

# Antarctic krill meal as an alternative protein source in pet foods evaluated in mink (*Neovison vison*). II. Growth

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**Background:** Antarctic krill meal has potential for use in pet food as a source of protein and lipids. An experiment was conducted in growing male and female mink to evaluate the safety of Antarctic krill meal as an ingredient for pet food.

**Materials and methods:** In the first growth period, the levels of krill were 0%, 8%, 17%, and 33% of dry matter. The levels were slightly less in the second growth period (0%, 8%, 16%, and 32% of dry matter). The four diets used were labeled K0, K8, K17, and K33 for both growth periods. The experiments included assessments of growth, hematology, clinical chemistry, tissue histology, liver and kidney mineral concentrations, and bone fluoride accumulation.

**Results:** Mink receiving Antarctic krill meal grew at the same rate as controls, suggesting that protein and energy values of the krill meal were comparable to the control fishmeal. Relative organ weights of animals were the same for the K0, K8, and K17 groups, whereas K33 animals showed higher values for weights of the stomach and rectum. Hematological, clinical chemistry, and morphological analyses did not differ between animals fed K0 and K8 diets. Animals in the K17 and K33 groups showed some histological changes in the liver and kidney, and a few alterations in some clinical chemistry and hematology values related to nutrient intake or metabolism. Joint/bone deformities were observed in K33 mink.

**Conclusion:** Antarctic krill meal has a similar nutritional value as good-quality fishmeal and produces no adverse effects in growing mink at levels up to 8% of dry matter. The results suggest that Antarctic krill meal can be safely included in pet food for growing animals.

**Keywords:** Antarctic krill meal, protein source, growth, mink

## Introduction

Antarctic krill (*Euphausia superba*) is receiving increased attention as a marine protein and lipid source for animals and man. Antarctic krill is an organism rich in the omega-3 ( $\omega$ -3) fatty acids eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3),<sup>1</sup> which have several reported benefits for human health.<sup>2</sup> In addition, the protein quality is comparable to that of good fishmeal.<sup>3</sup> Krill was examined as a nutrient source for fish feeds in the late 1970s and early 1980s,<sup>4</sup> but its use has been limited by challenges in processing.<sup>5</sup> The potential for use of krill as a feed ingredient for fish and other monogastric animals has recently been reconsidered because of advances in processing technology and limitations in the supply of fishmeal. Antarctic krill meal is expected to be a healthy feed ingredient for pets, providing beneficial protein and fatty acids.

A major impediment to the high dietary inclusion of krill is its naturally high fluorine content, which originates from the exoskeleton. The current European Union

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(EU) limits on fluorine in animal feeds vary with species and life stage.<sup>6,7</sup> For nonruminant mammals, the general current limit is 150 mg/kg in complete feeds. For feed ingredients derived from marine crustaceans, the limit is 3,000 mg/kg. Fluorine in whole Antarctic krill can vary from 1,000 mg/kg<sup>8,9</sup> to 2,400 mg/kg (dry weight).<sup>10</sup> The United States Department of Agriculture has not established a maximum recommended daily intake of fluorine. For adult humans, the US Institute of Medicine has set a tolerable upper intake level of 10 mg/day.<sup>11</sup> Fluorine content of krill can be reduced by separating the exoskeleton from the muscle fraction,<sup>12</sup> although some fluorine may leak from the exoskeleton to the muscle during storage.<sup>13,14</sup> A partial deshelling of the krill meal will also reduce the level of chitin, a long chain, nondigestible polymer of N-acetyl-glucosamine that exhibits properties similar to dietary fiber.<sup>15</sup> Another factor that may limit the use of krill in animal feeds is the naturally high copper content, which ranges from 13 mg/kg–81 mg/kg in whole krill.<sup>16–18</sup> In contrast to vertebrates (which use hemoglobin for oxygen and carbon dioxide transport), crustaceans such as krill use hemocyanin that utilizes copper as the prosthetic group,<sup>19</sup> which results in high copper levels in krill.<sup>16</sup> The EU currently allows up to 25 mg/kg copper in complete feedstuffs.<sup>20</sup>

Few studies have been performed to investigate Antarctic krill meal inclusion in feeds for terrestrial mammals. Studies performed over 30 years ago indicate that use of up to 25% “raw krill” in feed is well tolerated by mink and produces beneficial effects on fur quality.<sup>21,22</sup> The need for thorough studies is therefore urgent to assess nutritional value and possible health implications. Mink is considered a good model for evaluation of nutritional and toxicological aspects of feed ingredients for other mammals, including dogs, cats, and foxes.<sup>23–26</sup> Efficient and accurate standard procedures have been developed for nutritional studies. The current work used mink as a model for dogs and cats with the aim to characterize nutritional and safety aspects of Antarctic krill meal. In the feeding experiment, the krill meal partially replaced fishmeal and the nutritional, physiological, and histopathological effects were assessed.

## Materials and methods

### Test substance and diet formulation

The live animal work of the project and evaluation of nutritional characteristics of the diets were conducted at the Norwegian University of Life Sciences’ (NMBU’s) Department of Animal and Aquacultural Sciences, Ås, Norway, where feeds were also produced. The farm is under the supervision of the Norwegian Research Authority and Norwegian protocols of ethical standards concerning experiments involving animals were

followed. Evaluation of the health-related effects of the diets was conducted at NMBU’s School of Veterinary Medicine, Department of Basic Sciences and Aquatic Medicine, Oslo, Norway. The Antarctic krill meal product used in the experiment was Antarctic krill meal produced on June 1, 2010 and supplied by Aker BioMarine AS (Oslo, Norway). The control fishmeal was NorSeaMink meal (Norsildmel AS, Fyllingsdalen, Norway).

Antarctic krill meal was tested at four levels, including a control diet containing no Antarctic krill meal. Diet compositions are given in Table 1. The diets were formulated to have metabolizable energy (ME) content of 5 MJ/kg on a wet weight basis, and the percentages of ME from protein, lipid, and carbohydrates of 40%, 45%, and 15%, respectively. In the diets with Antarctic krill, the krill meal replaced a portion of the protein coming from fishmeal in the control diet. Protein from Antarctic krill meal accounted for 0%, 15%, 30%, and 60% of the protein in the four experimental diets and 0%, 9%, 17%, and 35% of dry matter (DM) of the diets in the first feeding period from June 23–August 1. In the second feeding period, from August 2–October 3, the energy density of the feed was planned to account for approximately 6.5 MJ/kg on a wet weight basis by including higher amounts of lard and soybean oil. The contribution of the krill to DM to each of the four diets used in the second period was approximately 0%, 8%, 16%, and 32%. Throughout this document, the respective diets are referred to as K0, K8, K17, and K33, although there were slight differences in the first and second feeding periods. The inclusion of protein sources other than krill was similar for the four diets, and krill protein as part of total protein did not change. The diets were produced weekly. The daily rations for the first 3 days were stored at 4°C until feeding, while the rations for the last 4 days were stored frozen (–20°C) and thawed the day before use. The animals were fed once daily and drinking water was given by a semiautomatic system (nipples). Daily feed consumption was recorded separately for each sex on a group basis.

