Paper II

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Abstract

The main objective of the present study was to evaluate the effect of using three different crustacean meals (*Tysanoessa inermis*, *Euphausia superba*, *Themisto libellula*) on product quality of Atlantic salmon (*Salmo salar* L.). In order to do this, a total of six iso-protein, iso-lipid and iso-carotenoid diets were prepared. Two experimental diet series were prepared. In the first series, a control feed (K0) was compared with diets where 20%, 40% and 60% of the fish meal protein were replaced with protein from Northern krill *T. inermis* (K20, K40 and K60, respectively). In the second series, control feed (K0) was compared with diets where 40% of the dietary protein was replaced by protein from *T. inermis* (K40), Antarctic krill *E. superba* (AK40) and the Arctic amphipod *T. libellula* (AMP40). The salmon groups were fed the various diets for 160 days and the average weight of the fish increased from 410 g to around 1500 g. Fish given diets containing krill displayed a general better growth compared with the ones given pure fish meal diet. Replacing fish meal protein with protein from the crustacean sources had, in general, only minor effects on the flesh quality measured both by technical and sensory methods. However, some significant effects were noted. Postmortem muscle pH was generally lower ($P < 0.05$), for K20, K40, AMP40 in fish fed crustacean diets compared with those receiving the control diet. Increasing the replacement level of non-fish meal protein from Northern krill (K20, K60) significantly reduced the rigor contraction. Fish given K20 had a slightly firmer meat texture, measured as resistance to post-rigor compression, especially when compared with K60 ($P < 0.05$). Fish from the K20 and AMP40 groups had a deeper red flesh coloration [both light reflection (A*-value and chroma) and flesh astaxanthin concentration] than fish fed K0 and higher inclusions of krill meal. The groups with the highest astaxanthin flesh content also showed the best growth and had the highest feed intake. Finally, a sensory panel analysis differed slightly from the technical measurements in that K0, rather than K20 was given the highest score for hardness and colour. In comparison with K0, AK40 got the lowest salty taste and hardness scores from the panellists relative to the control fish ($P < 0.05$). Despite minor effects on the present quality measures, it is concluded that meal from three different crustacean species can successfully replace fish meal up to 60% with Northern krill, and 40% of Antarctic krill and amphipod meal of dietary proteins.

KEY WORDS: amphipod, astaxanthin, crustacean meal, krill, muscle technical and chemical quality, sensory analysis, texture, zooplankton

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Introduction

Expected restrictions in protein resources accelerate the need to find sources for fish meal replacement (Waagbø *et al.* 2001). Crustaceans, at present represent an unexploited yet abundant marine biomass at lower trophic levels (Melk *et al.* 2004). They play a significant role in pelagic ecosystems as food for the fish, birds and marine mammals. They are highly nutritious, excellent sources of vitamins, minerals, essential amino acids, n-3 polyunsaturated fatty acids, as well as natural carotenoid pigments, nucleotides and organic acids

(Lee & Meyers 1997; Everson 2000). Krill has high potentiality to be used as feed ingredient, and recently performed growth studies have shown that crustaceans can partially or wholly substitute fish meal in Atlantic salmon diets (Olsen *et al*. 2006; Suontama *et al*. 2006).

However, if crustaceans are to be used in fish diets in the future, it is also essential that they do not lower or in any way negatively affect the final quality of the product. Earlier feeding studies with rainbow trout either did not affect sensory quality (Reinacher 1979) or improved the taste and texture of the fish when krill (*E. pacifica*) was included in the diets (Spinelli 1979). Furthermore, both feed technology, growth rates and a method for sensory analysis have developed significantly over the past 20 years and is unlikely that previous data are valid in the current modern settings. Zooplankton is also known to be a source of natural pigments (see review Storebakken 1988). Therefore, the aim of the current study was to examine the technical and sensory fillet quality as well as astaxanthin content in salmon muscle fed different crustacean species as fish meal replacement and source of natural pigment for muscle pigmentation for 160 days.

Materials and methods

Raw materials and feed production

Details of raw materials and their free and esterified astaxanthin content, meal and feed production are given in Suontama *et al*. (2006). In brief, Antarctic krill (*Euphausia superba*) were purchased block frozen from Superba Invest, Ltd, Ålesund, Norway while northern krill (*Thysanoessa inermis*) and an Arctic amphipod *(Themisto libellula*) were collected from the norwegian sea, frozen and transported to the pilot plant of the Norwegian Sea Institute of Fisheries and Aquaculture Research, Fyllingsdalen, Norway. After a minimum of thawing, the meal was produced and a total of six diets were then prepared using extrusion and similar production standards and additives as to commercial diets. For each experimental diet, fish meal was gradually replaced with krill meal. In one series, fish meal protein in the control diet (K0) was gradually substituted with 20%, 40% and 60% of fish meal protein (K20, K40, K60) with protein from *T. inermis*. In the other series, a 40% protein replacement was compared using protein from *T. inermis* (K40), *E. superba* (AK40), or the amphipod *T. libellula* (AMP40). All diets were iso-energetic and with similar protein concentration. As some of the krill meals contained natural astaxanthin esters, the diets were made iso-pigmented through the addition of Carophyll Pink® (CP) (astaxanthin 10% w/w) (Hoffman la Roche, Ltd, Basel, Switzerland). Astaxanthin esters present in crustacean meals were stable during meal preparation, and up to 70% of astaxanthin could be recovered from raw materials. The chemical compositions of the diets are shown in Table 1. Further details are given in Suontama *et al*. (2006).

