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GROWTH AND PHYSIOLOGICAL PROPERTIES IN WHITE TRUNK MUSCLE OF TWO ANADROMOUS POPULATIONS OF ARCTIC CHARR (*SALVELINUS ALPINUS*)

By

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ABSTRACT

Anadromous Arctic charr (*Salvelinus alpinus*) belonging to 2 different populations were compared with respect to growth, food utilization, and metabolic parameters of the white trunk muscle. The fish were 1.5 years old, but differed in body weight. The strain 'Skogseid' weighed 70 g and the strain 'Hammerfest' 300 g at the start of the feeding experiment which lasted for 49 days. Food intake was elevated in the fish of high body weight, while specific growth rate and percentage weight gain were the same for the 2 populations. Relative to live weight gain food intake was less for the large than for the small fish. Among the muscle metabolic variables, the RNA and glycogen content/g wet weight of muscle were significantly higher in the large size as compared with the small size fish. In the strain of high body weight the significant increase in acid proteinase activity was compensated for by the rise in RNA levels, suggesting an elevation in the capacity for protein synthesis in that strain. Unchanged variables included the protein and DNA content/g wet weight of tissue, as well as the myosin heavy chain portion of the total protein. In conclusion, the 2 populations differed in food intake and utilization. The change in muscle metabolic parameters was the result of the size and body weight of the fish explaining the phenotypic diversities of the 2 populations but not their genetic differences.

INTRODUCTION

The family *Salvelinus* is represented in Scandinavia as 3 species, *Salvelinus salvelinus*, *S. alpinus* and *S. stagnalis*. *S. alpinus* consists of 3 populations with slight genetic variation (Torrison and Barnung, 1991). In Norway, the strain 'Hammerfest' differs from that of 'Skogseid' in localization and size. The strain Hammerfest is a northern anadromous fish with a high growth rate. The southern Skogseid strain has anadromous potentials (Nordeng, 1983) and a low growth rate. Isozyme electrophoretic and

biometric analyses have been applied to Arctic charr populations in attempts to discover an underlying genetic basis for the phenotypic differences (Partington and Mills, 1988). Genetic differences have been described in the trypsin-like isozyme pattern (Torrison and Barnung, 1991).

In recent years farming of Arctic charr has become more widespread (Nordeng, 1983). The preference for low temperature makes the species an attractive object for farming in the northern regions with a climate less suitable for rearing Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*).

Interactions between food energy intake or food composition and genetic processes generate a network of physiological responses (Castro, 1987). The question arises whether the genetic difference in size at similar age of the 2 populations of Arctic charr (Skogseid and Hammerfest) is extended to muscle metabolic variables. In this study physiological characteristics of the white trunk muscle were compared between the 2 populations. The genetically dependent growth was reflected in food utilization and as a result in alterations of physiological properties of muscle normally associated with growth.

MATERIALS AND METHODS

Materials

All chemicals were of the highest purity available, supplied by Sigma Chemical Co., St. Louis, MO, and Serva, Heidelberg, Germany. The fish food Tess Elite Plus was obtained from Skretting A/S, Stavanger, Norway.

Fish and feeds

Arctic charr, 1.5 years old, of 2 populations, Skogseid and Hammerfest, were cultured in 1.3-m^3 tanks at a water temperature of 10.3±0.2°C. The Skogseid strain (n=60) was kept at a salinity of 6.8.±0.2 g/L and the Hammerfest strain (n=35) at 17.6±0.2 g/L. The aquaria were supplied with running water at 18–20 L/min and exposed to constant light. The fish were fed the Tess Elite Plus diet (Table 1) by an automatic device at an amount of 0.5% of total biomass per day. After an acclimatization period of 3 weeks the fish were weighed and the experiment started. After a further 49 days the fish were killed by a blow to the head and weighed. The white type of the epaxial muscle was dissected, sliced, wrapped into aluminium foil and frozen between 2 blocks of CO_2 and stored at –80°C (Lund and von der Decken, 1980).

Table 1. Composition of the diet[1,2] .

Composition, g × Kg⁻¹	
Herring (*Clupea harengus*) meal, low-temperature dried	500
Wheat	175
Extruded wheat	280
Mineral and vitamin mixture[3]	45
Analytical values, g × Kg⁻¹	
Water	95
Protein	415
Fat	170
Carbohydrate	260
Ash	60

[1] Tess Elite Plus, Skretting.

[2] Energy value, MJ × Kg^{1-} diet: Protein 7.5; fat 5.7; carbohydrates 3.2. The values of 18.0, 33.5 and 12.5 KJ × g^{-1} were used to calculate the digestible energy of protein, fat and carbohydrates, respectively (Brett and Groves, 1979).

[3] Mineral and vitamin mixtures, the details have been published previously (von der Decken *et al.*, 1992).

Analytical methods

The food protein (Nx6.25) was determined by a modified Kjeldahl procedure (Crooke and Simpson, 1971); lipids by gravimetry of the ethylacetate extract of the food; ash by gravimetry after ashing for 24 h at 660°C; water and dry matter by gravimetry before and after drying for 24 h at 105°C. Carbohydrate content was the difference between the sum of the above analytical results and the original weight of the food.

Preparation of muscle homogenate, separation into a sarcoplasmic and a myofibrillar fraction and the immunoassay have been described previously (von der Decken and Lied, 1992a,b; Nazar *et al.*, 1991). DNA was analysed by a fluorescent method using salmon DNA as a standard (Setaro and Morley, 1976). Proteins were analyzed by the Coomassie brilliant blue method using bovine serum albumin as a standard (Bradford, 1976). RNA was determined after alkaline digestion of the perchloric acid precipitate (Fleck and Munro, 1962). Glycogen was determined as described by Harris *et al.* (1974). The glycogen content was expressed as mg glucose/g wet weight of tissue. Acid proteinase activity was determined in the supernatant obtained after centrifugation of the muscle homogenate for 10 min at 1600 × g (Mommsen *et al.*, 1980). Haemoglobin was used as substrate and the tyrosine released was analyzed by a fluorescent method (Ambrose, 1974). The results are expressed as tyrosine released

from haemoglobin/h. Polyacrylamide gel electrophoresis was carried out in 10% acrylamide gel containing 0.1% SDS (Hames, 1981). After electrophoresis the gels were stained with Coomassie brillant blue R-250.

Statistical analysis

The results are expressed as mean values ±SEM. Student's t-test was used for statistical evaluation (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Arctic charr of low body weight consume less food than those of high body weight (Table 2). The specific growth rate and percentage weight gain were the same for the 70 g and 300 g fish. In Arctic charr the percentage weight gain and specific growth rate were higher at a lower food intake than in Atlantic salmon (*Salmo salar*) of 150 g body weight and kept under similar experimental conditions (von der Decken *et al.*, 1992). Comparing Arctic charr with Atlantic salmon at the initial 14 days in saltwater the charr were larger than the Atlantic salmon (Delabbio *et al.*, 1990). After 80 days there was no difference and after 130 days the Atlantic salmon had surpassed the Arctic charr in size.

No significant differences were noted between the 2 populations of the Arctic charr in the protein content/g wet weight of white trunk muscle (Table 3). Separation of the muscle into fractions enriched in sarcoplasmic and myofibrillar proteins showed a higher content of sarcoplasmic than of myofibrillar proteins. Electrophoretic analysis showed no differences in migration and composition between proteins of the 2 populations of Arctic charr (not shown here). The expression of myosin isoforms in the white muscle of Arctic charr is age dependent (Martinez *et al.*, 1991).

Table 2. Growth and food intake of 2 populations of Arctic charr of the same age but differing in body weight.

Strain	Skogseid (n=60)	Hammerfest (n=35)
Initial body weight, g	71.1±3.5	303.1±11.7
Final body weight, g	97.4±7.0	416.6±21.6
Average weight gain, %	36.9	37.4
Specific growth rate, % × day^{-1}	0.64	0.65
Food intake, g /live weight gain[1]	0.90	0.82
Protein retained /protein intake	0.37	0.43

The results are means ±SEM. (n) indicates the number of fish.

[1] Food intake, g /live weight gain = feed conversion ratio.

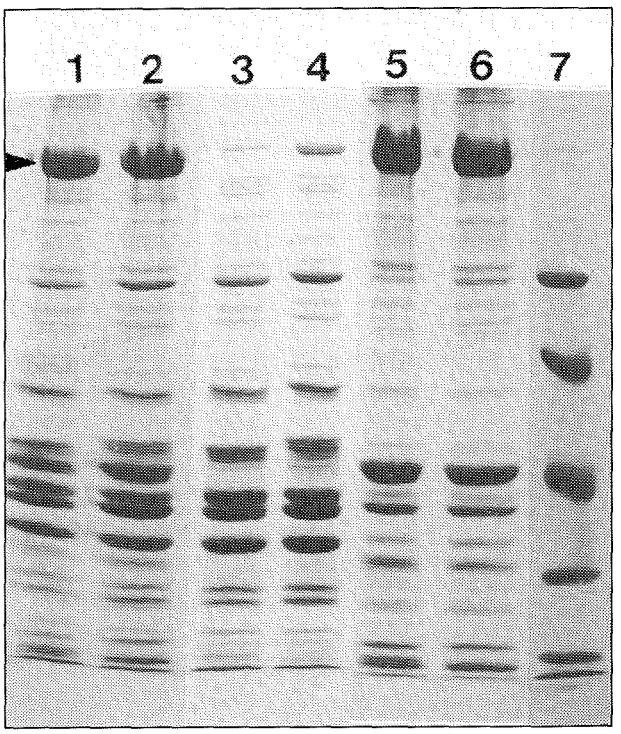

Fig. 1. SDS-polyacrylamide gel electrophoresis of proteins from white trunk muscle of Arctic charr and Atlantic salmon.

Lanes 1, 3 and 5, Arctic charr; lanes 2, 4 and 6, Atlantic salmon. Lanes 1 and 2, total muscle proteins; lanes 3 and 4, proteins of sarcoplasmic; lanes 5 and 6, proteins of myofibrillar origin. Lane 7, molecular weight marker, from above: 94K, 67K, 43K, 30K, 20K and 14K. The arrow indicates myosin heavy chain.

The isoforms are present in the myosin light chains. Comparison of the muscle proteins between Arctic charr and Atlantic salmon showed differences in the composition of sarcoplasmic proteins (Fig. 1). Analysis by the enzyme-linked immunosorbent method showed that myosin heavy chain was absent from the sarcoplasmic fractions (Persson *et al.*, 1991). The protein migrating at a slightly slower rate than myosin heavy chain did not belong to this type of contractile elements (Fig. 1, lanes 3,4).

As a representative part of the myofibrillar proteins myosin heavy chain was determined by an immunological assay (Table 4). No significant differences in content were noted between the 2 populations although the level was consistently lower in the large size than the small size fish. In pre-smolt Atlantic salmon some 23% of the total muscle proteins are analyzed as myosin heavy chain (Nazar *et al.*, 1991).

Table 3. Metabolic parameters of white trunk muscle in Arctic charr differing in body weight.

Fish, body weight	97 g	417 g
Parameter	Per g wet weight of muscle	
Total protein, mg	125.86 ±2.95	124.57 ±2.75
Sarcoplasmic protein, mg	64.20 ±0.91	66.54 ±0.78
Myofibrillar protein, mg	60.37 ±1.59	58.48 ±2.22
− Total RNA, mg	1.187±0.04[a]	1.322±0.04[b]
+ Sarcoplasmic RNA, mg	0.690±0.04[a]	0.852±0.04[b]
Myofibrillar RNA, mg	0.498±0.03	0.470±0.03
DNA, mg	0.748±0.03	0.818±0.02
RNA/DNA ratio	1.587±0.04	1.615±0.03
Glycogen (mg glucose)[1]	7.78 ±0.26[a]	8.62 ±0.37[b]
Acid proteinase activity[2]	0.255±0.01[a]	0.314±0.02[b]

The results are means ±SEM of 4 fish per group with 2 independent determinations per fish and each determination run in triplicate. Values with different superscript letters in one row are significantly different, $P<0.05$.

[1] Glycogen is analyzed as glucose.

[2] Activity expressed as mg tyrosine released/hour and g wet weight.

Deposition of glycogen in muscle increased with marginal significance ($P=0.08$) in the large size fish (Table 3). Intracellular water is associated with glycogen at a ratio of 4 to 1. The apparent diminished content of myosin heavy chain per g wet weight may be due to the increased water content and in reality be unchanged.

The RNA level was elevated in the strain of high body weight (Table 3). This was confined to the sarcoplasmic fraction giving significant difference between the groups. Total RNA contains predominantly ribosomal RNA and the rate of protein synthesis is related to the RNA content and expressed per unit RNA (Millward *et al.*, 1976). As compared with the small size fish the elevated level of RNA in the large size fish indicated a greater capacity for protein synthesis. DNA content was similar for the 2 strains and so was the RNA/DNA ratio. The slightly elevated level of DNA in the large size fish was responsible for the similarity in the RNA/DNA ratio of the 2 populations.

The significantly higher acid proteinase activity together with the unchanged protein content were indicative of an increased protein turnover in the large size as compared with the small size fish (Table 3). Increased food intake and muscle growth rates are associated with increased rates of both protein synthesis and protein degradation (Millward, 1989).

Table 4. Myosin heavy chain content in white trunk muscle of Arctic charr differing in body weight.

Fish, body weight	97 g	417 g	Percentage change with body weight
Myosin heavy chain, mg/			
mg protein	0.258±0.029	0.233±0.021	−9.4
g wet weight of muscle	32.43 ±3.73	29.13 ±2.70	−10.2
mg of DNA	43.36 ±5.11	35.61 ±3.38	−17.9

The results are means ±SEM of 4 fish per group with 2 independent determinations per fish.

In conclusion, the feed composition supported the specific growth rate and percentage weight gain equally well in populations of Arctic charr genetically differing in size and body weight. The strain dependent differences in muscle of Arctic charr included glycogen content as well as protein degradation as measured by the activity of acid proteinase. The elevated level in activity was counteracted by an increase in the capacity for protein synthesis as seen by the rise in RNA content. The change in protein degradation and RNA content were related to size and body weight explaining the phenotypic diversities between the 2 populations but not their genetic differences.

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A NOTE ON THE MOLECULAR DISTRIBUTION OF THE STOMACH PROTEIN IN ATLANTIC SALMON (*SALMO SALAR*) FED DIETS OF EITHER INTACT OR PEPSIN PRE-DIGESTED COD MUSCLE PROTEIN

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ABSTRACT

Atlantic salmon (*Salmo salar*) were fed cod muscle or cod muscle pre-digested with pepsin for 6 and 48 hours, respectively for four weeks.

The fish were then starved for 48h to ensure empty alimentary tracts, and fed to satiety for about one hour. Immediately after feeding and after 6, 12, 24 and 48h, samples of stomach contents were collected and analysed for its protein molecular weight distribution.

No differences in emptying time of the stomach contents were observed, but those fed Diet A (not pre-digested) had more high molecular weight protein in the stomach as compared to those fed the pepsin treated feeds.

INTRODUCTION

In a previous communication with Atlantic salmon it was reported that weight gains were less with an amino acid diet than with a fish meal diet (Espe and Njaa, 1991). Similar results were reported in experiments with carp (Yamada *et al.*, 1981a; Kaushik and Dabrowski, 1983) and rainbow trout (Stone and Hardy, 1986; Walton *et al.*, 1986; Stone *et al.*, 1989). Yamada *et al.* (1981b) reported faster passage of an amino acid diet through the stomach of rainbow trout than of a casein diet. Matthews and Adibi (1976) and Silk *et al.* (1979) working with mammals found slower absorption of amino acids from amino acid diets than from diets containing di- and tripeptides.

The present experiment was carried out to test to what extent pre-digestion of cod muscle protein affected the molecular weight distribution in the stomach at various times after feeding.

MATERIALS AND METHODS

Protein sources and diets

To 7 kilos freeze dried cod muscle protein dispersed in 40L water was added 200g pepsin, and pH adjusted to 4.2 with HCl. Muscle was pre-digested at 27°C for 6 and 48 hours, respectively, before it was filtered and freeze dried. The pre-digested proteins were compared to freeze dried cod muscle not treated with pepsin. The compositions of the diets used are given in Table 1. The amino acid analyses of the three feeds are given in Table 2.

Fish and feeding

Sixty Atlantic salmon (*Salmo salar*) of approximately 100g body weight were fed each of the experimental diets to satiety 5 days a week for 28 days. Fish were kept in aquaria of 1.5m^3 water volume supplied with running water with temperature and salinity of 8.6±0.6°C and 18.4±0.7

Table 1. Composition and analytical values for the experimental feeds. Diet A is cod muscle, Diets B and C are cod muscle proteolysed with pepsin for 6 and 48 h, respectively.