### Animals and housing

Sixty-four mink kits (black genotype, 52–53 days old, body weight [BW] range: 338 g–740 g) were allocated into four groups of eight males and eight females per group. The kits were offspring of females involved in a preceding reproduction trial, which was reported in a separate paper.<sup>27</sup> Two animals of the same sex were housed in each cage. The animals were kept in semioutdoor houses with natural daylight. Each pair of individuals was kept in a cage measuring 0.45 m in height × 0.27 m<sup>2</sup> floor area. The cages were arranged in two rows and pairs belonging

**Table I** Diet composition, chemical content, and energy of diets (g/kg)

Date <sup>a</sup>	K0		K8		K17		K33	
	6/23–8/1	8/2–10/3	6/23–8/1	8/2–10/3	6/23–8/1	8/2–10/3	6/23–8/1	8/2–10/3
<b>Ingredient (g/kg)</b>								
Krill meal	–	–	34.7	33.5	67.5	65.0	131	126
Fishmeal	153.2	148	117.5	113.5	80.5	77.5	12.8	12.0
Precooked carbohydrates	139	134	139	134	136	131.5	127	123
Cod scraps	139	134	139	134	135	130.5	126.5	122
Poultry by-products	139	134	139	134	135	130.5	126.5	122
Lard (pig fat)	13.9	35	13.9	35	13.5	35	12.6	35
Soybean oil	13.9	25	13.9	25	13.5	25	12.6	25
Vitamin/mineral mix <sup>b</sup>	2	2	2	2	2	2	2	2
Water	400	388	401	389	417	403	449	433
Sum	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
DM, g/kg	370	391	384	384	321	371	362	357
<b>Chemical content (kg DM)<sup>c</sup></b>								
Crude protein	441	414	438	411	424	420	434	406
Crude lipid	173	230	172	234	165	235	174	232
Carbohydrates <sup>d</sup>	272	266	286	259	305	248	295	269
Ash	114	90	104	96	106	97	97	92
ME <sup>e,e</sup> (MJ/kg DM)	16.1	17.5	16.2	17.7	16.0	17.8	16.3	17.6
<b>ME distribution<sup>c</sup></b>								
Protein	42	37	42	36	41	37	41	36
Lipid	38	47	38	48	32	47	38	47
Carbohydrates	20	16	20	16	27	16	21	17
<b>Mineral content (mg/kg DM)</b>								
Copper		11.5		15.6		20.8		31.3
Fluoride		73		291		419		626
Arsenic		2.7		2.7		2.5		1.8
Calcium		25,000		29,170		28,130		26,040

**Notes:** <sup>a</sup>Dates during which the diet formulation was fed to mink (month/day); <sup>b</sup>content per kg: vitamin A, 2,000,000 IU; vitamin D3, 200,000 IU; vitamin E, 50,000 mg; vitamin B1, 15,000 mg; vitamin B2, 3,000 mg; vitamin B6, 3,000 mg; vitamin B12, 19.5 mg; Ca–D- pantothenic acid, 3,332 mg; niacin, 5,005 mg; biotin, 30 mg; folic acid, 301 mg; ferrous sulfate, 610 mg; ferrous fumarate, 15,280 mg; Fe (chelated), 4,110 mg; copper sulfate, 1,250 mg; manganese oxide, 7,502; zinc oxide, 9,998 mg; Ca iodinate, 63.5 mg; Na selenite, 99.9 mg; cobalt carbonate, 60 mg; <sup>c</sup>chemical content and energy values are data from one sample per diet from the period 6/23–8/1 and two samples per diet from the period 8/2–10/3; <sup>d</sup>calculated value (Carbohydrate = Dry matter – [protein + fat + ash]); <sup>e</sup>ME content was determined using standard digestibility factors given by the Norwegian Fur Breeders' Association of 82%, 90%, and 68% for protein, fat, and carbohydrates, respectively, and the ME content of 18.8 kJ/g, 39.8 kJ/g, and 17.6 kJ/g of digestible protein, fat, and carbohydrates, respectively (Data from Hansen et al.<sup>28</sup>).

**Abbreviations:** DM, dry matter; ME, metabolizable energy; MJ, megajoules; IU, international units.

to the same group were placed in cages side by side, with an empty cage between different groups. The kits were continued in the present growth trial in the same groups as they were in the previous trial until weaning (control or low, mid, or high dose). The animals were weighed on day 0 (June 23), day 30 (July 22), day 57 (August 18), day 83 (September 14), and on the last day of the 102-day experiment (October 3).

## Sampling and sample treatment

At the end of the 15-week growth trial (October 5–7), animals were euthanized, necropsied, and sampled for laboratory analyses. Animals were rendered unconscious by electric shock using a Euthanatos 2 (Lima A/S, Sandnes, Norway) and immediately euthanized by cervical dislocation. Blood samples were taken after euthanasia by cardiac puncture. Pelts were removed and animals dissected. Organs were examined grossly and weighed. Tissue samples for histology were taken from the

stomach, jejunum, colon, rectum, liver, kidney, spleen, adrenal glands, and heart, fixed in neutral buffered formalin (4% formaldehyde; pH 7.4), and processed using routine methods (NMBU School of Veterinary Medicine). Tissue sections were stained with hematoxylin and eosin. Liver tissue was stained with periodic acid–Schiff (PAS) stain to identify glycogen. All collected organs from the control and high dosage animal groups; and liver, spleen, and kidney samples from all other groups were evaluated histologically. The blinded samples were evaluated under a light microscope in random order. For bone fluoride and kidney and liver trace metal analyses, samples from two animals in the same group were pooled (if possible). Blood and plasma samples were analyzed at the NMBU School of Veterinary Medicine for complete blood cell count and plasma biochemistry profiles using certified assays. The complete blood count included red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit

(HCT), mean cell volume (MCV), mean cell HGB (MCHC), red blood cell distribution width (RDW), platelet count, and total and differential white blood cell count (WBC).

## Chemical analyses

Diets were analyzed for dry matter (heating at 105°C for 16–18 hours), ash (combustion at 550°C to constant weight), crude protein as nitrogen  $\times 6.25$  (by the semimicro-Kjeldahl method; Kjeltec Auto System, Tecator AB, Hognas, Sweden), and lipid (diethyl ether extraction in a Fostec analyzer [Tecator™] after HCl hydrolysis). Carbohydrates were calculated by difference:

$$[\text{Carbohydrate} = \text{Dry matter} - (\text{protein} + \text{fat} + \text{ash})]$$

Diets were analyzed for fluorine, copper, arsenic, and calcium. Liver and kidney tissue homogenates were analyzed for copper, cadmium, arsenic, and zinc. Dried, defatted bone (left femur) was analyzed for fluoride. The analyses were performed at Eurofins Food and Agro Testing, Kambo, Norway, using standard procedures. All minerals except fluorine were analyzed by simultaneous inductively coupled plasma spectroscopy (ICP) on a Vista Pro CCD (NMKL 161). An ion-selective electrode detector was used for the fluorine analysis.

## Statistics

Means and standard deviations were calculated for all quantitative data. Data within groups were evaluated for homogeneity of variance and normality by Bartlett's test. Where Bartlett's test indicated homogeneous variances, treated and control groups were compared using a one-way analysis of variance (ANOVA), with the exception of BW data, which were compared using a two-way repeated measures ANOVA. Data for each sex were analyzed separately. When the results of ANOVA were statistically significant, a Tukey's test for multiple comparisons was performed to compare results of all groups. When variances were significantly different by Bartlett's test, groups were compared using a nonparametric method (Kruskal–Wallis nonparametric ANOVA). When the results of the nonparametric ANOVA were statistically significant, all groups were compared using Dunn's test (Prism 5.02, GraphPad Software, Inc., La Jolla, CA, USA). The critical value for significance of all comparisons was  $P < 0.05$ .

## Results

### Diet characteristics

Chemical composition and ME content of the diets are shown in Table 1. Nutrient contents and ME distribution between

protein, fat, and carbohydrates were similar in the four experimental diets within the two experimental periods. Diets containing Antarctic krill meal contained higher amounts of copper and fluorine and lower amounts of arsenic than did the control diet. Fluorine content was 8.5 times higher in the K33 diet than in the K0 diet. Calcium levels were similar in all diets.

## Growth and organ weights

All animals appeared healthy and showed good appetite throughout the experiment, except for one male that was fed the K33 diet – it died on July 14 because of an infection with *Streptococcus canis* in a wound on one hind leg. In addition, one male in group K0 died on September 5 of an unknown cause. The K33 kit that died early was replaced by a sibling, whereas the K0 kit was not replaced. For each sex, feed consumption (measured as g/day for the whole group) was similar among the groups. As expected, feed intake was higher in males (298–310 g/day) than females (210–225 g/day), regardless of krill meal inclusion. There was no effect of krill meal on BWs of male or female mink compared to controls (Figure 1). The only statistically significant differences between BWs were for males in the K8 group versus males in the K17 or K33 groups on day 102 ( $P < 0.05$ ) (Figure 1).

