, was correlated with FCE in Atlantic cod (Lemieux et al. 1999). Growth rate in Atlantic salmon has been shown to correlate with trypsin specific activity and T/C ratio in the pyloric ceca, but not with chymotrypsin specific activity (Sunde et al. 2001). The T/C ratio value is more sensitive and advantageous than trypsin specific activity as it is independent of external factors, i.e., changes in extract protein content unrelated to changes in enzyme concentrations, as seen between the values in the pyloric ceca and feces (Table 3). In addition, both trypsin and chymotrypsin specific activities are related to dietary protein concentration, while the T/C ratio is related to growth rate independent of the activity levels of the two enzymes when studying with different feed qualities (Rungruangsak-Torrissen et al. 2006a) and gene-manipulated fishes (Sunde et al. 2001; Blier et al. 2002). More details were described in Rungruangsak-Torrissen et al. (2006c). In Experiment 1, the T/C ratio values showed significant correlation with weight and cumulative SGR on day 126, but not on day 90, suggesting that the differences in growth between the fish groups fed with the different diets occurred after day 90. Since weight (Fig. 3A) and cumulative SGR (Fig. 3B) were not different between fish groups on day 0–110, the difference in weight and SGR had to have occurred during the last 2 weeks before the termination of the Experiment 1 on day 126, as high variations in the periodic SGR between the different fish groups were observed between day
110 and day 126 (Fig. 3C). This result corresponds with an earlier estimation of an effect of krill meal diets on growth by the \textit{in vitro} digestibility method that the krill meal diets would have an effect on fish growth after 3 months of feeding. The relationships seen in Experiment 1 between T/C ratio with weight and with cumulative SGR were not observed in Experiment 2. This indicates a possibility of increasing growth rate in Experiment 1 but not in Experiment 2, if the experiments were prolonged for another month. This expectation is also supported by the increases in the pyloric cecal protease values (trypsin \( P < 0.001 \), chymotrypsin [albeit insignificant] and the T/C ratio \( P < 0.02 \)) regardless of diet type at the end of Experiment 1 (Table 2, Fig. 5A), and by the decreases in these enzymatic values (trypsin \( P < 0.0001 \), chymotrypsin \( P < 0.001 \) and the T/C ratio [albeit insignificant]) in Experiment 2 (Table 3, Fig. 5B).

The T/C ratio values in the pyloric ceca were not statistically different among fish groups in any experiments, but it correlated to fish weight and cumulative SGR at the end of Experiment 1. Although these correlations were not found in Experiment 2 (may be because of less impact on fish growth of Antarctic krill meal, compared to Atlantic krill meal), the K20 fish groups with the highest weight had the highest T/C ratio value (Table 3). The positive relationships between T/C ratio, FCE and \textit{in vitro} digestibility in association with decreasing krill meal levels in the diets indicate a tendency of an adverse effect of krill meal at a certain level in the diets on the digestive efficiency and growth of the fish. General observations in both experiments in association with weight and/or FCE showed that the lower average T/C ratios (although not significant) in every studied growth periods may suggest adverse effects of Atlantic krill meal at 50\% replacement and Antarctic krill meal at 80–100\% replacements on feed utilization and growth of the fish (Tables 2 and 3). Trypsin itself does not directly induce growth, as illustrated by a low correlation coefficient value between the T/C ratio and growth (Sunde \textit{et al.} 2001; Rungruangsak-Torrissen \textit{et al.} 2006c; current work). A highly significant correlation between T/C ratio and growth (although at a low correlation coefficient value) indicates that an increase in trypsin expression in the digestion process is a mediator that could stimulate growth mechanisms in animals (Rungruangsak-Torrissen and Male 2000; Rungruangsak-Torrissen \textit{et al.} 2006c; current work).