Fish

In January 2004, 540 Atlantic salmon, *Salmo salar* smolts (NLA strain; Norwegian breeding programme), with an

Table 1 Composition of the diets used in the experiment

Diet	K0[1]	K20	K40	K60	AK40	AMP40
Ingredients (g kg^{-1} diet)						
Fish meal (291/02)	613	491	368	245	370	373
Krill meal	0	152	303	454	281	348
Fish-oil	220	201	183	163	212	204
Soybean lecithin	5	5	5	5	5	5
Mais suprex	146	136	126	117	116	54
Mineral mixture[2]	4	4	4	4	4	4
Vitamin mixture[3]	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
Carophyll Pink® (10%)	0.8	0.6	0.4	0.1	0.5	0.8
Chemical composition by analysis (g kg^{-1} diet)						
Moisture	67	75	78	92	77	79
Protein	459	459	458	457	460	461
Lipid	248	253	255	246	256	251
Ashes	80	81	81	82	93	128
Carbohydrate	144	129	120	113	103	52
Chitin	0	4	7	10	11	29
Energy (MJ kg^{-1})	24.0	23.9	23.9	23.8	23.8	23.2
Calculated carotenoids based on levels analysed in meals (mg kg^{-1} diet)						
Astaxanthin Carophyll Pink®	64.0	48.0	32.0	8.0	40.0	64.0
Astaxanthin free (from krill meal)	0.0	0.8	1.5	2.3	1.4	0.7
Astaxanthin esters (from krill meal)	0.0	16.4	32.7	49.1	26.3	2.7
Total astaxanthin	64.0	65.1	66.2	59.3	67.6	67.4

[1]K0, Control fish meal diet, K20 = 20% of the proteins from *T. inermis*, K40 = 40% of the proteins from *T. inermis*, K60 = 60% of proteins from *T. inermis*, AK40 = 40% of the proteins from *E. superba*, AMP40 = 40% of the proteins from amphipod *T. libellula*.

[2]Will supply diets with the following vitamins per 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%), 200 mg; calcium pantothenate, 60 mg; biotin, 1 mg; folic acid, 10 mg; niacin, 200 mg; vitamin B12, 0.05 mg; menadione bisulphite, 20 mg.

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

initial weight of 412 ± 5 g were divided into eighteen $1.5 \times 1.5 \times 1$ m^3 lid-covered fibreglass tanks at the Matre Aquaculture Research Station, Institute of Marine Research, Western Norway (61°N). All tanks were equipped with feed collectors, and supplied with aerated sea water [salinity 27–30 g L^{-1} (on the Practical Salinity Scale 1978)] at 10 ± 1.5 °C. The light regimes were 12 : 12 h daylight. After 160 days of feeding, mean weight ranged between 1486 and 1550 g (Suontama *et al*. 2006).

Fish handling

At the end of feeding, 15 fish were collected randomly from each of the six experimental groups (five fish from each replicate tank), anaesthetized with 20–30 mL Benzoak® VET (Euro-Pharma, Leknes, Norway) 100 L^{-1} water, stunned by a sharp blow to the head and bled in seawater. Five fish from each group were further eviscerated and stored on ice in styrofoam salmon boxes (Nesseplast AS, Balestrand, Norway) and shipped by airfreight to Norwegian Food Research Institute (Ås, Norway) for sensory evaluation 3 days after slaughter. Another five fish were used for chemical and technical quality assessment including muscle pH and texture analyses. The remaining five fish were hand filleted, and the right fillet assessed for instrumental flesh colour, while the left fillet (Norwegian Quality Cut, NS9401 1994) was used for chemical astaxanthin analysis by HPLC.

pH measurement

Muscle pH was measured by injection of a pH-meter probe (MP 120pH Meter; Mettler Toledo CH-8603, Schwerzenbach, Switzerland) into the left side of the fish muscle below the dorsal fin (Fig. 1). The same fish was used for texture analyses. Between measurements, the fish were stored in a temperature controlled room at 4 °C. The analysis was repeated on day 3, the same day as the sensory evaluation (see below).