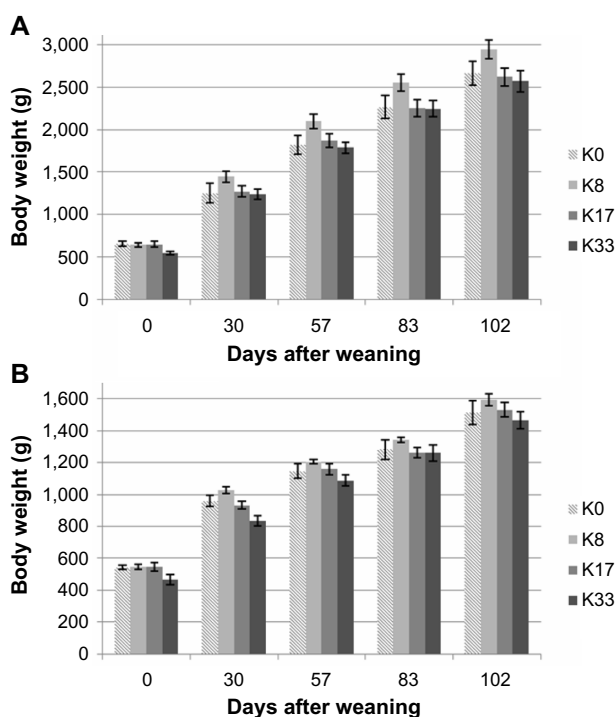


Figure 1 Body weights (g).

Notes: (A) Male and (B) female mink throughout the experiment as a function of the inclusion of Antarctic krill meal; data are presented as the mean  $\pm$  standard error.

Relative weights of the stomach (both sexes) and rectum (females only) increased with increasing dietary inclusion of krill (Table 2). The elevations were, however, small and statistically significant only for the K33 animals. The relative heart weight of the K33 females was higher than that of the K8 females ( $P<0.05$ ), but this was not significantly different from controls (Table 2). The relative weight of the liver decreased slightly with increasing krill level; however, the decrease was not statistically significant. No relationship was observed between dietary krill level and the relative weights of the kidneys, spleen, brain, adrenals, or gonads (Table 2).

## Organ structure

There was no clear relationship between dietary krill level and gross pathology, with the exception of joint/bone deformities in the K33 group (Table 3). The histological examination of the liver indicated reduced liver glycogen with increasing dietary Antarctic krill meal (Table 4) and staining (PAS) confirmed this finding (Figures 2 and 3). Reduced liver glycogen was found in males and females in the K17 and K33 groups. Glycogen staining had a distinct centrilobular distribution, but it also showed higher glycogen deposition around vessels and at the periphery of the liver lobe. Small focal to multifocal inflammatory lesions were also noted in the liver. The frequency of the observation (Table 5) in males and females of the K17 and K33 groups suggests a possible relationship with the dietary krill meal level. Lipoid-like vacuolization was noted in two to three animals in the control, low, and mid-dosage groups, and seven animals in the high dosage group. Crystals (basophilic crystalline material) were observed within tubules in the kidneys of controls and animals ingesting krill meal (Figure 4); however, they appeared

more frequently in males and females in the K17 and K33 groups and males in the K8 group (Table 6). There were no pathological changes associated with the crystallization, including inflammation. One male in the K8 group and three males in the K17 group exhibited “moderate” active lymphoid follicles in the spleen and seven animals from the K33 group (six males and one female) exhibited “moderate to marked” active lymphoid follicles in the spleen (Figure 5). Splenic nodules were observed during necropsy in two of the K33 males that exhibited active lymphoid follicles. There were no test material-related findings in the histopathology of the gastrointestinal tract, adrenal gland, or heart.

## Bone structure and fluoride concentration

Bone fluoride increased with increasing dietary Antarctic krill meal inclusion (Figure 6). The effect was consistent between males and females and fluoride accumulated to high levels in the K33 group. During preparation of the femur samples for fluoride analysis, several deformities were noted in the animals in the K33 group: 7/8 males and 1/8 females had deformities of the femoral neck or head (Figure 7). No bone deformities were noted in any of the other groups.

## Liver and kidney metal concentrations

Liver tissue concentrations of copper, cadmium, arsenic, and zinc are shown in Table 7. There was a positive relationship between krill level and copper and zinc concentrations in the livers of K33 males. Liver cadmium levels increased with increasing dietary krill, with values for K33 males and females significantly different from their respective controls. Arsenic decreased with increasing inclusion of krill meal, but

**Table 2** Organ relative weights (g/100 g body weight) of mink fed the experimental diets

	n	Stomach	Intestine	Rectum	Liver	Kidney	Spleen	Heart	Brain	Adrenal	Gonad
<b>Males</b>											
K0	7	0.44±0.09 <sup>a</sup>	2.38±0.83	0.18±0.04	2.82±0.64	0.55±0.17	0.24±0.03	0.57±0.13	0.48±0.08	0.005±0.001	0.044±0.005
K8	8	0.43±0.07 <sup>a</sup>	1.90±0.30	0.15±0.02	2.31±0.27	0.48±0.11	0.20±0.06	0.53±0.08	0.44±0.05	0.005±0.001	0.038±0.005
K17	8	0.46±0.06 <sup>a</sup>	2.03±0.21	0.18±0.02	2.37±0.26	0.49±0.08	0.18±0.03	0.56±0.07	0.47±0.06	0.005±0.001	0.042±0.010
K33	8	0.55±0.05 <sup>b</sup>	2.18±0.18	0.19±0.02	2.23±0.30	0.46±0.05	0.26±0.09	0.57±0.06	0.51±0.06	0.006±0.001	0.046±0.008
P-value		0.004	0.070	0.117	0.070	0.477	0.059	0.760	0.184	0.570	0.159
<b>Females</b>											
K0	8	0.47±0.04 <sup>a</sup>	2.13±0.24	0.17±0.01 <sup>a</sup>	2.51±0.19	0.53±0.07	0.23±0.05	0.59±0.09 <sup>a</sup>	0.68±0.08	0.006±0.001	0.006±0.002
K8	8	0.51±0.03 <sup>a</sup>	2.20±0.24	0.18±0.03 <sup>a,b</sup>	2.52±0.36	0.51±0.05	0.27±0.13	0.56±0.06 <sup>a,b</sup>	0.69±0.08	0.007±0.002	0.006±0.002
K17	8	0.51±0.05 <sup>a</sup>	2.22±0.13	0.19±0.02 <sup>a,b</sup>	2.36±0.26	0.49±0.05	0.22±0.03	0.60±0.06 <sup>a</sup>	0.69±0.07	0.006±0.001	0.006±0.002
K33	8	0.61±0.08 <sup>b</sup>	2.36±0.21	0.20±0.02 <sup>b</sup>	2.32±0.19	0.49±0.08	0.24±0.05	0.68±0.10 <sup>a,c</sup>	0.72±0.08	0.006±0.001	0.007±0.002
P-value		<0.0001	0.210	0.023	0.311	0.613	0.887	0.020	0.743	0.318	0.628

**Notes:** Values with different superscripts (letters) are significantly different from each other ( $P<0.05$ ). n=6 for K0 male gonad weight (no gonad weight was taken for one animal that had only one gonad).

**Abbreviation:** n, number of animals.

**Table 3** Gross abnormalities observed during necropsy or during sample preparation

Diet	Intestinal redness	Spleen pigmentation	Spleen – white nodules	Joint/bone deformities	Small left ventricle	Lung discoloration – gray	Lung discoloration – red
K0	3	1			1	2	
K8	1				3	1	
K17	1		4		2		
K33	1		2	8	1		2
	Kidney fibrosis	Kidney/bladder stones	Unilateral renal atrophy	Pale kidney	Red renal pelvis	Kidney – mottled appearance	Kidney cyst
K0	1			1			
K8	1	1			1		
K17						1	
K33			1				1

**Note:** The numbers represent the number of animals with the finding.

was significantly different from control only for K33 females. There was no effect of administration of krill meal at the 8% or 17% level on liver concentrations of copper, cadmium, or arsenic. While there was a significant increase in zinc content of the liver of K8 males ( $P < 0.05$ ), the increase was slight. There was no such increase in males in the K17 group. Kidney tissue concentrations of copper, cadmium, arsenic, and zinc are shown in Table 8. Copper concentrations were lower in the kidney compared to liver concentrations (as expected) and were not affected by ingestion of krill meal. Although zinc levels tended to increase in kidneys of K33 females, the effect was not statistically significant. Kidney cadmium levels increased with increasing dietary krill meal inclusion, and were significantly higher in K33 females than K8 females. Arsenic decreased linearly with increasing krill inclusion and was significantly lower in K33 males and females than their respective controls. There was no effect of administration of krill meal at the 8% or 17% level on kidney concentrations of copper, cadmium, arsenic, or zinc.