The relationship between specific activities of trypsin and chymotrypsin was observed not only in the pyloric ceca but also in the feces in Experiment 2 with significantly lower in both trypsin and chymotrypsin specific activities as well as the T/C ratio \( P < 0.0002 \) on day 168 than on day 76 (Fig. 4C), indicating a lower production of the digestive proteases during growth reduction (see Fig. 3F). In practice, sampling of feces is easier than pyloric ceca and prevents the fish from being killed. Using the protease specific activities of
trypsin and chymotrypsin in feces for estimating growth may still be in question as trypsin specific activity values in the pyloric ceca and in the feces were slightly correlated only on day 168 and T/C ratio values on day 76, while no correlation between chymotrypsin specific activities was found. In addition, positive relationships between trypsin specific activity and T/C ratio in feces (Fig. 5C) did not explain the growth status of a reduction in growth rate of the fish in the experiments as the negative relationships in the pyloric ceca did (Fig. 5A,B). Protease activities have been observed in the large intestine of salmonids (Torrissen 1984), probably including trypsin and chymotrypsin activity according to the optimal temperatures observed (Rungruangsak-Torrissen and Male 2000). We do not know whether the enzymes in the feces just come from the intestine itself or also from the normal bacteria in the gut (not under the scope of the current work). If the fish are needed alive for further research, trypsin specific activity and T/C ratio in feces may be used to compare digestive efficiency of the fish on different dietary qualities, but they cannot predict the growth status of the fish as the values in the pyloric ceca can. The enzyme specific activity in unit per mg protein (current work) is more suitable than the unit per fecal weight (Krogdahl et al. 2003), as the enzyme specific activity values would relate positively to the digestive efficiency and growth of the fish.

Muscle Growth and Muscle Quality

The significant decrease in muscle protein levels from 183.8 ± 3.04 mg/g before feeding the experimental diets to 169.6 ± 1.28 mg/g after 126 days of feeding on the experimental diets, regardless of the diet type in Experiment 1, seemed to have resulted from the differences in the protein and lipid concentrations in the feeds, as the krill meal diets (45% protein and 28% lipid) had less protein and higher lipid concentration than the commercial feed (Bio-Optimal SVEV, BioMar AS, with 46% protein and 22% lipid). Unfortunately, the change is not known in the white muscle protein concentration of the fish in Experiment 2, as they were not sampled at the start of the experiment. At the end of the experiments, the fish in Experiment 2 that were larger showed higher protein concentration in the white muscle (273.7 ± 4.46 mg/g) than the smaller fish in Experiment 1 (169.6 ± 1.28 mg/g), regardless of the diet type (Fig. 6A). As the concentrations of protein and lipid were similar between the two sets of experimental diets and fish growth rates were also similar, the differences in muscle protein concentrations between the fish in Experiments 1 and 2 may be because of the differences in krill meal qualities (not under the scope of the current work). Differences in white muscle protein concentration between small and large fish may also be a natural phenomenon, as 2-kg rainbow trout have a higher protein concentration in the white muscle than in
500-g trout fed with the same diet during their life cycle (Rungruangsak-Torrissen et al. 2006a). In both experiments (Experiment 1: not significant; Experiment 2: \( P < 0.01 \)), similar results of higher protein concentrations were observed in the white muscle of the fish fed with krill meal diets (pooled data), compared to those fed with control diets. High level of protein concentration in white muscle (as high as 30\%, compared to normal values of 15–20\%; reported in Jobling 2001) observed in the current study was due to complete solubilization of protein by using the newly developed method. The higher the solubilization of protein, the higher the concentration of protein detected.

The association between fish growth and RNA level or RNA/protein ratio observed in Experiment 1 indicates that increased white muscle RNA concentration and capacity for protein synthesis resulted in synthesis and growth (Mathers et al. 1992; Carter et al. 1993; Houlihan et al. 1993). This association was not found in Experiment 2, probably because no differences in SGR among the fish groups were seen, as less impact on fish growth were observed in Antarctic than Atlantic krill meal. It is interesting to note that, although no significant correlation was observed between the concentrations of RNA (\( x \)) and protein (\( y \)) in the white muscle, the correlation tendencies between them were seen in both experiments, positively on the individual basis (Experiment 1: \( y = 0.0023x + 158.7, r^2 = 0.01, n = 90 \); Experiment 2: \( y = 0.0232x + 214.8, r^2 = 0.02, n = 89 \)), but negatively on the group basis (Experiment 1: \( y = -0.0071x + 202.8, r^2 = 0.30, n = 6 \); Experiment 2: \( y = -0.0806x + 479.2, r^2 = 0.20, n = 12 \)). Protein growth occurred when protein synthesized exceeds the amount of protein retained (Houlihan 1991), and the efficiency of retention of synthesized protein is important for protein growth efficiency irrespective of the amount of protein synthesized (Carter et al. 1993). In addition, higher protein growth efficiency has been shown to associate with lower protein turnover rate (Hawkins 1991; Houlihan 1991; Rungruangsak-Torrissen et al. 1999), and small differences in protein turnover that did not attain the level of statistical significance could lead to the difference in protein growth efficiency (Carter et al. 1993). These findings suggest that although the control K0 and K00 groups had higher protein synthesis because of higher RNA concentrations, they had lower protein retention and higher turnover rate as lower muscle protein concentrations were observed, compared to the fish groups fed on diets containing krill meal (Tables 2 and 3). Fish grown at a slower rate usually have better fillet quality through the higher retention of protein in the white muscle (Rungruangsak-Torrissen and Fosseidengen 2007; current work). Similarly, the levels of RNA and protein synthesis capacity (RNA/protein ratio) in the white muscle were higher in small fish with lower muscle protein level in Experiment 1 than in large fish in Experiment 2 (Fig. 6B), indicating a lower retention of protein and a higher turnover rate in smaller fish in Experiment 1.
Oocyte Quality

