International Fishmeal & Oil Manufacturers Association

FRESHNESS OF FISH USED IN MAKING FISH MEAL FOR SALMONIDS AND THE EFFECTS OF BIOGENIC AMINES

Report of a Trial by J Opstvedt, H Mundheim and H Nygaard, SSF Institute, Bergen, Norway

RESEARCH REPORT NUMBER: 1996-6

October 1996

STRICTLY CONFIDENTIAL

EXECUTIVE SUMMARY

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Report of a Trial by the SSF Institute in Bergen

Background

The freshness of fish used to prepare fish meal has been found to affect the growth of salmon to which it is fed (see Research Reports 1991-2 and 1993-3). The feed intake was reduced with the fish meal prepared from stale fish, and this may have accounted for part or all of the lower growth rate. It was believed that biogenic amines produced during the spoilage of the fish may have been the cause.

Further confirmation of the effect of amines in depressing growth has been found in trials undertaken by the Icelandic Fisheries Laboratory. They subsequently undertook a trial on behalf of the Association to investigate specific biogenic amines and their effect on feed intake. Although originally the work was planned to include fish meals prepared from the same fish - fresh and stale, shortage of funds prevented the stale fish meal being prepared. Instead, the same fresh fish meal was spiked with combinations of the four main amines, histamine, cadaverine, tyramine and putrescine. Having added the four amines to the fresh meal, further treatments were incorporated where amines were systematically omitted one at a time (see Research Report 1994-5).

Whilst there was an indication that the addition of amines to the fresh fish meal may have depressed feed intake and growth, differences were unexpectedly small. Furthermore, the x-ray procedure used to measure feed intake resulted in large variability in the results.

Because of the importance of this work to the industry, it was recommended that this work should be repeated. Using their facilities for both producing experimental fish meals and doing feed intake/growth studies with salmon, the SSF Institute was invited to repeat the work.

Association Trials to Investigate Effects of Biogenic Amines on Feed Intake at the SSF Institute in Bergen

Using facilities at SSF, experimental fish meals were prepared from fresh fish and the same fish allowed to go stale. Both meals were analysed for amine content (see Table 1). These values were then used to calculate the quantities of amines that would have to be added to the meal from fresh fish to increase the levels to those in

the meal from stale fish (Table 2). The work was undertaken using the basic form of the four amines, histamine, cadaverine, putrescine and tyramine, in order to avoid any effects of acidic conditions. According to earlier work done by the Icelandic Fisheries Laboratory, their evidence suggested that acidic conditions may reduce the intake depressing effects of amines. By systematically removing one amine at a time from the mixture, it was considered that the effects of the absence of that amine would be assessed, still allowing for the possibility of interactions between the remaining amines. A comparison of the meal from fresh fish plus all four amines with the fish meal from the stale fish would indicate whether these amines fully account for any effect on feed intake.

Using the salmon rearing facilities at SSF's trial site at Austevol as well as monitoring feed intake by carefully hand feeding fish, growth rate was also to be assessed.

The Results

Growth achieved in the salmon receiving the fish meal prepared from fresh fish was good (Table 3). It compared favourably with the high levels of growth achieved by the better commercial fish farmers. Growth of the fish receiving the fish meal from stale fish was severely reduced as was feed intake. The addition of the biogenic amines to the "fresh" meal did not affect feed intake. In addition to the effects on growth and feed intake, the fish meal from stale fish meal also resulted in pathological changes in both the structure of the livers and the intestine wall structure of the fish (Table 4).

This work would appear to indicate that during the spoilage of fish, compounds are formed which are toxic to salmon. The absence of any effect of the biogenic amines tested per se indicate that these are not the toxic compounds. A possible explanation may be that microbially produced endotoxins could be responsible. Further studies are required to clarify the nature of these toxic materials.

Whilst the biogenic amines are not directly implicated in the depression of feed intake, they would appear to give an indication of the production of other compounds which are toxic during spoilage of the fish. It is recommended that the use of the levels of residual amines in fish meal as an indication of the freshness of the raw material should continue. It is further recommended that additional studies should be undertaken to try and identify the toxic compounds formed in spoiling fish. Ways might then be found of reducing their production. Furthermore, determination of these compounds might lead to a much more precise assessment of the state of the raw material used in producing the fish meal for quality control purposes.

Project B13A: Freshness of Fishmeal on Palatability in Salmon.

