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High inclusion of partially deshelled and whole krill meals in diets for Atlantic salmon (*Salmo salar*)

Jon Øvrum Hansen [a], Michael Penn [b], Margareth Øverland [a], Karl D. Shearer [a], Åshild Krogdahl [b], Liv Torunn Mydland [a], Trond Storebakken [a,*]

[a] Aquaculture Protein Centre, CoE, Department of Animal and Aquaculture Sciences, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway
[b] Aquaculture Protein CoE, Department of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Science, P.O. Box 8146 Dep, NO-0033 Oslo, Norway

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ABSTRACT

The aim of this study was to investigate how diets containing partially deshelled or whole krill meals affected growth, digestibility of main nutrients, faecal excretion of minerals, fluoride accumulation, and organ indices and health parameters in Atlantic salmon (*Salmo salar*). Three extruded diets were fed for 100 days to salmon with an average weight of 550 g, distributed into 9 tanks equipped with flow through sea water. The dietary treatments comprised a control diet based on high-quality fish meal (FM) and two experimental diets where the FM was substituted with either partially deshelled krill meal (PDKM) or whole krill meal (WKM). Shell removal reduced the chitin content from 28 to 8 g kg^{-1} dry matter (DM), while fluoride was only reduced from 940 to 631 mg kg^{-1} DM.

Growth rate for fish fed WKM was significantly lower than for salmon fed control diet whereas the PDKM diet did not appear to alter growth during the first feeding period. Digestibility of lipid tended to be higher for PDKM and lower for WKM compared to the FM control. No significant difference was seen for digestibility of nitrogen, but fish fed the FM diet had higher digestibility of threonine, serine, glutamine, histidine and lysine compared to fish fed the WKM diet. No major differences in plasma were seen for triacylglycerols, free fatty acids, glucose, total protein, albumin, globulin, urea, and total bilirubin. Trypsin activities in the pyloric and mid intestine were lower in fish fed the WKM diet compared to FM. Bile acid concentration in the pyloric intestine were significantly lower in fish fed the WKM diet compared to FM and PDKM. Fish fed both diets containing krill meal had signs of mild to moderate nephrosis. To conclude, PDKM could successfully replace FM as a sole protein source for Atlantic salmon, whereas the WKM slightly reduced growth rate compared to the FM and the PDKM diet.

1. Introduction

Farming of marine carnivorous fishes is increasing; implying a comparable increase in the demand for high-quality protein feed ingredients. Antarctic krill (*Euphausia superba*) is receiving increased attention as a marine protein source. Knox (2007) describes the difficulty of obtaining a reliable estimate of the Antarctic krill biomass but suggests that this biomass is between 10^8 and 10^9 tons. Krill was examined as a source for fish feeds in the late 70's and early 80's (Storebakken, 1988), but it's commercial use did not increase due to the economics of the krill fisheries, problems with krill processing and increased supplies of fish meal (FM) (Ichii, 2000).

A limiting factor for use of krill in European fish feeds has been EU's restriction on fluoride level in feed of 150 mg kg^{-1} (Commission dir. 2002/32/EC). A recent EU directive increased the allowable fluoride level in complete feed for fish to 350 mg kg^{-1} (Commission dir. 2008/76/EC). Fluoride is mainly located in the exoskeleton of the krill and the fluoride level in whole Antarctic krill has been shown to be between 1000 mg kg^{-1} (Boone and Manthey, 1983; Zhang et al., 1993) and 2400 mg kg^{-1} (dry wt.) (Soevik and Braekkan, 1979). A reduction of fluoride concentration in krill meal can be obtained by separating the exoskeleton from the muscle fraction prior to meal production. The fluoride, however, is able to leach from the exoskeleton to the muscle fraction during cold storage in a temperature dependent manner (Christians and Leinemann, 1983; Adelung et al., 1987). A partial deshelling of the krill meal will also reduce the level of chitin, a long-chain polymer of N-acetyl-glucosamine that is known to reduce growth rate similarly to that of dietary fiber (Tharanathan and Kittur, 2003). Another limiting factor for use of krill is the natural high level of copper in krill; EU's allows a maximum of 25 mg kg^{-1} in complete feedstuff (Commission dir. 2003/100/EC).

Feeding rainbow trout (*Salmo gairdneri*) with krill resulted in reduced growth rate and feed efficiency when average quality FM was

* Corresponding author. Tel.: +47 64965263; fax: +47 64965101.
E-mail address: trond.storebakken@umb.no (T. Storebakken).

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completely replaced with krill meal (Beck et al., 1977; Koops et al., 1979). Feeding Atlantic salmon (*Salmo salar*) with Antarctic krill, a complete replacement of high-quality FM also gave significantly lower final weights compared to fish fed a diet with 200 g kg^{-1} krill meal replacement (Rungruangsak-Torrissen, 2007). These experiments suggest that krill meal might contain components that reduce growth rate at high dietary inclusions. In contrast, Yoshitomi et al. (2007) reported no negative effect on growth in small rainbow trout reared in fresh water and fed graded levels, up to complete replacement of a brown FM with a low fluoride krill meal, produced by deshelling the meal after it was dried.

The aims of this study were to examine nutritional, physiological and histo-pathological responses to diets where krill meals, with and partly without shell, fully replaced FM as the source of protein.

2. Materials and methods

2.1. Krill meal and diet production

The experimental krill meals were produced at the Norwegian University of Life Sciences (UMB) (Ås, Norway) by a technology proprietary to Krillsea Group AS, using frozen Antarctic krill, *Euphausia superba* (United Ocean Co., Ltd, South Korea). Two types of krill meal were produced. One was a partially deshelled krill meal (PDKM), where parts of the shells had been mechanically removed prior to heating. The other was a WKM, with shells (Table 1). Both meals contained krill water solubles ("stickwater"), which is shown to be responsible for some of the palatability properties in krill meal (Tibbetts et al., 2010). The shell removal process reduced the chitin level from 28 to 8 g kg^{-1} dry matter (DM), while the fluoride level was only reduced from 940 to 631 mg kg^{-1} DM. The experimental krill meals were lower in ammonium and volatile nitrogen compared to the FM (Table 1). The levels of biogenic amines in all three meals were within the quality specifications for Norse Eco LT FM from Norsildmel A/L, Fyllingsdalen, Norway. The WKM contained higher level of the biogenic amine tyramine compared to the FM, but putrescine, cadaverine, and histamine levels were higher in the FM compared to the krill meals.

Three extruded diets were produced at the Centre for Feed Technology at UMB (Table 2). Yttrium oxide (Y_2O_3) was used as an inert marker for determination of digestibility (Austreng et al., 2000). The diets were mixed, ground, conditioned, extruded, dried and vacuum coated by the same equipment and procedures as used by Aslaksen et al. (2007).

After cooling, the coated pellets were packed in plastic bags and stored at 5 °C. The diets were formulated to be isonitrogenous and isolipidic (Table 3). Because FM and krill meals differed in lipid content, different levels of oil were added during vacuum coating to obtain similar dietary lipid levels.

