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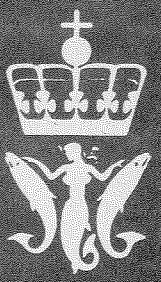

DIRECTORATE OF FISHERIES
BERGEN - NORWAY

EDITORIAL NOTE

Dear reader

The issue of Fiskeridirektoratets skrifter, Serie ernæring you now have in hand is the last one of this journal.

The forerunner of the present journal, Fiskeridirektoratets skrifter, Serie Teknologiske undersøkelse (Directorate of Fisheries, Reports on Technological Research conserning Norwegian Fish Industry), was first published in 1936 and came irregularly up to 1973. The first paper from the Institute of Nutrition in this journal was published in 1952 by O. R. Brækkan and G. Lambertsen. It was in Norwegian and discussed several different methods for determination of vitamin A. Further papers were on vitamins in Norwegian fish and on problems related to the preservation of herring for fish-meal production. From 1958 onwards papers were presented in English, altogether about 30 papers were published in the series Teknologiske Undersøkelser.

The time between 1972 and 1976 was turbulent because of the reorganisation of fisheries research. Fiskeridirektoratets Ernæringsinstitutt (Institute of Nutrition, Directorate of Fisheries) was established separate from The Institute for Chemical and Technological Research in 1975.

Fiskeridirektoratets skrifter, Serie ernæring was first published in 1976. Georg Lambertsen was editor from the start and up to his retirement in 1990. Then Leif R. Njaa took over as editor and in 1993 Amund Maage joined him as a co-editor. The journal was in the beginning published irregularly, depending on the availability of suitable papers. Volume 1 comprised one issue in 1976, 2 issues in 1977, one issue in 1978 and one issue in 1981. Volume 2 comprised one issue in 1981, 2 issues in 1982, and one issue each in 1983, 1984, 1986 and 1989.

From 1990 it was decided to publish regularly 2 issues per year as papers available justified this. This new routine was followed through 1992 but in 1993 only one issue of that years volume was printed. The second issue of that volume is the present one.

Our original plan was to upgrade the journal gradually by introducing "peer reviews" and to change the name to Journal of Fish Nutrition. Regular publication of 2 issues per year of this international journal was anticipated. Letters were written to established scientists around the world to ask them to join the Editorial

advisory board. There were essentially positive answers and it was decided to continue along these lines.

Our plans were thus rather widely known which probably was the reason why we were contacted by Blackwell Scientific Publishers Ltd., (BSP) in Oxford, England. U.K. They had similar plans and suggested that we went into collaboration. The result was a scientific journal called Aquaculture Nutrition with the editorial office at the institute. L. R. Njaa was appointed by the Institute of Nutrition, Directorate of Fisheries, to be Editor in Chief and Ø. Lie as one of the assistant editors. The other assistant editor chosen in collaboration with BSP, was dr. Kim Jauncey from the University of Stirling, Scotland.

In this last issue of Fiskeridirektoratets skrifter, Serie ernæring we take the opportunity to thank all those who have submitted scientific papers to the journal. We have been happy to observe that some of the papers have become real "best-sellers" as they have regularly been referred to in international publications.

We also wish to thank those of you who have subscribed to the journal and those who have been our readers.

We have great hopes for the new journal and we hope that former subscribers and readers will be among the subscribers to Aquaculture Nutrition.

Leif R. Njaa *Amund Maage*

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NUTRIENT CONTENT IN FISH AND SHELLFISH

by

ØYVIND LIE, EINAR LIED, AMUND MAAGE, LEIF R. NJAA
and KJARTAN SANDNES.
Institute of Nutrition, Directorate of Fisheries, P.O. Box 1900 N 5024, Bergen.

INTRODUCTION

In later years the relation between diet and health has been in focus. In this connection food from the sea is important. Fat from fish has received much attention because of their content of n- 3 fatty acids which may protect against cardiovascular diseases and are vital for the development of the brain and the nervous system. Further these fatty acids are necessary for foetal development and recent research indicates that they are also involved in the protection against chronic infection, diabetes and certain types of cancer. Several of these aspects were discussed during the Fourteenth Marabou Symposium (1992).

Fish is an excellent source of protein with a well-balanced amino acid composition and seafood is also a good source for some of the B-vitamins, particularly for vitamin B_{12}. Fish fat is one of the very few natural food sources of vitamin D and contains important amount of vitamins A and E (α-tocopherol). Fish and shellfish are very good sources of selenium, iodine and fluorine, but most of the mineral elements required in nutrition are present in appreciable amounts.

In the present communication are compiled result on the nutrient content of fish and shell-fish obtained over several years in this institute. Also results on the contents of unwanted mineral elements are reported.

MATERIALS

The names (English, Latin and Norwegian) of the species analysed are given in Tables 1a, 1b and 1c for bony fishes, cartilaginous fishes and shellfish, respectively. Only the edible parts of the samples were analysed. In Table 1a fish species traditionally eaten as well as species not generally used for food are included. Except for sprat, which was cured, all fillets were analysed raw.

Table 1 a. Bony fishes

	English	Latin	Norwegian
1.	Angler fishes	*Lophius spp.*	Breiflabb
2.	Ballan wrasse	*Labrus berggylte*	Berggylt
3.	Blue ling	*Molva dypterygia (el. byrkelange)*	Blålange
4.	Blueseacat	*Anarhichas denticulatus (el. Latifrons)*	Blåsteinbit
5.	Bluefin tuna	*Thunnus thunnus*	Tunfisk
6.	Catfish	*Anarchiaslupus*	Steinbit
7.	Chars	*Salvelinus spp.*	Røye
8.	Cod	*Gadus morhua*	Torsk
9.	Common sole	*Solea solea*	Sjøtunge
10.	Conger	*Conger conger*	Havål
11.	Cuckoo wrasse	*Labrus ossiphagus, (el. bimuaculatus)*	Blåstål
12.	Eel	*Anguilla anguilla*	Ål
13.	Gold sinny wrasse	*Ctenolabrecs rupestris*	Bergnebb
14.	Greenland halibut	*Reinhardtius hippoglossoides*	Blåkveite
15.	Grey gurnard	*Trigla gurnardus*	Knurr
16.	Haddock	*Melanogrammus aeglefinus*	Hyse
17.	Halibut	*Hippoglossus hippoglossus*	Kveite
18.	Herring	*Clupea harengus*	Sild
19.	Lemon sole	*Microstomus kitt*	Lomre
20.	Ling	*Molva molva*	Lange
21.	Mackerel	*Scomber scombrus*	Makrell
22.	Mora	*Mora moro*	Mora
23.	Plaice	*Pleuronectes platessa*	Rødspette
24.	Pollack	*Pollachius pollachius*	Lyr
25.	Rainbow trout	*Oncorhynchus mykiss*	Ørret (oppdrett)
26.	Redfish	*Sebastes marinus*	Uer
27.	Rocklings	*Gaidropsarus spp.*	Tangbrosme
28.	Roughhead grenadier	*Macrurus berglax*	Isgalt
29.	Saithe	*Pollachius virens*	Sei
30.	Salmon	*Salmo salar*	Laks (vill)
31.	Salmon	*Salmo salar*	Laks (oppdr.)
32.	Sculpins	*Cottidae*	Ulke
33.	Sprat**	*Sprattus sprattus*	Brisling
34.	Turbot	*Scopthalmus maximus*	Piggvar
35.	Tusk	*Brosme brosme*	Brosme

** cured

Sharks marked with asterisk (Table 1b) were obtained from the Institute of Marine Research, Directorate of Fisheries and were from a pilot fishing expedition to the Caribbean Sea and the West Atlantic Ocean in 1965 (Myklevold, 1966).These samples as well as samples of picked dogfish and of porbeagle caught in Norwegian waters were analysed raw for the B-vitamins, but they

were acetone-dried for the amino acid analyses. The reason for this was that shark meat was considered an interesting raw material for the production of a protein concentrate for human consumption. It was therefore necessary to reduce the content of urea.

The sharks not marked with asterisks were from a pilot fishing expedition in

Table 1 b. Cartilaginous fishes

English		Latin	Norwegian
36.*	Basking shark	*Cetorhinus maximus*	Brugde
37.	Birdbeak dogfish	*Deania calceus*	Gråhå
38.*	Blue shark	*Prionace glauca*	Blåhai
39.*	Brown shark	*Carcharhinus milberti*	Brunhai
40.*	Bullshark	*C. leucas*	Oksehai
41.*	Common hammerhead	*Sphyrna zygaena*	Vanlig hammerhai
42.*	Common thresher	*Alopias vulpinus*	Vanlig revehai
43.*	Dusky shark	*C. obscurus*	Mørkhai
44.	Greater lantern shark	*Etmopterus princeps*	Storsvarthå
45.*	L. Black tipped shark	*C. maculipinis*	Stor svartuggehai
46.	Lanthern shark		Lanternehai
47.	Leafscale gulper shark	*Centrophorus squamosus*	Brunhå
48.*	Mako	*Isurus oxyrinchus*	Makrell hai
49.¤	Picked dogfish	*Squalus acanthias*	Pigghå
50.¤	Porbeagle	*Lamma nasus*	Håbrand
51.	Portuguese dogfish	*Centroscumnus coelipsis*	Dypvannshå
52.	Ray	*Rajidae*	Skate
53.*	Sharpnose shark	*Scolodion terra novae*	Spiss-snutehai
54.*	Sickle shark	*C. falciformis*	Myklehai
55.*	Silky shark	*C. floridanus*	Silkehai
56.*	Whitetipped shark	*C. longimanus*	Hvituggehai

Table 1c. Shell-fishes

English		Latin	Norwegian
57.*	Crab	*Cancer pagurus*	Krabbe
58.*	Lobster	*Homarus vulgaris*	Hummer
59.*	Norway lobster	*Nephrops norvegicus*	Sjøkreps
60.	Oyster	*Ostrea edulis*	Østers
61.	Scallop	*Pecten maximus*	Kamskjell
62.*	Shrimp	*Pandalus borealis*	Reke

* cooked

Norwegian waters arranged by the Directorate of Fisheries in 1993. The catch also included picked dogfish and porbeagle.

Samples taken of fillets (meat) from sharks and skate are listed Table 1b, and samples of the edible parts of shellfish are listed in Table 1c.

Shellfish (Table 1c) included oysters and scallops which were analysed raw, and crab, lobster, Norwegian lobster and shrimps which were cooked in salt water (about $30gL^{-1}$) before the meat was taken.

METHODS

Dry matter was determined by freeze-drying to a constant weight at 105°C or y freeze-drying. Proteins (N x 6.25).was determined in Kjeldahl-digests either by destilation of ammonia or colorimetrically as described by Crooke and Simpson (1971). For some of the shark samples the protein content so calculated was higher than the content of dry matter. In these cases protein was calculated on the assumption that dry matter contained only protein (160mg N g^{-1})and urea (467mg N g^{-1}).

Fat was determined after extraction of dry samples with ethyl acetate and weighing after evaporation.

Ash was determined by ashing at 550 °C.

For the analysis of fatty acids the samples were homogenised and extracted as described by Lie and Lambertsen (1990). The lipid fractions were evaporated and saponified, 19:0 fatty acid was added as internal standard and the fatty acids were esterified in 12 % BF_3 in methanol. The methyl esters were separated using a Carlo Erba 2900 gas chromatograph ("on column" injection) equipped with a 50 m CP-sil 88 (Chromopack) fused silica capillary column (id: 0.32 mm).The fatty acid composition was calculated using a Maxima 820 (Chromatography Workstation, installed in an IBM-AT), connected to the GLC and identification ascertained by standard mixtures of methyl esters (Nu-Chek, Elysian, USA).

Amino acids were determined chromatographically by either Technicon Amino Acid Analyzers or by the Waters system after hydrolysis with 6M HCl. Tryptophan was determined after $Ba(OH)_2$ hydrolysis either chromatographically as described by Slump and Schreuder (1969) or colorimetrically as described by as described by Sachse (1981) .

For element analysis the samples were digested in $HNO_3/HClO_4$ (9 + 1) as described by Julshamn *et al* (1982). All the elements were measured by atomic absorption spectrophotometry (ASS), except sodium and potassium, which were analysed by atomic emission spectrophotometry. The elements sodium, magnesium, potassium, calcium ,manganese, iron, copper and zink were

determined by flame-AAS (Perkin Elmer Model 3030 AAS). Standard curves were used for the calculation of the element in question.

Phosphorus, arsenic, selenium, cadmium and lead were determined by platform electrothermal AAS (Perkin Elmer Model 5000 AAS equipped with deuterium background corrector, a Perkin Elmer HGA-500 graphite furnace and a Perkin Elmer AS-40 autosampler).For the analyses of arsenic and selenium nickel was used as matrix modifier (Maage *et al.*, 1991) and for the analysis of lead ammoniundihydrogenphosphate was used as matrix modifier.The procedure used for phosphorus and selenium, and for arsenic and cadmium were described by Maage *et al* (1991) and Maage and Julshamn (1987), respectively. All platform electrothermal AAS elements were calculated using standard addition procedure, except for phosphorus where the standard curve procedure was used . Total mercury was analysed by cold vapour AAS as described by Egaas and Julshamn (1978).

Retinol (vitamin A), α-Tocopherol (vitamin E) and cholecalciferol (vitamin D_3)were analysed by methods described by Lambertsen (1983), Lie *et al.*(1994) and Horvli and Lie (1994), respectively.The B-vitamins were determined microbiologically, thiamine with *Lactobacillus mesenteroides* (ACTT 12706) ,riboflavin with *Leuconostoc mesenteroides* (ATCC 10100), niacin, pantothenic acid and biotin with *Lactobacillus plantarum* (ACTT 8014) and vitamin B_{12} with *Lactobacillus leichmani* (ACTT 4797) according to standard methods in current use at the Institute of Nutrition.

RESULTS

In Tables from 2a to 2d are given results for bony (*teleost)* fish. In Table 2a are given the proximate composition, fat soluble vitamins and B-vitamins. The fatty acid compositions and contents of n-3 fatty acids and cholesterol in 100g fillet portions are given in Table 2b. Amino acid compositions are given in Table 2c and macro- and micro element contents in Table 2d.

Results for the sharks caught in the 1993 expedition (Table 1b) are listed in Tables 3a to 3d results for the sharks caught in the 1993 expedition (Table 1b) are listed in Tables 3a to 3d.

In Table 3a proximate compositions and vitamins A and D are given in this table are also given analyses of lead.

Fatty acid compositions and n-3 fatty acids in 100g fillet portions are listed in Table 3b. Amino acid compositions and macro- and micro elements are given in Table 3c and 3d, respectively.

The shark samples from the 1965 expedition (Table 1b) were frozen raw and kept for proximate analyses and for analyses of the B-vitamins. These results are given in Table 4b. As the meat was considered as a source for production of

Table 2a. Dry matter (DM). protein (prot). fat and glycogen (glyc). fat soluble vitamins and B-vitamins in fillets from bony fishes.

		Angler fishes 1	Blue ling 3	Blue seacat 4	Bluefin tuna 5	Cat-fish 6	Chars 7	Cod 8	Common sole 9	Conger eel 10	Eel 12
DM	g/kg	-	179	83	261	224	269	196	163	246	541
Prot.	g/kg	-	157	62	240	186	161	181	148	180	173
Fat	g/kg	-	1	10	10	25	71	3	5	52	325
Glyc.	g/kg	-	-	-	<1	1	1	<1			
Vit. A	µg/kg	800	20	-	-	270	-	20	<50	-	6000
Vit. D	µg/kg	10		-	16	18	69	15	-	-	
Vit. E	mg/kg	5	2	-	-	11	13	11	6	-	28
Thi.	mg/kg	-	0.5	-	1.6	0.7	0.9	0.5			
Rib.	mg/kg	-	1.2	-	1.6	0.8	0.2	1.1	-	-	0.4
Niac.	mg/kg	-	25	-	91	22	61	20	-	-	35
Pant.	mg/kg	-	3	-	7	6	9	2	-	-	2
Vit. B_6	mg/kg	-	3	-	-	3	6	2	-	-	-
Vit. B_{12}	µg/kg	-	20	-	48	20	100	10	-	-	-

Table 2a. forts.

		Greenland halibut 14	Haddock 16	Halibut 17	Herring 18	Lemon sole 19	Ling 20	Mackerel 21	Mora 22	Plaice 23	Pollack 24
DM	g/kg	285	192	279	326	191	197	220-500	185	177	184
Prot.	g/kg	176	166	162	152	160	175	140-180	178	134	160
Fat	g/kg	132	2	104	140	3	2	30-300	3	14	2
Glyc.	g/kg	< 1	< 1	1	1	-	< 1	1	-	1	<1
Vit. A	µg/kg	50	20	<20	60	<20	20	140	16	40	20
Vit. D	µg/kg	114	n.d.	180	115	-	34	34-125	-	90	22
Vit. E	mg/kg	22	5	10	6	5	3	6	2	6	7
Thi.	mgtkg	0.6	0.5	0.4	0.4	-	0.5	1.1	-	1.5	0.5
Rib.	mg/kg	0.8	1.1	0.6	3.0	-	0.8	3.6	-	0.9	1.0
Niac.	mg/kg	15	40	44	40	-	23	94	-	35	19
Pant.	mg/kg	3	3	4	10	-	3	10	-	7	3
Vit. B_6	mg/kg	5	5	5	5	-	3	8	-	3	2
Vit. B_{12}	µg/kg	10	20	10	120	-	5	120	-	100	10

Table 2a. forts.