Protein synthesis capacity was higher in the oocytes than in the white muscle (Fig. 6B). A possible higher breakdown of oocyte protein was observed in the presence of protease, as trypsin-like specific activity was inversely correlated with protein content in the oocytes (Fig. 8). At the same time, trypsin-like specific activity was related to protein synthesis capacity (RNA/protein ratio) in the oocytes (Fig. 8). Both degradation and synthesis of protein have to be under control during oocyte development. Embryo hatching and outgrowth are the first critical steps on the way to a successful reproduction, and it is generally known that serine proteases are responsible for this process although the exact mechanisms of the action are not clearly understood (O’Sullivan et al. 2002). Embryonic-hatching enzymes have been studied in different species and are mainly metalloproteases. Most have trypsin-like activity (Lubbering and Hofmann 1995; Daniello et al. 1997; O’Sullivan et al. 2001) and are very sensitive to trypsin-specific inhibitors (Yamazaki et al. 1994; Daniello et al. 1997; Fan and Katagiri 2001). A higher trypsin-like specific activity is related to less oocyte development as it is higher in the oocytes than in the mature eggs (Rungruangsk-Torrissen et al. 2006b). A high level of protease activity is also associated with ovarian cancer (Shigemasa et al. 2000), suggesting an abnormal ovary development. Thus, the significant increase \( P < 0.003 \), Table 3\) in trypsin-like specific activity in the oocytes from the fish in K80 and K100 groups \( (0.57 \pm 0.05 \mu \text{mol p-nitroaniline produced/h/mg protein}) \) compared to the other groups \( (0.38 \pm 0.03 \mu \text{mol p-nitroaniline produced/h/mg protein}) \) may impact egg quality through less or abnormal development. Moreover, the water uptake during final oocyte maturation seems to be driven osmotically, not only by the uptake of ions but also by the hydrolysis of yolk protein to free amino acids (Craik and Harvey 1987; Thorsen et al. 1993, 1996). If these are the case, different levels of oocyte protease specific activity may affect oocyte development and egg quality, although the concentrations of RNA, protein, and RNA/protein ratio were similar among the oocytes from the different fish groups (Table 3). According to these criteria, Antarctic krill meal levels at 60% replacement of fish meal did not seem to have any effect on the quality of the oocytes during the experimental period, in association with the other parameters studied.

CONCLUSIONS

FCE, not the growth rate, is the most important criterion for growth efficiency, especially when the fish were fed \textit{ad lib}. The observed relationships among the FCE value, \textit{in vitro} digestibility and pyloric cecal T/C ratio (at the
time of growth differences) indicated that the in vitro digestibility and the pyloric cecal T/C ratio should be considered as indicators for studying dietary quality in general (not only for protein quality), especially when feed consumption is difficult to measure or cannot be measured in growth studies. The methods could probably be applicable in other animal species. The relationships of these parameters with inverse levels of krill meal in the experimental diets indicated that krill meal at a certain level would impact feed utilization and growth of the fish. This work also indicated that variations in the pyloric cecal T/C ratio values that did not attain statistical significance could lead to differences in feed utilization and growth of the fish. In addition, when growth was limited or reduced, increased chymotrypsin specific activity was observed in the pyloric ceca resulting in an inverse relationship between trypsin specific activity and the T/C ratio. There were changes in the biological conditions and growth of the fish fed with diets containing krill meal, especially in Experiment 1. Although it was concluded that krill meal at 50–60% fish meal replacements would be acceptable for use in aquaculture feed, and replacement of fish meal by krill meal would also improve fillet quality through increased protein concentration in the white muscle, the Antarctic krill meal used in Experiment 2 seemed to show better quality than the Atlantic krill meal in Experiment 1; at similar levels of replacement (50–60%), Antarctic krill meal did not significantly affect the biological conditions and growth of the fish in comparison to the control diet, whereas Atlantic krill meal did. If these effects are prolonged until harvest or maturation, Antarctic krill meal could be a better resource for future fish feed production at least at 60% replacement of fish meal and not ≥80%.

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