Composition g kg^{-1}:	Diet A	Diet B	Diet C
Cod muscle	528	-	-
6h cod muscle	-	528	-
48h cod muscle	-	-	528
Capelin oil	185	185	185
Extruded wheat	205	205	205
Mineral mixture[1]	50	50	50
Vitamin mixture[2]	2	2	2
Gelatine	30	30	30
Analytical values:			
Dry matter (g kg^{-1})	964	960	964
Protein (g kg^{-1} dry wt)	517	486	492
Fat (g kg^{-1} dry wt)	183	178	180
Ash (g kg^{-1} dry wt)	49	72	74
NPN (g kg^{-1} total N)	134	230	396

[1] *Mineral mixture*: $CaHPO_4 \cdot 7H_2O$ 562.5g, KH_2PO_4 450.0g, NaCl 300.0g, $MgSO_4$ 150.0g, $FeSO_4 \cdot 7H_2O$ 15.0g, $ZnSO_4 \cdot 7H_2O$ 15.0g, $MnSO_4 \cdot 4H_2O$ 3.0g, $CuSO_4 \cdot 5H_2O$ 0.6g, KI 0.45g and partly dextrinized potato starch was added to 1500g.

[2] *Vitamin mixture*: Thiamin 0.143g, Riboflavin 0.143g, Pantothenic acid 0.143g, Niacin 0.286g, Pyridoxin 0.571g, Biotin 0.357g, Folic acid 0.286g, B_{12} 0.286g, Inositol 7.143g, Ascorbic acid 14.285g, Choline 78.571g, Vit E 5.714g, Vit A 0.024g and Vit D_3 0.004g.

Table 2. Amino acids (mg g^{-1} protein) in the experimental feeds. For Diets A to C, see Table 1.

A.a./Diets	Diet A	Diet B	Diet C
Asp	105.6	105.1	109.6
Glu	169.3	170.3	175.5
OH-pro	8.8	10.0	10.3
Ser	48.2	49.3	50.8
Gly	59.5	60.5	61.6
His	26.4	25.4	25.9
Arg	69.1	70.4	72.6
Thr	48.4	49.4	51.3
Ala	67.0	66.4	68.4
Pro	46.2	47.2	48.9
Tyr	39.3	38.9	41.6
Val	51.6	52.2	54.5
Met	33.0	33.8	35.2
Cys	2.2	1.5	2.0
Ile	49.9	49.6	52.1
Leu	87.7	89.5	91.6
Phe	43.4	43.2	44.8
Lys	100.5	100.2	103.4

Trp was not analysed.

gL^{-1}, respectively. Constant light was used over the aquaria during the experiment. After 28 days on the experimental feeds, fish were starved for 48h to empty the alimentary tract. They were then fed for about one hour at which time satiation occured. Immediately after feeding (0h) and after 6, 12, 24 and 48h six fish from each feeding group were killed. Pooled samples of the stomach contents from three fish (i.e. 2 pooled samples from each tank at each sampling time) were collected in 0.9% NaCl. EDTA (10mM) was added to each sample to prevent further hydrolysis and they were freeze dried and weighed. The molecular weight distribution in the stomach contents was determined.

Analytical methods

Total dry matter was determined by freeze drying. The samples were then suspended in 10 ml 0.02M phoshate buffer (pH 7.6). Fat was removed by ethylacetate (1:2, vol), fat free samples were freeze dried, and resuspended in 0.02M phosphate buffer (pH 7.6). Separation of molecular weight were carried out by successive ultrafiltration (Amicon filters, Danvers MA USA) using cut-offs 2kD and 0.5kD. Protein in the

filtrates was determined using a modified Lowrey method (Biorad D_C Protein assay).

Proximal and non-protein nitrogen analyses of the feed samples were carried out as described by Espe *et al.* (1989). Amino acids in the feed samples were determined on a Waters HPLC after pre-derivatisation with phenylisothiocyanate according to Cohen et al. (1989).

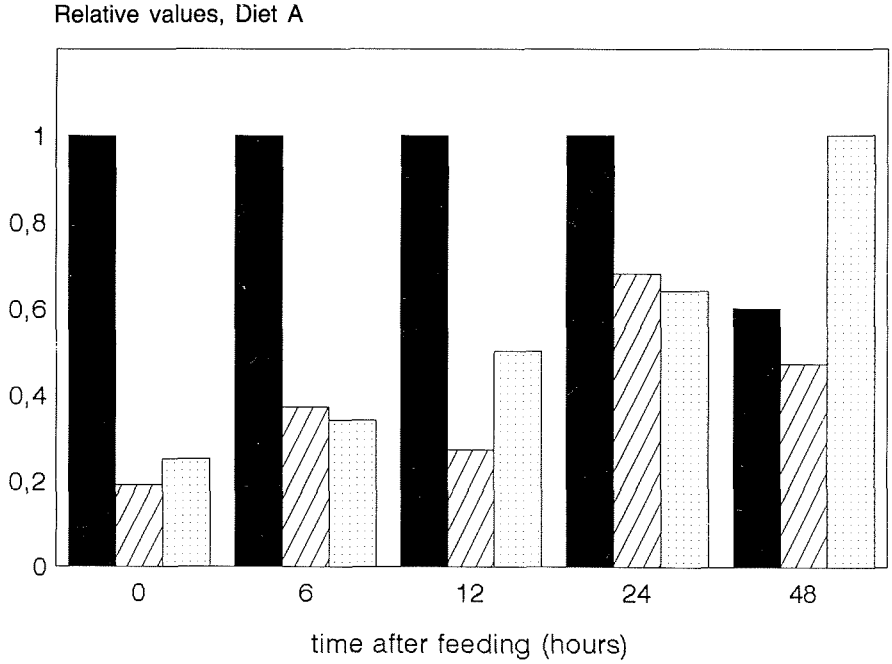
As pooled samples were analysed, no statistical treatment of the data were done.

RESULTS AND DISCUSSION

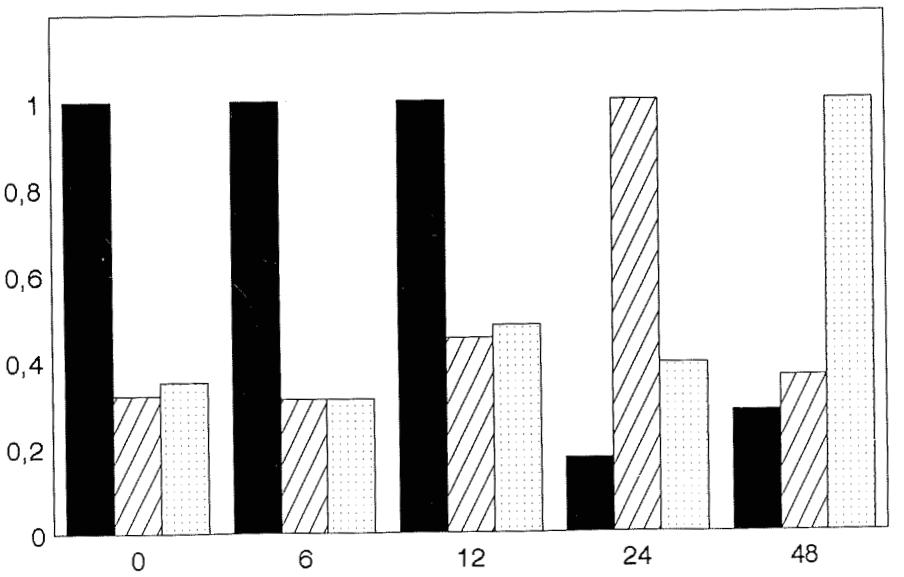
Solubility of the feeds used.

Non protein nitrogen (NPN) increased from 134 to 396 g kg^{-1} total nitrogen in feed A to C (Table 1) which was due to the protein being hydrolysed to peptides by the added pepsin. This gave a feed containing partly solubilized protein. In contrast Yamada *et al.* (1981b) compared diets containing a mixture of free amino acids and casein.

Figure 1. Distribution of the water soluble proteins in stomach contents in fish fed diets of increasingly pepsin pre-digested cod muscle protein. The highest value at each sampling time is set equal to one, and the others calculated relative to this. (■), (□) and (▨) are mw>2kD, 2kD<mw>0.5kD and mw<0.5kD, respectively.



Relative values, Diet B



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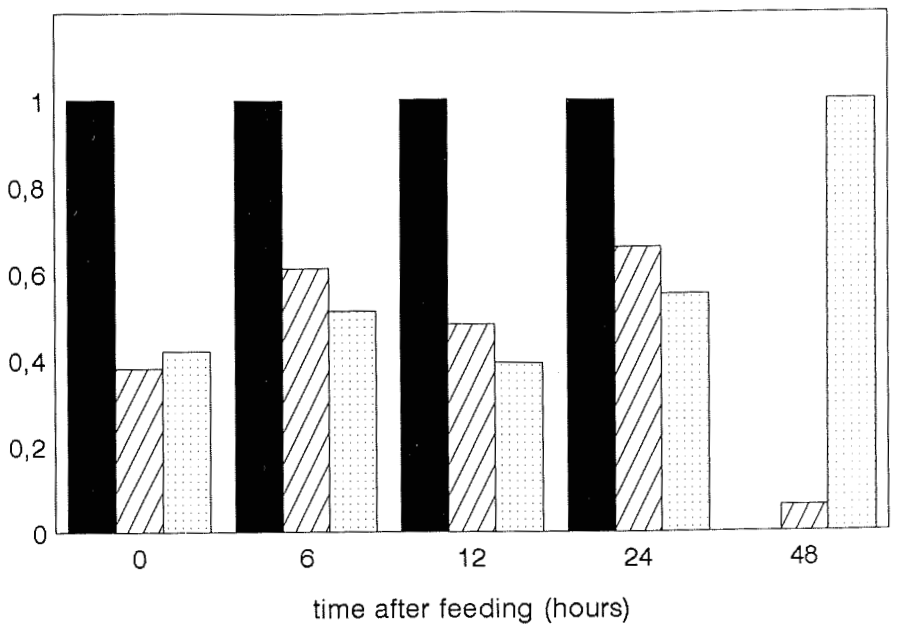

Table 3. Dry matter and water soluble protein and molecular distribution of the water soluble protein. Values are means of two samples±deviation from the mean.

Diets	0h	6h	12h	24h	48h
Diet A					
Dry matter (mg)	919±199	677±215	420±79	190±16	64±12
Soluble protein (mg)	103± 25	116± 12	87±37	126±23	60±31
mw>2kD %	68± 9	58± 7	57±20	43±11	29±29
2<mw>0.5 %	14± 7	22± 2	15±16	29± 5	23±19
mw>0.5kD %	18± 7	20± 2	28± 7	28± 3	48± 3
Diet B					
Dry matter (mg)	500± 12	572± 76	389±82	125±31	62±33
Soluble protein (mg)	292± 19	204± 33	185±70	74±19	49±43
mw>2kD %	60± 2	62± 7	53±12	11± 8	18±17
2<mw>0.5 %	19± 1	19± 5	23±16	64± 8	22±13
mw<0.5kD %	21± 4	19± 3	24±11	25±11	60±58
Diet C					
Dry matter (mg)	791±252	583±110	384±47	136±12	69±24
Soluble protein (mg)	390± 95	465± 47	242±18	130±16	37± 4
mw>2kD %	56± 9	47± 7	53± 2	45± 2	0± 0
2<mw>0.5 %	21± 8	29± 1	26± 5	30±12	6± 6
mw<0.5kD %	23± 6	24± 2	21± 1	25± 3	94± 6

For Diets A to C, see Table 1.

Distribution of the low molecular weight protein within the stomach

The fish showed an average weight gain of 0.5 g day^{-1} in the pre-experimental period. In experiment with whiting (*Merlangius merlangus*) fed natural feed (sandeels) Bromley (1988) reported the passage time through the alimentary tract to be approximately 48h. According to this the fish in the present experiment were starved for 48h prior to the experiment to insure an emty alimentary tract.

The results are given in Table 3 and Figure 1. Most of the dry matter had passed the stomach at 24h after feeding. It did not seem to be any differences in the passage time through the stomach, but more of the protein was present as soluble nitrogen in the fish fed the pre-digested feeds as compared to those fish fed the non pre-digested feed (Table 3). In Figure 1 the molecular weight class at each sampling time being present in highest amount are set equal to one, then the other two molecular weight classes are calculated relative to the highest one. I, II and III shows the results of fish fed Diets A, B and C, respectively. The intention in doing so was to examine to what extent the pre-digestion of the dietary protein affected the size distribution of the protein within the

stomach in time intervals after feeding. Less than 10 percent of the initial amount was left 48 h after feeding. It did not seem to be any differences in the passage time through the stomach, but more of the pre-digested feeds had higher amounts of low molecular peptides and less of peptides with molecular weights higher than 2kD in the stomach. As time vent on the low molecular weight peptides dominated the high molecular weight proteins. Yamada *et al.* (1982b) reported differences in emtying time of the stomach of rainbow trout fed either casein or a diet consisting of free amino acids. This also have been reported for carp fed similar diets (Kaushik and Dabrowski, 1983). The reason this was not found in the present experiment probably was due to that by using free amino acids the feed do not have to be digested at all, and probably will 'leak' rapidly into the alimentary tract. Partly pre-digested feed will have to be digested to some degree prior to being passed further on. Feeding the free amino acid diet then probably will result in a somewhat greater pressure and competition upon the absorption mechanisms, only the absorption mechanisms for free amino acids being available. Pre-digested feeds on the other hand may also use the absorption mechanisms for di-and tripeptides.

One should then expect that lesser amounts of dietary nitrogen from a pre-digested feed than from a diet of free amino acids might be lost in the faeces. A great faecal loss of free amino acids when feeeding juvenile carp free amino acid diets have previously been reported (Kaushik and Dabrowski, 1983).

Although the present results indicate that fish fed the pre-digested feeds had more more low molecular weight peptides in the stomachs in shorter time after feeding as compared to those fed the intact protein source, further experiments with better control of the amount feed offered are required to determine if this is beneficial for the fish in that more of the available proteins might be absorbed in the time span of passage through the alimentary tract.

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CAROTENOID ACCUMULATION IN ATLANTIC SALMON FED DIETS WITH MAIZE GLUTEN, PEA OR RAPESEED

By

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ABSTRACT

Triplicate groups of Atlantic salmon were fed canthaxanthin containing diets with 30% maize gluten or peas or 45% rapeseed or canola rapeseed for 10 weeks. All diets were supplemented with 50 mg canthaxanthin/kg. The maize gluten diet contained zeaxanthin and lutein in addition to canthaxanthin. Canthaxanthin was the predominant carotenoid in the other diets.

The total carotenoid content in flesh was lower in the fish receiving maize gluten and rapeseed than in those given the pea diet. No significant differences in the relative proportions of canthaxanthin and its metabolites were seen among the various groups of fish.

INTRODUCTION

The diet composition is among the factors affecting pigmentation of salmonids (Torrissen *et al.*, 1989; Storebakken and No, 1992). Investigations have been made to elucidate the effects of fat content and quality, and of vitamin A and vitamin E levels in the diet, as reviewed by Storebakken and No (1992). However the effects of different protein sources on pigmentation have not been studied before.

Plant proteins represent interesting alternatives as substitutes for fish meal in feeds for salmon, due to abundant supply and stable, often low prices. However, antinutritional factors present in most plants, may

reduce their value as fish feed ingredients (Higgs *et al.*, 1988; Krogdahl, 1990). For example, 10% or more dietary fiber depressed growth in juvenile rainbow trout (Hilton *et al.*, 1983). In rapeseed, high levels of glucosinolates (goitrogens), erucic acid, phytic acid and tannins limit its value as a protein source for salmonids. Canola is the name for new rapeseed breeds developed to contain less glucosinolates and erucic acid. This genetic selection has enhanced its value as an animal feed ingredient. Nevertheless, inclusion of canola meal in diets for salmonids still leads to reduced growth and protein utilization, mainly due to the antithyroid effect of glucosinolates (Higgs *et al.*, 1988). Among the antinutrients present in legumes like the peas, are indigestible oligosaccharides and lectins. The former cause flatulence in warm blooded animals, and the latter, also called haemagglutinins, are plant proteins with the ability to agglutinate cells in a manner similar to that of antibodies.