Objectives: To investigate to what extent reduced performance in Atlantic salmon due to fish meal made from stale fish is caused by biogenic amines

To investigate whether reduced performance in Atlantic salmon due to fishmeal made from stale fish is mediated through reduced feed consumption,i .e. through reduced palatability

To investigate whether the ingestion of fish meal made from stale fish or biogenic amines causes pathological changes in the salmon, i.e. toxic effects

Introduction

A previous study conducted by IFOMA showed reduced growth of Atlantic salmon smolt and fry when fed fish meal processed from herring (1) or anchovy (2) stored for increased length of time compared to that of salmon fed fish meal processed from absolutely fresh fish. Fish meal processed from stored fish had increased content of water-soluble protein and higher levels of volatile nitrogen and the biogenic amines histamine, cadaverine, putresine and tyramine. The freshness of the fish raw material did not affect the protein digestibility of the fish meal as determined in mink. The suggested reasons for the reduced performance of the fish fed fish meal made from stale fish was due to the content of biogenic amines. It was unclear whether reduced performance was due to depressed feed intake and/or physiological (toxic effects).

Earlier Icelandic experiments indicated that the growth reduction seen in salmon fry fed fish meal processed from stale capelin was due to histamine, cadaverine, putresine and tyramine formed during storage of capelin (Bjarnason, personal communication). However subsequent small scale Icelandic experiment with Atlantic salmon smolt using X-ray technic to measure feed consumption gave inconclusive results (3).

Existing knowledge indicates that fish meal made from stale fish (i.e. fish where the content of volatile nitrogenous compounds is significantly increased) causes reduced performance in Atlantic salmon compared to fish meal made from fresh fish, although the digestibility of the protein is not reduced. However, it was unclear if this effect of stale fish was due to the elevated content of biogenic amines or to other compounds formed during spoilage of the fish or during processing of spoiled fish. Neither is it clear whether the reduced performance of the fish is a consequence of reduced palatability or due to toxic factors. Consequently the present study was conducted in which Atlantic salmon were fed diets with fish meal processed from absolutely fresh herring or from herring allowed to go stale, or diets with fish meal made from the fresh herring to which were added biogenic amines to provide dietary levels similar to those found in the diet made from fish meal made from stale fish . The experiment monitored growth and feed consumption with the intention to reveal whether one or both were affected. In addition the fish were subjected to morphological and histological examination to study possible toxic effects.

Experimental

Fish raw material and processing.

Absolutely fresh North Sea herring (Clupea harangues) with 16.6 % fat, 18.1 % non-fat DM and a TVN content of 10 to 15 mg per 100 g and a temperature of about 0.5 °C was processed at a commercial factory to a semi-dry (abt. 30 % DM) Norse-LT 94 meal 1 to 1 ½ days after catch, or stored until stale before being processed in a pilot plant. A sample of the semi-dried meal was taken from the processing line in the commercial factory and dried in an ultra rotor pilot dryer, inlet temperature 300 °C, outlet temperature 80° C , meal outlet temperature 75° . The meal was chilled immediately after drying, added 100 mg EMQ per 1000g and stored in paper bags at ambient temperature (FM1). The fish to become stale was stored for 9 days at ambient temperature (10-15 °C) when the TVN content had increased to about 110 mg per 100 g before being processed in a pilot plant. The fish was cooked in a conveyer belt cooker at a temperature of about 85° C before being pressed and the press liquor desludged, separated into an oil and a water phase and the water phase concentrated in a falling film evaporator. The mixed presscake, sludge and concentrated stickwater was dried in a pilot plant dryer as previously described (FM2). The proximate composition, TVN content, content of water-soluble protein and biogenic amines and the protein digestibility of the fish meals are shown in Table 1. Fish meal made from stale herring had about the same proximate composition as that made from fresh herring, but had elevated levels of TVN, water-soluble protein and biogenic amines. There was no significant difference in protein digestibility, as determined in mink, between fish meal made fresh and stale herring. Thus the intention to compare fish meal from fresh and stale herring that deviated only in those parameters being a direct result of the deterioration of the fish raw material was achieved.

Diets

The composition and chemical content of the experimental diets are shown in Table 2. The fish meals were the sole protein sources to provide 45 % protein in all diets. All diets were iso caloric and were added the same amounts of minerals and vitamins. Diet 1 used fish meal made from absolutely fresh herring with a low content of biogenic amines, diet 2 fish meal made from stale herring with a high content of biopgenic amines. To diets 3 to 6 were added various combinations of biogenic amines to give the same concentration as that provided by the fish meal made from stale fish in diet 2. Diet 3 were added the four amines hitamine, cadaverine, putrecine and thyramine. In diets 4 to 6 one or two amines at the time were excluded from the complete mixture used in diet 3 in order to explore if certain amines were more detrimental than others. The results of the chemical analysis show that the intended content of nutrients and biogenic amines were achieved.