**Table 4** Glycogen content of liver sections as determined using PAS staining

	n	Absent	Low	Moderate	High
<b>Males</b>					
K0	7	0	0	1	6
K8	8	0	0	3	5
K17	8	1	1	3	4
K33	8	1	3	2	2
<b>Females</b>					
K0	8	0	0	1	7
K8	8	0	1	1	6
K17	8	0	1	3	4
K33	8	2	1	2	3

**Note:** The numbers represent the number of animals with the finding.

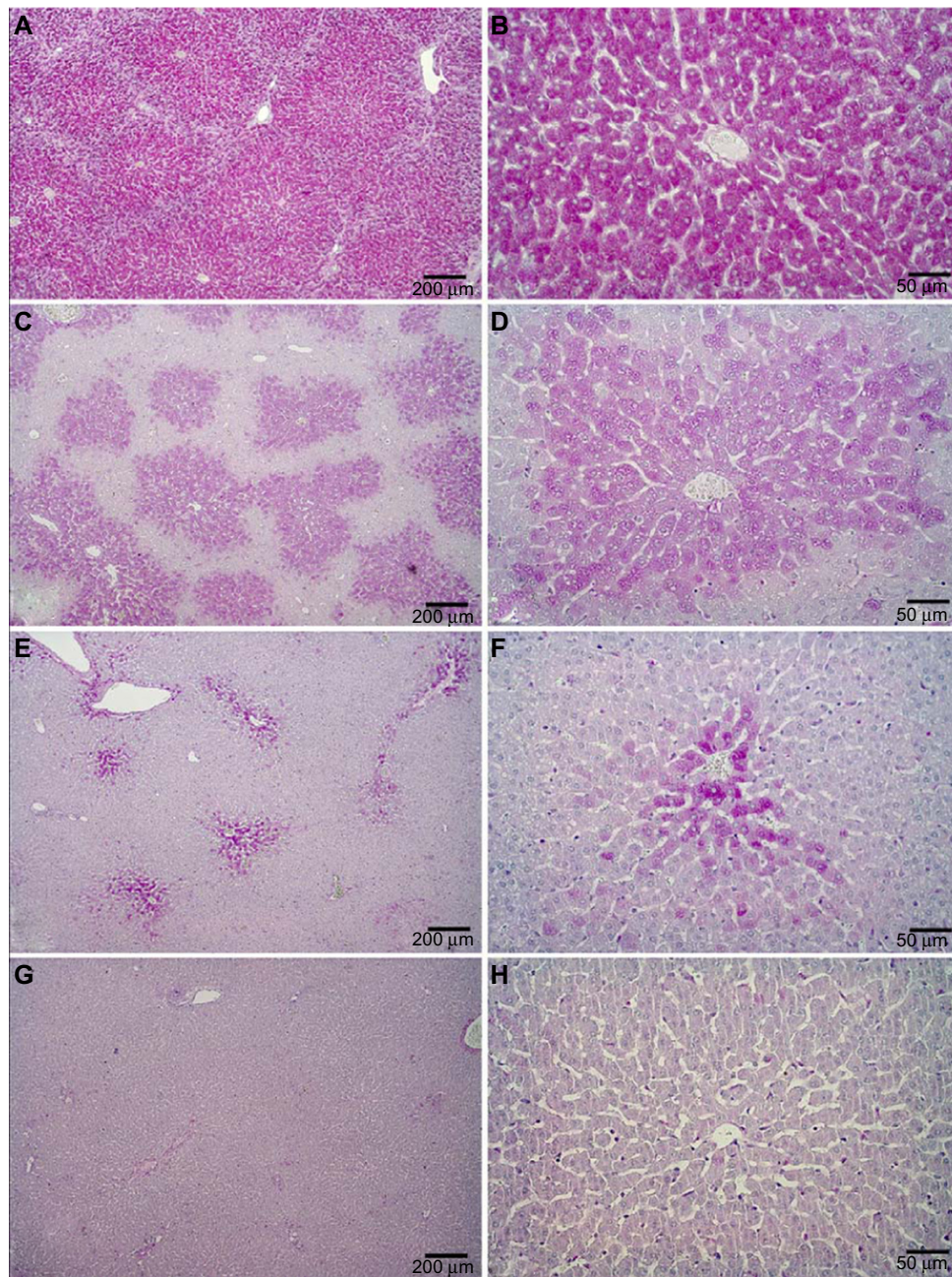
**Abbreviations:** PAS, periodic acid–Schiff; n, number of animals.

## Blood characteristics

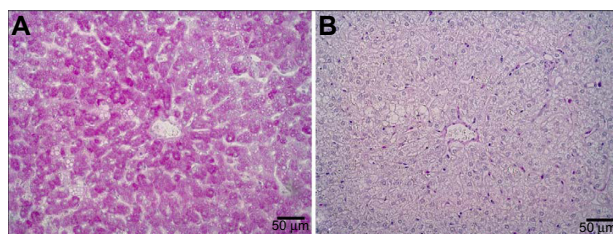
There was no effect of Antarctic krill on RBC, HGB, HCT, MCV, or platelet count (Table 9). MCHC decreased and RDW increased in group K33 animals; however, only the MCHC of K33 females and the RDW of K33 males were significantly different from controls ( $P < 0.05$ ). There were no significant differences in the total or differential WBC between treated animals and controls (Table 10). The blood biochemistry profiles yielded similar results in males and females. There was no effect of Antarctic krill on plasma electrolytes (inorganic P, Ca, Na, K, or Cl) (Table 11). Notable clinical chemistry findings included higher alkaline phosphatase (AP) and amylase in K33 males, decreased urea and creatinine in K33 males and females, and decreased bile acids in K33 females ( $P < 0.05$ ) (Table 12). There was no significant effect of Antarctic krill on cholesterol, triglycerides, or glucose in males or females (Table 13). Free fatty acids increased in K17 and K33 males, but not in any female groups.

## Discussion Growth

In the current study, the safety of Antarctic krill meal in feeds for juvenile mink at various inclusion levels was investigated. The animals grew well during the experimental period and the BWs at the end of the trial were similar to those reported for black mink of similar age,<sup>29</sup> indicating favorable environmental conditions. There are only a few published studies on Atlantic krill as feed for animals and none with mink, dogs, or cats. Results of a study conducted with Atlantic salmon are consistent with the present study showing that, regarding the production indicators such as growth and feed utilization, Antarctic krill can replace good fishmeal at high proportions.<sup>30</sup>



**Figure 2** Illustration of the evaluation criteria used for semiquantification of periodic acid-Schiff staining indicating glycogen accumulation in the liver.  
**Notes:** (A and B) High; (C and D) moderate; (E and F) low; and (G and H) absent.



**Figure 3** Periodic acid-Schiff staining of diastase pretreated sections confirming that the periodic acid-Schiff staining in the liver was due to glycogen.  
**Notes:** (A) From a female fed the K0 diet; (B) from a female fed the K17 diet.

