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Reduced growth and feed consumption of Atlantic salmon (*Salmo salar* L.) fed fish meal made from stale fish is not due to increased content of biogenic amines

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Abstract

Atlantic salmon (*Salmo salar* L.) smolts were fed for 11 weeks a diet based on fish meal made from fresh herring (Diet 1) or a diet based on fish meal made from herring stored until stale (Diet 2) in addition to three diets made by adding to Diet 1 combinations of biogenic amines to a level comparable to that found in Diet 2: cadaverine, histamine, putrescine plus tyramine (Diet 3), cadaverine, putrescine plus tyrosine (Diet 4), histamine, putrescine plus tyrosine (Diet 5) and cadaverine plus histamine (Diet 6). Salmon fed diets based on fish meal made from stale herring had reduced growth and feed consumption and impaired efficiency of feed utilisation compared with those fed fish meal made from fresh herring. Furthermore, salmon fed fish meal made from stale herring showed gross and histological changes in the liver and intestines that were either not found or found to a lesser extent in fish fed fish meal from fresh herring. Addition of biogenic amines to the diet based on fish meal made from fresh herring neither affected production performance nor led to pathological changes in the gastrointestinal tract. Reduced production performance resulting from fish meal produced from stale herring most probably has a multiple

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background. Reduced palatability due to compounds formed in stale fish or during processing of stale fish reduces feed intake and thereby growth and feed utilisation. Bacterial action in the fish raw material during unfavourable storage conditions reduces the content of essential amino acids and leads to diminished protein supply with reduced growth and impaired efficiency of feed utilisation as a consequence. Finally and probably most significant, toxic compounds formed in stale fish or in processing of stale fish cause pathological changes in vital organs that results in reduced growth. These compounds are not the biogenic amines histamine, cadaverine, putrescine and tyramine. However, the content of biogenic amines may be indicator for freshness of the fish raw material and thereby serve as a quality criterion for fish meal. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Biogenic amines; Histidine; Cadaverine; Putrescine; Tyramine; Fish meal; Atlantic salmon (*Salmo salar* L.)

1. Introduction

It is known that salmon fed fish meal made from stale fish have reduced growth and impaired efficiency of feed utilisation (i.e. increased feed conversion ratio = feed offered/weight gain) compared to salmon fed fish meal made from fresh fish although the fish meals may be similar with regard to proximate composition and protein digestibility (Pike, 1991; Pike and Hardy, 1997; Tryggvadottir et al., 1994a,b; Anderson et al., 1997). Since it is also known that stale fish has increased content of biogenic amines compared to fresh fish (Pan and James, 1985), and that histamine is toxic to several animal species (Askar and Treptow, 1986), it has been assumed that the reduced growth seen in fish fed diets containing stale fish was due to biogenic amines or products formed therefrom, i.e. gizzerosine (Pan, 1988). However, when rainbow trout were fed diets to which high levels of crystalline histamine had been added, the effect on growth was small although the amine led to pathological changes in the stomach (Watanabe et al.,1987; Fairgrieve et al., 1994).

The present study was conducted with the aim of elucidating whether the reduction in growth and efficiency of feed utilisation seen in salmon fed fish meal made from stale herring is due to the biogenic amines formed in spoiled fish or caused by other factors. In addition, we wanted to study whether the reduction in production criteria is caused by a negative effect on feed intake or to a toxicological effect.

2. Material and methods

2.1. Experimental design and diets

The study was conducted according to a completely randomized statistical design with six treatments, each consisting of three replicate groups (tanks).The six treatments were diets based on fish meal made from fresh (Diet 1) or from stale herring (Diet 2)

and four additional diets composed of fish meal made from fresh herring and added cadaverine (C), histamine (H), putrescine (P) and tyramine (T) up to the levels found in Diet 2 (Diet 3) or the same diet devoid of H addition (Diet 4), C addition (Diet 5) or additions of P plus T (Diet 6).

Fresh North Sea Herring (*Clupea harengus*) with 16.6% fat and 18.1% non-fat dry matter (DM) and with a total volatile nitrogen (TVN) content of 10–15 mg/100 g and a temperature of 0.5°C was processed at a commercial factory into a semi-dry (about 30% DM) Norse-LT 94 fish meal 1–1.5 days after catch (FM1), or stored for an additional 9 days at ambient temperature (10–15°C) until the TVN content had increased to 110 mg/100 g before being processed into presscake and stickwater in a pilot fish meal plant (FM2). The semi-dried meal from the commercial plant (FM1) and the presscake–stickwater mixture from the pilot plant (FM2) were dried in an ultra rotor pilot drier with inlet air temperature of 300°C, outlet air temperature of 80°C, and outlet meal temperature of 75°C. Proximate composition, TVN-content and contents of water-soluble protein and biogenic amines in the fish meals are shown in Table 1 together with their true protein digestibility as determined in mink (Skrede, 1979).