Fish and handling

Atlantic salmon smolt, about 10 months post hatching (0-years smolt) and about 100 g of weight, were obtained from a commercial hatchery and distributed on 18 glassfibre tanks (2x2x1 m) with 165 fish per tank. The tanks were supplied with saltwater taken from 50 m depts. Water temperature was about 10 °C and decreased to 8.5 °C the last 10 days of the experiment. Salinity was 32-33 ‰ throughout the experiment. The photoperiod was 24 hrs

light. The fish was fed from automatic feeders at amounts equal to 20% above the Akvaforsk growth tables (Aust reng *et al.*, 4), and adjusted according to assumed biomes and appetite. Feeding periods were of 20 seconds duration intervened by 200 seconds and lasted from 07.00 to 12.00, 13.30 to 18.00 and 19.30 to 24.00 hrs respectively. In addition, the fish was handfed to satiety once per day. The fish was kept for acclimatizion and fed a commercial feed for 4 weeks before three tanks were randomly allotted to each diet. The fish was monitored daily for appetite and general appearance. Total fish weight and fish number in each tank were recorded at the start of the experiment, after 6 weeks and at the termination of the experiment after 11 weeks of experimental feeding. At the termination of the experiment 30 fish from each tank from diets 1,2 and 3 were randomly taken and their weight and length measured for determination of the condition factors. Further at termination 10 fish with the higher condition factor and 10 with the lower were selected for morphological examination and tissue sampling of lever, intestines, pancreas, kidneys and muscular tissue (muscularis longissimus dorsi, 1 cm caudal to the back fin). Immediately after being satisfactorily anesthesized, the actual organ tissues were cut out and fixed in 4% formaldehyde in phosphate buffer. The fish was also dissected and examined for clinical signs of abnormalities. A further 5 fish were randomly selected from each of groups 1,2 and 3 for inspection of the alimentary canal, and 3 fish from each group were used for further histological examination of the alimentary tract.

Histological examination

Organ tissues from 5 fish randomly taken from each of 10 fish with low and 10 fish with high condition factor (i.e. Weight/Length) in experimental groups 1,2 and 3 were embedded in paraffin, sectioned to approximately 5 ym and stained with HES. Examination was done by light microscopy at 100x magnification. Five further fish from each of groups 1,2 and 3 were taken for morphological examination of the alimentary canal after dissection from the oesophagus to the rectum. From 3 of these fishes a section of the intestine, in the transition between the anterior and posterior part, was taken out and fixed for later histological examination as previously described.

Analytical methods

Crude protein (Nx6.25)was determined by the Kjeldahl method (ISO 5983-1979) and moisture (ISO 6496-1983) and ash (ISO 5984-1978) gravimetrically after drying for 4 hrs at 105 oC and after combustion for 16 hrs at 450 oC, respectively. Lipids were determined according to the Soxhlet method by petroleumbenzin extraction (AOCS Ba-38). TVN (Total Volatile Nitrogen) was determined by destilation (AOAC, Methods of Analysis, 1984,2.065). Biogenic amines were determined at the Torry Research Station by a modification of the method described by Seiler and Knødgen (5) using an improved reversed-phase high-performance liquid chromatography system. For determination of watersoluble protein 10.0 g of fish meal was added 200 ml of destilled water and kept in boiling water for 30 minutes, filtered and protein in the supernatant determined by the Kjeldahl method.

True protein digestibility

True protein digestibility was determined in mature male mink as described by Skrede (6).

Statistical Methods

Test for statistical significance between dietary groups for the parametric observations was conducted by single classification analysis of variance and determination of LSD (least significant difference) (Snedecor and Cochran,7). Observations on histopathology did not follow a normal distribution and were considered to be non-parametric and tested by the chi-square test.

Results

Growth and feed consumption.

The overall results from each individual tank are shown in Appendix Table 1. The experiment was uneventfully conducted. Losses of fish were small in all tanks. No abnormal behaviour was detected. Differences between tanks within diets were low. Table 3 shows mortality, growth and feed consumption and feed conversion in the different groups for the first 6 weeks (period 1) and the last 5 weeks (period 2) and for the entire experimental period. Mortality was highest in group 2 fed fish meal made from stale fish and lowest in group 1 fed fish meal made from fresh fish but the differences to the other groups fed diets with added biogenic amines were small. There were no differences in growth rate between fish fed fish meal made from fresh fish and those fed the same fish meal with added biogenic amines, all having growth rates about 30% above the Akvaforsk growth table (Aust reng *et al.* ,4). Fish fed fish meal made from stale fish grew on an average at only 53 % of the rate (61 % in period 1 and 48 % in period 2) and significantly slower than those fed fish meal from fresh fish. There were no differences in amount of offered feed between the different groups in period 1, but feed consumed per unit weight increase was more than 150 % and significantly higher in group 2 compared to the other groups. It is likely that the fish in group 2 was overfed and that a significant amount of the offered feed was lost through the drains, unobserved due to the small amount of feed being eaten at this time. Due to reduced feed intake the amount of feed offered to group 2 in period 2 was less than 50 % and significantly lower than that of the other groups. There were no differences in feed conversion between the different groups in period 2. For the whole experimental period, significantly less feed was offered and feed conversion was significantly poorer in group 2 compared to group 1 and the other groups between which there were no differences. The condition factor (weight for length) was significantly lower in group 2 fed fish meal made from stale fish compared with fish fed fish meal made from fresh fish or this fish meal added biogenic amines.