2.2. Biological experiment and facilities

A total of 225 Atlantic salmon, weighing 550 g on average, were randomly distributed to nine fibreglass tanks of 1.5 m^3 each and kept in 34 g l^{-1} sea water supplied at 22 l min^{-1}. The mean water temperature was 8.1 °C for days 1–56 and 7.0 °C for days 57–100. The tanks were under constant light 24 h d^{-1}. Each diet was fed to triplicate groups of fish. Feed was supplied in excess of appetite in order to ensure maximum voluntary feed intake.

2.3. Sampling procedure

All fish were weighed at the start of the experiment and at days 56 and 100. At the end of the experiment, the fish were anaesthetized with metacaine (MS-222™; 50 mg l^{-1} water). Weight was recorded for all fish. Randomly selected fish from each tank were sampled for analyses. All fish in each tank, except those sampled for histology and gut enzymes, were stripped for faeces. Three fish per tank were homogenized and pooled prior to analyzing for whole body composition. Another five fish per tank were sampled for weight of the gastrointestinal tract (GIT), liver and carcass. Blood was drawn from six fish by caudal venipuncture using vacutainers containing lithium heparin. The gastrointestinal tracts were removed and carefully cleaned of adherent adipose. The intestine was then divided into four regions: stomach (ST), pyloric caeca (pyloric intestine, PI), mid intestine (MI) and distal intestine (DI). Digesta was collected from the PI (distal half), MI and DI (proximal half) for analysis of bile acid concentration and lipase and trypsin activities. The tissue of each region was individually weighed and samples taken for histology (fixed in neutral buffered formalin) and the remainder frozen in liquid N_2 for brush border enzyme activity analyses. Additional samples for histology were taken from the liver (LI) and trunk (filtrative) kidney (KI). The Norwegian quality cut, NQC (NS, 1994) was sampled from three fish per tank. A standard piece, approximately 10 g of the white dorsal muscle from the NQC was sampled with a cork punch. The remainder was microwaved in sealed plastic bags until the bone easily separated from the muscle. The bone and muscle were freeze dried and pooled within tank, ground with a pestle and mortal prior to fluoride analysis. Three whole fish from each tank were frozen and later thawed for radiography.

Table 1
Chemical composition of fish meal (FM), partly deshelled krill meal (PDKM), and whole krill meal (WKM) used in the experimental diets.

	FM	PDKM	WKM
Composition, g kg^{-1}			
Dry matter	969	964	925
Crude protein[b]	708	610	594
Crude lipid	88	187	151
Ash	139	118	134
Chitin	0.2	8	28
Ammonium nitrogen	26	8	9
Total volatile nitrogen	0.1	<.1	<.1
Volatile fatty acids, % of total 18:1	nd. [a]	29	31
Peroxide value	nd.	0.7	4.1
Amino acids[c], g kg^{-1}			
Total AA[d]	519.6	390.6	381.5
Cysteine	5.7	5.2	4.8
Methionine	16.1	11.7	11.0
Aspargine	54.7	43.8	43.4
Threonine	25.4	19.7	19.2
Serine	25.3	17.8	17.1
Glutamine	83.9	58.1	57.9
Proline	23.1	22.1	23.0
Glycine	30.8	20.2	21.1
Alanine	32.6	22.0	22.3
Valine	26.4	20.5	20.2
Isoleucine	23.7	20.6	19.7
Leucine	42.0	31.8	30.7
Tyrosine	15.2	14.0	13.1
Phenylalanine	22.0	18.4	17.9
Histidine	11.8	8.3	8.6
Lysine	45.5	31.4	30.0
Arginine	35.3	24.9	21.5
Minerals, mg kg^{-1}			
Fluoride	176	631	940
Copper	5.2	78	81
Zinc	63	58	55
Biogenic amines, mg kg^{-1}			
Tyramine	215	92.6	529
Putrescine	122	12.4	21.1
Cadaverine	496	<1	<1
Histamine	7.0	<1	<1

[a] nd. = not determined.
[b] Adjusted for chitin nitrogen.
[c] Presented in dehydrated form.
[d] Total sum of amino acids without tryptophan.

Table 2
Diet formulation.

Diet	FM [a]	PDKM [b]	WKM [c]
Formulation, g kg^{-1}			
Partly deshelled krill meal	0	752	0
Whole krill meal	0	0	689
Fish meal[a]	641	0	0
Wheat flour[d]	108	105	107
Vitamin and mineral premix[e]	5.0	5.0	5.0
Vitamin C[f]	0.60	0.60	0.60
MCP[g]	0	18.0	18.3
Yttrium oxide[h]	0.1	0.1	0.1
Fish oil[i]	245	119	180

[a] Norse Eco LT, Norsildmel, Egersund, Norway.
[b] PDKM; partly deshelled krill meal.
[c] WKM; whole krill meal.
[d] Felleskjøpet, Kambo, Norway.
[e] Farmix, Trouw Nutrition, LA Putten, The Netherlands. Per kg feed. Retinol 2500.0 IU, Cholecalciferol 32400.0 IU, α-tocopherol SD 0.2 IU, Menadione 40.000 mg, Thiamin 15.0 mg, Riboflavin 25.0 mg, d-Ca-Pantothenate 40.003 mg, Niacin 150.003 mg, Biotin 3000.0 mg, Cyanocobalamin 20.0 mg, Folic acid 5.0 mg, Pyridoxine 15.0 mg, Ascorbate polyphosphate 0.098 g, Cu: CuSulfate 5H_2O 11.998 mg, Zn: ZnSulfate 89.992 mg, Mn: Mn(II)Sulfate 34.993 mg, I: K-Iodide 1.999 mg, Se: Na-Selenite 0.200 mg, Cd Max. 0.003 mg, Pb Max. 0.028 mg, Ca 0.915 g, K 1.380 g, Na 0.001 g, Cl 1.252 g.
[f] Stay-C® 35. DSM Nutritional Products, Basel, Switzerland.
[g] Bolifor® MCP-F. KPP Oy, Animal Nutrition, Helsingborg, Sweden.
[h] Y_2O_3. Metal Rare Earth Limited, Shenzhen, China.
[i] NorSalmOil, Norsildmel, Egersund, Norway.