The chemical composition of the experimental diets is shown in Table 2. Fish meal was the sole source of protein ($N \times 6.25$) in all diets. All diets were isocaloric and isonitrogeneous with the addition of the same amounts of vitamins and minerals, and differed only in fish meal source and additions of biogenic amines. The biogenic amines were used in their free base form. Histamine and tyramine were added to the vitamin mixture and mixed into the diets. Putrescine and cadaverine were heated to 50°C and dissolved in fish oil before being mixed into the diets. The chemical analyses confirmed that the intended nutrient composition and contents of biogenic amines were mainly achieved. The differences between the analysed content of biogenic amines and that calculated from the content in the fish meals in Diets 1 and 2 were not greater than may be explained by sampling and analytical error.

Table 1
Chemical composition and protein digestibility of fish meals

Fish meal	FM1 (fresh)	FM2 (stale)
Protein ($N \times 6.25$), %	73.4	73.4
Moisture, %	7.6	6.1
Ash, %	11.1	9.7
Fat (Soxhlet), %	8.8	11.3
TVN[a], %	0.16	0.20
Water-soluble protein, percentage of total protein	24.5	27.5
Protein digestibility (mink), %	92.7	92.2
Biogenic amines, mg / 100 g		
Tyramine	2.1	133.7
Putrescine	7.3	146.5
Cadaverine	7.9	325.4
Histamine	0.9	276.3

[a]TVN = total volatile nitrogen.

Table 2
Ingredient content and chemical composition of experimental diets

Diet	1	2	3	4	5	6
Fish meal	FM1	FM2	FM1	FM1	FM1	FM1
Amine added[a]	–	–	CHPT	CPT	HPT	CH
Fish meal, %	61.31	61.31	61.31	61.31	61.31	61.31
Fish oil[b], %	20.60	19.07	20.60	20.60	20.60	20.60
Suprex maize[c], %	16.15	17.68	15.62	15.79	15.82	15.79
Soya lecithin[d], %	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin mixture[e], %	1.00	1.00	1.00	1.00	1.00	1.00
Mineral mixture[f], %	0.40	0.40	0.40	0.40	0.40	0.40
Carophyl pink 8%[g], %	0.04	0.04	0.04	0.04	0.04	0.04
Tyramine, mg/100 g	–	–	81	81	81	–
Putrescine, mg/100 g	–	–	85	85	85	–
Cadaverine, mg/100 g	–	–	195	195	–	195
Histamine, mg/100 g	–	–	169	–	169	169
Chemical composition (as analysed)						
Protein ($N \times 6.25$), %	48.9	47.2	48.1	48.2	48.2	48.3
Moisture, %	3.7	3.7	4.3	4.5	4.4	4.4
Ash, %	7.7	6.7	7.5	7.5	7.7	7.6
Fat (Soxhlet), %	25.3	26.0	26.0	25.9	25.5	25.9
Tyramine, mg/100 g	4.4	86.6	72.0	70.3	74.9	6.2
Putrescine, mg/100 g	6.7	82.8	62.4	62.7	72.7	7.6
Cadaverine, mg/100 g	10.4	215.6	164.9	172.4	13.7	142.4
Histamine, mg/100 g	4.8	174.2	158.4	6.1	162.8	142.3

[a] C = cadaverine, H = histamine, P = putrescine, T = tyramine.
[b] Norsalmoil, Norsildmel, Bergen, Norway.
[c] Precooked maize, Cadrico, Rotterdam.
[d] Denofa, Norway.
[e] Vitamin mixture provides per kilogram of feed: vitamin A, 3000 IU; vitamin D3, 600 IU; vitamin E, 160 mg; thiamin, 12 mg; riboflavin, 24 mg; pyridoxine, 12 mg; vitamin C, 60 mg; pantothenic acid, 48 mg; biotin, 0.6 mg; folic acid, 6 mg; niacin, 120 mg; vitamin B12, 0.24 mg; vitamin K, 12 mg.
[f] Mineral mixture provides per kilogram of feed: Mg, 500 mg; Cu, 5 mg; Mn, 10 mg; K, 400 mg; Zn, 80 mg; Fe, 50 mg.
[g] Hoffmann-L Roche, Switzerland.