		Rainbow trout 25	Redfish 26	Rough head grenadier 28	Saithe 29	Salmon. wild 30	Salmon. farmed 31	Sculpins 32	Sprat 33	Turbot 34	Tusk 35
DM	g/kg	302	210	176	200	343	310	162	491	209	181
Prot.	g/kg	172	171	165	165	197	184	123	124	159	161
Fat	g/kg	102	28	1	3	115	99	1	176	24	2
Glyc.	g/kg	1	< 1	-	3	< 1	1	-	1	1	<1
Vit. A	µg/kg	100	30	-	20	<50	220	<100	-	40	20
Vit. D	µg/kg	329	n.d.	-	7		80	-	187	17	-
Vit. E	mg/kg	27	10	-	6	13	30	440	12	6	3
Thi.	mg/kg	1.0	1.0	-	0.5	2.0	2.1	-	0.8	-	0.5
Rib.	mg/kg	2.1	1.1	-	2.0	1.5	1.4	-	1.5	-	1.5
Niac.	mg/kg	52	20	-	34	70	82	-	47	-	28
Pant.	mg/kg	20	4	-	4	7	13	-	6	10	3
Vit. B_6	mg/kg	6	2	-	5	6	9	-	2	3	3
Vit. B_{12}	µg/kg	50	10	-	40	-	90	-	70	-	10

Table 2b. Fatty acid composition (% of total lipid) of the lipid extracts of fish fillets from bony fishes. and amounts of omega 3 fatty acids in 100g fillet portions.

	Angler fishes 1	Ballan wrasse 2	Blue-ling 3	Bluefin tuna 5	Catfish 6	Char 7	Cod 8	Common sole 9	Conger eel 10	Cuckoo wrasse 11	Eel 12
14:0	1.0	2.3	2.4	0.1	3.7	4.6	2.0	4.1	4.3	0.8	3.4
16:0	17.7	16.1	19.2	13.2	13.2	12.3	16.3	18.4	18.4	21.6	16.8
∑ 16:1	2.1	5.8	2.7	1.9	10.0	9.2	2.5	8.0	9.2	4.5	8.5
18:0	6.8	5.2	4.0	9.2	2.7	1.5	2.9	3.2	3.0	5.0	4.5
∑ 18:1	14.0	17.9	13.3	13.2	20.9	17.0	9.0	15.4	35.5	10.9	32.4
18:2n-6	1.2	1.0	1.0	1.9	1.2	3.4	1.2	0.9	0.8	0.9	5.2
18:3n-3	0.2	0.8	0.5	0.3	1.7	0.7	0.3	0.5	0.4	0.2	6.0
18:4n-3	0.3	1.3	0.8	0.3	1.7	2.2	1.1	0.8	0.3	0.2	0.5
∑ 20:1	2.5	4.5	3.4	3.2	5.2	12.1	4.7	6.3	4.3	1.2	1.2
20:4n-3	0.1	0.8	0.5	0.3	0.6	0.9	0.4	0.8	0.8	-	1.5
20:4n-6	4.3	5.1	2.4	5.8	4.4	0.4	1.8	3.0	2.1	5.0	2.4
20:5n-3	6.9	13.3	8.5	3.6	10.8	6.4	14.5	5.9	3.6	9.0	2.8
∑ 22:1	0.7	1.7	3.4	2.2	2.1	11.8	1.3	4.0	1.2	-	-
22:5n-3	1.3	2.5	1.5	1.4	1.8	1.3	1.2	5.0	3.6	2.4	2.5
22:6n-3	34.2	16.9	30.8	26.9	10.1	11.0	36.8	16.2	8.9	36.5	3.1
∑ sat.	26.6	23.6	26.2	24.9	21.5	19.2	22.2	26.9	26.5	27.3	26.1
∑ mon.	20.2	29.9	23.5	20.9	38.5	50.7	17.8	33.7	50.2	16.6	42.8
∑ n-3	43.0	35.6	42.5	32.8	26.7	22.5	54.3	29.2	17.6	48.3	17.1
∑ n-6	5.6	6.1	3.6	8.2	6.5	4.1	3.1	4.3	3.3	5.9	8.8
n-3 g/100g*	0.6	0.2	0.1	0.3	0.7	1.6	0.2	0.2	0.9	-	5.6
Chol. mg/100g*	-	-	-	41.0	55.0	61.0	58.0	-	-	-	-

* Contents in 100 g portions of edible product.

Table 2b. forts.

	Gold sinny wrasse 13	Greenland halibut 14	Grey gurnard 15	Haddock 16	Halibut 17	Herring 18	Lemon sole 19	Ling 20	Mackerel 21	Mora 22
14:0	1.7	4.2	2.5	1.1	4.5	7.1	2.4	0.6	5.3	0.8
16:0	22.2	11.0	19.7	20.1	9.0	14.0	16.8	18.1	14.9	20.0
∑ 16:1	4.7	10.5	4.5	1.6	8.7	6.1	5.5	1.1	4.0	1.6
18:0	5.3	2.0	5.0	4.0	1.9	1.0	4.4	5.8	3.1	4.1
∑ 18:1	14.4	24.6	12.9	11.0	24.4	8.6	11.5	12.1	13.9	10.5
18:2n-6	1.1	0.9	1.0	0.9	1.3	1.2	1.0	0.8	1.7	1.0
18:3n-3	0.3	0.4	-	0.2	0.5	1.0	0.4	0.2	1.1	0.1
18:4n-3	0.3	0.9	0.5	0.3	0.7	3.3	0.7	0.2	2.5	0.1
∑ 20:1	4.3	18.1	0.7	1.6	18.5	11.3	4.1	2.2	9.8	2.8
20:4n-3	0.6	0.3	0.3	0.4	0.4	0.5	0.5	0.5	0.9	0.4
20:4n-6	6.1	0.3	1.6	4.6	0.4	0.3	5.6	3.3	0.5	3.5
20:5n-3	7.5	3.0	7.5	16.1	2.7	9.4	13.4	8.3	5.7	7.6
∑ 22:1	0.4	14.3	0.2	0.7	19.6	19.4	0.9	0.4	16.3	0.8
22:5n-3	3.4	0.7	1.6	2.0	0.6	0.7	4.7	2.0	1.2	2.5
22:6n-3	22.2	4.2	39.4	31.4	2.9	9.9	19.2	41.6	13.7	37.7
∑ sat.	29.3	17.8	27.2	26.0	16.1	23.1	26.5	24.8	24.2	25.9
∑ mon.	23.9	68.7	18.5	15.3	72.4	46.5	23.8	16.2	45.7	15.9
∑ n-3	34.4	9.5	49.3	50.3	7.7	24.8	38.9	52.9	25.0	48.5
∑ n-6	7.2	1.6	2.6	5.7	2.1	1.7	7.1	4.1	2.5	4.8
n-3 g/100g*	-	1.3	-	0.1	0.8	3.5	0.1	0.1	0.8-7.5	0.1
Chol. mg/100g*	-	40.0	-	46.0	49.0	68.0	-	46.0	68.0	-

* Contents in 100 g portions of edible product.

Table 2b. forts.

	Plaice 23	Pollack 24	Rainbow trout 25	Redfish 26	Rough head grenadier 28	Saithe 29	Salmon. wild 30	Salmon. farmed 31	Sculpins 32	Turbot 34	Tusk 35
14:0	3.3	0.8	4.4	5.6	2.7	2.5	4.1	5.1	0.9	4.5	1.0
16:0	17.3	19.1	15.5	13.0	14.2	17.1	13.7	14.1	19.5	15.4	19.8
∑ 16:1	8.9	1.0	7.9	7.1	6.3	3.7	5.3	7.9	2.9	6.6	1.3
18:0	4.1	4.4	2.8	2.0	3.1	3.8	2.7	2.6	6.2	1.9	4.5
∑ 18:1	12.3	7.8	21.2	17.0	14.7	15.3	21.9	19.5	14.7	23.3	11.6
18:2n-6	1.1	0.8	4.0	1.6	1.3	1.8	1.2	4.4	1.3	4.2	1.1
18:3n-3	1.0	0.3	0.9	0.9	0.6	1.1	0.7	0.9	0.3	2.2	0.3
18:4n-3	0.7	0.3	1.5	2.4	1.6	0.8	0.9	1.5	0.4	2.1	0.3
∑ 20 1	4.0	1.6	10.2	12.2	8.0	4.6	13.1	11.5	3.6	1.3	3.4
20:4n-3	0.4	0.4	1.0	0.5	0.5	0.6	0.6	1.3	0.3	1.3	0.4
20:4n-6	6.6	2.3	0.4	0.5	2.2	1.9	1.3	0.4	3.0	1.3	2.4
20:5n-3	17.0	11.6	4.6	8.3	10.6	10.7	5.0	5.6	8.4	8.1	6.3
∑ 22:1	0.5	0.2	8.5	12.2	6.1	3.1	12.7	11.2	1.4	0.4	1.1
22:5n-3	4.8	1.5	1.7	0.8	2.3	1.4	2.4	2.1	1.3	3.1	1.6
22:6n-3	10.4	45.1	12.8	11.3	17.0	29.0	10.8	9.0	31.7	18.6	41.6
∑ sat.	26.6	24.9	23.4	21.4	20.0	24.0	21.5	22.5	27.4	23.0	26.0
∑ mon	26.5	11.2	48.1	49.5	35.7	27.2	53.2	50.3	22.8	31.9	18.1
∑ n-3	34.3	59.2	22.4	24.2	32.5	43.6	21.0	20.4	42.4	35.9	50.3
∑ n-6	8.2	3.2	4.6	2.3	3.5	3.6	2.1	5.2	4.5	6.2	3.5
n-3 g/100g*	0.2	0.1	2.2	1.2	-	0.1	2.4	2.0	0.1	0.7	0.1
Chol. mg/100g*	54.0	40.0	59.0	43.0	-	49.0	-	66.0	-	54.0	53.0

* Contents in 100 g portions of edible product.

Table 2c. Amino acid compositions (g/kg protein) of fillets from bony fishes

	Bluefin tuna 5	Catfish 6	Char 7	Cod 8	Eel 12	Greenland halibut 14	Haddock 16	Halibut 17	Herring 18	Ling 20
Ala.	63	48	75	55	58	51	60	56	59	63
Arg.	50	54	62	55	58	57	66	62	59	63
Asp.	79	86	99	77	92	85	102	99	92	109
Glu.	125	140	56	133	121	142	157	130	164	177
Gly.	38	38	50	33	69	34	42	43	46	40
His.	71	22	37	22	29	17	30	25	26	29
Ile.	42	38	50	39	40	40	42	43	46	46
Leu.	71	70	87	72	69	80	84	80	72	86
Lys.	67	86	87	88	81	80	84	105	92	103
Met.	25	27	31	28	23	23	30	31	26	29
Phe.	42	43	56	39	35	45	48	37	39	51
Pro.	29	27	37	28	46	34	30	37	33	34
Ser.	33	38	43	39	40	40	42	43	33	46
Thr.	42	43	143	39	40	45	42	43	39	46
Trp.	13	10	12	11	12	11	12	19	13	11
Tyr.	29	32	37	33	29	34	36	37	33	40
Val.	42	38	56	39	46	40	48	49	53	51

Table 2c. forts.

	Mackerel 21	Mora 22	Plaice 23	Pollack 24	Rainbow trout 25	Redfish 26	Saithe 29	Salmon. farmed 31	Sprat 33	Turbot 34	Tusk 35
Ala.	55	61	67	63	81	64	67	76	81	50	62
Arg.	57	76	75	63	76	70	67	71	89	57	62
Asp.	89	113	112	81	116	105	103	103	145	82	99
Glu.	133	184	187	163	180	170	158	163	202	132	143
Gly.	44	47	52	44	52	47	48	49	65	44	43
His.	44	19	22	25	47	23	24	43	32	19	19
Ile.	44	47	52	44	52	47	48	60	73	38	43
Leu.	74	90	97	81	99	94	85	92	113	69	75
Lys.	86	127	82	81	99	111	91	92	105	82	99
Met.	30	38	30	31	35	29	30	33	40	25	31
Phe.	38	46	52	50	64	58	55	54	81	44	43
Pro.	37	36	37	31	47	35	36	38	56	31	37
Ser.	38	60	52	44	47	47	42	43	56	38	43
Thr.	46	50	52	44	58	53	48	49	65	44	43
Trp.	-	9	7	13	12	12	12	11	0	13	12
Tyr.	36	37	37	38	41	41	36	38	56	31	37
Val.	54	50	52	50	58	47	48	60	73	38	50

Table 2d. Macro- and micro elements in fillets from bony fishes.

		Angler fishes	Blue ling	Bluefin tuna	Catfish	Char	Cod	Common sole	Conger	Greenland halibut	Haddock	Halibut	Herring sole	Lemon	Ling
		1	3	5	6	7	8	9	10	14	16	17	18	19	20
Na	g/kg	0.82	1.04	0.40	0.82	0.48	0.82	0.98	0.56	0.82	0.96	0.90	1.18	0.99	0.72
K	g/kg	3.42	3.12	4.75	3.63	4.20	4.55	3.63	4.08	3.60	3.35	3.63	4.63	3.53	3.60
P	g/kg	0.02	1.60	2.66	2.12	2.67	-	2.00	2.15	1.80	1.90	2.00	2.90	1.90	2.00
Ca	mg/kg	490	90	110	130	160	83	290	130	85	190	60	380	260	280
Fe	mg/lcg	3	1	23	2	2	1	1	1	1	1	2	10	1	2
Se	mg/kg	2.9	0.4	2.0	0.5	0.3	0.3	0.3	0.9	0.2	0.3	0.4	0.5	0.4	0.3
Zn	mg/kg	33	4	6	9	4	5	5	14	4	3	3	5	3	4
Mn	mg/kg	<5	<0.5	<0.7	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Mg	mg/kg	1890	230	390	200	250	290	250	220	190	270	160	380	220	240
Cu	mg/kg	<6	<0.6	1.0	<0.6	0.7	<0.6	<0.6	2.0	2.0	<0.6	<0.6	<0.6	<0.6	<0.6
Hg	mg/lcg	0.00	1.00	0.10	0.10	0.03	0.08	0.05	0.40	0.10	0.07	2.00	0.08	0.08	0.20
Cd	mg/kg	0.0050	<0.0005	0.0200	0.0010	n.d.	<0.08	n.d.	<0.001	<0.001	0.0010	0.0020	0.00300	n.d.	n.d.
Pb	mg/kg	0.20	0.02	0.05	0.02	0.02	<0.08	<0.002	0.06	0.07	0.01	<0.002	0.05	0.01	0.01
As	mg/kg	102.0	9.0	-	9.0	4.0	2.0	4.0	34.0	4.0	11.0	3.0	2.0	105.0	2.0

Table 2d. forts.

		Mackerel 21	Mora 22	Plaice 23	Pollack 24	Rainbow trout 25	Redfish 26	Saithe 29	Salmon. wild 30	Salmon. farmed 31	Sprat 33	Turbot 34	Tusk 35
Na	g/kg	0.75	-	0.95	0.65	0.75	1.08	0.77	0.63	0.57	27.90	0.73	1.18
K	g/kg	3.80	-	2.60	3.85	4.17	3.78	3.96	4.82	4.41	2.09	2.90	2.94
P	g/kg	2.40	-	1.40	2.20	2.44	1.90	2.30	2.62	2.45	1.20	1.60	1.70
Ca	mg/kg	120	-	340	80	200	210	75	83	120	470	160	370
Fe	mg/kg	9	-	1	1	2	2	1	4	4	8	2	1
Se	mg/kg	0.3	-	0.3	0.3	0.3	0.5	0.3	0.5	0.3	0.1	0.3	0.3
Zn	mg/kg	6	-	6	3	4	3	7	4	4	9	6	4
Mn	mg/kg	<0.5	-	<0.5	<0.5	<0.1	<0.5	<0.5	<0.1	<0.1	< 1	<0.5	<0.5
Mg	mg/kg	270	-	190	230	280	260	220	300	280	160	190	230
Cu	mg/kg	<0.6	-	< 1	<0.6	<0.1	1.0	<6	<0.1	<0.1	<2	0.7	<0.6
Hg	mg/kg	0.50	0.03	0.04	0.09	0.09	0.20	0.10	0.09	0.07	0.02	0.10	0.20
Cd	mg/kg	0.0070	-	0.0006	0.0002	<0.004	0.0030	0.0010	<0.004	<0.004	0.0900	<0.001	0.0006
Pb	mg/kg	0.04	-	0.03	0.00	<0.03	0.04	0.01	<0.03	<0.03	<0.002	0.04	0.02
As	mg/kg	2.0	-	14.0	3.0	10.0	4.0	1.0	2.0	7.0	2.5	0.3	1.0

Table 3a. Dry matter (DM). protein (prot), fat, Hg and fat soluble vitamins in fillets from cartilaginous fillet portions.

		Bird-beak dogfish 37	Greater lantern shark 44	Lan-thern shark 46	Leaf-scale gulper 47	Picked dogfish 49	Por-beagle 50	Porto-guese dogfish 51	Ray 52
DM	g/kg	209	227	192	200	243	213	197	148
Prot.	g/kg	228	248	213	224	179	203	207	140
Fat	g/kg	4	11	7	7	64	4	15	2
Hg	mg/kg	0.9	0.6	0.4	0.9	0.1	0.4	1.9	1.0
Vit. A	µg/kg	<47	<45	<49	<48	<20	<50	<46	<50
Vit. E	mg/kg	48	76	36	45	20	6	122	7

Table 3b. Fatty acid composition (% of total lipid) of the lipid extracts of fillets from cartilaginous fishes, and amounts of omega-3 fatty acids in 100g fillet portions.