2.4. Chemical and physical analyses

Diets and freeze-dried faeces were ground with a pestle and mortar prior to analysis. Diets and faeces were analyzed for dry matter by drying to constant weight at 104 °C (Commission dir. 71/393/EEC), protein using Kjeldahl nitrogen (Commission dir. 93/28/EEC) × 6.25, lipid by HCl hydrolysis followed by diethyl ether extraction (Commission dir. 98/64/EC), starch (AOAC enzymatic method 996.11), ash (Commission dir. 71/250/EEC), minerals (ICP-AES/ICP-MS) (Nordic Committee on Food Analysis (NMKL) method 161), and yttrium oxide (ICP-AES) (NS-EN ISO 11885). Starch gelatinization was analyzed by the BioLab Analyse (Nofima Ingredients, Bergen) using a modification of Chiang and Johnson (1977) glucoamylase methodology. Biogenic amines were analyzed by using HPLC according to Smělá et al. (2003). Amino acids were analyzed according to Commission dir. 98/64/EC on a Biochrom 30 amino acid analyzer (Biochrom Ltd., Cambridge, UK). Chitin in diets was determined as glucosamine-residues after post-column derivatization with ninhydrin on the same Biochrom 30 analyzer as used for the amino acid analyses. In brief, the 100 mg chitin samples were hydrolyzed at 110 °C for 4 h in Duran-Schott GL-18 screw capped tubes in a Labtherm® heating block (Liebisch Labortechnik GmbH, Bielefeld, Germany) using 10 ml of 6 M HCl. After cooling to room temperature, 8 ml of 7.5 M NaOH was gently added and the hydrolysates were diluted to 100 ml using 0.2 M sodium citrate loading buffer, pH 2.2 (Biochrom Ltd., Cambridge, UK). An aliquot (1 ml) of the hydrolysates was micro-filtrated (0.45 µm Spartan membrane filter, Schleicher & Schuell, Dassel, Germany) prior to injection (40 µl). All data were analyzed against external standards of glucosamine-HCl (Sigma Chemical, St. Louis, Mo., U.S.A.) using the Chromeleon® Chromatography Management Software (Dionex Corporation, Sunnyvale, CA). Fluoride concentration was determined using a fluoride ion selective combination electrode (VWR symphony, model 14002-788) using the procedure given by Malde et al. (2001).

Physical pellet quality was tested for durability, hardness and expansion 14 days after production. Pellet durability was tested with a DORIS pellet tester (AKVAsmart, Bryne, Norway) using 100 g of presieved pellets. After the pellets passed through the machine the pellet collector was emptied on a 4 mm screen with a collector and sieved for 60 s at 0.5 amplitude with a Retsch AS 200 Control (Haan, Germany). The material remaining on the 4.0-mm screen after sieving was weighed. The durability was defined as the percentage of pellets remaining on the screen. The testing was conducted in triplicate for each diet. Hardness was determined with a Texture Analyzer TA-XT2 (SMS Ltd, Surrey, UK), equipped with a 5 kg load cell as described by Øverland et al. (2009). The length of each pellet tested was recorded with an electrical caliper and the pellet width was recorded by the texture analyzer. Expansion was calculated as: ((pellet width − die diameter) × die diameter^{-1}) × 100.

2.5. Plasma clinical chemistry

The Central Laboratory at The Norwegian School of Veterinary Science performed plasma analyses according to standard methods. These methods are based on Mulder et al. (1983) for free fatty acids and Trinder (1969) and Allain et al. (1974) for cholesterol. The analyses for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), total protein, albumin, globulin, urea, creatinine, glucose, triglycerides, free fatty acids, bile acids, total bilirubin, inorganic phosphorus, Ca, Na, K, and Cl, are based on Tietz (1995). Bile acid in plasma was determined using the Enzabile® test kit (Cat. No. 550101, Bio-Stat Diagnostic Systems, Cheshire, UK). The concentrations of 3α-hydroxy bile acids were calculated from a standard curve generated using bovine serum based standards containing glycohenodeoxycholic acid.

Table 3
Chemical composition of the diets.

	FM	PDKM[a]	WKM[b]
Dry matter (DM), g kg^{-1}	959.8	955.9	951.1
In DM, kg^{-1}			
Crude protein, g	484.4	494.0	464.9
Total protein[c], g	484.4	490.4	455.9
Lipid, g	323.0	284.6	310.2
Starch, g	72.9	67.0	66.2
Ash, g	103.7	108.4	112.1
Chitin, g	0.1	8.5	20.9
Gross energy, MJ	23.6	22.6	22.9
Amino acids[d], g kg^{-1}			
Total AA[e]	336.0	315.8	296.9
Cysteine	3.7	4.3	3.8
Methionine	10.1	9.2	8.4
Aspargine	35.2	35.3	33.4
Threonine	16.2	15.9	14.9
Serine	16.6	14.8	13.6
Glutamine	56.5	48.4	46.6
Proline	16.1	18.5	18.1
Glycine	20.2	16.0	16.3
Alanine	20.8	17.6	17.3
Valine	16.7	16.1	15.5
Isoleucine	14.8	16.0	14.8
Leucine	26.8	25.5	23.6
Tyrosine	9.8	11.4	10.1
Phenylalanine	14.4	14.9	14.0
Histidine	7.5	6.7	6.3
Lysine	27.9	24.8	23.1
Arginine	22.7	20.4	16.9
Minerals, kg^{-1}			
Phosphorus, g	14.5	14.5	12.3
Copper, mg	10.0	72.0	64.0
Iron, mg	13.0	22.0	14.0
Zinc, g	0.19	0.20	0.19
Fluoride, mg	110	470	640
Yttrium, mg	82.0	87.0	84.0

[a] PDKM; partly deshelled krill meal.
[b] WKM; whole krill meal.
[c] Adjusted for chitin nitrogen.
[d] Presented in dehydrated form.
[e] Total sum of amino acids without tryptophan.

2.6. Histological evaluation

Samples were fixed in neutral buffered formalin (40 g formaldehyde l^{-1}; pH 7.4) for 24 h and subsequently transferred to 70% ethanol until processing. Initially three fish sampled from each tank were processed for tissue histology (n = nine per treatment). Tissues were processed at the Pathology Laboratory, NVH using standard histological methods. Sections for routine histological analysis were stained with haematoxylin and eosin (H&E). When initial screening indicated differences among groups (i.e. in kidney samples) additional samples were examined (n = 18 total per treatment).

2.7. Brush border enzyme activities

Brush border membrane bound alkaline phosphatase (ALP), leucine aminopeptidase (LAP) and maltase activities were determined in homogenates of intestinal tissue. The tissues were thawed, weighed and homogenized (1:20) in ice-cold 2 mM Tris/50 mM mannitol, pH 7.1, containing the serine protease inhibitor phenyl-methyl-sulphonyl fluoride (Sigma no. P-7626; Sigma Chemical Co., St. Louis, MO, USA). Aliquots of homogenates were frozen in liquid nitrogen and stored at −80 °C until analysis. Enzyme activities were determined colorimetrically as previously described by Krogdahl et al. (2003). Incubations were performed at 37 °C. Enzyme activities are expressed as mol substrate hydrolyzed min^{-1} and related to kg fish (specific activity).

2.8. Trypsin and lipase activities and bile acid content of digesta

Trypsin and lipase activities and bile acid concentration were determined on pooled, freeze dried digesta from the distal PI, MI and proximal DI. Trypsin activity was determined colorimetrically (Kakade et al., 1973) using the substrate benzoyl-arginine-*p*-nitroanilide (BAPNA) (Sigma no. B-4875) and a curve derived from a standardized bovine trypsin solution.

