Paper III

Growth, feed conversion and chemical composition of Atlantic salmon (*Salmo salar* L.) and Atlantic halibut (*Hippoglossus hippoglossus* L.) fed diets supplemented with krill or amphipods

Growth, feed conversion and chemical composition of Atlantic salmon (*Salmo salar* L.) and Atlantic halibut (*Hippoglossus hippoglossus* L.) fed diets supplemented with krill or amphipods

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KEY WORDS: krill, quality, fish meal replacement, Atlantic salmon, Atlantic halibut, attractants.

Abstract

The effects of partial replacement of fish meal with meal made from northern krill (*Thysanoessa inermis*), Antarctic krill (*Euphausia superba*), or Arctic amphipod (*Themsto libellula*) as protein source in the diets for Atlantic salmon (*Salmo salar* L.) and Atlantic halibut (*Hippoglossus hippoglossus* L.) on growth, feed conversion, macro-nutrient utilisation, muscle chemical composition and fish welfare, were studied. Six experimental diets were prepared using a low-temperature fish meal (FM) diet as control. The other diets included northern krill where 20, 40, or 60% of the dietary fish meal protein were replaced with protein from northern krill, and two diets where the fish meal protein where replaced with protein from Antarctic krill or Arctic amphipod at 40% protein replacement level. All diets were iso-nitrogenous and iso-caloric. Atlantic salmon grew from 410 g to approximately 1500 g during the 160 days experiment, and Atlantic halibut grew from 345 g to 500-600 g during the 150 days experiment. Inclusion of krill in diets enhanced specific growth rate (SGR) in salmon, especially during the first 100 days ($P < 0.01$), and in a dose-response manner in halibut for the whole 150 days feeding period ($P < 0.05$). Feed conversion ratio did not differ between dietary treatments, neither did dry matter or protein digestibilities, nor fish muscle composition. Good growth rates, blood parameters within normal ranges, and low mortalities in all experimental treatments indicted that fish health was not affected both Atlantic salmon or Atlantic halibut fed the various zooplankton diets.

Introduction

The demand for feed in intensive aquaculture production has increased over recent years in parallel with increase in total production volumes (FAO, 2004). To sustain further growth, matching increases in feed ingredients are required. Until recently, the feed industry has relied on fish meal and fish oil as the major sources of dietary macronutrients. However, as most fisheries are exploited to the maximum level, it is not likely that further increase in fish feed production can be sustained by harvesting of fish stocks (FAO, 2004). Further growth in the aquaculture industry will thus depend on new sustainable feed resources becoming available.

The only unexploited marine resources of significant biomass are those found at lower tropic levels. For instance, estimates suggest that the standing biomass of Antarctic krill (*E. superba*) is 44 million tonnes, and the CCAMLR Scientific Committee, has suggested a precautionary catch limit of 4 mill. tonnes krill for the Antarctic Sector (Area 48) (Hewitt *et al.* 2002). In 2003/2004 less than 120 000 tonnes were harvested (CCAMLR 2005). Recent studies have shown that meal from krill species can replace fish meal in salmonid diets without having any negative effect on growth rate (Julshamn *et al.* 2004; Olsen *et al.* 2006) or product quality (Suontama *et al.* 2006).

Northern waters also contain large amounts of krill, amphipods and other groups of zooplankton that may be used as feed resources for carnivorous fish species (Storebakken, 1988; Melle *et al.* 2004). The standing stock of krill and amphipods of the Norwegian Sea has been estimated at 42 and 29 mill. tonnes, respectively, and estimates of the annual production to be about twice these numbers (Melle *et al.* 2004; Skjoldal *et al.* 2004). The standing stock of the most abundant herbivorous zooplankton species of the Norwegian Sea, *Calanus finmarchicus*, has been estimated at 48 mill. tonnes while annual production probably exceeds 290 million tonnes (W. Melle, pers. comm.). The Norwegian Sea in this context is defined as 1 million km^2, while the Nordic and Barents Seas are about 3.1 million km^2 (Skjoldal, 2004). If economically profitable catch methods can be developed, the resources for harvesting are available. However, the ecological consequences of harvesting at lower trophic levels are currently not understood.

The aim of the present study was to elucidate the suitability of krill and amphipod meals from catches in northern waters as fish meal replacements for farmed Atlantic salmon (*Salmo salar* L.) and Atlantic halibut (*Hippoglossus hippoglossus* L.). Focus was laid on growth and utilisation, as well as selected health parameters.

Materials and methods

Raw materials and feed preparation

Antarctic krill (*Euphausia superba*) were purchased block frozen from Superba Invest, Ltd, Ålesund, Norway. The Arctic amphipod, *Themisto libellula* (20–40 mm), were collected in Arctic water of the Norwegian Sea in June 2002, and the northern krill species, *Thysanoessa inermis*, (20–24 mm), were collected in the vicinity of the Arctic front of the Norwegian Sea, in May 2003. Both samples were taken by pelagic trawl north and south of Jan Mayen, respectively. They were immediately frozen after catch and transported to a pilot plant for meal and diet production. Total catches consisted of pure animals from krill and amphipods swarms, as observed by echo sounder and determined by inspection of subsamples of the catches, and weighed about 800 kg of each species.

Production of meal was carried out from all species after a minimum of thawing. A detailed description of the krill meal preparation is described elsewhere (Olsen *et al.* 2006). For amphipod the press liquid was concentrated in an evaporator without sieving and oil separation. The meals were stabilized by adding 100 mg kg^{-1} FEQ500 (LL Chemie AB, Helsingborg, Sweden) (mixture of ethoxyquin and formic acid) both to the press cake and the meal. The chemical composition of the raw materials is given in Table 1.

The diets (4 mm diameter pellets) were produced by extrusion using a Wenger (TX-52, Sabetha, USA) co-rotating twin-screw pilot scale extruder. After extrusion and drying (Paul Klöckner, type 200.2 carousel dryer, Nistertal, Germany), the diets were coated with fish-oil in a vacuum oil coater (Dinnissen, Sevenum, Netherland) to give a total lipid level of 30%. A total of 6 diets were then prepared using similar standards and additives as in the commercial diets (Table 2). A standard fish meal diet (FM) (low-temperature-dried, Norse-LT 94®, Norsildmel, Bergen, Norway, from 62% blue whiting and 38% capelin) was used as control. Three diets were made from northern krill meal, replacing 20, 40 or 60% of the fish meal protein (150, 300 and 460 g kg^{-1} of the total diet, denoted K20, K40, K60). Further, two diets were produced where 40% of the fish meal protein was replaced with protein from either Antarctic krill (280 g kg^{-1} of the total diet), or the Arctic amphipod (350 g kg^{-1} of the total diet) (denoted AK40, AMP40, respectively). Increasing the level of krill gave less expansion of the pellet and it was necessary to add an increasing level of oil in the mash to obtain the desired fat level in the final feed. For diet FM and K20 fish oil was added

126 to obtain 130 g kg^{-1} fat in mash (on d.w. basis), for diets K40, AK40 and AMP40, 140 g kg^{1} and for
127 diet K60 150g kg^{-1}. All diets were initially designed to be iso-lipid and iso-proteinous. However, as
128 the protein level in the meals differed somewhat, it was decided to include starch as filler in order to
129 maintain the desired protein and lipid levels. This did cause a minor difference in total energy
130 content varying from 23.2 MJ kg^{-1} for the amphipod diet (APM40) to 24.0 MJ kg^{-1} for the pure fish
131 meal diet (FM) (Table 2). As the content of astaxanthin varied between the various meals,
132 astaxanthin (Carophyll pink®) was added to all diets in order to obtain a balanced final concentration
133 of minimum 60 mg kg^{-1}.
134