2.2. Fish and handling

Atlantic salmon (*Salmo salar* L.) smolts, about 10 months post hatching (0-years smolts) and about 100 g average weight, were obtained from a commercial hatchery and distributed randomly to 18 fiberglass tanks ($2 \times 2 \times 1$ m^3) with 165 fish per tank. The tanks were supplied with 75 l/min of seawater taken from a depth of 50 m. Water temperature was about 10°C at the start of the experiment and decreased to 8.5°C at the end. Salinity was 3.2–3.3%, and the photoperiod 24 h light throughout the experiment.

The fish were fed by automatic feeders at amounts equal to 120% of the AKVAFORSK growth tables (Austreng et al., 1987) adjusted according to assumed biomass

and appetite. Feeding periods were of 20 s duration intervened by 200 s and lasted from 0700 to 1200, 1330 to 1800 and 1930 to 2400 h. In addition, the fish were hand-fed to satiety once per day. The fish were kept for acclimatisation and fed a commercial feed for 4 weeks before three tanks were randomly allotted to each diet. The main feeding experiment lasted for 11 weeks from 3 October to 18 December. The fish were monitored daily for appetite and general appearance.

Total fish weight and fish number in each tank were determined at the start of the experiment, after 6 weeks of experimental feeding and at termination of the experiment. At termination, 30 fish from each tank from Diets 1, 2 and 3 were randomly taken for weight and length measurements and for determination of condition factor ($C =$ weight/lenght^3). Twenty fish from each diet were subjected to gross pathological inspection and 10 for histological examination (see Section 2.3). These observations were not taken from fish fed Diets 4, 5 and 6 since they appeared to be similar with regard to weight and appearance to those fed Diet 3. As a consequence of possible pathological changes observed in the gastrointestinal tract after 11 weeks of experimental feeding, fish fed Diets 1, 2 and 3 were fed their respective diets for an additional period of 2 weeks after which a pathological examination of the anterior intestine was carried out.

2.3. Pathological examination

From the 30 fish sampled for determination of condition factor from each tank of Diets 1, 2 and 3 after 11 weeks of experimental feeding (see Section 2.2), 10 fish from each diet having higher than average condition factor and 10 having lower were selected for examination of gross pathology. From these 10, five fish (10 from each diet) were randomly sampled for histological examination of pancreas, skeletal muscle (dorsal muscle 1 cm caudal to the dorsal fin), kidney and liver. Furthermore, after an additional feeding period of 2 weeks, five fish from each tank fed Diets 1, 2 and 3 were randomly sampled for gross pathological examination of the gastrointestinal tract. From three of these fish, samples of the anterior intestines, 2–3 cm posterior to the pyloric caeca region, were obtained for histological examination. All tissue samples were taken immediately after the fish were killed with an overdose of benzocain and were fixed in 4% formaldehyde in phosphate buffer. The tissues were embedded in paraffin, sectioned to 5 μm and stained with haematoxylin, eosin and safran. Examination was done by light microscopy at 100 × and 400 × magnifications.

2.4. Chemical analysis

In fish meals, feeds and faeces, crude protein ($N \times 6.25$) was determined by the Kjeldahl method (ISO 5983-1979) and moisture (ISO 6496-1983) and ash (ISO 5984-1978) gravimetrically after drying for 4 h at 105°C and after combustion for 16 h at 450°C, respectively. Fats in fish meal and feeds were determined by the Soxhlet method using petroleum benzene extraction (AOCS Ba-38). TVN in fish meal was determined by distillation (AOAC, Method of Analysis, 1984, 2.065). Biogenic amines in fish meals

and feeds were determined at the Torry Research Station, Scotland, by a modification of the method described by Seiler and Knødgen (1985) using a reversed-phase high-performance liquid chromatography system. For determination of water-soluble protein, 200 ml of distilled water was added to 10.0 g of fish meal and heated in a boiling water bath for 30 min, filtered and the protein content in the supernatant determined by the Kjeldahl method. True protein digestibility was determined in mature male mink as described by Skrede (1979).

2.5. Statistical methods

The test for statistical significance between dietary groups for parametric observations was conducted by single classification analysis of variance and determination of LSD (least significant difference) (Snedecor and Cochran, 1989). Observations on histopathology and mortality were considered non-parametric and tested by the chi-square test (Snedecor and Cochran, 1989).

3. Results

3.1. Effect of fish raw material freshness on proximate composition and quality criteria in fish meal

Storage of the fish before processing until stale had only minor effects on the proximate composition of the fish meal made therefrom, but fish meal made from stale fish had higher contents of TVN and water-soluble protein indicating autolysis of the protein and breakdown of amino acids (Table 1). Fish meal made from stale fish also had significantly higher contents of the four biogenic amines determined showing a considerable degree of decarboxylation of amino acids. Raw material freshness had only a limited negative effect on protein digestibility, and both fish meals showed protein digestibility in excess of that found in high-quality commercial fish meal.