Lipase activity was analyzed in freeze dried digesta (1.25 mg ml^{-1} sonicated suspension in 25 mM Tris-buffer, pH 8.0) spectrophotometrically by hydrolysis of 4-nitrophenol-myristate (4-NPM) as described by Gjellesvik et al. (1992). The reaction rate was measured at 37 °C and pH 8.0. Bile acid concentration was determined using the same method described for plasma chemistry.

2.9. Calculation and statistical analysis

Specific mechanical energy (SME) during extrusion was calculated as: $(2\times\pi\times60^{-1})\times(S_{rpm}\times T_{kNm}\times T_{t/h}^{-1})$, where S_{rpm} is screw speed, T_{kNm} is torque and $T_{t/h}$ is throughput. Specific growth rate (SGR) was calculated as: SGR = 100 × (ln(end wt) − ln(start wt)/Δt), where end wt = end weight of fish, start wt = start weight of fish, Δt = number of experimental days. Apparent digestibility coefficients (%) of individual nutrients were calculated as follows: ADC = $100\times(1-(D_i\times F_i^{-1}\times F_n\times D_n^{-1}))$, where; D_i and F_i represent the concentrations of inert marker in the diet and faeces, and D_n and F_n represent the concentrations of nutrients in the diet and faeces, respectively. Faecal excretion for minerals was calculated as follows: $(-100\times(1-(D_i\times F_i^{-1})\times(F_n\times D_n^{-1})))+100$.

The results were statistically analyzed by one-way analysis of variance to differentiate between the diets (SAS, 1990). Results are presented as means and pooled standard errors of means (s.e.m.). Significant ($P<0.05$) differences among means were ranked by Tukey's multiple range test and are indicated in the tables by different superscripts [a,b,c]. Tendency is indicated for $0.10>P\geq0.05$.

Table 4
Processing parameters and physical pellet quality and starch gelatinization in diets based on fish meal (FM), partially deshelled krill meal (PDKM) and whole krill meal (WKM).

	FM	PDKM	WKM	s.e.m[1]	*P*-value
Feed mash					
Lipid content [2], g kg^{-1}	77.9	162	130		
Moisture content [3], g kg^{-1}	268	162	185		
Extrusion					
Section 3, °C	118	129	124		
SME [4], Wh kg^{-1}	37.0	45.0	35.0		
Physical pellet quality					
Hardness, N	16.7[b]	29.9[a]	31.4[a]	0.23	0.0002
Durability, %	64.4[c]	83.8[b]	92.2[a]	0.77	<.0001
Expansion, %	23.6[a]	15.0[b]	8.6[c]	1.12	<.0001
Starch gelatinization [5]	91.3	73.4	84.1		

[1] Pooled standard error of mean. Different letters denote significant ($P<0.05$) difference among diets. n = 3 replicates per treatments. Tukey multiple range test is used. [2] Lipid content in feed mash prior to processing. No lipid was added before vacuum coating. [3] Total moisture content, calculated based on analyzed moisture in raw materials and added amounts of steam and water in the conditioner. [4] Specific mechanical energy, W h kg^{-1}. [5] Percent of total starch content in finished feed.

Table 5
Growth for Atlantic salmon fed diets with fish meal (FM), partially deshelled krill meal (PDKM) or whole krill meal (WKM).

	FM	PDKM	WKM	s.e.m.[1]	*P*-value
Start weight, g	544	554	553	3.38	0.17
Mid weight 56 d, g	758[ab]	809[a]	711[b]	12.2	0.004
End weight, 100 d, g	1060[ab]	1100[a]	956[b]	32.0	0.045
SGR, 1–56 d	0.76[a]	0.86[a]	0.57[b]	0.035	0.003
SGR 1–100 d	0.66	0.69	0.55	0.033	0.055

[1] Pooled standard error of mean. Different letters denote significant ($P<0.05$) difference among diets. n = 3 replicates per treatments.

3. Results

3.1. Feed production and pellet quality

Different amounts of water and steam were added into the extruder to obtain equal conditions between the dietary productions, because the amount of lipid in mash prior to extrusion differed among the diets (Table 4). The SME varied from 35 to 45 for the experimental diets (Table 4). The krill meal based diets had increased pellet hardness and durability compared to the FM diet, while expansion was lower in the krill diets. The degree of starch gelatinizing was 91.3, 73.4, and 84.1% of total starch for the FM, PDKM, WKM diets, respectively (Table 4).

3.2. Growth performance

No fish died during the experimental period and there were no differences among treatments for initial weights. The fish grew from an average of 550 g to between 956 and 1100 g during 100 days of feeding (Table 5). During the first 56 days of feeding, growth rates were significantly higher for the fish fed PDKM and FM than fish fed WKM. For the entire 100 days of feeding, there was a strong tendency for reduced growth rate for the WKM diet compared to the PDKM and FM diets.

3.3. Nutrient digestibility and mineral faecal excretion

There was a clear tendency for reduced lipid digestibility (Table 6), whereas the fish fed the WKM were ranked below the PDKM and FM treatments. Replacement of FM with WKM did not appear to affect starch digestibility, whereas PDKM reduced starch digestibility. Nitrogen digestibility did not significantly differ from the two dietary krill diets compared to the FM control. However, digestibility of several amino acids was affected. Cysteine digestibility ranged from 80.2 to 83% and tended to be higher for the PDKM fed fish compared to the FM fed fish. Fish fed the WKM diet had significantly lower

Table 6
Apparent digestibility (%) of main nutrients, amino acids and faecal excretion of minerals in Atlantic salmon fed diets with fish meal (FM), partially deshelled krill meal (PDKM) or whole krill meal (WKM).

	FM	PDKM	WKM	s.e.m.[1]	*P*-value
Crude lipid	93.3	95.2	89.9	1.26	0.062
Nitrogen	84.2	85.6	83.5	0.81	0.23
Starch	74.3^{b}	59.4^{a}	66.2^{ab}	2.16	0.008
Amino acids					
Total	90.5	90.4	88.8	0.44	0.065
Cysteine	80.2	83.0	81.5	0.73	0.089
Methionine	90.3	91.2	89.2	0.51	0.079
Aspargine	85.1	84.4	82.2	0.72	0.068
Threonine	89.8^{a}	88.6^{ab}	86.8^{b}	0.50	0.015
Serine	89.9^{a}	89.3^{a}	86.9^{b}	0.48	0.001
Glutamine	93.0^{a}	91.6^{ab}	90.2^{b}	0.35	0.004
Proline	90.1^{b}	93.3^{a}	92.4^{a}	0.31	0.0009
Glycine	88.3	90.2	89.4	0.44	0.062
Alanine	92.0	92.5	91.3	0.34	0.12
Valine	90.5	90.5	89.2	0.45	0.15
Isoleucine	90.2	90.3	88.7	0.50	0.10
Leucine	92.0	91.8	90.2	0.44	0.057
Tyrosine	89.7	91.0	89.1	0.44	0.062
Phenylalanine	89.4	90.2	88.8	0.52	0.25
Histidine	89.2 ^{a}	86.7 ^{b}	84.6 ^{b}	0.56	0.003
Lysine	93.3 ^{a}	92.5 ^{ab}	91.4 ^{b}	0.35	0.026
Arginine	92.5 ^{ab}	93.9 ^{a}	91.9 ^{b}	0.33	0.013
Faecal excretion of minerals, % of intake					
Phosphorus	68.3	57.5	54.9	3.27	0.059
Copper	77.4	93.2	81.2	5.13	0.15

[1] Pooled standard error of mean. Different letters denote significant ($P<0.05$) difference among diets. $n=3$ replicates per treatments.

digestibilities for threonine, serine, glutamine, histidine and lysine compared to fish fed the FM control.