	Bird-beak dogfish 37	Greater lantern shark 44	Lan-thern shark 46	Leaf-scale gulper 47	Picked dogfish 49	Por-beagle 50	Porto-guese dogfish 51	Ray 52
14:0	0.2	0.2	0.1	0.1	2.1	0.8	0.1	1.0
16:0	14.8	16.8	17.0	14.8	15.9	20.2	16.8	21.5
∑ 16:1	1.2	1.3	1.3	1.4	3.8	2.1	1.5	2.0
18:0	5.3	4.9	5.1	6.9	2.8	5.5	5.4	5.6
∑ 18:1	11.5	11.5	15.7	12.9	17.0	13.1	13.8	11.8
18:2n-6	0.3	0.4	0.5	0.4	1.6	0.6	0.2	1.2
18:3n-3	-	-	-	-	0.8	-	-	0.2
18:4n-3	-	-	-	-	1.1	-	-	0.2
∑ 20:1	1.8	1.6	1.9	1.6	8.7	2.6	1.0	2.3
20:4n-3	0.1	0.1	0.1	0.1	0.9	0.2	-	0.3
20:4n-6	2.9	3.5	5.0	5.9	2.8	5.3	7.1	5.1
20:5n-3	1.2	2.3	2.9	2.2	8.0	3.7	1.8	5.2
∑ 22:1	0.6	0.6	0.4	0.2	7.8	-	0.3	1.1
22:5n-3	3.9	2.1	2.4	3.9	2.2	7.2	1.2	5.3
22:6n-3	39.0	39.1	35.5	37.6	18.3	33.6	36.4	32.7
Z sat.	21.7	22.9	22.9	23.3	21.6	27.0	23.0	29.0
∑ mon.	15.6	15.3	19.6	16.7	37.8	19.2	16.8	18.4
∑ n-3	44.4	43.7	41.2	44.0	31.4	44.6	39.7	44.0
∑ n-6	3.4	4.0	5.6	6.4	4.8	6.2	7.3	6.5
n-3 g/100g*	0.2	0.5	0.3	0.3	2.0	0.2	0.6	0.1

* Contents in 100 g portions of edible product. Z Sum of isomers.

Table 3c. Amino acid compositions (g/kg protein) of fillets from cartilagiorons fishes.

	Birdbeak dogfish 37	Greater lantern shark 44	Lanthern shark 46	Leafscale gulper shark 47	Portoguese dogfish 51
Ala.	46	46	47	46	44
Arg.	58	48	59	55	48
Asp.	79	78	79	81	73
Glu.	133	131	130	129	121
Gly.	46	47	44	44	43
His.	16	15	19	12	16
Ile.	35	36	35	35	36
Leu.	68	68	70	67	71
Lys.	78	71	74	78	66
Met.	24	23	26	26	23
Phe.	32	33	32	32	31
Pro.	31	30	31	33	30
Ser.	45	41	43	40	37
Thr.	37	37	40	38	34
Trp.	7	7	7	7	7
Tyr.	25	24	26	26	23
Val.	34	33	32	33	34

Table 3d. Macro- and micro elements in fillets from cartilaginous fishes.

		Picked dogfish 49	Por beagle 50	Ray 52
Na	g/kg	1.00	1.60	1.18
K	g/kg	2.89	2.42	2.32
P	g/kg	2.2	2.0	1.5
Ca	mg/kg	55	86	830
Fe	mg/kg	3	5	2
Se	mg/kg	0.2	0.3	0.4
Zn	mg/kg	3	4	3
Mn	mg/kg	<0.5	<0.5	<0.5
Mg	mg/kg	180	190	180
Cu	mg/kg	<0.6	<0.6	<0.6
Hg	mg/kg	0.1	0.4	1.0
Cd	mg/kg	0.005	0.001	0.001
Pb	mg/kg	0.03	n.d.	0.08
As	mg/kg	10	10	39

Table 4a. Dry matter (DM), total nitrogen (Tot. N), fat and ash, and B-vitamins in shark fillets.

		Basking shark 36	Blue shark 38	Brown shark 39	Bull shark 40	Common hammer head 41	Com-mon tresher 42	Dusky shark 43	L. Black tipped shark 45	Mako 48	Picked dogfish 49	Por-beagle 50	Sharp nose shark 53	Sickle shark 54	Silky shark 55	White tipped shark 56
DM	g/kg		162	215	209	245	225	232	208			227	232	211	232	22[illegible]
Tot. N			35	39	38	45	40	43	38			41	43	39	42	41
Prot.*	g/kg		126	190	185	215	202	203	183			202	203	185	206	296
Urea*	g/kg		36	25	24	30	23	29	25			25	29	26	26	27
Fat	g/kg		3	2	2	2	1	2	2			5	3	2	2	3
Ash			14	14	13	13	11	14	13			12	14	14	13	13
Thi.	mg/kg	0,34	0,25	0,13	0,13	0,29	0,28	0,18	0,26	-	-	0,80	0,13	0,13	0,27	0,14
Rib.	mg/kg	1,10	0,58	1,10	1,20	0,75	0,58	0,75	0,62	0,95	1,40	1,00	0,71	0,85	0,81	1,10
Niac.	mg/kg	54,0	44,1	54,6	68,3	90,6	73,3	77,4	55,4	99,4	52,0	70,0	89,9	60,6	80,6	55,8
Pant.	mgSkg	2,60	2,40	2,28	2,90	1,65	1,03	2,32	2,04	2,50	6,90	3,30	2,61	2,42	1,38	2,18
Vit. B_6	mg/kg	3,35	3,38	3,30	5,75	7,55	4,94	6,53	5,80	-	5,35	6,38	6,21	6,79	7,83	4,06
Vit. B_{12}	µg/kg	8,6	8,3	5,3	2,9	12,1	13,5	5,3	7,4	12,0	18,0	26,0	4,2	3,9	4,8	3,0
Biotin	µg/kg	-	10,0	7,4	10,0	15,0	17,0	9,7	8,1	-	110,0	39,0	9,0	8,4	8,5	9,1

* Prot. and Urea by calculatiou.

Table 4c. Amino acid compositon (g/kg protein) in aceton- dryed fillets of 15 species of shark.

	Basking shark 36	Blue shark 38	Brown shark 39	Bull shark 40	Common hammer head 41	Com- mon tresher 42	Dusky shark 43	L. Black tipped shark 45	Mako 48	Picked dogfish 49	Por- beagle 50	Sharp nose shark 53	Sickle shark 54	Silky shark 55	White tipped shark 56
Prot.	912	928	887	919	917	953	904	933	906	904	932	927	909	928	916
Urea	4	5	5	5	5	7	5	5	5	-	6	5	5	5	6
Ala.	58	54	56	57	59	56	57	56	61	54	54	61	56	58	57
Arg.	58	64	58	62	59	56	61	58	61	54	54	60	63	58	62
Asp.	89	86	93	92	98	90	91	89	89	88	87	95	65	92	85
Glu.	143	147	149	143	142	143	139	141	141	144	126	144	143	141	144
Gly.	45	46	46	42	46	43	48	45	45	65	48	43	43	44	46
His.	24	23	26	24	29	27	25	25	30	18	25	26	25	25	25
Ile.	46	44	51	50	53	50	49	47	52	44	47	48	50	50	47
Leu.	74	75	81	78	81	75	80	75	78	75	76	78	79	78	76
Lys.	84	87	91	88	95	91	90	90	91	85	93	91	89	85	89
Met.	24	28	29	28	27	27	27	27	25	28	25	28	27	27	28
Phe.	36	41	40	44	40	44	41	44	41	43	38	39	41	42	40
Pro.	39	39	38	34	49	35	35	36	39	40	35	47	37	36	38
Ser.	36	37	37	37	37	38	37	39	37	40	37	39	37	38	39
Thr.	45	44	45	46	48	47	44	46	45	39	42	45	44	45	44
Trp.	-	-	11	12	12	13	13	12	-	-	-	12	12	12	10
Tyr.	32	35	35	36	35	35	34	36	35	32	37	34	36	34	33
Val.	46	45	47	49	51	48	50	48	53	46	48	52	49	51	47

a protein concentrate, the amino acid analyses were done with samples from which most of the urea present in shark meat was removed by acetone extraction. The results of the amino acid analyses are given in Table 4c.

The analyses of shellfish are presented in Tables 5a to 5d: proximate analyses and vitamins in Table 5a, fatty acids and n-3 fatty acids and cholesterol in Table 5b, amino acids in Table 5c and macro and micro elements in Table 5d.

COMMENTS

The fat fishes herring, mackerel and Atlantic salmon are excellent sources of the n-3 fatty acids. Fishes with medium fat contents *e.g.* Greenland halibut and halibut are also good sources of these fatty acids. Further both groups are good sources for vitamins A and D.

The amino acid compositions of bony fishes and cartilaginous fishes are very similar when they are calculated by taking into account that cartilaginous fishes contain appreciable amounts of urea.

The contents of n-3 fatty acids in shellfish are low; the amino acid compositions are similar to those of bony fishes.

The fillets of fat fishes : char, herring, mackerel plaice, salmon and sprat

Table 5a. Dry matter (DM), protein (prot), fat and glycogen (glyc), fat soluble vitamins and B-vitamins in meat of cooked crab, lobster, Norway lobster and shrimp, and in raw whole oyster and scallop.

		Crab 57	Lobster 58	Norway lobster 59	Shrimp 62	Oyster 60	Scallop 61
DM	g/kg	276	216	147	319	147	139
Prot.	g/kg	229	152	107	233	90	91
Fat	g/kg	18	6	13	8	17	4
Glyc.	g/kg	10	< 1	5	1	-	10
Vit. A	µg/kg	40	<20	<50	20	100	<20
Vit. D	µg/kg	n.d.	n.d.	n.d.	35	31	42
Vit. E	mg/kg	63	43	32	53	5	3
Thi.	mg/kg	0.5	1.0	-	-	-	-
Rib.	mg/kg	4.0	0.6	0.6	0.7		
Niac.	mg/kg	17	18	24	23	-	-
Pant.	mg/kg	7	17	41	23		
Vit. B_6	mg/kg	2	-	2	1	-	-
Vit. B_{12}	µg/kg	135	10	30	46	-	-

showed appreciably higher values for vitamin B_{12} than the fillets of lean fishes. Also crab among the shellfishes showed a high vitamin B_{12} value.

Fish fillets are known to be good sources of the elements iodine and selenium. Our results confirm this for selenium which is found in concentrations around 0.5 mg / kg wet weight

Up to now we have not had an efficient method for iodine determination,but we are working with this problem.

Shellfish are good sources for most of the essential elements. Oyster is a very good source of zink.

The toxic elements mercury and arsenic are generally found in higher concentrations in food of marine than in food of terrestrial origin (3d and 5d).

Table 5b. Fatty acid composition (% of total lipid) extracts of meat of cooked crab. lobster. Norway lobster and shrimp, and in raw whole oyster and scallop.

	Crab 57	Lobster 58	Norway lobster 59	Shrimp 62	Oyster 60	Scallop 61
14:0	2.5	1.5	1.5	2.4	2.6	4.1
16:0	15.2	13.9	17.3	14.4	12.7	14.6
∑ 16:1	3.0	7.3	7.4	5.8	7.5	4.3
18:0	4.4	4.3	4.7	2.3	3.2	6.7
∑ 18:1	6.8	20.7	22.5	20.1	17.8	3.6
18:2n-6	1.5	1.0	17.3	0.9	1.2	0.6
18:3n-3	1.5	0.3	7.1	0.3	0.8	0.9
18:4n-3	2.8	0.4	-	0.3	1.4	4.7
∑ 20:1	6.6	6.3	-	7.0	5.6	2.4
20:4n-3	0.9	-	-	0.3	0.5	1.5
20:4n-6	2.1	4.0	5.3	1.2	3.1	1.3
20:5n-3	15.6	19.1	6.0	17.4	11.0	20.4
∑ 22:1	-	1.9	-	5.1	2.8	
22:5n-3	1.2	1.4	-	1.0	1.5	1.3
22:6n-3	16.5	12.8	1.5	16.0	13.9	21.8
∑ sat.	25.0	20.9	25.3	19.9	20.3	27.3
∑ mon.	16.9	36.7	30.2	38.8	34.3	11.8
∑ n-3	38.5	34.0	14.6	35.3	29.2	50.5
∑ n-6	4.0	6.1	23.9	2.4	5.4	2.4
n-3 g/100g*	0.5	0.2	0.2	0.3	0.5	0.2
Chol. mg/100g*	-	93	125	184	-	126

* Contents in 100 g portions of edible product.

∑ sum of isomers.

Table 5c. Amino acid composition (g/kg protein) of meat of cooked crab. lobster. Norway lobster and shrimp. and in raw whole oyster and scallop.

	Crab 57	Lobster 58	Norway lobster 59	Shrimp 62	Oyster 60	Scallop 61
Ala.	35	56	53	47	33	55
Arg.	61	103	99	77	33	77
Asp.	66	112	92	77	56	88
Glu.	105	178	145	116	78	143
Gly.	39	47	79	82	33	99
His.	17	19	20	17	22	11
Ile.	31	47	39	39	22	44
Leu.	52	93	72	64	33	77
Lys.	52	84	53	56	33	77
Met.	17	28	20	21	11	33
Phe.	35	56	46	39	22	44
Pro	35	37	59	52	22	33
Ser.	35	47	46	39	22	44
Thr.	35	47	39	34	33	44
Trp.	9	9	7	9	4	11
Tyr.	31	37	33	26	22	11
Val.	35	47	39	34	22	44

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Table 5d. Amino acid composition (g/kg protein) of meat of cooked crab, lobster, Norway lobster and shrimp, and in raw whole oyster and scallop.

		Crab 57	Lobster 58	Norway lobster 59	Shrimp 62	Oyster 60	Scallop 61
Na	g/kg	5.50	6.80	7.65	6.00	1.10	0.56
k	g/kg	2.44	1.16	1.06	2.39	2.65	1.61
P	g/kg	4.50	1.50	1.50	1.50	1.61	1.50
Ca	mg/kg	5510	1380	2220	610	220	67
Fe	mg/kg	18	7	24	40	31	6
Se	mg/kg	2.0	0.9	0.3	0.3	1	0.2
Zn	mg/kg	65	41	24	10	420	18
Mn	mg/kg	0.3	0.2	3.1	<0.6	0.2	1.0
Mg	mg/kg	630	450	330	430	190	190
Cu	mg/kg	16.0	22.0	19.0	6.0	9.0	1.0
Hg	mg/kg	0.07	0.09	0.07	0.10	0.02	0.01
Cd	mg/kg	1.0000	0.2000	0.07000	0.05000	0.500	0.0900
Pb	mg/kg	0.06	0.05	0.07	0.05	0.07	0.40
As	mg/kg	21.0	4.0	0.4	10.0	2.0	0.5

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WATER-SOLUBLE HISTIDINE IN CANNED BRISLING AND SILD SARDINES IN RELATION TO MATURATION

by

ROLV RAGÅRD[1], LEIF REIN NJAA[2] and BJØRN BJERKENG[1]
[1] NORCONSERV, P.O. BOX 327, N-4001 , STAVANGER. (Norway)
[2] Institute of Nutrition , P.O. Box 1900, Nordnes, N-5024 Bergen (Norway)

ABSTRACT

Total and water-soluble histidine were determined in raw materials and in canned products of sprat «brisling» *(Sprattus sprattus)*, small herring *(Clupea harengus)*, pilchard *(Sardina pilchardus)* and mackerel *(Scomber scombrus)*.
In Norwegian Brisling sardines water soluble histidine was generally higher than in Norwegian Sild sardines.
Judged by an arbitrary maturation score the degree of maturation of the canned product seemed to be positively associated with the level of watersoluble histidine. This observation is discussed.

INTRODUCTION

Routine analyses have shown that brisling (sprat) (*Sprattus sprattus)* contains more histidine in free (water-soluble) form than small herring (*Clupea harengus*). These species are used for production of canned Norwegian Brisling and Sild sardines, respectively.

It appears that Brisling sardines "mature" after canning whereas Sild sardines do not. The latter retains the taste of herring whereas the former develops a characteristic taste and consistency. After sterilisation and a few months of storage in the can, the following characteristics are often observed:

1: A light pink colour appears along the backbone. This colour darkens very soon after the belly has been opened Ronold and Jakobsen (1947). This is not the situation with the well known brown colour developed in connection with staleness of the raw material.
2: A special taste, often described as nutty, appears.
3: A soft delicious texture develops.

The changes continue and become more distinct during storage of the sardines, especially when they are packed in oils like olive oil, vegetable oils or cod liver oil.

This maturing process has been observed for decades and it occurs predominantly in brisling caught during the summer season. It has been suggested that it is related to a reduction of trimethylamine oxide (TMA-O) to trimethylamine,Ronold and Jakobsen (1947).

The maturing process might be related to the level of free histidine in the raw material. The Research Laboratory of the Norwegian Canning Industry (HL) in Stavanger (now NORCONSERV) therefore tested whether addition of histidine in the cans would induce maturing in canned Sild sardines. This was only partially successful.

In connection with these studies in 1968-1971 free histidine levels in canned Brisling sardines and Sild sardines were determined in collaboration between HL and the Vitamin Laboratory, Directorate of Fisheries (VL) in Bergen (now: Institute of Nutrition). A few samples of raw materials and intermediary steps in the production were included in the studies. Some other canned fish products were also included. The results from these studies are presented in this communication.

MATERIALS

Canned products and some corresponding raw materials and intermediary products were procured by HL. Samples of juvenile sprat and herring were obtained from the Institute of Marine Research, Directorate of Fisheries.

Nineteen samples of canned Brisling sardines and 13 of Sild sardines together with raw materials for 13 and 4 of these, respectively, were analysed. Further, Brisling sardines produced from Baltic Sea sprat and from Scottish sprat, and Portuguese sardines from pilchard (*Sardina pilchardus*) were obtained together with raw materials. Also 4 samples of canned mackerel (*Scomber scombrus*) produced in different seasons and the raw materials for 3 of these were analysed.

Eighteen samples of juvenile sprat caught during four expeditions by the Institute of Marine Research, and three samples of juvenile herring from one of these, were analysed.

METHODS

Fat was determined by weighing after extraction with trichloroethane and evaporation of the solvent; protein (N* 6.25) by a macro Kjeldahl technique followed by titration. Total volatile nitrogen (TVN), trimethylamine nitrogen (TMA-N) and trimethylamine-oxide nitrogen (TMAO-N) were determined

by methods described by Conway and Byrne (1933) and by Hjorth-Hansen (1952). Water soluble histidine was determined at VL as follows: Ten g minced sample was stirred with 50 mL water, pH was adjusted to 5.0. Twenty mL trichloroethane were added and stirring was continued for five minutes. The mixture was centrifuged in a high-speed centrifuge (20000 r.p.m.), the supernatant was decanted through a White bond filter paper (Schleicher & Schüll). Trichloroethane was removed by suction. The solids were extracted two more times with 50 mL water but without added trichloroethane. The extracts were combined and diluted to 200 mL. Protein was determined by a micro-Kjeldahl technique followed by titration. Histidine was determined microbiologically with *Leuconostoc mesenteroides* as described by Brækkan and Boge (1962). Total amino acids were determined on a Technicon Amino Acid Analyzer in hydrolysates using 6 M HCl for 20 hours and with norleucine as internal standard.