3.2. Production criteria

Mortality, growth, feed intake, feed conversion ratio, and condition factor are shown in Table 3. Mortality was low with no significant ($P \approx 0.50$) differences between the diets. Fish meal made from stale herring caused a significant ($P < 0.01$) growth depression compared to fish meal made from fresh herring. Thus, the fish fed Diet 2 had a growth rate that was only 47% (39% in the first 6 weeks and 52% in the last 5 weeks) of that of Diet 1 that gave a growth rate 30% above the AKVAFORSK growth table (Austereng et al., 1987). Addition of various combinations of biogenic amines to the diet based on fish meal from fresh herring (Diets 3–6) had no significant ($P > 0.05$) effect

Table 3
Production performance of Atlantic salmon fed diets with fish meals made from fresh or stale herring or from fresh fish with added biogenic amines
Values not followed by the same letter are significantly different at $P < 0.05$.

Diet	1	2	3	4	5	6	SEM[a]
Fish meal[b]	FM1	FM2	FM1	FM1	FM1	FM1	
Biogenic amines[c]	–	–	CHPT	CPT	HPT	CH	
Body weight at start, g	99	99	100	100	100	101	1[d]
Body weight at 6 weeks, g	179a	130b	189a	185a	185a	188a	4***
Body weight at 11 weeks, g	312a	198b	330a	333a	326a	319a	8***
Mortality, %	0.2	1.6	1.0	0.2	0.4	0.6	[d]
Growth 1st period, g/fish	80a	31b	89a	85a	85a	87a	5**
Growth 2nd period, g/fish	132a	68b	141a	147a	141a	131a	7**
Growth, total g/fish	212a	99b	230a	233a	226a	218a	12**
Specific growth rate[e], %	1.55a	0.94b	1.61a	1.62a	1.60a	1.55a	0.06**
Feed offered 1st period, g/fish	85	87	90	85	88	87	1[d]
Feed offered 2nd period, g/fish	105a	49b	113a	111a	116a	112a	6*
Feed offered total, g/fish	190a	135b	202a	196a	204a	200a	6*
Feed conversion ratio[f], 1st period	1.06a	2.82b	0.99a	1.01a	1.05a	1.01a	0.11***
Feed conversion ratio[f], 2nd period	0.80	0.73	0.80	0.75	0.82	0.86	0.03[d]
Feed conversion ratio[f], total period	0.90a	1.37b	0.88a	0.84a	0.90a	0.91a	0.03***
Condition factor[g]	1.32a	1.20a	1.32a	–	–	–	0.01**

[a] SEM = standard error of means.
[b] FM1 produced from fresh raw material, FM2 produced from stale raw material.
[c] Biogenic amines: C = cadaverine, H = histamine, P = putrescine, T = tyramine.
[d] ns = $P > 0.05$.
[e] Specific growth rate = 100 (ln final weight − ln initial weight)/no. of days.
[f] Feed conversion ratio: feed offered/gain.
[g] Condition factor: weight/length^3 × 100.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

on growth rate compared to the diets with no additions (Diet 1), but it is noteworthy that the fish fed Diets 3–6 grew from 3% to 10% faster than those fed Diet 1.

There was no difference among the different groups in amount of feed offered in the first 6 weeks. If offered feed is assumed to represent consumption, feed conversion ratio was more than 150% and significantly ($P < 0.05$) higher in fish fed fish meal from stale herring (Diet 2) compared with that of those fed fish meal from fresh herring (Diet 1). It can, however, not be excluded that the fish fed Diet 2 were overfed, and that a significant amount of the offered feed was lost through the drains, unobserved due to the small amount of feed being fed at that time. There were no differences in feed offered

and feed conversion ratio between the fish fed fish meal from fresh herring (Diet 1) and those fed that fish meal with the additions of various combinations of biogenic amines (Diets 3–6). During the last 5 weeks, fish fed fish meal made from stale herring (Diet 2) had seriously reduced feed intake, and feed offered was only 50% and significantly ($P < 0.05$) lower than that offered to the other groups, between which there were no significant ($P > 0.05$) differences. It is, however, worth noting that the fish fed additions of crystalline amines had from 6% to 10% higher feed intake than those fed the same diet without additions. There were no differences between the different diets in feed conversion ratio in the last 5 weeks. Thus, although fish meal made from stale herring caused a severe reduction in feed intake and growth, it did apparently not affect the efficiency of feed utilisation. For the whole experimental period, significantly ($P < 0.05$) less feed was offered, and feed conversion ratio was significantly ($P < 0.05$) higher in the fish fed fish meal from stale herring compared to the other groups, between which there were no significant ($P > 0.05$) differences, although additions of biogenic amines tended to improve both criteria. The condition factor (weight for length) was significantly ($P < 0.05$) decreased in the fish fed fish meal made from stale herring compared to those fed fish meal made from fresh herring without and with the addition of biogenic amines. However, that may be an indirect affect caused by weight reduction.