Faecal excretion of phosphorus tended to be higher in the two diets with krill meal than the FM diet (Table 6). Faecal excretion of copper was not significantly affected by dietary treatment.

3.4. Whole body and liver mineral, tissue fluoride concentrations, and radiography

There were no clear trends among fish fed the dietary treatments for whole body and liver minerals (Table 7). The muscle tissue fluoride level ranged from 5.7 to 11.5 mg kg^{-1} on a dry weight basis with no effect of dietary treatment (Table 7**).** There appeared to be more fluoride accumulation in bone compared to muscle, varying from 7.3 to 18.4 mg kg^{-1}. The salmon fed the WKM diet contained significantly more bone fluoride than the other dietary treatments. Radiography showed no differences in bone density or incidence of skeletal deformities that could be specifically ascribed to dietary treatment.

3.5. Blood plasma parameters, tissue somatic indices, intestinal enzyme activities and bile acid levels

Salmon fed the diets with krill meal had significantly lower plasma cholesterol and creatinine levels than fish fed the FM diet (Table 8). No significant differences were seen for TG, FFA, glucose, bile acids, total protein, albumin, globulin, or urea. The same applied to the enzymes AST, ALT, and AP. Inorganic phosphorous was significantly higher in fish fed the two diets with krill meal compared to the FM control, while calcium, sodium, potassium and chloride plasma levels were similar among treatments.

The tissue somatic indices of stomach, PI and DI were higher for fish fed the WKM compared to fish fed the FM control (Table 9). There was no significant difference among treatments for liver and MI somatic index.

Table 7
Fluoride content in muscle tissue, bone tissue and faeces (mg kg^{-1} dry wt.), minerals in whole body and liver of Atlantic salmon fed diets with fish meal (FM), partially deshelled krill meal (PDKM) or whole krill meal (WKM).

	Start	FM	PDKM	WKM	s.e.m.[1]	*P*-value
Fluoride, mg kg^{-1}						
Muscle		10.3	5.65	11.5	2.43	0.27
Bone		8.64^{b}	7.27^{b}	18.4^{a}	1.98	0.014
Faeces		519^{b}	2308^{a}	2417^{a}	156	0.0002
Whole body minerals, kg^{-1}						
Calcium, g	4.49	4.49	4.71	5.00	0.31	0.56
Phosphorus, g	5.2	5.46	5.45	5.53	0.13	0.89
Magnesium, mg	558	440	450	450	4.01	0.53
Zinc, mg	65.9	61.8	57.7	59.3	3.32	0.69
Copper, mg	3.64	2.53	2.31	2.27	0.09	0.19
Liver minerals, mg kg^{-1}						
Zinc		2.51	2.67	2.64	0.05	0.13
Copper		11.1	11.0	11.0	0.60	0.99

[1] Pooled standard error of mean. Different letters denote significant ($P<0.05$) difference among diets. $n=3$ replicates per treatments. Tukey multiple range test is used.

Brush border enzyme (ALP, LAP and maltase) activities are shown in Table 9. Fish fed the krill containing diets had higher ALP and LAP activities in the DI compared to fish fed FM. Similar findings were observed in the PI, but the differences only tended to be significant for the ALP activity. In contrast, there was lower maltase activity in fish fed the WKM diet compared to FM, though this effect was only significant in the PI.

Trypsin activity in digesta was lower in fish fed the WKM diet compared to fish fed the FM diet, particularly in the PI and MI (Table 10). Lipase activity was lower in fish fed the PDKM diet compared to the WKM and the FM diet in the MI. Bile acid levels in the PI were lower in fish fed the WKM diet compared to FM control, and both dietary treatments with krill meal resulted in reduced bile acid levels in the DI when compared to FM.

3.6. Histology of the GIT, liver and kidneys

No histological differences were found in the intestines and livers between fish from the different dietary groups. In the trunk kidney,

Table 8
Blood chemistry in Atlantic salmon fed diets with fish meal (FM), partially deshelled krill meal (PDKM) or whole krill meal (WKM).

	FM	PDKM	WKM	s.e.m.[1]	*P*-value
Metabolites [2]					
Cholesterol, mM	15.7^{a}	11.1^{b}	10.5^{b}	0.30	<.0001
TG, mM	2.58	2.68	2.69	0.05	0.31
FFA, mM	0.53	0.47	0.48	0.04	0.57
Glucose, mM	4.60	5.03	4.84	0.11	0.082
Bile acids, μM	13.9	6.2	6.0	2.64	0.13
Total protein, g l^{-1}	48.2	50.2	48.8	1.22	0.53
Albumin g l^{-1}	24.5	25.8	25.1	0.65	0.41
Globulin g l^{-1}	23.8	24.4	23.6	0.69	0.70
Urea, mM	1.07	1.05	1.05	0.05	0.96
Creatinine, μM	55.1^{a}	19.2^{b}	19.0^{b}	3.23	0.0003
Enzymes [2], U l^{-1}					
AST	362	458	385	42.4	0.32
ALT	23.1	25.0	28.1	3.89	0.68
AP	207	224	202	7.45	0.18
Electrolytes [2], mM					
Inorganic P	3.1^{a}	4.2^{b}	4.1^{b}	0.05	<.0001
Calcium	3.0	3.1	3.0	0.03	0.47
Sodium	162	164	163	0.89	0.35
Potassium	2.8	2.9	2.8	0.16	0.75
Chloride	138	138	138	1.02	0.79

[1] Pooled standard error of mean. Different letters denote significant ($P<0.05$) difference among diets. $n=3$ replicates per treatments. [2]Abbreviations: TG: triglycerides, FFA: free fatty acids, AST: aspartate aminotransferase, ALT: alanine aminotransferase, AP: alkaline phosphatase, Inorganic P: inorganic phosphorous.

Table 9
Tissue somatic indices, activities of alkaline phosphatase (ALP), leucine aminopeptidase (LAP) and maltase for Atlantic salmon fed diets with fish meal (FM), partially deshelled krill meal (PDKM) or whole krill meal (WKM).