Carotenoids are lipid soluble and the uptake of carotenoids may be associated with the digestion and uptake of lipids. This includes emulsification, incorporation into mixed miscelles in the intestine, mucosal uptake and blood transport associated with lipoproteins. Hence, any factor disturbing lipid digestion and absorption may affect carotenoid utilization. Plant antinutrients may strongly affect lipid digestibility in fish (Krogdahl, 1990), and possibly reduce the carotenoid uptake in a similar way. Components such as fibers, oligosaccarides, lectins, tannins and saponins are among those that might disturb the lipid digestion and absorption. In chicken, 7% inclusion of several fibers in the diet, significantly reduced β-carotene utilization, measured as liver retinol (Erdmann *et al.*, 1986). This experiment was designed to study the effects of some alternative vegetable protein sources for salmon on the accumulation of canthaxanthin, its reductive metabolites (i.e. echinenone and β-carotene; Schiedt *et al.*, 1988) and other carotenoids from the feed.

Canthaxanthin was chosen in order to be able to separate the carotenoids originating from the experimental diets from those ingested prior to the experiment.

MATERIALS AND METHODS

Feeding experiment

The experiment was carried out at AKVAFORSK at Sunndalsøra, Norway, in a 10 week period from late August to mid November 1989. Atlantic salmon (*Salmo salar)* with an initial average weight of 218 g, were used as the experimental fish. Initially, 23 fish were distributed into each of 12 outdoor fibre-glass tanks (1.4 × 1.4 × 0.4 m) arranged in three

randomized blocks. The tanks were supplied with seawater with an average temperature of 10°C during the feeding period.

The formulation and chemical composition of the experimental diets are given in Table 1. Triplicate groups of fish were fed each diet in excess by automatic feeders every 10. minute. Prior to the experiment the salmon had been fed a commercial diet (TESS Elite Pluss, T. Skretting A/S, Stavanger, Norway) containing astaxanthin as the major carotenoid.

The fish in each tank were weighed together at the beginning of the experiment, and after 2, 6 and 10 weeks of feeding. For digestibility

Table 1. Composition of the diets.

Diet #	1 maize gluten	2 peas	3 rapeseed	4 canola
Ingredients, g/kg				
Fish meal (Norse Lt-94)	250	450	300	300
Wheat bran	200	0	0	0
Maize gluten	300	0	0	0
Pea (ground)	0	300	0	0
Rapeseed	0	0	450	0
Canola rapeseed	0	0	0	450
Blood meal	30	30	30	30
Skimmed milk powder	30	30	30	30
Fish oil (NorSalmOil)	140	140	140	140
Finnsteam[1]	5	5	5	5
Lignosulphonate[2]	20	20	20	20
NaCl	5	5	5	5
Ground limestone	10	10	10	10
Vitamin and mineral premix[3]	10	10	10	10
Ascorbic acid	1	1	1	1
Lysine	2	0	0	0
Carophyll Red[4]	0.5	0.5	0.5	0.5
Chemical composition, g/kg				
Dry matter (DM), g/kg	949	929	946	938
Protein, g/kg DM	438	419	408	403
Fat, g/kg DM	176	156	181	177
Ash, g/kg DM	82	96	100	94
Starch, g/kg DM	74	117	30	36
Indigestible fibers, g/kg DM[5]	110	80	170	160

[1] Betain-rich byproduct from beat-sugar production, Finnsugar, Helsinki, Finland (Virtanen *et al*, 1989).

[2] Borrebond, Borregård, Fredrikstad, Norway.

[3] Storebakken and Austreng (1987).

[4] Canthaxanthin, 10% formulation, Hoffmann-La Roche Ltd., Basel, Switzerland.

[5] Calculated from the contents of the ingredients.

studies, faeces were obtained by stripping according to Austreng (1978) after two weeks, and pooled for each tank. Pooled samples of flesh, skin and liver were taken for carotenoid analyses. Ten fish were sampled at the start of the experiment, and five fish from each tank were taken at the end. The samples were frozen immediately and stored at –80 °C until analysis.

The percentage weight distribution on flesh (55%), skin (11%) and liver (1.4%) of total body weight was determined in 6 salmon of about the same size as the experimental fish at the end of the experiment.

Analyses

Apparent digestibilities of fat and protein were determined according to Austreng (1978) using Cr_2O_3 as an inert indicator. The chromium content of the diets and faeces were analysed by atomic absorption according to Williams *et al.* (1962).

Chemical composition of the diets were analysed as described by Storebakken and Austreng (1987). Indigestible fiber content was calculated from the contents in the feed ingredients. The ingredients were analysed according to Prosky *et al.* (1985).

Carotenoid analyses were carried out as described by No and Storebakken (1991). The visible absorption spectra of skin and liver extracts were recorded immediately after extraction, while flesh extracts were analysed after concentration and dehydration. Carotenoid composition was analysed by HPLC with a nitrile column (250 × 4.6 mm I.D. stainless steel Spherisorb S-5CN, Phase Separation Ltd., UK). In order to achieve separation of the hydroxycarotenoids astaxanthin, lutein and zeaxanthin, HPLC was carried out with a silica column (250 × 4.6 mm I.D. stainless steel Brownlee Labs SS-5A Spheri-5, Applied Biosystems Inc., USA) in addition to the nitrile column. Skin extracts were pooled for each treatment prior to saponification and HPLC. Prior to HPLC analysis with the silica column, the extracts from other tissues were also pooled for each treatment.

The results were subjected to two-way analysis of variance. Significant differences between treatments were ranked using Duncan's multiple range test. Significant differences are indicated for $P < 0.05$.

RESULTS

The total carotenoid concentration and composition of the four experimental diets are shown in table 2. The maize gluten diet (diet 1) differed from the other three in containing considerable amounts of hydroxycarote-

Table 2. Carotenoid content and composition of the diets.

Diet #	1 maize gluten	2 peas	3 rapeseed	4 canola
Total carotenoid concentration, µg/g	88	50	46	40
Carotenoid composition,%				
Canthaxanthin	50	93	95	96
Hydroxycarotenoids[1]	37	4	3	1
β-carotene	4	3	1	2
Unidentified	9	–	–	–

[1] Mainly lutein and zeaxanthin in diet 1, astaxanthin in all other diets.

noids, mainly lutein and zeaxanthin, besides canthaxanthin. Canthaxanthin constituted more than 90% of the total carotenoid content in all the other diets.

The growth of the salmon varied between 50 and 110 g/fish in average per treatment. This is lower than reported for salmon of the same size by Austreng *et al.* (1987). No significant differences in growth were observed among groups. The mortality varied between 17 and 32% and seemed to be size selective as big fish showed higher survival than small ones. Mortality did not differ significantly among diet groups.

The digestibility of nitrogen (Table 3) was the lowest in the salmon

Table 3. Growth, nutrient digestibility and carotenoid content in the fish[1].

Diet #	1 maize gluten	2 peas	3 rapeseed	4 canola
Initial weight, g/fish	219±0	218±2	217±2	218±2
Weight increase, g/fish	93±19	102±30	50±3	61±17
Digestibility				
Nitrogen	80.7 ± 0.9^{c}	85.4 ± 0.6^{d}	72.7 ± 1.1^{a}	77.5 ± 0.5^{b}
Fat	82.8 ± 1.8^{a}	89.3 ± 0.6^{b}	88.8 ± 1.4^{b}	90.1 ± 1.4^{b}
Carotenoid content, µg/fish[2]				
Flesh	87 ± 3^{a}	196 ± 40^{b}	152 ± 23^{ab}	101 ± 12^{a}
Skin	243±11	283±34	228±16	198±14
Liver	32±7	43±14	49±12	39±4

[1] Mean±s.e., n=3 tanks. Significant ($P<0.05$) differences are indicated by different superscripts^{abcd}.

[2] The carotenoid content of the initial sample was: flesh, 14 µg; skin, 51 µg; liver, 5 µg.

Table 4. Carotenoid composition[1].

Diet #	1 maize gluten	2 peas	3 rapeseed	4 canola
Total carotenoid concentration in flesh, μg/g[2]	0.5±0.0[a]	1.1±0.1[b]	1.0±0.2[b]	0.7±0.0[a]
Composition,% of total				
Canthaxanthin	78±2[a]	88±1[b]	89±1[b]	76±3[a]
Hydroxycarotenoids[3]	22±2[b]	12±1[a]	11±1[a]	24±3[b]
Total carotenoid concentration in skin, μg/g[2]	6.4±0.1	7.2±0.5	7.0±0.5	5.8±0.3
Composition,% of total				
Canthaxanthin	6	11	7	5
Hydroxycarotenoids	4	2	5	7
Echinenone	21	29	22	20
β-carotene	61	51	59	61
Unidentified	8	6	5	7
Total carotenoid concentration in liver, μg/g[2]	7.5±2.0	9.3±2.3	13.0±3.2	10.1±1.4
Composition,% of total				
Canthaxanthin	52±5	62±12	61±16	38±2
Hydroxycarotenoids	9±2[b]	3±0[a]	0±0[a]	3±2[a]
Echinenone	17±3	14±6	14±6	21±1
β-carotene	14±3	14±7	17±11	30±1
Unidentified	9±0	7±3	8±1	8±2

[1] Footnotes, see Table 3.

[2] The carotenoid concentration of the initial sample was: flesh, 0.1 μg/g; skin, 1.9 μg/g; liver, 1.6 μg/g.

[3] Lutein, zeaxanthin and astaxanthin.

fed the rapeseed diet (diet 3) and the highest for pea (diet 2), while digestibility of fat was lower for the maize gluten diet than for the three others.

As can be seen in Tables 3 and 4, the carotenoid content was significantly different between diets only in flesh. The fish fed the diets with maize gluten and canola rapeseed (diet 4) contained less carotenoids than those from the other two groups.

Canthaxanthin made up a greater part, and hydroxycarotenoids a smaller part, of the flesh carotenoids in the pea and rapeseed groups, than in the two other groups (Table 4). HPLC with the silica column indicated that astaxanthin, zeaxanthin and lutein constituted 8, 6 and 6%, respectively, of the total flesh pigment in the maize gluten group. In liver, the fish fed maize gluten had the highest relative content of

hydroxycarotenoids. No other differences in carotenoid composition were observed among treatments.

Echinenone and β-carotene constituted about 80% of the total carotenoid content in skin (Table 4). In liver, these two compounds made up about 30% of the carotenoids. They were present in about equal amounts in liver, while in skin β-carotene concentration was about three times that of echinenone.

DISCUSSION

The low total carotenoid concentration in flesh and the high concentration in skin (Table 4) are in keeping with the report of Storebakken *et al.* (1987) with salmon of about the same size. The carotenoid concentration in liver was much higher than reported in rainbow trout by different authors (Guillou *et al.*, 1989; Hata and Hata, 1975; No and Storebakken, 1991, 1992). This may be due to difference in both species and size and age of the fish.

Dietary protein source did affect carotenoid accumulation, since salmon fed the diets with maize gluten and canola rapeseed contained less carotenoids than fish from the other two groups. The low carotenoid concentration in the maize gluten group coincided with low fat digestibility. However, this experiment does not give sufficient information to draw conclusions about eventual effects of specific components in the maize gluten on fat digestibility and pigment accumulation. The maize gluten diet was the only one with added wheat bran, but the total fiber content was to low compared to the other diets, to explain the lower fat digestibility obtained. Earlier feeding of salmon with diets containing wheat bran at this institute have not led to any reduction in fat and protein digestibilities.

The low carotenoid concentration in flesh in the canola rapeseed group may in part be due to lower carotenoid concentration in the diet (Table 2). However, this difference is to small to explain the whole difference in carotenoid concentration.

The content of hydroxycarotenoids (astaxanthin, lutein and zeaxanthin) relative to canthaxanthin in the maize gluten group was lower in flesh than in the diet. This is in agreement with earlier results with rainbow trout, showing that canthaxanthin is more efficiently utilized for flesh pigmentation than zeaxanthin (Schiedt *et al.*, 1985).

The relatively high hydroxycarotenoid content in flesh from fish in the canola rapeseed group may be due to previous feeding with astaxanthin and low accumulation of canthaxanthin during the experiment.

The presence of echinenone and β-carotene in skin is in agreement

with earlier findings in salmon fed canthaxanthin (Schiedt *et al.*,1988). However, the ratio of echinenone and β carotene to canthaxanthin in this experiment (approximately 1:3:8 of canthaxanthin, echinenone and β-carotene respectively) were much higher than in the study mentioned above (approximately 1:1:2 of canthaxanthin, echinenone and β-carotene respectively). Since Schiedt *et al.* (1988) used Atlantic salmon of about the same age as in this experiment, lower growth, canthaxanthin intake and final body weight in this experiment may be the reason for the difference.

Considerable amounts of echinenone and β-carotene were present also in the liver. The relative proportions of canthaxanthin, echinenone and β-carotene in this organ were 4:1:1. This observation might indicate that the reductive metabolism of canthaxanthin is taking place in the liver. If this is true, β-carotene seemes to be most easily deposited in skin, followed by echinenone and canthaxanthin.

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THE INFLUENCE OF DIETARY LIPID SOURCES AND VITAMIN E ON IRON STATUS IN POSTSMOLT ATLANTIC SALMON (*SALMO SALAR*)

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ABSTRACT

Atlantic salmon, *Salmo salar*, were given diets with three different lipid sources: soyabean oil, capelin oil and sardine oil. The diets were used with and without addition of 300 mg α-tocopheryl acetate per kg diet.

After 12 months of feeding no significant differences were observed between the experimental groups in iron concentrations in liver, serum, spleen and muscle, and in blood hemoglobin concentration. However, hepatic iron tended to be higher in fish given soyabean oil than in fish given the fish oils. Blood hemoglobin concentration was positively correlated to the hepatic iron concentration in the low vitamin E groups. Vertebrae iron content showed variations according to both dietary lipid source and vitamin E.

INTRODUCTION

Iron metabolism in salmonids is poorly understood. The minimum requirement of iron in feed for Atlantic salmon (*Salmo salar*) has been found to be in the range of 60 to 100 mg/kg (Lall, 1989; Andersen *et al.*, 1992; Bjørnevik, 1992).

Iron deficiency symptoms in fish due to low dietary iron concentration are rarely seen. However, antioxidant vitamin deficiencies (vitamin C and E) may induce iron deficiency-like symptoms (Maage *et al.*, 1990; Hamre, pers. comm.). On the other hand, it has been suggested that iron metabolism in Atlantic salmon is poorly regulated and that excess dietary iron may result in oxidative damages *in vivo* (Rørvik *et al.*, 1991). This has led to a reduction in the level of iron supplementation in Norwegian commercial fish feeds.

It is therefore of interest to obtain information on how the composition of the diet may influence the utilization of dietary iron. The present study

reports on results obtained in an experiment in which three lipid sources with different levels of (n-3) polyunsaturated fatty acids were compared. Addition of α-tocopherol acetate to the diets was also studied. The growth of the fish and its chemical composition, as well as the utilization of zinc and selenium, were described in previous communications (Maage and Waagbø, 1990; Waagbø *et al.*, 1991).

MATERIALS AND METHODS

Experimental conditions

Three diets varying in (n-3) polyunsaturated fatty acids (PUFA) and with and without vitamin E were fed to duplicate groups of Atlantic salmon (*Salmo salar*) for 12 months. Initially 750 fish per tank (1.5m × 1.5m × 1.5m), weighing 31 g were set up. After 5 months 450 fish per group were transferred to sea cages (3m × 6m × 6m) and fed the experimental diets until sampling. The fish were fed by automatic feeders in the tanks and fed by hand to satiety twice a day in the net pens.

The diets were produced by coating extruded pellets with 16% soyabean oil, capelin oil or sardine oil, with or without addition of 300 mg α-tocopheryl acetate per kg diet. By analysis, the diets contained 19, 35 and 60 g (n-3) PUFA per kg diet, respectively. The mean vitamin E content in the non-supplemented diets was approx. 60 mg/kg compared to 270 mg/kg in the supplemented diets (Waagbø *et al.*, 1991). The diets were supplemented with a mineral mix containing iron (II) sulphate and the iron concentration in the diets was 193 mg/kg. Further details on diet compositions and procedures are given by Maage and Waagbø (1990) and Waagbø *et al.* (1991).

Five fish were randomly sampled from each cage medio March 1989 as described by Maage and Waagbø (1990). Mean water temperature in March was 8°C.

Iron concentration was measured in liver, serum, vertebrae, spleen and muscle, and blood haemoglobin concentration was recorded.