135 ## *Fish*

136 *Atlantic salmon*
137 In January 2004, 540 Atlantic salmon post-smolts averaging 412 ± 5 g (NLA strain - Norwegian
138 breeding program), were anaesthetized (for details see later) and individually tagged with T-Bar
139 anchor tags (Floy Tag & MFG. Inc., Seattle, WA, USA) at the Institute of Marine Research (Matre,
140 61°N, Norway). They were divided randomly into eighteen round (1.5 m diameter, 1 m water depth)
141 indoor fiberglass tanks (triplicate groups for each diet) each containing 30 fish and equipped with
142 feed collectors and artificial light sources. The tanks were supplied with aerated sea water
143 (10 ± 1.5 °C), salinity 29.5 ± 1.5 g L^{-1} and kept under a 12 : 12 h daylight regime. During this
144 period, the fish continued to be fed with a commercial feed (4 mm, Nutra Svev, Skretting, Norway).
145 The fish were then allowed to adapt to the new conditions for two weeks. Three days prior to
146 initiation of the experiment, fish were starved, and one day prior to initiation, fish were
147 anaesthetized and weighed (grams) and length measured (cm). During the experimental period
148 salmon were fed the experimental diets in slight excess (about 10% excess) daily from 08:00 until
149 12:00 using automatic disc feeders (Storvik, Sunndalsøra, Norway). Weight and length were also
150 measured after 100 days and at the end of the feeding period (160 days). Mortality was low or absent
151 in experimental groups, and did not vary significantly between treatments.
152

153 *Atlantic halibut*
154 In November 2003, 480 Atlantic halibut averaging 330 ± 80 g were transferred into twelve outdoor
155 tanks (duplicate groups) (3 m diameter, water depth 1 m), each containing 40 fish. The halibut,
156 hatched in 2002, were produced at the Institute of Marine Research (Austevoll, 60°N, Norway). The

tanks were supplied with aerated seawater (8 ± 1.5 °C), salinity 35.2 ± 0.15 and were equipped with feed collectors. All groups were reared under natural light conditions and the tanks were covered with nets to reduce light intensity. At start, after 78 days and at the end of the feeding period (150 days) all fish were anaesthetized, digitally photographed, and measured to the closest weight in grams and length in cm. The fish were fed with automatic feeders (Betten, Vågland, Norway) in slight excess (about 10%) using the same diets as for Atlantic salmon. Mortality was low or absent in experimental groups, and did not vary significantly between treatments.

Fish sampling and slaughter

Anaesthezation of fish by 4 g L^{-1} benzocaine (Norsk Medisinaldepot AS, Bredtvet, Norway) was used prior to all handling and sampling. At the end of the experiment, all fish were anaesthetized and stunned by a sharp blow to the head, weighed and length measured as described above. Blood was withdrawn from the caudal vessels (*Vena caudalis*) of five fish per tank using heparinised syringes. Sub-samples of whole blood were stored at 4 °C and analysed for haematocrit (HCT), haemoglobin (HB) and red blood cells (RBC) within 24 h after collection. From another sub-sample, plasma was immediately separated by centrifugation at 3000 × ***g*** for 5 minutes and stored at –80 °C for later analysis of plasma nutrients and leakage of organ specific enzymes. Additionally, white muscle samples used for muscle dry matter and fatty acid analysis were eviscerated using scalpel from an area below the dorsal fin from five fish per tanks. Muscle samples were immediately frozen in liquid nitrogen and stored at –80 ºC until analysis. From the same five fish the intestine was eviscerated with a scalpel and faeces collected from the distal part of the intestine according to Ringø (1991). Faeces samples were mixed to one pooled sample per tank and frozen in liquid nitrogen and stored at –80 ºC until analysed.

Analyses

Crude protein in the meals was determined by the combustion method (ISO/DIS 16634, 2004); water soluble protein by the Kjeldahl method (ISO 5983:1997) and ash by ISO 5984:1978. Total lipid in the fish meal was quantified by the Soxhlet method (AOCS Ba 3–38) while the Bligh & Dyer (1959) procedure was used for the krill and amphipod meals. Moisture content was obtained after drying at 103 °C (ISO 6496:1999) until stable weight was obtained, and salt content by AOAC 937.09. Total

187 astaxanthin was analysed by normal phase chromatography on a Si60 column (Schierle & Härdi,
188 1994).

189 The chitin content was assessed by the Kjeldahl method after having the protein removed. In
190 brief, the meal was first demineralised using 50 g L^{-1} HCl at 50 °C for 30 min and then deproteinised
191 using 40 g L^{-1} NaOH at 80 °C for 90 min. Nitrogen in the insoluble residue was then determined by
192 the Kjeldahl method and total chitin content estimated using the formulae N × 14.51 (Merck & Co.
193 Inc. 1996). Total protein content in the raw material was then calculated by subtracting estimated
194 chitin content from the estimated Kjeldahl protein content. True protein digestibility of the meals in
195 mink (*Mustela vison*) was performed using the method modified from Skrede (1979) as described by
196 Opstvedt *et al.* (2003) and was based on analyses for nitrogen in feed and faeces and protein
197 calculated as N × 6.25.

198 Total lipid was extracted from diets and muscle according to Folch *et al.* (1957). After
199 evaporation to dryness *in vacuo* at room temperature, total lipid was measured gravimetrically,
200 before being re-dissolved in chloroform/methanol (2:1, by vol.) and stored in nitrogen at –80 ºC
201 prior to analysis. Total lipid fatty acid composition of muscle and diets were determined according
202 to Olsen *et al.* (2004).

203 The total amino acid composition in the feed was assessed with HPLC according to Olsen *et*
204 *al.* (2006).

205 Homogenized samples of diets, faecal matter and muscle were analyzed for dry matter by
206 means of standard reference methods: moisture by difference after drying at 103 ˚C for 24 h (until
207 stable weight was obtained), total nitrogen content was determined on homogenized freeze-dried
208 samples using a nitrogen element analyser/(LECO, FP-428; system 601-700-500, St. Joseph, MI,
209 USA). Protein was then calculated as N × 6.25. Faecal samples were analyzed for yttrium oxide
210 content as described previously by Otterå *et al.* (2002).

211 HCT, HB and RBC were analysed according to Sandnes *et al.* (1988). The plasma enzymes
212 aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), plasma glucose, and total
213 protein concentrations were determined as described by Hemre *et al.* (1995).

214

215 ## *Calculations and statistical treatment*

216 Specific growth rate (SGR) was estimated according to the formula:

217 $$SGR = 100 \times (\ln(W_{end}) - \ln(W_{start}) / \Delta t)$$

where W_{end} and W_{start} denotes end and start weight of fish and Δt experimental days.

Apparent digestibility coefficient (ADC) was estimated according to the formulae:

$$ADC = 100 - 100 \times ((Y_{feed} / Y_{faeces}) \times (N_{faeces} / N_{feed}))$$

where Y_{feed} = yttrium oxide in feed, Y_{faeces} = yttrium in faeces, N_{faeces} = nutrient in faeces, N_{feed} = nutrient in feed. All data were based on calculated dry weight of the samples.

k-factor was calculated as:

$$k\text{-factor} = 100 \times (W / L^3)$$ where W is the fish weight in grams and L fork length in cm.

Feed conversion ratio (FCR) was estimated as:

$$FCR = \Delta \text{ feed} / \Delta \text{ growth}$$

where Δ feed is the amount of feed in grams consumed by the fish in the tank between each weighing of the fish, and Δ growth is the increase of fish weight in grams during the same period.

Data used for FCR and ADC are pooled samples from each tank, resulting in $n = 3$ for Atlantic salmon, and $n = 2$ for Atlantic halibut. Data intended for use in parametric statistics were checked for homogeneity of variances by the Levene test and normality using the Lilliefors test (Statistica v. 7.1, StatSoft, Inc., www.statsoft.com). Whenever necessary, statistical analysis was carried out using arcsine transformations on percentage data, or ln transformations for remaining samples. Correlations between variables were checked with Pearson bivariate (2-tailed) correlation and significance level was accepted at $P < 0.05$. Dietary effects were analysed using one-way ANOVA (blood and plasma parameters) or nested ANOVA (length, weight, *k*-factor and SGR, tanks nested under diet), followed by the Tukey HSD post hoc test if significant at the 5% level.

Results

Composition of raw materials and diets

Fish meal had the highest content of crude protein (732 g kg^{-1}) and lowest content of salt (23 g kg^{-1}) (Table 1). The amphipod and krill meals ranged between 524-641 g kg^{-1} for crude protein and 38-59 g kg^{-1} for salt. Northern krill meal had the lowest level of ash (104 g kg^{-1}) followed by fish meal (124 g kg^{-1}) and that of Antarctic krill (151 g kg^{-1}). Amphipod differed from the other species by

248 having very high ash content (222 g kg^{-1}). Lipid content in meals ranged from 75 g kg^{-1} in fish meal
249 to 93 and 99 g kg^{-1} in the meals from Antarctic krill and amphipod respectively. The highest lipid
250 content (182 g kg^{-1}) was found in the meal from northern krill.