3.3. Pathology

Table 4 shows the results of the histological examination. No differences in gross pathology were seen between fish with high and low condition factor, and both groups

Table 4
Organ histology in Atlantic salmon fed diets with fish meal made from fresh or stale herring or from fresh herring with added biogenic amines

Diet	1	2	3	Significance
Fish meal	FM1	FM2	FM1	
Added biogenic amines	–	–	CHPT[a]	
Incidence (number affected / total numbers)				
Pancreas	0/10	1/10	0/10	$P > 0.05$
Muscle	0/10	0/10	0/10	$P > 0.05$
Kidneys	0/10	0/10	0/10	$P > 0.05$
Liver	3/10	7/10	2/10	$P < 0.05$
Intestines	0/3	3/3	0/3	$P < 0.01$
Numbers of fish in each category				
Lever lesion severity, grade[b]				
0	7	3	8	
1	3	0	2	
2	0	7	0	$P < 0.001$
3	0	0	0	

[a] For explanation, see Table 2.

[b] 0 = Normal. 1 = Minor changes; most probably reversible to normal. 2 = Significant changes; most probably affecting the function of the organ. 3 = Severe pathological changes with necrosis.

are therefore treated together. Pancreas, kidneys, muscle, liver and stomach from all fish showed no gross pathological changes. Also the gastrointestinal tract of fish fed Diets 1 and 3 based on fish meal made from fresh herring without or with the addition of the biogenic amines H, C, P and T appeared normal. All fish fed Diet 2 based on fish meal made from stale herring showed an abnormal, distinct, whitish colour of the gastrointestinal tract ranging from the pyloric portion to the anterior part of the posterior intestines. Also the diameter of the intestines appeared to be increased and the mucosa had a coarse, granular texture. The changes appeared to be less pronounced from the anterior to the posterior part of the affected area.

Tissues of muscle, kidney and pancreas showed no histopathological changes in any of the groups. Histopathological changes were found in the liver of fish from all three

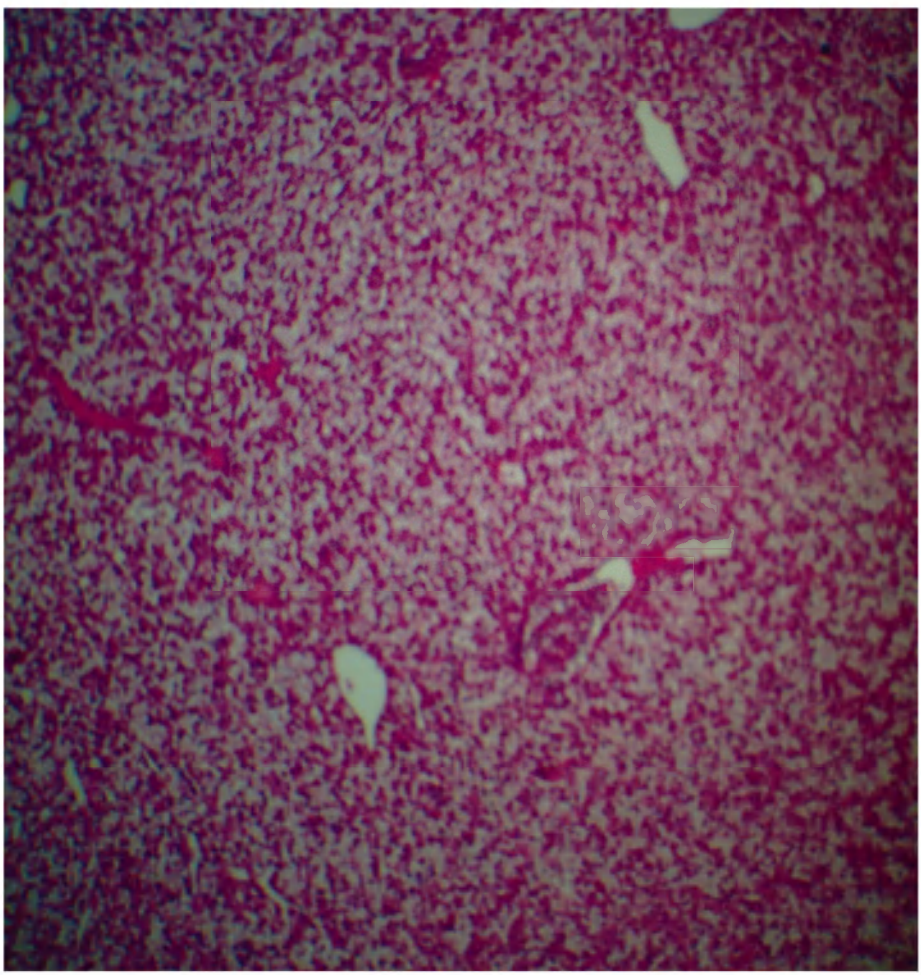

Fig. 1. Richly vacuolized hepatocytes. Diet 2, severity 2 in Table 4. 100× magnification.

