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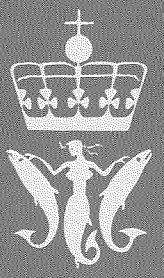

DIRECTORATE OF FISHERIES
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# THE STABILITY AND BIOLOGICAL AVAILABILITY OF DIFFERENT FORMS OF VITAMIN C IN FEED FOR ATLANTIC SALMON *(SALMO SALAR)*.

By

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## ABSTRACT

Vitamin C losses due to feed processing and storage, and subsequent efficiencies as vitamin C sources in Atlantic salmon *(Salmo salar)* were studied using six commercial available vitamin C compounds. These were crystalline ascorbic acid (AA), the coated products Davitin C80 and Aqua Stable, and the AA derivatives Ester C calcium ascorbate, ascorbate-2-sulphate (AS) and ascorbate polyphosphate (ApP).

The best stability in the feeds during processing and storage was found for ApP and AS. Davitin C80 was fairly stable during storage when coated by spraying onto the pellets in capelin oil suspension, but the extrusion process enhanced the breakdown of this product. None of the other vitamin C forms showed acceptable stability throughout processing and storage.

Atlantic salmon were fed diets to which were added the vitamin C forms suspended in the oil after extrusion and stored for 11 weeks at 20 °C. Ascorbate polyphosphate was superior to the other forms in terms of liver AA retention.

Parts of this material was presented as a poster at The Third International Symposium on Feeding and Nutrition in Fish, Toba, Japan, 1989.

## INTRODUCTION

Several approaches have been tried to overcome the problem of oxidation loss of ascorbic acid (AA) in practical fish diets. Coated products containing AA and chemical derivatives of AA have been introduced. For inclusion in fish feeds the compounds should be stable during processing and storage and possess vitamin C bioactivity.

An ethyl cellulose coated AA product has been reported to be more stable than crystalline AA during cold pelleting/drying, steam pelleting and extrusion (Hilton *et al.*, 1977; Lovell and Lim, 1978; Sandnes and Utne, 1982). Triglyceride coated AA and AA coated with a synthetic polymer have been reported to be more stable than crystalline AA during feed processing and storage, and were found to be efficient as vitamin C sources (Soliman *et al.*, 1987; Skelbaek *et al.*, 1990).

Among the chemical AA derivatives, ascorbyl palmitate (Albrektsen *et al.*, 1988), ascorbate-2-sulphate (AS), ascorbate polyphosphate (ApP) and ascorbate monophosphate (AmP) have been studied. These compounds have been shown to exhibit good feed stability, but as recently reviewed by Sandnes (1991) the phosphate derivatives are superior to the sulphate as regards bioactivity in fish (Sandnes, 1991).

The aim of the present study was to compare processing and storage stability of some coated AA products and AA derivatives, integrated with a bioactivity study in Atlantic salmon *(Salmo salar)*.

## MATERIALS AND METHODS

*Feed formulation and processing*

A commercial salmonid fish feed formulation (Tess Edel, Skretting A/S, Norway) without any vitamin C supplementation was applied as the basal mash in this study.

The following forms of vitamin C were tested: Crystalline AA, Davitin C80 (coated), Aqua Stable (coated), Ester C calcium ascorbate (derivative), ascorbate-2-sulphate (derivative), ascorbate polyphosphate (derivative). A non – supplemented feed was used as control.

The vitamin C forms were supplemented in the feeds at a level equivalent to 500 mg AA/kg complete diet, either a) in the dry mix before extrusion of the feeds or b) in a suspension of capelin oil sprayed (coated) onto the pellets after extrusion.

The feeds were extruded using a pilot extruder (Clextral BC-45). The process lasted 13 to 14 seconds. The temperature in the sylinder material varied between 103 °C and 121 °C and in the outlet approx. 116 °C, and the pressure was 22 bar.

After extrusion the pellets were dried for 13 minutes, and the temperature at the end of the drying process was 57 °C.

The pellets were coated with a) capelin oil only or b) capelin oil containing a suspension of the vitamin C forms (mixed at 30 °C until a homogenous suspension was obtained) in a feed mixer in 30 kg portions. All feeds were stored at 4 °C and 20 °C in black plastic bags.

*Feed sampling and ascorbate analyses*

During processing samples were collected of the dry unsupplemented feed mash, mash including vitamin C forms and extruded pellets prior to and after oil coating. The feeds were stored at 4 °C and 20 °C for 11 weeks. Feed samples were collected initially and after 1, 5 and 11 weeks of storage.

*Feeding experiment with Atlantic salmon*

The feeds coated with capelin oil containing vitamin C which had been stored at room temperature (20 °C) and a control diet devoid of AA were used in a bioavailability study with Atlantic salmon. As the pellet size was too large to be fed directly (5 mm), the feeds were gently crushed and a proper particle size of 3 mm was sifted out. During the feeding study the experimental feeds were stored at –20 °C and portions of the feeds were taken out daily.

Atlantic salmon were fed the control diet for 8 weeks before feeding the experimental diets (in duplicate) for 4 weeks at a water temperature of 12 °C. Fish weight at start of the pre-experimental period was approximately 25 g. Samples of fish were collected initially (n = 38) and thereafter 10 fish from each tank were sampled weekly for analysis of AA in liver tissue.

*Analytical methods*

Ascorbic acid was determined in feeds (3 replicates) and liver tissue by means of a high performance liquid chromatography (HPLC) method described by Schüep *et al.* (1984) and AS was assayed by the same method modified according to Sandnes *et al.* (1990). Analyses of ApP in the feeds were carried out by Rangen Inc., USA (blind coded samples) according to a method using enzymatic cleavage of the phosphate moieties (unspesific phosphatase) prior to determination of AA (Wang *et al.*, 1988).

## RESULTS AND DISCUSSION

*Feed processing and storage*

Of the vitamin C forms tested, ApP and AS showed best stability in the feeds during processing and storage (Tables 1 and 2). Davitin C80 was fairly stable during storage when coated onto the pellets, but the extrusion process enhanced the breakdown of this product. Ester C was the least stable of the chemical derivatives, regardless of the way of supplementation. The retention of Aqua Stable after the storage period was low when stored at 20 °C, but somewhat better at 4 °C.

*Table 1.* Retention of vitamin C sources in fish feed after processing and storage for 11 weeks at 20 °C.

| | Sources added in mash before extrusion (corrected for lipid content) % retained of supplemented | | | | | Sources suspended in the oil added after extrusion % retained of supplemented | | |
|---|---|---|---|---|---|---|---|---|
| Source of vitamin C | | Weeks of storage | | | | Weeks of storage | | |
| | MASH | 0 | 1 | 5 | 11 | 1 | 5 | 11 |
| Crystalline AA . | 112 | 34 | 22 | 5 | 3 | 95 | 12 | 3 |
| Davitin C80 ..... | 91 | 49 | 35 | 7 | 2 | 64 | 45 | 28 |
| Aqua Stable ..... | 101 | 39 | 28 | 6 | 4 | 56 | 12 | 4 |
| Ester C ..... | 88 | 27 | 17 | 5 | 4 | 70 | 19 | 5 |
| AS ..... | 59 | 66 | 72 | 83 | 77 | 58 | 66 | 55 |
| ApP[1]) ..... | 69 | 86 | 97 | –[2]) | 105 | 90 | – | 102 |

[1]) Analysed by Rangen Aquaculture Research Center, USA.
[2]) Not analysed.

*Table 2.* Retention of vitamin C sources in fish feed after processing and storage for 11 weeks at 4 °C.

| | Sources added in mash before extrusion (corrected for lipid content) % retained of supplemented | | | | | Sources suspended in the oil added after extrusion % retained of supplemented | | |
|---|---|---|---|---|---|---|---|---|
| Source of vitamin C | | Weeks of storage | | | | Weeks of storage | | |
| | MASH | 0 | 1 | 5 | 11 | 1 | 5 | 11 |
| Crystalline AA . | 112 | 34 | 22 | 13 | 11 | 91 | 47 | 40 |
| Davitin C80 ..... | 91 | 49 | 37 | 23 | 18 | 43 | 43 | 37 |
| Aqua Stable ..... | 101 | 39 | 26 | 18 | 15 | 85 | 51 | 68 |
| Ester C ..... | 88 | 27 | 16 | 10 | 9 | 72 | 37 | 30 |
| AS ..... | 59 | 66 | 76 | 78 | 83 | 60 | 68 | 40 |
| ApP[1]) ..... | 69 | 86 | –[2]) | – | – | – | – | – |

[1]) Analysed by Rangen Aquaculture Research Center, USA.
[2]) Not analysed.

The preferred way to supplement vitamin C in fish diets would be to include it in the mash before processing. However, due to loss during extrusion, unstable vitamin C forms have been suspended in the lipid which has been coated onto the pellets after processing. Regardless of technology, excess vitamin C has been added to compensate for losses during feed processing and storage in practice.