Fillet colour

Immediately after filleting, flesh colour was measured by means of a portable Hunterlab Miniscan/EX instrument (10° standard observer, Illuminant D65, Hunterlab Associates Laboratory Inc., Reston, VA, USA) calibrated to a white and black standard. The L^*-variable represents lightness (i.e. increasing lightness with increasing positive values), the A^*-scale represents the intensity in red/green (i.e. increasing positive values indicates increasing red hue) and the B^*-scale represents the intensity in yellow (Hunter & Harold 1987). Chroma-values (colour intensity) were calculated using the formula: chroma = sqrt($A^{*2} + B^{*2}$). Fillet colour was measured on five fish from each dietary group three points above and three points below lateral line, in the rostral caudal plane, consisting of six measurements for each fillet. Results are given as means of the six readings for each fish.

Chemical composition of the fish and feed

Astaxanthin analysis Five fish per diet group were used for chemical astaxanthin analyses. NQC (Norwegian Quality Cut, NS9401 1994) was cut out from the fillets (Fig. 1), skin removed, homogenized and kept frozen at −80 °C until

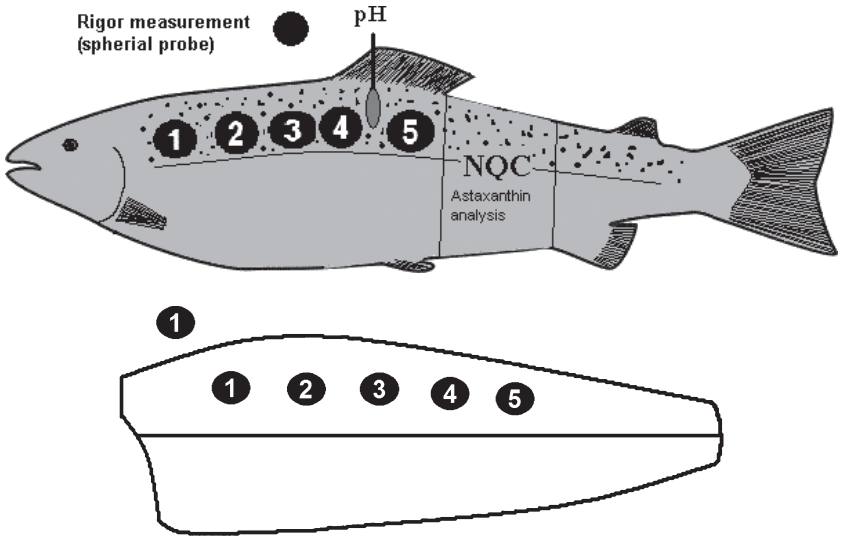

Figure 1 Sampling was performed using whole fish for rigor measurement (measured on points 1–5), pH and chemical muscle astaxanthin analyses from NQC (picture above). Salmon fillet was used for postmortem texture analysis (measured on points 1–5, picture below).

analysis. Upon analysis, homogenates were extracted (three parallels) three times into acetone with vitamin C and BHT (100 mg L^{-1}) added as antioxidants. Moisture was removed by means of Na_2SO_4 and stored at −80 °C until analysed. The HPLC system consisted of the following equipment (Hewlett Packard, palo Alto, CA, USA): an automated sample injector (G1329A ALS), a G1315A DAD diode array detector and G1316A ColComp column temperature controller maintained at a constant temperature of 4 °C. Separation was performed using tandem installed Chromspher 5 μm C_{18} columns (100 mm × 3 mm I.D.) with a guard column of C_{18} material (Chromsep guard column SS) preceding the main column. The mobile phase was acetonitrile–dichlormethane–methanol–propionic acid–water (61 : 20 : 7.6 : 5.7 : 5.7), which was filtered before use. Vitamin C (263 mg L^{-1}) was added to the mobile phase as an antioxidant. Elution was isocratic and at the rate of 1 mL min^{-1}. Both column and auto-injector temperatures were maintained at 1 °C. Peaks were detected at 476 nm and subsequently quantified with reference to authentic standards. Each sample was analysed in triplicate. Data were stored and processed using HP Chemstation software.

Crustacean meal astaxanthin was extracted according to 'Analytical methods for Vitamin and Carotenoids in Feed, Index no. 2101' (Hoffmann la Roche, Ltd), evaporated to dryness, dissolved in solvents and analysed by HPLC as described by Yuan *et al.* (1997). Using this procedure, Carophyll Pink® (CP) was added in to complete diets to give a total astaxanthin (sum of esterified and synthetic astaxanthin) a minimum of 60 mg kg^{-1} (Table 1).