	FM	PDKM	WKM	s.e.m.[1]	*P*-value
Tissue somatic indices					
Stomach	0.45[b]	0.50[ab]	0.50[a]	0.01	0.023
Pyloric intestine	1.61[b]	1.84[a]	2.06[a]	0.05	0.003
Mid intestine	0.25	0.21	0.25	0.01	0.11
Distal intestine	0.38[b]	0.46[ab]	0.50[a]	0.02	0.028
Liver	1.17	1.21	1.19	0.03	0.70
ALP activity, μmol min^{-1} kg^{-1} fish					
Pyloric intestine	418	610	622	50.9	0.052
Mid intestine	80.0	94.3	107	11.3	0.31
Distal intestine	147[b]	242[a]	241[a]	16.4	0.010
LAP activity, μmol min^{-1} kg^{-1} fish					
Pyloric intestine	166[b]	230[ab]	246[b]	15.8	0.026
Mid intestine	21.0	24.7	29.6	2.65	0.15
Distal intestine	57.8[b]	87.2[a]	88.5[a]	5.28	0.010
Maltase activity, μmol min^{-1} kg^{-1} fish					
Pyloric intestine	48.3[a]	44.4[ab]	37.6[b]	1.94	0.022
Mid intestine	32.2	36.0	32.6	2.05	0.42
Distal intestine	33.9	33.3	28.1	1.47	0.060

[1] Pooled standard error of mean. Different letters denote significant ($P<0.05$) difference among diets. $n=3$ replicates per treatments.

degeneration and mild to moderate apoptosis and necrosis of renal tubule cells with concomitant exfoliation into the tubular lumen (Fig. 1) were observed in fish fed the krill containing diets. The renal glomeruli appeared normal.

During sampling grossly observable lesions were noted in the stomachs of between three and eight (of 18) fish per treatment (Fig. 2A). There was no apparent correlation to dietary treatment. The lesions appeared as focal to multifocal (2–3) round to oblong, pale, raised, slightly soft masses. Some of the lesions had a central depression, but no discoloration was noted. Histologically the lesions exhibited altered tissue architecture (Fig. 2B). The mucosa appeared as long folds extending to the basal lamina propria. The epithelia consisted of a single layer of low columnar cells. In more advanced lesions the normal glandular tissue was completely absent and epithelial sloughing was observed.

4. Discussion

The observed trend for increased pellet hardness and pellet durability with decreased pellet expansion with krill meal inclusion in the present experiment is in keeping with previous findings (Lue et al., 1990; Hansen and Storebakken, 2007). Increased lipid in the mash prior to extrusion has been shown to increase the lubrication of the extruder barrel resulting in reduced dough temperature (Lin et al., 1997) and to function as an insulating agent preventing water from being absorbed by the starch granules resulting in reduced starch gelatinization (Schweizer et al., 1986). This is in line with the present results, where increased lipid and reduced water in the mash reduced starch gelatinization, resulting in lower starch digestibility. This is in line with earlier results for rainbow trout (Bergot and Breque, 1983) and European sea bass (*Dicentrarchus labrax*) (Peres and Oliva-Teles, 2002).

Reduced growth in salmon fed the WKM diet agrees with data of Beck et al. (1977) and Koops et al. (1979), who completely replaced FM with WKM in diets for fresh water raised rainbow trout and observed reduced growth and feed efficiency. Olsen et al. (2006) reported the same effect, while Yoshitomi et al. (2006) reported decreased growth of rainbow trout fed 30% replacement of FM with WKM. Also in line with our results, Yoshitomi et al. (2007) found no effect on growth in rainbow trout when replacing all of the FM with a deshelled krill meal. These data indicate that the krill shell fraction can partially explain the reduced growth when a high fraction of FM is replaced by WKM.

The chitin content of whole Antarctic krill is approximately 30 g kg^{-1} dry weight (Nicol and Hosie, 1993). In the present experiment the PDKM and the WKM diets had chitin levels of 8.5 and 20.9 g kg^{-1} diet, respectively. Lindsay et al. (1984) reported reduced growth in juvenile rainbow trout fed 40, 100 and 250 g kg^{-1} dietary chitin compared to a control diet without chitin. Shiau and Yu (1999) reported that feeding

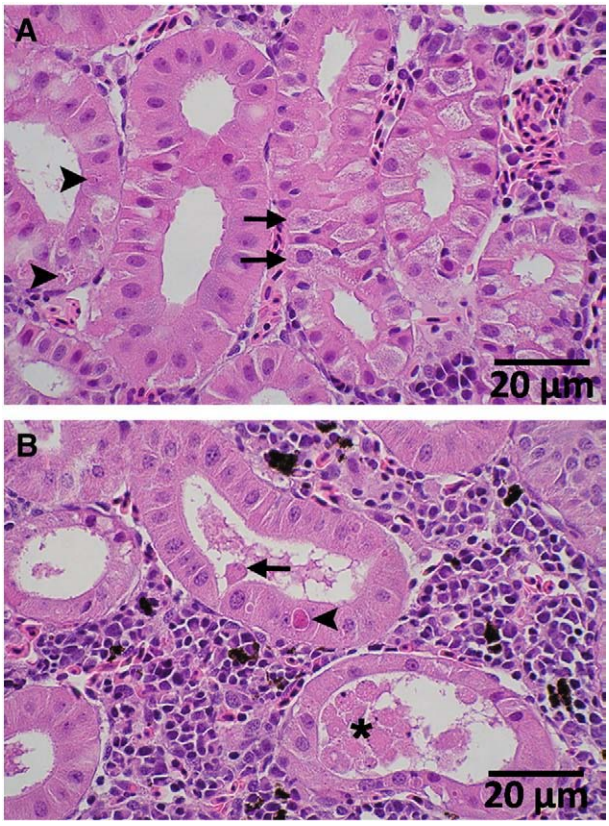

Fig. 1. Mild to moderate nephrotic changes of fish fed WKM (A) and PDKM (B) diets. Panel A shows degenerative renal tubule epithelial cells (arrows) and apoptotic bodies (arrowheads). Panel B shows extrusion of a renal tubule epithelial cell (arrow), apoptotic bodies (arrowhead) and cellular debris within the tubule lumen (asterisk).

Table 10
Dry matter, trypsin and lipase activity, bile acids concentration in the digesta of Atlantic salmon fed diets with fish meal (FM), partially deshelled krill meal (PDKM) or whole krill meal (WKM).