251 The diets were well balanced with regard to protein and lipid levels (Table 2). Analysed
252 protein level was close to 460 g kg^{-1}, lipid level about 250 g kg^{-1}, and with minor differences
253 between diets. Chitin content in diets increased in a dose dependent manner as the amount of meal
254 from northern krill increased (up to 10 g kg^{-1} in K60). AK40 had slightly higher chitin content than
255 K40, whereas the chitin content of AMP40 was three to four times higher (29 g kg^{-1}) than AK40 and
256 AMP40. Total astaxanthin level in diets was balanced with addition of Carophyll pink® to a
257 minimum level of 59.3 mg kg^{-1}.

258 The dominant dietary fatty acids were 16:0, 20:1n-9, 22:1n-11 and 22:6n-3 (Table 3),
259 followed by 18:1 n-9 and 20:5n-3 in all diets. Marginal differences were detected only for the level
260 of total saturated (SAT), both polyunsaturated (PUFA) and *n*-3 fatty acids were similar. There was
261 however, a general decline in monoenoic (MONO) fatty acids content, mainly 22:1n-11 and 20:1n-9,
262 with increased levels in northern krill along with a corresponding decrease in 22:6n-3. Comparing
263 zooplankton sources at the 40% protein inclusion levels, especially the level of 20:1n-9 was higher
264 in amphipod diet, resulting in the highest MONO content of all diets.

265 The essential amino acid levels in diets are given in Table 4. Only minor differences were
266 observed between the diets. The levels of histidine, methionine and lysine showed small decreases as
267 fish meal was replaced with meal from northern krill while levels of arginine, isoleucine and
268 phenylalanine increased. At the 400 g kg^{-1} replacement level, the variation was small and
269 insignificant for most compounds. The only amino acids found to vary were isoleucine and lysine,
270 but even in this case, variation was relatively small in absolute terms. According to NRC (1993) all
271 diets held levels of essential amino acids above described requirements for optimal growth.

272

273 *Atlantic salmon growth*

274 Salmon grew from around 410 g to approximately 1500 g during the experimental period (Fig. 1A).
275 There were no significant size differences between the dietary treatments or tanks at experimental
276 start (nested ANOVA, $P > 0.05$). Replacing fish meal protein with protein from northern krill,
277 generally increased fish weight, length, *k*-kactor and SGR (0.87 to 0.91-0.92), from start to 100 days
278 of feeding (Fig. 1A, B, C & D), but the differences were not significant at the 5% level. From 100

279 days to termination of feeding at day 160, no variation in growth pattern was detected between diet
280 groups. Average SGR, when calculated for the whole experimental period, was not significantly
281 affected by inclusion of northern krill. SGR values ranged between 0.80 and 0.82, and final weights
282 between 1486–1550 g (Fig. 1A). The *k*-factor increased from 1.13–1.14 at start, reaching 1.32-1.35
283 after 100 days of feeding, which was similar to what was observed after 160 days (Fig 1C). The K20
284 diet resulted in significantly higher *k*-factor than the K60 diet after both 100 and 160 days (nested
285 ANOVA, $P < 0.05$).
286 Replacing 400 g kg^{-1} of the fish meal protein with proteins from Antarctic krill or Arctic
287 amphipod significantly increased weight and SGR compared to the FM from start to 100 days of
288 feeding ($P < 0.01$; Fig. 1A & 1D). Also, fish fed with AK40 and AMP40 increased in length when
289 compared to fish fed FM, but only significant between AK40 and FM (Fig 1B). From 100 to 160
290 days of feeding fish fed AK40 and AMP40 did not differ from fish fed FM diet (Fig 1D). Overall (0-
291 160 days), for both AK40 and AMP40 groups SGR and final weight increased compared to fish fed
292 FM, though only AMP40 fed fish had a significantly higher SGR compared to FM group. The *k*-
293 factor increased from 1.13 to 1.36 and 1.35 during 160 days feeding period in FM and AK40 groups,
294 respectively. At termination of the experiment *k*-factor was significantly higher in fish fed AMP40
295 diet (1.39) compared to K40, K60 and AK40; Fig. 1C).
296
297 *Atlantic halibut growth*
298 The Atlantic halibut grew from around 345 g to 500-600 g during the 150 days experiment (Fig.
299 2A). There were no significant differences in weight between the dietary treatments or tanks at
300 experimental start (nested ANOVA, $P > 0.05$). Replacing fish meal protein with protein from
301 northern krill increased SGR ($P < 0.05$) linearly. During the first feeding period (0–78 days) K60
302 had significantly higher SGR compared to the FM and K20 groups (Fig. 2D). During the second
303 feeding period (up to day 150) these were tendencies, but not being statistically significant.
304 However, as a result of this, SGR during the total 150 days feeding resulted in fish fed diet K60 to
305 grow significantly faster compared to fish fed solely fish meal protein (0.39 vs 0.33). The *k*-factor
306 showed a similar pattern as growth and *k*-factor, resulting in a significantly higher *k*-factor in fish fed
307 diet K60 compared to FM after 78 days feeding (Fig. 2C). The difference in *k*-factor was mainted
308 throughout the study ($P < 0.01$). The *k*-factor increased or showed similar pattern in fish fed diet
309 AK40 as fish fed FM diet (Fig. 2D). The only difference in *k*-factor was noticed at the end of the 150

310 days feeding period, where fish fed diet AK40 had significantly lower *k*-factor compared to fish fed
311 diet K60. Fish fed diet AMP40 showed the slowest growth (SGR), weight- and length gains when
312 compared to all the other experimental groups, however, the growth difference was not significant
313 different from fish fed FM diet. The *k*-factor did not differ in this group when compared to the FM
314 group (Fig. 2C). Diet treatment AMP40 resulted in significantly lower SGR compared to groups
315 K40 and K60 for the whole experimental period (0.30 vs 0.39, 0.36; Fig. 2D). The *k*-factor was also
316 significantly lower in AMP40 fed fish after 78 days feeding than those fed diets K60 and K40 and
317 also after 150 days of feeding in group K60.
318

319 *Atlantic salmon flesh composition, FCR, ADC, blood and plasma parameters*

320 The muscle fatty acid composition was dominated by 16:0, 18:1n-9, 20:1n-9, 20:5n-3, 22:1n-11 and
321 22:6n-3 (Table 5) independent of dietary treatment. In general, differences between dietary
322 treatments were rather small. The only significant differences found were marginal increases in
323 levels of 18:1n-9, 18:1n-7 and 20:5n-3 as the level of meal from northern krill increased ($P < 0.001$,
324 $P < 0.001$ and $P < 0.05$, respectively) along with increases in 18:1n-7 in fish fed AK40 diet. In each
325 case fatty acids in muscle mirrored fatty acids in the diets.

326 No differences in FCR were detected between start and 100 days of feeding, varying between
327 0.85-0.93 (Table 6). During 100-160 days of feeding, FCR increased in all groups, although no
328 significant tendency towards increased FCR was noticed.

329 The protein content in faeces remained stable varying from 279 g $kg^{-1}$and 293 g kg^{-1}, faecal
330 moisture content was around 850 g kg^{-1} for all groups except for a significant lower moisture level in
331 the AMP40 group (833 g kg^{-1}; $P < 0.05$, Table 6). Lipid content in faeces was highest in fish fed FM
332 (102 g kg^{-1}) and lowest in fish fed K20 (58 g kg^{-1}). No significant differences were observed
333 between diet groups (Table 6).

334 The ADC of dry matter varied between 95.3% and 96.5%. The ADC of protein was very
335 similar in all groups and varied from 84.2 (FM) to 85.7% (K60). Lipid ADC was stable and varied
336 from 98.8 to 99.1% (Table 6).