Rigor mortis measurements Five fish of each diet group were used for measurement of *Rigor mortis* (rigor). Rigor was characterized over time by resistance (force expressed as Newton) to constant compression of muscle tissue at 90° angle (Sørensen *et al.* 2001) at a speed of 1 mm s^{-1} to a depth of 5 mm at predetermined locations along the epaxial muscle on the left side of whole fish using a spherical probe (Ø 20 mm) using the TA.XT2 (Stable Micro Systems, Surrey, UK) texture analyzer, equipped with a calibration cell load of 5 kg and the software Texture Expert version 1.0 *SMS* for PC (Stable Micro Systems) as described in detail by Kiessling *et al.* (2006). The analysis started at the neck region, moving stepwise towards the caudal region at time intervals of 12 h (Fig. 1). The fish were kept in a temperature controlled room at 4 °C on a styrofoam board (the same board used during analysis) between measurements to avoid handling the fish between measurements).

Texture analysis The fillet hardness was measured on day 3 after slaughter (56 h postmortem) using the right side of the same fish that was used for the rigor measurements described above. The texture profile analysis (TPA) (Bourne 1978) was carried out using flat-ended cylinder (Ø 12.5 mm, test speed 1 mm s^{-1}) that was pressed 80% of fillet thickness above the lateral line at five places (Fig. 1). The total force (Newton, N) needed to penetrate the fillet surface total force was recorded. Results are given as mean values (N) of each dietary group.

Sensory evaluation Upon arrival at the Norwegian Food Research Institute (Ås, Norway) the fish were stored on ice in a refrigerated room (4 °C) until day 2 of collection when 2-cm wide cutlets were cut from each fish. The cutlets were packed in diffusion-proof plastic bags and stored at 4 °C overnight. The following day (3 days postmortem) the bags were heated in a 75 °C water bath for 30 min, opened and the samples evaluated by 11 trained assessors [Quality-Descriptive-Analyse, ISO 6564:1985E, described in detail by Einen & Skrede (1998) using a scale from 1 to 9 (1 = low intensity and 9 = high intensity)] for the following attributes: Odour: intensity, acidulous-, rancid-, sea- and metallic odour. Colour: colour intensity, whiteness and hue (from red = 1 to yellow = 9). Flavour: intensity, acidulous, salty, bitter, metal, sea and rancid flavour. Texture: coarseness, hardness, fatness and juiciness. Prior to this evaluation, the sensory panel used the fish from the control group and the fish fed diets containing either *T. inermis*, *E. superba* or *T. libellula* to calibrate. Deviating smell or taste was evaluated according to these diets.

Calculations and statistical treatment Data are given as mean ± SD of five fish per diet unless otherwise stated in the text or tables. The results of sensory tests were analysed by variance analysis (one-way ANOVA) and by Tukey's multiple range tests if significant differences were found. Significance was accepted at $P < 0.05$.

Data from technical quality analysis and rigor contractions were analysed using the Statistical Analysis System (SAS) for Windows (version 6.12). The effects of the main variables were tested by a main factorial model (GLM procedure for unbalanced data). Groups were compared by the *ad hoc* variance test (*F*-test) using the least-squares means procedure when significant effects were found in the main model. To obtain partial regression coefficients that include the main factors, factors were added or withdrawn from the model using the GLM procedure (SAS Institute Inc., Cary, NC, USA). All measured variables were analysed as continuous responses with diet inclusion of krill (0, 20, 40 and

60) and source (K40, AK40 and AMP40) as categorical main factors.

Results from the sensory panel were analysed with variance analysis (ANOVA) following Tukey's test, where significance was accepted at $P < 0.05$.

Results and discussion

Salmon flesh quality was evaluated both by technical and sensory panel methods. Although some significant differences in quality parameters between salmon fed fish meal and krill meal were found, there was a general trend that these differences were of minor importance for quality. The set of scores from the sensory panel are summarized in Fig. 2 and further detailed in Table 2, while the chemical and technical measurements are given in Table 3, and Figs 3–6. Whiteness was assessed both by the sensory panel and instrumental analysis (Hunterlab L^*). While the sensory test panel generally gave all krill diets slightly higher whiteness score (sign. for AK40) (Table 2), the L^*-value obtained of the Hunterlab gave the highest lightness score to the fish meal diet, where significantly lower values were found in K20, K40 and AMP40 fed fish (Table 3). Furthermore, the sensory panel generally judged flesh colour (colour hue, colour intensity) to be lower in the krill supplemented diets (sign. for K40, K60 and AK40) (Table 2), while both redness (A^*), chroma and chemically assessed muscle astaxanthin content showed a trend for increased values in K20 and AMP40 groups compared with fish given only fish meal (Table 3 and Fig. 3).

