Paper V

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Element concentrations in meals from krill and amphipods, — Possible alternative protein sources in complete diets for farmed fish

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Abstract

In the marine environment, organisms from lower trophic levels seem as a good alternative to the traditional meal and oil sources. In the present study, meals were produced from Antarctic krill (*Euphausia superba*), Arctic krill (*Thysanoessa inermis*) and the Arctic amphipod *Themisto libellula*. Diets were then prepared for Atlantic salmon and Atlantic cod where up to 100% of the fish meal protein was replaced by protein from these organisms. Concentrations of copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) were determined by ICP-MS in the various krill and amphipod meals, complete diets and muscle samples from fish fed these diets. The element concentrations were related to growth and general fish health as well as present EU legislations on feed ingredients and complete diets. The cod showed no difference in growth during the trial, while salmon fed diets where 40% of the fish meal protein was replaced with Arctic krill or amphipod meal shoved improved SGR during the first period of feeding (first 100 days). No adverse effects on growth rate or fish health were observed in any fish species or treatment. Nevertheless, high levels of Cu were found in the meal from Antarctic krill (46 mg kg^{-1} dry matter (dm)) resulting in a dietary level of Cu exceeding the upper limit for complete feedingstuff set by EU. Furthermore, the Cd level found in the meal from amphipod (12 mg kg^{-1} dm) was 6 times higher than EU's upper limit. This indicates limitations for the use of certain zooplanktons as alternative protein sources in feed for farmed fish, unless future processing methods yield lower levels of these unwanted elements.

Keywords: Krill; Amphipods; Copper; Zinc; Arsenic; Cadmium; Mercury; Lead; Protein source

1. Introduction

Marine zooplankton including amphipods and krill are natural food organisms for many wild fish during parts of their life cycle (Dalpadado and Bogstad, 2004). Furthermore, meals from marine zooplankton have been suggested as alternative protein sources in fish diets (Virtue et al., 1995). Krill as feed for salmonids was focused in several studies in the late 1970s and 1980s (Storebakken, 1998). Although being inconclusive, the results were generally regarded as promising. However, the initial studies were

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not followed up partly because of the high fluorine content found in the krill species of interest (Boone and Manthey, 1983; Søvik and Brækkan, 1979). Fluorine is a part of the exoskeleton of the krill and the natural fluorine content of krill has been reported to vary between 1000 and 6000 mg/kg dry matter (dm) (Søvik and Brækkan, 1979; Sands et al., 1998). As opposed to birds and terrestrial mammals, marine fish species seem to tolerate extraordinary large amounts of fluorine from feed materials without suffering any harm. Present legislation has set the maximum fluorine level in raw material from krill as fish feed ingredients to 3000 mg kg^{-1} based on 88% dm. Some krill meal producers appear to deliver products within this limit; hence the krill meal can be used as long as the fluorine level is below the maximum allowed level in complete diets, currently set at 150 mg kg^{1} (88% dm). Another challenge for some of the alternative marine protein sources is the presence of other undesirables. Previous studies have shown that cadmium (Cd) levels are relatively high in amphipods (Ritterhoff and Zauke, 1998), while copper (Cu) concentrations may be high in both Arctic and Antarctic krill species (Ritterhoff and Zauke, 1997; Nygård et al., 2001). The levels of lead (Pb) and mercury (Hg) as well as arsenic (As) and zinc (Zn) seem to be low in most zooplanktons (Ritterhoff and Zauke, 1997; Nygård et al., 2001; Edmonds, 2003). These reports are, however, based on results from analyses conducted on raw ingredients. Processing the zooplankton will most likely alter the content of some of these compounds. It is therefore important to acquire knowledge on the element levels in meals processed from different zooplankton and compare them with existing legislation. Maximum allowable levels of Cu, Zn, As, Cd, Hg and Pb in feed ingredients and complete feedingstuff are given in Tables 5 and 6, respectively (EU, 2002, 2003, 2005).