337 Flesh composition and blood parameters are shown in Table 7. Muscle dry matter varied
338 between 323 and 332 g kg^{-1} and muscle lipid content between 234 and 303 g kg^{-1} of muscle dry
339 weight. Blood haemoglobin was fairly stable between groups varying from 80 (K20) to 92 (K40) g
340 L^{-1}, HCT and RBC showed a similar pattern, varying between 39 and 43 g L^{-1} and between 1.0 and

341 1.2 1×10 ^{12} L^{-1}, respectively. Plasma glucose varied between 4.9 and 5.7 mM and protein between 56
342 and 62 g L^{-1} with no differences between dietary groups. Plasma ALAT varied between 18 and 40 U
343 L^{-1} being lowest in K40 and K60 diets and highest in fish fed the AMP40 diet. The same pattern was
344 shown in plasma ASAT values that varied between 337 and 737 U L^{-1} being lowest in fish fed diet
345 K40 and highest in fish fed diet AMP40.
346
347 *Atlantic halibut flesh composition, FCR, ADC, blood and plasma parameters*
348 Muscle fatty acid composition (Table 8) was remarkably stable regardless of diet, with minor
349 changes that appeared to be related to dietary fatty acid profiles. The most noticeable effects were
350 that fish fed FM had higher muscle level of 16:0 (170 g kg^{-1}) compared to fish fed AK40 (135 g
351 kg^{-1}; $P < 0.01$). The level of 20:5n-3 (EPA) in muscle increased from 100 g kg^{-1} in the control group
352 up to 146 g kg^{-1} in fish fed diet K60 ($P < 0.05$). The level of 22:6n-3 (DHA) in muscle dominated in
353 all groups, being the highest in fish fed AK40 (340 g kg^{-1}) and AMP40 (348 g kg^{-1}) diets and lowest
354 in fish fed FM (285 g kg^{-1}).
355 The total *n*-3 PUFA level in muscle did not vary significantly between groups fed different
356 diets, but showed somewhat lower values (461 g kg^{-1}) in the FM group and somewhat higher values
357 (535 g kg^{-1}) in fish fed diet AK40. The level of SAT was significantly higher in fish fed FM
358 compared to fish fed AK40.
359 No significant differences were found in FCR values, which varied from 0.78 in FM fed fish
360 to 0.99 in AMP40 fed fish, when calculated for the whole feeding period (Table 6).
361 The protein content in faeces varied from 281 g kg^{-1} to 309 g kg^{-1} (Table 6). The moisture
362 content in faeces varied from 861 g kg^{-1} in FM fed fish to 887 g kg^{-1} in fish fed K60.
363 ADC of dry matter varied between 94 and 96% showing a slight tendency to increase from
364 FM to K60 along with increased northern krill inclusion, and being the lowest in fish fed AMP40.
365 Protein ADC ranged from 74 (K20) to 79% (K60) (Table 6).
366 No statistical differences in total fat, or blood and plasma values were found in Atlantic
367 halibut fed the different experimental diets. Blood haemoglobin values ranged between 6 and 7 g
368 100mL $^{-1}$ in all diet groups (data not shown). Total lipid in the muscle (on a dry weight basis) varied
369 from 43 g kg^{-1} in fish fed K60 to 58 g kg^{-1} in fish fed AK40 with no significant differences between
370 dietary groups (Table 6). Muscle dry matter was also similar in all groups varying from 211 to 219
371 g kg^{-1} (Table 6).

Discussion

The nutritional value of northern krill (*Thysanoessa inermis*), Antarctic krill (*Euphausia superba*), and Arctic amphipod (*Themsto libellula*) was for the first time shown to support growth and feed utilisation as well as or even better than, when Atlantic salmon and Atlantic halibut were fed fish meal diets. The content of lipids, essential fatty- and amino acids from northern krill, Antarctic krill and amphipod were in the range of that found in the fish meal used. Northern krill was particularly rich in lipid (182 g kg^{-1}). The levels of biogenic amines in the meals were low and may indicate low enzymatic degradation grade of raw materials before processing (Pike, 1993; Aksnes & Mundheim, 1997).

The lower protein level of the zooplankton meals was particularly evident for the amphipod meal, showing values of 200 g kg^{-1} less protein when compared to fish meal. Consequently, a larger zooplankton mass is required in all zooplankton diets, compared to fish meal based diets, in order to obtain adequate levels of protein. This may pose a problem as ash and chitin dilute energy. Numerous studies with salmonids have shown that energy-dense diets stimulates growth and feed utilisation (Hillestad, 2001), still no adverse effects were detected in any of the present evaluated ingredients. High chitin amounts may also decrease lipid absorption as shown by Olsen *et al.* (2006) where the digestibility of lipid showed reduction, when high levels (> 80% of diet proteins) of Antarctic krill was included in the diets. However, fish meal replacement up to 60% of crude protein did not reduce lipid digestion or increase lipid amount in faeces in any of the present diet groups, even if approximately similar dietary chitin level was used in this study 29 vs 27 g kg^{-1} by Olsen *et al.* (2006). In the present study the highest dietary chitin level originated from amphipods, whereas Olsen *et al.* (2006) used Antarctic krill. Different nutrient profile of the zooplankton species evaluated, or variable growth rates in fish may have resulted in differences in lipid digestibility.

The present results on dry matter ADC in Atlantic salmon agreed with values obtained for Antarctic krill by Olsen *et al.* (2006). The somewhat reduced ADC_{DM} in Atlantic halibut (0.5-1.4% lower) when compared to salmon, are still within acceptable values, especially when compared to Hatlen *et al.* (2005) who reported significantly lower ADC_{DM} in Atlantic halibut when fed diets varying in protein and carbohydrate levels, but without any krill in diets. Fish size in the study from Hatlen *et al.* (2005) varied between 258 and 1580 g. Furthermore, stripping of faeces (Hevrøy *et al.* 2005) or the faeces collecting technique as described by Windell *et al.* (1978), and Choubert *et al.*

403 (1979) may affect deviations resulting in different moisture content to faecal material. Similar
404 moisture content in the faeces of Atlantic halibut was observed in this study as reported by Hatlen *et*
405 *al.* (2005). Olsen *et al.* (2006), however, reported lower faecal moisture content in Atlantic salmon
406 when compared to this study when same fish species and size was used, this might partly be
407 explained by the different faecal sampling techniques used.

408 True protein digestibility, evaluated in mink, was highest for the fish meal diet and appeared
409 to decrease with increasing amount of chitin in the diets, as was the case when the various krill
410 species increased in concentration in our experimental diets. The higher chitin level in the plankton
411 based meals, in particular the amphipod diet, influencing protein digestibility in a negative manner,
412 agree with findings showing that the fibre fraction of a fish diet highly influences digestibility of
413 macronutrients (Krogdahl *et al.* 2005). However, inclusion of zooplankton in the diets did not result
414 in lowered protein ADC in fish. Krill meal amino acid profile is reported to be well balanced with
415 respect to requirements of cultured fish (Nicol *et al.* 2000). Protein digestibility values for Atlantic
416 salmon were somewhat lower than observed in the experiment with Atlantic salmon of similar size
417 (Olsen *et al.* 2006). Hatlen *et al.* (2005) reported higher protein digestibility (N × 6.25) for Atlantic
418 halibut, which varied between 81 and 83%.

419 Olsen *et al.* (2006) reported 5-9% lower lipid digestibility values with similar fish size, and
420 comparable ingredient composition, when compared to the present study with Atlantic salmon.

421 Under the present experimental conditions, the data show that exchanging fish meal protein
422 with meal from three different zooplankton protein sources does not have adverse effects on fish
423 growth and agree with previous findings for Antarctic krill in diets for rainbow trout (*Oncorhynchus*
424 *mykiss* Walbaum) (review; see Storebakken, 1988), chinook salmon (*Oncorhynchus tshawytscha*;
425 Anderson *et al.* 1997) with Atlantic salmon (Julshamn *et al.* 2004; Olsen *et al.* 2006) and Atlantic
426 cod (Moren *et al.* 2006). In the present study, there was however a general tendency of fish to
427 respond to increased krill meal in diets by ingesting more of the diet to maintain growth. These
428 results are in accordance with the earlier study by Bromley & Adkins (1984) where they found that
429 undigested α-cellulose up to 300g kg^{-1} of the feed for rainbow trout was compensated by increased
430 intake. The slightly higher growth rate during the first period of feeding has also been observed
431 previously (Olsen *et al.* 2006) and could relate to natural attractants present in the zooplankton
432 meals. Atlantic salmon responded differently to various krill ingredients when compared to Atlantic
433 halibut. This can be concluded when comparing growth pattern in the two species, with an initial

SGR stimulation in salmon, but a continuous SGR stimulation throughout the 160 days of feeding in halibut. The two species responded also in a different manner on the various zooplankton species. Additionally SGR was greater in smaller Atlantic salmon, showing a reduction as fish size increased, in agreement with (Brett, 1979; Jobling, 1988; Brander, 1995), while Atlantic halibut had higher SGR in this study from 78 to 150 days compared to the first feeding period. This might also be a result from a need for longer time for Atlantic halibut to adapt to a new environment, or taste, when compared to Atlantic salmon. Krill products are known to be excellent feed attractants in fish diets, e.g. for rainbow trout (Oikawa & March, 1997), and it is possible that this caused the fish to accept the experimental diets more readily during the early feeding phase in Atlantic salmon. Similar results were obtained with largemouth bass, where krill meal diet was accepted first and showed to be the most attractive starter diet (Kubitza & Lovshin, 1997). Increased palatability with krill diets may also explain the growth enhancement found in the 56 days feeding trial in Chinook salmon juveniles (Anderson *et al.* 1997) and improved diet ingestion rates in yellow perch (*Perca flavescens*) and lake whitefish larvae (*Coregonus clupeaformis*) (Kolovski *et al.* 2000). Growth enhancement effects with whole krill meal was also reported by Allahpichay & Shimizu (1984), where whole krill was included in red sea bream, Japanese eel and grey mullet diets. High level of the amino acids glycine, glucosamine and proline together with nucleotides, nucleosides and ammonium present in krill meal are suggested to enhance chemical stimuli in fish when included in feed (Kolkovski *et al.* 2000).