In agreement with the sensory evaluation, all technical colour measurement ranged K40, K60 and AK40 lowest among fish given krill diets. Considering the above, it is therefore not surprising that all the technical colour measurements relate to fillet astaxanthin concentration and fish size. Different isomers and bindings will significantly affect the reflection properties of astaxanthin, resulting in differences in colour perception (Foss *et al.* 1987; Storebakken *et al.* 1987). Both meals from Antarctic krill and *T. inermis* contained significant amounts of astaxanthin in the form of astaxanthin esters. As the level of these meals were reduced in diets, increasing amounts of free astaxanthin in the form of Carophyll Pink® (CP) was added with the aim of reaching a target level of 64 mg kg^{-1} in the diets. In the case of *T. inermis*, CP decreased from 64 mg kg^{-1} in the control diet (K0), to only 8 mg kg^{-1} in the K60 diet, i.e. an almost 100% replacement. Despite this, flesh pigmentation was similar in all feeding groups (except being higher in fish fed K20) with no significant deviation from the controls (K0) (Fig. 3). This is in parity with previous observations in our laboratories where flesh pigmentation in Atlantic salmon was similar when they were fed free or ester bound astaxanthin (Olsen, personal communication). This appears to contrast reports in rainbow trout where feeding astaxanthin esters generally appears to reduce pigmentation when compared with free astaxanthin (e.g. Sommer *et al.* 1992) and probably indicates that there may be significant species differences in pigment availability. Intestinal hydrolysis (White *et al.* 2002) or cleavage (Choubert & Heinrich 1993) may be a limiting step for the absorption and deposition of astaxanthin from ester form. Significantly higher values in astaxanthin content, and A^*- and chroma values found in K20 and AMP40, compared with fish fed K40 and K60 diets may relate to higher amount of ingested free astaxanthin in K20 and AMP40 fed fish, as these groups showed also bigger body weight and hence higher feed intake compared with other groups.

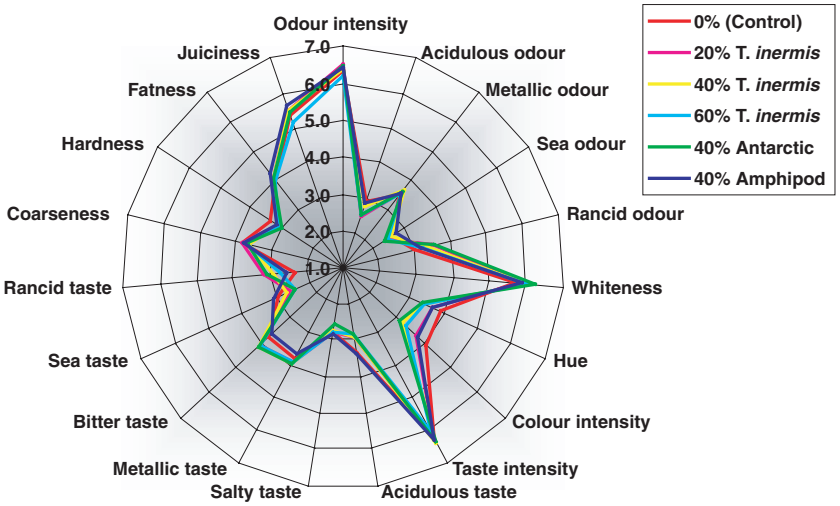

Figure 2 Sensory attribute scores in Atlantic salmon cutlets from the experimental groups (see legend). Scores from 1 to 9 are used where 1 = low intensity and 9 = high intensity.

Table 2 Sensory evaluation of Atlantic salmon cutlets fed the control and five experimental diets for 160 days (scores 1–9 for low to high intensities) (mean ± SD, $n = 11$). Significant effects were determined by one-way ANOVA and Tukey multiple range test

Diet	Whiteness	Colour hue	Colour intensity	Acidulous taste	Salty taste	Bitter taste	Hardness
K0*	5.67 ± 0.25^{a}	3.89 ± 0.13^{b}	4.09 ± 0.26^{b}	3.31 ± 0.16^{a}	2.77 ± 0.08^{b}	3.77 ± 0.15^{ab}	3.32 ± 0.15^{b}
K20	5.86 ± 0.16^{ab}	3.64 ± 0.19^{ab}	3.70 ± 0.45^{ab}	2.91 ± 0.41^{a}	2.74 ± 0.13^{ab}	4.08 ± 0.18^{ab}	3.11 ± 0.16^{ab}
K40	5.98 ± 0.20^{ab}	3.39 ± 0.24^{a}	3.19 ± 0.35^{a}	2.92 ± 0.36^{a}	2.70 ± 0.11^{ab}	4.11 ± 0.26^{b}	3.09 ± 0.12^{ab}
K60	5.95 ± 0.24^{ab}	3.41 ± 0.19^{a}	3.31 ± 0.29^{a}	2.77 ± 0.27^{a}	2.73 ± 0.06^{ab}	4.10 ± 0.26^{ab}	3.08 ± 0.09^{ab}
AK40	6.20 ± 0.25^{b}	3.35 ± 0.19^{a}	3.12 ± 0.40^{a}	2.78 ± 0.35^{a}	2.53 ± 0.12^{a}	4.14 ± 0.18^{b}	2.96 ± 0.13^{a}
AMP40	5.85 ± 0.23^{ab}	3.65 ± 0.26^{ab}	3.75 ± 0.43^{ab}	3.38 ± 0.31^{a}	2.79 ± 0.08^{b}	3.64 ± 0.18^{a}	3.13 ± 0.13^{ab}
c^{1}	0.455	0.386	0.735	0.704	0.237	0.461	0.301