*Retention of AA in Atlantic salmon*

All chemically or otherwise modified essential nutrients should be carefully evaluated for their ability to replace their native forms. The bioactivity of the vitamin C compounds studied was therefore evaluated in a feeding experiment with Atlantic salmon. The fish were fed the diets with the vitamin C forms suspended in the oil added to extruded pellets and thereafter stored for 11 weeks at 20 °C. Percent vitamin C retention in these feeds are given in Table 1, and the actual levels (AA equivalents, mg/kg feed) were: 13 (AA), 141 (Davitin C80), 22 (Aqua Stable), 14 (Ester C), 276 (AS) and 512 (ApP).

The initial liver AA status (27 μg/g w.w.) indicates a physiological status near to what has been suggested as marginal for fish (Sandnes, 1991). The retention of total AA in the livers (Table 3) was already after the first week of feeding significantly higher in fish fed ApP than any other form of vitamin C. After 3 weeks the liver AA status in fish fed ApP reached a steady state.

*Table 3.* Total ascorbic acid (μg/g w.w.) in the liver of fish fed the diets with the vitamin C forms suspended in the oil added to the feeds after extrusion and storage for 11 weeks at 20 °C (see Table 1).

| Source of vitamin C | Weeks | | | |
|---|---|---|---|---|
| | 1[1]) | 2 | 3 | 4 |
| Crystalline AA | 23 ± 2[2]) | 28 ± 5 | 13 ± 2 | 24 ± 4 |
| Davitin C80 | 19 ± 4 | 31 ± 4 | 23 ± 3 | 26 ± 3 |
| Aqua Stable | 44 ± 4 | 30 ± 3 | 32 ± 3 | 29 ± 3 |
| Ester C | 15 ± 3 | 16 ± 2 | 22 ± 2 | 30 ± 3 |
| AS | 20 ± 4 | 46 ± 4 | 43 ± 4 | 37 ± 3 |
| ApP | 114 ± 9 | 126 ± 7 | 158 ± 7 | 156 ± 7 |
| No vitamin CH | 18 ± 2 | 22 ± 4 | 19 ± 3 | 26 ± 4 |

[1]) Initial value (week 0) in all groups: 27 ± 14 μg/g (X ± SD, n=38)
[2]) Mean ± SEM, n=20

Although the feed supplemented with AS contained a high level in terms of vitamin C equivalents (276 mg/kg), salmon fed AS showed only a minor increase in liver total AA concentration compared to fish fed the other forms (except ApP) which were present in low levels in the diet. Calculated from liver AA concentrations and analysed feed levels (AA equivalents), ApP was found to be 7 times more effective than AS as regards liver retention of AA.

AS has been claimed to be bioactive in some fish species and to be a storage form of AA in fish (Halver *et al.*, 1975; Tucker and Halver, 1984 a,b;

1986). However, recent studies have demonstrated a low utilization of AS as a vitamin C source in fish (Sandnes *et al.* 1990; Dabrowski *et al.*, 1990). In an *in vitro* experiment using extracts from Atlantic salmon intestinal content as enzyme source, Sandnes and Waagbø (1991) reported that phosphatase activity was present and able to hydrolyse ascorbate-2-monophosphate, while AS was unaffected. The bioactivity and metabolism of AS and phosphate derivatives of AA in fish were recently reviewed by Sandnes (1991).

The Davitin C80 test feed retained a significant amount of AA (141 mg/kg) at the end of the storage period, but after crushing and sieving the pellets in order to obtain a proper particle size, less than 10 mg/kg was recovered in the experimental feed given to the fish. This explains the low retention of total AA in fish fed this diet, as earlier studies have confirmed a good bioavailability of this product (Skelbaek et *al.*, 1988). The results point at a possibility that the Davitin C80 particles did not move into the interior parts of the pellet during coating, but were remaining on the surface with concomitant extensive loss during crushing and sieving.

In summary, ApP was superior to the other compounds tested with regard to the combined effects of feed stability and bioavailability in Atlantic salmon.

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# GROWTH AND CHEMICAL COMPOSITION OF ATLANTIC SALMON *(SALMO SALAR)* GIVEN A FISH MEAL DIET OR A CORRESPONDING FREE AMINO ACID DIET

By

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## ABSTRACT

An experiment with Atlantic salmon (*Salmo salar*) fed a diet containing LT-fish meal []: fish meal dried at low temperature, as the sole protein source and an amino acid diet of similar amino acid composition as the fish meal was run. The growth rate was significantly higher for the fish given the fish meal diet than for the fish on the free amino acid diet. Feed conversion ratio was also better on the fish meal diet. Fish given the free amino acids lost dry matter and fat compared to starter fish and fish given the fish meal diet.
Fat accumulated in the liver of fish given the free amino acid diet.

## INTRODUCTION

The nutritional value of proteins depends on the amino acid content, the protein digestibility and the utilization of the amino acids after absorption. The utilization may be for protein synthesis or for energy production.
To determine the amino acid requirements in fish, diets with graded amounts of free amino acids have been used (Cowey 1979, Wilson 1985, Walton *et al.* 1986). In such experiments the weight gain was used as criterium for protein utilization. However weight gain is not always correlated with protein utilization as fat and glycogen storage may contribute.

In salmonid fish, the time required to reach maximum concentration of free amino acids in blood is much shorter with free amino acid diets than with intact protein diets. Also the concentration is declining to prefeeding values faster than is the concentration in plasma of fish given intact protein (Plakas and Katayama 1981, Yamada *et al.* 1981 a, Cowey and Walton 1988, Murai *et al.* 1987). Similar results were obtained with carp (Plakas *et al.* 1980, Plakas and Katayama 1981).

When hydrolysed protein is fed, there seems to be a preference for the absorption of the essential amino acids (Yamada *et al.* 1981 a, Plakas and Katayama 1981, Plakas *et al.* 1980). Trout fed large doses of free amino acids were reported to have greater hepatosomatic indices than trout fed intact proteins (Walton *et al.* 1982). This may indicate that the free amino acids in such diets may be stored as fat or glycogen in the liver. Thus the utilization for protein synthesis may be impaired.

The present experiment intended to study if "protein" in the form of free amino acid is less well utilized for growth and protein incorporation than is intact protein.

## MATERIAL AND METHODS

### *Fish and feeds*

Duplicate groups of Atlantic salmon (*Salmo salar*) of approximately 88 g body weight were used. 100 fish were randomly distributed in each tank of approximately 1.5$m^3$ water volume. Temperature and salinity were 10.7±0.6°C and 15±1 $gL^{-1}$, respectively throughout the experimental period of 4 weeks. The fish were given the experimental diets daily by use of automatic feeders. The composition and the proximate analyses of the diets are given in Table 1. Minerals were added to the amino acid diet in amounts corresponding to the ash content in the fish meal. No minerals were added to the fish meal diet.

At the end of the experiment, all the fish were anaesthetized with benzocaine before they were weighed. Five fish from each tank were used for carcass analyses. Blood samples from the caudal vein in 5 other fish from each tank were centrifuged and the serum deproteinized by addition of 5% sulpho-salicylic acid (1:1), and analysed for total ammonium-N. These 5 fish were dissected and liver and muscle were analysed for dry matter, total protein, total fat and ash.

Content of carbohydrate, which probably mostly is glycogen was determined by difference.

### *Analytical methods*

Crude protein (N*6.25), ash, dry matter and fat were determined as described by Espe *et al.* (1989). Ammonia-N was determined in the deproteinized plasma by the diffusion method described by Haaland and Njaa (1988). Leaching of feed nitrogen was determined by keeping approximately 3 g of pellets in 200 ml distilled water for 30, 120 and 180 minutes. Samples of water were withdrawn and analysed for crude protein. Feed conversion ratio (FCR= fed diet g dw/liveweight gain) were calculated.

*Table 1.* Composition of the experimental diets.

| | Diet A | Diet B |
|---|---|---|
| *Composition (g kg$^{-1}$)* | | |
| LT-fish meal | 0 | 694 |
| Amino acid mixture[1]) | 502 | 0 |
| Extruded wheat | 188 | 188 |
| Capelin oil | 172 | 96 |
| Alginate | 20 | 20 |
| Vitamin mixture[2]) | 2 | 2 |
| Mineral mixture[3]) | 82 | 0 |
| Water | 34 | 0 |
| *Analytical values (g kg$^{-1}$ diet)* | | |
| Dry matter | 931 | 911 |
| Protein (dry wt) | 515 | 528 |
| Fat (dry wt) | 170 | 181 |
| Ash (dry wt) | 81 | 91 |

[1]) AA-mixture(g kg$^{-1}$ feed): Asp/Asn 22.1/22.1, Glu/Gln 34.6/34.6, OH-pro 3. 5, Ser 20.1, Gly 30.1, His*HCl 11.0, Arg*HCl 30.1, Thr 20.6, Ala 30.1, Pro 20.6, Tyr 15.6, Val 24.6, Met 15.1, Ile 21.6, Leu 37.6, Phe 20.1, Lys*HCl 91.7, Trp 5.0, Cys 5.0, Urea 20.0 and $NaHCO_3$ 16.5.