The fatty acid composition, in particular of depot lipids, is known to be heavily influenced by the diets (Sargent *et al.* 2002; Bell *et al.* 2004). This was especially the case for salmon in this study. However, the fish also has capacity to modify fatty acids to fit a narrower and more preferred composition (Greene & Selivonchick, 1990; Olsen *et al.* 1999). This was also evident in the present study where muscle differences for salmon were less in magnitude than that of the diets. For Atlantic halibut, the differences were even less pronounced. This probably reflects two situations. Firstly, the fish may actively modify fatty acids prior to incorporation into muscle as for salmon. Secondly, the high PUFA content indicate that the fish had low lipid depots, which would indicate that a majority of the fatty acids in this tissue were phospholipids and part of structural lipids (Greene & Selivonchick, 1990; Olsson *et al.* 2003; Rosenlund *et al.* 2005). Also, 16:0, 20:5n-3 and 22:6n-3 were the most abundant fatty acids in muscle phospholipids in small fish (Bell & Dick, 1991), the same fatty acids were the dominant fatty acids present in Atlantic halibut muscle in our study. These membrane lipids are under even more stringent control than depot lipids, and it is not likely that the

465 moderate changes in diet in the present study could have major impact on such structures. That is in
466 accordance with the study where small Atlantic halibut (0.6–1.5 kg) was fed varied amount of lipid
467 in the diet (180-380g kg^{-1} of diet dm) without affecting muscle total lipid composition (Berge &
468 Storebakken, 1991). However, the highest body total lipid content is generally found in red muscle
469 near the lateral line tissue and at the belly flap, as shown by Exler *et al.* (1975) and Nortvedt &
470 Tuene (1998). In this study the white muscle samples of Atlantic halibut were cut from the middle of
471 the thick part of the muscle below the dorsal fin, where lipid depots normally are low.
472 The main trends seen in the present were that diet level of 18:1n-9 showed increase from
473 8.1% to 10.1% concomitant with increased level of northern krill in diets. The same fatty acid level
474 increased in Atlantic salmon muscle from 12.2% in FM group to 14.6% in K60 group. In Atlantic
475 halibut the level of 18:1n-9 remained more stable showing only a slight increase from 4.9% in FM to
476 5.5% in K60. The level of 18:3n-3 and 18:4n-3 decreased in the diet with increasing level from
477 northern krill. The muscle levels of these fatty acids were lower in both fish species compared to the
478 levels in the diet, and may indicate elongation and desaturation of these fatty acids as discussed by
479 Stubhaug *et al.* (2005). The levels of long chain monoenes 20:1n-9 and 22:1n-11 were high in the
480 diets, but low in body tissues, especially in Atlantic halibut. These fatty acids have been reported to
481 be reduced in muscle due to metabolic modification of dietary lipids Hemre *et al.* (1992), and
482 preferentially to be oxidised and not retained in the body (Henderson & Sargent, 1985; Lie &
483 Lambertsen, 1991; Bell *et al.* 2001; Stubhaug, 2005). The level of 20:5n-3 increased in both Atlantic
484 salmon and Atlantic halibut compared to the level in diets. This fatty acid together with 18:2n-6 has
485 shown the greatest uptake and retention in Atlantic salmon (Vegusdal *et al.* 2004; Stubhaug *et al.*
486 2005). Consequently, the level of essential fatty acids 20:5n-3 (EPA) and 22:6n-3 (DHA) was
487 upconcentrated in muscle tissue showing higher values compared to the diets. The level of EPA
488 showed a linear increase in both Atlantic salmon and Atlantic halibut muscle when fed diets with
489 increasing level of northern krill in agreement with increasing dietary levels.
490 The amount of essential and total free amino acids in muscle from Atlantic salmon and
491 Atlantic halibut, decreased concomitant as fish meal was replaced by zooplankton diet (data not
492 shown). A further study of free amino acids in Atlantic salmon and Atlantic halibut muscle fed
493 zooplankton is presently going on.
494 The tendency towards poorer FCR with increased levels of zooplankton in the feed (not
495 significant) may be related to the increased content of indigestible compounds like chitin in those

496 diets. In the present study, fish seemed to respond to this condition by eating more, and thus
497 maintaining a relatively high growth rate. This compensative response appeared to be sufficient to
498 maintain muscle lipid level as indicated by similar muscle dry weight and lipid level in all groups
499 examined. Furthermore this muscle lipid level was in the range of previous literature of large salmon
500 fed diets of similar lipid content (Bell *et al.* 1998) and small halibut (Aksnes *et al.* 1996; Nortvedt &
501 Tuene, 1998). Muscle dry matter content in Atlantic halibut was similar in all groups (211-219 g
502 kg^{-1}) independent of the protein source used, in accordance with results from Aksnes *et al.* (1996),
503 where muscle dry matter remained unaffected, even though slightly higher, (263-272 g kg^{-1})
504 regardless of dietary protein level. Atlantic salmon had 11-12% higher dry matter values in this
505 study compared to Atlantic halibut. Olsen *et al.* (2006) reported 4-5% lower muscle dry matter in
506 Atlantic salmon when compared to this study.

507 All blood parameters measured were within normal ranges for Atlantic salmon (Sandnes *et*
508 *al.* 1988), and Atlantic halibut (Hemre *et al.* 1992) indicating no adverse health effects even at very
509 high krill inclusion levels. This confirms the earlier results obtained for Atlantic salmon (Olsen *et al.*
510 2006) and Atlantic cod (Moren *et al.* 2006) where no differences were found in blood parameters
511 when using zooplankton in diets. This was further confirmed by no variation in ASAT or ALAT
512 levels between diet groups, since these organ-specific enzymes would leak to the plasma
513 compartment in case of liver or kidney damage (Sandnes *et al.* 1988). Further, feeding sub-optimal
514 diets for prolonged time most probably would result in elevated plasma glucose that was not found
515 in the present study (Hemre & Sandnes, 1998). Additionally, the plasma total protein indicated good
516 nutritional condition of fish (Hemre *et al.* 1995).

517 In conclusion, zooplankton can partially replace fish meal in diets to Atlantic salmon and
518 Atlantic halibut without causing any adverse effects on growth, it may even results in stimulation of
519 appetite and growth. No effect was detected on feed conversion or fish health / welfare parameters,
520 and the marine fatty acid profiles in fish muscles were maintained or even improved.
521
522

523 ## Acknowledgement

524 The work was in part supported by grants from the Norwegian Research Council (”Krill as feed
525 source for fish”, 146871/120). We are also indebted to Ivar Helge Matre and Anor Gullanger and the
526 staff at the Institute of Marine Research Austevoll for technical assistance.
527

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Table 1 Composition (g kg^{-1}) of the fish- and krill meal used in the experiments

	Fish meal[1]	Northern krill	Antarctic krill	Amphipod
Crude protein	732	591	641	524
Moisture	72	75	67	66
Ash (including NaCl)	124	104	151	222
Lipid	75	182	93	99
Salt (NaCl)	23	44	59	38
Total volatile nitrogen	1.3	1.7	1.0	0.5
Water soluble protein (of total protein)	296	532	517	363
Biological digestible protein (%) (of total protein) (mink)[2]	92	90	90	81
Chitin (g kg^{-1})	0	30	40	100
Biogenic amines (g kg^{-1})				
Putrescin	0.28	<0.1	0.2	<0.1
Cadaverin	0.75	<0.1	0.4	<0.1
Histamin	0.12	<0.1	<0.1	<0.1
Astaxanthin (mg kg^{-1})				
Free	0	<5	5	2
Esters	0	109	94	8
Total	0	<114	99	10

[1] Norse-LT 94® from 620 g kg^{-1} blue whiting and 380g kg^{-1} capelin.