Histopathology

The results of the histopathological examination are shown in Table 4. The pathologist's comments are given in Appendix 1. Pathological changes related to diets were found in the liver and intestines. Numbers of fish with affected livers were 7 out of 10 in group 2 fed fish meal produced from stale fish which was significantly ($P < 0.05$) higher than the 3 out of 10 found in group 1 fed fish meal made from fresh fish or 2 out of 10 in group 3 fed fish meal made from fresh fish with added biogenic amines. Also lesions in the livers were significantly ($P<0.001$) more severe in group 2 compared to the other groups. Thus 7 out of 10 fish in group 2 had lesions that were characterised as most probably affecting the function of the liver. No differences between fish with high and low condition factor were found.

Three out of three fish in group 2 fed fish meal made from stale fish showed severe pathological changes in their intestines as compared to none in the groups fed fish meal made from fresh fish or this fish meal added biogenic amines, the difference between groups being highly significant ($P<0.001$)

Discussion

The present study confirm the findings in previous experiments conducted by IFOMA (1,2) that fish meal made from stale fish reduces performance in salmon compared to fish meal made from fresh fish. In the present experiment growth in salmon fed fish meal from stale fish was reduced by 53 % compared to that of salmon fed fish meal made from fresh fish. This is somewhat higher than the 43 % reduction found in the previous trial with herring (1) and 30 % in the trial with anchovy (2). However the degree of spoilage may not have been the same in the different trials.

It was the intention in the present study to try to separate effects of raw material freshness on growth and feed consumption, i.e. appetite. The results from the present experiment confirm earlier experience of the difficulties involved in measuring feed consumption in fish. In the first priod no difference in feed consumption between groups could be noted and the different groups were offered the same amount of feed, which resulted in severely depressed feed utilisation in the group fed the fish meal made from the stale fish. In the second period it was possible to better observe feed consumption and the amount of feed offered could be adjusted accordingly, and no difference in feed conversion was seen between the various groups. Since an organotoxic effect of fish meal made from stale fish is shown in this study, the question whether this effect is first seen on growth or on appetite is of little practical importance.

It has been believed that the reduced performance found in salmon fed fish meal made from stale fish was due to the content of biogenic amines. The present study shows conclusively that the reduced performance is not caused by biogenic amines, but is due to other hitherto unknown factors that are formed in spoiling fish or by processing of spoiled fish and are present in the meal at a level that correlate with the content of biogenic amines.

The present study showed conclusively that the reduced performance experienced in fish fed fish meal made from stale fish is the results of a toxic effect leading to pathological changes in vital organs like the liver and the intestines.

Proposal for further studies.

It is now evident that fish meal made from stale fish possesses a factor(s) that is toxic for fish and that this factor(s) is formed during storage of the fish or during processing of stale fish. It is of great importance to the fish meal industry to identify this factor(s), to learn how it is formed and in which fraction of the meal it is found. Further studies should be conducted to elaborate these questions.

Literature.

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Table 1. Chemical composition and protein digestibility of the fishmeals.

FISHMEAL	FM1 (fresh)	FM2 (stale)
Protein (Nx6,25), %	73.4	73.4
Moisture, %	7.6	6,1
Ash,%	11.1	9.7
Fat (Soxhlet), %	8.8	11.3
TVN, %	0.16	0.20
Water soluble protein, % of total protein	24.5	27.5
Protein digestibility (mink) %	92.7	92.2
Biogenic amines. g/kg:		
Tyramine	0.021	1.337
Putrescine	0.073	1.465
Cadaverine	0.079	3.254
Histamine	0.009	2.763

Table 2. Ingredient content and chemical composition of experimental diets.

Diet	1	2	3	4	5	6
FISH MEAL (FM)	FM1	FM2	FM1	FM1	FM1	FM1
AMINE ADDED	-	-	CHPT	CPT	HPT	CH
FISH MEAL, %	61.31	61.31	61.31	61.31	61.31	61.31
FISH OIL[2], %	20.60	19.07	20.60	20.60	20.60	20.60
SUPREX MAIZE [3],%	16.15	17.68	15.62	15.79	15.82	15.79
Soyalecithin [4], %	0.50	0.50	0.50	0.50	0.50	0.50
Vit.mix.[5], %	1.00	1.00	1.00	1.00	1.00	1.00
Min.mix. [6], %	0.40	0.40	0.40	0.40	0.40	0.40
Charophyl pink 8%,%	0.04	0.04	0.04	0.04	0.04	0.04
Biogeminc amines, g/kg Tyramine	-	-	0.81	0.81	0.81	-
Putrescine	-	-	085	0.85	0.85	-
Cadaverine	-	-	1.95	1.95	-	1.95
Histamine	-	-	1.69	-	1.69	1.69
Chemical composition (as analysed): Potein (Nx6.25),%	48.0	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, g/kg.	0.044	0.866	0.720	0.720	0.749	0.062
Putrescine, g/kg	0.067	0.828	0.624	0.627	0.727	0.076
Cadaverine, g/kg	0.104	2.156	1.649	1.724	0.137	1.424
Histamine, g/kg	0.048	1.742	1.584	0.061	1.628	1.423