Table 1a. Amino acid contents of minced canned Brisling sardines and of aqueous extracts of the mince. Analysed 1, 4, and 7 months after canning (Sample BRF 38), and 8 years after canning (Sample 115) (g amino acid per kg sardine protein).

Sample	BRF 38 one month Sardine	Extract	BRF 38 four months Sardine	Extract	BRF 38 seven months Sardine	Extract	115 eight years Sardine	Extract
Asp	88	6.9	84	9.7	90	7.7	87	5.8
Thr	41	3.8	38	5.0	41	3.6	40	2.8
Ser	36	4.4	37	5.9	39	4.7	36	3.2
Glu	127	12.4	123	17.2	129	9.9	128	9.8
Pro	56	5.7	45	12.1	38	6.4	47	10.8
Gly	48	13.3	47	19.8	51	13.9	49	13.6
Ala	59	7.8	56	11.8	66	8.9	61	8.6
Val	51	3.8	48	6.1	55	3.9	51	2.9
Cys	6.7	0.8	12.6	0.4	11.1	-	4.9	0.6
Met	31	1.1	28	2.5	29	0.5	30	1.1
Ile	41	2.2	41	3.8	45	2.2	41	1.8
Leu	72	5.5	69	7.7	74	5.5	71	3.9
Tyr	33	1.7	31	2.7	33	1.7	33	1.1
Phe	39	3.2	38	4.0	38	2.7	37	1.9
Lys	79	8.2	73	10.5	80	8.4	72	6.3
His	29	9.8	30	9.9	30	9.5	29	8.9
Arg	55	6.3	52	7.3	55	6.5	55	5.3

Table 1b. Amino acid contents of minced canned Sild sardines (MO 39) and of aqueous extracts of the mince. Analysed 1, 4, and 7 months after canniing (g amino acid per kg sardine protein).

	One month Sardines	Extract	Fourmonths Sardines	Extract	Seven months Sardines	Extract
Asp	82	4.8	82	7.4	82	5.5
Thr	42	2.5	41	37	39	2.2
Ser	36	4.1	36	4.8	37	3.9
Glu	126	8.1	122	11.0	123	8.2
Pro	-	6.1	46	11.3	36	6.4
Gly	51	16.6	51	21.0	55	17.0
Ala	62	8 .9	58	11.9	60	9 .4
Val	48	2.2	46	3.5	48	2.7
Cys	7.0	-	11.8	0.3	6.2	-
Met	28.7	1.0	26.6	13	29.7	0.2
Ile	40	1.2	39	2.1	41	1.5
Leu	69	2.9	68	4.3	74	3.8
Tyr	30	0.6	31	1.1	32	0.9
Phe	36	1.7	36	2.5	38	2.1
Lys	76	3.9	72	5.5	81	4.4
His	20	1.9	21	2.1	17	1.2
Arg	43	5.1	52	6.0	51	4.9

RESULTS AND DISCUSSION

Total amino acid analyses of two samples of canned Brisling sardines and of one sample of Sild sardines are given in Table 1a and Table 1b. One of the Brisling sardine samples and the Sild sardine sample were analysed 1, 4 and 7 months after canning, the other Brisling sardine sample (115) was 8 years old when analysed. In all samples amino acids were also determined in the extracts .

Amino acids concentrations in Brisling and Sild sardines and in extracts were similar except for histidine which was higher in the Brisling than in the Sild samples .This was mainly due to the higher content of soluble histidine in brisling.

Histidine in extracts of raw material and canned products

Raw materials were available for eight samples of canned Brisling sardines, three of Sild sardines ,and in three cases for brined and smoked fish . In Table 2 the data for soluble protein and histidine as percent of protein for these samples are given. The amounts of soluble protein were about the same in the raw materials as in the canned products (about 20% of total protein). Soluble histidine was higher in Brisling than in Sild raw materials but the differences between them

Table 2: Protein content (g / kg) of raw materials and of canned products and levels of extract protein and histidine (% of total protein)

	Raw material			Canned product				Raw material			Canned product		
Saw	Prot	Prot %	His %	Prot	Prot %	His %	Smpl.	Prot	Prot %	His %	Prot %	Prot %	His %
H1A	170	20.7	1.19	226	25.0	1.04	H1B	144	16.4	0.56	192	25.2	0,46
H2A	144	19.9	1.17	199	23.4	1 12	H2B	161	20.1	0.62	218	19.8	0,38
H3A			1.96	205	21.0	1.54	H3B	161	20.9	0.95	217	21.4	0,63
H4A	150	20 .5	1.46	163	17 . 7	1 12	brined	168	20 .4	1 .00			
	151	20.6	1.65				smoked	223	19.6	0.77			
smoked	208	13 .2	1 .26				M 7/8	158	20.9	0 .85			
H6A	158	21.7	1.54	207	17.8	0.86							
B5/6 69	150	21 .7	1.41	206	21 .2	1 .00							
B5/6 70	150	21 .4	1 .18	217	16 .1	0 .81							
B29/6 70	157	24 .2	1 32	225	18 .4	0 .55							
brined	147	19.6	1.24										
smoked	248	13.6	0.70										

Table 3. Protein content (g / kg) of canned Brisling sardines and Sild sardines and levels of extract protein and histidine (% of total protein.

	Brisling sardines				Sild sardines		
	Protein	Protein %	Histidine %		Protein	Protein %	Histid
BR 1	240	17.9	1.08	MR 2	207	17 .9	0.38
BR 3	244	16.4	0.94	MR 3	217	17 .0	0 .44
BR 14	223	17.5	0.98	MR 4	211	16 .8	0 .27
BS 27	222	16.8	1.08	MT 4	210	16.1	0 .10
46 /BR /34	199	17 .0	1.21	MT 5	205	17.9	0 .19
BRF 38	197	18 .5	1.07	MT 6	196	15 .4	0 .34
115	25o	19 .6	1.44	MR 4	215	16.2	0.27
255	199	16 .9	1 .44	MR 2	235	20.4	0.18
B 3 /8 70	201	18 .3	1.36	MRF /21	255	18.0	0.51
BS	204	18.7	0.95	MRF /26	250	17 .7	0 .44
BV	190	20 .1	0 .56	Mo /39	223	17.4	0 .21

were less than in the samples referred to in Tables 1a and 1b. There seemed to be some loss of free histidine during canning, but whether this observation is due to an artifact is difficult to assess as there was also a clear effect of canning on the protein content of the product.

Histidine in extracts of canned brisling and sild sardines

Eleven samples each of canned Brisling sardines and Sild sardines for which there was no knowledge about the raw materials were analysed for soluble protein and histidine.(Table 3).The level of free histidine was from two to ten times higher in Brisling sardines than in Sild sardines, the mean was about three times higher. Within each group there was great variation as indicated by the standard deviations. In two instances low values for soluble histidine in canned Brisling sardines were found: 0.55% and 0.56% of total protein in samples B29/ 6 70 (Table 2) and BV(Table 3), respectively. Similarly, rather high values , 0.58% and 0.51%, were found in canned Sild sardines in samples H3B (Table 2) and MRF/21 (Table 3).

Sample BV was produced from wintercaught sprat, Brisling sardines from this type of raw material are held to not mature in the can. However, a low value was found in a canned sample (B29/6 70) produced from a raw material with a high level of histidine.

Relationship between chemical data and maturing

Table 4 shows the available data for assessment of the degree of maturing and for the chemical analyses performed. Data were available for five Brisling sardines,

Table 4. Analyses of raw materials of sprat ,small herring, pilchard and mackerel together with protein contents of canned products (g / kg) levels of extract protein and histidine (% of total protein). (Values in parenteses refer to the canned products).

Brisling sardines

Sample	Fat g/ kg	TVN mg/ 100 g	TMA-N mg /100 g	TMAO-N mg/100 g	Prot. g / kg	Prot. g / kg	Extr. Prot. %	Extr. Prot. %	Extr. His. %	Extr. His. %	Mat-uratin score
H2A Sept	174	10.5	0	10 .5	143	(191)	20.7	(17.6)	1.19	(1.14)	2
H3A Oct.	172	19.0	2.2	20.4	175	(224)	19.9	(19.2)	1.17	(1.61)	5
H4A Oct	187	11.5	1.1	17.4	150	(170)	23.3	(17.3)	1.96	(1.18)	5
BS sum.	103	17.6	4.4	19.7	154	(204)	-	(18.7)	0.95	(0.95)	4
VS vin.	130	16.9	6.3	35.1	147	(190)	-	(20.1)	-	(0.56)	1
Sild sardines.											
H1B Aug	103	10.3	3.0	12.0	144	(192)	16.4	(25.2)	0.56	(0.46)	1
H2B Oct	48	16.0	1.5	29.2	161	(218)	20.1	(19.8)	0.62	(0.38)	1
Sprat											
Sprat, Bs	87	9.1	1.4	20.1	167	(179)	18.6	(17.8)	0.76	(0.44)	3
Sprat, Se	115	14.0	17.6	7.4	182	(197)	-	(19.3)	-	(0.83)	1
Pilchard											
Pilch, Nov	75	13.6	2.2	26.6	75	(196)	-	(17.5)	-	(0.44)	3
Pilch.	161	55.5	22.8	11.7	254	(241)	-	(19.5)	-	(1.85)	5
Mackerel											
Mc, May	109	22.5	0	16.7	182	(232)	-	(13.5)	-	(1.44)	1
Mc, Aug.	186	19.4	2.5	42.2	177	(272)	-	(13.1)	-	(1.88)	5
Me .Sept.	-	-	-	-	-	(245)	-	(14.8)	-	(2.45)	5
Mc, Febr.	125	103.2	0	159.4	171	(212)	-	(14.7)	-	(1.09)	1

two Sild sardines, two Pilchard sardines, one Baltic Sea Brisling sardines, one Scottish sprat sardines and four samples of canned mackerel for which the raw materials were fish caught in one of the four seasons of the year. Evaluation of the results indicates no clear-cut conclusion. For canned Brisling sardines the two best samples as judged by the maturing score showed high levels of free histidine in the product (Sample H3A) or in the raw material (Sample H4A) and as mentioned before in the product from winter sprat(Sample BV).

The two Sild sardine samples both scored low as did the product from Scottish sprat. One pilchard sample scored high and one intermediate. Canned mackerel scored high for summer and autumn caught fish and low for spring and winter caught fish. This is in rather good agreement with the free histidine levels in the canned products. The high level of TVN in the winter sample of mackerel is curious because of the low value of TMA-N.

What causes the maturing process has remained unresolved for many years. One of the most characteristic features in matured Brisling sardines is the rapid transformation of the pink colour to a brown colour along the backbone when the fish is opened. The initial production of the greyish brown colour in newly canned Brisling and Sild sardines is probably related to the oxidation of ferrohaemochrome to ferrihaemochrome (Fe^{2+} to Fe^{3+}) (Wong, 1989). This oxidation is likely to proceed at a higher rate at lower pH, as shown for the oxidation of oxyhaemoglobin to methaemoglobin (Mal and Chatterjee, 1991). The production of ferrohaemochrome during the maturation of Brisling sardines requires a reducing environment not present in for instance Sild sardines. Observations at HL show that the maturing process is accelerated in the presence of sulphites. The addition of $FeCl_2$ also seemed to increase the maturation rate, indicating the involvement of iron in the process. The reduction of TMA-O during maturation (HL, unpublished results) give additional indication for the for the presence of a reducing environment. Factors responsible for the conversion of TMA-O to DMA (dimethylamine) and formaldehyde may be present in the kidney (Regenstein *et al.* 1982). Candidates for creating reducing power are: NAD(P)H, glutathione, free cysteine, tocopherol and to a lesser extent ascorbic acid. The redox potential of the Fe^{2+}/ Fe^{3+} pair is reduced when the iron is complexed to different ligands *e.g.* ethylenediaminetetraacetic acid (EDTA) (Mahoney and Graf, 1986). Histidine can also complex to iron (Huang *et al.* 1993)

Different tuna fish species producing an attractive red colour after canning are known to contain high levels of water-soluble histidine and short histidine-containing peptides (1- 2 % of muscle weight) (Okuma and Abe, 1992) retained after thermal processing (Perez-Martin *et al.* 1988). A lowered reduction potential for the reduction of Fe^{3+} to Fe^{2+} due to complexing iron species with histidine or histidine-containing peptides may facilitate the reduction process

Table 5. Protein (g / kg) and water soluble protein and histidine (% of total protein) in juvenile sprat and herring. (Herring values in parentheses).

	Novem 1969						Januar 1970			April 1970			Novem 1970		
	Protein	Protein	Extract Protein	Extract Protein	Extract His.	Extract His.	Protein	Extract Protein	Extract His	Protein	Extract Protein	Extract His.	Protein	Extract Protein	Extract His.
1	167	(170)	23 .2	(22.4)	1 .52	(1.11)	161	19.8	0 .89	152	16 .5	1 .01	165	32 .1	1.53
2	169	(169)	23 .0	(21 .4)	2.08	(1 .01)	161	21.3	0 .94	161	15 .5	1.23	167	23 .2	1.58
3	164	(169)	24 .8	(22 6)	1 .35	(0.90)	146	23 .0	1 .05	165	17 .8	1 .23	161	25 .7	1.48
4	170		23.8		1.56		162	20.8	1.11	145	15.0	0.88	166	22.9	1.52
5	154		21.5		1.20		168	20.8	0.99						

caused by sulphydryl reductants. This suggests a possible role for histidine in the maturing of Brisling sardines and other fish species . Future comparative studies of reducing potential, reductants, iron speciation and ligands in Sild and Brisling sardines could prove fruitful in elucidation of the factors governing the maturing process.

It has been the hope of the canning industry that it might be possible to produce Sild sardines with the same or similar taste and consistency as found in Brisling sardines. It is not possible on the basis of the present results to conclude that free histidine is important for the maturing process taking place in Brisling sardines but only very seldom in Sild sardines. Unpublished results at HL showed that histidine added before canning reduced the fishy taste of Sild sardines . However, we are now aware of the fact that the resulting bitter taste the products may have been caused by L-histidine itself (Haefeli and Glaser, 1990).

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VITAMIN CONTENTS OF THE ROTIFER *BRACHIONUS PLICATILIS*

by

KJARTAN SANDNES, ØYVIND LIE, HERBORG HAALAND and YNGVAR OLSEN
Institute of Nutrition, Directorate of Fisheries, P. O. Box 1900, N-5024 Bergen, Norway.
SINTEF Applied Chemistry, Aquaculture research group, N-7034 Trondheim, Norway.

The rotifer *Brachionus plicatilis* is the dominating live food organism used in the early rearing of marine fish larvae prior to the Artemia stage. It can be cultivated in high densities (>1000 individuals per ml), in large volumes (>5 m3), and it is fairly resistant to handling.

Experiments have been conducted on the role of vitamins related to zooplankton growth and ecology (Poulet et al., 1989; Yu et al., 1989), and specific studies concerning vitamin requirements for optimal growth of rotifers have been reported (Satuito and Hirayama, 1986, 1991; Scott, 1983). However, little effort has been made to evaluate the vitamin contents of rotifers with respect to their suitability as live food for fish larvae, and on establishing vitamin enrichment procedures.

Rotifers were cultivated (experiment A) using baker's yeast and Super Selco (Artemia Systems, Belgium), or baker's yeast and capelin oil (experiment B) as feed (Olsen et al., 1993). The proportion (on wet weight basis) of yeast and Super Selco/capelin oil was 10:1. The rotifers were grown in tanks holding 280 L of water at 20 g/L salinity at 20 °C with continuous air bubbling. Rotifers grown to early stationary phase were collected using a 70 mm filter. The samples were kept at -80 °C until analysed.

Analyses of ascorbic acid (Roy et al., 1976), vitamin A and vitamin E (Lambertsen, 1983) were performed chemically. The vitamins of the B-group were analysed using microbiological methods according to AOAC (1990), modified and adjusted to routines and equipment at the Institute of Nutrition, Bergen.

Table 1. Contents of vitamins (mg/g d.w.) in *Brachionus plicatilis* fed Baker's yeast and super Selco (A) or Baker's yeast and capelin oil (B). NRC (1981) requirements (mg/Kg dry diet) for coldwater fish are shown in the table.

	A	B	C
Ascorbic Acid	167	267	100
Thiamin	16	16	10
Riboflavin	26	23	20
Pantothenic Acid	107	118	40
Niacin	214	167	150
Pyridoxine	7	2	10
Biotin	3	3	1
Vitamin B_{12}	2	1	0,02
Vitamin A (retinol)	4	<1	0,8
Vitamin E (α-tocoperol)	640	122	30

All analyses were carried out on wet samples. Dry matter was determined (60°C, 24 hours) on filtered samples. The rotifer density at sampling was approximately 170 rotifers per ml, and the number of eggs per rotifer was 0.20.

The results are given in Table 1. As the vitamin contents of the media were not known the results can only be used as indicators for those batches of cultivated rotifers grown under the conditions described. Thus notifers may contain substantially different amounts of Vitamin E and of ascorbic acid. Among the B-vitamins determined only pyridoxine seemed to be different between the two samples. The results indicate that the vitamin contents of the rotifer *Branchionus plicatilis* may be manipulated. The factors responsible for the differences observed should be investigated.

Although the comparison is not directly relevant, the present findings suggest that the NRC (1981) recommendations for vitamin requirements in coldwater fish are met by rotifers from experiment A with the exception of pyridoxine. Rotifers from experiment B showed even further reduced levels of pyridoxine compared to experiment A. However, the requirements for vitamins in marine fishes are not known at early developmental stages. The daily weight increase may exceed 12 % in cod (*Gadus morhua*), 10 % in halibut (*Hippoglossus hippoglossus*) and 25 % in turbot (*Scophthalmus maximus*) larvae, and this is markedly higher than in adult fish. Further, the vitamin requirement of larvae may be influenced by a variety of factors. The relative importance of factors as absorption mechanisms, chemical composition of the feed, leaching and microbial activity may differ between larvae and the later stages of development.