The resource situation for traditional fish meal and fish oil at present, and as expected in the future (FAO, 2004), and the numerous results showing great limitations in alternative plant resources (Krogdahl et al., 2005), points to the necessity to search for alternatives also in the marine environment. Meal from Antarctic krill is a commercial product used in a variety of products, from human health promoters to fish diets. Current estimates suggest that the standing biomass is 44 million tons, and Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) Scientific Committee (CCAMLR, 2005), has suggested a precautionary catch limit of 4 million tons krill for the Atlantic Sector (Area 48) (Hewitt et al., 2002). In 2003/2004 less than 120,000 tons were exploited. Feeding studies with fish has shown that a certain amount of krill meal inclusion in feed for fish (Julshamn et al., 2004) results in improved appetite, which may be explained by the level of attractants (Oikawa and March, 1997). A small inclusion may therefore enhance feed intake. Krill meal also contains relatively high levels of phospholipids (Saito et al., 2002) which may be utilised in diets for marine fish, in particular larvae, which require high levels of marine phospholipids (Salze et al., 2005; Hamre et al., in press). In addition, egg and larval quality has been shown to be improved by including krill in the diets to the brood stock of red sea bream and the results suggest that the polar lipid content in the krill ingredient is at least a part of the reason for this improvement (Watanabe and Kiron, 1995).

To evaluate the quality of krill and amphipod meals as substitutes for fish meal in diets for fish, one feeding study with salmon and one with cod were conducted. The present paper reports on element concentrations in samples from the meals, diets and fish muscles from these feeding studies. Also fish health has been evaluated as a result of exposure to krill and amphipod meals in both cod and salmon.

2. Materials and methods

2.1. Fish experiments and diets

2.1.1. Cod trial

The cod feeding trial was carried out at Austevoll Aquaculture Research Station, Storebø, Norway. A number of 840 Atlantic cod (*Gadus morhua*) with initial body weight of 122 ± 27 g were randomly distributed into 12 tanks (3 m Ø, 1 m water depth) covered with light reducing shades. The experiment commenced on April 25th, 2003 and was terminated on July 9th, 2003 (75 days). The tanks were supplied with aerated seawater with an average water temperature of 7.7 ± 0.2 °C and average salinity of 34.6 g l^{-1}. The fish were kept under natural light regime. During the experiment the fish were fed in duplicates with six different experimentally produced diets, where 0, 20, 40, 60, 80 or 100% of the fish meal protein (Norse-LT 94, Norsildmel, Bergen, Norway, 38% blue whiting, 30% sandeel, 20% Norway pout, 12% herring) was replaced with protein from Antarctic krill (*Euphausia superba*). The diets were produced as 4 mm pellets by the Norwegian Institute of Fisheries and Aquaculture Research, Titlestad, Norway. The composition of the diets used in the study is presented in Table 1.

All components, except the meal source, were kept at a constant level. The fish were fed daily in slight excess (approximately 15%) by automatic feeders. Mortality was low or absent and did not vary among the experimental groups.

Table 1
Experimental feed ingredients and analysed nutritional composition of diets fed cod and salmon. 0, 60 or 100% of the fish meal protein was replaced by protein from Antarctic krill meal from *Euphausia superba* in the cod diets (c-*Es* 0, 60 and 100)

% fish meal substituted	Diets for cod			Diets for salmon			
	c-*Es* 0	c-*Es* 60	c-*Es* 100	s-*Ti* 0	s-*Ti* 40	s-*Es* 40	s-*Tl* 40
Ingredients (g kg^{-1} diet, dry wt.)							
Fish meal	726	299	0	613	368	370	373
Krill meal	0	494	840	0	303	281	348
Fish oil	81	68	58	220	183	212	204
Soy lecithin	5	5	5	5	5	5	5
Starch (corn)	173	119	81	146	126	116	54
Mineral mix**	4	4	4	4	4	4	4
Vitamin mix*	10	10	10	10	10	10	10
Inositol	0.3	0.3	0.3	0.3	0,3	0,3	0,3
Betaine	1	1	1	1	1	1	1
Analysed (g/100 g dry wt.)							
Moisture	6.9	6.7	6.5	5.9	6.2	5.7	5.6
Crude protein	54.6	54.6	54.7	45.9	45.8	46.0	46.1
Crude fat	14.1	14.2	14.2	27.1	27.0	27.1	27.2
Ash	9.5	11.7	13.2	8.0	8.1	9.3	12.8