1) C = Cadaverine, H = Histamine, P=Putrescine, T=Tyramine
2) Norsalmoil, Norsildmel, Bergen - Norway
3) Precooked maize, Cadrico B.V. Rotterdam
4) Denofa, Norway
5) Vit.mix. support per kg of feed:
Vit. A 3000 IU, Vit D3 600IU, Vit.E 160 mg, B1 12 mg, B2 24 mg, B6 12 mg, Vit.C 60 mg, Calpan 48 mg, Vit.H 0.6 mg, Foline 6 mg, Niacin 120 mg, Vi. B12 0.024 mg, MPB/K3 12 mg.
6) Min.mix. support per kg of feed:
Mg 500 mg, Cu 5 mg, Mn 10 mg, K 400 mg, Zn 80 mg, Fe 50 mg.

Table 3. Performance of salmon fed diets with fishmeal made from fresh or stale fish or made from fresh fish with added biogenic amines.

Diet	1	2	3	4	5	6	
Fishmeal [1)]	FM1	FM2	FM1	FM1	FM1	FM1	
Biogenic amines[2)]	-	-	CHPT	CPT	HPT	CH	SEM[6)]
Mortality %	0.2	1.6	1.0	0.2	0.4	0.6	-
Growth 1. period g/fish	80a	31b	89a	85a	85a	87a	5xx
Growth 2. period g/fish	132a	68b	141a	147a	141a	131a	7xx
Growth, Total g/fish	212a	99b	230a	233a	226a	218a	12xx
Specific growth rate[3)], %	1.55a	0.94b	1.61a	1.62a	1.60a	1.55a	0.06xx
Feed offered 1.period g/fish	85a	87a	90a	85a	88a	87a	1
Feed offered 2.period g/fish	105a	49b	113a	111a	116a	112a	6x
Feed offered, total, g/fish	190a	135b	202a	196a	204a	200a	6x
Feed conversion[4)], 1. period	1.06a	2.82b	0.99a	1.01a	1.05a	1.01a	0.17x
Fedd conversion, 2. period	0.80a	0.73a	0.80a	0.75a	0.82a	0.86a	0.02
Feed conversion, Total	0.90a	1.37b	0.88a	0.84a	0.90a	0.91a	0.05x
Condition factor[5)]	1.32a	1.20b	1.32a	-	-	-	0.01xx

1) FM1 produced from fresh raw material, FM2 produced from stale raw material.
2) Amines: C=Cadaverine, H=Histamine, P=Putrescine, T=Tyramine
3) Specific growth rate: = 100 (ln final weight - ln initial weight)/no. days.
4) Feed conversion: Feed offered/gain.
5) Condition factor: $\frac{\text{weight}}{\text{length}^3} \times 100$
6) SEM = Standard error of means. xx $P<0.01$, x $P<0.05$.

Values not followed by a letter are significantly different.

Table 4. Organ histology in Atlantic salmon fed dies with fish meal produced from herring of varying freshness and added biogenic amines.

Diet	1	2	3	Significance
Fish meal	FM 1	FM 2	FM1	
Added amines	-	-	CHPT[1)]	
		Incidence (number affected/total numbers)		
Pancreas	0/10	1/10	1/10	P >0.05
Muscle	10/10	10/10	10/10	P >0.05
Kidneys	1/10	0/10	0/10	P >0.05
Liver	3/10	7/10	2/100/10	P <0.05
Intesines	0/10	10/10		P <0.001
		Lesion severtiy [2)]		
Grade				
0	7	3	7	
1	3	0	2	
2	0	7	0	P <0.001
3	0	0	0	

1) For explanation see Table 2

2) 0 = Normal
1 = Minor changes. Most probably reversible to normal
2 = Significant changes. Most probably affecting the function of the organ
3 = Strong changes. Irreversible. Necrosis

APPENDIX 1

PATHOLOGIST'S COMMENTS

Pancreas muscle. kidney and liver

Classification

The changes found by the histological examinations might be categorised as follows:

0 - Normal features
1 - Minor changes - most probably reversible to normal
2 - Significant changes - Most probably affecting the function of the organ
3 - Strong changes - Irreversible - Necrosis