[1] Critical value (when two numbers difference are $>c$ they are significantly different and marked with different superscript letters).

Table 3 Colour measurements from Hunterlab and Chroma ($n = 5$, mean of six measured values in five fish of each diet group) after 160 days of feeding (±SD)

Diet	*L**	*A**	Chroma
K0	51.1 ± 0.4^{a}	23.1 ± 0.5^{a}	34.9 ± 0.5^{ad}
K20	49.9 ± 0.4^{bc}	24.6 ± 0.4^{b}	36.4 ± 0.5^{b}
K40	49.7 ± 0.4^{bc}	22.4 ± 0.4^{a}	33.6 ± 0.5^{c}
K60	50.7 ± 0.4^{ab}	22.0 ± 0.5^{a}	33.5 ± 0.5^{c}
AK40	50.5 ± 0.4^{ab}	22.5 ± 0.5^{a}	33.9 ± 0.5^{cd}
AMP40	49.1 ± 0.4^{c}	24.6 ± 0.5^{b}	36.0 ± 0.5^{ab}
P_{Level}	0.048	0.0005	0.0002
P_{Type}	0.045	0.0007	0.002
R^2	0.17^{ns}	0.41**	0.38**

$^{**}P < 0.005$.

Sensory panel found no deviations in odour parameters, while two of seven investigated flavour parameters differed slightly giving AK40 fed fish lower salty taste than fish fed control fish meal diet and AMP40 diet, and giving K40 and AK40 fed fish higher bitter taste compared with fish fed AMP40 diet (Table 2). Differences in sensory attributes between all experimental groups were totally set marginal (Fig. 2). Similarly, no effects on sensory quality of rainbow trout was found when fed with krill as reported by Reinacher (1979), whereas Spinelli (1979) reported enhanced sensory taste and texture in fish fed krill.

Furthermore, substituting fish meal to krill meal did not appear to influence muscle hardness to any major extent. Sensory texture analysis scored AK40 group to be lower than control fish meal group, but was similar with other experimental groups (Table 2). Technically measured fillet hardness was the highest in K20 fed fish being significantly higher than K60 group, which displayed the softest tissue hardness (Fig. 4).

Growth rate in conjugation with slaughter is reported to affect tissue hardness (Einen *et al.* 1999; Robb 2001). Our data agree with such interpretation, i.e. that the underlying cause for the observed differences stems from differences in growth (Table 4) rather than from differences in dietary protein source. Such interpretation also explains why AMP40 fish had the same tissue hardness as K20 fish, as fish given these two diets displayed the best overall growth rate.

Following slaughter, the endogenous energy reserves are consumed by the actomyosin complex contraction and muscle fibres contract, forming stiff and permanent binding between the contractile proteins, myosin and actine, and is termed *Rigor mortis* (rigor) (Tornberg *et al.* 2000). Later, the tissue is softened by proteolysis as the fish slowly exits rigor. This process is characterized by a reduction in pH as glycogen is metabolized to lactic acid via pyrovaic acid. We could not detect any differences in final muscle pH that related to

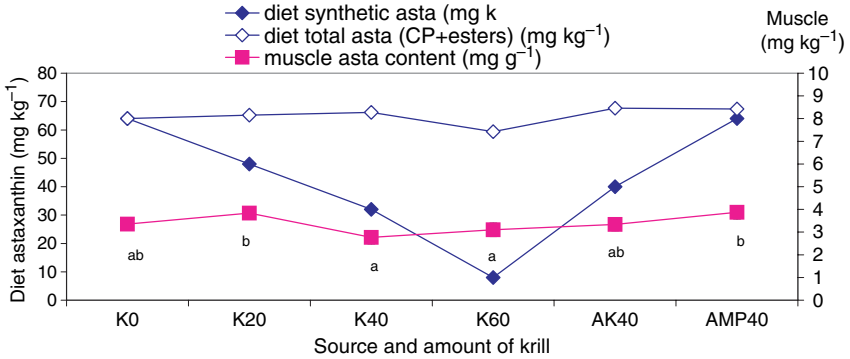

Figure 3 Diet added CP level (mg kg^{-1}) and muscle total pigment content (from CP + mainly astaxanthin esters from crustaceans) (mg kg^{-1}) (chemical analysis) after 160 days of feeding $n = 5$ (±SE). The R^2 of the statistical model (GLM) = 0.22 ($P < 0.01$) included level ($P = 0.02$) and type ($P = 0.0007$) of krill as main factors.