Analyses

Blood samples were treated and analysed for haemoglobin as described by Sandnes *et al.* (1988). Serum samples were stored at –80°C until analysed for iron.

Freeze dried samples of muscle, spleen, liver and vertebrae as well as sera and feed were digested in nitric/perchloric acid (9:1) according to Julshamn and Andersen (1982).

Iron analyses were performed by flame atomic absorption spectroscopy (Perkin Elmer 3030), and were controlled by concomitant analyses of oyster tissue and bovine liver standards from National Institute of Standards and Technology, USA.

Statistical analyses

The data were statistically evaluated by using a CSS: Statistica ™ software (StatSoft, Inc., 1991). A two way ANOVA analysis was performed, and main effects of dietary (n-3) PUFA and vitamin E were tested as well as interactive effects based on mean values (n=5) from each cage. Correlations of analysed parameters on individual fish were included.

RESULTS AND DISCUSSION

No significant effects of lipid sources, vitamin E and interaction between the two were found as regards iron concentrations in liver, serum and muscle. There were no correlations between liver, muscle and serum iron concentrations in individual fish grouped according to dietary n-3 PUFA and vitamin E concentration. However, liver iron concentration seemed to be higher in fish fed soyabean oil than in fish receiving the marine oils (Table 1).

Liver iron concentration was shown to respond readily to changes in dietary iron concentration in salmonids (Walker and Fromm, 1976; Desjardins *et al.*, 1987; Bjørnevik, 1992; Andersen *et al.*, 1992). The

Table 1. Iron status indicators (Means ± SEM, n=10). The concentrations of blood haemoglobin (Hb, g/100mL), serum iron (mg/L), liver iron concentration (mg/Kg wet weight) and iron concentration in vertebrae (mg/Kg wet weight), muscle (mg/Kg wet weight) and spleen (mg/Kg dry weight) of Atlantic salmon given different dietary lipids and two different vitamin E levels.

Dietary lipid source (g n-3 PUFA/Kg diet)	SOYABEAN OIL (19)		CAPELIN OIL (35)		SARDINE OIL (60)	
Vitamin E addition (mg/Kg)	0	300	0	300	0	300
Blood Hb	8.9±0.4	9.1±0.2	8.8±0.4	9.0±0.3	8.2±0.2	8.9±0.3
Serum Fe	1.9±0.4	2.5±0.5	2.1±0.6	2.0±0.3	1.8±0.3	2.0±0.2
Liver Fe	62±5	71±3	61±8	58±6	55±7	54±3
Vertebrae Fe	4.2±0.4	2.8±0.3	3.8±0.3	3.0±0.3	2.6±0.2	2.7±0.3
Muscle Fe	3.7±0.3	3.1±0.3	4.2±0.8	3.9±0.3	4.1±0.6	3.7±0.4
Spleen Fe[1]	860	590	860	1025	870	810

[1] Mean of two pooled samples from five fish each.

levels of liver iron (54–71 mg/kg wet weight) in this experiment were lower than reported by Lie *et al.* (1986) and Hjeltnes and Julshamn (1992) in young and adult Atlantic salmon fed commercial diets (with capelin oil), and in wild Atlantic salmon (Maage *et al.* 1991). The iron concentration in organs from Atlantic salmon fed diets containing between 30 and 190 mg iron/kg for 8 weeks showed only minor differences in all organs except in the liver, which varied in the range 65 to 118 mg/kg wet weight (Bjørnevik, 1992).

As reported earlier there were no differences in weight gain and mortality in the present experiment. Fish from all groups grew from 31 gram to about 400 gram during the 12 months period (for details see Maage and Waagbø, 1990 and Waagbø *et al.*, 1991). Desjardins *et al.* (1987) did not find effects of increasing dietary iron and lipid quality on iron status, growth and physiological responses in groups of rainbow trout (*Oncorhynchus mykiss*) fed 50, 250 and 1250 mg iron/kg feed.

Haematological analyses have been used to study disturbances in iron metabolism in fish (Kawatsu, 1972; Gatlin and Wilson, 1986; Sakamoto and Yone, 1978). Despite no significant effects or interactions of the dietary factors on blood haemoglobin concentration, fish fed the sardine oil diet without addition of vitamin E seem to have somewhat lower haemoglobin concentration compared to the other groups (Table 1). Experiments on fish immunity performed parallel to the present experiment (Waagbø *et al.*, 1992b) also showed moderately lower Hb concentration and increased erythrocyte osmotic fragility in fish when fed these diets in indoor tanks. This points to a possible selection of younger erythrocytes after removal of erythrocytes damaged by lipid peroxidation. This effect is probably related to a marginal vitamin E status as minor differences in erythrocyte total fatty acid composition were found between fish fed the capelin oil diet and the sardine oil diet (Waagbø *et al.*, 1992b). This view is supported by a significant positive correlation ($p < 0.029$) between liver iron and blood haemoglobin concentrations in the low vitamin E groups only.

The iron concentration in the vertebrae decreased when fish were fed increased amounts of dietary (n-3) PUFA without vitamin E supplementation (Table 1). Both dietary parameters imposed significant effects on vertebrae iron concentration, while an interactive effect was not apparent ($p < 0.10$). Besides contributing to the mineralization and in osteocyte iron compounds, the function of iron in the vertabrae is not clear.

The intestinal absorption of iron is susceptible to interactions with other micronutrients as well as other feed components (Hilton, 1989; Maage *et al.*, 1990). Vitamin C and E deficiency has been shown to negatively interact with iron absorption and/or utilization, causing iron deficiency-

like symptoms despite a massive accumulation of iron in the liver and spleen (Maage *et al.*, 1990; Hamre, pers. comm.). On the other hand, increasing dietary iron (10 to 410 mg/kg) has been shown to decrease liver vitamin C concentration to marginal levels (< 5 mg/kg) concomitant with an increased liver iron concentration (Andersen, pers. comm.).

Other studies on (n-3) PUFA and vitamin E have shown that liver total lipid and (n-3) PUFA (Waagbø *et al.*, 1991), liver vitamin E and vitamin A (Waagbø *et al.*, 1992a) and selenium (Maage and Waagbø, 1990) concentrations were affected by the present diets. This illustrates the complexity of studying the causal relations as regards interactive nutrients and physiological responses. Thus, care should be taken not to emphasize single factors as responsible for experimental findings in interaction studies.

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THE CONTRIBUTION OF SUPPLEMENTARY SEA WATER TO THE MINERAL BALANCE OF ATLANTIC SALMON ALEVINS

by

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ABSTRACT:

The concentrations of Ca,K, Mn, Mg, Na, P, and Zn were monitored in eggs and larva of Atlantic salmon (*Salmo salar*) incubated in buffered freshwater and buffered freshwater to which 2% seawater was added. Ca concentrations were significantly higher in the larva reared in the seawater supplemented water, but did not differ in the incubating eggs. The levels of the other elements did not differ in the eggs or larva. Mortalities in the larva reared in freshwater increased after hatching but remained low in the seawater-added group. The results suggest that the low Ca concentration present in the acid waters of western Norway may be responsible for increased mortality and retarded development in Atlantic salmon fry.

INTRODUCTION:

The low water pH of fresh water causes severe problems for the salmon hatcheries in western Norway (Ulgenes and Torrissen, 1989). The survival is impaired by the resulting high aluminium level, particulary under low calcium conditions (Sadler and Lynam, 1988). In sensitive areas, the hatcheries need to adjust the pH of the fresh water in order to achieve a satisfactory survival of salmon eggs and fry (Rosseland and Skogheim, 1986). Adjustments are usually done by adding a solution of aqueous NaOH or a slurry of $CaCO_3$ (lime). Even though the pH is kept at safe levels, above 6.5, the alevins tend to turn dark and increased mortalities occur especially during the green egg stage and also during the last two weeks prior to feeding. This weakening of the alevins is avoided by

addition of small amounts of sea water to the fresh water. In this experiment, we studied the effect of sea water addition on the mineral status of eggs and alevins of Atlantic salmon (*Salmo salar*).

MATERIAL AND METHODS:

Fertilized eggs of Atlantic salmon (*Salmo salar*) were divided into twelve lots and were incubated in two hatching trays supplied with pH buffered (NaOH) water from the Matre River or with riverwater to which 2% seawater (0.6–0.7‰ salt) had been added. Samples, consisting of 10 randomly selected eggs or larvae from the 6 riverwater and the 6 seawater supplemented groups, were collected every seven days from fertilization until yolk sac absorption on day 154.

The conductivity of the fresh water was in the range of 10–15 μmho and total hardness 1.5–2.5 ppm as CaO. In the sea water supplemented system the conductivity was 1900–2100 μmho. The average aluminium level in the fresh water is 70–80 μg/l, but may peak 300 μg/l during periods with heavy rainfall or snow melting (Ulgenes and Torrissen, 1989).

The eggs were rinsed with deionized water, were dried at 105° C to constant weight and were then ashed at 550° C overnight. The ash was dissolved in HNO_3 and HCl and appropriately diluted with deionized water. Elemental measurements were made using an inductively coupled argon plasma emission spectrophotometer (Jarrell-Ash Atomcomp, Fisher Scientific, Waltham, Ma.)

RESULTS AND DISCUSSION

The eggs incubated in 2% seawater-supplemented freshwater had a significantly ($P < 0.001$) higher survival rate prior to the eyed stage than the eggs incubated in pure pH adjusted river water (Fig. 1). After the eyed stage, mortality was similar in both treatments. The seawater-supplemented groups tended to be more active than the groups in fresh water. These results correspond to the experience of commercial hatcheries, where supplementation of seawater is regarded as the most efficient way to prevent or treat problems due to the acidic water supply. Seawater is assumed to make complexes with the inorganic monomeric forms of aluminium present in the fresh water, and so reducing the toxicity of the aluminium.

Figure 2 shows the concentrations of Mn, P, K, Na, Mg and Zn during the experiment. No significant differences were observed in Mn, P, K, Na and Mg. Zinc levels were similar with the exception of the last two

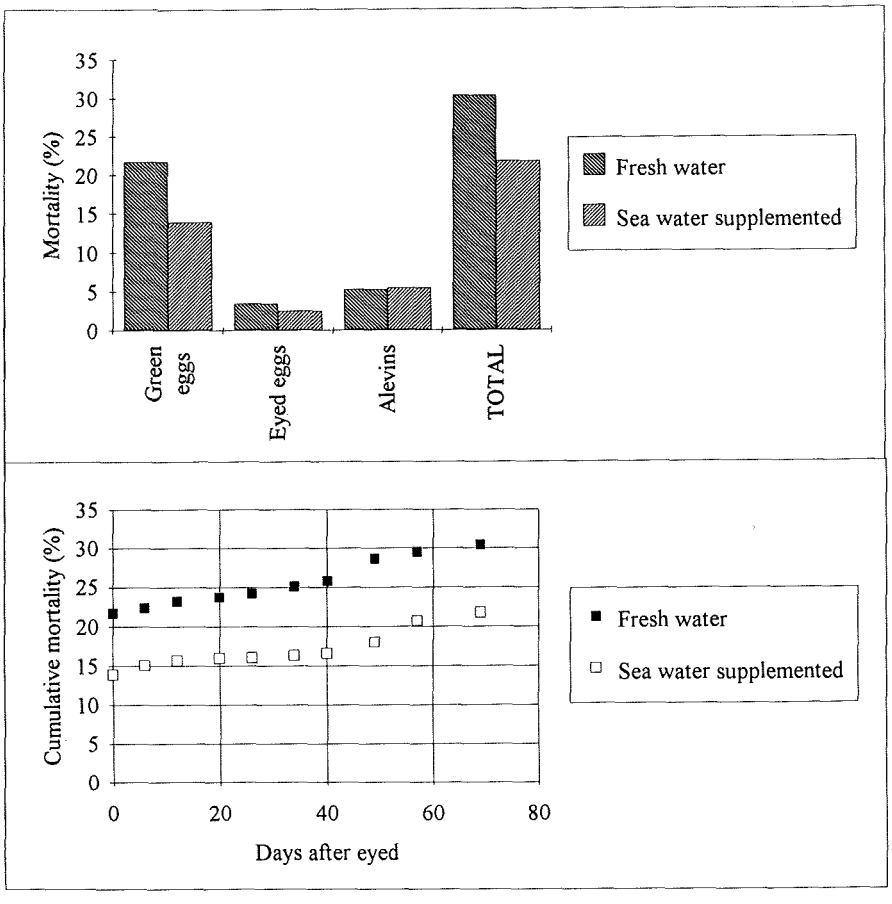

Figure 1. Mortality of Atlantic salmon eggs and alevins incubated in fresh water and fresh water supplemented with 2% seawater.

samplings where the level of Zn in the pure fresh water group increased compared to the seawater-supplemented groups.

The calcium levels were identical in the two treatments prior to hatching (Fig. 3). During the alevin period, the amount of Ca in the seawater-supplemented groups increased while the level in the freshwater groups remained constant. An increase in Ca concentration during this period is observed in rainbow trout (*Oncorhynchus mykiss*)(Ogino and Yasuda, 1962; Zeitoun *et al.*, 1976). Reader *et al.* (1988) reported that aluminium impaired gross development of brown trout (*Salmo trutta* L.) and net uptake of calcium, potasium and sodium. Our results on calcium confirm this observation, but we did not find corresponding results for Na and K.

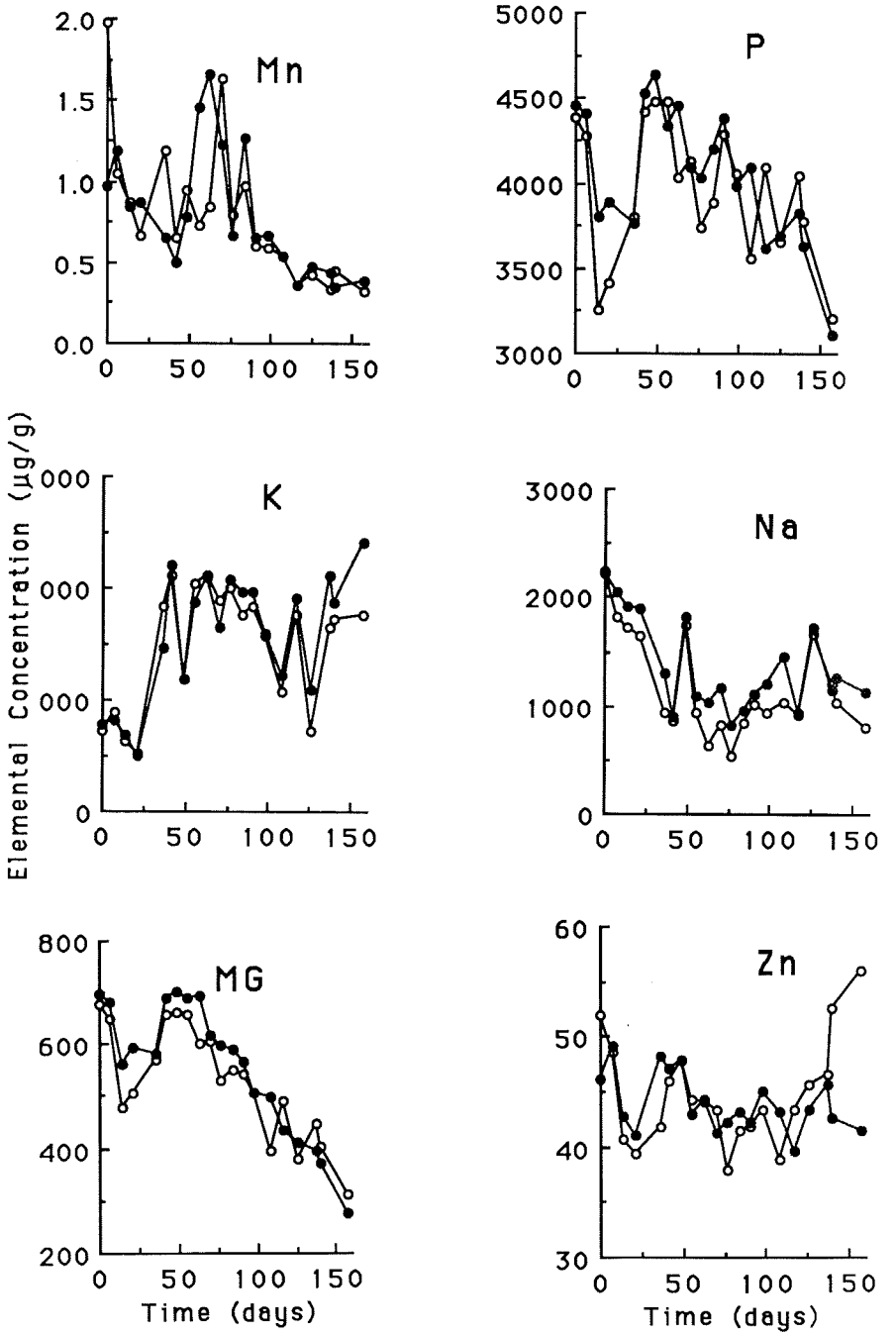

Figure 2. Concentrations of Manganese, Potassium, Sodium, Magnesium and Zinc in eggs and alevins of Atlantic salmon incubated in freshwater and seawater (2%) supplemented fresh water.