[2] The biological protein digestibility is nitrogen digestibility and calculated as: $N_{consumed}$ - (N_{faeces} - $N_{endogenous}$) x1000/$N_{consumed}$ where $N_{consumed}$ = consumed nitrogen, N_{faeces} = nitrogen in faeces, $N_{endogenous}$ = endogenous nitrogen in faeces. No correction was made for chitin in feed or faeces.

Table 2 Composition (g kg^{-1}diet) of the diets used in the experiment

Diet[1]	**FM**	**K20**	**K40**	**K60**	**AK40**	**AMP40**
Fish meal[2]	613	491	368	245	370	373
Krill meal	0	152	303	454	281	348
Fish-oil (Norsalmoil)	220	201	183	163	212	204
Soybean lecithin	5	5	5	5	5	5
Mais suprex	146	136	126	117	116	54
Mineral mixture [3]	4	4	4	4	4	4
Vitamin mixture [4]	10	10	10	10	10	10
Inositol	0.3	0.3	0.3	0.3	0.3	0.3
Betaine	1	1	1	1	1	1
Carophyl pink® (10%)	0.8	0.6	0.4	0.1	0.5	0.8
Chemical composition by analysis (g kg^{-1} of diet)						
Moisture	69	75	78	92	77	79
Protein	459	459	458	457	460	461
Lipid	248	253	255	246	256	251
Ash	80	81	81	82	93	128
Carbohydrate	144	129	120	113	103	52
Chitin	0	4	7	10	11.6	29
Energy (MJ kg^{-1})	24	23.9	23.9	23.8	23.8	23.2
Calculated Carotenoids (mg kg^{-1} diet)						
Astaxanthin Carophyll pink®	64	48	32	8	40	64
Astaxanthin free (from krill meal)	0	0.8	1.5	2.3	1.4	0.7
Astaxanthin esters (from krill meal)	0	16.4	32.7	49.1	26.3	2.7
Total astaxanthin	64	65.1	66.2	59.3	67.6	67.4

[1] FM, Fish meal diet; K20, K40 and K60, 20-60% of the diet proteins from *T. inermis*; AK40 and AMP40, 40% of the diet proteins origins from *E. superba* and *T. libellula*, respectively.

[2] Norse-LT 94® from 62% blue whiting and 38% capelin.

[3] will supply diets with the following vitamins pr kg diet: vitamin D3, 3000 I.E; vitamin E (Rovimix, 50%), 160 mg; thiamin, 20 mg; riboflavin, 30 mg; pyridoxine-HCl, 25 mg; vitamin C (Rovimix Stay C 35%), 200mg; calcium pantothenate, 60 mg; biotine, 1 mg; folic acid, 10 mg; niacin, 200 mg; vitamin B 12, 0,05 mg; menadione bisulphite, 20 mg.

[4] will supply the diet with the following minerals pr kg diet: magnesium, 500 mg; potassium, 400 mg; zinc 80 mg; iron, 50 mg; manganese, 10 mg; copper, 5 mg.

Table 3 Total lipid content (g kg^{-1} of dry weight) and fatty acid composition (g kg^{-1} of total fatty acids) of the experimental diets fed to Atlantic salmon and Atlantic halibut during 160 days (n = 4).

	FM	**K20**	**K40**	**K60**	**AK40**	**AMP40**
Total lipid	248	251	255	246	254	256
Fatty acid						
14:0	33	28	26	29	37	36
16:0	118	124	122	126	132	117
16:1n-9	49	45	40	40	47	46
18:1n-9	81	88	95	101	83	77
18:1n-7	23	25	28	31	29	21
18:2n-6	29	28	26	24	27	28
18:3n-3	13	13	12	11	13	12
18:4n-3	41	38	36	34	39	37
20:1n-9	102	95	87	72	92	104
20:4n-6	6	6	5	5	6	6
20:5n-3	88	95	105	108	93	78
22:1n-11	126	120	109	88	115	118
22:5n-3	7	7	6	5	7	6
22:6n-3	98	93	90	79	95	88
SAT	201	203	205	223	221	212
MONO	462	463	458	444	444	471
PUFA	337	334	337	338	333	337
n-3	288	285	293	290	286	284
n-6	48	49	45	48	47	53

SAT = saturated fatty acids, MONO = mono unsaturated fatty acids
PUFA = poly unsaturated fatty acids
Totals include some minor components not shown.

Table 4 Essential amino acids in diet (g kg^{-1} protein) (n = 3) and sum of essential amino acids, ∑ EAA

	FM	**K20**	**K40**	**K60**	**AK40**	**AMP40**
Arginine	75	76	79	78	77	77
Histidine	24	23	23	21	23	23
Isoleucine	41	42	44	44	43	42
Leucine	43	44	44	43	44	43
Lysine	79	78	77	73	74	76
Methionine	28	28	28	27	28	28
Phenylalanine	42	42	43	43	42	41
Threonine	43	44	44	44	44	45
Valine	49	51	51	51	51	50
∑ EAA	424	428	433	425	426	425

Tryptophane was not detectable with the method used.

Table 5 Muscle fatty acid composition (g kg^{-1} total fatty acids) of Atlantic salmon (*Salmo salar* L.) fed with six experimental diets ± SD (n = 3). Significant effects were determined by one-way ANOVA and Tukey multiple range test.

	FM	K20	K40	K60	AK40	AMP40	P
14:0	24±6	25±9	21±5	25±7	19±0	27±4	ns
16:0	130±18	124±14	140±7	142±5	128±1	127±11	ns
16:1n-9	44±2	48±3	42±2	44±4	43±0	44±1	ns
18:1n-9	122±8^{ab}	135±13^{bc}	139±2^{bc}	146±7^{c}	133±2^{abc}	111±4^{a}	**
18:1n-7	25±1^{a}	30±1^{b}	31±0^{b}	33±2^{b}	32±0^{b}	25±1^{a}	***
18:2n-6	25±2	27±1	25±1	25±1	27±1	26±1	ns
18:3n-3	11±1^{ab}	11±0^{ab}	10±0^{a}	10±0^{a}	12±0^{b}	11±1^{ab}	**
18:4n-3	22±2	24±1	22±0	22±1	25±1	24±1	ns
20:1n-9	87±5^{abc}	85±8^{abc}	84±1^{ab}	72±5^{a}	88±1^{bc}	99±4^{c}	**
20:4n-6	4±0	4±0	4±0	4±0	4±0	4±0	ns
20:5n-3	65±1^{ab}	71±3^{ab}	71±3^{ab}	76±8^{b}	68±4^{ab}	59±2^{a}	*
22:1n-11	94±5^{ab}	90±11^{ab}	92±3^{ab}	75±7^{a}	95±1^{ab}	103±6^{b}	**
22:5n-3	25±1^{ab}	26±0^{abc}	26±1^{abc}	27±1^{c}	27±0^{bc}	24±1^{a}	**
22:6n-3	124±6	120±5	116±0	114±1	124±5	126±10	ns
SAT	216±26	197±7	218±7	226±6	201±4	201±9	ns
MONO	448±17	459±16	456±9	438±8	459±4	455±32	ns
PUFA	336±15	343±23	326±2	336±3	340±1	324±16	ns
n-3	291±13	295±19	282±2	291±2	295±0	279±13	ns
n-6	45±2	49±4	43±0	45±4	45±1	45±4	ns
n-3/n-6	70±2	65±2	70±0	70±5	70±2	66±3	ns

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$
ns = not significant
Common superscript $^{a, b}$ indicates significant difference between groups
Totals include some minor components not shown.
Values bearing different superscript letter are significantly different ($P< 0.05$).

Table 6 Feed conversion ratio (FCR), composition of faeces and apparent nutrient digestibility of Atlantic salmon and Atlantic halibut fed krill and fish meal diets (mean ± SD, *n* = 3) Significance were determined by one-way ANOVA and Tukey multiple range test.