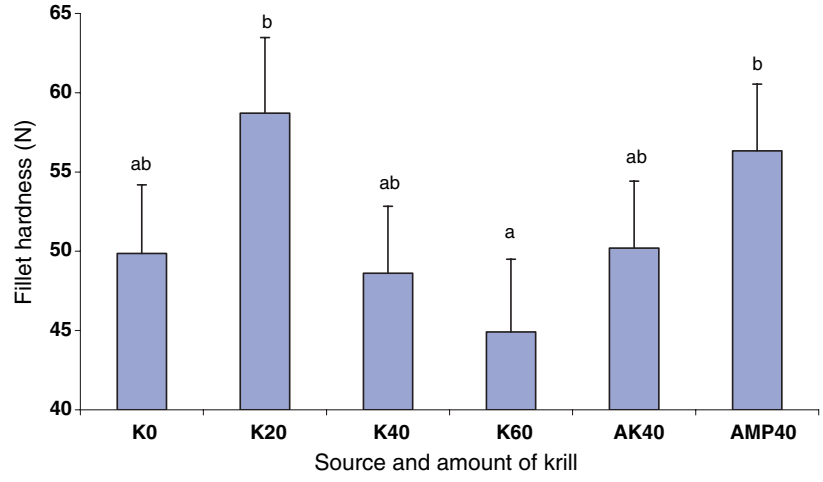

Figure 4 Fillet hardness (mean of the five measurement points ± SE) measured postmortem as total force after 3 days ice storage in salmon fed different levels and types of krill. The R^2 of the statistical model (GLM) = 0.34 ($P < 0.01$) included level ($P < 0.01$) and type (P = ns) of krill as main factors and fish body weight as covariate ($n = 30$). The same lower case letter indicates no significant difference ($P > 0.05$) between the different treatments.

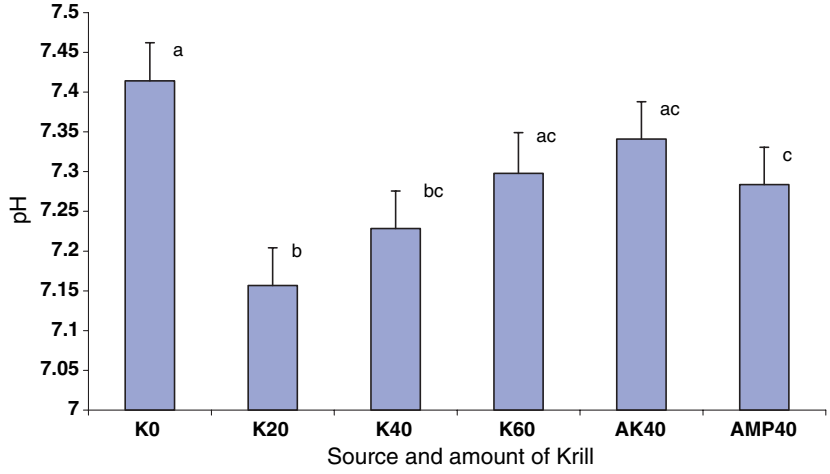

Figure 5 pH (mean ± SE) in muscle immediately after slaughter of salmon fed either different levels or different types of krill. The total R^2 of the statistical model (GLM) = 0.44 ($P = 0.025$) included level ($P = 0.006$) and type (P = ns) of krill as main factors and fish body weight as covariate ($n = 30$). The same lower case letter indicates no significant difference ($P > 0.05$) between the different treatments.

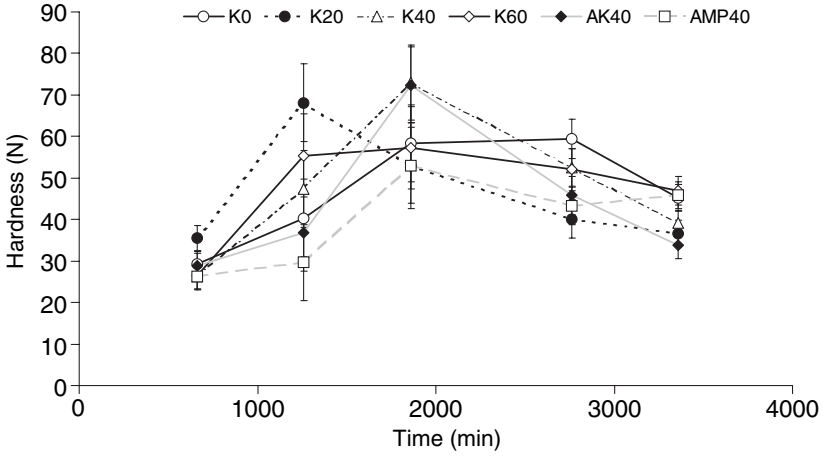

Figure 6 Changes in resistance to compression (N, mean ± SD) in whole fish during rigor contraction of Atlantic salmon fed the different krill diets, measured by tissue resistance by use of a texture analyzer TA-XT2 (*SMS* Stable Micro Systems, Surrey, UK) equipped with a spherical plastic probe, Ø 20 mm.