[2]) Vitamin mixture(g kg$^{-1}$ dry diet): A 12000IU, D 2000IU, E 100, K 6, $B_1$ 10, $B_2$ 20, niacin 150, pantothen acid 50, $B_6$ 10, $B_{12}$ 0.03, folic acid 4, biotin 0.8, choline 1000, C 400 and inositol 300.

[3]) Mineral mixture (g kg$^{-1}$ dry diet): $CaHPO_4*H_2O$ 30, $CaCO_3$ 3, NaCl 15, $K_2SO_4$ 20, $MgSO_4$ 10, $FeSO_4*7H_2O$ 0.7, $MnSO_4*H_2O$ 0.3, $CuSO_4*5H_2O$ 0.16, KI 0.015.

Differences between means of body weight and contents of dry matter, protein, fat and ash in the fish analysed at the start of the experiment and the corresponding individual values at the end of the experiment were used as measures of growth and storage in whole body, muscle and liver.

### *Statistical methods*

Differences between dietary treatments on growth, feed conversion and protein productive value were evaluated by simple ANOVA. Differences in the means of whole fish, muscle and liver at start and after feeding the two diets were evaluated by F-tests (Sokal and Rohlf 1969).

## RESULTS AND DISCUSSION

Leakage of nitrogenous compounds from feed to water in Experiment 1 is given in Figure 1. The diet containing free amino acids leaked much more

N-compounds to water than did the fish meal diet. In experiment with partly hydrolysed proteins in diets for carp, Wood *et al.* (1985) reported similar N-leakage differences.

The results of the feeding experiment are given in Tables 2 and 3. There were no significant differences between duplicates, they were therefore treated as one group. Weight gain during the 4 weeks of the experiment differed significantly ($p<0.010$), the fish given the fish meal showing the greatest weight gain. Also the feed conversion ratio (FCR) was significantly ($p<0.05$) better for these fish. Reduced weight gain by feeding part of the total feed protein as free amino acids relative to an intact protein source was reported by Aoe *et al.* (1970), Walton *et al.* (1982), Wood *et al.* (1985), Walton *et al.* (1986) and Cowey and Walton (1988). The lower FCR found for fish given the free amino acid diet is in agreement with results reported for mirror carp when fed partly hydrolysed proteins as fish silage (Wood *et al.* 1985) and in carp fed either free amino acid diet or casein (Yamada *et al.* 1981 b).

Analysis of whole fish at the start and the end of the experiment showed a significant ($p<0.001$) reduction in total body fat for those fish given the free amino acid diet. These fish also had significantly ($p<0.01$) less dry

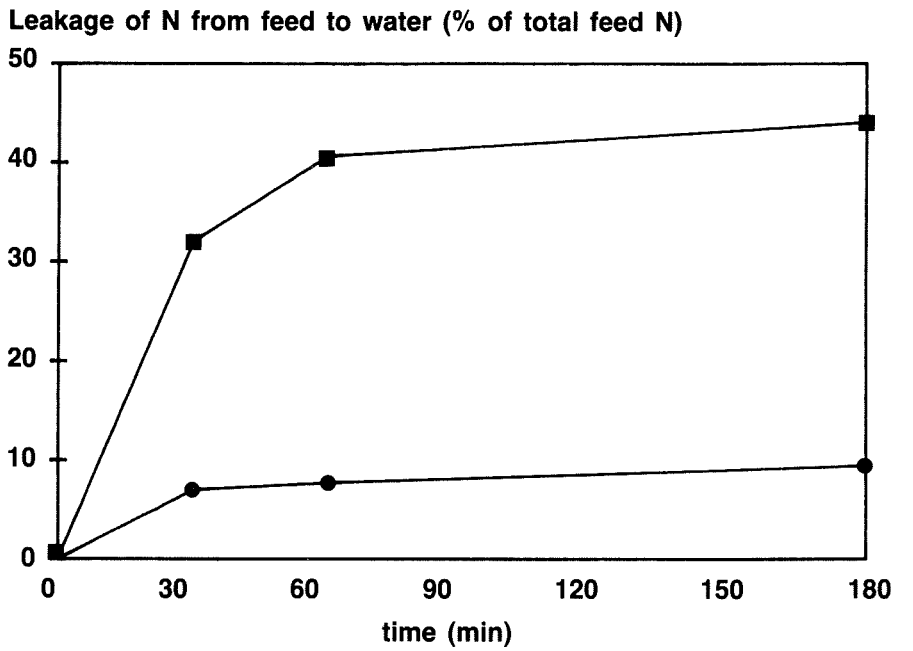


*Figure 1.* Leakage of nitrogenous compounds from feed to water. (■) is diet A in which the protein source is free amino acids, and (●) is diet B in which the protein source is low temperature dried fish meal. Water was analysed for N*6.25 after storing the feed pellets in water for 30, 60 and 180 minutes.

*Table 2.* Total weight gain and feed conversion ratio (FCR)[1]) of fish given either a diet of free amino acids simulating fish meal, diet A, or a fish meal diet, diet B, for 4 weeks.

| | Diet A | Diet B | ANOVA |
|---|---|---|---|
| Final weight (g)[2] | 100 ± 13 | 141 ± 19 | |
| Weight gain (g) | 12 | 53 | ** |
| FCR(n=2) | 1.5 ± 0.2 | 0.9 ± 0.1 | * |

Initial weight 88 ± 11g
*=$p<0.05$, **=$p<0.010$

[1]) FCR= feed intake (g dry wt) / liveweight gain.
[2]) Average from duplicate tanks.

*Table 3.* Dry matter and protein, fat and ash (g $100g^{-1}$ dry weight) in whole fish, muscle and liver at start of the experiment and after 4 weeks of feeding the experimental diets. Hepatosomatic indices (HSI) are also given for the liver. Each value is the mean ± SEM, n=10. Diet A is the amino acid diet and diet B is the LT-fish meal diet. Differences in statistics are indicated.

| | At start | After 4 weeks with experimental diets | |
|---|---|---|---|
| | | Diet A | Diet B |
| *Whole fish* | | | |
| Dry matter | 26.6 ± 0.2$^{A}$ | 25.2 ± 0.4$^{B}$ | 25.9 ± 0.2$^{A}$ |
| Protein | 69.9 ± 0.5$^{A}$ | 72.8 ± 0.7$^{B}$ | 70.5 ± 0.7$^{A}$ |
| Fat | 21.6 ± 0.4$^{A}$ | 14.9 ± 1.0$^{B}$ | 18.9 ± 0.5$^{A}$ |
| Ash | 9.4 ± 0.3$^{A}$ | 9.1 ± 0.3$^{B}$ | 8.0 ± 0.2$^{C}$ |
| Carbohydrate | 0.8 ± 1.5 | 3.2 ± 2.1 | 2.6 ± 1.2 |
| *Muscle* | | | |
| Dry matter | 23.7 ± 0.2$^{A}$ | 22.6 ± 0.3$^{B}$ | 23.5 ± 0.3$^{A}$ |
| Protein | 76.9 ± 0.6$^{A}$ | 83.2 ± 0.7$^{B}$ | 79.1 ± 0.9$^{A}$ |
| Fat | 10.2 ± 0.7$^{A}$ | 3.7 ± 0.6$^{B}$ | 10.0 ± 0.8$^{A}$ |
| Ash | 6.3 ± 0.1$^{A}$ | 6.2 ± 0.1$^{A}$ | 5.8 ± 0.1$^{B}$ |
| Carbohydrate | 6.5 ± 0.7 | 6.9 ± 1.3 | 5.1 ± 1.3 |
| *Liver* | | | |
| Dry matter | 20.9 ± 0.3$^{A}$ | 19.9 ± 0.4$^{B}$ | 21.7 ± 0.4$^{A}$ |
| Protein | 61.4 ± 1.4$^{A}$ | 57.6 ± 1.3$^{A}$ | 63.3 ± 1.7$^{A}$ |
| Fat | 15.8 ± 1.4$^{A}$ | 25.7 ± 1.8$^{B}$ | 14.0 ± 0.5$^{A}$ |
| Ash | 4.7 ± 0.2$^{A}$ | 3.8 ± 0.3$^{B}$ | 5.3 ± 0.2$^{C}$ |
| Carbohydrate | 18.1 ± 5.5 | 12.9 ± 2.3 | 17.4 ± 4.8 |
| HSI | nd | 1.1 ± 0.1 | 1.1 ± 0.1 |

Horizontal lines with different superscripts differ significantly.
Carbohydrate is determined by difference.
n.d. = not determined

matter. For protein and ash only minor differences were seen. The same tendency was also found for muscle. A significant ($p<0.001$) increase in muscle protein content was found in fish given the free amino acids (Table 3). Analyses of livers showed a significant ($p<0.001$) increase in the fat content for the fish given free amino acids. Also less dry matter ($p<0.01$) and ash ($p<0.001$) were found (Table 3). Despite the increase in hepatic fat content in the free amino acid group, no increase in hepatosomatic indices (HSI) were found. This is probably due to the short duration of the experiment and the very slow growth of the fish in this group compared to the fish meal group.