## Organ weights

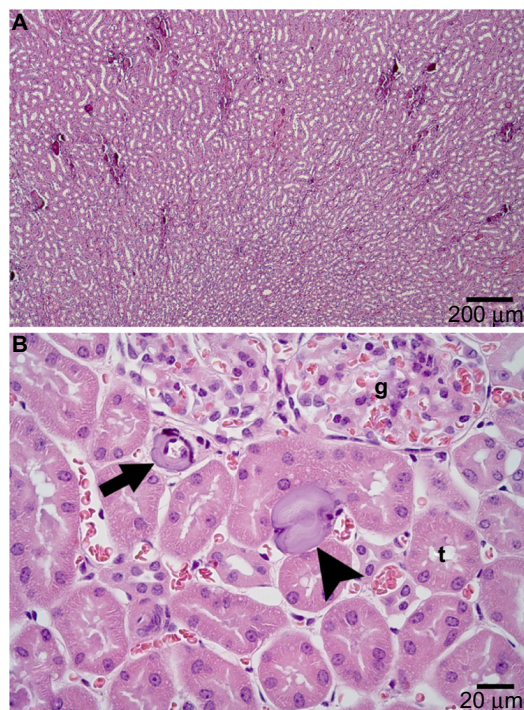
Relative weights of the stomach of males and females and rectum of females were significantly higher in the K33 group than in controls. It is possible that increased gastrointestinal organ weights are related to the presence of chitin, an indigestible polyglucosamine that exhibits properties similar to those of dietary fiber. Fiber has been shown to increase gastrointestinal tract relative weights in a variety of animals, including rats and swine.<sup>31-33</sup> Fiber may stimulate gut growth

**Table 5** Frequency of focal inflammatory lesions in the liver

	n	None	Focal (1)	Multifocal (2-3)	Multifocal ( $\geq 4$ )
<b>Males</b>					
K0	7	5	1	1	0
K8	8	4	3	1	0
K17	8	2	1	2	3
K33	8	2	1	1	4
<b>Females</b>					
K0	8	8	0	0	0
K8	8	6	1	1	0
K17	8	3	2	3	0
K33	8	4	1	0	3

**Note:** The numbers represent the number of animals with the finding.

**Abbreviation:** n, number of animals.

**Figure 4** Crystalline material observed within the kidney of a male fed the K17 diet.

**Notes:** (A) Crystalline material was most often located in the t near the corticomedullary junction. (B) Crystalline material was occasionally observed outside of the t (arrowhead) or the surrounding blood vessels (arrow).

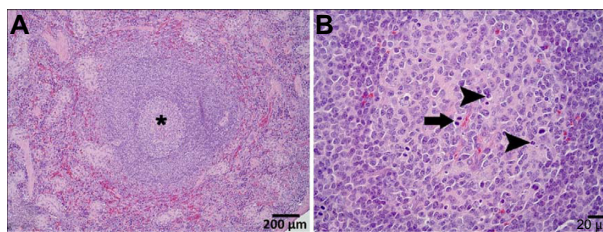
**Abbreviations:** g, glomerulus; t, tubule.

**Table 6** Frequency of crystalline material within kidney tissue

	n	None	Rare	Occasional	Numerous
<b>Males</b>					
K0	7	1	3	3	0
K8	8	1	1	1	5
K17	8	0	0	0	8
K33	8	0	0	3	5
<b>Females</b>					
K0	8	1	5	2	0
K8	8	0	6	0	2
K17	8	0	0	2	6
K33	8	0	1	1	6

**Note:** The numbers represent the number of animals with the finding.

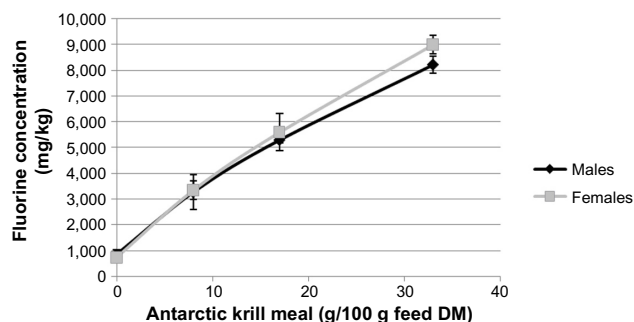
**Abbreviation:** n, number of animals.

**Figure 5** A reactive lymphoid follicle in the spleen of a male fed the K33 diet.

**Notes:** (A) The germinal center (asterisk) is apparent. (B) Mitotic figures (arrow) and apoptotic bodies (arrowheads) within the germinal center.

by supplying substrate to microbiota for the production of short chain fatty acids.<sup>34</sup> The reason for the slight, but statistically significant, increase in the relative heart weight of females in the K33 group is unknown, but that result was not considered toxicologically relevant because of lack of other findings in heart.

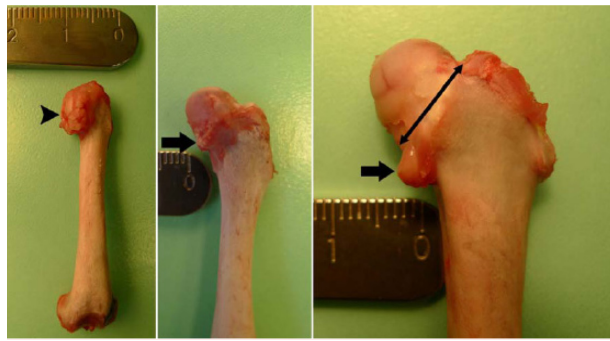
The amount of glycogen in the liver decreased in K17 and K33 animals, as indicated by the histological examination. The relevance of decreased liver glycogen is unclear. Plasma glucose concentrations in all groups were normal, indicating that low liver glycogen did not significantly affect the ability to maintain blood glucose levels. The findings were similar to those of the previous study in adult female mink provided 35% krill meal in the diet during pregnancy and lactation.<sup>27</sup> Although liver weights tended to decrease (particularly in the K33 group), there were no significant differences between liver weights of any groups of animals provided Antarctic krill compared to controls. The mechanism for the effect of high dosages of Antarctic krill on liver glycogen in mink is unknown, but it may be due to lower energy assimilation, increased glycogenolysis, or some combination thereof. The fact that serum amylase was increased in the K33 group suggests that glycogenolysis was stimulated by inclusion of krill meal at the 33% level. Fluorine can affect various enzyme systems,<sup>35</sup> including those involved in glucose

**Figure 6** Bone (left femur) fluoride concentration showing a clear increase with increasing dietary fluoride concentration.

**Note:** Data are presented as the mean  $\pm$  standard deviation.

**Abbreviation:** DM, dry matter.





**Figure 7** Bone deformities of the left femur.

**Notes:** Femoral head malformation (arrowhead), calcification of the joint capsule (arrows), or thickening of the femoral neck (double-ended arrow) were found in most males and one female in the K33 group.

phosphorylation<sup>36</sup> and glucose tolerance.<sup>37</sup> Fluorine may also interfere with glycogen deposition by impeding glucose entry into cells.<sup>36</sup>

## Organ metals

The dietary copper level for the K33 diet (31.1 mg/kg) exceeded the EU limit of 25 mg copper/kg in complete feed-stuffs for animals. The level of copper would have been above this limit even if the mineral mix had been free of copper because it would have only reduced the dietary copper level by 1.8 mg/kg. Males ingesting the K33 diet had significantly higher amounts of copper in liver than study controls and healthy ranch mink of similar age fed on a diet containing approximately 20 mg copper/kg diet on a dry weight basis (14.2±8.48 ppm).<sup>38</sup> The level of zinc in the liver of K33 males

**Table 7** Cu, Cd, As, and Zn concentrations in liver tissue

	n*	Cu (mg/kg)	Cd (mg/kg)	As (mg/kg)	Zn (mg/kg)
<b>Males</b>					
K0	4	7.2±1.6 <sup>a</sup>	0.029±0.005 <sup>a</sup>	0.79±0.18	31.2±3.4 <sup>a</sup>
K8	4	15.0±4.2 <sup>a</sup>	0.030±0.003 <sup>a</sup>	0.66±0.17	37.5±3.1 <sup>b,c</sup>
K17	4	14.5±2.6 <sup>a,b</sup>	0.035±0.004 <sup>a</sup>	0.61±0.15	34.2±2.1 <sup>a-c</sup>
K33	4	37.0±11.6 <sup>b</sup>	0.051±0.002 <sup>b</sup>	0.46±0.22	46.2±1.9 <sup>b</sup>
P-value		0.0002	<0.0001	0.135	<0.0001
<b>Females</b>					
K0	4	15.8±5.0	0.033±0.003 <sup>a</sup>	0.69±0.11 <sup>a</sup>	32.0±2.9
K8	4	28.8±11.5	0.034±0.005 <sup>a,b</sup>	0.64±0.21 <sup>a</sup>	37.0±2.2
K17	4	22.5±5.7	0.040±0.004 <sup>a,b</sup>	0.42±0.02 <sup>a,b</sup>	35.8±3.5
K33	4	20.8±5.1	0.052±0.015 <sup>b</sup>	0.28±0.12 <sup>b</sup>	37.0±2.2
P-value		0.150	0.008	0.0001	0.075

**Notes:** Values are presented as the mean ± standard deviation; Cu data for males and Cd and As data for females were analyzed using a Kruskal–Wallis test. All other data were analyzed by ANOVA; values with different superscripts are significantly different from each other ( $P<0.05$ ); \*samples were pooled (two individuals per sample) prior to analysis, with the exception of one of the samples from K0 males due to  $n=7$  in this group.