Results

Experimental group 1:

Big fishes,	5 pancreas:	0-0-0-0-0
	5 muscle:	1-1-1-1-1
	5 kidney:	1-0-0-0-0
	5 Liver:	1-0-0-0-0
Small fishes,	5 pancreas:	0-0-0-0-0
	5 muscle:	1-1-1-1-1
	5 kidney:	0-0-0-0-1
	5 Liver:	1-0-1-0-0

Experimental group 2:

Big fishes,	5 pancreas:	0-1-0-0-0
	5 muscle:	1-1-1-1-1
	5 kidney:	0-0-0-0-0
	5 Liver:	2-2-0-2-2
Small fishes,	5 pancreas:	0-0-0-0-0
	5 muscle:	1-1-1-1-1
	5 kidney:	0-0-0-0-0
	5 Liver:	2-2-2-0-0

Experimental group 3:

Big fishes,	5 pancreas:	0-0-0-0-0
	5 muscle:	1-1-1-1-1
	5 kidney:	0-0-0-0-0
	5 Liver:	0-0-1-1-0
Small fishes,	5 pancreas:	0-0-0-0-0
	5 muscle:	1-1-1-1-1
	5 kidney:	0-0-0-0-0
	5 Liver:	0-0-0-0-0

Conclusion

The observed changes might be described as follows:

Pancreas:
1 big fish from group 2 had mild degeneration characteristics in the form of oedema. Most pancreas tissues had peritonitis.
This is caused by the vaccine with which the fish at an earlier stage of life were injected with intraperitoneally.

Muscle:
All tissues had more or less obvious signs of degenerations. This consisted of mucus piling and more significant changes as destruction of muscle fibres. It is uncertain what causes muscle degeneration in fish. It is often observed in connection with pancreas necrosis, but is also quite common in periods of rapid growth of the fish.

Kidney:
1 big fish from group 1 showed mild degeneration characteristics as shrinkage of glomerules and degenerated cells in nephrotubuli.

Liver:
In our opinion we found the most significant changes in the livers. In group 2, 6 out of 10 livers had significant degenerations: Thickly vacuolized liver cells with pyknotic nuclei. In some of the livers the cell structures were partially disintegrated. These changes are reminiscent of what is described to develop during prolonged toxic exposure. In group I there were degenerative changes in 3 out of 10 livers. These changes had in general the same features as for group 2, but significally milder. In group 3 there were changes in 2 out of 10 livers. These changes differed from those observed in group 1 and 2. In group 3 there were regular infiltrations of fatty cells in the liver parenchyma. The liver cells were normal.

Gastro-Intestinal tract

Group 1:

All fish appeared to be clinically normal.

Group 2:

All fish showed signs of abnormal changes. From the pylorus portion to the anterior part of the posterior intestines there was an abnormal whitish colour and the intestine itself was thickened. The whitish colour was also shown on some of the pylorus caeca. It seemed as if these changes became less prominent from the anterior to the posterior part of the affected area. Inside the intestine the affected areas seemed to have a particulate structure of the mucosa

Group 3:

All fishes seemed to be clinically normal.

Histological examination:

In the affected areas, the epithelial cells of the mucosa were degenerated. Most significant features were strongly vacuolized cells with condensed mucins, which had migrated towards the basal end of the cells. The mucosa of the affected areas were generally oedematous.

Bergen, February 1996
Hans Aase, fish pathologist

Appendix table 1. Growth rate, offered feed and growth in individual fish tanks.

DIET	1			2			3			4			5			6		
FISHMEAL[1)]	FM1			FM2			FM1			FM1			FM1			FM1		
BIOGENIC AMINES[2)]	-			-			CHPT			CPT			HPT			CH		
Tank no.	5	11	17	6	12	18	7	13	19	8	14	20	9	15	21	10	16	22
No. fish at start	165	165	165	165	165	165	165	165	165	165	165	165	165	165	165	165	165	165
No. fish at end	165	165	164	161	164	162	163	165	162	165	165	164	164	164	165	165	163	164
Bodyweight at start, g	100	101	97	99	98	100	100	100	100	100	101	100	99	99	102	100	100	104
Bodyw. after 6 weeks, g	185	180	174	134	124	132	196	191	181	186	188	182	172	193	191	181	193	190
Bodyweight at end, g	325	314	298	208	195	192	344	335	313	350	322	327	307	333	339	315	324	319
Feed offered 1. period g/fish	88	87	81	85	86	87	87	92	87	84	84	88	84	87	94	88	83	91
Feed offered 2. period «	110	107	99	50	49	49	120	128	92	113	107	112	108	121	118	108	115	114
Feed offered Total «	198	194	180	135	135	136	207	220	179	197	191	200	192	208	212	196	198	205
Condition factor [3)]	1.32	1.29	1.35	1.19	1.25	1.15	1.36	1.31	1.30	-	-	-	-	-	-	-	-	-

1) FM1 = Produced from fresh fish. FM2 = Produced from fresh stale fish.