Savitz (1971) reported a decrease in both body protein and fat contents in Bluegill sunfish *(Lepomis macrochirus* Rafinesque) starved for 28 days compared to non starved fish. This may indicate that the fish given the free amino acid diet in the present experiment were in a state of starvation, with inadequate supply of protein to support maximal growth. Consequently body fat and body protein were used for energy.

The fact that amino acids are absorbed faster from free amino acid diets than from intact protein diets, may probably lead to scarcity of some amino acids relative to others. This may explain the reduced utilization of the free amino acid "protein" in the present study as all amino acids have to be present simultaneously for protein synthesis to occur (Geiger 1947). Plakas *et al.* (1980) reported higher concentrations of ammonia-N in plasma of carp fed a diet of free amino acids compared to a casein diet and postulated that this was a result of stimulated protein catabolism. In this experiment, however, the ammonia-N concentration in deproteinized plasma was $0.9\pm0.1$ $\mu g$ $ml^{-1}$ and $0.7\pm0.2$ $\mu g$ $ml^{-1}$ for salmon given the fish meal diet and the free amino acid diet, respectively. The higher amount of ammonia-N in plasma of fish given the fish meal may indicate somewhat higher protein turnover in these fish compared to those given the free amino acid diet, while no increased catabolism of "proteins" from the free amino acid diet is indicated. This is in accordance with results reported for Rainbow trout given diets deficient in one amino acid which did not reduce catabolism to save the lacking amino acid (Walton *et al.* 1986).

Excretion of amino acids through the gills is reported when feeding free amino acids (Murai *et al.* 1984). Concomitant with leaching of nitrogen from diet to water (Figure 1) this may be responsible for the starving-like condition found in fish given the free amino acid diet in the present experiment. Probably the leaching have been of minor importance for the reduced growth, because fish do eat the feed offered to them in relative short periods after pelletts are put into the water.

The results of this experiment show that Atlantic salmon does not utilize the "protein" from a free amino acid diet as well as the protein from a fish

meal diet for growth. More fat is stored in the liver when free amino acids are fed. No signs of increased catabolism could be detected. Further studies to determine why free amino acids are less well utilized than intact proteins is required, but before doing this it is required to develop a diet in which the problem of leakage is reduced.

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# A COMPARISION OF TISSUE LEVELS OF FOUR ESSENTIAL TRACE ELEMENTS IN WILD AND FARMED ATLANTIC SALMON *(SALMO SALAR)*

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## INTRODUCTION

The increasing production of Atlantic salmon (*Salmo salar*) has established salmon nutrition as a subject of significant academic and economic interest. An important aspect in fish nutrition has been to establish the requirements of the essential minerals and trace elements (Lall, 1989). In this connection indicators of element status in terms of tissue levels in the fish are much needed and the hepatic concentration has been widely used for several elements as such status indexes e.g. selenium, iron and copper. However, when organ concentrations are needed, one should be aware of that the elemental status of an element in terms of organ concentration reflects the combined effects of the general physiological status, the dietary intake and the exposure through water.

Several contributions have shown that there exists a relationship between dietary components and trace element concentrations in tissues of farmed salmonids (Hilton *et al.*, 1980; Wekell *et al.*, 1983; Maage *et al.*, 1989 and 1990; Julshamn *et al.*, 1990). Very little is, however, known about the tissue concentrations of essential trace elements in the wild populations of Atlantic salmon.

The wild species of Atlantic salmon are hatched in rivers and live there for 3–6 years before they enter the sea water as smolts. The wild stocks of Norwegian salmon feed and migrate along the Norwegian coast until they approach sexual maturation. They then migrate back to their native river to spawn.

As a part of our work to elucidate the trace element requirements in farmed Atlantic salmon we assumed that evaluation of the element status in the wild species might give important information about trace element contents and bioavailability in their natural food.

## MATERIALS AND METHODS

### *Fish*

Five wild Atlantic salmon were caught as they were approaching a salmon river near Stamnes in the county Hordaland in Western Norway in 1984. The fish were weighed and organs dissected.

In addition, twelve Atlantic salmon were obtained from Austevoll Fiskeindustri A/S in July, 1985. These fish had been caught by drift nets in the open sea. They were weighed and the livers were dissected and weighed.

For comparison, organs were also dissected from four farmed Atlantic salmon obtained from a commercial fish farm.

After dissection the organs were freeze dried, homogenized and stored at –20 °C before trace element analyses.

### *Analyses*

All samples were digested in a mixture of concentrated nitric- and perchloric acids (suprapure; 9:1) as described by Julshamn *et al.* (1982) and the elements were then analysed by atomic absorption spectrometry (AAS). The analyses of iron, copper and zinc were carried out by flame AAS as described by Julshamn *et al.* (1978). Selenium analyses were carried out by graphite furnace AAS and the procedure, apparatus and instrument settings are described in details by Maage *et al.* (1990).

The accuracy of the element analyses were tested in an intercalibration study arranged by ICES (Berman, 1984) as well as by analysing standard reference material from the National Institute of Standards and Technology (NIST). All methods were found to be satisfactory.

## RESULTS

As shown in Table 1 the weight range of the wild Atlantic salmon caught at sea varied from 1.6 to 6.0 kilogram.

Tables 2, 3 and 4 show the element concentrations of the organs analysed in salmon caught in the fjord and in the open sea and in farmed salmon, respectiveley.

*Table 1.* Weight, liver weight, hepatosomatic index (HSI*) and dry weight of twelve Atlantic salmon caught in the open sea.

| Fish | Fish weight | Liver weight | HSI | Dry matter |
|---|---|---|---|---|
| 1 | 1.6 | 13.7 | 0.86 | 24.3 |
| 2 | 2.3 | 26,5 | 1.15 | 26.0 |
| 3 | 2.6 | 34.3 | 1.32 | 27.2 |
| 4 | 2.7 | 30.4 | 1.13 | 25.3 |
| 5 | 2.8 | 41.3 | 1.48 | 39.0 |
| 6 | 3.4 | 53.3 | 1.57 | 31.0 |
| 7 | 3.4 | 45.8 | 1.35 | 29.3 |
| 8 | 3.5 | 50.7 | 1.45 | 22.9 |
| 9 | 4.2 | 55.1 | 1.31 | 26.6 |
| 10 | 5.1 | 74.1 | 1.45 | 25.2 |
| 11 | 5.4 | 40.6 | 0.75 | 26.6 |
| 12 | 6.0 | 71.0 | 1.18 | 25.3 |
| Mean | 3.6 | 44.7 | 1.25 | 27.4 |
| S.D. | 1.3 | 17.6 | 0.25 | 4.2 |

* 100 l liver weight/fish weight

*Table 2.* Concentration of zinc, iron, copper and selenium (mg/kg wet weight) and percent dry weight in selected organs from cultured Atlantic salmon. Mean ± S.D. (N=4).

| Organ | Dry matter % | Zinc | Iron | Copper | Selenium |
|---|---|---|---|---|---|
| Liver | 21.4 ± 2.0 | 23.7 ± 3.0 | 69.8 ± 42.6 | 35.7 ± 19.1 | 1.29 ± 0.16 |
| Kidney | 20.1 ± 2.1 | 18.2 ± 5.8 | 132 ± 69 | 2.4 ± 0.3 | 0.93 ± 0.15 |
| Gills | 29.2 ± 2.0 | 130 ± 75 | 37.6 ± 21.1 | 2.2 ± 0.8 | 0.15 ± 0.02 |
| Gonads | 35.9 ± 1.3 | 43.5 ± 10.5 | 31.7 ± 9.1 | 7.5 ± 1.5 | 1.29 ± 0.16 |

*Table 3.* Concentration of zinc, iron, copper and selenium in liver (mg/kg wet weight) from wild Atlantic salmon caught in the open sea. Mean ± S.D. (N=12).

| Organ | Dry matter % | Zinc | Iron | Copper | Selenium |
|---|---|---|---|---|---|
| Liver | 27.4 ± 4.2 | 39.5 ± 3.5 | 157 ± 61 | 195 ± 66 | 13.8 ± 4.2 |

Significant differences were found between the hepatic element concentrations, the greatest differences were between the farmed and the wild salmon. The selenium concentration showed a ten-fold difference, copper a five-fold, iron about a three-fold and zinc about a two-fold concentration in wild compared to farmed salmon.