*Yields 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; biotine, 1 mg; folic acid, 10 mg; niacin, 200 mg; vitamin B 12, 0,05 mg; menadione bisulphite, 20 mg.
**Yields per kg diet: magnesium, 500 mg; potassium, 400 mg; zinc 80 mg; iron, 50 mg; manganese, 10 mg; copper, 5 mg.
In the diets fed salmon 40% of the fish meal protein was replaced with either arctic krill meal (*Thysanoessa inermis*) (s-*Ti*), Antarctic krill meal (s-*Es*) or amphipod meal (*Themisto libellula*) (s-*Tl*). One salmon diet (s-*Ti* 0) had 100% fish meal.

2.1.2. Salmon trial

The salmon feeding trial was carried out at the Institute of Marine Research, Matre Aquaculture Research Station, Matredal, Norway. A number of 360 Atlantic salmon (*Salmo salar*) with initial body weight 412±5 g (NLA strain — Norwegian breeding programme) were randomly distributed into 12 covered round fibre glass tanks (1.5 m Ø, 1 m water depth). The tanks were supplied with aerated seawater. Average temperature was 10±2 °C, while salinity ranged from 30 to 33 g l^{-1}. The fish were kept under a 12:12 h dark:light regime. During the experiment, the salmon were fed the different diets in triplicate, where the fish meal in four of the diets was substituted with 0 or 40% of protein from the Arctic krill (*Thysanoessa inermis*), while 40% of the protein from fish meal (Norse-LT 94, see subchapter above for details) content was substituted with either protein from Antarctic krill or amphipod meal (*Themisto libellula*) in two of the diets. The composition of these diets is presented in Table 1. The fish were fed daily in slight excess (about 10%) with the six experimental diets using disc feeders. Mortality was low or absent in all experimental tanks, and did not vary significantly among treatments.

Table 2
Instrumental settings for Agilent 7500c ICP-MS

ICP-MS settings	
RF power (W)	1550
Carrier gas flow (l/min)	1.2
Plasma gas flow (l/min)	15
Auxiliary gas flow (l/min)	1.0
Nebuliser	Babington nebuliser
Spray chamber	Water cooled double pass
Spray chamber temperature (°C)	2
Interface cones	Platinum
Lens voltage (V)	2–3
Mass resolution (u)	0.8
Integration time (s)	1000

2.2. Sampling

At the end of both experiments, all fish were anaesthetized with 30 ml 100 l^{-1} Benzoak® VET (Euro-Pharma, Norway) and killed by a blow to the head. Blood from five fish was withdrawn from the caudal vessels using heparinised syringes. Sub-samples of whole blood were stored at 4 °C and analyzed for haemoglobin and red blood cell counts within 24 h of collection. From another sub-sample, plasma was immediately prepared by centrifugation at 3000 ×*g* for 5 min and stored at −80 °C for later analysis of plasma nutrients and enzyme activities. Samples of fillet were taken from three fish per tank and stored at −20 °C for metal analysis.

Table 3
Results from the analysis of the certified reference material Tort-2 (Lobster hepatopancreas) for copper, zinc, arsenic, cadmium, mercury and lead

Element	Number of analysis (N)	Mean (μg/g)	2 s (μg/g)	RSD (%)	Certified (x±2 s) (μg/g)
Copper	15	66	15	11	71.6±1.6
Zinc	15	160	35	12	180±6
Arsenic	15	21.5	3.6	8.4	21.6±1.8
Cadmium	15	26.5	2.2	4.1	26.7±0.6
Mercury	15	0.28	0.06	11	0.27±0.06
Lead	15	0.37	0.07	9.5	0.35±0.13

All results in μg/g (dry mass)±2 s (n=20).