In order to optimize intensive production of marine fish fry, further studies are needed to obtain knowledge on nutrient requirements of vitamins and to establish procedures to secure the supply of these essential nutrients in live and formulated feeds. The present data suggest that vitamin enrichment is possible in rotifers as indicated by differences in vitamin contents when different diets are fed.

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FATTY ACID COMPOSITION OF ORGANS IN TURBOT *(Scophthalmus maximus)* DURING SEXUAL MATURATION

by

ØYVIND LIE, ANDERS MANGOR-JENSEN*, GRETHE ROSENLUND** and RAGNAR NORTVEDT.

Institute of Nutrition, Directorate of Fisheries, P.O. Box 1900, N-5024 Bergen. *Institute of Marine Research, Austevoll Aquaculture Research Station, N-5392 Storebø. **BP Nutrition Aquaculture Research Centre Stavanger.

ABSTRACT

480 maturing turbot brood fish were fed a commercial dry feed *ad.lib* five days a week in a 50m^3 tank at ambient temperature at natural light cycle. Ten fish were sampled each month from January to August, resulting in 63 females and 18 males. Weight, length and organ weights were recorded, and organ indices were calculated. Feed, liver, fillet, gonads and plasma were analysed for fatty acid composition.

The lipid reserves in fillets were utilised during gonadal development in the females, whereas the protein level in fillets and protein and lipid levels in livers were not influenced by the maturation of the ovaries. The fatty acid composition of tissue lipids from immature and mature turbot differed so clearly that the fatty acid composition of the tissues could be used in the overall classification of immature and maturing turbot. The feed fatty acid composition differed from all the tissue classes, but generally showed the best fit to that of the immature tissues, suggesting that the fatty acid composition of the dietary lipids had more influence on the immature turbot than on the maturing female turbot.

INTRODUCTION

There are species specific feeding behaviour in fish: e.g. Atlantic salmon (*Salmo salar*) cease eating several weeks before spawning whereas red sea bream (*Chrysophrys major*) eat during spawning. The influence of maternal nutrition upon fecundity and egg viability as well as upon larval development through the yolk sac period have been reported (Sandnes *et al.*, 1984; Luquet and Watanabe, 1986; Hardy *et al.*, 1989; Mangor-Jensen *et al.*, 1994). The reproduction of red sea bream is greatly affected by the n-3 fatty acid content of the broodstock diet. These fatty acids are necessary to achieve normal rates of hatching (> 90%) and to produce larvae without deformities (Watanabe *et al.*, 1984ab, 1985). According to Shimma *et al.* (1977) the hatchability of the eggs of common carp (*Cyprinus carpio*) was low when the level of 22:6n-3 in the eggs

was below 10% of the total fatty acids. The effect of the fatty acid composition of the parental diet on the fatty acid composition of individual phospholipids in eggs and milt of cod (*Gadus morhua*) was reported by Lie (1991) and effects of these on egg quality (Lie *et al.*, 1993) have also been reported.

However, little is known about the nutrition of brood fish, particularly regarding the interaction between nutrients and general reproductive physiology. The objective of the present study was to elucidate how the fatty acid composition of plasma, liver, fillet and gonads was influenced by the diet and the maturation process in turbot. The results presented in this paper is a part of a large broodstock experiment on turbot (Lie *et al.*, 1992)

MATERIALS AND METHODS

Experimental setup

The broodstock was 480 fish (male and female, 1-2 Kg), reared at ambient temperature (6.5 - 8.5 °C) and in a 50m^3 tank (salinity 33g/L) at Austevoll Aquaculture Research Station. Detailed information is given by Lie *et al.* (1992). The fish were given a commercial dry feed for turbot (T. Skretting, Norway) to satiation once daily five days a week. Dry content matter of the diet was 903 g/kg , protein and lipid levels were 513 g/kg and 205 g/kg, respectively. The fatty acid composition of the dietary lipid is given in Table 1.

Sampling

The experiment lasted from January to August 1991 and ten fish (12 in March, one excluded), preferentially females, were taken for analyses each month throughout the experimental period, further details are given by Lie *et al.* (1992).

Chemical analyses

Liver, gonads, fillet and blood were taken from each fish and stored on dry ice. They were analysed for fatty acid composition of total lipids. The contents of dry matter, protein and fat in liver and fillet were determined. The lipid was extracted with ethyl acetate and weighed after evaporation of the solvent. The fatty acid composition was analysed according to the methods described by Lie and Lambertsen (1991). Total nitrogen was determined by a Nitrogen analyser (LECO, FP-428; System 601-700-500) and protein was calculated as N*6.25.

Statistics

This study was correlative. Therefore single pairwise correlation tests were performed between all fatty acids and gonadosomatic index (weight of gonad * 100/

body weight of fish, GSI) and time, respectively to reveal a minimum of association between the internal biochemical status and the maturation of females. The males were not included in the detailed data analysis (Lie *et al.* 1992). Fatty acids significantly (α = 0.05) positively or negatively correlated with GSI were chosen for further studies. Fatty acids significantly correlated with time exclusively, were omitted from further evaluation.

Biological systems are multivariate, and due to high intercorrelation between fatty acids, the mapping of significant main effects were treated as a multivariate problem. Data were pretreated and analyzed by the SIRIUS programme (Version 3.0) (Kvalheim and Karstang 1987) and SIRIUS for Windows (Ver. 1.0). The fatty acid composition of total lipids from different tissues and from the diet were used in the multivariate analysis to elucidate if there were differences in composition between tissues from immature and mature turbot (Jan.-July) and if the maturation status could be related to the feed composition. Data from immature and maturing organs were defined as single classes (Lie *et al.*, 1992).

A $\log_{10}(x+1)$ transformation was performed on each class, to avoid negative numbers. The transformation was carried out to avoid spurious results from masking of real variance in minor fatty acid constituents with low total variance compared to major fatty acid constituents with relatively high variance, according to Brakstad (1992). Principal component analyses (PCA) (Wold *et al.* 1987) was performed in each data matrix from the respective organs. The purpose of PCA is to express the main information in the variables by a lower number of variables, the so-called principal components (PC1, PC2, ...). Only two significant principal components were found in each PCA in the present investigation. PC1 represents a vector along the longest axis in the multidimensional "cloud" of data points and usually explains the dominant part of the actual variance. The second component (PC2) runs perpendicular to PC1. The final unexplained variance of each sample is gathered in a residual vector, perpendicular to the significant components.

The principal components may be understood as perpendicular sides in a window frame. The information from the multivariate room is displayed in the window by score and loading plots. The projection of the samples onto their PC's gives their respective scores (coordinates). An eigen vector from the variance-covariance matrix is associated along each component. The projection of the eigen vectors onto the variable axes gives the loadings of the respective variables. A high positive or negative loading reveals a significant variable in the actual PCA model. Score plots from the PCA explored the main trends in the data and their respective loadings revealed the significant fatty acids.

Organs (fillet, liver, gonad and plasma) from immature fish (Jan. - March) and maturing fish (May - July) were classified according to the Simca method (Wold 1976), based upon the total data matrix from Lie *et al.* (1992). An F-

Table 1. Fatty acid composition of total lipid of diet and fillet in female turbot during the reproductive cycle.

	Diet (n=7)		Jan (n=7)		Feb. (n=8)		Mar. (n=5)		Apr. (n=7)		May (n=9)		June (n=9)		July (n=9)	
	Mean	±CI*	Mean	±CI*	Mean	±CI*	Mean	±CI*	Mean	±CI*	Mean	±CI*	Mean	±CI*	Mean	±CI*
14:0	6,4	0,1	4,9	0,6	4,1	0,9	4,7	0,7	3,3	0,6	3,7	0,4	3,2	0,4	3,0	0,3
15:0	0,4	0,0	0,4	0,0	0,3	0,0	0,3	0,0	0,3	0,0	0,3	0,0	0,3	0,0	0,3	0,0
16:0	14,1	0,2	16,4	1,2	16,3	1,7	15,9	1,7	16,5	0,7	15,8	0,3	17,4	1,2	17,5	0,6
16:1n-9	0,2	0,0	0,3	0,0	0,3	0,1	0,3	0,0	0,2	0,0	0,3	0,0	0,3	0,0	0,3	0,0
16:1n-7	6,7	0,7	4,3	0,6	3,9	1,0	4,4	0,8	3,2	0,7	3,7	0,5	3,2	0,5	2,9	0,3
17:0	0,4	0,1	0,8	0,1	0,8	0,1	0,8	0,0	0,2	0,0	0,5	0,1	0,4	0,0	0,5	0,0
18:0	1,8	0,1	2,3	0,2	2,6	0,5	2,4	0,5	2,9	0,3	2,5	0,3	2,6	0,2	2,6	0,2
18:1n-11	0,3	0,2	0,6	0,1	0,6	0,1	0,6	0,1	0,9	0,1	0,9	0,1	1,0	0,1	0,9	0,1
18:1n-9	11,3	0,2	9,9	0,6	9,4	0,9	10,1	0,7	8,8	0,5	9,7	0,3	9,1	0,8	8,5	0,6
18:1n-7	3,3	0,1	2,7	0,3	2,6	0,2	2,9	0,2	2,6	0,2	3,0	0,1	2,8	0,3	2,8	0,2
18:2n-6	3,7	0,6	5,1	0,3	5,0	0,2	5,1	0,3	4,9	0,2	4,6	0,2	4,7	0,2	5,1	0,3
18:3n-3	1,0	0,1	0,8	0,1	0,8	0,2	0,8	0,1	0,7	0,1	0,7	0,1	0,5	0,1	0,5	0,1
20:1n-11	0,5	0,2	0,8	0,4	0,7	0,1	0,6	0,2	0,7	0,1	0,8	0,1	0,8	0,1	0,8	0,1
20:1n-9	10,1	0,6	6,3	0,8	5,5	0,8	6,8	1,3	5,4	0,6	6,0	0,5	5,8	0,9	5,5	0,4
20:1n-7	0,3	0,1	0,1	0,1	0,1	0,0	0,1	0,1	0,2	0,0	0,1	0,0	0,1	0,0	0,1	0,0
18:4n-3	2,7	0,2	1,6	0,4	1,6	0,5	1,8	0,4	1,1	0,3	1,2	0,2	0,8	0,1	0,7	0,1
20:2n-6	0,2	0,0	0,3	0,1	0,4	0,0	0,4	0,1	0,4	0,0	0,3	0,0	0,3	0,0	0,3	0,0
20:3n-6	0,0	0,0	0,0	0,0	0,1	0,0	0,1	0,1	0,1	0,0	0,1	0,0	0,1	0,0	0,1	0,0
20:3n-3	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,0
20:4n-6	0,4	0,0	1,2	0,1	1,4	0,3	1,2	0,2	1,6	0,2	1,3	0,2	1,6	0,2	1,8	0,1
22:1n-11	14,3	0,4	5,4	1,0	4,6	1,2	6,1	1,4	4,4	0,9	4,8	0,8	4,2	1,3	3,2	0,4

22:1n-9	0,6	0,3	0,3	0,1	0,3	0,1	0,3	0,4	0,3	0,1	0,3	0,0	0,3	0,1	0,2	0,0
20:4n-3	0,5	0,1	1,0	0,1	0,9	0,1	0,9	0,1	0,8	0,1	0,8	0,0	0,7	0,0	0,7	0,1
20:5n-3	6,9	0,3	7,4	0,7	8,0	0,5	7,2	1,0	8,5	0,6	8,6	0,3	9,1	1,1	9,3	0,7
24:1	0,4	0,2	0,3	0,1	0,3	0,1	0,4	0,1	0,3	0,0	0,3	0,0	0,2	0,0	0,2	0,0
22:5n-3	0,7	0,1	2,7	0,2	2,7	0,1	2,5	0,2	2,8	0,2	2,8	0,2	2,7	0,2	2,9	0,1
22:6n-3	8,5	0,4	20,7	2,3	22,6	3,6	20,0	2,9	25,0	2,5	22,5	1,4	24,3	2,4	25,1	2,1
Div.	2,8	0,4	3,1	1,1	2,9	0,2	2,4	0,8	3,2	0,2	3,1	0,3	2,9	0,2	3,5	0,1
Sum saturated	23,2	0,5	24,8	1,1	24,4	1,4	24,2	1,4	23,4	0,5	23,0	0,4	23,9	1,0	24,0	0,8
Sum 16:1	7,2	0,7	4,8	0,7	4,4	1,1	4,9	0,8	3,7	0,7	4,3	0,4	3,7	0,5	3,4	0,3
Sum 18:1	14,9	0,2	13,2	0,9	12,6	1,0	13,6	0,8	12,3	0,7	13,7	0,4	12,8	1,2	12,3	0,8
Sum 20:1	10,9	0,5	7,2	0,6	6,3	0,9	7,5	1,2	6,3	0,7	6,9	0,6	6,8	1,0	6,3	0,4
Sum 22:1	14,9	0,4	5,8	1,0	4,9	1,3	6,4	1,7	4,7	0,9	5,1	0,8	4,5	1,4	3,4	0,4
Sum monoenes	48,6	1,6	31,7	3,1	28,7	4,4	32,9	4,5	27,3	2,9	30,4	1,8	28,1	3,9	25,8	1,7
Sum n-3	20,9	0,5	34,2	2,1	37,0	3,1	33,4	3,4	39,0	2,2	36,9	1,3	38,3	2,6	39,3	1,8
Sum n-6	4,2	0,5	6,6	0,4	6,9	0,3	6,8	0,3	6,9	0,3	6,4	0,3	6,7	0,4	7,3	0,3
Sum polyenes	25,5	0,9	40,8	1,8	44,0	3,2	40,5	3,4	46,0	2,4	43,5	1,5	45,1	2,9	46,7	1,7
n-3/n-6	5,0	0,7	5,2	0,6	5,4	0,4	4,9	0,5	5,7	0,2	5,7	0,2	5,8	0,2	5,4	0,4

* CI = Confidence values (95%)

test, based upon mean standard deviation of class (RSD), was used to remove outliers (α = 0.05). The Simca method includes a test for whether each single sample may belong to a certain class of data. Each fish and the feed were treated as samples in the present investigation and could consequently be fitted to the defined organ classes. The squared residual distances of the fitted samples to the organ classes were calculated and corrected for the loss of degrees of freedom. The residual distance of the feed was compared to a critical residual value and showed if the fatty acid composition of the feed could be associated with the fatty acid composition of a particular organ. Discrimination power (Albano *et al.* 1981) between organ class and organ class + feed was calculated to reveal which variables matched (discrimination power < 1.0) or discriminated (discrimination power > 3.0) between the feed and the respective organs.

RESULTS AND DISCUSSION

Mean protein and fat values in fillets from females ranged between 181 and 208 and 3 and 11 g/kg, respectively, and levels of lipids in liver varied between 131 and 173 g/kg. A negative correlation ($p < 0.05$) between fillet fat and GSI was observed, whereas no significant correlation was found between liver fat and GSI. These results suggest that the fillet lipid reserves are utilised during the gonadal development in the females and that the liver lipids do not seem to be used in a similar way.

The fatty acid composition of the diet (Table 1) showed a high level of the long chain monounsaturated fatty acids 20:1n-9 (10.1%) and 22:1n-11 (14.3%), the sum of monoenes constituted about 50% of the fatty acids. The n-3 fatty acids accounted for more than 20% of the fatty acids which according to recognised requirements (Sargent *et al.* 1989) should be an adequate amount of essential n-3 fatty acids. The role of n-6 fatty acids is uncertain, but a small amount of 20:4n-6 is considered to be required (Sargent *et al* 1989).

The fatty acid composition of the fillet lipids is given in Table 1. The mean levels of 22:6n-3 and 20:5n-3 ranged between 20.0 and 25.1 and 7.1 and 9.3%, respectively, and the level of 20:4n-6 ranged between 1.2 and 1.8%. There were neither correlation between GSI and total n-3 fatty acids nor between GSI and total n-6 fatty acids in the fillets, indicating no massive transport of these essential fatty acids from the fillet to the developing ovary. The brood fish were classified in two main groups, one of immature (0.5 < GSI < 2.8) and one of maturing females (6.2 < GSI < 16.0) (Lie *et al.*, 1992). The score plot (PC1 versus PC2) of the samples of fatty acids in fillets showed two main classes in the data, but the diet lipid did not fit to any of them (Fig. 1). The explained variance (76.8%) along the main component (PC1) was mainly due to the separation of the fish lipid from the feed lipid, irrespective of maturation status. Hence, even if there

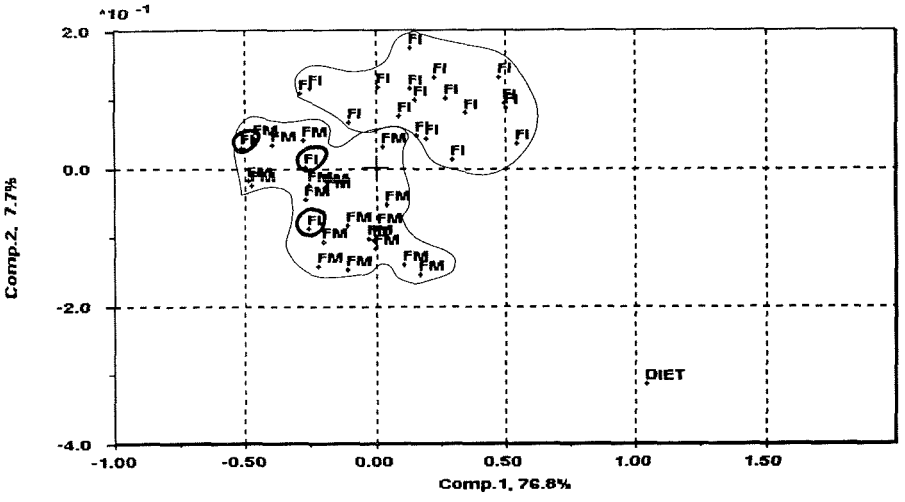

Figure 1: Score plot of all the fatty acid samples from immature fillet (FI), maturing fillet (FM) and the diet. Explained variance from the PCA model is noted along the respective PC1 and PC2 axes.

was a marked difference in fatty acid composition between immature and maturing fish, the diet was roughly equally adjusted to all the fish. The maturation status of the fillets, with respect to the fatty acid composition could consequently not be directly related to this particular feed composition. One class contained only immature fish and the other contained maturing females and three immature females. The loadings from this analysis revealed that 18:4n-3 had high positive loading along both PC1 and PC2, whereas 18:1n-11 had the highest negative loading along PC2. This reflected the higher level of 18:4n-3 in the feed and its decrease in relative occurrence in fillet during maturation. However, the change in 18:1n-11 is difficult to interprete, because a small absolute decrease in other fatty acids might cause a relative large increase in the percent value of this minor component during maturation. The long chain monoenes 22:1n-11 had the highest positive loading along PC1 and 22:6n-3 had the highest negative loading, and were the main reason for the separation of the diet from the two groups of fillet. Further, the level of 22:1n-11 in fillet was negatively correlated to GSI, suggesting a specific utilisation of this fatty acid as an energy source. Similar results are reported by several authors (Yu *et al.*, 1977; Henderson and Sargent, 1985; Lie *et al.*, 1986,1991)

The liver lipid fatty acid composition is given in Table 2. The mean levels of 20:5n-3 and 22:6n-3 varied between 4.5 and 6.2% and 8.9 and 13.4% of total fatty acid composition, respectively. The levels of arachidonic acid (20:4n-6) were low, the mean values varied between 0.4 and 0.8%. Negative correlations between the levels of 20:5n-3, 22:5n-3 and 22:6n-3 in the liver of the females

Table 2. Fatty acid composition of total lipid of liver in female turbot during the reproductive cycle.