	FM	PDKM	WKM	s.e.m.[1]	*P*-value
Dry matter (g 100 g^{-1})					
Pyloric intestine	11.8[b]	11.7[b]	14.1[a]	0.36	0.005
Mid intestine	14.9	14.3	14.5	0.53	0.76
Distal intestine	16.7[a]	15.2[b]	15.1[b]	0.33	0.026
Trypsin (U mg^{-1} dry matter)					
Pyloric intestine	216.1[a]	221.1[a]	129.3[b]	6.42	<.0001
Mid intestine	174.5[a]	151.0[ab]	120.7[b]	7.51	0.007
Distal intestine	80.8	62.0	60.1	7.49	0.18
Lipase (U mg^{-1} dry matter)					
Pyloric intestine	0.044	0.041	0.044	0.003	0.56
Mid intestine	0.038[a]	0.028[b]	0.037[a]	0.002	0.018
Distal intestine	0.035	0.024	0.032	0.004	0.19
Bile acid (mg g^{-1} dry matter)					
Pyloric intestine	127.0[a]	107.5[ab]	82.0[b]	5.95	0.005
Mid intestine	115.6	111.0	67.2	15.6	0.12
Distal intestine	53.2[a]	38.8[b]	34.9[b]	2.39	0.004

[1] Pooled standard error of mean. Different letters denote significant ($P<0.05$) difference among diets. $n=3$ replicates per treatments.

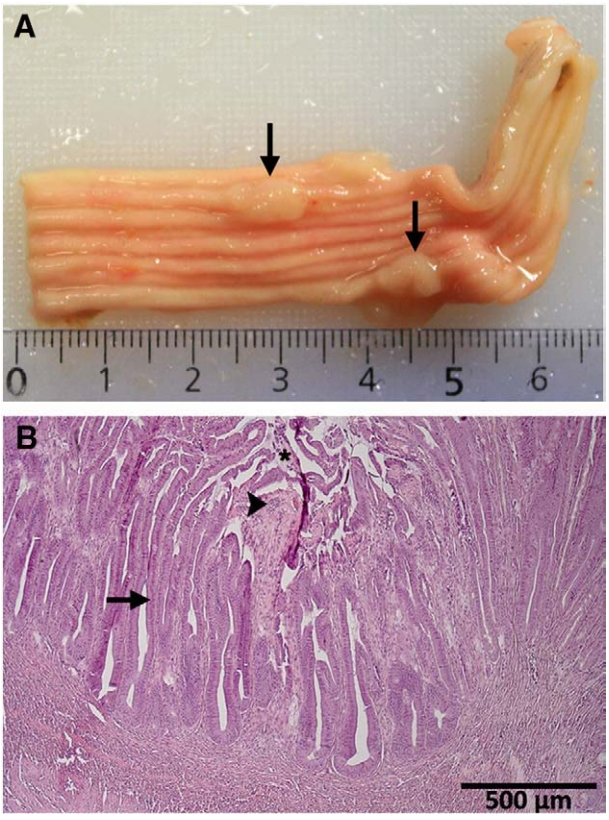

Fig. 2. Gross (A) and microscopic (B) appearances of stomach lesions. Histologically the lesions exhibited altered tissue architecture. The mucosa appeared as long folds (arrow) extending to the basal lamina propria. The epithelia consisted of a single layer of columnar cells. There is an area of widened lamina propria and leukocyte infiltration (arrowhead) accompanied by epithelial necrosis and sloughing (asterisk). The normal glandular tissue is completely absent. This condition appeared both for the FM and krill meal dietary treatments.

juvenile tilapia (*Oreochromis niloticus*×*O. aureus*) diets with 0, 20, 50 and 100 g kg^{-1} chitin resulted in a linear decrease in weight gain. Moreover, adding 10 g kg^{-1} chitin to diets depressed growth in juvenile carp compared to a control diet (Gopalakannan and Arul, 2006). Thus, it appears that even small amounts of dietary chitin can depress growth in fish.

The tendency of reduced lipid digestibility in fish fed the WKM diet in the present experiment indicates a negative effect of the krill shell fraction on lipid digestibility. Similarly, Olsen et al. (2006) found a tendency toward reduced lipid digestibility when all of the FM was replaced by WKM and suggested that the chitin produced a diarrhoea-like effect in the gastrointestinal tract. However, no such diarrhoea-like effect was observed in the present study, although a small difference in faecal dry matter content was noted among fish fed with FM and those fed diets with krill meal. Furthermore, Shiau and Yu (1999) reported decreased lipid digestibility (from 96.1 to 89.5%) in tilapia fed diets with increasing levels of dietary chitin (varying from 0 to 100 g kg^{-1}). Chitin is a natural biopolymer found in exoskeletons of crustaceans and has characteristics in common with insoluble plant fibre. However, the chemical properties of chitin differ mainly in its ability to form strong hydrogen bonds between the amino acetyl and hydroxyl groups (Minke and Blackwell, 1978). These chemical properties make chitin able to reduce activity of digestive enzymes as demonstrated for porcine pancreatic lipase (Kilinç et al., 2006). Furthermore, Zacour et al. (1992) reported that rats fed 5% chitin showed higher levels of triglycerides in faeces, suggesting that chitin may interfere with lipid absorption in the intestine. Our results did not reveal reduced lipase activity at high dietary chitin level, and thus, we cannot explain the reduction in lipid digestibility for fish fed the WKM diet by chitin's lipase inhibitory effects. The explanation for the lack of effect of WKM on lipase activity may be the fact that fish lipase are of the bile salt dependent type and as such different from the co-lipase dependent porcine pancreatic lipase (Gjellesvik et al., 1992). Rather, the decreased bile acid level in the pyloric intestine for fish fed the WKM diet might contribute to the reduced lipid digestibility because the pyloric region is the main area for lipid digestion and bile acids are essential for lipase activation as well as for efficient fatty acid absorption.

The reduction in digestibility of threonine, serine, glutamine, histidine, and lysine in fish fed the WKM compared to the FM fed fish may be related to the low trypsin activity seen in the fish fed the WKM diets compared to the control fed fish. A study by Rungruangsak-Torrissen (2007) supports our results by showing a significant reduction of *in vitro* amino acid digestibility with increased dietary WKM. One possible reason for this could be that chitin from a partially digested krill shell fraction is able to immobilize proteolytic enzymes as shown in previous experiments (Muzzarelli, 1980; Spagna et al., 1998).

In contrast to fish, that use hemoglobin for oxygen transport, crustacean blood utilizes respiratory proteins, with copper as the oxygen binding atom. This, results in high copper content in krill meals. Information on copper concentration in whole Antarctic krill, from several publications has been summarized by Locarnini and Presley (1995). The review shows variation from 12 to 82 mg copper kg^{-1} krill. The results of the present study are in line with previous studies showing 81 mg copper kg^{-1} of WKM. The high copper level was, however, not considered to be a factor in the observed growth reduction as Berntssen et al. (1999) found no effects on growth in juvenile Atlantic salmon fed up to 500 mg copper kg^{-1} diet.