2.3. Analysis

The diets were analysed for proximate composition (Table 1) using standard methods. Moisture was determined gravimetrically after drying at 103 °C for 24 h. Total nitrogen was determined in homogenized, freeze-dried samples using a nitrogen analyser (LECO, FP-428; system 601-700-500, St Joseph, MI, USA). Protein was calculated as N×6.25 (the amount of chitin was withdrawn from the total N concentration). Lipid was determined gravimetrically after ethyl-acetate extraction (Lie and Lambertsen, 1991).

Plasma samples were analysed using a Technicon RA1000 analyser together with the standard material, SETpoint Chemical Calibrator (Technicon Instruments, Tarrytown, NY, USA). Technicon method No. SM4-0143K85 was used for plasma glucose determination, plasma protein was analysed according to the Biuret method, standardised for RA1000 (Technicon method No. SM4-0147K82). Plasma total lipid, cholesterol levels and liver dehydrogenase complex (LDH) activity were quantified according to Technicon method No. SM4-0173H88, No. SM4-0139K82 and No. SM4-0145K82, respectively. The methods described by Sandnes et al. (1988) were used to determine blood haematocrit (Hct) and red blood cell count (RBC), and plasma aspartate aminotransferase (ASAT) and plasma alanine aminotransferase (ALAT) activities.

For the element determinations, the samples were wet digested using a Milestone microwave laboratory system (Milestone, Sorisole, Italy) (Julshamn et al., 2000). An Agilent quadrupole ICP-MS 7500c instrument (Yokogawa Analytical System Inc., Tokyo, Japan) was run in the standard mode, and used as an element specific detector for Cu, Zn, As, Cd, Hg and Pb. The sample introduction system consisted of an ASX-500 Auto sampler (CETAC Technologies, Omaha, NE, USA), a peristaltic pump, a concentric nebuliser (CPI International, Amsterdam, The Netherlands) and a water-cooled (2 °C) spray chamber. The ICP-MS settings are given in Table 2. Data were collected and processed using Agilent Chemstation ICP-MS software. Rhodium was used as an internal standard. For the determination of mercury, gold was added, in order to stabilise the element. Internal calibration was used for the calculation of the element concentrations. The accuracy and precision obtained for the methods used were tested by analysing certified reference materials as well as by participation in proficiency tests. The results from the analysis of the certified reference material Tort-2 (Lobster hepatopancreas) are shown for the elements Cu, Zn, As, Cd, Hg and Pb in Table 3. The limits of quantification (LOQ) were based on 10 times the standard deviation of 20 blind samples and the following LOQs were found (given in mg kg^{-1} dry wt.) for Cu: 0.3, Zn: 1.5, As: 0.03, Cd: 0.008, Hg: 0.003 and Pb: 0.02.

2.4. Statistics and calculations

Weight, length, and blood parameters from the cod feeding trial, were tested using simple linear regression ($n=6$), p-plot was used to test normality in the data (StatSoft "Statistica", ver. 7.0 Tulsa, OK, USA). Transformation of glucose ($1/x$) and ALAT ($\log(x)$) data was necessary to achieve normality. The specific growth rate (SGR) was calculated using following formula:

$$\mathrm{SGR} = \left(e^{(\ln W2 - \ln W1)/(T2-T1)} - 1\right)^{*} 100,$$

where $W1$ and $W2$ denotes fish weights at $T1$ (start) and $T2$ (final), respectively.

3. Results

3.1. Growth and health parameters

No significant differences were observed for cod in either length or weight increases due to increased levels of Antarctic krill meal in diets. Initial average weight was

Table 4
Average specific growth rate (SGR)±std ($n=15$), and average plasma levels of protein, glucose, triacylglycerol, aspartate aminotransferase (ASAT) and plasma alanine aminotransferase (ALAT) activity, cholesterol and LDH±std ($n=2$), in cod fed 0–100% protein from Antarctic krill meal