*Table 4.* Concentration of zinc, iron, copper and selenium in organs and tissues (mg/kg wet weight) from wild Atlantic salmon caught near a spawning river. Mean ± S.D. (N=5).

| Organ | Dry matter % | Zinc | Iron | Copper | Selenium |
|---|---|---|---|---|---|
| Liver | 21.7 ± 0.6 | 45.2 ± 9.0 | 326 ± 98 | 296 ± 129 | 12.0 ± 3.0 |
| Kidney | | 21.8 ± 1.6 | 227 ± 44 | 1.7 ± 0.7 | 1.5 ± 0.8 |
| Gills | | 355 ± 93 | 35.8 ± 4.0 | 1.2 ± 0.8 | 0.3 ± 0.1 |
| Gonads | | 44 ± 21 | 14.4 ± 7.2 | 8.8 ± 2.0 | 1.1 ± 0.5 |
| Heart | | 22.5 ± 7.5 | 65 ± 52 | 2.8 ± 1.3 | 0.44 ± 0.12 |
| Spleen | | 32.2 ± 15.5 | 475 ± 130 | 1.0 ± 0.5 | 1.0 ± 0.5 |
| Intestine | | 2528 ± 558 | 52 ± 19 | 2.0 ± 1.6 | 0.7 ± 0.1 |
| Skin | | 171 ± 72 | 9.6 ± 2.0 | 3.7 ± 3.6 | 0.40 ± 0.04 |
| Brain | | 13.4 ± 1.5 | 16.9 ± 6.4 | 1.1 ± 0.5 | 0.28 ± 0.09 |
| Dark muscle | | 5.6 ± 4.4 | 8.9 ± 8.3 | 1.1 ± 0.6 | 0.21 ± 0.03 |
| Light Muscle | | 4.7 ± 2.7 | 3.6 ± 1.3 | 1.6 ± 1.9 | 0.19 ± 0.03 |
| Vertebrae | | 28.1 ± 8.2 | 9.2 ± 2.8 | 2.1 ± 0.4 | n.a. |
| Eyes | | 73.6 ± 24.4 | 12.9 ± 6.3 | 0.55 ± 0.07 | 0.42 ± 0.19 |
| Scales-front | | 145 ± 87 | 4.6 ± 2.5 | 1.6 ± 0.8 | n.d.* |
| Scales-back | | 104 ± 88 | 6.1 ± 3.9 | 1.9 ± 0.2 | n.d.* |
| Adipose fin | | 96 ± 17 | 8.2 ± 7.4 | 0.5 ± 0.2 | 0.31 ± 0.07 |
| Ventral fin | | 56 ± 16 | 8.0 ± 7.1 | 1.3 ± 0.6 | n.a. |

* n.d. = not determined.

## DISCUSSION

The main finding in this work is the great differences in the hepatic concentrations of the essential elements analysed for farmed and wild Atlantic salmon. The differences ranged from about a two-fold hepatic eleement concentration for zinc to a ten-fold for selenium. In the report by Poppe *et al.* (1986) it was also shown that hepatic concentrations of selenium and copper were higher in wild than in farmed Atlantic salmon, but they found no significant differences in iron and zinc concentrations.

Difficult questions are whether there were depressed element concentrations in the farmed salmon, whether the concentrations in the wild salmon were unphysiologically high and whether the observed values were within normal range. Regardless of the answer to the first question we also have to ask whether the different levels have any impact on health of either of the fish groups. In elucidating this one must bear in mind that even though all the four elements are essential for living organisms they are also toxic at high levels.

In a recently published work where we used fish meal with no minerals and trace elements added we found similar liver and kidney concentrations as those reported in this report for farmed salmon of these four elements

also in juvenile salmon in fresh water (Maage *et al.*, 1990). In commercial fish farms there has been added gradually more trace elements to the feeds during the last five years and today the values would probably be higher in farmed salmon.

Relationships between dietary element concentration and hepatic element concentration have been found for iron Bjørnevik and Maage (not published), selenium (Hilton *et al.*, 1980; Julshamn *et al.*, 1990), zinc (Maage and Julshamn, 1991) and copper (Julshamn *et al.*, 1988).

Hansen and Pethon (1985) showed that wild Atlantic salmon predominately eat fish and crustaceans in the sea. The latter (e.g. euphausids) are known to be higher in trace elements than whole fish and this could therfore account for the differences.

Another possible explanation of the differences found is that the life histories of the wild and farmed salmon are so different. At the time of slaughter the farmed salmon is about 3 years old and have a weight of about six kilos, while the wild salmon at this age have still not left the river and have hardly reached 100 grams.

Only about 10 percent of the ingested energy is normally used for growth so through a life span the wild salmon eat more and of course show a slower growth. So even if the wild and farmed salmon would have similar diets there would probably develop a difference in trace element concentration.

In conclusion we have found large differences in tissue levels of farmed and wild Atlantic salmon. We assume that a combination of different growth rates and different feeding regimes are the main reason for the differences found. We are, nevertheless, very careful not to draw conclusions based on these comparisons on whether the farmed salmon gets enough trace elements in their diets.

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# DIGESTIBILITY DETERMINATION IN ATLANTIC HALIBUT *(HIPPOGLOSSUS HIPPOGLOSSUS)*.

By

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## ABSTRACT

Experiments were carried out with Atlantic halibut *(Hippoglossus hippoglossus)* in order to adapt the digestibility assay with chromic oxide ($Cr_2O_3$) as an indigestible indicator for studies with this species. Macro anatomy of the digestive tract was studied, and two methods of collecting faecal samples were evaluated. Manual stripping proved to be the best method. An experiment was carried out to assess optimal time for stripping related to last feeding. Faeces was obtained at all investigated points of time (24, 28, 32 and 36 hours), but most at 28 and 36 hours after feeding. There was a large individual variation in amount of faeces, and the only reliable way to get sufficient samples seemed to be using a large number of fishes in the experiment. No simple relationship was found between amount of faeces collected and temperature, feed intake or time of stripping. The digestibility experiments showed a successive digestion and absorption throughout the intestine, and digestibility coefficients ranged from 75 to 88 for protein, from 78 to 87 for fat and from 0 to 23 for carbohydrates.

## INTRODUCTION

Nutrient digestibilities are important quality indicators in feed evaluation and useful variables in other nutritional studies with fish. Several methods have been developed for analyses of nutrient digestibility. The indicator method, using chromic oxide ($Cr_2O_3$) (Edin, 1918) as an indigestible indicator, together with stripping of faeces from the hind gut (Austreng, 1978), is well established for salmonids. This method allows the fish to be kept in its normal environment with normal feeding and care. It also has the advantage that leaching of nutrients from faeces into the water is avoided. The method does not require killing of the experimental fish, and the sampling procedure is simple. It is, therefore, possible to include a larger number of fish in the

experiments, and thus reduce the effect of individual differences and give a more reliable estimate of digestibility.

Before the indicator method can be used for a new species, such as Atlantic halibut *(Hippoglossus hippoglossus)*, several factors must be examined. The principles of the method should be applicable, but, as the halibut differs from salmonids in anatomy and feeding pattern, adjustments must be made concerning the practical aspects of the experiments, to ensure useful results.

The material presented here is drawn from a number of experiments that were conducted to gain information on how to use this method for halibut. First the anatomy of the gastrointestinal tract was studied. Then the best method for collecting faeces and the optimal time for stripping related to the last feeding were investigated. After this information was collected, a digestibility experiment was carried out. Some individuals were killed to compare the digestibility coefficients obtained from stripped faeces with digestibility coefficients obtained in different segments of the gastrointestinal tract.

## MATERIAL AND METHODS

### *General experimental conditions*

All experiments were carried out at AKVAFORSK, Sunndalsøra, during the summer of 1987 and 1988. Experimental fish were Atlantic halibut caught in the wild, and fish size varied from 0.3 to 5.0 kg. The fish were kept in circular fibreglass tanks, 2m diameter, with a water level of 80–90 cm. The tanks were supplied with sea water (salinity 32g/L) with the natural variation in temperature (7–12 °C). At most, 24 fishes were used for the experiments. The fish were fed a moist diet made from 50% herring, 25% coalfish filleting offals, and 25% binder meal, and when needed, chromic oxide (1% of dry matter). The fish were hand fed every second day, according to appetite. For stripping or other handling, the fish were anaesthetized using 30% chlorobutanol in etanol. This solution was mixed in sea water to a concentration of 1:220. Deviations from the general procedure are indicated below.

### *Anatomy of the gastrointestinal tract*

The anatomy of the gastrointestinal tract was examined in 5 individuals who died shortly after arrival at AKVAFORSK. The cause of death was most likely catching and handling stress. The fish were kept frozen until examination. We examined the location and relative size of different organs to determine how to collect faeces from live fish without causing injury.

*Collection of faeces*

Faeces were collected only from fish that had been observed eating. The fish were anaesthetized about 30 hours after feeding. This point of time was chosen after previous observations of the halibut, and references to fishes with a similar feeding pattern (De Groot, 1971). Routine resident time in the anaesthetic bath was by experience fixed to 2.5 minutes. An extension of this period would eventually cause some of the fish to defaecate in the water. Two methods for collecting faeces were compared, the use of vacuum and a pipette into the anus of the fish, and manual stripping. The former method was unsuccessful whereas the latter, which is a common method of collecting faeces from salmonids, appeared to be a useful method. The fish was put on a table, mucus and water was dried off the anus, and the sampling tube was held ready. Some fish would defaecate within 30 seconds without further treatment, but most fish needed a slight pressure over the rectum. Some fishes had empty rectum at the time of stripping.