	Jan (n=7)		Feb. (n=8)		Mar. (n=5)		Apr. (n=7)		May (n=9)		June (n=9)		July (n=9)	
	Mean	±CI*	Mean	±CI*	Mean	±CI*	Mean	±CI*	Mean	±CI*	Mean	±CI*	Mean	±CI*
14:0	5,7	0,2	6,0	0,5	6,4	0,7	6,5	0,5	6,2	0,5	6,6	0,4	5,8	1,1
15:0	0,4	0,1	0,3	0,1	0,3	0,1	0,3	0,1	0,4	0,0	0,4	0,0	0,5	0,1
16:0	13,0	0,8	12,8	1,2	13,4	1,0	13,7	1,1	15,0	0,8	15,9	1,1	17,5	0,8
16:1n-9	0,5	0,1	0,6	0,2	0,5	0,1	0,7	0,3	0,9	0,2	0,9	0,2	0,9	0,3
16:1n-7	7,0	0,4	7,8	1,0	8,6	1,8	8,1	1,0	7,6	0,5	7,8	0,4	6,9	1,6
17:0	0,9	0,1	0,4	0,2	0,8	0,2	0,4	0,1	0,9	0,1	0,8	0,1	0,8	0,2
18:0	1,3	0,2	1,6	0,2	1,6	0,2	1,6	0,2	1,5	0,4	1,4	0,2	1,6	0,3
18:1n-11	1,6	0,3	1,0	0,4	0,6	0,8	2,1	0,6	1,9	0,6	2,0	0,7	2,0	0,6
18:1n-9	15,1	2,2	17,7	1,9	19,2	2,0	16,3	0,9	16,3	1,0	17,4	1,5	14,6	2,7
18:1n-7	3,8	0,2	4,1	0,2	4,4	0,4	4,3	0,2	4,4	0,2	4,6	0,2	4,3	0,5
18:2n-6	5,9	0,5	5,1	0,8	4,4	1,1	4,6	0,9	5,0	0,6	4,8	0,4	5,2	0,4
18:3n-3	1,0	0,1	0,9	0,2	0,8	0,2	0,9	0,2	0,9	0,1	0,8	0,1	0,7	0,1
20:1n-11	1,3	0,2	1,3	0,3	1,1	0,5	1,4	0,2	1,4	0,2	1,7	0,2	1,3	0,4
20:1n-9	5,5	0,2	5,5	0,4	6,5	0,5	6,2	0,5	5,8	0,6	6,1	0,4	5,7	0,5
20:1n-7	0,3	0,0	0,2	0,0	0,2	0,0	0,2	0,1	0,2	0,0	0,2	0,0	0,2	0,0
18:4n-3	1,0	0,1	1,0	0,2	0,9	0,3	1,0	0,2	1,0	0,1	0,9	0,1	0,8	0,0
20:2n-6	0,9	0,1	0,9	0,1	0,8	0,1	0,7	0,1	0,7	0,1	0,6	0,1	0,6	0,1
20:3n-6	0,1	0,0	0,1	0,0	0,1	0,0	0,1	0,0	0,1	0,0	0,1	0,0	0,1	0,0
20:3n-3	0,4	0,0	0,4	0,1	0,3	0,0	0,3	0,0	0,3	0,0	0,2	0,1	0,1	0,1

20:4n-6	0,5	0,1	0,5	0,1	0,5	0,1	0,4	0,1	0,5	0,2	0,5	0,1	0,8	0,4
22:1n-11	3,7	0,4	3,3	0,7	4,0	1,0	4,5	0,9	3,7	0,9	3,9	0,5	3,3	0,7
22:1n-9	0,7	0,1	0,8	0,1	0,7	0,4	0,9	0,1	0,8	0,2	0,8	0,1	0,6	0,2
20:4n-3	1,7	0,2	1,9	0,2	1,7	0,1	1,6	0,2	1,3	0,1	1,0	0,2	0,9	0,2
20:5n-3	6,2	0,8	5,3	0,8	4,6	0,8	5,2	0,9	5,5	0,8	4,8	0,6	5,6	1,7
24:0	0,8	0,2	0,9	0,1	0,4	0,1	0,5	0,2	0,2	0,1	0,3	0,2	0,2	0,1
24:1	0,5	0,0	0,5	0,0	0,4	0,1	0,4	0,1	0,4	0,0	0,3	0,0	0,3	0,0
22:5n-3	2,8	6,9	2,6	0,3	2,2	0,2	1,9	0,3	1,8	0,3	1,4	0,4	1,7	0,7
22:6n-3	13,4	1,8	11,4	1,2	10,9	1,7	10,6	1,4	10,5	2,0	8,9	1,7	12,7	4,7
Div.	3,6	0,3	4,4	0,5	3,4	0,3	3,8	0,5	4,1	0,4	4,0	0,1	3,6	0,3
Sum saturated	22,1	0,9	22,0	1,7	22,8	1,5	23,0	1,6	24,3	0,8	25,5	1,5	26,5	1,4
Sum 16:1	7,7	0,4	8,5	1,0	9,2	1,8	9,0	0,8	8,7	0,5	8,9	0,5	7,9	1,8
Sum 18:1	20,6	2,4	22,7	1,7	24,3	2,2	22,8	1,4	22,6	1,2	24,0	1,4	20,9	3,6
Sum 20:1	7,2	0,4	7,0	0,6	7,7	0,8	7,9	0,6	7,3	0,8	8,0	0,6	7,2	0,8
Sum 22:1	4,4	0,5	4,1	0,8	4,7	1,3	5,4	1,1	4,5	1,1	4,7	0,5	3,8	0,8
Sum monoenes	40,9	2,9	43,0	2,5	46,5	2,6	45,6	2,5	43,8	3,3	46,2	2,3	40,4	6,2
Sum n-3	26,5	2,2	23,7	2,6	21,4	2,8	21,5	2,6	21,5	2,6	18,2	2,6	22,7	6,7
Sum n-6	7,4	0,6	6,6	1,0	5,7	1,1	5,8	1,0	6,2	0,7	6,0	0,5	6,7	0,6
Sum polyenes	33,9	2,5	30,5	3,6	27,3	3,8	27,6	3,4	27,9	3,2	24,3	2,9	29,5	7,3
n-3/n-6	3,6	0,3	3,7	0,3	3,8	0,4	3,8	0,6	3,5	0,3	3,1	0,4	3,3	0,7

* CI = Confidence values (95%)

and GSI were found, suggesting a depletion of these fatty acids from the liver during the development of the ovary. No such effect was found for the n-6 fatty acids. However, as the lipid contents in the liver were influenced by the maturation process to a small extent these correlations seem to be of minor importance and closely related to the two outlying maturing females discussed below.

Two main classes were seen from the score plot (PC1 versus PC2) of the liver data (Fig.2). The mature females were separated from the immatures, however, two mature females had different composition excluding them from both classes. Further, the feed was separated from both liver classes, but showed the best fit to the immature liver. The analysis showed a high positive PC1 loading of 22:6n-3, 22:5n-3 and 20:5n-3 due to the outliers and a high negative loading of 14:0 and sum of C16 and C18 monoenes. The separation of the main classes and the feeed was explained by a high negative loading along PC1 and PC2 of 18:1n-11 and 16:1n-9 and a high positive loading of 22:1n-11 along both PC1 and PC2 were found.

The mean levels of 22:6n-3 of total lipids in the gonads were between 16.9 and 20.5% (Table 3), but no systematic variation due to maturation was found. The levels of 20:5n-3 and 22:5n-3 in the gonads were negatively correlated ($p < 0.05$) to GSI, while the total n-3 level was not correlated to GSI. It is noteworthy, that both the level of 20:4n-6 and the total level of n-6 in female gonads were negatively correlated with GSI, indicating that these specific fatty acids were utilised during the maturation of the gonads.

A score plot (PC1 versus PC2) of the fatty acid composition of gonad lipids from the immature and mature females divided the material into two classes, one with the immature and one with the mature fish, with the diet separated from both classes (Fig. 3). The loadings of the principal components (1 and 2) showed that 20:4n-6 had the highest negative loading and 22:1n-11 the highest positive loading along PC1 and both had high negative loading along PC2. The monoenes 16:1n-7 and 18:1n-11 had high positive loading along PC2. High loading along PC1 reflected the separation of the gonads from the diet , whereas high loading along PC2 reflected both the separation of immature and maturing fish and the separation of the gonad from the diet. 16:1n-7 showed the best fit between feed and the maturing gonad (discrimination power < 1.0) whereas the effect of 18:1n-11 is believed to be of minor importance, due to its low level in the gonad (0.8 - 1.6 %). The fatty acids 16:0, 18:1n-9, 18:2n-6 and 20:5n-3 showed the best match between the immature gonad and the feed , whereas 16:0 also matched the maturing gonad with the feed. The composition of the diet lipid consequently resembled that of the immature gonad lipid more than that of the maturing gonad lipid, suggesting that the diet composition is of minor importance in the maturation of the gonads. This further point to the

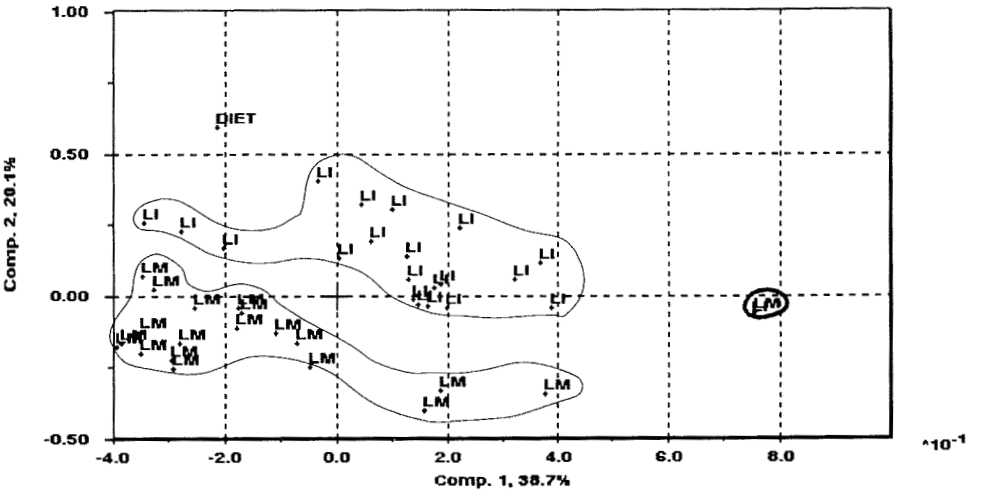

Figure 2: Score plot of all the fatty acid samples from immature liver (LI), maturing liver (LM) and the diet. Explained variance from the PCA model is noted along the respective PC1 and PC2 axes.

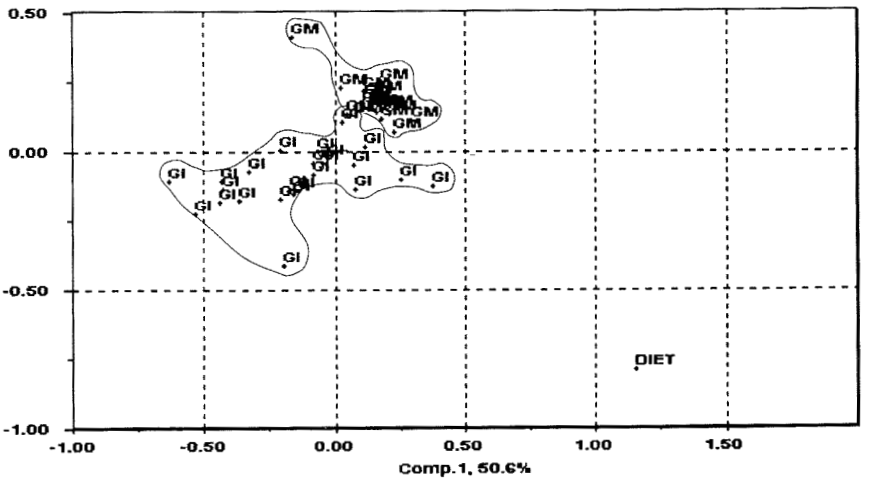

Figure 3: Score plot of all the fatty acid samples from immature gonad (GI), maturing gonad (GM) and the diet. Explained variance from the PCA model is noted along the respective PC1 and PC2 axes.

utilisation of available body reserves in the maturation period in wild stocks, independent of food availability.

The plasma lipid fatty acid composition is presented in Table 4. The level of 20:4n-6 was negatively correlated to GSI, while no correlation between GSI and 20:5n-3 or between GSI and 22:6n-3 were found. The score plot (PC1 versus PC2) of the plasma fatty acid composition gave four main classes (Fig.

Table 3. Fatty acid composition of total lipid of gonad in female turbot during the reproductive cycle.

	Jan (n=7)		Feb. (n=8)		Mar. (n=5)		Apr. (n=7)		May (n=9)		June (n=9)		July (n=9)	
	Mean	±CI*	Mean	±CI*	Mean	±CI*	Mean	±CI*	Mean	±CI*	Mean	±CI*	Mean	±CI*
14:0	3,4	0,5	4,0	0,5	3,9	0,9	4,6	0,4	4,5	0,3	4,1	0,5	3,8	0,5
15:0	0,3	0,0	0,3	0,0	0,3	0,0	0,3	0,0	0,3	0,0	0,3	0,0	0,3	0,0
16:0	17,8	1,3	15,2	0,9	16,0	1,8	14,7	0,7	14,7	0,6	14,9	0,4	15,4	0,5
16:1n-9	0,4	0,0	0,4	0,0	0,4	0,1	0,4	0,1	0,5	0,1	0,5	0,1	0,5	0,1
16:1n-7	3,8	0,5	5,3	0,5	5,5	1,3	6,5	0,6	6,4	0,5	6,0	0,5	5,8	0,7
17:0	1,2	0,1	1,0	0,1	0,6	0,5	0,2	0,1	0,6	0,1	0,6	0,0	0,6	0,0
18:0	3,5	0,4	2,7	0,3	3,1	0,6	2,5	0,3	2,5	0,1	2,7	0,1	2,8	0,3
18:1n-11	1,1	0,2	1,3	0,1	0,8	0,6	1,4	0,1	1,4	0,1	1,6	0,1	1,5	0,2
18:1n-9	12,0	1,2	13,8	0,8	13,6	1,6	13,9	0,5	13,5	0,6	13,4	0,2	13,0	0,6
18:1n-7	3,2	0,3	3,6	0,1	3,8	0,5	4,0	0,1	4,0	0,2	4,1	0,1	3,9	0,3
18:2n-6	5,4	0,4	5,7	0,6	5,1	0,2	5,2	0,3	5,2	0,2	4,8	0,2	4,6	0,3
18:3n-3	0,7	0,1	0,8	0,2	0,6	0,2	0,8	0,1	0,9	0,0	0,7	0,1	0,6	0,1
20:1n-11	0,6	0,1	0,7	0,1	0,6	0,1	0,9	0,1	0,7	0,1	0,8	0,1	0,8	0,1
20:1n-9	4,0	0,7	4,9	0,4	5,5	1,0	6,2	0,8	5,3	1,0	5,5	0,4	5,0	0,4
20:1n-7	0,1	0,1	0,1	0,0	0,1	0,0	0,2	0,0	0,2	0,0	0,2	0,0	0,1	0,0
18:4n-3	0,8	0,2	1,1	0,2	0,9	0,3	1,2	0,2	1,2	0,1	1,0	0,2	1,0	0,2
20:2n-6	0,4	0,0	0,5	0,0	0,4	0,1	0,4	0,0	0,4	0,0	0,4	0,0	0,4	0,0
20:3n-6	0,1	0,1	0,1	0,0	0,1	0,0	0,1	0,0	0,1	0,0	0,1	0,0	0,1	0,0
20:3n-3	0,0	0,0	0,1	0,1	0,0	0,0	0,1	0,1	0,1	0,0	0,1	0,0	0,1	0,0

20:4n-6	3,2	0,5	2,2	0,5	2,5	1,2	1,5	0,6	1,2	0,2	1,3	0,4	1,6	0,7
22:1n-11	1,6	0,2	1,7	0,2	1,8	0,6	2,0	0,5	1,4	0,4	1,5	0,2	1,4	0,5
22:1n-9	0,1	0,1	0,2	0,0	0,1	0,1	0,2	0,0	0,2	0,1	0,2	0,0	0,2	0,1
20:4n-3	0,8	0,1	1,0	0,1	0,8	0,1	0,9	0,1	1,0	0,1	0,9	0,1	0,8	0,1
20:5n-3	9,9	1,3	8,8	0,6	8,9	1,6	8,4	0,4	8,4	0,2	8,4	0,2	8,3	0,6
24:1	0,3	0,1	0,3	0,0	0,2	0,1	0,3	0,0	0,3	0,0	0,3	0,0	0,3	0,1
22:5n-3	2,7	0,2	2,6	0,2	2,4	0,2	2,3	0,1	2,3	0,2	2,4	0,2	2,5	0,2
22:6n-3	19,6	0,9	17,8	1,2	18,2	1,4	16,9	1,3	18,6	2,2	19,9	1,4	20,5	1,0
Div.	2,4	0,5	3,0	0,5	3,4	0,9	3,0	0,4	3,1	0,1	2,7	0,2	3,2	0,3
Sum saturated	26,5	1,6	23,6	0,8	23,8	1,8	22,4	0,7	22,7	0,5	22,7	0,7	23,0	0,6
Sum 16:1	4,4	0,6	5,9	0,5	6,0	1,3	7,2	0,6	7,2	0,5	6,7	0,5	6,6	0,7
Sum 18:1	16,3	1,6	18,7	0,9	18,3	1,9	19,3	0,5	18,9	0,7	19,1	0,4	18,4	1,0
Sum 20:1	4,7	0,9	5,7	0,5	6,2	1,1	7,3	0,8	6,2	1,2	6,5	0,4	5,9	0,5
Sum 22:1	1,7	0,3	1,9	0,2	1,9	0,6	2,2	0,5	1,6	0,5	1,7	0,2	1,6	0,6
Sum monoenes	28,1	3,3	32,5	1,8	32,7	4,5	36,4	2,1	34,4	2,6	34,4	1,0	33,0	1,4
Sum n-3	34,5	2,0	32,2	1,2	31,8	2,4	30,7	1,5	32,7	2,2	33,4	1,1	33,9	1,0
Sum n-6	9,1	0,4	8,5	0,2	8,1	0,9	7,2	0,5	6,9	0,3	6,6	0,4	6,7	0,5
Sum polyenes	43,6	2,1	40,8	1,2	40,1	3,0	38,2	1,7	39,9	2,4	40,2	1,4	40,8	1,2
n-3/n-6	3,8	0,3	3,8	0,2	3,9	0,4	4,2	0,3	4,7	0,2	5,0	0,2	5,1	0,3

* CI = Confidence values (95%)

Table 4. Fatty acid composition of total lipid of plasma in female turbot during the reproductive cycle.