**Abbreviations:** n, number of samples; ANOVA, analysis of variance; Cu, copper; Cd, cadmium; As, arsenic; Zn, zinc.

**Table 8** Cu, Cd, As, and Zn concentrations in kidney tissue

	n*	Cu (mg/kg)	Cd (mg/kg)	As (mg/kg)	Zn (mg/kg)
<b>Males</b>					
K0	4	3.48±0.30	0.044±0.005	0.78±0.09 <sup>a</sup>	19.2±0.9
K8	4	3.50±0.18	0.042±0.007	0.67±0.15 <sup>a,b</sup>	20.2±0.5
K17	4	3.28±0.17	0.053±0.006	0.58±0.13 <sup>a,b</sup>	19.8±2.2
K33	4	3.90±1.40	0.059±0.014	0.40±0.16 <sup>b</sup>	20.5±1.7
P-value		0.680	0.066	0.012	0.663
<b>Females</b>					
K0	4	3.78±0.22	0.050±0.006 <sup>a,b</sup>	0.81±0.08 <sup>a</sup>	20.2±1.5
K8	4	3.80±0.18	0.049±0.003 <sup>a</sup>	0.78±0.22 <sup>a</sup>	21.2±2.6
K17	4	3.78±0.05	0.062±0.007 <sup>a,b</sup>	0.55±0.11 <sup>a,b</sup>	22.0±1.4
K33	4	3.88±0.33	0.070±0.017 <sup>b</sup>	0.33±0.10 <sup>b</sup>	23.8±3.1
P-value		0.973	0.030	0.001	0.223

**Notes:** Values are presented as the mean ± standard deviation; Cu and As data for females were analyzed using a nonparametric Kruskal–Wallis test due to significantly different standard deviations; all other data were analyzed by ANOVA; values with different superscripts are significantly different from each other ( $P<0.05$ ); \*samples were pooled (two individuals per sample) prior to analysis, with the exception of one of the samples from K0 male due to  $n=7$  in this group.

**Abbreviation:** n, number of samples; ANOVA, analysis of variance; Cu, copper; Cd, cadmium; As, arsenic; Zn, zinc.

also was higher than that of control males. The reason for this is unclear because the zinc content of Antarctic krill<sup>17</sup> is typically lower than that of fishmeal (90 ppm).<sup>39</sup> The slight increase in zinc content of the liver was not considered to be toxicologically relevant. Animals in the K33 group also exhibited slight, but statistically significant, increases in kidney and liver cadmium compared to control. The reason for this increase is unclear, because the cadmium content of Antarctic krill is typically <1 ppm.<sup>17</sup> Animals receiving Antarctic krill meal showed dose-dependent decreases in liver and kidney arsenic (a beneficial effect), suggesting that the arsenic content of the krill meal was lower than that of the fishmeal used in the control diet.

## Bone structure and fluoride concentration

Fluorine levels in all diets with krill exceeded the current EU limit for fluorine in animal feeds. The effects of dietary fluorine (as sodium fluoride [NaF]) on mink have been previously investigated to a limited extent.<sup>40,41</sup> NaF is rapidly absorbed in the acidic environment of the stomach as hydrogen fluoride, with absorption reported as high as 99% in the fasted state.<sup>42</sup> The bioavailability of fluorine in krill, although lower than NaF, remains high, although reports vary. In rats, the absorption of fluorine from krill can reach 80%.<sup>43</sup> However, substances in feeds can affect fluorine absorption. The presence of food reduces the efficiency of fluorine absorption to 50%–80%.<sup>42</sup> Calcium can bind fluorine, a well-known phenomenon in caries prevention,<sup>44</sup> forming an

**Table 9** Red blood cell indices of mink fed the experimental diets

	n	RBC ( $\times 10^{12}/L$ )	HGB (g/L)	HCT (L/L)	MCV (fL)	MCHC (g/L)	RDW (%)	PLT ( $\times 10^9/L$ )
<b>Males</b>								
K0	7	9.88 $\pm$ 0.69	183 $\pm$ 14	0.60 $\pm$ 0.04	60.1 $\pm$ 3.7	308 $\pm$ 12	13.6 $\pm$ 0.8 <sup>a</sup>	686 $\pm$ 178
K8	8	9.48 $\pm$ 0.31	178 $\pm$ 9	0.58 $\pm$ 0.03	61.2 $\pm$ 2.4	306 $\pm$ 8	13.4 $\pm$ 0.7 <sup>a</sup>	624 $\pm$ 112
K17	8	10.04 $\pm$ 0.47	184 $\pm$ 5	0.60 $\pm$ 0.03	60.2 $\pm$ 3.1	304 $\pm$ 11	13.3 $\pm$ 0.6 <sup>a</sup>	607 $\pm$ 118
K33	8	9.68 $\pm$ 1.08	169 $\pm$ 20	0.57 $\pm$ 0.07	58.7 $\pm$ 2.2	298 $\pm$ 8	14.9 $\pm$ 0.8 <sup>b</sup>	769 $\pm$ 204
P-value		0.421	0.317	0.434	0.412	0.166	0.0004	0.181
<b>Females</b>								
K0	6	9.68 $\pm$ 0.61	187 $\pm$ 8	0.59 $\pm$ 0.03	61.0 $\pm$ 2.3	318 $\pm$ 16 <sup>a</sup>	13.3 $\pm$ 0.4	533 $\pm$ 153
K8	7	10.18 $\pm$ 0.75	186 $\pm$ 9	0.60 $\pm$ 0.04	59.4 $\pm$ 1.7	308 $\pm$ 14 <sup>a,b</sup>	13.4 $\pm$ 0.6	659 $\pm$ 175
K17	7	9.62 $\pm$ 0.79	180 $\pm$ 9	0.59 $\pm$ 0.03	61.3 $\pm$ 2.4	306 $\pm$ 10 <sup>a,b</sup>	13.4 $\pm$ 0.2	653 $\pm$ 97
K33	8	9.54 $\pm$ 0.53	164 $\pm$ 24	0.56 $\pm$ 0.06	58.6 $\pm$ 4.6	292 $\pm$ 14 <sup>b</sup>	15.5 $\pm$ 2.1	765 $\pm$ 314
P-value		0.298	0.044	0.300	0.295	0.011	0.037	0.440

**Notes:** Values are presented as the mean  $\pm$  standard deviation. Values for some female animals (two K0, one K8, and one K17) were not obtained because the blood sample was not taken or was not sufficient for the analysis. RBC and HGB data for males and HGB, RDW, and platelet data for females were analyzed using a Kruskal–Wallis test; all other data were analyzed by ANOVA; values with different superscripts are significantly different from each other ( $P < 0.05$ ).

**Abbreviations:** n, number of animals; RBC, red blood cell count; HGB, hemoglobin concentration; HCT, hematocrit; MCV, mean cell volume; HCHC, mean cell hemoglobin concentration; RDW, red blood cell distribution width; PLT, platelet count; ANOVA, analysis of variance.