groups, as richly vacuolized hepatocytes with condensed, pycnotic nuclei (Fig. 1). The frequency of these changes was significantly ($P < 0.05$) higher and the lesions significantly ($P < 0.001$) more severe in fish fed Diet 2 based on fish meal made from stale herring compared to fish fed Diets 1 and 3 based on fish meal made from fresh herring without or with the addition of the biogenic amines C, H, P and T, respectively. No

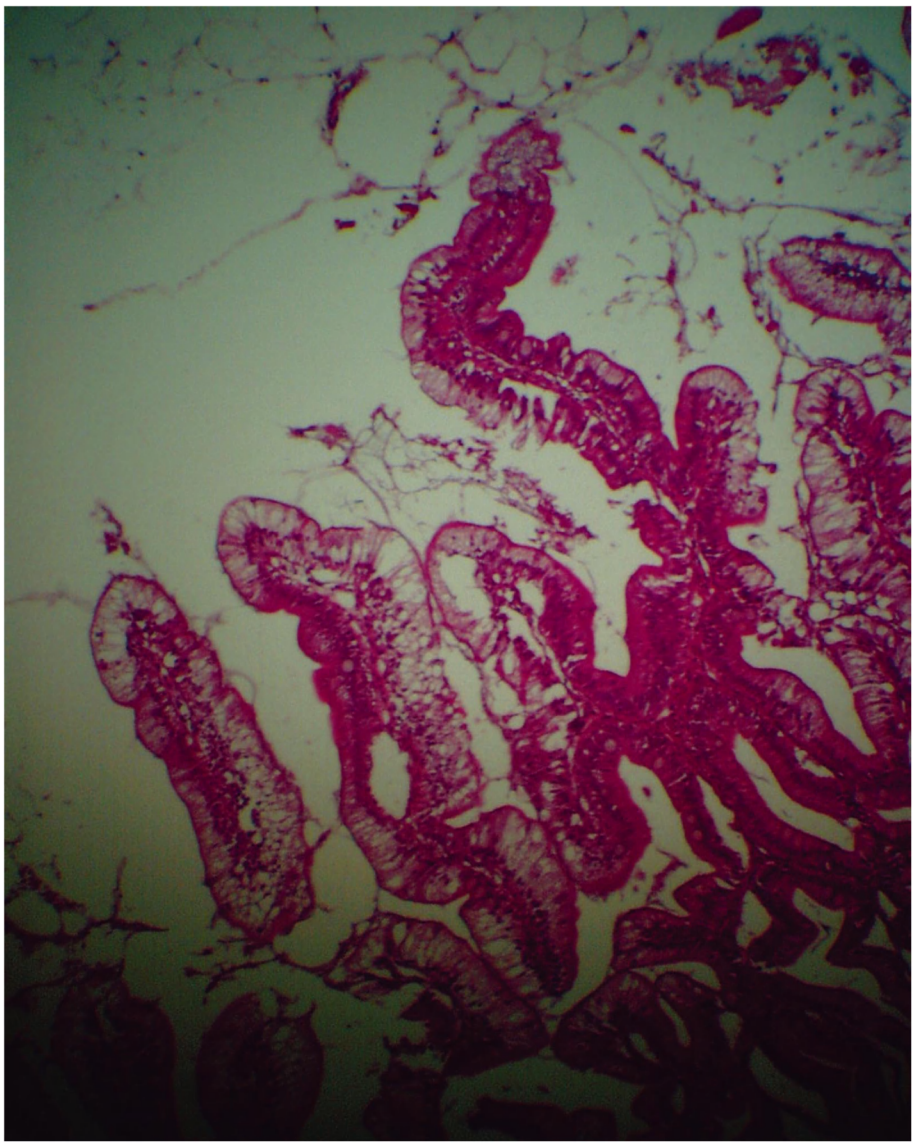

Fig. 2. Degenerated cylindrical epithelial cells in mucosa of anterior intestines. Diet 2 in Table 4. 100× magnification.

histopatological changes were found in the anterior intestines of fish fed diets based on fish meal made from fresh herring, without or with added biogenic amines (Diets 1 and 3). Intestines from fish fed diets based on fish meal made from stale herring (Diet 2) showed changes in mucosa. The cylindrical epithelial cells were extensively vacuolized and the nuclei were condensed and located at the base portion of the cells (Fig. 2). Some tissues from this group also showed sloughing of the mucosa.