Diet groups	c-*Es* 0	c-*Es* 20	c-*Es* 40	c-*Es* 60	c-*Es* 80	c-*Es* 100
SGR	1.0±0.4	0.9±0.4	0.9±0.4	1.0±0.4	1.0±0.5	1.0±0.4
Protein (g/100 g)	34±1	41±1	40±4	43±3	42±2	43±2
Glucose*	9±3	5±1	4±1	3±1	4±1	4±1
TG	5.8±0.5	6.9±0.7	7±1	7.0±1.1	6.1±0.9	6.1±0.8
ASAT	210±100	180±190	98±97	57±28	180±160	117±39
ALAT	46±10	26±13	16±13	8±4	22±15	23±6
Cholesterol	10±1	10±1	10±1	9±1	9±1	8±1
LDH	2900±1900	2500±3400	2100±1000	580±380	1900±1900	1400±700

* Significant correlation between diets and glucose levels, $r=0.2$ $P=0.004$.

Table 5
Contents of copper, zinc, arsenic, cadmium, mercury and lead in fish meal (Norse-LT 94, Norsildmed, Bergen, Norway) and meals produced from Arctic krill, Antarctic krill and Arctic amphipod (mg kg^{-1} diet, dry wt.) together with EU's upper limit for feed ingredients (mg kg^{-1} diet, 88% dry wt.)

	Cu	Zn	As	Cd	Hg	Pb
Norse-LT 94	4	80	14	0.19	0.08	0.09
Antarctic krill (*Es*)	46	51	4.0	0.61	0.008	0.09
Arctic krill (*Ti*)	22	81	9.4	1.4	0.009	0.22
Amphipod (*Tl*)	8.2	58	11	12	0.04	0.16
EU's upper limits	25*	200*	15	2	0.5	10

*Upper limits for copper and zinc do only appear for complete feeds and these levels are therefore identical to what is printed in Table 5.

120 g and the initial average length was 22 cm (n=840). Final average weight was 250 g (n=195) and average length was 27.2 cm, while mean SGR was 1.0 (Table 4). Final weight in the salmon feeding trial varied between 1486 and 1550 g. Replacing 40% of the protein from fish meal with protein from Antarctic krill or Arctic amphipod meal significantly increased salmon SGR (0.87 to 0.91–0.92) compared to the fish group fed 100% protein from fish meal from start to 100 days of feeding (P<0.0019). From 100 to 160 days of feeding however, the salmon SGR was similar for all groups. Only the amphipod supplemented group grew significantly better compared to fish meal group (P<0.0018). But there was also a general tendency of fish fed s-*Ti*-40 and s-*Es*-40 to be heavier than those fed only fish meal protein. These growth data are presented with the courtesy of Suontama, J., whom discusses this more thoroughly in relation to feed and muscle quality in Suontama et al. (submitted for publication).

Plasma levels of protein, glucose, triacylglycerol and cholesterol, ranged as shown in Table 4. Only in one of the groups (c-*Es*-0) elevated plasma glucose level was detected, and averaged 9 mM. The levels of the enzymes aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT), and the values for red blood cells (RBC), haemoglobin (HB) and haematocrit (HCT) were within normal expected variation (Table 4). Plasma nutrients, enzyme activities, cholesterol and LDH from the cod trial were tested using linear regression and only the levels of ALAT gave an r-value significantly different from zero.

3.2. Elements

The content of the elements in all meals used is given in Table 5 as are current EU upper limits for these elements in feed ingredients. The content of zinc (Zn), arsenic (As), mercury (Hg) and lead (Pb) were well below EU limits. The highest content of Hg was found in the amphipod meal (0.04 mg kg^{-1} dm) but still being only 10% of the EU limit (0.5 mg kg^{-1} 88% dm). On the other hand, levels of Pb were higher in the krill and amphipod meals compared to the fish meal. Nevertheless, the highest level was 0.22 mg Pb kg^{-1} (dm) in Arctic krill, being around 5% of the EU's upper limits. Similarly, the content of Zn and As were in the range 51–81 and 4–14 mg kg^{-1} (dm), respectively, which was lower than the current maximum levels for complete feedingstuff (200 and 15 mg kg^{-1} 88% dm, respectively). The upper limits were exceeded for copper (Cu) and cadmium (Cd). In the case of Cu, Antarctic krill-meal