*Optimal time of stripping*

Twelve individually tagged fish were kept in each of two tanks. They were fed the standard feed Monday, Wednesday and Friday. The fish were hand fed according to appetite. Pellet weight was standardized to 15g, and individual recording of feed intake was attempted. Fish were stripped 24, 28, 32 and 36 hours after feeding, three fish were sampled from each tank at each point of time. Individual fish weight was recorded together with weight of faeces. The following variables were calcuated:

Percent feed intake = (feed ingested (g))/fish weight (g) x 100%
Percent faeces collected = (faeces (g))/fish weight (g) x 100%
The experiment was repeated four times at varying temperatures (8.4 °C, 8.7 °C, 10.0 °C and 12.6 °C).

One way analysis of variance was used to evaluate differences among the four points of time for stripping in amount of faeces obtained. Linear regression analyses was performed to see wether the amount of faeces (percent faeces) was dependent on fish weight, time of stripping, feed intake or temperature.

*Digestibility determination*

Over a period of 6 months, four halibut were fed seven different diets in subsequent periods. The diets were formulated from mackerel, argentine, squid, capelin oil and binder meal (FK-EWOS ST 58, Pellet-mix 35%) in

*Table 1.* Proximate composition (g/100g) of feeds used for digestibility determination.

| | DIET 1 | DIET 2 | DIET 3 | DIET 4 | DIET 5 | DIET 6 | DIET 7 |
|---|---|---|---|---|---|---|---|
| Dry matter | 61.1 | 61.1 | 56.3 | 64.7 | 66.5 | 60.4 | 62.7 |
| Protein | 24.4 | 24.4 | 26.0 | 24.8 | 25.5 | 23.6 | 23.9 |
| Fat | 20.2 | 18.6 | 10.6 | 23.4 | 23.0 | 19.8 | 20.6 |
| Carboh.[1]) | 11.1 | 13.0 | 14.6 | 11.1 | 12.8 | 12.0 | 13.4 |
| Ash | 5.4 | 5.1 | 5.1 | 5.4 | 5.2 | 5.0 | 4.6 |

[1]) Carbohydrates = dry matter – (protein + fat + ash)

different amounts, and with added chromic oxide. Chemical composition is given in Table 1. Pooled samples of faeces corresponding to each feed were collected, to examine in which range digestibility would be.
Apparent digestibility coefficients were calculated as follows:

Digestibility = 100 * (D–F)/D;
D is nutrient to Cr ratio in the diet.
F is nutrient to Cr ratio in the faeces.

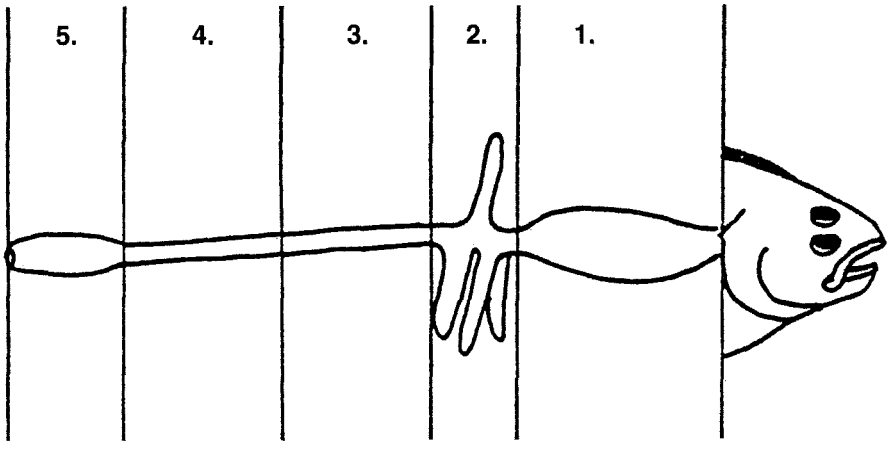
Later 24 fishes were kept in two tanks and fed a moist pellet containing chromic oxide. Feed composition is given in Table 2 . The fish were stripped and faeces was pooled into two samples. Five fishes were killed, the gastroin-

*Table 2.* Composition (g/100g) of the feed used to determine digestibility in faeces and content of different segments of the gut.

| | |
|---|---|
| *Formulation:* | |
| herring | 35 |
| coalfish filleting offals | 37 |
| capelin oil | 3 |
| binder meal 1) | 25 |
| ascorbic acid | 0.02 |
| *Analyses:* | |
| Dry matter | 43.5 |
| Protein | 19.1 |
| Fat | 8.3 |
| Carbohydrates | 11.7 |
| Ash | 4.4 |

[1]) Tess Salmomix 25%, T. Skretting A/S, Stavanger, Norway: 50% fishmeal (NorSeaMink, Norsildmel, Bergen, Norway), 40% carbohydrate feedstuffs, 10% vitamins, minerals and binder.

*Figure 1.* Schematic outline of the gastrointestinal tract in Atlantic halibut.




1. Stomach
2. Pyloric caeca
3. Anterior intestine
4. Posterior intestine
5. Rectum

testinal tracts were dissected and divided into five segments as indicated in Figure 1. Chyme from each segment was collected for analyses. The faeces, chyme from the different gut segments and a feed sample were analysed for dry matter (freeze drying), ash (600 °C over- night), chromic oxide (atomic absorption modified according to Williams *et al.,* 1962), protein (semi micro-Kjeldahl, Kjeltec-Auto system) and fat (Folch *et al.,* 1957). The contents from the pyloric caeca and rectum were too small to allow any analyses. Digestibility coefficients were calculated for stripped faeces and for contents of the stomach, anterior intestine and posterior intestine.

## RESULTS AND DISCUSSION

*Anatomy of the gastrointestinal tract*

Dissection of the halibut, coloured side up, showed a large stomach, four pyloric caeca connected to the intestine posterior to the gastrointestinal sphincter. The rectum was a somewhat widened part of the gut, with a sphincter in each end. The gut was coiled in the peritoneal cavity. The rectum was located close to the head, together with the liver. When stripping the fish, care should be taken not to damage the liver by adding pressure to this region.

Our recommendations for the methods of collection are to put a slight pressure by thumb and forefinger right over the rectum, 2–5 cm (depending on the size of the fish) from the anus, and slide fingers in the anus direction. This procedure was triedsuccessfully with a number of fish. The amount of faeces obtained from each fish varied widely, from nothing to more than 5 g in one sample. The faeces had a rather fluid consistency. Dry matter content ranged from 12 to 15%.

*Optimal time of stripping*

In the experiment designed to determine the optimal point of time of stripping, we were able to collect samples of faeces at all the chosen points of time. Results from the experiment are given in Table 3, which includes data on temperature, fish weight, amount of feed ingested and faeces collected. The amount of faeces obtained at different points of time differed significantly ($p<0.05$). Stripping at 28 and 36 hours after feeding gave more faeces than stripping after 24 and 32 hours. The reason for this finding may be that the halibut empty the gut in portions, but more comprehensive data

*Table 3.* Time of stripping, water temperature, fish weight, feed ingested, and amount of faeces collected (means ± s.e.m.).

| Time of stripping, hours after feeding | temperature °C | fish weight kg | feed ingested[1]) | faeces collected[1]) |
|---|---|---|---|---|
| 24 | 8.4 | 1.83 ± 0.34 | 3.19 ± 0.48 | 0.20 ± 0.06 |
| | 8.7 | 1.41 ± 0.13 | 4.47 ± 0.49 | 0.09 ± 0.04 |
| | 10.0 | 1.71 ± 0.31 | 2.24 ± 0.75 | 0.02 ± 0.01 |
| | 12.6 | 1.48 ± 0.12 | 5.69 ± 1.90 | 0.09 ± 0.04 |
| 28 | 8.4 | 1.50 ± 0.44 | 4.52 ± 2.00 | 0.29 ± 0.13 |
| | 8.7 | 1.97 ± 0.50 | 2.73 ± 1.00 | 0.08 ± 0.05 |
| | 10.0 | 1.59 ± 0.17 | 6.30 ± 1.73 | 0.32 ± 0.07 |
| | 12.6 | 2.11 ± 0.49 | 2.96 ± 1.02 | 0.20 ± 0.07 |
| 32 | 8.4 | 1.83 ± 0.49 | 6.78 ± 1.33 | 0.13 ± 0.06 |
| | 8.7 | 1.97 ± 0.50 | 3.00 ± 1.02 | 0.12 ± 0.05 |
| | 10.0 | 2.19 ± 0.49 | 2.82 ± 0.50 | 0.13 ± 0.06 |
| | 12.6 | 1.36 ± 0.23 | 3.88 ± 1.06 | 0.15 ± 0.07 |
| 36 | 8.4 | 0.84 ± 0.15 | 5.77 ± 1.17 | 0.34 ± 0.06 |
| | 8.7 | 1.03 ± 0.24 | 7.70 ± 3.24 | 0.12 ± 0.10 |
| | 10.0 | 1.36 ± 0.23 | 7.28 ± 2.34 | 0.23 ± 0.10 |
| | 12.6 | 1.78 ± 0.50 | 5.67 ± 0.03 | 0.20 ± 0.10 |

[1]) % of fish weight

should be collected before concluding. Unpublished results show that it is possible to obtain large amounts of faeces as late as 60 hours after the last feeding (Strømsnes, pers. comm.).