4. Discussion

In the present study, Atlantic salmon fed a diet based on fish meal made from stale herring grew at less than half the rate of salmon fed a diet based on fish meal made from fresh herring, and also showed reductions of similar magnitude in feed consumption and efficiency of feed utilisation was impaired. Further, they had pathological changes in the liver and the gastrointestinal tract that were either not seen or appeared at significantly less severity in salmon fed fish meal made from fresh herring. Addition of the biogenic amines, histidine, cadaverine, putrescine and tyrosine, in different combinations and at levels to give similar contents to those found in the diets based on fish meal made from stale herring did not have a significant effect on the observed parameters, although they tended to improve growth and efficiency of feed utilisation.

The growth reduction from fish meal made from stale fish found in the present study confirms previous results with salmon smolts (Pike, 1991; Anderson et al., 1997) and fry (Tryggvadottir et al., 1994a,b), halibut (Aksnes and Mundheim, 1997) and shrimp (Ricque-Marie et al., 1998), and our pathological findings confirm previous observations in halibut (Aksnes and Mundheim, 1997). Contrary to these findings, Watanabe et al. (1987) reported increased growth in rainbow trout fingerlings fed fish meal made from moderately stale mackerel (66.9 mg histamine/kg) compared to that of fish fed fish meal made from absolutely fresh mackerel (3.9 mg histamine/kg). It appears that the mackerel used by Watanabe et al. (1987) was less stale than the herring used in this and previously reported studies with similar outcome, which may explain the different results.

Due to the design of the present study, it is not possible to conclude whether the reduction in growth seen in the salmon fed the fish meal from stale fish was due to diminished appetite and reduced feed consumption, to reduced nutrient content or availability or to toxic factors. The possibility cannot be ruled out that the diet based on fish meal made from stale herring was less palatable than that based on fish meal from fresh herring. Reduced palatability may have been caused by loss of feed attractants or formation of undesirable or toxic compounds during spoilage. Bacterial action in stored fish leads to formation of biogenic amines and ammonia from amino acids and reduction of trimethylamine oxide (TMAO) to trimethylamine (TMA) (Groninger, 1959; Pan and James, 1985; Aksnes, 1989). The results from the present study show no negative effects of biogenic amines, while previous studies have shown that TMA may depress feed intake. Thus, Hughes (1991) found that addition of 591 mg (0.01 M) TMA/kg of diet reduced feed intake in first feeding chinook salmon, and similar findings have been reported for turbot (Mackie and Adron, 1978) and plaice (Mackie, 1982). The content of

TMA was not analysed in the present experiment but previous studies at our institute have shown that fish meal made from moderately stale herring may contain 2000–2500 mg TMA/kg, while fish meal made from absolutely fresh herring is virtually free of the compound (Høstmark, personal communication). Assuming a level 2000–2500 mg TMA/kg in the fish meal made from stale herring (FM2), the content in Diet 2 would have been 1200–1500 mg/kg, which is far in excess of that found by Hughes (1991) to cause reduced feed intake in chinook salmon. It is therefore conceivable that the increased content of TMA in the fish meal produced from stale herring caused a reduction in feed intake. However, to the best of our knowledge TMA has never been found to cause pathological changes in fish or other animals.

The fish meal made from stale herring had only marginally lower protein digestibility than that made from fresh herring, which implies that the availability of the amino acids were not much different in the two meals. We did not determine the content of total amino acids, but in previous studies (Aksnes, 1989), reductions in total content of amino acids in stale herring ranging from nil (valine) and up to 41% (histidine) have been found. Losses of amino acids in stale fish are due to microbial decarboxylation and formation of biogenic amines and deamination and formation of ammonia (Toyama et al., 1981; Pan and James, 1985; Aksnes, 1989). In the present study, increments in the content of putrescine, cadaverine, tyramine and histamine represented a loss of about 6% of the amino acids arginine, lysine, tyrosine and histidine, respectively. Since ammonia is lost during processing of fish meal, there is no analytical criterion in fish meal that can show the extent of deamination in the fish raw material prior to processing. However, previous studies (Aksnes and Brekken, 1988) indicate that losses of essential amino acids due to deamination in moderately stale fish are relatively limited. In conclusion, it is possible that the loss of total amino acids may have contributed to the reduction in growth seen in the fish fed fish meal from stale herring, but it is unlikely that it had a major effect. Furthermore, reduced protein quality would not be expected to give pathological changes. It therefore appears that a toxic compound(s) formed in the stale herring or during the processing of stale herring was the main cause for the reduction in growth and also led to pathological changes.