2) C = Cadaverine, H = Histamine, P=Putrescine, T=Tyramine

3) Condition factor = $\frac{\text{weight}}{\text{length}^3} \times 100$

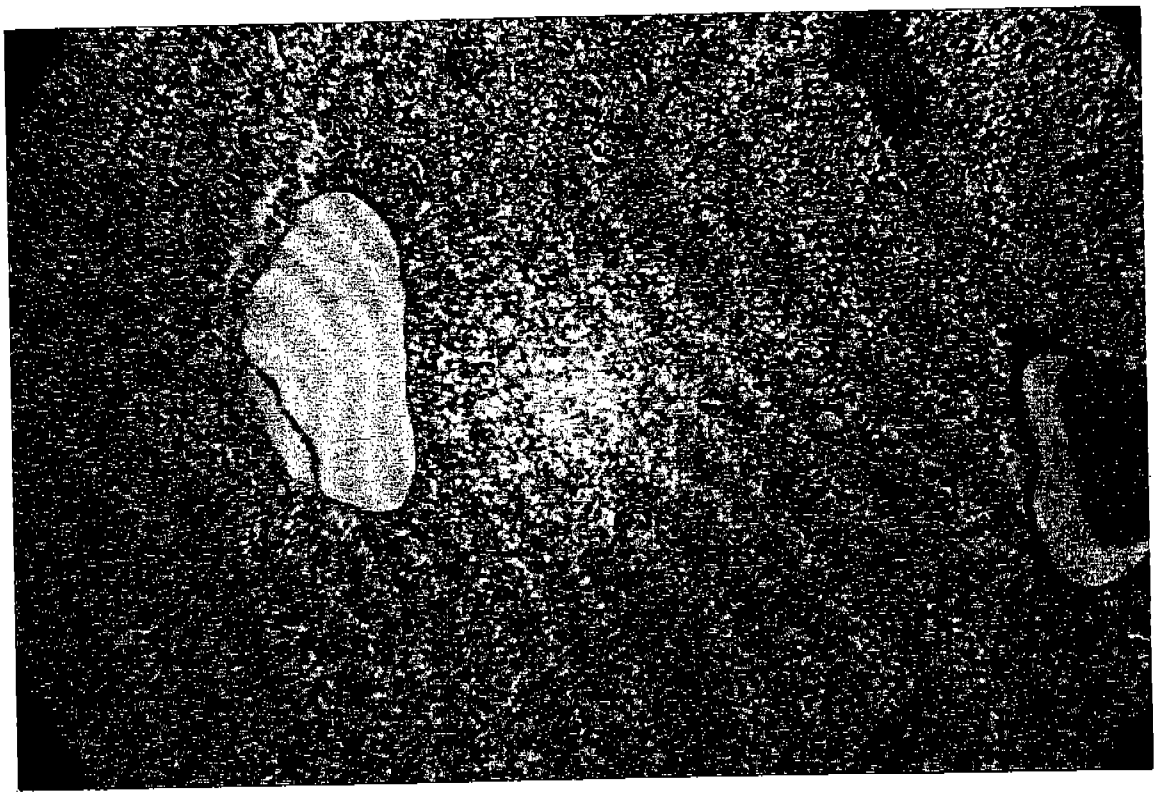

Plate 1. Generally degenerated liver tissue. Heavily vacuolized cells and pycnotical nuclei