Regression analysis showed no significant linear relationship between amount of faeces obtained, temperature, feed intake and time of stripping.

The temperature increased during the four repetitions of this experiment. Other investigations have indicated that gastric evacuation rate may increase when temperature increases (Jobling *et al.*, 1977; Jobling, 1980, Jobling and Spencer Davies, 1979; Fauconneau *et al.*, 1983). According to these previous studies, it was expected that the optimal time for stripping was closer to the last feeding at the highest temperatures. This was not observed in the present experiment. The reason might be that the temperature intervals were too small to get significant effects in this study. According to other investigations, fish with the highest feed intake were also expected to give samples of faeces sooner after feeding and over a longer period (Jobling *et al.*, 1977; Jobling and Spencer Davies, 1979). This was not observed in our experiment. Fish weight showed significant effect ($p<0.05$), and explained 16% of the variation in amount of faeces. The smallest fish gave the relatively largest amounts of faeces (percent faeces collected), but there was, however, large individual variation. Since the halibut also may be a portion defaecator, it seems that the only reliable way to get sufficient samples of faeces is to have a large number of fish in the experiment.

*Digestibility determinations*

Digestibility coefficients for the seven different feeds ranged from 75 to 88 for protein, from 78 to 87 for fat and from 0 to 23 for crude carbohydrates. For comparison, corresponding digestibility coefficients for salmonids are normally found to be from 79 to 87 for protein, from 83 to 95 for fat and from 27 to 28 for carbohydrates (Austreng, 1978; Austreng, 1979; Austreng *et al.*, 1979), depending on the source of nutrients.

Results from the present study of digestibility in different gut segments compared to stripped samples are given in Table 4. In contents from the stomach, the digestibility coefficients were negative, while the values varied throughout the intestine. A possible explanation of this may be a selectively faster transport of chromic oxide compared to the remaining matter from the stomach to the intestine (Dabrowski and Dabrowska, 1981). This would alter the distribution of chromic oxide and be a source of error in estimation of digestibility coefficients in the different segments of the gut. Experience from ruminants shows that the passage rate is different in the liquid and the solid phase of the chyme (Faichney, 1975). Another possible explanation might be related to the secretion of endogenous material from the stomach.

*Table 4.* Apparent digestibility of nutrients in different gut segments compared to stripped faeces, means ± sem.

| | Stomach | Anterior intestine | Posterior intestine | Faeces |
|---|---|---|---|---|
| Segment nr, fig.1 | 1 | 2 | 3 | 4 |
| Protein | − 17.8 ± 6.5 | 39.1 ± 11.1 | 73.3 ± 2.1 | 82.0 ± 0.2 |
| Fat | − 15.1 ± 0.4 | 72.4 ± 4.7 | 87.5 ± 2.5 | 95.9 ± 0.6 |
| Carbohydrates | − 8.3 ± 0.03 | − 62.6 ± 25.5 | 21.9 ± 4.8 | 22.5 ± 2.2 |

The general tendency was, however, a successive digestion and absorbtion of nutrients throughout the intestine. Our results indicate that fat is absorbed to a greater extent in the anterior part of the intestine than is protein. The digestibility coefficients in stripped faeces were higher than in the posterior intestine, which should mean that active absorption occured in the posterior part of the intestine and possibly in the rectum. Our samples from the rectum were too small to allow analyses, but investigations of other species indicate that absorption may take place in the rectum (MacDonald, 1987; Georgopoulou *et al.*, 1988). Consequently, stripping should be carried out as late after feeding as possible to obtain samples of maximum absorption, with the risk of losing portions of faeces.

The method chosen for collection of faeces has certain sources of error. When stripping and collecting faeces from the fish, it was very difficult to avoid urine contamination of the faecal samples. This may result in underestimation of protein digestibility. However, if the halibut excrete most of the nitrogen as ammonia through the gills like other fish (Forster and Goldstein, 1969), this error would be minor. The present method appeared to be suitable for evaluation of feedstuffs, and also in experiments studying the effect of different factors on digestiblity.

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# A NOTE ON THE AMINO ACID DISTRIBUTION IN FISH SILAGE LAYERS

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## ABSTRACT

Fish silages stored without stirring formed three layers containing protein. Tyrosine, but also cystine and tryptophan accumulated in the sediment. These amino acids were low in the clear aqueous layer. In a capelin silage stored for 1 month tyrosine crystals were formed.

## INTRODUCTION

Silages from raw fish and fish offals liquefy fast due to autolysis (Haaland and Njaa, 1989). In commercial practice silages are stored in tanks with continuous stirring. If they are left without stirring a layering will take place. From the topp down there will be an oil layer, a layer of floating solids (Layer 1), a clear soluble layer (Layer 2) and a sediment (Layer 3). In the present communication are presented amino acid analyses of layers from some silages prepared in the laboratory and left unstirred at ambient temperatures.

## MATERIALS AND METHODS

Silages were prepared from fresh small cod *(Gadus morhua)* from which the livers were discarded, from fillets from small cod, from small fresh saithe (*Pollachius virens* livers discarded), from small fresh saithe kept 9 days on ice, from fresh herring and herring offal *(Clupea harengus)* and from fresh, frozen capelin *(Mallotus villosus)*.

The fish were minced and mixed with formic acid and stored in wide-necked plastic bottles in the laboratory at ambient temperatures for the times specified. Samples from the layers were obtained by suction. Three layers were obtained from small cod, fresh saithe and fresh, frozen capelin. From cod fillets only layers 2 and 3 were obtained, and from fresh saithe stored on ice and from herring and herring offal only layers 1 and 2 were obtained. In the capelin silage, crystals were seen to collect on the wall of the plastic bottle. A sample containing these crystals was scraped off, air dried and analysed for amino acids. An absorption curve of a solution in 0.1 M HCl was read around the absorption maximum for tyrosin at 274.5 nm and compared with the curve obtained for tyrosin (Figure 1). Samples were also obtained from a commercial formic acid silage stored in a tank with continuous stirring. One sample was taken from the bottom of the tank. Stirring was then stopped for 16hrs, and samples were taken from the top and from the bottom of the tank.

Protein (N*6.25) and amino acids were analysed as described by Espe *et al.* (1989).

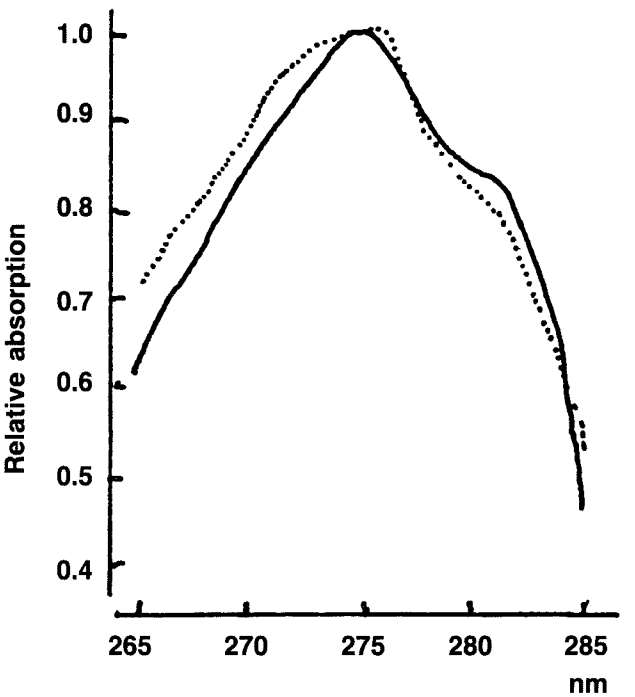


*Figure 1.* Absorption relative to maximum absorption for tyrosine (full drawn line) and for crystal containing sample taken from capelin silage left unstirred for one month (broken line).

## RESULTS AND DISCUSSION

The amino acid analyses of the layers obtained and the relevant experimental conditions are given in Tables 1-3. The outstanding differences between the amino acid composition of the layers from whole fish were very high levels of tyrosine in the sediment (Layer 3) and low levels in the clear soluble layer (Layer 2). In Layer 1 from cod, fresh saithe, herring and capelin the tyrosine levels were about the same as are usually found for whole fish. For saithe stored on ice the tyrosin level was high in Layer 1. A sediment was not obtained and it seems that much of what is usually sedimented floated in this sample. Except for cystine and tryptophan, the other amino acids showed small differences between layers. Cystine and tryptophan were high in Layer 3 and low in Layer 2. In the sediment from cod fillet silage (Table 1) the tyrosine was lower than in the other sediments but higher than in the clear layer. This may be due to fillets containing less proteolytic enzymes active around pH 4.0 than whole fish.