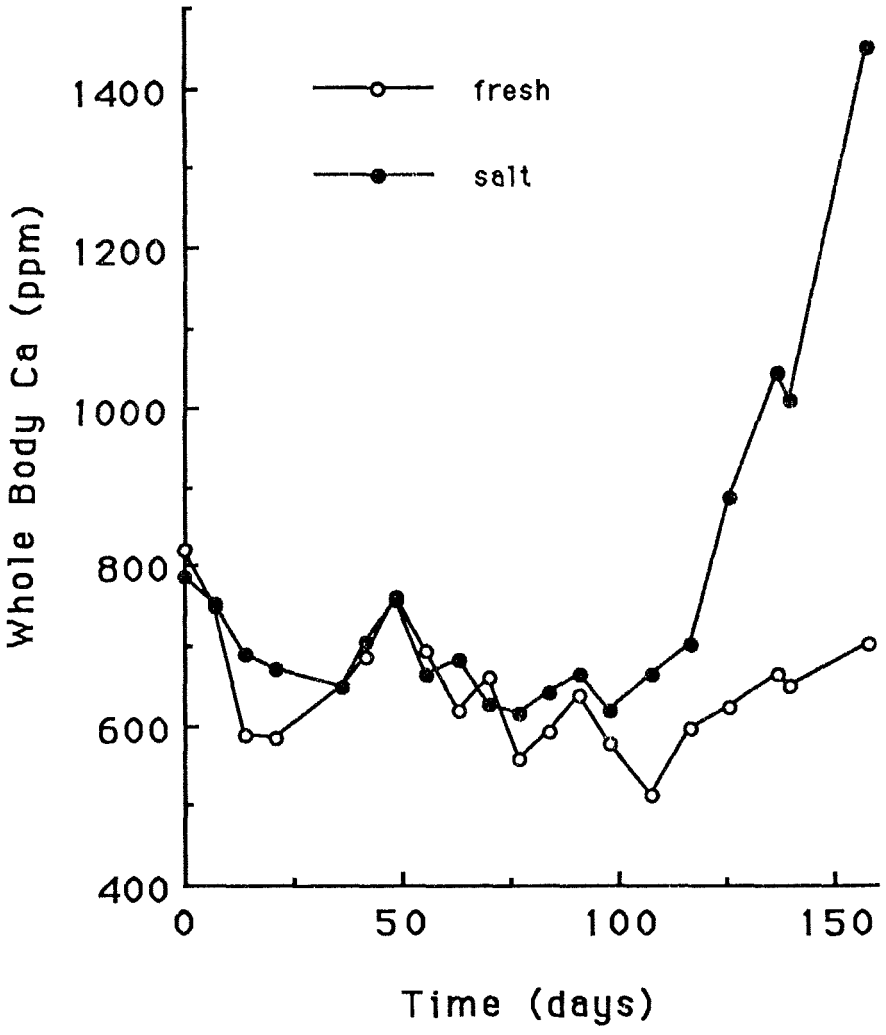

Figure 3. Concentrations of Calcium in eggs and alevins of Atlantic salmon incubated in freshwater and seawater (2%) supplemented freshwater.

Our results indicate that not only does the seawater contribute to the osmoregulatory function of the Atlantic salmon eggs and larvae, but it also supplies Ca for structural development and improve its availability in soft, acidic fresh water.

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HAEMATOLOGICAL VALUES AND CHEMICAL COMPOSITION OF HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS* L.) FED SIX DIFFERENT DIETS

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ABSTRACT

Results from an experiment with reared wild caught halibut are reported using six different dietary treatments. Haematocrit and red blood cell count were within normal ranges from 24–30% and 1.95–2.11 × $10^{12}L^{-1}$, respectively. Glucose levels were low and ranged within normal levels from 1.1 to 2.8 mmol/L. Low and stable fillet glycogen were found with no variation between dietary treatments. Also liver glycogen levels were low and ranged from 13 to 39 g/Kg. The results indicate no negative effects of feeding formulated feeds with approximately 10% starch on a dry matter basis to Atlantic halibut. Muscle protein levels were stable, while quite large variations were found in muscle dry matter and lipid contents. The variations of dry matter and lipid contents depended on dietary regime and on where the muscle samples were taken. The fatty acid compositions of the fillet lipids reflected those of the feeds, and only minor variations were observed between the groups. The lowest fraction of n-3 fatty acids was found in the fillet part with the highest lipid content. Only small variations were found in proximate composition of the halibut liver.

INTRODUCTION

Atlantic halibut is a cold water fish found in the North Atlantic from Norway to the East Coast of Canada on depths ranging between 20 and 2000 meters. Its diet as a juvenile consists of sand eels, flatfishes and crustaceans and the adult diet consist of a variety of marine fishes and larger crustaceans (Gray 1964, Wheeler 1978). The Atlantic halibut stores its surplus energy in the muscle, liver and subcutanous lipid depots (Berge and Storebakken 1991).

As indicated by Gray (1964) and Wheeler (1978) the natural diet for the Atlantic halibut contains little carbohydrate, and teleologically this species would not be expected to have a system to digest or metabolize large amounts of dietary carbohydrate. However, both α-amylase and α-glucosidase activities have been identified in Atlantic halibut intestines (Glass *et al.* 1987). This indicates that starch may be used in formulated feeds but practical levels have not been investigated. Glucose is a pivotal energy supply to 'glucose-dependent' tissues in vertebrate animals. In addition glucose oxidation occurs in other organs as a smaller and variable fraction of the energy requirement (Hemre 1992). Blood glucose levels are well regulated ($<$ 7 mmol/L) in mammals, and the same regulatory mechanisms have been shown to exist in fish (Plisetskaya *et al.* 1986; 1991, Plisetskaya 1989), however the levels differ (Hertz *et al.* 1989, Hung 1991). Generally, lower blood glucose values are found in sluggish than in highly active fish (Larsson *et al.* 1976, Gutierrez *et al.* 1987, 1991), the flatfishes being in the category sluggish.

Carbohydrate is stored as an energy reserve in the liver and muscle of all animals in the form of glycogen. Varying amounts of liver glycogen have been measured in different fish species. In plaice (Cowey *et al.* 1975) and rainbow trout (Hilton and Atkinson 1982) the stores increased when dietary starch increased, while in the cod there seemed to be an upper limit of glycogen storage (Hemre *et al.* 1992). Data on how feed carbohydrate affects glycogen storage in the halibut have not been reported.

Growth rate and feed conversion data from the present experiment were published by Bjørnsson *et al.* (1992). The natural diets (diet groups I – III) varied in terms of lipid/protein ratios from 0.5 to 1.5, while the formulated feeds varied in dry matter content, but not in terms of the ratio between protein, lipid and starch (diets IV – VI). No differences were found between growth rates for the whole experimental period. However, quite large variations were found in feed utilization, the feed conversion factor ranging from 1.0 to 3.0 (Bjørnsson *et al.* 1992). The main conclusions were that feed for the ongrowing of halibut may have a lipid/protein ratio of 1.5 without any negative effects on growth or feed utilization and that lipid had a pronounced protein sparing effect.

The aim of the present study was to evaluate further the effect of feed composition upon haematological values in halibut, muscle and liver composition, as well as the effect of dietary carbohydrate on plasma glucose and glycogen retention. In addition the halibut fillet was divided into 4 parts and analysed for proximate and fatty acid composition, these results were correlated to dietary treatment.

MATERIALS AND METHODS

Fish and diets

Six groups of halibut were fed to satiation six days a week on the following diets: I – lean capelin (*Mallotus villosus*), II – fat capelin, III – lean and fat capelin (alternations every third day), IV – moist feed from capelin silage, V – dry salmon feed and VI – moist feed from ground capelin, from 7th November 1988 to 31st May 1990. Exact diet composition and weight data are given by Bjørnsson *et al.* (1992). In diet I and II lipid levels were 6 and 15%, respectively, the variation in dry matter followed the variation in lipid content while protein and carbohydrate were stable at 10% and 0.1%, respectively. Diet IV, V and VI varied in dry matter content, on a dry matter basis all feeds contained 43–46% protein, 20–23% lipid and 10–11% starch. Constant temperature (7° C) and salinity (32.5 gL^{-1}) were maintained throughout the experiment.

Sampling

10 fish from each tank were collected for analyses at the end of the experiment. The fish were knocked dead with a blow on the head just before blood samples were withdrawn from *Vena caudalis* by a medical syringe and put into heparinized tubes. From each tank 5 fish were dissected, and liver and muscle samples were stored at –20° C until analyses for glycogen, proximate and fatty acid composition, and 5 fish were frozen for analyses of whole fish. For fatty acids and proximate composition, muscle from individual fish were divided into 4 parts (Figure 1), pooled samples of these parts were used for glycogen analyses.

Analytical methods

Haematocrit (hct) and red blood cell count (rbc) were determined on heparinized blood, hct within one hour of sampling. Vitrex Pari microhaematocrit tubes were used for hct determinations as described by Sandnes *et al.* (1988). Blood samples were centrifuged at 3000 rpm and plasma was thereafter deproteinized with 0.33 M $HClO_4$. Glucose was measured spectrophotometrically in deproteinized samples as NADPH at 340 nm after a hexokinase reaction in an automated analyser.

Samples of the four muscle parts and of liver and whole fish were analysed for dry matter, protein, lipid, fatty acids and glycogen. Protein (Nx6.25) was analysed according to Crooke and Simpson (1971) and lipid was measured gravimetrically using ethyl acetate extraction. The fatty acid composition of total lipids of fillet and liver was analysed as described

by Lie and Lambertsen (1991). Glycogen in muscle and liver samples were analysed using an enzymatic method as described by Hemre *et al.* (1989).

Statistics

Correlations were obtained by a non parametric regression model (Spearman R CSS Statistica).

RESULTS AND DISCUSSION

Weight, growth and hepatosomatic index of the fish sampled for analysis are given in Table 1. Detailed discussion regarding growth and feed conversion is given by Bjørnsson *et al.* (1992).

The haematological parameters, haematocrit (hct) and red blood cell count (rbc), are given in Table 1. Hct ranged between 24 and 30% (except for group IV) and rbc varied between 1.95 and 2.19 × $10^{12}L^{-1}$ showing a normal variation in groups of healthy fish (Sandnes et al., 1988). Haematological data from halibut seem to be scarce in the literature, while such data are available for other fish species. In turbot and plaice hct and rbc ranged between 28–41% and 1.3–1.8 × $10^{12}L^{-1}$, respec-

Table 1. Growth, hepatosomatic index and haematological values in halibut fed six different diets.

Diet	I	II	III	IV	V	VI
Initial weight, kg	2.30	2.40	2.49	2.46	2.25	2.49
SD	0.33	0.54	0.27	0.25	0.46	0.71
Final weight, kg	4.69	5.31	4.98	4.61	4.22	4.71
SD	1.46	1.63	1.26	0.56	1.25	1.27
Growth, kg	2.40	2.91	2.49	2.14	1.97	2.22
SD	1.29	1.33	1.10	0.52	0.89	0.96
Hepatosomatic index[1]	1.9	2.1	1.9	2.3	2.0	2.0
SD	0.4	0.2	0.4	0.2	0.1	0.3
Haematocrit	27	30	30	24	30	29
SD	0	4	2	2	2	3
Rbc[3]	2.11	2.10	2.19	1.95	2.11	2.07
SD	0.15	0.17	0.14	0.11	0.27	0.08

[1] Hepatosomatic index = liver weight×100 / weight of fish
[2] Haematocrit = percent red blood cells (%)
[3] Rbc = red blood cell count ($10^{12}L^{-1}$).

tively (Aldrin *et al.* 1982; Larsson *et al.*, 1976). In cod hct ranged between 23–33% and rbc beween 1.24–1.56 × $10^{12}L^{-1}$ (Lie *et al.* 1990), and in Atlantic salmon values between 44–49% and 0.85–1.10 × $10^{12}L^{-1}$ for hct and rbc, respectively, were reported (Sandnes *et al.* 1988). According to Larsson *et al.* (1976) these interspecies variations are probably due to an evolutionary physiological adaptation to the mode of life and ecological habitat. Haematological tests and analysis of serum constituents have proved useful in the detection and diagnosis of metabolic disturbances and disease processes in fish (Waagbø *et al.* 1988), therefore knowledge of normal values of these parameters may be useful for halibut farming.

Proximate composition of the four parts of the fillet are given in Table 2. The abdominal part of the muscle (A) (Figure 1) had significantly higher dry matter and lipid levels, and lower protein levels than all other segments of the muscle. The results support that there is an interdependence between dietary lipid levels and storage of surplus energy in the form of lipid in this part of the fish which is one of the largest lipid depots of the halibut. In Atlantic salmon also an

Table 2. Proximate composition (g/kg) of muscle (part A, B, C and D) from halibut fed six different diets (I–VI).

	Initial	I	II	III	IV	V	VI
PART A							
Dry matter	316	498	589	536	491	550	543
Protein	174	127	105	101	76	137	120
Lipid	107	340	437	400	391	358	376
Ash	12	5	5	5	3	7	6
PART B							
Dry matter	249	245	260	249	251	245	245
Protein	194	213	213	217	221	215	216
Lipid	68	2	16	3	2	2	2
Ash	14	14	13	14	12	10	12
PART C							
Dry matter	236	244	258	247	243	241	200
Protein	187	216	223	216	215	213	176
Lipid	10	3	6	4	2	2	3
Ash	12	9	15	12	12	9	10
PART D							
Dry matter	254	267	266	261	279	252	257
Protein	183	182	185	184	186	188	186
Lipid	36	49	45	40	58	35	39
Ash	12	13	12	13	11	11	11

interdependence between dietary lipid levels and muscle lipid levels have been found (Lie *et al.* 1988a). The halibut fed the lean capelin diet had the lowest average lipid values in the abdominal part of the muscle (A) (34%) compared to fish fed with either fat (44%) or alternating fat and lean capelin (40%). In wild caught halibut (2–4 years) a lipid level of about 40% (of dry weight) in this tissue has been reported (Haug *et al.* 1988, Berge and Storebakken 1991).

The back (B) and tail (C) muscle segments had the highest protein contents, with no differences between dietary treatments. Intermediate protein levels were found in the head (D) muscle segment, compared to the abdominal part of the muscle (A) with the lowest protein level. Very low lipid levels were found in part C and B (less than 2%), although higher lipid values were measured also in these segments in fish fed fat capelin compared to the other dietary fish groups. In part D lipid levels varied between 3.5 and 4.9% with no correlation to dietary lipid levels, in this segment dry matter levels followed the variation in lipid levels. These results indicate that the variations in muscle composition were more dependent on where the samples ('steaks') were collected than from what dietary regime the reared halibut was taken. The same variation in proximate muscle composition was seen at the start of the experiment (initial, Table 2). The levels were, however, different and lower lipid levels were measured in part A. These results are consistent with results found in other fish species storing its surplus energy in the muscle and adipose tissue (Lie *et al.* 1988a, Berge and Storebakken 1991). Very high liver lipid levels were found in halibut from all dietary treatments, however, this organ is of small size and is therefore considered of minor importance as an energy reserve in the halibut, in contrast to what is found in lean fish such as the cod (Lie *et al.* 1986, 1988b). The liver proximate compositions could not be correlated to variations in proximate compositions of the diets.

In whole body homogenates (Table 3) the lipid levels were higher ($p < 0.05$) in the fish fed fat and fat and lean capelin than in fish fed the other diets. Dry matter levels reflected lipid levels, while protein was constant irrespective of dietary treatment. The results are in agreement with whole body analyses of other fish species (Lie *et al.* 1988a).

Low muscle glycogen levels were measured in the present experiment in line with measurements in other fish species (Hemre *et al.* 1991, 1992). No measurements of digested or absorbed amount of glucose were made in the present experiment. However, Atlantic halibut has intermediate activities of starch hydrolyzing enzymes in the intestinal tract (Glass *et al.* 1987). The halibut fed fat capelin and fat and lean capelin had significantly ($p < 0.05$) lower liver glycogen levels than the fish fed either lean

Table 3. Proximate composition (g/kg) of liver and whole body from halibut fed six different diets(I–VI).