	FM	K20	K40	K60	AK40	AMP40	P
Atlantic salmon							
FCR							
0-100 d	0.89± 0.02	0.85± 0.01	0.92± 0.04	0.92± 0.01	0.92± 0.04	0.93± 0.03	ns
100-160 d	0.92± 0.02	0.92± 0.00	0.96± 0.11	1.05± 0.10	1.00± 0.07	1.08± 0.04	ns
Chemical composition of faeces by dry weight (g kg^{-1})[1]							
Protein	279± 6	287± 15	293± 3	287± 3	282± 7	283± 9	ns
Moisture	850± 22^{b}	847± 8^{ab}	850± 2^{b}	860± 6^{b}	847± 20^{ab}	830± 13^{a}	*
Lipid	102± 17	58± 19	63± 5	69± 4	99± 13	86± 23	ns
ADC[2] (%)							
Dry matter	95.9± 0.5	95.7± 0.2	96.1± 0.2	96.5± 0.2	96.2± 0.2	95.3± 0.3	ns
Protein	84.2± 0.5	84.8± 0.5	84.7± 0.7	85.7± 0.5	85.5± 0.9	84.3± 0.1	ns
Lipid	98.3± 0.4	99.1± 0.4	99.1± 0.1	99.1± 0.1	98.6± 0.3	98.6± 0.5	ns
Atlantic halibut							
FCR							
0-150 d	0.78± 0.02	0.97± 0.01	0.87± 0.22	0.95± 0.03	0.88± 0.19	0.99± 0.00	ns
Chemical composition of faeces by dry weight (g kg^{-1})[1]							
Protein	286± 17	293± 8	282± 12	298± 13	309± 16	290± 6	ns
Moisture	861± 31	877± 21	882± 4	887± 15	875± 3	878± 1	ns
ADC[3] (%)							
Dry matter	94.5± 0.7	94.6± 0.6	95.0± 0.7	96.0± 1.2	94.8± 0.5	94.4± 0.1	ns
Protein	78.1± 0.9	74.0± 2.6	76.1± 1.6	79.4± 4.2	74.6± 1.6	75.2± 1.0	ns
Muscle (g kg^{-1})							
Total lipid	44± 6	44± 5	43± 4	43± 4	58± 15	45± 3	ns
Dry matter	217± 3	219± 6	214± 2	211± 12	217± 4	212± 4	ns

* $P < 0.001$
ns=not significant ($P > 0.05$)
Common superscript [a, b] indicates significant difference between groups
[1] Chemical composition of faeces by dry weight (g kg^{-1}) $n = 9 \pm$ SD
[2] ADC, Apparent digestibility coefficient of nutrients (g kg^{-1}) $n = 3 \pm$ SD.
[3] ADC, Apparent digestibility coefficient of nutrients (g kg^{-1}) $n = 2 \pm$ SD.

Table 7 Mean (± SD) of Atlantic salmon flesh composition, blood values of haemoglobin (HB), red blood cells (RBC) and haematocrit (HCT), and plasma values of ASAT, ALAT, glucose and protein for the different dietary treatments. The data were analysed by nested ANOVA. Lack of common superscript indicates no significant difference between groups. *n* = 9 for flesh composition and 15 for other parameters.

	Muscle		Blood			Plasma			
	Dry matter ($g\ kg^{-1}$)	Lipid ($g\ kg^{-1}$)	HB ($g\ L^{-1}$)	RBC ($1\times10^{12}\ L^{-1}$)	HCT (%)	Protein ($g\ L^{-1}$)	Glucose ($mmol\ L^{-1}$)	ALAT ($U\ L^{-1}$)	ASAT ($U\ L^{-1}$)
FM	327 ± 14	234 ± 51	84 ± 6^{ab}	1.1 ± 0.1^{ab}	42 ± 4	62 ± 6	5.4 ± 0.7	36 ± 19^{b}	472 ± 283^{ab}
K20	323 ± 13	277 ± 70	80 ± 8^{a}	1.0 ± 0.1^{a}	39 ± 5	61 ± 7	5.7 ± 1.4	35 ± 17^{ab}	637 ± 363^{ab}
K40	332 ± 10	277 ± 61	92 ± 14^{b}	1.2 ± 0.1^{b}	42 ± 4	56 ± 7	5.4 ± 0.8	18 ± 21^{a}	337 ± 90^{a}
K60	327 ± 11	265 ± 72	88 ± 10^{ab}	1.1 ± 0.2^{ab}	43 ± 5	56 ± 9	4.9 ± 1.4	22 ± 31^{ab}	489 ± 263^{ab}
AK40	331 ± 9	303 ± 40	83 ± 11^{ab}	1.0 ± 0.2^{ab}	39 ± 7	57 ± 7	5.1 ± 1.3	28 ± 48^{ab}	435 ± 309^{ab}
AMP40	329 ± 13	277 ± 86	83 ± 11^{ab}	1.0 ± 0.2^{ab}	41 ± 5	59 ± 5	5.3 ± 1.5	39 ± 46^{ab}	737 ± 382^{b}
P	ns	ns	*	**	ns	ns	ns	**	**

* P < 0.05. ** P < 0.01.
ns=not significant (P > 0.05)

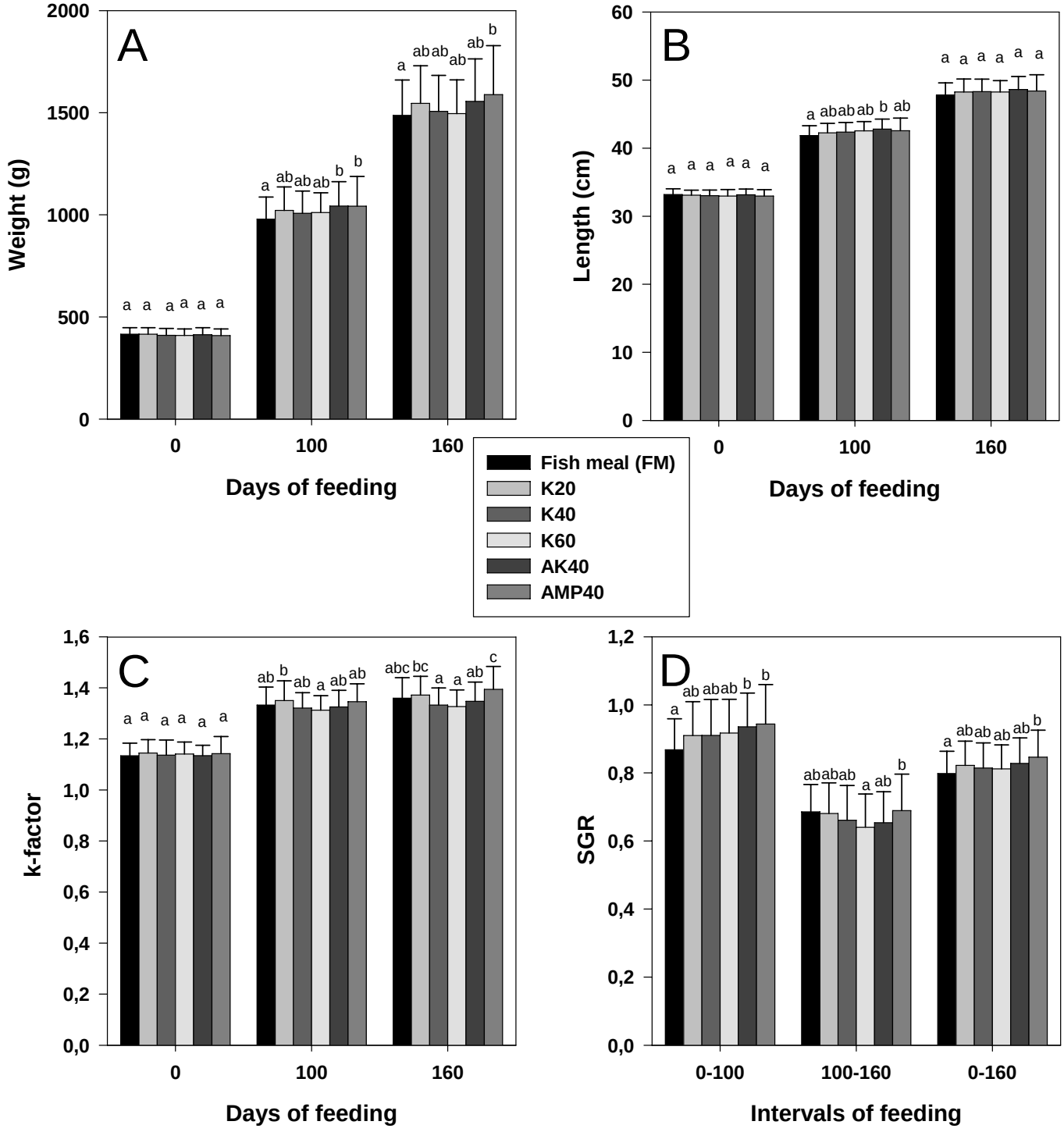
Table 8 Muscle fatty acid composition (g kg $^{-1}$ total fatty acids) of Atlantic halibut (*Hippoglossus hippoglossus* L.) fed with six experimental diets ± SD (n = 3). Significant effects were determined by one-way ANOVA and Tukey multiple range test.