*Table 1.* The content of amino acids (mg $g^{-1}$ protein) in layers of silage made from cod and cod fillets stored at ambient temperature for 8 months.

| | whole cod | | | | | | cod fillets | |
|---|---|---|---|---|---|---|---|---|
| Formic acid (%, w/w) | 2.5% | | | 3.0% | | | 2.5 | |
| pH | 4.4 | | | 4.1 | | | 3.9 | |
| Layer | 1 | 2 | 3 | 1 | 2 | 3 | 2 | 3 |
| Protein (g $kg^{-1}$) | 217 | 175 | 225 | 210 | 175 | 218 | – | 213 |
| Asp | 90 | 100 | 87 | 88 | 105 | 93 | 96 | 124 |
| Thr | 43 | 48 | 44 | 42 | 51 | 45 | 44 | 56 |
| Ser | 50 | 48 | 56 | 49 | 61 | 56 | 45 | 53 |
| Glu | 130 | 152 | 127 | 128 | 159 | 132 | 144 | 171 |
| Gly | 75 | 91 | 70 | 76 | 98 | 74 | 55 | 52 |
| Ala | 67 | 78 | 63 | 67 | 81 | 66 | 61 | 65 |
| Cys | 9 | 4 | 12 | 9 | 4 | 13 | 9 | 10 |
| Val | 48 | 53 | 47 | 47 | 56 | 49 | 44 | 53 |
| Met | 28 | 29 | 28 | 29 | 30 | 31 | 27 | 35 |
| Ile | 43 | 47 | 42 | 42 | 49 | 45 | 36 | 50 |
| Leu | 60 | 66 | 60 | 64 | 74 | 69 | 73 | 88 |
| Tyr | 30 | 13 | 104 | 24 | 18 | 99 | 29 | 44 |
| Phe | 35 | 35 | 42 | 34 | 40 | 44 | 31 | 46 |
| Lys | 76 | 71 | 72 | 76 | 94 | 77 | 104 | 103 |
| His | 19 | 21 | 18 | 19 | 23 | 20 | 22 | 24 |
| Arg | 58 | 65 | 55 | 57 | 70 | 58 | 61 | 67 |
| Trp | 6 | 3 | 20 | 7 | 6 | 10 | – | – |
| Pro | 48 | 61 | 48 | 49 | 64 | 50 | – | – |

*Table 2.* The amino acid content (mg $g^{-1}$ protein) in layers of silage made from saithe and herring stored for 6 months at ambient temperatures.

| | Saithe | | | | | Herring | |
|---|---|---|---|---|---|---|---|
| | fresh | | | 9 days on ice | | fresh | |
| Layer | 1 | 2 | 3 | 1 | 2 | 1 | 2 |
| Protein (g $kg^{-1}$) | 171 | 172 | 182 | 140 | 147 | 160 | 163 |
| Asp | 102.6 | 104.5 | 100.8 | 98.4 | 104.5 | 101.5 | 101.5 |
| Thr | 50.0 | 49.3 | 48.3 | 49.2 | 50.7 | 48.9 | 51.5 |
| Ser | 51.6 | 52.8 | 50.7 | 50.0 | 52.2 | 45.4 | 47.8 |
| Glu | 148.0 | 151.3 | 141.6 | 139.7 | 147.9 | 133.7 | 146.6 |
| Gly | 59.3 | 78.3 | 71.8 | 70.0 | 77.6 | 60.7 | 66.5 |
| Ala | 66.6 | 67.8 | 64.3 | 63.3 | 67.8 | 62.8 | 68.8 |
| Cys | 12.5 | 6.4 | 10.2 | 13.7 | 6.0 | 11.9 | 4.5 |
| Val | 54.2 | 52.4 | 52.2 | 54.4 | 52.5 | 55.0 | 60.0 |
| Met | 34.1 | 31.4 | 32.7 | 33.0 | 31.7 | 29.5 | 32.7 |
| Ile | 46.4 | 45.3 | 45.4 | 45.4 | 45.3 | 45.3 | 47.5 |
| Leu | 74.7 | 77.6 | 72.8 | 72.4 | 74.1 | 71.2 | 76.2 |
| Tyr | 35.3 | 12.0 | 60.6 | 64.7 | 13.2 | 43.7 | 9.5 |
| Phe | 40.4 | 37.3 | 42.0 | 43.7 | 36.1 | 39.3 | 37.3 |
| Lys | 91.0 | 95.3 | 88.2 | 86.6 | 92.3 | 87.3 | 99.1 |
| His | 22.4 | 22.1 | 21.5 | 22.2 | 23.1 | 30.7 | 34.6 |
| Arg | 63.8 | 66.1 | 62.2 | 60.0 | 63.7 | 57.8 | 63.6 |
| Trp | 9.4 | 4.1 | 10.2 | 11.9 | 4.8 | 7.8 | 7.8 |
| Pro | 45.7 | – | 46.9 | 44.6 | 49.7 | 43.8 | 44.4 |

In Table 3 are given data on the amino acid composition of the crystallized fraction scraped off from the wall of the bottle.

The very high tyrosine content (778 mg $g^{-1}$ dry sample) together with the absorption curve of the extract in 0.1 M HCl indicate that crystals of tyrosine may have precipitated. Together with cystine tyrosine are the least soluble of the natural amino acids. Thus, it is possible that the high tyrosine and cystine levels in the sediments are due to precipitation of these amino acids as they are liberated during autolysis. However, this explanation does not hold for tryptophan.

Gildberg and Raa (1977) reported higher tyrosin contents in the sediment in a cod viscera silage than in the soluble aqueous phase, and Strøm and Eggum (1981) found lower tyrosin values in the aqueous phase in viscera silages stored for about 3 weeks than in freshly prepared silages. Gildberg and Raa (1977) also found a high cystine value in the sediment and a very low value in the aqueous phase and suggesfed that some amino acids precipifated upon storage.

The results presented here show that when it is intended to have an amino acid composition in fish silage equivalent with that in whole fish, stirring to avoid layering is important. It is also considered to be important to have the fatty phase evenly distributed in the product.

The results of the analyses of the commercial silage are given in Table 3. They show that in this case no difference between top and bottom sample was found, and that a sample taken from the bottom of the tank before stirring was stopped also showed very similar amino acid composition.

The amino acid compositions of the layers formed in unstirred fish silage were very similar, except for the contents of tyrosine, cystine and tryptophan. The importance of variations brought about by insufficient stirring for the use of fish silage as a feed ingredient, will depend upon to what extent these amino acids will be limiting factors for the total feed protein. If it can be verified that tyrosin may crystalize in silages it may be of importance to study its bioavailability in that form.

Table 3. The content of amino acids (mg $g^{-1}$ protein) in layers of capelin silage stored 1 month at ambient temperatures, pH 3.96 and in samples of herring/herring offal silage taken from bottom of a tank with continous stirring and from bottom and top of the tank without stirring (16 hrs).

| Silage | Capelin | | | | Herring/herring offal | | |
|---|---|---|---|---|---|---|---|
| Sample | Layer 1 | Layer 2 | Layer 3 | Crystalized fraction | Bottom stirred | Top nonstirred | Bottom |
| Protein (g $kg^{-1}$) | 142 | 123 | 495 | – | 123 | 121 | 121 |
| Asp | 90 | 102 | 101 | 2.0 | 89 | 91 | 88 |
| Thr | 45 | 49 | 44 | 1.6 | 44 | 46 | 44 |
| Ser | 42 | 47 | 57 | 1.6 | 43 | 44 | 42 |
| Glu | 128 | 150 | 142 | 4.3 | 128 | 132 | 125 |
| Gly | 52 | 61 | 71 | 1.1 | 59 | 60 | 58 |
| Ala | 56 | 66 | 57 | 2.3 | 61 | 63 | 59 |
| Cys | 8 | 9 | 12 | 2.4 | 14 | 13 | 11 |
| Val | 50 | 54 | 50 | 2.4 | 48 | 50 | 48 |
| Met | 28 | 30 | 32 | 2.0 | 26 | 26 | 26 |
| Ile | 44 | 45 | 44 | 1.7 | 39 | 40 | 38 |
| Leu | 74 | 77 | 71 | 4.6 | 72 | 75 | 73 |
| Tyr | 43 | 31 | 137 | 778.1 | 33 | 34 | 34 |
| Phe | 42 | 36 | 52 | 48.3 | 36 | 37 | 37 |
| Lys | 77 | 95 | 74 | 3.6 | 84 | 85 | 82 |
| His | 20 | 23 | 23 | 1.2 | 23 | 24 | 23 |
| Arg | 53 | 63 | 70 | 1.9 | 60 | 61 | 60 |

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