	Initial	I	II	III	IV	V	VI
LIVER							
Dry matter	545	624	630	614	548	586	624
Protein	62	74	87	80	80	73	60
Lipid	417	513	493	494	412	455	523
Ash	6	14	14	15	11	15	14
WHOLE BODY							
Dry matter	324	352	368	372	346	339	342
Protein	159	166	154	158	163	166	163
Lipid	117	151	182	179	144	142	144
Ash	19	16	20	17	22	18	20

capelin or formulated feeds, higher liver glycogen levels were found at the start of the experiment (Table 5). However, all levels were low and indicate no negative effects of feeding formulated feeds with approximately 10% starch on a dry matter basis. Also all measured plasma glucose levels were low and within normal ranges which further supports this conclusion. These results are in line with results obtained for cod, another carnivorous marine fish species, which tolerates approximately 10% dietary starch but has problems to efficiently metabolize higher starch amounts (Hemre *et al.* 1989, 1990, 1991, 1992). The very low plasma glucose levels in the present experiment are in agreement with results on

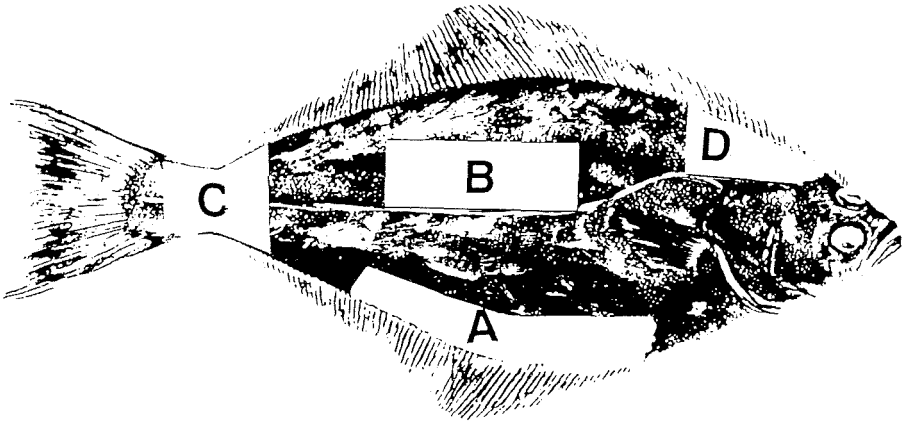

Figure 1. Segmentation of muscle in the present investigation. The muscle was divided into four parts: A = abdominal, B = back, C = tail and D = head.

Table 4. Glycogen in liver and muscle (g/kg), and plasma glucose values (mmol/L) of halibut fed with no added carbohydrate (group I, II and III) and with 10% carbohydrate (formulated feeds – groups IV, V and VI).

	Initial	I	II	III	IV	V	VI
g/kg							
Liver	33	21	15	11	28	25	24
Muscle	2	2	2	2	2	2	2
mmol/L							
Plasma		1.2	1.8	4.7	2.8	2.8	2.6
S.E.M.		0.2	0.2	1.0	0.4	0.5	0.6

S.E.M. (standard error of the mean) values for liver and muscle was below 0.5 for all samples.

active versus sluggish fish species (Larsson *et al.* 1986, Gutierrez *et al.* 1987; 1991).

The fatty acid composition of all feeds were quite similar due to the use of capelin both as lipid and protein sources, with a characteristic high level of the two long chain monoenoic fatty acids 20:1 and 22:1. The fatty acid composition of the fillet lipids reflected those of the feeds, and only minor variations were observed between the groups. However,

Table 5. Fatty acid composition (%) of total lipids, mean values of the diets, the different parts of the fillets (A,B,C and D) and liver of the halibut fed the experimental diets.

	DIET	FILET				LIVER
		A	B	C	D	
14:0	6.3 (0.2)*	6.0 (0.1)	4.3 (0.2)	4.6 (0.2)	6.1 (0.2)	4.8 (0.2)
16:0	12.3 (0.4)	11.1 (0.2)	15.1 (0.4)	13.9 (0.2)	13.1 (0.2)	11.0 (0.1)
18:0	1.3 (0.2)	1.2 (0.1)	2.6 (0.2)	2.4 (0.2)	1.8 (0.1)	1.4 (0.1)
SUM SAT.[A]	20.5 (0.4)	18.7 (0.2)	22.4 (0.4)	21.4 (0.1)	21.5 (0.1)	18.7 (0.2)
SUM 16:1	9.2 (1.0)	9.9 (0.6)	7.5 (0.8)	8.2 (0.9)	11.2 (0.6)	12.4 (0.3)
SUM 18:1	13.4 (1.2)	14.8 (0.7)	14.0 (0.3)	15.4 (0.5)	17.8 (0.7)	23.0 (1.4)
SUM 20:1	14.8 (1.0)	13.6 (0.3)	9.7 (0.5)	11.1 (0.5)	12.9 (0.2)	8.6 (0.9)
SUM 22:1	17.7 (1.3)	14.3 (0.4)	8.2 (0.5)	9.9 (0.3)	10.9 (0.3)	6.7 (1.0)
SUM MON.[B]	56.1 (1.9)	53.1 (0.7)	39.6 (1.6)	44.8 (1.6)	53.2 (0.8)	51.4 (2.5)
20:5n-3	7.8 (0.6)	6.7 (0.4)	10.6 (0.1)	8.8 (0.2)	7.9 (0.4)	10.6 (1.6)
22:6n-3	6.7 (0.6)	5.3 (0.2)	20.5 (1.3)	17.9 (1.5)	10.5 (0.5)	10.7 (0.5)
SUM POL[C]	20.4 (1.4)	17.4 (0.7)	35.5 (1.3)	31.8 (1.5)	23.6 (0.8)	27.3 (2.4)

* S.E.M. (standard error mean) values are given in parantheses
[A] SAT. = SATURATED
[B] MON. = MONOENES
[C] POL. = POLYENES

some differences between the different parts of fillets were found. The fatty acid composition of feed, liver and fillet (average for the four parts) of the different feeding groups are presented in Table 5. Due to metabolic modification of dietary lipids an increased level of 18:1 and reduced levels of 20:1 and 22:1 were measured. The lowest level of n-3 fatty acids was found in the part of the fillet with the highest lipid content (A), mainly due to a higher proportion of neutral lipids. Neutral lipids contain lower levels of n-3 fatty acids than phospholipids (Lie and Lambertsen 1991).

The wide interest in the nutritional value of n-3 polyenoic fatty acids from fish warrants a proper choice of lipids in the feeds. Using capelin oil in the diets, 100 gram of fillet A gave 4–6 gram of n-3 fatty acids, while 100 gram of fillet B gave only 0.1–0.5 gram of n-3 fatty acids.

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FATTY ACID COMPOSITION OF GLYCEROPHOSPHOLIPIDS AND NEUTRAL LIPIDS IN SIX DIFFERENT TISSUES OF HALIBUT (HIPPOGLOSSUS HIPPOGLOSSUS) FED CAPELIN AT CONSTANT TEMPERATURE

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ABSTRACT

Halibut, were fed at constant temperature (7±° C) intervals with lean and fat capelin for 5 months. The fish had a mean weight of 3.7 Kg at sampling.

White muscle, liver, gill, heart and spleen and red blood cells were collected. The lipids were extracted and the fatty acid composition of the glycerophospholipids: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS) was analyzed.

The pattern of fatty acid distribution within each of the individual phospholipids from the different tissues of halibut showed some general similarities. PC had the highest level of 16:0; PE the highest total PUFA; PI showed the highest level of 18:0 and 20:4n-6 and had the lowest n-3/n-6 ratio. PS had lowest ratio of 20:5n-3/22:6n-3.

INTRODUCTION

Numerous reports on the fatty acid composition of total lipids of many species of fish and organs from fish have been published during the last 30 years. However, the fatty acid composition of individual phospholipids has received limited attention. The major phospholipids of marine animals are dominated by 20:5n-3 and 22:6n-3, giving a high (n-3)/(n-6) ratio, with the exception of phosphatidylinositol which is unusual in being rich in 20:4n-6 (Bell *et al.*,1983,1985; Tocher and Sargent, 1984; Lie *et al.*, 1989, 1992 and Lie and Lambertsen 1991). Relatively few studies of lipid composition in marine fish have included fatty acid compositions of individual phospholipids and still the roles of n-3 and n-6 long chain polyunsaturated fatty acids (PUFA) in marine phospholipids are not yet fully understood.

Phospholipids have important structural functions in fish and occupy a key role as a bridge between the dietary pool and the metabolic requirement for fatty acid precursors. According to Greene and Selivonchick (1987) 'it should be the goal of the next decade to take a comprehensive view of lipid metabolism in fish and delineate more clearly the ways in which lipids in fish function analogously to those in mammalian systems and to demarcate where the pathways diverge'. They suggest that the phospholipids should provide a promising starting point.

This paper present the fatty acid composition of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS) from six different tissues in halibut.

MATERIALS AND METHODS

Fish and diets

Wild caught halibut, were fed *ad lib* lean (Mon.–Wed.) and fat (Thurs–Sat.) capelin for 5 months in circular tanks indoors, 2.9m in diameter and the bottom was covered with sand. The fish had a mean weight of 3.7 Kg at sampling. The temperature and salinity were constant, 7 $\pm 0.2°$ C and 32.5 $\pm$ 0.5 gL^{-1}, respectively.

Sample collection

Pooled samples from five fish (0.7–1.0 Kg) of white muscle, liver, gill, heart and spleen and red blood cells (RBC) were collected and blood samples were collected from *Vena caudalis*. The organs were immediately frozen on dry ice and stored at –80° C until further analysis.

Analytical methods

The lipids were extracted and the glycerophospholipids were separated by HPLC according to Lie and Lambertsen (1991). The column (25 × 0.46 cm) was packed with silica gel (LiChrosorb 5μm, Merck, Darmstadt, FRG) and all solvents were of HPLC grade from Rathburn Chemicals Ltd (Walkerburn, Scotland).

The identity and purity of the isolated phospholipids were verified by thin layer chromatography (Merck, Darmstadt, FRG Kieselgel 60) using standard phospholipids in a solvent system of ethyl acetate:n-propanol: chloroform:methanol:0.25% aqueous KCL (15:25:25:10:4.5, by volume). No cross – contamination of the phospholipid classes was seen. The

phospholipid standards, PC, PI, PE and PS were purchased from Sigma (St. Louis, USA). The phospholipid fractions were evaporated, saponified, 19:0 was added as internal standard and used to calculate the relative levels of the different phospholipids by means of the sum of fatty acids in each fraction.

The fatty acids were esterified in 12% BF_3 in methanol. The methyl esters were separated in a Carlo Erba 2900 gas chromatograph ('on column' injection) equipped with a 50m CP-sil 88 (Chrompack, Middelburg, The Netherlands) fused silica capillary column (id: 0.32 mm). The fatty acid composition was calculated using a Maxima 820 (Chromatography workstation, installed in a IBM-AT), and identification ascertained by reference to a standard mixture of methyl esters (Nu-Chek, Elysian, USA).

RESULTS AND DISCUSSION

Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) accounted for 50–68 and 20–39%, respectively of the relative levels of the glycerophospholipids, while phosphatidylinositol (PI) and phosphatidylserine (PS) made up 6–15 and 3–14%, respectively (Table 1). Similar results are reported for cod by Lie and Lambertsen (1991).

Table 2 details the fatty acid composition of the neutral lipids of the different tissues. 16:0 was the main saturated fatty acid, while 18:1n-9 was the main monoenoic fatty acid. However, the two long chain monoenes, 20:1n-9 and 22:1n-11, accounted for 7.4–14.1% and 5.4–12.3% of the neutral fatty acids. About 30% of the fatty acids were of the n-3 series, mainly 20:5n-3 and 22:6n-3, except for the fillet lipid. Lipids in marine organisms are characterized by high ratios of (n-3)/(n-6) polyenes, and high contents of n-3 polyenes particularly in the phospholipids, whereas storage forms of lipids, mainly triacylglycerols in fish, has lower levels of polyunsaturated fatty acids than the phospholipids. Halibut stores surplus lipid partly in the fillet (abdominal part) as well as in subcuta-

Table 1. Relative levels of the glycerophospholipids in the different tissues of halibut fed capelin.

	RBC	Gills	Spleen	Heart	Liver	Fillet
PC	68	52	50	51	50	62
PE	20	21	38	39	22	28
PI	6	13	6	7	15	7
PS	6	14	6	4	13	3

Table 2. The fatty acid composition (%) of neutral lipids of different tissues of halibut fed capelin.

	RBC	Gills	Spleen	Heart	Liver	Fillet
14:0	4.0	3.8	3.7	3.8	4.5	6.2
15:0	0.3	0.2	0.3	0.3	0.2	0.3
16:0	10.7	10.1	11.3	12.0	10.7	11.6
16:1n-9	0.7	0.6	0.5	0.6	0.6	0.3
16:1n-7	6.1	6.6	5.7	8.0	10.2	11.1
17:0	0.5	0.9	0.2	0.5	0.6	0.3
18:0	2.8	3.6	4.1	2.1	1.2	1.5
18:1n-11	1.0	0.8	1.0	1.5	n.d.	n.d.
18:1n-9	10.1	10.7	8.9	12.1	17.1	13.4
18:1n-7	3.5	4.3	4.2	4.6	4.2	3.9
18:2n-6	1.1	1.4	1.1	1.4	1.1	1.2
18:3n-3	0.1	0.3	0.2	0.3	0.3	0.3
20:1n-11	0.6	0.5	0.2	0.6	n.d.	n.d.
20:1n-9	9.7	7.4	8.5	8.2	8.9	14.1
20:1n-7	0.6	0.7	0.5	0.5	0.6	0.8
18:4n-3	0.9	0.8	0.6	0.6	0.7	1.8
20:4n-6	0.8	2.9	1.9	0.8	0.2	0.1
22:1n-11	9.6	5.4	8.9	6.5	6.8	12.3
22:1n-9	1.5	0.8	1.2	1.1	1.6	1.4
20:4n-3	0.2	0.4	0.4	0.6	0.7	0.5
20:5n-3	12.8	11.5	11.6	10.1	9.9	7.4
22:5n-3	1.2	1.8	1.5	1.6	1.8	1.0
22:6n-3	14.4	18.9	14.1	17.3	11.8	6.6
Others	6.6	5.6	9.2	4.9	6.0	4.0
Sum saturated	18.9	18.6	19.6	19.2	17.8	19.8
Sum monoenes	44.8	38.8	41.0	45.1	51.7	58.6
Sum n-3	29.7	33.7	28.4	30.5	25.5	17.5
Sum n-6	1.9	4.6	3.1	2.3	1.6	1.6
n-3/n-6	15.8	7.3	9.3	13.4	16.2	11.1

neous depots (Hemre et al., 1992). The halibut in this experiment were fed whole capelin. The mean fatty acid composition of the diet is given by Hemre *et al.* (1992), the sum of saturated fatty acids was 20.5%, sum of monoenes was 56.1% and sum of n-3 was 18.2%. The corresponding values of the neutral lipids of fillet was 19.8, 58.6 and 17.5% and point to that halibut store surplus dietary lipids with small modifications in the fillet. This was not the case for neutral lipids of the other tissues. In these tissues, neutral lipids are only minor parts of the total lipids.

Table 3. The fatty acid composition (%) of phosphatidylcholine (PC) of different tissues of halibut fed capelin.