**Table 10** WBC of mink fed the experimental diets

	n	WBC ( $\times 10^9/L$ )	Neutrophils ( $\times 10^9/L$ )	Lymphocytes ( $\times 10^9/L$ )	Monocytes ( $\times 10^9/L$ )	Eosinophils ( $\times 10^9/L$ )	Basophils ( $\times 10^9/L$ )
<b>Males</b>							
K0	7	9.20 $\pm$ 5.07	4.06 $\pm$ 2.75 <sup>a,b</sup>	4.23 $\pm$ 2.04	0.54 $\pm$ 0.34	0.31 $\pm$ 0.07	0.03 $\pm$ 0.05
K8	8	6.88 $\pm$ 2.03	2.89 $\pm$ 0.91 <sup>a</sup>	3.40 $\pm$ 1.37	0.26 $\pm$ 0.11	0.26 $\pm$ 0.13	0.06 $\pm$ 0.07
K17	8	9.58 $\pm$ 6.30	4.26 $\pm$ 3.48 <sup>a,b</sup>	4.19 $\pm$ 2.26	0.52 $\pm$ 0.29	0.49 $\pm$ 0.58	0.25 $\pm$ 0.55
K33	8	11.95 $\pm$ 5.58	5.12 $\pm$ 1.37 <sup>b</sup>	5.52 $\pm$ 3.58	0.58 $\pm$ 0.29	0.61 $\pm$ 0.57	0.60 $\pm$ 0.67
P-value		0.272	0.026	0.398	0.102	0.820	0.139
<b>Females</b>							
K0	6	5.87 $\pm$ 2.16	1.98 $\pm$ 0.58	3.32 $\pm$ 1.63	0.25 $\pm$ 0.16	0.28 $\pm$ 0.15	0.02 $\pm$ 0.04
K8	7	6.54 $\pm$ 3.05	3.03 $\pm$ 2.18	2.84 $\pm$ 1.05	0.30 $\pm$ 0.21	0.30 $\pm$ 0.21	0.03 $\pm$ 0.05
K17	7	5.79 $\pm$ 3.56	2.26 $\pm$ 1.09	3.01 $\pm$ 2.43	0.33 $\pm$ 0.20	0.19 $\pm$ 0.13	0.01 $\pm$ 0.04
K33	8	4.14 $\pm$ 2.22	2.01 $\pm$ 1.05	1.68 $\pm$ 0.97	0.26 $\pm$ 0.15	0.10 $\pm$ 0.08	0.06 $\pm$ 0.11
P-value		0.408	0.730	0.245	0.849	0.054	0.672

**Notes:** Values are presented as the mean  $\pm$  standard deviation; values for some female animals (two K0, one K8, and one K17) were not obtained because the blood sample was not taken or was not sufficient for the analysis; neutrophil, eosinophil, and basophil data for males and neutrophil and basophil data for females were analyzed using a Kruskal–Wallis test; all other data were analyzed by analysis of variance; values with different superscripts are significantly different from each other ( $P < 0.05$ ).

**Abbreviations:** WBC, white blood cell count; n, number of animals.

**Table 11** Plasma electrolytes of mink fed the experimental diets

	n	Inorganic P (mmol/L)	Ca (mmol/L)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
<b>Males</b>						
K0	7	2.29 $\pm$ 0.52	2.87 $\pm$ 0.10	160.1 $\pm$ 4.2	7.24 $\pm$ 0.88	116.6 $\pm$ 2.8
K8	8	2.06 $\pm$ 0.47	2.84 $\pm$ 0.21	159.5 $\pm$ 3.6	7.24 $\pm$ 1.32	116.1 $\pm$ 3.9
K17	8	2.02 $\pm$ 0.35	2.89 $\pm$ 0.11	159.0 $\pm$ 3.3	6.94 $\pm$ 0.82	117.1 $\pm$ 1.9
K33	8	2.41 $\pm$ 0.34	2.92 $\pm$ 0.10	160.3 $\pm$ 4.0	7.36 $\pm$ 0.86	117.5 $\pm$ 1.6
P-value		0.236	0.652	0.903	0.850	0.756
<b>Females</b>						
K0	7	1.89 $\pm$ 0.45	2.74 $\pm$ 0.11	158.9 $\pm$ 3.7	7.56 $\pm$ 1.44	117.0 $\pm$ 3.0
K8	7	1.48 $\pm$ 0.54	2.77 $\pm$ 0.10	158.4 $\pm$ 3.1	6.73 $\pm$ 1.16	116.6 $\pm$ 2.2
K17	8	2.20 $\pm$ 0.58	2.78 $\pm$ 0.07	158.8 $\pm$ 3.2	7.55 $\pm$ 1.54	116.4 $\pm$ 2.5
K33	8	2.31 $\pm$ 0.40	2.76 $\pm$ 0.05	158.6 $\pm$ 2.6	7.49 $\pm$ 1.52	117.5 $\pm$ 2.3
P-value		0.206	0.888	0.995	0.643	0.820

**Notes:** Values are presented as the mean  $\pm$  standard deviation; values for some female animals (one K0 and one K8) were not obtained because the blood sample was not taken or was not sufficient for the analysis; all data were analyzed by analysis of variance; no values were significantly different from each other ( $P < 0.05$ ).

**Abbreviations:** n, number of animals; P, phosphorus; Ca, calcium; Na, Sodium; K, potassium; Cl, chloride.

**Table 12** Blood biochemistry profile of mink fed the experimental diets

	Diet				P-value
	K0	K8	K17	K33	
Males (n)	7	8	8	8	
AST (U/L)	166±115	176±126	110±46	110±65	0.286
ALT (U/L)	249±229	261±210	100±30	112±73	0.092
AP (U/L)	107±15 <sup>a</sup>	126±49 <sup>ab</sup>	146±52 <sup>ab</sup>	234±99 <sup>b</sup>	0.013
CK (U/L)	641±460	882±617	1,067±809	1,242±1,624	0.597
Amylase (U/L)	84±18 <sup>a</sup>	89±15 <sup>ab</sup>	95±14 <sup>ab</sup>	109±13 <sup>b</sup>	0.016
Lipase (U/L)	25±3	24±2	28±8	27±3	0.211
Total protein (g/L)	67.9±3.4	66.5±2.8	66.6±3.6	67.6±2.5	0.771
Albumin (g/L)	39.9±4.6	40.5±2.4	40.4±2.4	38.4±2.2	0.481
Globulin (g/L)	28.0±1.9	26.0±2.4	26.2±2.8	29.2±2.8	0.052
Urea (mmol/L)	10.6±1.9 <sup>a</sup>	8.8±3.2 <sup>ab</sup>	8.1±2.5 <sup>ab</sup>	6.2±1.1 <sup>b</sup>	0.010
Creatinine (μmol/L)	112±17 <sup>a</sup>	102±34 <sup>ab</sup>	78±20 <sup>ab</sup>	68±9 <sup>b</sup>	0.006
Bile acids (μmol/L)	9.4±4.6	5.5±4.3	5.0±3.6	4.2±3.4	0.082
Total bilirubin (μmol/L)	0.6±0.5	0.8±0.5	0.5±0.5	0.4±0.5	0.534
Females (n)	7	7	8	8	
AST (U/L)	156±132	209±291	333±328	212±216	0.585
ALT (U/L)	153±139	203±197	428±506	236±244	0.605
AP (U/L)	105±7	93±30	118±20	139±43	0.034
CK (U/L)	1,620±2,493	1,001±1,421	857±717	1,436±1,101	0.491
Amylase (U/L)	82±7	87±14	87±16	102±20	0.090
Lipase (U/L)	31±3	30±6	30±2	31±4	0.991
Total protein (g/L)	63.9±3.3	67.1±1.7	65.4±2.1	63.9±2.5	0.056
Albumin (g/L)	41.0±1.3	40.1±5.4	40.6±1.4	38.6±2.9	0.090
Globulin (g/L)	22.9±2.4	27.0±6.3	24.8±1.8	25.2±3.3	0.096
Urea (mmol/L)	10.0±1.7 <sup>a</sup>	9.4±2.1 <sup>ab</sup>	7.5±2.1 <sup>ab</sup>	7.2±1.4 <sup>b</sup>	0.018
Creatinine (μmol/L)	93±19 <sup>a</sup>	90±14 <sup>a</sup>	79±19 <sup>ab</sup>	63±12 <sup>b</sup>	0.004
Bile acids (μmol/L)	11.1±4.6 <sup>a</sup>	10.7±8.1 <sup>ab</sup>	5.6±3.1 <sup>ab</sup>	4.4±1.8 <sup>b</sup>	0.032
Total bilirubin (μmol/L)	0.6±0.5	0.6±0.5	0.4±0.5	0.5±0.5	0.872

**Notes:** Values are presented as the mean ± standard deviation; values for some female animals (one K0 and one K8) were not obtained because the blood sample was not taken or was not sufficient for the analysis; AST, ALT, AP, CK, lipase, and creatinine data for males and ALT, AP, CK, albumin, globulin, and bile acid data for females were analyzed using a Kruskal–Wallis test; all other data were analyzed by analysis of variance; values with different superscripts are significantly different from each other ( $P < 0.05$ ).