any dietary groups (pH range 6.28–6.32; Table 4). On the other hand, significant differences were observed in initial muscle pH measured directly after slaughter. K20 and in part K40 fed fish displayed significantly reduced initial muscle pH compared with all other dietary groups (Fig. 5). AMP40 fish displayed a significantly lower pH than the control group

Table 4 Body weight, final muscle pH (3 days postmortem, and muscle total fat in the Atlantic salmon muscle after 160 days of feeding (mean ± SD)

Diet	pH_{end}	Body weight (g kg^{-1})	Muscle total fat (g kg^{-1} wet weight)
K0	6.32 ± 0.1	1492 ± 174	76 ± 20
K20	6.30 ± 0.1	1613 ± 160	89 ± 27
K40	6.30 ± 0.1	1495 ± 204	91 ± 24
K60	6.28 ± 0.0	1470 ± 210	88 ± 28
AK40	6.31 ± 0.0	1612 ± 272	100 ± 16
AMP40	6.30 ± 0.0	1630 ± 237	91 ± 34
P_{Level}	ns	0.10	ns
P_{Type}	ns	0.10	ns
R^2	–	0.10	–

(K0). The underlying rationality for this observation is difficult to explain, but the high growth of the K20 fish may render them more sensitive to stress. However, the initial pH values were within the same range reported by Roth *et al.* (2005).

Stress at slaughter is well known to exhaust muscle endogenous energy reserves, resulting in reduced muscle pH (Kestin & Warris 2001). Stress at slaughter also affects tissue hardness, even though this effect differs between studies (see Kiessling *et al.* 2004) making a direct comparison between our data and that of other studies difficult.

Because of its direct coupling to slaughter stress and thereby mobilization of muscle glycogen, initial postmortem muscle pH is often related to the rigor process. Muscle glycogen level was not measured in this study, but lower postmortem initial pH, especially in K20 fed fish, reflects lower endogenous energy reserves in that group, and has shown to lead to a faster initiation of rigor process (Stien *et al.* 2005), a phenomenon that was observed in the present study as whole fish muscle hardness (Fig. 6). Following the development in hardness in whole fish over time, and the subsequent tissue softening, it became evident that the fish from diet K20 displayed an increased hardness earlier than fish from the other treatments and tallies well with the observation with stress at slaughter. The rigor process, measured as whole muscle hardness (Fig. 6), showed great variation in the other experimental groups, in agreement with their higher initial pH and thereby larger intact endogenous energy reserves after death.

The approach to measure texture at five locations from anterior to caudal regions (see Fig. 1 and Materials and methods) revealed an interesting observation of a more technical and general nature. Namely that measurements made at one morphological location is not predictive of the hardness of the whole fillet. This observation is in agreement with earlier reports by Bjørnevik *et al.* (2004) and Espe *et al.* (2004), who compared hardness at two sites located either anterior or caudal to the dorsal fin. Fillet fat and physical properties are known to differ in different fillet locations resulting variations in muscle texture (Sigurgisladottir *et al.* 1999; Mørkøre *et al.* 2002).

Based on the present study we conclude that up to 60% of fish meal protein can be replaced by krill or amphipod meal in diets to salmon without causing any quantitatively important impacts on flesh technical or sensory quality. Similarly, Atlantic cod fed Antarctic krill up to 100% of diet proteins did not change technical or sensory quality compared with fish fed with fish meal diet (Karlsen *et al.* 2006). Replacing synthetic free astaxanthin (CP) with astaxanthin esters from macrozooplankton resulted in lower sensory colour hue and – intensity experience evaluated by sensory panel, but left muscle chemical astaxanthin content unaffected. Contribution of pigment from amphipod meal was low, and pigment had to be added in a synthetic form in the diet. Based on this study, it can be concluded that krill meal can be used as a pigment source and contribute muscle pigmentation. Different amounts of ingested feed, and thereby deposited astaxanthin gave some minor chances in quality variables in this study. Other aspects of using krill meal in fish feed, such as feed levels and retention of unwanted substances (fluorine, heavy metals and organic pollutants) will be considered in other communications (Moren *et al.* 2006).

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