	RBC	Gills	Spleen	Heart	Liver	Fillet
14:0	3.1	5.9	3.7	2.3	3.4	1.7
15:0	0.4	0.7	0.4	0.4	0.5	0.4
16:0	21.6	24.6	18.1	22.8	18.5	26.1
16:1n-9	0.6	1.6	0.6	0.6	1.1	0.7
16:1n-7	2.5	5.1	3.7	2.1	2.0	1.1
17:0	0.5	0.6	0.4	0.6	0.2	0.4
18:0	3.6	9.5	3.8	3.2	6.4	2.1
18:1n-11	0.5	0.7	0.6	0.5	0.8	1.4
18:1n-9	4.2	12.9	11.9	6.2	4.5	4.1
18:1n-7	2.4	5.0	3.3	2.8	2.7	1.4
18:2n-6	1.5	1.5	1.4	0.9	1.5	0.9
18:3n-3	0.2	n.d.	0.1	0.1	0.1	n.d.
20:1n-11	n.d.	n.d.	0.1	n.d.	n.d.	n.d.
20:1n-9	3.7	5.4	3.3	3.2	4.2	1.4
20:1n-7	0.3	1.4	0.2	0.2	0.6	n.d.
18:4n-3	0.3	n.d.	0.3	0.2	0.2	n.d.
20:4n-6	1.0	1.9	1.6	1.2	0.6	0.9
22:1n-11	1.3	0.8	1.5	0.9	1.0	0.3
22:1n-9	n.d.	n.d.	0.4	n.d.	n.d.	n.d.
20:4n-3	0.2	n.d.	0.2	0.2	0.4	0.2
20:5n-3	18.7	9.2	15.8	14.1	19.8	16.5
22:5n-3	1.7	1.2	2.2	1.4	1.5	1.6
22:6n-3	28.6	9.3	22.2	33.3	26.0	35.1
Others	2.8	2.8	4.3	2.8	3.9	3.5
Sum saturated	29.3	41.2	26.4	29.4	29.3	30.7
Sum monoenes	16.5	33.2	26.7	17.3	17.6	10.9
Sum n-3	49.8	19.6	40.9	49.3	48.0	53.4
Sum n-6	2.5	3.4	3.3	2.3	2.3	1.8
n-3/n-6	20.1	5.7	12.6	21.4	21.0	29.6

A characteristic high content of 16:0, 18–26%, was seen in PC in all organs, more than double the average content in all other phospholipid fractions (Table 3). The monoenes accounted for 11–33% of the fatty acids, being lowest in fillet PC. 18:1n-9 was the major monoene, exceeding 12% in the gills, whereas only small amounts of 22:1 isomers were present. The n-3 polyenes dominated all samples, with the exception of the gills, which contained only 19.6%. The sum of the two major long chain n-3 fatty acids 22:6 and 20:5 exceeded 45% in fillet, liver, heart

and red blood cell PC. High levels of n-3 fatty acids resulted in high (n-3)/(n-6) ratios, ranging between 5.7 and 29.6.

In PE (Table 4), the monoenes, primarily 18:1 and 20:1, accounted for 18–36% of total fatty acids while the saturates ranged from 12 to 27%. Highest levels were found in liver PE, and lowest levels in heart PE. Similar to PC, PE was characterized by high levels of polyenes, lowest in gills and liver. The major polyunsaturated fatty acid was 22:6n-3. This gave a (n-3)/(n-6) ratio between 6 and 34. Low gas chromatograph-

Table 4. The fatty acid composition (%) of phosphatidylethanolamine (PE) of different tissues of halibut fed capelin.

	RBC	Gills	Spleen	Heart	Liver	Fillet
14:0	0.7	1.7	0.7	0.5	2.7	1.4
15:0	0.2	1.0	0.1	0.2	0.5	0.1
16:0	6.5	11.8	5.2	9.9	10.2	9.2
16:1n-9	0.5	1.2	0.1	0.2	0.8	0.6
16:1n-7	1.2	2.2	0.9	0.7	4.9	2.1
17:0	0.1	2.8	0.7	0.2	0.2	0.2
18:0	4.2	9.4	7.5	6.5	6.0	4.1
18:1n-11	n.d.	0.4	0.5	0.5	1.3	1.2
18:1n-9	4.7	7.3	3.5	2.8	9.2	5.6
18:1n-7	5.7	6.5	5.7	3.8	4.6	3.7
18:2n-6	1.1	1.8	0.8	0.5	1.0	0.7
18:3n-3	0.1	n.d.	n.d.	n.d.	0.2	0.1
20:1n-11	0.2	n.d.	n.d.	n.d.	0.4	0.4
20:1n-9	11.8	6.2	7.8	7.2	8.3	7.7
20:1n-7	0.6	0.8	0.5	0.4	1.0	0.3
18:4n-3	n.d.	n.d.	n.d.	n.d.	0.4	0.3
20:4n-6	1.7	2.9	1.7	1.5	0.8	0.6
22:1n-11	5.7	1.8	2.5	2.0	4.0	2.4
22:1n-9	0.4	n.d.	0.2	0.1	0.8	0.2
20:4n-3	0.3	n.d.	0.2	0.2	0.4	0.3
20:5n-3	16.6	9.8	11.4	8.3	12.3	8.0
22:5n-3	1.5	1.8	2.7	1.8	1.5	1.7
22:6n-3	31.1	17.6	35.6	46.4	22.8	39.8
Others	5.0	13.1	11.7	6.1	5.8	9.3
Sum saturated	11.8	26.7	14.2	17.4	19.9	15.1
Sum monoenes	31.3	26.2	21.9	17.9	35.8	24.5
Sum n-3	49.6	29.3	49.9	56.8	37.6	50.2
Sum n-6	3.1	4.7	2.7	2.2	2.1	1.5
n-3/n-6	16.2	6.2	18.2	26.0	18.2	33.9

recoveries of known fatty acids, particularly in the gills and spleen, may be explained by the presence of plasmalogens in the PE. In the microsomal fraction isolated from the gills of cod, Bell *et al.* (1983) found dimethyl acetals only in the PE fraction, implying that approximately one third of PE was present as plasmalogens.

In PI (Table 5) the saturated fatty acids accounted for 30–41% of total fatty acids, as in PC, but in contrast to the high 16:0 in PC, 18:0 was the major saturated fatty acid in PI (17–31%). The monoenes represented

Table 5. The fatty acid composition (%) of phosphatidylinositol (PI) of different tissues of halibut fed capelin.

	RBC	Gills	Spleen	Heart	Liver	Fillet
14:0	0.7	1.6	0.7	1.3	2.6	1.6
15:0	0.2	0.8	0.2	0.3	1.3	0.4
16:0	8.5	9.4	6.9	8.9	9.2	7.8
16:1n-9	0.8	1.1	0.4	1.0	1.0	1.4
16:1n-7	0.8	1.1	0.7	1.6	4.6	1.6
17:0	0.4	n.d.	0.5	0.4	0.3	0.3
18:0	27.8	27.1	29.2	27.0	17.0	30.7
18:1n-11	n.d.	n.d.	0.5	n.d.	1.3	1.4
18:1n-9	5.0	6.3	5.1	6.5	10.5	5.9
18:1n-7	2.4	3.3	3.1	2.6	2.7	2.0
18:2n-6	1.0	1.6	1.1	1.1	1.3	0.8
18:3n-3	n.d.	n.d.	0.1	0.2	0.3	n.d.
20:1n-11	n.d.	n.d.	n.d.	0.1	0.4	0.2
20:1n-9	2.7	2.7	2.9	3.1	4.6	3.7
20:1n-7	n.d.	0.5	0.2	0.2	0.4	n.d.
18:4n-3	n.d.	n.d.	n.d.	n.d.	0.4	0.2
20:4n-6	8.4	15.1	21.3	16.4	13.8	5.7
22:1n-11	1.2	1.4	0.9	1.1	2.6	1.5
22:1n-9	n.d.	n.d.	n.d.	n.d.	0.7	n.d.
20:4n-3	n.d.	n.d.	n.d.	0.1	0.4	n.d.
20:5n-3	16.9	8.1	13.4	15.2	11.5	10.7
22:5n-3	1.3	1.1	1.4	1.0	1.0	1.3
22:6n-3	13.2	8.0	9.5	10.8	7.5	20.6
Others	8.6	10.7	2.1	1.3	4.8	2.4
Sum saturated	37.7	39.0	37.4	37.8	30.5	40.7
Sum monoenes	13.3	16.6	13.9	16.3	29.5	17.5
Sum n-3	31.4	17.2	24.4	27.2	20.9	32.7
Sum n-6	9.4	16.7	22.7	17.5	15.3	6.6
n-3/n-6	3.4	1.0	1.1	1.6	1.4	5.0

13–18% of the fatty acids except for 30% in the liver PI. The fatty acid composition of PI was characterized primarily by high levels of arachidonic acid (6–21%). In gills, spleen, heart and liver 20:4 n-6 was the major polyene, and consequently, PI had the lowest ratio of (n-3)/(n-6), of all the phospholipids. 20:5n-3 was also a prominent PUFA in PI, with higher relative levels than 22:6n-3 in all tissues except for fillet.

Table 6 details the fatty acid composition of PS of the different tissues.

Table 6. The fatty acid composition (%) of phosphatidylserine (PS) of different tissues of halibut fed capelin.

	RBC	Gills	Spleen	Heart	Liver	Fillet
14:0	0.7	1.8	1.1	2.0	2.6	1.6
15:0	0.3	0.3	0.2	0.5	0.5	0.5
16:0	9.2	9.6	8.6	13.6	16.3	11.7
16:1n-9	1.1	1.2	0.7	2.2	1.2	2.4
16:1n-7	1.2	1.2	0.8	2.0	1.2	1.7
17:0	0.2	2.4	0.4	0.6	0.2	0.2
18:0	9.6	22.5	19.9	16.7	8.7	18.3
18:1n-11	n.d.	0.2	0.2	n.d.	0.7	0.9
18:1n-9	4.4	6.3	3.9	7.7	4.7	7.5
18:1n-7	3.8	4.1	4.7	3.0	3.1	2.1
18:2n-6	1.0	1.6	0.9	2.2	1.6	1.3
18:3n-3	n.d.	n.d.	0.1	0.2	0.2	n.d.
20:1n-11	0.2	0.1	n.d.	n.d.	n.d.	0.2
20:1n-9	11.7	8.0	8.9	6.5	5.2	7.0
20:1n-7	0.8	1.7	1.0	0.6	0.8	0.3
18:4n-3	0.1	n.d.	n.d.	n.d.	0.2	n.d.
20:4n-6	0.7	2.1	0.7	0.6	0.7	0.5
22:1n-11	10.8	2.7	1.5	1.8	1.2	2.9
22:1n-9	0.9	0.7	0.1	n.d.	0.2	0.2
20:4n-3	n.d.	n.d.	n.d.	n.d.	0.3	n.d.
20:5n-3	4.8	3.9	3.3	3.6	17.0	3.4
22:5n-3	1.3	2.5	3.5	2.4	1.4	1.9
22:6n-3	33.9	19.8	36.6	31.1	28.4	30.2
Others	3.5	7.2	3.0	2.7	3.7	5.4
Sum saturated	19.9	36.6	30.2	33.6	28.4	32.4
Sum monoenes	35.9	26.7	22.2	23.7	19.1	25.7
Sum n-3	40.0	26.2	43.4	37.2	47.4	35.5
Sum n-6	1.7	3.7	1.7	2.8	2.4	1.7
n-3/n-6	23.7	7.0	25.6	13.4	19.4	20.9

The saturated fatty acids accounted for 20 to 37%. The monoenes varied between 20 and 37%, 20:1n-9 was the main monoenes, except for heart and fillet where about equal levels of 18:1n-9 were found. A notable high level of 22:1n-11 was found in red blood cell PS (11%). The n-3 polyenoic fatty acids (26–47%) were again dominated by 22:6n-3, with contents several times higher than 20:5n-3 in all organs. The (n-3)/(n-6) ratio varied between 7 and 24.

Literature data for individual phospholipids fatty acid composition of halibut seem not to have been presented. However, in general, the compositions of PC, PE, PI and PS confirm the results reported on the fatty acid composition of tissues of several other fish species. One difference between halibut and cod was the higher level of linoleic acid, 18:2 n-6, in cod phospholipids (Lie and Lambertsen, 1991). But cod were fed a commercial diet where 18:2n-6 accounted for about 6% of the total lipids, whereas the capelin fed to the halibut only contained trace levels of 18:2n-6. These results point to the influence of the diet on the phospholipid composition. These aspects are discussed in details by Lie *et al.*, 1992.

PC and PE of several tissues contained more than 50% polyenoic fatty acids of the total fatty acids. These values imply the existence of di-PUFA molecular species in PC and PE. Di-PUFA molecular species of PC and PE have been reported in ripe roe of cod (Bell, 1989). Tocher and Harvie (1988), also found that the fatty acid composition of PE from fish retinas, especially from cod, implied the existence of di-PUFA and even di-22:6 n-3 molecular species. Such molecular species may be of particular importance for the regulation of localized membrane structure and functionality in actively metabolizing tissues. Nevertheless, the presence of di-PUFA molecular species is contrary to earlier ideas on phospholipid structure, and their properties and role in biomembrane function remain to be elucidated (Bell, 1989).

N-6 PUFA and their eicosanoid metabolites are very important in osmoregulatory physiology in mammals (Benabe *et al.*, 1982; Hansen and Jensen, 1983; Wertz *et al.*, 1986). The gills, which constitute the osmoregulatory tissue in teleost fish, have been extensively examined in cod by Bell *et al.* (1983). Overall fatty acid composition of the gills was in general accordance with previous reports.

Fish bone does not contain marrow for haemopoiesis and the haemopoietic sites in fish are primarily the spleen (and kidney). The erythrocyte phospholipids, particularly PS had somewhat elevated levels of the long chain monoenes 20 and 22, as also reported in erythrocytes from cod (Lie *et al.*, 1989).

The fatty acid composition of PI of organs and tissues in halibut warrants special attention. PI from marine fish contains arachidonic acid,

20:4n-6 as a major fatty acid, whereas phospholipids generally are dominated by n-3 PUFA (Bell *et al.*, 1983,1985; Tocher and Sargent, 1989; Lie *et al.*, 1989,1992; Lie and Lambertsen 1991). The major molecular species, 18:0/20:4n-6 of PI (Bell *et al.*, 1986; Bell 1989), corresponds well with that found in terrestrial mammals and suggests that the fatty acid composition in PI is strictly controlled. The high arachidonic acid level in fish PI points to the pivotal metabolic role of this minor phospholipid (Berridge, 1984), as the source of arachidonic acid for eicosanoid synthesis in marine species. However, the presence also of substantial amounts of 20:5n-3 in PI in halibut tissue phospholipids suggests an eicosanoid production of the n-3-series. In the gill, spleen, heart and liver PI, 20:4n-6 dominated over 20:5n-3 and only in fillet PI, 22:6n-3 was found in higher levels than 20:5n-3.

The halibut were fed whole capelin which contain high levels of 20:1 (15%) and 22:1 (18%). Considering the high levels of 22:1 in the diet, only minor amounts of this fatty acid were found in the phospholipids of halibut. This fatty acid seems to play a minor role in the phospholipids, with a notable exception of erythrocytes and particularly in phosphatidylserine. Dietary 22:1 has previous been suggested to be utilised preferentially as an energy source by fish (Henderson *et al.*, 1982; Lie *et al.*, 1986, 1992; Lie and Lambertsen 1991b).

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BIOLOGICAL AVAILABILITY TO RATS OF SELENIUM FROM COD (*GADUS MORHUA*) AND SELENOMETHIONINE RELATIVE TO SODIUM SELENITE[1].

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ABSTRACT

Biological availability of selenium (Se) in cod fillets, selenomethionine and selenite were compared for restoring glutathione peroxidase activity in serum and liver of rats. Groups of Se deficient, weanling, male rats, were given diets containing 0.20 ± 0.02 mg Se kg^{-1} diet. Glutathione peroxidase (EC 1.11.1.9.) activities were measured in serum and liver. The Se concentrations were measured in faeces, urine, serum, liver and femur of the experimental animals. All measurements were done after 3, 6 and 12 days of Se supplementation. Glutathione peroxidase activities in serum and liver and the hepatic Se concentrations were similar in all groups after 12 days. Serum Se concentration was highest in the group given cod fillets. The groups given cod fillets and selenomethionine had significantly higher femur Se concentration than the group given selenite.

The study showed, that Se in cod fillets was as biological available to rats as selenomethionine and selenite.

Key words: Selenium, glutathione peroxidase activity, relative biological availability, organ retention, total apparent retention.

INTRODUCTION

Marine fish and other seafood are amongst our most selenium-rich foods (0.2–0.6 mg Se kg^{-1} wet weight). Previous studies with rats reported a lower biological availability of Se from fish products than from other Se-containing foods such as beef kidney and wheat (Douglass *et al.*, 1981; Alexander *et al.*, 1983). Selenomethionine and selenite are also reported to be more available for chicks than Se from fish solubles and fish meals (Miller *et al.*, 1972; Gabrielsen and Opstvedt, 1980).

In the studies on the biological availability of Se from fish, tuna was the species used (Douglass *et al.*, 1981; Alexander *et al.*, 1983). In a previous study (Lorentzen, 1990) Se from fish species commonly used in Norway showed a good biological availability to rats. In the present experiment the biological availability of Se from cod fillets (*Gadus morhua*) was compared with availabilities of sodium selenite and seleno-L-methionine.