	Jan (n=7)		Feb. (n=8)		Mar. (n=5)		Apr. (n=7)		May (n=9)		June (n=9)		July (n=9)	
	Mean	_±CI*	Mean	_±CI*	Mean	_±CI*	Mean	_±CI*	Mean	_±CI*	Mean	_±CI*	Mean	_±CI*
14:0	3,8	0,7	3,8	0,2	4,1	0,5	4,1	0,3	4,5	0,3	3,9	0,4	3,1	0,3
15:0	0,3	0,0	0,3	0,0	0,3	0,0	0,3	0,0	0,3	0,0	0,3	0,0	0,4	0,0
16:0	13,7	0,6	13,7	0,7	14,3	0,6	13,8	0,3	14,7	0,6	16,3	0,7	18,0	0,6
16:1n-9	0,3	0,0	0,3	0,0	0,3	0,0	0,4	0,1	0,5	0,1	0,6	0,1	1,2	0,9
16:1n-7	3,3	0,7	3,8	0,3	4,4	0,6	4,3	0,5	6,4	0,5	4,1	0,6	2,4	1,1
17:0	0,4	0,1	0,4	0,1	0,7	0,2	0,7	0,1	0,6	0,1	0,7	0,1	0,8	0,1
18:0	2,4	0,2	2,5	0,2	2,5	0,3	2,3	0,2	2,5	0,1	2,4	0,2	2,6	0,2
18:1n-11	0,7	0,1	0,7	0,1	0,7	0,2	0,9	0,1	1,4	0,1	1,4	0,3	2,3	2,0
18:1n-9	10,0	0,6	9,7	0,5	10,3	0,3	9,9	0,3	13,5	0,6	10,1	0,6	8,0	2,4
18:1n-7	2,5	0,3	2,8	0,2	3,0	0,2	3,0	0,1	4,0	0,2	3,2	0,2	2,7	0,3
18:2n-6	5,0	0,4	4,0	0,2	3,8	0,1	3,7	0,1	5,2	0,2	3,3	0,2	3,3	0,2
18:3n-3	0,8	0,1	0,6	0,0	0,6	0,1	0,6	0,1	0,9	0,0	0,5	0,1	0,3	0,0
20:1n-11	0,5	0,1	0,5	0,1	0,4	0,2	0,7	0,1	0,7	0,1	0,7	0,1	0,6	0,1
20:1n-9	5,0	0,9	6,0	0,3	6,8	0,8	7,0	0,7	5,3	1,0	5,3	1,0	3,7	0,3
20:1n-7	0,1	0,0	0,2	0,0	0,2	0,1	0,2	0,0	0,2	0,0	0,2	0,0	0,2	0,0
18:4n-3	1,7	0,3	1,5	0,1	1,4	0,4	1,5	0,3	1,2	0,1	0,9	0,2	0,5	0,1
20:2n-6	0,5	0,1	0,5	0,0	0,4	0,0	0,4	0,0	0,4	0,0	0,4	0,0	0,4	0,0
20:3n-6	0,1	0,1	0,0	0,0	0,1	0,1	0,0	0,0	0,1	0,0	0,1	0,0	0,1	0,0
20:3n-3	0,0	0,0	0,0	0,0	0,0	0,1	0,1	0,0	0,1	0,0	0,1	0,0	0,0	0,0
20:4n-6	1,6	0,3	1,4	0,1	1,3	0,2	1,0	0,3	1,2	0,2	1,1	0,2	1,5	0,2
22:1n-11	5,6	1,6	6,7	0,4	7,1	1,7	7,9	1,5	1,4	0,4	4,9	2,0	2,3	0,4

22:1n-9	0,4	0,1	0,4	0,0	0,3	0,2	0,5	0,1	0,2	0,1	0,5	0,1	0,3	0,1
20:4n-3	1,0	0,1	0,9	0,1	0,9	0,1	0,8	0,0	1,0	0,1	0,6	0,1	0,5	0,1
20:5n-3	9,2	0,9	9,0	0,5	9,2	0,6	9,4	0,3	8,4	0,2	9,3	0,6	9,5	0,5
24:0	0,7	0,4	0,8	0,1	0,2	0,2	0,3	0,1	0,1	0,0	0,2	0,1	0,3	0,0
24:1	0,3	0,0	0,3	0,0	0,4	0,0	0,5	0,2	0,3	0,0	0,3	0,0	0,2	0,0
22:5n-3	2,6	0,4	2,4	0,2	2,3	0,2	2,1	0,2	2,3	0,2	2,3	0,3	2,5	0,1
22:6n-3	24,2	3,3	20,9	1,4	20,4	2,8	18,7	2,3	18,6	2,2	22,3	2,6	28,2	2,0
Div.	2,8	0,4	5,1	4,3	3,0	0,4	3,7	0,7	3,1	0,1	3,4	0,2	3,8	0,5
Sum saturated	21,4	0,9	21,6	1,0	22,1	0,6	21,6	0,4	22,7	0,5	23,9	0,8	25 1	0,6
Sum 16:1	3,8	0,7	4,3	0,3	4,9	0,6	4,8	0,5	7,2	0,5	4,9	0,6	3,8	0,4
Sum 18:1	13,2	0,9	13,2	0,7	14,1	0,5	13,7	0,4	18,9	0,7	14,8	0,9	13,0	1,1
Sum 20:1	5,7	1,0	6,7	0,3	7,3	0,8	7,9	0,8	6,2	1,2	6,2	1,1	4,5	0,4
Sum 22:1	5,9	1,6	7,1	0,4	7,5	1,6	8,5	1,6	1,6	0,5	5,3	2,0	2,6	0,5
Sum monoenes	29,0	4,0	31,8	1,2	34,3	3,1	35,6	3,2	34,4	2,6	31,6	3,8	24,1	1,9
Sum n-3	39,5	4,3	35,5	2,2	34,9	2,3	33,6	2,4	32,7	2,2	36,1	3,1	41,6	1,9
Sum n-6	7,1	0,5	5,8	0,4	5,5	0,3	5,1	0,3	6,9	0,3	4,9	0,4	5,2	0,2
Sum polyenes	46,7	4,7	41,5	2,5	40,7	2,4	39,1	2,7	39,9	2,4	41,1	3,5	46,9	1,9
n-3/n-6	5,5	0,4	6,1	0,2	6,3	0,4	6,6	0,2	4,7	0,2	7,4	0,2	8,0	0,5

* CI = Confidence values (95%)

4). The mature females were grouped into 3 classes and one subgroup was included in the class containing the main part of the immature females. The loading plot revealed that 22:1n-11 had high positive PC1 loading reflecting the higher content in the feed, whereas 18:1n-11 and 16:1n-11 had the highest negative loading. Further, 16:1n-11 and 18:1n-9 showed high PC2 loading and 22:6n-3 a high negative loading along PC2. High loading along PC1 reflected both the separation of the plasma lipid from the diet lipid, particularly in plasma from the maturing fish, and the separation of the immature from the maturing plasma lipid. Separation of the maturing fish into several plasma lipid groups along PC2 is difficult to interprete on the basis of these data. However, as appetite is observed to vary greatly in maturing fish, the observed differences may reflect differences in time from last intake of feed between individual fish.

In general there were differences in the fatty acid composition of tissue lipids from immature and mature turbot. This confirms the main trend discussed by Lie *et al.* (1992) and suggests that the fatty acids in the different tissues have a significant impact on the overall classification of immature and maturing turbot. In several ways the essential fatty acids 20:5n-3, 22:6n-3 and 20:4n-6 seemed to be involved. The roles of the essential fatty acids are complex as they are involved in many processes, directly or indirectly, and one cannot point to a single site of action. However, they are certainly important during the development of the ovary. Some of the EFA, as 20:4n-6 and 20:5n-3, serve as precursors for the eicosanoids. These substances are formed in minute amounts in tissues and have very short lifetimes, but physiological effects are powerful. The modulatory effect in mammals of n-3 fatty acids on eicosanoid formation from 20:4n-6 has been focused by Sargent *et al.* (1989).

This aspect seems to be important also in marine fish as in these species, n-3 PUFA greatly exceeds the n-6 PUFA. This points to the need to optimise the ratio between and the levels of these fatty acids in the diets for marine fish species. It is important that the diets cover the requirements at the different life stages of the fish, and in brooder diets it is also important to satisfy the needs for nutrients to the developing gonads and thereby secure the production of healthy offspring.

Fatty acids present in small relative levels partly caused the difference between tissues of immature and maturing turbot. Because a small absolute decrease in other fatty acids might cause a relative large increase in the percent value of these minor components during maturation, the significance of the minor components is not stressed in the present investigation. The fatty acid composition of tissue lipids are influenced by a number of factors and is the momentary net result of complex dynamic interrelationships between them. The major factors are: dietary fatty acid intakes, rates of oxidative catabolism of the fatty acids, kinetics of desaturation and elongation reactions, competitive

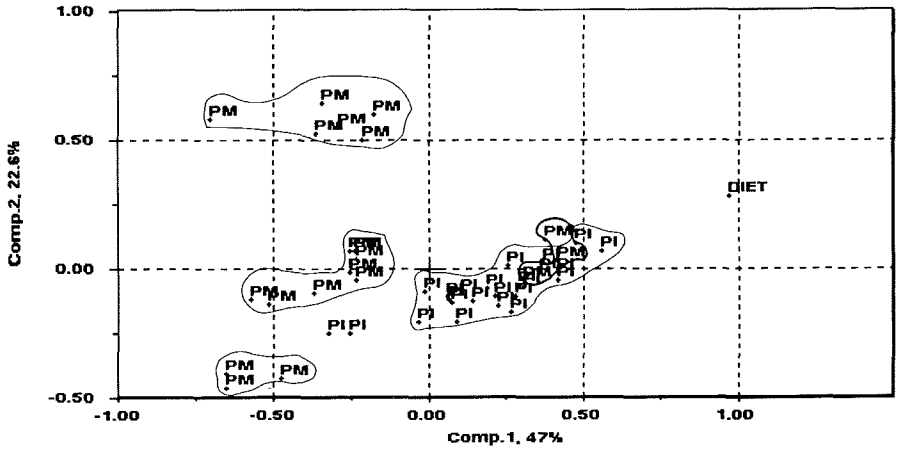

Figure 4: Score plot of all the fatty acid samples from immature plasma (PI), maturing plasma (PM) and the diet. Explained variance from the PCA model is noted along the respective PC1 and PC2 axes.

incorporation and retroconversions among fatty acids, but the details are not fully understood.

In the present experiment the diet lipid differed from the different tissue lipids in all the score plots, and this was mainly caused by the high levels of the long chain monoenes 22:1n-11 in the diet (discrimination power > 3.0). This points to the metabolism of this particular acid, because the levels in all tissues were considerably lower than in the diet. Similar observations have been reported in rainbow trout fed high levels of 22:1n-11 (Sargent *et al.*, 1979) and in cod (Lie *et al.*, 1986, 1991). In these experiments 22:1 was not over represented in faeces, thus a specific excretion of the high quantities of 22:1n-11 ingested does not seem to exist. The suggestions are still speculative, but points to a specific catabolism of 22:1n-11.

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ORGAN DISTRIBUTION OF VITAMINS A AND E DURING THE BROODSTOCK PHASE OF FEMALE TURBOT *(Scopthalmus maximus).*

by

HEMRE G.-I. , MANGOR-JENSEN A.[2], ROSENLUND G.[3] and LIE Ø.,

Institute of Nutrition, Directorate of Fisheries, Strandgaten 229, N-5024 Bergen,
[2] Institute of Marine Research, Austevoll Aquaculture Research Station, N-5392 Storebø,
[3]BP-Nutrition Aquaculture Research Centre, P.O.Box 532, N-4001 Stavanger, Norway .

ABSTRACT

Retinol and α-tocopherol levels were determined during monthly sampling of 5 to 9 (totally 63) female brood turbot fillets, livers, gonads and plasma during a six month experiment. The fish were fed a commercial diet containing ave. 5.2 μg/g vitamin A equivalents and ave. 285 μg/g vitamin E equivalents. The retinol (vitamin A) concentration in fillet was found to be from 0.03 to 0.14 μg/g. In the livers the level ranged from 45 - 199 μg/g, increasing as daylength increased. No correlation was found between liver retinol and gonadal weight relative to body weight. In spite of high vitamin A contents in feed and liver, low and decreasing concentrations of retinol were detected in gonads as maturation proceeded. In plasma the levels were from 0.09 to 0.23 μg/g with no significant variation between samplings.

Fillet, liver and plasma α-tocopherol concentrations decreased moderately while the total amount of vitamin E in gonads increased as the size of the gonads increased, indicating a net transport of vitamin E from the female fish to the gonads. The final concentration of gonadal vitamin E was found to be high (ave. 101.7 μg/g w.w.) as judged from dietary requirements described for other fish.

The results indicate that the dietary vitamin A concentration was sufficient to support maturing turbot. With regard to vitamin E, however, a net transport from the tissues to the gonads was evident. This may indicate that the dietary level was suboptimal, but further studies are warranted to confirm this.

INTRODUCTION

Turbot is a relatively new species in Norwegian aquaculture. The breeding of eggs to the larval stage is a critical step which assumedly depends on the nutrition of the brood fish (Watanabe 1985, Luquet and Watanabe 1986, Bromage *et al.* 1992). Little is known on the nutrition of turbot.

Vitamins A (retinol) and E (α-tocopherol) have been related to many aspects

of the development of eggs in fish and in other animals (Watanabe *et al.* 1981, Shim and Tan 1990, Sper *et al.* 1990). Few works do, however, describe the distribution of vitamin A. Data giving contents in roe are found for two species, herring (*Clupea harengus*) which contains about 2.5 - 5.7 μg/g vitamin A_1-aldehyd and 0.3-0.9 μg/g retinol (Brækkan *et al.* 1960), and cod (*Gadus morhua*) which contains about 0.3 μg/g retinol (Statens ernæringsråds matvaretabell, 1984).

The distribution and effect of vitamin E in diets for maturing fish and shrimp *(Penaeus indicus)* have been investigated (Luquet and Watanabe 1986, Sanchai-Sutjaritvongsanon 1987, Waagbø, unpublished results, Fakhfakh *et al.* 1991, Watanabe *et al.* 1991). However, still little information regarding requirements of brood turbot are available, which lessens the possibilities to secure and optimise the production of healthy juveniles by broodstock nutrition.

To obtain insight into how nutrients from the diet is distributed in the organs of brood turbot, a comprehensive study was performed at this institute (Lie *et al.* 1992). The present communication reports on the distribution of vitamins A and E in female turbot during the growth of the ovaries.

Table 1. Contents of vitamins A and E in the diet (µg/g w.w.)

Month	Vitamin A	Vitamin E
January	5.3	219
February	5.2	308
March	5.5	307
April	5.1	295
May	4.7	306
June	5.9	298
July	4.9	262

Table 2. Contents of vitamins A and E (µg/g w.w.) in fillets from female turbot during the brood phase. Conf.int.= Confidence interval (95%).

Month	Vitamin A	Conf.int.	Vitamin E	Conf.int.
January (n=7)	0.08	0.06	9.5	1.8
February (n=8)	0.05	0.02	6.8	1.9
March (n=5)	0.04	0.02	12.2	3.8
April (n=7)	0.07	0.03	10.5	3.4
May (n=9)	0.07	0.02	7.8	1.0
June (n=9)	0.04	0.01	7.2	1.5
July (n=9)	0.03	0.02	7.7	1.7
August (n=9)	0.14	0.02	10.5	1.3

MATERIALS AND METHODS

Experimental design

The experimental fish were 480 male and female turbot weighing 1-2 kg. They were hatched and reared at Austevoll Aquaculture Research Station and kept in a circular tank, 7 m in diameter, containing about 50 m^3 water with a salinity of 33 g/L. The ambient temperature was 6.5 - 8.5 °C. Natural light cycle was maintained, but about 70% of the light was shaded with a net covering the tank. The fish were fed to satiation five days a week on a commercial dry feed for turbot containing a high level of poly-unsaturated fatty acids (PUFA) (T. Skretting A/S, Stavanger, Norway).