**Abbreviations:** n, number of animals; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AP, alkaline phosphatase; CK, creatine kinase.

insoluble complex and reducing fluorine absorption from krill.<sup>43</sup> Bone fluoride concentrations corresponded well with dietary krill inclusion and fluorine concentrations. At the highest dietary krill level, clear indications of skeletal fluorosis were observed and fluoride levels in dried defatted bone exceeded 8,000 mg/kg in males and reached 9,000 mg/kg in females. Deformations of the femoral head and neck were also observed in 7/8 males and 1/8 females in this group. Even at the lowest dietary krill inclusion level, bone fluoride reached levels of approximately 3,300 mg/kg. Aulerich et al<sup>40</sup> reported similar fluoride concentrations in ashed bone of 3-month-old mink fed diets containing between 33 mg NaF/kg and 194 mg NaF/kg for 382 days. Schupe et al<sup>41</sup> reported fluoride concentrations of 5,110 mg/kg in the dried defatted femurs of mink kits fed a diet containing 111.5 mg fluorine/kg for 7 months, and 4,716 mg fluoride/kg in the femurs of adult males fed a diet containing 287 mg fluorine/kg

for 8 months. These findings led the authors to recommend maximum fluorine concentrations in diets for mink of 50 mg/kg for breeding stock and 100 mg/kg in animals raised for fur.<sup>41</sup>

## Blood characteristics

Values of some blood chemistry variables in K17 and K33 animals were significantly different from control ( $P < 0.05$ ). AP activity in blood plasma was increased in K33 males, which may be related to the high fluorine content of the krill. Serum AP has been reported to be elevated in rats given 50 ppm fluoride in drinking water for 60 days,<sup>45</sup> and mink fed diets containing 194 ppm supplemental fluorine (from NaF).<sup>40</sup> Increased AP activity is associated with increased osteoblast activity<sup>46</sup> and thus may indicate increased bone deposition and/or mineralization. AP is not, however, specific for bone, but it can also indicate

**Table 13** Plasma cholesterol, triglycerides, free fatty acids, and glucose of mink fed the experimental diets

	n	Cholesterol (mmol/L)	Triglycerides (mmol/L)	Free fatty acids (mmol/L)	Glucose (mmol/L)
<b>Males</b>					
K0	7	6.3±0.7	1.6±0.8	0.2±0.1 <sup>a</sup>	8.9±2.1
K8	8	6.1±1.2	1.5±0.9	0.6±0.4 <sup>ab</sup>	9.8±2.9
K17	8	6.5±1.3	1.4±0.6	0.6±0.4 <sup>b</sup>	8.4±1.7
K33	8	7.4±1.4	1.2±0.4	0.8±0.5 <sup>b</sup>	7.8±1.5
P-value		0.198	0.715	0.004	0.329
<b>Females</b>					
K0	7	6.9±0.4	1.7±0.3	0.3±0.3	8.3±1.2
K8	7	6.9±0.9	1.6±0.4	0.6±0.5	8.9±2.4
K17	8	7.3±0.5	1.6±0.5	0.4±0.2	9.0±2.0
K33	8	7.0±0.6	1.4±0.5	0.6±0.4	8.5±1.7
P-value		0.626	0.556	0.455	0.852

**Notes:** Values are presented as mean ± standard deviation; values for some female animals (one K0 and one K8) were not obtained because the blood sample was not taken or was not sufficient for the analysis; free fatty acid data for males and data for females were analyzed using a Kruskal–Wallis test; all other data were analyzed by analysis of variance; values with different superscripts are significantly different from each other ( $P < 0.05$ ).

**Abbreviation:** n, number of animals.

liver injury. Elevations in intestinal and plasma AP are observed in rats ingesting high concentrations of dietary lipid.<sup>47–50</sup> Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were not affected by the dietary krill level, suggesting that the livers were functioning normally and that damage to the liver was not the cause of elevated plasma AP.

The reason for the increase in plasma free fatty acids in K17 and K33 males was unclear. It is possible that the decrease in stored glycogen in the liver may have caused a compensatory shift to catabolism of lipid, which would be expected to cause an increase in the plasma fatty acid level. Plasma urea and creatinine levels were also decreased in K33 animals, indicating that high dosages of the krill meal may have influenced protein intake or metabolism. The slight decrease in crude protein level of the K33 diet may be responsible for the changes in plasma urea and creatinine. The decrease in plasma bile acids levels with increasing krill inclusion may have been due to the sterol binding capacity of chitosan (a deacetylated metabolite of chitin), a characteristic this polysaccharide has in common with many other nonstarch polysaccharides.<sup>51</sup> It could not be determined whether the pancreas or the kidneys were the source of the statistically significant but modest elevation of plasma amylase in K33 males.

The results of the hematological examination were unremarkable with the exception of a slight decrease in MCHC and an increase in the RDW of K33 animals. The

cause for this is unknown, but it may be related to increased dietary fluorine. Inconsistent effects of fluorine on hematological variables have been reported in rats and humans.<sup>52,53</sup> Reported findings include reduced RBC, HGB, HCT, and MCHC. However, Khandare et al<sup>54</sup> reported no adverse effects of fluorine on hematological variables in dogs. Likewise, Ersoy et al<sup>55</sup> did not observe effects on any hematological variables in humans with endemic fluorosis. Few studies have reported on the effects of dietary krill meal on blood indices. Zaleska-Freljan and Cywińska<sup>56</sup> examined the effect of different krill meals fed to laboratory rats. The mean values of HCT and HGB levels, and the mean corpuscular thickness and volume were lower in rats fed with krill carapace (shell) meal, but not in rats fed low chitin or standardized krill meal. The authors concluded that the changes observed were the result of excess fluorine in the diet.

There were no significant effects of krill meal on WBC parameters with the exception of a slight increase in neutrophils in K33 males. However, the average WBC in K17 and K33 males was higher than normal (approximately  $5.5–8.0 \times 10^9/L$ ).<sup>57</sup> High numbers of WBCs were observed in one male in the K17 group ( $24.6 \times 10^9/L$ ) and in three males in the K33 group ( $15.2–22.8 \times 10^9/L$ ), and the individual variation was reflected in the high standard deviation in these groups. The total WBC was not attributed to any cell type, and the differential cell counts did not differ between treatment groups or sex (data not shown). In one of the three high dosage group males exhibiting an increased WBC, mild inflammation was observed in rectal tissue, but in the other males, no specific cause for an inflammatory response was found.

## Histopathology

Focal inflammatory changes in the liver and crystalline material in tubules of the kidneys occurred frequently in all groups of animals (including controls). The incidences of both of these findings increased in K17 and K33 animals. Although animals from the K8 group did not exhibit an increased incidence of focal inflammatory lesions in the liver, the frequency of finding crystalline material in the kidneys was slightly higher in K8 males than controls. The etiology and toxicological relevance of the focal inflammatory lesions in the liver are unclear based on the relative lack of changes in clinical chemistries related to the liver (AST and ALP) or other histopathological findings in the liver. Similarly, urea, creatinine, and creatine kinase were not increased in any groups of treated animals, which indicates that the observed increased frequency of crystalline material in the kidney

tubules of the treated animals was not pathological in nature. As the finding of crystalline material in the tubules was a common finding in control animals consuming a fishmeal diet, it was concluded that mink may be predisposed to developing crystalline material in the kidneys regardless of diet.

## Conclusion

The protein and energy value of Antarctic krill meal appeared to be comparable to that of the fishmeal used as a control diet, as indicated by feed intake and growth results. However, some effects that could be considered adverse were observed when krill meal was included in the diet at the medium concentration (K17). Effects noted at the K33 concentration were adverse and consistent with fluorosis. At the lowest inclusion level (K8), the only effects noted were an increased level of fluoride in bone (which had no effect on bone integrity) and a slight (nonsignificant) increase in the frequency of crystalline material in kidney tubules (which was not associated with clinical evidence of toxicity to the kidney). Therefore, it was concluded that 8% (based on dry matter) is the no observable adverse effect level of krill meal for growing mink.

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