The criterium used for assessing biological availability of Se was based on the ability to restore the activity of the Se dependent enzyme gluthatione peroxidase (EC 1.11.1.9) in serum and liver of Se deficient rats.

The Se retentions in liver, serum and femur were measured and total apparent Se retention was calculated.

EXPERIMENTAL

Three experimental diets were prepared and specified as Fish-Se, Selenomethionine and Selenite. The compositions of the diets are given in Table 1A. Ground and freeze-dried cod fillets were used as the protein- and Se source in the Fish-Se diet. Se-free Torula yeast (Bio-Lux, Belgium)

Table 1A. Composition of the experimental diets (g kg^{-1}).

Ingredients	Experimental diets		
	Fish-Se	Selenomethionine	Selenite
Cod[a]	230	–	–
Torula-yeast[b]	–	440	440
Dextrine[c]	520	310	310
Sucrose	100	100	100
Cellulose	50	50	50
Soyaoil[d]	50	50	50
Mineral mix[e]	40	40	40
Vitamin mix[f]	10	10	10

[a] Ground, freeze dried fillets of cod (*Gadus morhua*).
[b] 0.4 g DL-methionine kg^{-1} diet was added to the Selenomethionine and Selenite diets.
[c] Dextrinized potato starch.
[d] To prevent rancidity 200 mg Ethoxyquin kg^{-1} fat was added.
[e] The mineral mix provided (g kg^{-1} diet): 14.4 $Ca(PO_4)$, 3.8 $CaCO_3$, 8.2 KCl, 2 NaCl, 7.4 $Na_2HPO_4 \times 2H_2O$, 2.8 $MgSO_4$, 0.18 $MnSO_4 \times H_2O$, 0.176 Fe-citrat$\times 5H_2O$, 0.03 $ZnCO_3$, 0.02 $CuSO_4$, 0.0012 KIO_3.
[f] The vitamin mix provided (mg kg^{-1} diet): 22 Vitamin A/D_3 5200/100 IU/g, 60 vitamin E(α-tocoferolacetat) (50%)), 2 Thiamin-mononitrat, 2.5 Riboflavin, 3 Pyridoxin-HCl, 5 Niacin, 60 Ca-Pantothenic acid, 0.2 Cobalamin (B_{12}), 930 Choline-tartrate, 0.75 Menadione.

was used as the protein source in the Selenomethionine and the Selenite diets. To improve the amino acid composition of the Torula yeast based diets, 4 g DL-methionine kg^{-1} diet (from Koch-Light Laboratories) was added. The Selenomethionine and the Selenite diets were supplemented with 0.2 mg Se kg^{-1} diet as seleno-L-methionine and disodium selenite, respectively.

Chemical analyses of the diets are given in Table 1B.

The diets were stored at –20° C for three weeks, prior to the start of the experiment.

Forty-five Se deficient, male Wistar-Møll rats weighing 45 grams were randomly distributed into three groups. The rats (Møllegaard, Denmark) were born of Se deficient mothers and had not received Se in their feed before the start of the experiment.

The rats had free access to the feed, which was given daily in equal amounts to all rats. The feed intake was about 9 grams per day for all rats during the experiment. Distilled, deionized water was given *ad lib.* The rats were caged individually in plexiglass cages, with stainless steel wire-mesh tops and bottoms, in a room maintained at 20 ± 2° C and with an ambient humidity of about 60%.

After 3, 6 and 12 days, five rats from each group were killed by intraperitoneal injection of Mebumal, 0.1 ml per 100 grams body weight. Blood samples were drained from the heart.

Reference data were obtained from five rats killed at the start of the experiment. Urine and faeces were collected throughout the experimental period. The rats were weighed at the start and after 3, 6 and 12 days on the diets.

Table 1B. Proximate and mineral analysis of the experimental diets.

	Experimental diets		
	Fish-Se	Selenomethionine	Selenite
Crude protein g kg^{-1}	264	257	243
Crude lipid g kg^{-1}	52	51	51
Se (mg kg^{-1})	0.2	0.2	0.2
Zn (mg kg^{-1})	18.1	82.3	83.2
Fe (mg kg^{-1})	40.4	101	107
Cu (mg kg^{-1})	7.3	8.8	8.6
Mg (mg kg^{-1})	814	1150	1097
Hg (mg kg^{-1})	< 0.005	< 0.005	< 0.005
Ca (g kg^{-1})	6.8	8.9	8.9
P (g kg^{-1})	4.2	4.9	4.9

ANALYTICAL METHODS

Blood was sampled in glass tubes and left at room temperature for one hour to coagulate. The tubes were centrifuged at 1100 × g in a Wifug 500 S centrifuge for 10 minutes. Serum was decanted and stored at –80° C until analyzed. Femurs were thoroughly cleaned and freeze-dried. The livers were divided in two parts. One part was freeze-dried and homogenized for Se analyses, and the other part was stored at –80° C until enzymatic analyses.

Samples to be analyzed for Se (ca. 0.1 g) were digested in 2 ml of a mixture of concentrated nitric and perchloric acids (9+1; Merck suprapure quality) as described by Maage *et al.* (1991). Se was analyzed by electrothermal atomic absorption spectrometry (ETAAS) using 0.5% Ni as $Ni(NO_3)_2$ as a matrix modifier. Serum and liver samples were analyzed using Deuterium arc background correction. The samples of femur, urine and faeces were analyzed by using Zeeman background correction. The accuracy and precision of the ETAAS methods were tested by analyzing SRM 1577a Bovine Liver and 1566 Oyster Tissue from the National Institute of Standards and Technology (NIST), and Seronorm TM Trace Elements, Serum batch no 105, and Urine batch no. 108, from Nycomed-Pharma, Norway. All methods used were found satisfactory.

For the enzymatic analysis, the liver samples were homogenized in 9 volumes phosphate buffer with 1mM EDTA and pH 7, in a Potter-Elvehjem homogenizer. This suspension was then centrifuged for 10 minutes at 1100 × g. The supernatant was decanted and centrifuged in a MSE Pegasus 65 ultracentrifuge at 105.000 × g for 60 minutes. The serum samples were analyzed after dilution with the phosphate buffer.

The coupled method of Paglia and Valentine (1967), modified by Flohé and Günzler (1984), was used for the determination of GSH-px activity. Enzyme units are reported as μmoles NADPH oxidized per minute per g liver or per mL serum. The substrate was 12 mM tert-butyl-H_2O_2 (from Aldrich-Chemie, Germany) for serum- and 1.5 mM H_2O_2 for liver assays.

All data were analyzed by one-way analysis of variance (ANOVA), followed by Sheffe's multiple range test (STSC, 1987) to assess treatment differences between groups.

RESULTS

The weight gains after 12 days were not significantly different between the groups (52 ± 4, 43 ± 2 and 36 ± 4 g ± SEM for the Fish-Se, the Selenomethionine and the Selenite groups, respectively).

Serum GSH-px activity (Table 2) showed a slow increase in the Seleno-

Table 2. Activity of GSH-Px in serum (U mL^{-1})* and liver (U g^{-1} wet weight) in selenium deficient rats fed different selenium sources for 3 and 6 days (Means±SEM, N=5).

Organ	Diet	Days of repletion		
		3	6	12
Serum	Selenite	1.0±0.1[a]	1.6±0.2[ab]	2.0±0.3
	Fish-Se	1.0±0.1[a]	1.9±0.2[a]	2.5±0.3
	Selenomethionine	0.8±0.1[b]	1.1±0.1[b]	2.0±0.2
Liver	Selenite	18.2±0.6[a]	28.7±2.8	33.3±0.8
	Fish-Se	14.0±0.4[b]	28.2±2.9	36.6±1.7
	Selenomethionine	9.5±1.2[c]	25.7±2.2	33.0±1.9

* Units (U) are defined as μmoles NADPH oxidized per minute.

[2] The GSH-Px activity measured at the start of the study (0-group) in: Serum: 0.13 ± 0.04, Liver: 3.34 ± 0.23.

[a] Same superscript in same column indicates that results are not significantly different ($p>0.05$).

methionine group compared to the Fish-Se group, but after 12 days there were no significant differences in serum GSH-px activity between any of the experimental groups.

After three days of repletion, the Selenite group showed the highest hepatic GSH-px activity ($p < 0.05$), but after 6 and 12 days no significant differences were found between the experimental groups (Table 2).

Biological availability as defined by Gregory and Kirk (1981) is the part of total amount ingested of a nutrient that is involved in a physiological response. In the present study, the term biological availability refers to the ratio between GSH-px activities in serum or in liver and the

Table 3. Relative biological availability (RBA)* of selenium from diets containing cod fillets or selenomethionine as the selenium source, as related to selenite (100%).

Organ	Diet	Days of repletion		
		3	6	12
Serum	Fish-Se	95	113	111
	Selenomethionine	79	67	100
Liver	Fish-Se	77	89	100
	Selenomethionine	56	96	100

* RBA = Glutathione peroxidase activity in serum or in liver (U) divided by total amount selenium ingested.

amounts of Se ingested. Relative biological availability (RBA) for Se from cod fillets and selenomethionine is given as related to 100% for selenite (Table 3).

The serum RBA was low in the Selenomethionine group after 3 and 6 days (79 and 67%) but equal to the Selenite group after 12 days (100%). The serum RBA in the Fish-Se group was higher than in the Selenite group after 6 and 12 days, 113 and 111%, respectively.

Liver RBA was highest in the Selenite group after 3 and 6 days, but all groups were equal after 12 days (100%) ($p > 0.05$).

The Se concentrations in serum, livers and femurs are given in Table 4. The Se concentrations in serum were similar in all groups after 3 days, but after 12 days, the serum Se concentration in the Fish-Se group had increased to a significantly higher level than the other groups. The hepatic Se levels were not significantly different between the experimental groups after 3 and 12 days ($p > 0.05$), but after 6 days the Selenite group had the highest liver Se concentration.

The femur Se concentrations increased gradually in the Fish-Se group and in the Selenomethionine group throughout the experimental period but not in the Selenite group. After 12 days of repletion the Selenite group had a significantly lower femur Se concentration than the other groups ($p < 0.05$).

Table 4. Selenium concentrations in serum (mg L^{-1}), livers and femurs (mg kg^{-1} dry weight) in selenium deficient rats fed different selenium sources for 3, 6 and 12 days* (Means ± SEM; N = 5)

Organ	Diet	Days of repletion		
		3	6	12
Serum	Selenite	0.24±0.02	0.30±0.04[ab]	0.30±0.01[a]
	Fish-Se	0.19±0.04	0.40±0.01[b]	0.40±0.02
	Selenomethionine	0.17±0.01	0.28±0.02[a]	0.34±0.02[a]
Liver	Selenite	0.97±0.1	2.27±0.1	2.62±0.2
	Fish-Se	0.83±0.0	1.64±0.1[a]	3.05±0.1
	Selenomethionine	0.84±0.1	1.56±0.1[a]	2.78±0.1
Femur	Selenite	0.24±0.03	0.19±0.02[a]	0.24±0.01[a]
	Fish-Se	0.27±0.03	0.31±0.03[b]	0.33±0.04[b]
	Selenomethionine	0.27±0.05	0.28±0.01[ab]	0.33±0.02[b]

* Se-concentration at start of the study (0-group) in: Serum (mg L^{-1}): 0.04 ± 0.005, Liver (mg kg^{-1}): 0.20 ± 0.05, Femur (mg kg^{-1}): 0.04 ± 0.02

[a] Same superscript in same column indicates that results are not significantly different ($p>0.05$).

Table 5. Total apparent retention* of selenium in% of intake, in selenium deficient rats fed different selenium sources for 3, 6 and 12 days (Means ± SEM; N = 5). Days of repletion

Diet	Days of repletion		
	3	6	12
Selenite	29 ± 9[a]	42 ± 6	53 ± 7
Fish-Se	61 ± 6[b]	63 ± 3	60 ± 6
Selenomethionine	56 ± 8[ab]	53 ± 8	63 ± 6

* Apparent retention = Se intake (μg) - (Se in urine (μg) + Se in faeces (μg)).

[a] Same superscript in same column indicates that results are not significantly different ($p>0.05$).

The total Se retention was determined as the difference between Se intake and the sum of Se in faeces and in urine. It was, however, not corrected for endogenous Se losses. Thus, the Se retention (percent of intake) is given as apparent values (Table 5). Apparent Se retention after 12 days were 53%, 60% and 63% for the Selenite diet, the Fish-Se diet and the Selenomethionine diet, respectively (Table 5). For the two last diets the apparent retention was remarkably constant during the experimental period whereas the retention of Se from the Selenite diet was low after 3 days and then increasing. After 6 and 12 days the Se retention were similar in all groups.

DISCUSSION

Se is an essential constituent of the enzyme glutathione peroxidase (GSH-px). It is logical, therefore, to use the effect of Se sources on this enzyme as an indicator of biological availability of Se found in chemicals and food items. This approach was used by Gabrielsen & Opstvedt (1980), and Cantor *et al.* (1981) who worked with chickens. It was also used by Douglass *et al.* (1981); Alexander *et al.* (1983); Mutanen *et al.* (1986); Deagen *et al.* (1987) and Knight & Sunde (1988) who used rats.

The problem approached in the present study and in a previous study (Lorentzen, 1990) was to compare the biological availability of Se found in cod fillets (edible part of fish) with Se in sodium selenite and selenomethionine. As judged by the criterium restoration of GSH-px activity in serum and in liver of Se-deficient rats, the biological availability of Se from the three sources tested were similar. This is in agreement with the results of Mutanen *et al.* (1986) for Baltic herring and shrimps. They found, however, that Se from oyster and crab was less well utilized. As

mentioned earlier Douglass *et al.* (1981) and Alexander *et al.* (1983) found that Se in tuna was less available for rats than Se in other Se-rich foods. The reason for this is not known, but it may be related to the high contents of Hg usually found in tuna (Ganther *et al.*, 1972). In the present experiment the level of Hg in the diets was very low (Table 1B).

The availability of Se in fish meals (capelin and mackerel) was studied in experiments with chicks by Gabrielsen and Opstvedt (1980). They measured GSH-px activity in blood plasma and found that fish meal-Se was utilized less than 50% as effective as selenite in restoring enzyme activity in Se deficient chicks. Their experiment lasted for 9 days. Besides that these results were obtained with chicks and the present results with rats, it is possible that whole fish may contain some Se in a chemical form that is less available than Se in fish fillets. It is also possible that there are other constituents in whole fish that reduces the availability of Se.

In the present study, Se from the Fish-Se and the Selenite diets seemed to restore the enzyme activity more rapidly than Se from the Selenomethionine diet. After absorption, different chemical forms of Se ingested must be transformed to the form available for incorporation in GSH-px. It is possible that the transformation time is dependent on the chemical form of the element ingested.

Selenite was found to replenish the liver more rapidly and the femur slower than cod-Se and selenomethoinine. An explanation of this could be that the different chemical forms of Se are metabolized differently.

It is possible that all chemical forms of Se will be utilized for GSH-px over time. The duration of a feeding experiment for assessing biological availability will therefore be of importance. Deagan *et al.* (1987), found no difference in the ability to restore GSH-px activity when selenomethionine, selenocysteine or selenite were given to rats for 9 weeks. Knight and Sunde (1988) found in an experiment with Se-depleted rats that the GSH-px activity after 14 days of repletion with 0.5 mg Se kg^{-1} diet as selenite, was not different from Se-adequate rats, while repletion with 0.1 mg kg^{-1} diet only gave a significant increase in GSH-px activity. Their data show that also the Se level in the diet is of importance for assessing biological availability. From the present study with 0.2 mg Se kg^{-1} diet a repletion period of twelve days seems suitable, as GSH-px activity reached a plateau at this time.

During the years of Se studies, several criteria have been used to assess the biological availability of Se: The protective effects of Se against liver necrosis in rats, exudative diathesis and muscular dystrophy in chicks, whole body retention of Se, or retention in various organs and the ability of Se to restore GSH-px activity (Cantor *et al.*, 1981). Different species of experimental animals may metabolize the various chemical forms of

Se differently. The method chosen for assessing the biological availability, the duration of the study, the chemical form of Se used and the concentration of Se in the diet, will be of significant importance for the results obtained.

From the present study with Se deficient rats fed 0.2 mg Se kg^{-1} diet for 12 days, it is concluded that Se from cod fillets is as biological available as the chemical forms selenite and selenomethionine.

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