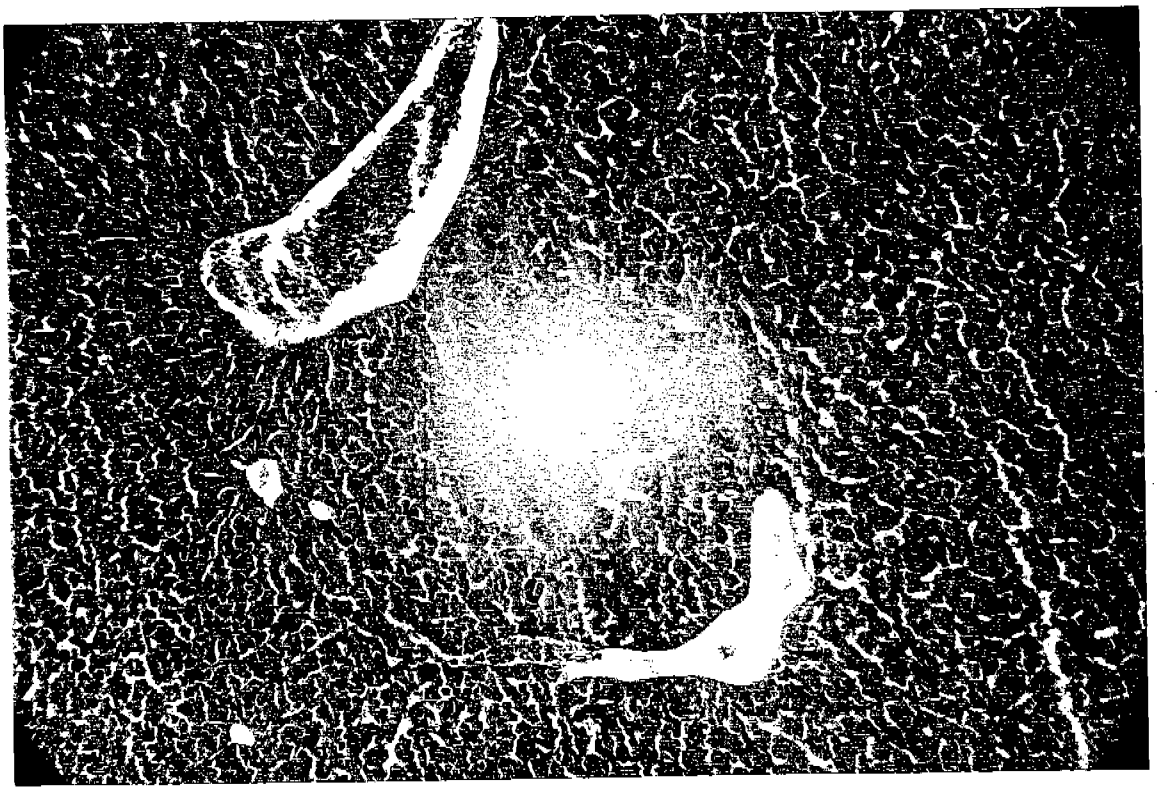

Plate 2 Normal liver

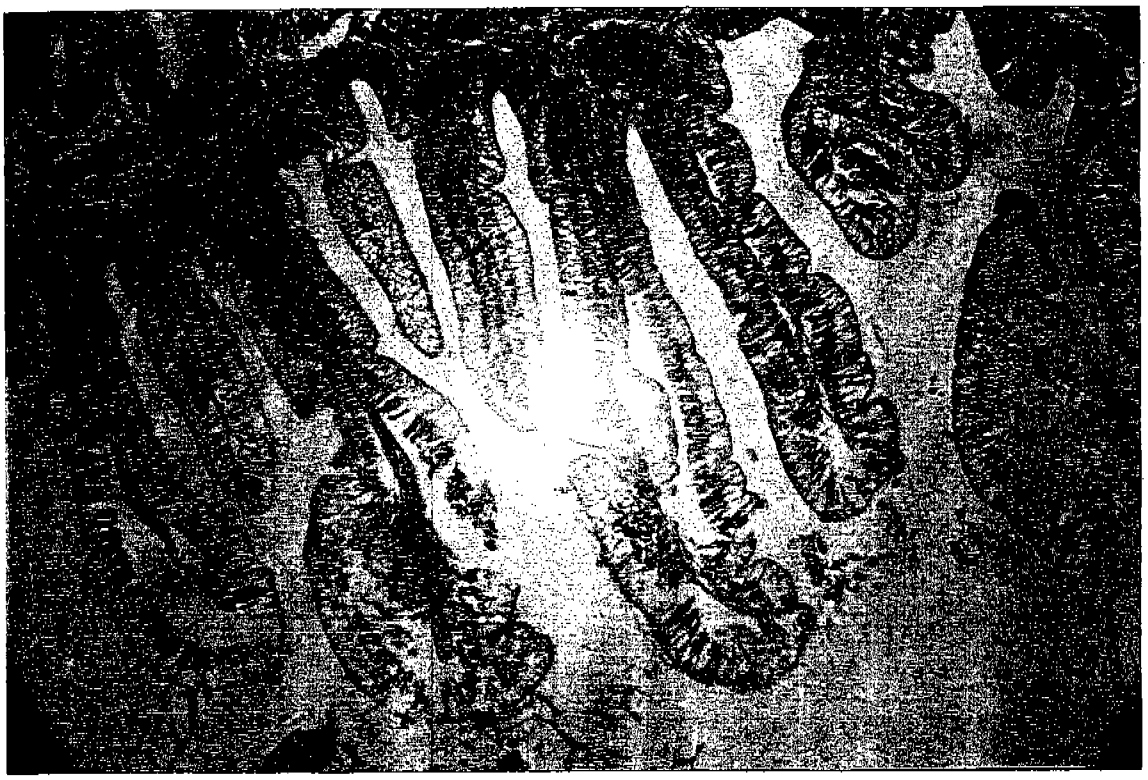

Plate 3 Intestinal tissue. Heavily degenerated cylindrical epithelial cells