Several studies have aimed at elucidating the factors in fish meal made from stale fish that cause reduction in fish growth. Much attention has been directed to studying the effect of biogenic amines. Several of the biogenic amines, including histamine, have strong pharmacological effects and are toxic when ingested at higher levels in mammals and birds (Askar and Treptow, 1986), while their effects may be more variable in other vertebrate species (Reite, 1969a,b). When some species of pelagic fatty fish are processed into fish meal at high temperatures, a histamine derivative, gizzerosine (GE = 2-amino-9-(4-imidazolyl)-7-azanonanoic acid) is formed by a reaction between the ε-amino group of lysine with the imidazolethyl group of histidine or histamine (Okazaki et al., 1983). Gizzerosine is toxic to chicks causing ulceration and cellular necrosis in the gizzard (gizzard erosion, black vomit disease) (Okazaki et al., 1983), but to the best of our knowledge, toxicity in mammals has not been conclusively demonstrated.

Watanabe et al. (1987) fed rainbow trout fingerlings diets with graded levels from 1000 to 10,000 mg/kg of added histamine free base for 10 weeks and found that the

fish developed a black body colour and the gastric folds of the inner walls disappeared in an increasing number of fish fed 5000 mg or more histamine per kilogram. Histological observations on the stomach showed erosion, necrosis and edema of mucosal epithelium and also necrosis of the gland cells. However, addition of histamine did not lower growth after 30 days of feeding. In subsequent studies, Watanabe et al. (1987) showed that heating fish meal with high content of histamine caused gizzard erosion in chicks and caused severe histopathological changes in the stomach of rainbow trout fingerlings, characterised by necrosis and pycnosis of gastric gland cells together with necrosis of the lamina propria. These findings were confirmed in part in later studies on rainbow trout by Fairgrieve et al. (1994). Thus, juvenile rainbow trout fed diets containing 2000 mg/kg of added histamine had distended stomachs, but these authors did not find other pathological changes. Tryggvadottir et al.(1994a,b) found varying effects when feeding Atlantic salmon parr and fry diets to which biogenic amines had been added, and claimed that negative effects were only found when the amines were added as the free base and were due to reduced palatability. Nile tilapia fed GE-positive fish meal had reduced growth but had no pathological changes of the liver, stomach or intestines (Reyes-Rosa and Castellanos-Molina, 1995).

There is no apparent explanation why the addition of histamine in the present study did not give pathological changes in the gastrointestinal tract similar to those reported by Watanabe et al. (1987) and Fairgrieve et al. (1994). However, it is worth noting that the levels of addition were lower in our study than in the previously reported experiments (1587 vs. 2000–5000 mg/kg). Furthermore, we did not give histamine as a single addition but together with other amines, but it is unlikely that this had a preventive toxicological effect. Finally, it is also possible but not likely that salmon smolts are more resistant to histamine toxicity than rainbow trout fingerlings. Therefore, when all observations are taken together, our results strongly indicate that the fish meal made from stale herring contain factor(s) that are toxic to Atlantic salmon but that these factors are not biogenic amines. We did not determine the content of gizzerosine since to the best of our knowledge, it has never been found in fish meal made from fish caught in the northern hemisphere. We therefore conclude that it is unlikely that the toxic factor was gizzerosine.

In conclusion, this study has confirmed previous findings that fish meal made from stale herring causes reduced growth and feed intake in Atlantic salmon. It is likely that part of this effect is due to reduced appetite and feed intake caused by appetite depressive factor(s) formed in spoiling fish or during processing of such fish to fish meal, possibly TMA. It is further conceivable that reduction in content of essential amino acid caused by bacterial action may impair protein supply and thereby reduce growth. The likelihood is, however, that the reduced production performance mainly is due to pathological changes in vital organs caused by toxic factors being formed during unfavourable storage conditions of the fish prior to processing or during processing of such fish into fish meal. The present study has shown conclusively that the toxic factors are not the biogenic amines tyramine, putrescine, cadaverine or histamine and probably not gizzerosine. Although storage of fish raw material prior to processing under the conditions used in this study hardly occurs in commercial production of fish meal for fish feed today, the results underline the importance of proper handling of the fish raw

material in fish meal production. Furthermore, although it was shown that the biogenic amines histamine, cadaverine, putrescine or tyrosine do not reduce production performance in salmon, they may be indicators for the freshness of the fish raw material used for production of fish meal, and thus serve as a quality criterion for fish meal.

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