Table 6
Contents of copper, zinc, arsenic, cadmium, mercury and lead in diets containing meal from Arctic krill, Antarctic krill and amphipod meal (mg kg^{-1} (dm)±std, n=2)

	Cu (mg kg^{-1})	Zn (mg kg^{-1})	As (mg kg^{-1})	Cd (mg kg^{-1})	Hg (mg kg^{-1})	Pb (mg kg^{-1})
Diets fed cod						
c-*Es* 0**	31±1	230±11	12±1	0.21±0.03	0.09±0.03	<LOQ*
c-*Es* 60	43±1	200±10	5.9±0.9	0.42±0.03	0.09±0.03	<LOQ
c-*Es* 100	52±1	180±9	3.5±0.9	0.51±0.03	<LOQ	<LOQ
Diets fed salmon						
s-*Ti*-0	11±1	147±5	8.6±0.9	0.12±0.03	0.11±0.03	0.09±0.04
s-*Ti* 40	17±1	135±3	7.9±0.9	0.53±0.03	<LOQ	0.18±0.04
s-*Es* 40	27±2	134±4	6.4±0.9	0.29±0.03	<LOQ	0.11±0.04
s-*Tl* 40	12±1	132±8	9.1±0.9	4.4±0.3	0.10±0.03	0.12±0.04
EU's upper limits	25	200	6	1	0.1	5

*LOQ (see Materials and methods for LOQ).
**Experimental feed ingredients and analysed nutritional composition of diets fed cod and salmon. 0, 60 or 100% of the fish meal was replaced by Antarctic krill meal from *Euphausia superba* in the cod diets (c-*Es* 0, 60 and 100). In the diets fed salmon 40% of the fish meal protein was replaced with protein from either arctic krill meal (*Thysanoessa inermis*) (s-*Ti*), Antarctic krill meal (s-*Es*) or amphipod meal (*Themisto libellula*) (s-*Tl*). One salmon diet (s-*Ti* 0) had 100% of the protein from fish meal.
EU's upper limits of allowed levels in complete feeds (mg kg^{-1} diet 88% dry wt.) are given.

Table 7
Contents of copper, zinc, arsenic, cadmium, mercury and lead in filet of cod and salmon fed diets with krill meal (mg kg^{-1} muscle±std dm, $n=2$)

	Cu	Zn	As	Cd	Hg	Pb
Cod						
c-*Es* 0**	0.24±0.08	4±3	4.5±0.6	<LOQ	0.05±0.03	<LOQ*
c-*Es* 60	0.21±0.08	4±3	1.0±0.3	<LOQ	0.03±0.03	<LOQ
c-*Es* 100	0.24±0.08	5±3	0.4±0.3	<LOQ	0.03±0.03	<LOQ
Salmon						
s-*Ti*-0	0.37±0.08	4±3	4.1±0.4	<LOQ	0.03±0.03	<LOQ
s-*Ti* 40	0.33±0.08	4±3	0.7±0.3	<LOQ	0.02±0.03	<LOQ
s-*Es* 40	0.35±0.08	3±3	1.0±0.3	<LOQ	0.02±0.03	<LOQ
s-*Tl* 40	0.34±0.08	3±3	1.9±0.3	<LOQ	0.03±0.03	<LOQ

*LOQ (see Materials and methods for limit of quantification).
**Experimental feed ingredients and analysed nutritional composition of diets fed cod and salmon. 0, 60 or 100% of the fish meal protein was replaced by Antarctic krill meal from *Euphausia superba* in the cod diets (c-*Es* 0, 60 and 100). In the diets fed salmon 40% of protein was replaced with either arctic krill meal (*Thysanoessa inermis*) (s-*Ti*), Antarctic krill meal (s-*Es*) or amphipod meal (*Themisto libellula*) (s-*Tl*). One salmon diet (s-*Ti* 0) had 100% protein from fish meal.

contained 46 mg kg^{-1} (dm), almost twice current EU limits (25 mg kg^{-1} 88% dm). Although Arctic krill meal contained less Cu (22 mg kg^{-1} dm), the level was very high compared to the fish meal (4 mg kg^{-1} dm). The meal from amphipods was, however, in the lower range of these meals (8.2 mg kg^{-1} dm), but this situation was changed for Cd. In the case of Cd, amphipod-meal had six times current EU limits (2 mg kg^{-1} 88% dm) while the other meal types were well within the upper limit.