In the present study, dietary krill inclusion did not affect muscle fluoride content, which is in line with previous experiments with Atlantic salmon, rainbow trout, Atlantic halibut (*Hippoglossus hippoglossus*) and Atlantic cod (*Gadus morhua*) (Julshamn et al., 2004; Moren et al., 2007). In contrast, there was an increase in bone fluoride level in the fish fed the WKM diet with a dietary fluoride level of 640 mg kg^{-1} in the present study. This increase in bone fluoride was not seen in rainbow trout, Atlantic halibut, Atlantic cod or Atlantic salmon reared in sea water and fed krill diets containing up to 1080 mg fluoride kg^{-1} (Moren et al., 2007). The reason for increased bone fluoride in fish fed the WKM diet in the present study remains unclear. However, the amount of fluoride accumulated in the salmon bones in the present study was small (18.4 mg kg^{-1} dry bone) compared to fluoride accumulation found in rainbow trout fed krill in fresh water (2400 mg kg^{-1} dry bone) (Yoshitomi et al., 2006). The possible negative effect of a high content of fluoride in diets for fish has not been well examined. Yoshitomi, et al. (2006) found a significant reduction in growth for fresh water raised rainbow trout fed diets with 30% replacement of FM with WKM containing 444 mg fluoride kg^{-1} diet compared to a FM control group. In contrast, up to a complete replacement of FM with a deshelled krill meal (222 mg fluoride kg^{-1} diet) did not depress growth in rainbow trout reared in fresh water (Yoshitomi et al., 2007). Yoshitomi et al. (2007) suggested increased fluoride deposition in bone could depress fish growth. However, Landy (1988) showed reduction in feed intake and growth in fresh water raised rainbow trout fed 4450 mg fluoride kg^{-1} diet during the first four weeks of feeding, but obtained similar feed intake and growth as the control group after six weeks of the total 30 weeks feeding period. Growth was similar in fish fed <30, 450 and 2250 mg fluoride kg^{-1} diet during the total feeding period.

The blood parameters for Atlantic salmon fed the three experimental diets are within normal levels (Sandnes et al., 1988; Stoskopf, 1993). The reason for the decrease in blood cholesterol seen in krill fed fish is uncertain. It has been shown that chitin can prevent uptake of TG and reduce liver cholesterol for rats (Zacour et al., 1992). However, this seems not to be a pure effect of chitin, because the

PDKM and WKM diet contained 8.45 and 20.9 g chitin kg^{-1} diet and gave the same plasma cholesterol level. In contrast, Olsen et al. (2006) did not see any differences in plasma cholesterol level of Atlantic salmon when WKM totally replaced FM. However, blood lipid parameters are highly dependent on sampling and feeding time, thus, it is difficult to draw any firm conclusions. The plasma enzymes AST, ALT, AP can serve as indicators of liver and kidney functions in fish (Sandnes et al., 1988). The present results for these enzymes indicate that replacing all the FM with a PDKM or WKM for Atlantic salmon did not affect liver or kidney functions.

Increased concentrations of creatinine and urea in plasma may reflect renal structural damage or kidney dysfunction (Bernet et al., 2001). The reason for the elevated creatinine plasma levels of the FM compared to the krill fed fish is uncertain, however, all values were close to normal levels (Sandnes et al., 1988). The levels of inorganic phosphorous in plasma of fish fed the krill diets reflect the reduced faecal excretion values of phosphorous in these diets. The decreased faecal excretion of phosphorous in the krill diets, which were supplemented with mono calcium phosphate, indicates that phosphorous from FM is less available than phosphorous from this phosphorus source. This is in line with previous results (Nordrum et al., 1997).

Intestinal brush border enzyme activities can be sensitive indicators of enterocyte alterations. Marked reductions in intestinal brush border enzyme activities are observed in the DI of salmon fed soy-based diets (Krogdahl et al., 2003; Kraugerud et al., 2007; Øverland et al., 2009) indicating enterocyte dysfunction or lack of maturation. Differences in brush border enzyme activities of alkaline phosphatase (ALP) and leucine aminopeptidase (LAP) were found between dietary treatments in the current work in the PI and DI. However, activities were higher in krill fed fish compared to FM fed fish. While the exact cause of the increased activities is not clear, it may be related to increased tissue mass as indicated by higher organosomatic indices for PI and DI in krill fed fish compared to FM fed fish.

The lesions observed in the stomach are consistent with intestinal metaplasia, based on hematoxylin and eosin staining. In humans, intestinal metaplasia of the stomach is most often associated with chronic gastritis, and is generally considered to be a pre-neoplastic change. No signs of chronic gastritis, however, were observed in the present experiment. Whether or not these lesions are pre-neoplastic in Atlantic salmon is unknown. To our knowledge there are no reports describing this type of lesion in salmon. Spontaneously occurring as well as chemically induced gastro-intestinal neoplasms have been reported in fish (Bunton, 1996; Spitsbergen and Kent, 2003; Dale et al., 2009) though very little is known regarding their behavior. The lesions observed in the present experiment did not appear to be correlated with inclusion of krill meal in the diets. If any correlation with diet exists, then it is more likely to be associated with a common component(s) or characteristic all diets.

The histological findings in the kidney are consistent with mild to moderate nephrosis. Nephrosis has been associated with nephrotoxic therapeutants (antibiotics, anti-inflammatory agents), heavy metals (mercury, uranium, arsenic, nickel and cadmium) and xenobiotic pollutants (Reimschuessel and Ferguson, 2006). The fish in the current study have no history of treatment with potential nephrotoxic therapeutants. No analysis for xenobiotic pollutants was performed. Based on the reported levels of heavy metals in krill (Gasparics et al., 2000; Moren et al., 2006), levels in the diets were expected to be less than current EU limits (Commission dir. 2002/32/EC, 1334/2003, 2005/87/EC), except for copper. Copper levels exceeded EU limits in both the krill diets. However, neither whole body nor liver copper tissue levels were higher in fish fed diets containing krill meal compared to fish fed the FM control, nor were any significant histological changes observed in the liver. Excessive dietary fluoride has been shown to produce morphological changes in the rat kidney (Ogilvie, 1953), and renal tissue apoptosis and necrosis in both rabbits (Shashi et al., 2002) and pigs (Zhan et al., 2006). The fresh water teleost *Labeo rohita* exhibited renal damage after 30, 60, 90, and 120 days of exposure to water-borne fluoride at a level of 6.8 mg l^{-1} (Bhatnagar et al., 2007). However, in all of these studies the authors note changes in both renal glomeruli and tubules, whereas in the current study only tubular changes were observed. Even if there was no, or a low level, of fluoride accumulation in bone in fish fed krill, the dietary fluoride may explain the changes in kidney histology of fish fed the two krill diets. Additional studies are necessary to clarify the cause(s) of the observed kidney changes.

To conclude, replacing fish meal with a whole krill meal to Atlantic salmon reared in sea water gave reduced growth rate and reduced digestibility of several amino acids compared to a fish meal control. Replacing fish meal with a partially deshelled krill meal, on the other hand, generally resulted in similar or better growth performance and similar nutrient digestibilities than fish fed the fish meal control. Both krill containing diets gave signs of mild to moderate nephrosis.

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