	FM	K20	K40	K60	AK40	AMP40	P
14:0	16±5	8±7	8±3	8±4	6±2	8±2	ns
16:0	170±3^{b}	150±13^{ab}	154±8^{ab}	163±10^{b}	135±9^{a}	160±9^{ab}	*
16:1n-9	17±5	15±8	13±7	11±2	13±6	10±2	ns
18:1n-9	49±3	54±12	51±10	55±3	59±12	46±3	ns
18:1n-7	17±0	19±3	19±2	22±1	22±2	17±1	*
18:2n-6	22±0	22±3	22±4	19±1	22±3	21±2	ns
18:3n-3	4±0	3±2	3±2	3±1	3±3	2±1	ns
18:4n-3	6±2	7±6	6±5	3±2	6±8	3±1	ns
20:1n-9	37±3	36±13	34±9	30±3	44±12	40±3	ns
20:4n-6	15±2	16±3	17±2	16±1	17±4	15±1	ns
20:5n-3	100±13^{a}	132±8^{ab}	126±10^{ab}	146±4^{b}	142±26^{ab}	115±7^{ab}	*
22:1n-11	29±3	23±15	20±11	18±4	32±18	23±6	ns
22:5n-3	17±1	18±2	18±1	18±2	19±3	17±1	ns
22:6n-3	285±8	328±39	326±51	310±37	340±35	348±22	ns
SAT	276±3^{b}	231±11^{ab}	241±15^{ab}	254±23^{ab}	210±17^{a}	246±16^{ab}	*
MONO	215±9	188±52	185±36	181±14	208±55	181±7	ns
PUFA	509±12	582±63	573±42	565±37	582±45	573±21	ns
n-3	461±12	528±60	522±47	517±36	535±45	525±21	ns
n-6	48±0	54±2	52±6	48±3	47±1	48±2	ns
n-3/n-6	106±0	104±8	111±21	115±7	119±12	117±6	ns

* $P < 0.05$.
ns=not significant ($P > 0.05$)
Common superscript $^{a, b}$ indicates significant difference between groups
Totals include some minor components not shown.
Values bearing different superscript letter are significantly different ($P< 0.05$).

Figure 1 Growth of Atlantic salmon fed diets with fish meal (FM) and various amounts of krill meal from *T. inermis* (20-60% of the diet protein) and 40% of *E. superba* and *T. libellula*, respectively. Increase in (**A)** weight, (**B)** length, (**C)** *k*-factor, (**D)** SGR between 0 and 100 days, 100 and 160 days and whole period 0-160 days. $n = 90 \pm$ SD.

Figure 2 Growth of Atlantic halibut fed diets with fish meal (FM) and various amounts of krill meal from *T. inermis* (0-60% of the diet protein) and 40% of *E. superba* and *T. libellula*, respectively. Increase in (**A)** weight, (**B)** length, (**C)** *k*-factor, (**D)** SGR between 0 and 78 days, 78 and 150 days and whole period 0-150 days. $n = 80 \pm$ SD.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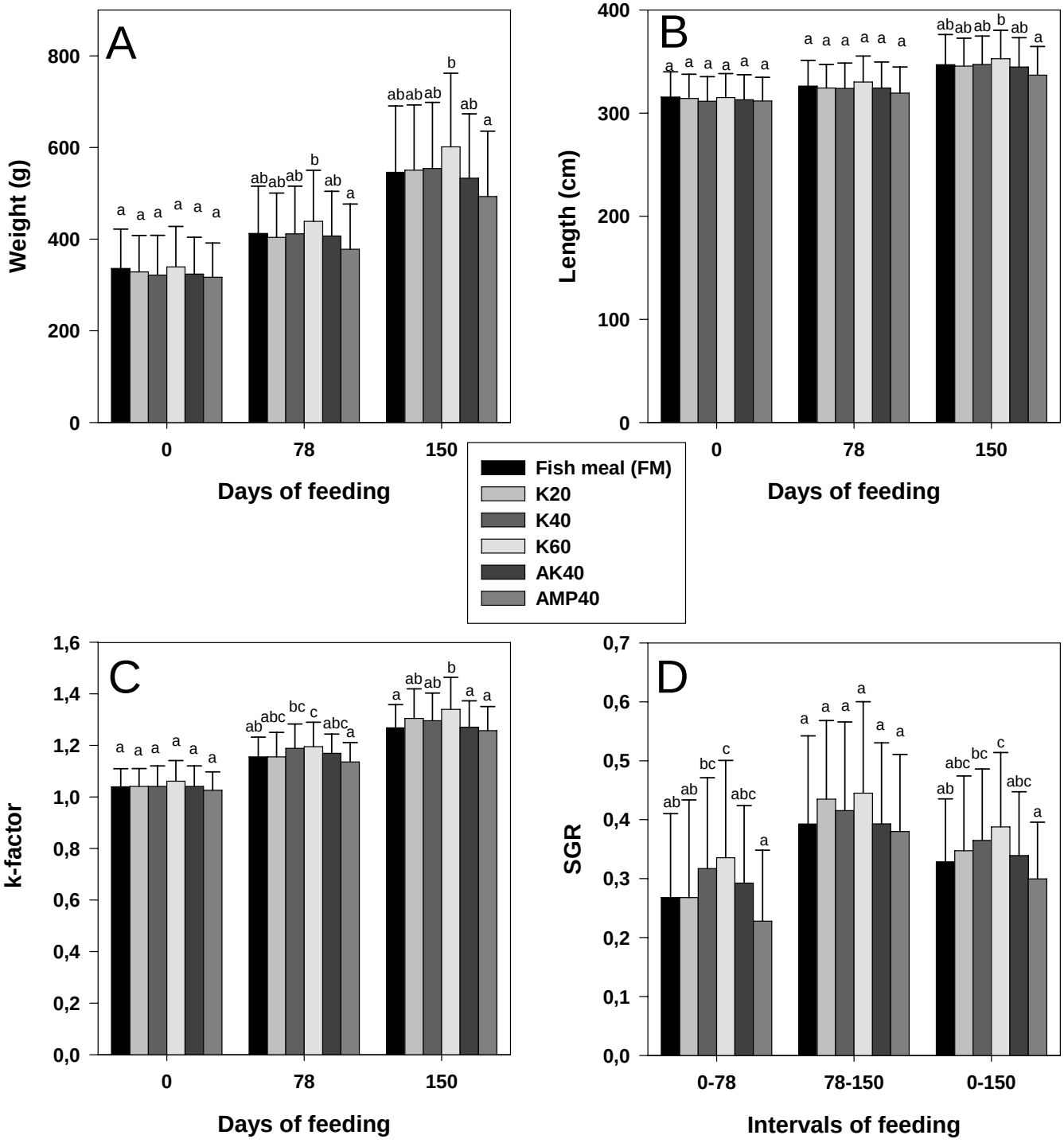
1500
1000
500
0
0
100
160
Days of feeding
B
Length (cm)
60
50
40
30
20
10
0
Days of feeding
Fish meal (FM)
K20
K40
K60
AK40
AMP40
C
k-factor
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
Days of feeding
D
SGR
0-100
100-160
0-160
Intervals of feeding


A
Weight (g)
800
600
400
200
0
a a a a a a
ab ab ab b ab a
ab ab ab b ab a
0
78
150
Days of feeding
B
Length (cm)
400
300
200
100
0
a a a a a a
a a a a a a
ab ab ab b ab a
0
78
150
Days of feeding
Fish meal (FM)
K20
K40
K60
AK40
AMP40
C
k-factor
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
a a a a a a
ab abc bc c abc a
a ab ab b a a
0
78
150
Days of feeding
D
SGR
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
ab ab bc c abc a
a a a a a a
ab abc bc c abc a
0-78
78-150
0-150
Intervals of feeding