When fish diets were prepared from these meals, the metal levels in the meals generally influenced levels in the diets (Table 6). In particular, increasing the amount of Antarctic krill meal in the cod diets caused the final content of copper to be approximately twice the allowed upper limit in the 100% krill meal diet (EU's upper limit: 25 mg kg^{-1} 88% dm). This was also the case for Cd where the 40% amphipod diet for salmon had 4.4 mg Cd kg^{-1} (dm), almost 10 times the upper limit in complete diets (0.5 mg kg^{-1} 88% dm). The cod diet produced from fish meal (c-*Es* 0, Table 6) had relatively high Cu, As and Zn concentrations (31, 11 and 230 mg kg^{-1} dm, respectively), resulting in a discrepancy between the level of Cu in the fish meal and the cod diet (c-*Es*-0: 4 mg Cu kg^{-1} dm). No such discrepancy was seen in the salmon diets (s-*Ti*-0) where the same fish meal was used. The level of Cu in the cod diets was above the EU's upper limit, this was not seen in commercial diets. The salmon diet had Cu levels within expected ranges due to level in the fish- and krill meals.

Nevertheless, the levels in the different diets reflected the levels found in the krill and amphipod meals, hence the Cu levels increased with increasing inclusion of krill meal, while the As and Zn levels decreased (Table 7).

4. Discussion

The increase in SGR during the first 100 days in salmon fed diets with 40% amphipod or Arctic krill meal may also explain the higher feed conversion ratio (FCR) although the difference was not significant (Suontama et al., submitted for publication). This may imply that the inclusion of krill or amphipod meal yields a better feed utilisation, perhaps due to a higher level of attractants in these meals (Oikawa and March, 1997). Improved growth rates are often connected to improved feed utilization, resulting especially from high protein qualities and amino acid balance of the fish diet (Nordgarden et al., 2003; Espe et al., 2006). Plasma levels of total protein, triacylglycerol, cholesterol and glucose were according to earlier findings on Atlantic cod for fish at similar sizes (Rosenlund et al., 2004), except for the elevated plasma glucose in the fish meal group (c-*Es*-0), which was in the upper range of normal values (Hemre et al., 1991). This may indicate a mild stress condition in this group compared to the others (Hemre et al., 1991), indicating a good potential for krill inclusions in cod diets. Although high krill meal inclusion results in relatively high amounts of chitin (Suontama et al., submitted for publication), we observed no lowering of the cholesterol levels with increasing krill meal inclusion in cod. Earlier studies with cod where diets had up to 44% plant proteins clearly showed a cholesterol lowering effect from increasing dietary fibre (Hansen et al., in press). Chitin may not yield the same effect as plant fibre. Leakage of the organ specific ASAT and ALAT to the plasma compartment did not occur in cod, confirming that both liver and kidney functions were normal (Sandnes et al., 1988). The variation in RBC, HB and HCT in all groups further confirmed a good health status of fish fed krill based diets, independent of krill level, and in accordance with earlier reported normal haematological values (Sandnes et al., 1988; Rosenlund et al., 2004). Similar results were also obtained for salmon fed various krill added diets. These are presented and discussed by Suontama et al. (submitted for publication). Overall health status was not affected by the diets in either species during the study.

The levels of Zn, As and Pb were in the range of what has been reported for unprocessed krill and amphipods (Ritterhoff and Zauke, 1997; Zydeman and Jarman, 1998; Edmonds, 2003; Li et al., 2005). Further, these levels, including Hg, were similar or lower than what we found for fish meals. Accordingly, the content of these metals do not pose any potential problems for the evaluation of these meals as alternative protein sources in fish diets. The

reported levels of Hg, ranging from 0.12 to 0.23 mg kg^{-1} in Arctic species is higher than what we found in the meals studied (Ritterhoff and Zauke, 1997), which may imply seasonal, regional or annual variation, or that some Hg is removed from the meal fraction during processing. The Cu levels found in the two krill meals were high but still within the range of previous' literature data, ranging from 32 to 65 mg kg^{-1} dm (Ritterhoff and Zauke, 1997; Zydeman and Jarman, 1998). The level in the amphipod meal, however, was especially low compared to what Ritterhoff and Zauke (1997) found in *T. libellula* from the Greenland Sea (26 mg kg^{-1} dm). The reason for this variation is unknown, but it may be related to the area of capture, season or other factors. The highest level of Cd was found in the meal from Arctic amphipods (12 mg kg^{-1} dm). Ritterhoff and Zauke (1997) found great interspecific heterogeneity in the levels of cadmium in zooplankton from the Greenland sea and Fram Strait, where the level of cadmium in calanoid species were in the range of what was found in Arctic krill while species from the genus *Themisto* contained up to 34 mg kg^{-1} dm. They further suggest that the investigated *Themisto* species may possess an effective detoxification system with metal-binding proteins which can cause relatively high levels of metal concentrations (Ritterhoff and Zauke, 1998). The Cu concentration in the Antarctic krill meal and the Cd level in amphipod meal are above EU's upper limits and if such levels are common, these species may be difficult to utilise in fish feed production.

As the inclusion of Antarctic krill meal increased in the cod diets, the concentration of As fell below EU's upper limit for As in complete feeds. Arsenic levels are often high in fish meals, resulting in diet concentrations ranging from 3.6 to 9.1 mg kg^{-1} (dm) (Julshamn et al., 2000). Although the main portion of diets were below EU's upper limit of 6 mg kg^{-1} (88% dm) (Julshamn et al., 2002), inclusion of krill meal may improve the utilisation of fish meals high in As which otherwise results in As levels that exceed the limit for complete diets.

The high level of Cu in Antarctic krill meal was reflected in the diets for cod and the salmon diet where this meal was used (s-*Es*-40). These diets had Cu concentrations above EU's upper limit. The unexplained Cu contribution found in the cod diet with 100% fish meal strengthens the negative outcome of krill meal inclusion in the cod diets. Nevertheless, this krill meal should still be used in smaller amounts to avoid violation of the present legislation. The use of the amphipod meal processed in this study would not be possible and the levels found in the diet S-*Tl*-40 confirms this as the Cd concentration exceeded EU's upper limit. Berntssen et al. (2000) showed that feeding 34 mg Cu kg^{-1} diet (dm) to salmon negatively affected the lipid peroxidation and selenium and gluthatione levels, while one needed a level 204 mg Cd kg^{-1} diet to see similar effects, indicating a difference in salmon tolerance towards these two metals. Existing legislation will, however, always have to be considered when choosing alternate feed ingredients.

Cu and Cd are elements that are accumulated in fish liver and kidney (Berntssen et al., 1999, 2000) which explain why high levels in the diets was not retrieved in the muscle samples. Further, the muscle levels of Zn, Hg and Pb were not altered with the diet alterations. As levels decreased considerable, being the only element in the muscle samples that was affected during these feeding trials, which is positive as the levels of As in fish often is relatively high compared to meat from terrestrial animals (reviewed by Amlund, 2005).

5. Conclusion

The cod showed no difference in growth during the trial, while salmon fed diets where 40% of the fish meal protein was replaced with Arctic or Amphipod meal had a better SGR during the first period. Further, no adverse effects in either cod or salmon were observed during the trials. Nevertheless, high levels of Cu in meal processed from Antarctic krill (46 mg kg^{-1} dry matter (dm)) resulted in a dietary Cu level exceeding the upper limits for complete diets set by EU. Furthermore, the Cd level found in the meal processed from the amphipods (12 mg kg^{-1} dm) is 6 times higher that of EU's upper limit. These results indicate limiting factors for the use of certain zooplanktons as alternative protein sources.

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