Sampling

The experiment was carried out from January to August 1991. Ten fish were collected each month throughout the experimental period. Only females, 5 to 9 per sampling, where considered. Throughout the study a total of 63 female fish were analysed. During the experimental period gonadal and liver sizes were recorded, the data are given by Lie *et al.* (1992). At sampling the fish were anaesthetized with metomidate and killed by a sharp blow on the head. Blood samples were immediately withdrawn from *Vena caudalis* and plasma was separated by centrifugation (3000 RPM). Fillet, liver and gonads were dissected and stored frozen at -20°C until analyses were carried out.

Chemical analyses

Vitamin A (retinol) and vitamin E (α-tocopherol) were determined in feed and organ samples by a HPLC method modified after Lambertsen (1983) and Lie *et al.* (1994).

Statistics

Due to the comparative design of this study single pairwise correlation tests were performed for all variables versus gonado somatic index (GSI) and versus time. Due to high intercorrelation between variables the mapping of significant main effects were treated as a multivariate problem, using the software programme SIRIUS (version 3.0, Kvalheim and Karstang 1987). Data in the tables are given as mean values with confidence intervals. In Lie *et al.* (1992) a detailed description of data treatment is given.

Table 3. Contents of vitamins A and E (μg/g w.w.) in livers from female turbot during the brood phase. Conf.int.=Confidence interval (95%)

Month	Vitamin A	Conf.int.	Vitamin E	Conf.int.
January (n=7)	114	39	172	56
February (n=8)	45	6	131	30
March (n=5)	124	31	115	36
April (n=7)	153	26	142	36
May (n=9)	112	23	171	44
June (n=9)	122	22	164	42
July (n=9)	199	49	180	50
August (n=9)	147	21	196	63

Table 4. Contents of vitamins A and E (μg/g w.w.) in gonads from female turbot during the brood phase. Conf.int.=Confidence interval (95%).

Month	Vitamin A	Conf.int.	Vitamin E	Conf.int.
January (n=7)	0.23	0.19	45.3	17.3
February (n=8)	2.25	0.71	53.7	7.5
March (nå)	0.13	0.07	50.9	27.0
April (n=7)	0.27	0.07	61.9	12.0
May (n=9)	0.43	0.12	48.2	4.6
June (n=9)	0.14	0.12	57.1	29.6
July (n=9)	0.22	0.10	61.9	29.9
August (n=9)	0.67	0.44	101.7	34.2

Table 5. Contents of vitamins A and E (μg/g) in plasma from female turbot during the brood phase. Conf.int.=Confidence interval (95%).

Month	Vitamin A	Conf.int.	Vitamin E	Conf.int.
January (n=7)	0.23	0.03	34.7	7.9
February (n=8)	0.18	0.03	35.7	10.8
March (nå)	0.16	0.03	41.4	5.9
April (n=7)	0.23	0.07	39.0	6.5
May (n=9)	0.23	0.05	28.2	6.7
June (n=9)	0.09	0.02	26.9	8.1
July (n=9)	0.18	0.02	18.6	2.1
August(n=9)	0.21	0.04	23.4	2.5

RESULTS AND DISCUSSION

Gonadal weight increased relative to body weight from 2 to 15% during the experiment. Also liver weight relative to body weight increased with increasing GSI (gonado somatic index), indicating no net use of liver stores as maturation proceeded. Liver and gonadal indices in the present experiment are given by Lie *et al.* (1992).

Vitamin A

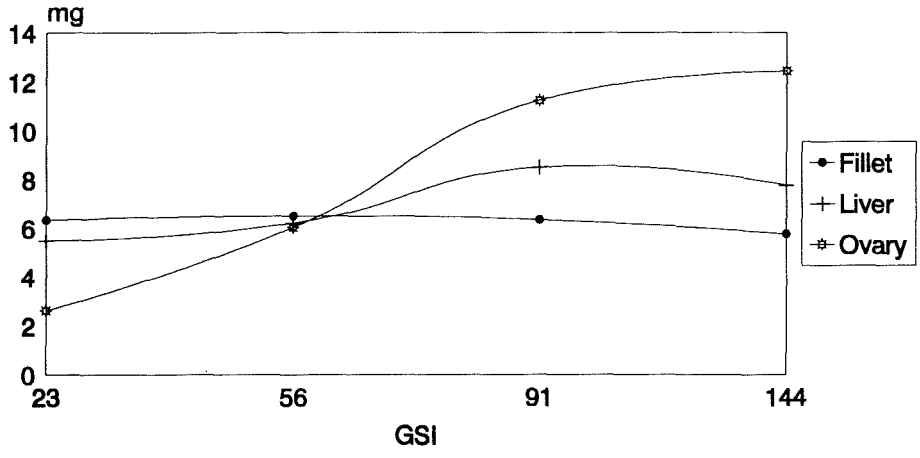
The content of vitamin A in the feed was 5.2 ± 0.4 μg/g or 17 IU/g (Table 1). The exact dietary requirement for vitamin A for turbot is not established (NRC 1991). For comparison maturing guppy (*Poecilia retinculata*) showed a minimum dietary requirement of 2 - 4 IU/g (Shim and Tan 1990) for normal growth and gonadal development. Several fish species show, however, high body levels of vitamin A without dietary supplementations; analyses ranging from 20 IU/g (6 μg/g) whole body mass (w.w. salmon, *Salmo salar*) to 150 IU/g (45 μg/g) whole body mass (w.w. herring) (Dierenfeld *et al.*, 1991). Independent of sampling time the ranges of vitamin A levels determined in the present study were 0.03 - 0.14 μg/g w.w. for fillets (Table 2) and 0.09 - 0.23 μg/g w.w. for plasma (Table 5). In the livers the range was from 45 to 199 μg/g w.w. (Table 3). In the latter case the increase was significantly correlated to time of year ($r=0.51$, $p < 0.05$, $n=63$) but not to GSI. The results indicate that 1) the liver was the main depot for vitamin A in turbot as was also found for other species (Brækkan *et al.* 1969: Lambertsen and Brækkan 1969: Blomhoff *et al.*1991) and 2) the turbot liver retinol depots increased during maturation when the feed contained 17 IU/g. Following absorption from the intestine vitamin A is stored primarily in the liver as retinyl esters. Such stores may be hydrolysed to yield free retinol which is bound in serum by specific retinol binding proteins and distributed throughout the body (Blomhoff *et al.* 1991). For gonads the retinol levels were from 0.14 to 2.25 μg/g w.w. (Table 4), with significant decreased levels from January to August ($r=-0.37$, $p < 0.05$, $n = 63$), as GSI increased from 2 to 15% (Lie *et al.* 1992). These results indicate some use of retinol in the gonads during maturation, maybe as a function in protection against toxic substances, as described for retinol in herring gull eggs (Sper *et al.* 1990). Low and decreasing levels of retinol in gonads may be critical for the developing larvae, as vitamin A is found to be directly involved in control of gene expression and cell proliferation (DeLuca 1977, Takase *et al.* 1979, Petkovich *et al.* 1987). However, there may be significant amounts of other forms of vitamin A than retinol, such as retinoic acid (Guillon *et al.* 1989) or retinal (Brækkan *et al.* 1960) in the eggs to cover the needs. Retinoic acid is suggested to be the regulatory form of vitamin A in the developing ferret (Wang *et al.* 1993) and rat (Audouin-

Chevallier *et al.* 1993). The aspectes of other forms of vitamin A were not considered in the present experiment. As vitamin A has an essential role in early development it is likely that ova of turbot contain oxidized forms of vitamin A as was found for herring (Brækkan *et al.*, 1960). Analyses of only retinol probably are not a good indicator of the role of vitamin A's in turbot gonadal development. Analyses of other forms of vitamin A will in future work at this institute be done in relation to maturing fish, and optimisation of live feed for marine larvae.

Vitamin E

The content of vitamin E in the feed averaged 285 ± 33 μg/g (Table 1). The minimum dietary requirement is not determined for turbot. It was estimated to be 60 μg/g for salmon fry (Hamre and Lie 1994). The range of vitamin E levels in the present study was 6.8 - 12.2 μg/g w.w. in fillet (Table 2), 115 - 196 μg/g w.w. in liver (Table 3), 45.3 - 101.7 μg/g w.w. in gonads (Table 4) and 18.6 - 41.4 μg/g w.w. in plasma (Table 5). The fillet levels of α-tocopherol decreased significantly as GSI increased ($r = -0.25$, $p < 0.05$, $n=63$). Also α-tocopherol in the liver decreased significantly as GSI increased ($r = -0.37$, $p < 0.05$, $n=63$). These results suggest that both fillet and liver functioned as depots of vitamin E for transport to ovaries, in line with results in maturing Atlantic salmon (Lie *et al.* 1994), and maturing female shrimp (Alverz-del-Castillo *et al.*1988). As the maturing turbot in the present study was fed during the brood phase, decreased organ depots suggests that the feed levels possibly were in the lower range at this stage of the life cycle. Plasma vitamin E levels were significantly reduced as maturation proceeded ($r = -0.56$, $p < 0.05$, $n=63$) and GSI increased ($r = -0.35$, $p < 0.05$, $n=63$), which indicates that the main vitamin E transport to gonads was early in the maturation phase. Vitamin E levels in gonads were positively correlated to maturing cycle, with no correlation to GSI, but the total amount of vitamin E in the whole organ increased as GSI increased (Figure 1). The highest levels measured (102 μg/g w.w.) in gonads indicate good nutritional status, and high enough to cover the metabolic needs in early life stages (Hamre and Lie 1994). High levels of vitamin E in gonads must be considered to be an advantage as reduced phagocytic index and B and T-cell responses have been found in rainbow trout marginally deficient in vitamin E (Blazen and Wolke 1984). Graded intakes of vitamin E above the minimum requirement also had a differential effect on the immune system in both fish (Hardie *et al.* 1993) and humans (Tengerdy 1989), and positive effects have been measured with 100 - 200 mg/kg α-tocopherol supplementation in feed for maturing red sea bream (*Pagrus major*) (Watanabe *et al.* 1991) and goldfish (*Carassius auratus*) (Sanchai-Sutjaritvongsanon 1987).

Figure 1. Total α-tocopherol (mg/total organ) in fillet, liver and ovary with increasing GSI (gonado somatic index).



Alpha-tocopherol is the main vitamin E compound in animal nutrition, and its biological function as a lipid soluble antioxidant is well established in fish (Hamre *et al.* 1994). In the present study high PUFA diets were used. The polyenes accounted for 36% of the lipids, which amounts to 7% of the dietary dry weight (detailed data are given by Lie *et al.* 1992). The factor best documented as interacting with vitamin E turnover is the dietary level of polyunsaturated fatty acids (PUFA), with increased requirement of vitamin E as the PUFA-levels increase (Watanabe *et al.* 1981, Roem *et al.* 1990). The high PUFA levels in the present study may explain the low organ levels of vitamin E in spite of quite high dietary vitamin E levels. In addition to dietary PUFA, also factors such as developmental stage has been found to influence the turnover and requirement of this vitamin (Dierenfeld *et al.* 1991). It may be suggested that higher dietary vitamin E supplements before start of maturation will lead to higher levels of α-tocopherol in the maturing turbot. Confirmation of this will need further studies. However, no effect on survival rates of eggs and fry when feeding high levels of vitamin E has been found (Eskelinen 1989). The increased gonadal amount of vitamin E in spite of deminished levels in all other organs suggests that the brood fish "takes care of" the outcome independent of dietary levels or lowered brood fish depots.

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SPRAY-DRIED BLOOD IN DIETS TO ATLANTIC SALMON *(SALMO SALAR L.)*

By

K. J. RASMUSSEN
daka a.m.b.a., Ribevej 53, DK-8723 Løsning (Denmark)

ABSTRACT

Spray-dried blood was tested as a substitute for part of low temperature processed (LT-) fish meal in a salmon diet.

Isonitrogenous inclusions of spray-dried blood at 24 and 48 g/kg feed showed no adverse effect on growth. At 95 g/kg there was a slight growth reduction. The inclusions of spray-dried blood showed no effects on salmon lice infestation and the occurrence of winter-ulcers.

The increased dietary iron levels from the inclusion of up to 48 g spray-dried blood/kg feed had a positive effect on the haemoglobin values. No untoward effects were observed at dietary iron levels at 210 mg/kg.

The experiment indicated, that in diets to post-smolt Atlantic salmon 1 kg spray-dried blood might substitute between 1.2 and 1.3 kg of LT fish meal at inclusion rates up to 50 g/kg feed with no adverse effects on growth and health.

INTRODUCTION

Salmon diets usually contain a large amount of fish meal. This is an expensive protein source, and the feed industry has been searching for cheaper substitutes (e.g. refined soya products). Spray-dried blood is a good and cheap source of protein, but the inclusion of this raw material in the diets will inevitably increase the iron content. A high dietary iron level was suggested by Rørvik (1990) and Rørvik *et al.* (1991a) to be associated with the degree of lice infestation and winter-ulcers. This experiment was carried out in order to test, whether a high quality spray-dried blood (BLOSOL) could be included in diets to Atlantic salmon without untoward effects.

MATERIALS AND METHODS

Experimental conditions

Atlantic salmon smolts were obtained from the Institute for Aquaculture Research Ltd. at Sunndalsøra, Norway, and adapted to sea water at the

experimental site, the Institute for Aquaculture Research Ltd. at Averøy, Norway. At the start of the experiment, July 10th 1992, 4.050 smolts were transferred to fifteen, (3 x 3 x 3)m^3 net-pens. The mean weight of the 270 individuals in each pen was 125 g. The experiment lasted for 30 weeks, until February 6th, 1993.

Each of the five diets was fed to salmon in three pens. None of two pens, side-by-side, received the same diet. The pens were under daily surveillance and dead fish were removed when observed. The water temperature was measured dayly at a depth of 2 m. The mean temperatures (°C) in the five periods of approximately six weeks, were 14.2° (July 10th - August 19th), 13.8° (August 20th - September 30th), 9.8° (October 1st - November 17th), 6.8° (November 18th - December 22th) and 5.2° (December 23th - February 6th).

Feeding was automatic and the amount of feed given to all pens in the first 12 weeks was 20% above and in the following six weeks according to the standard feeding table for Atlantic salmon (Austreng *et al.*, 1987) calculated from the highest mean fish weight of the diet groups. Afterwards the amount of feed was increased to 20% above the table values and the mean weights in each diet group used in the calculation. This feeding in excess was established in order to allow the fish to feed according to requirements.

Every six weeks 100 fish per pen were weighed and examined for salmon lice and winter-ulcers. After thirty weeks all fish were counted and weighed in bulk. Blood samples and whole livers were taken from 50 fish at the start of the experiment and from 10 fish/pen at week 12 and after thirty weeks. De-lousing of the fish while staying in the pens on July 29th and October 23th was done by use of Nuvan®(Ciba Geigy.

The feed

The commercial diet (Bio-Optimal, produced by Biomar A/S, Norway) was isocaloric to the four, isonitrogenous test diets.

The formulation of the test diets, Table 1, the selection of raw materials and the choice of feed production equipment were the author's responsibility. Fish meal of low temperature processed (LT-) quality was the main protein source in the test diets. The diets contained 0 (no. 1), 24 (no. 2), 48 (no. 3) and 95 g/kg (no. 4) of BLOSOL, spray-dried blood from Danish Export Slaughterhouses, produced by daka a.m.b.a., Denmark. Fish oil was used as the main fat source.

The feed pellets were produced on a Clextral BC 45 extrudor with two dies of 3.5 mm and sprayed with fish oil before cooling. Pellet diameters were from 4.3 to 4.5 mm. As the test diets were low in pyridoxine, a mixture of pyridoxine hydrochloride and wheat-middlings was added to the pellets, previously coated with cod-liver oil in order to bind the mixture. The pyridoxine coating was

initiated on October 5th. From January 5th increased floating of the commercial pellets was reduced by coating with a mixture of cod-liver oil and an emulgator (Panodan™ 100, Grindsted Products A/S, Denmark). Among the test diets only no. 4 showed some floating, especially in the last six weeks of the experiment. All diets were stored at -20°C before use.

Analyses

Analyses of random samples of the feed were performed by the National Institute of Animal Science, Research Center Foulum, Denmark (amino acids) and the Institute for Aquaculture Research Ltd., Aas, Norway (fish blood haemoglobin, hepatic iron, dietary total iron, crude fat, fatty acids and peroxide value). The analyses not mentioned above, but presented in Table 1, were performed by the Biotechnological Institute, the Academy of Technical Sciences, Kolding, Denmark.

The analysis of not protein-bound iron was as follows: 5 g of ground feed and 100 ml 0.1 M HCl were shaken for thirty minutes. Protein was precipitated with sulpho-salicylic acid and filtered on a common paper filter. Iron was determined in the solution. Fish blood haemoglobin was determined by the standard cyanomethaemoglobin method. Stored liver iron was determined as follows: None-haeme iron was extracted according to Brückmann and Zondek (1940) and determined on an atom-absorptionspectrophotometer.

Statistical analyses

The General Linear Model in SAS was used in the statistics employed. One way ANOVA analysis was performed with body weight, blood haemoglobin, stored liver iron and growth for the five diets ($p<0.05$).

RESULTS

The ingredients used in the test diets and the chemical composition of all diets are shown in Table 1. As was to be expected from the increasing amounts of spray-dried blood included in the test diets the levels of total and protein-bound iron increased from test diet no. 1 to test diet no. 4. Except for iron, protein, vitamin E and ethoxyquin the chemical composition of the test diets and the commercial diet were similar. The difference in vitamin C was, presumably, reflected by the difference in the levels of ascorbic acid added.

The dietary levels of C20:5 n-3 (eicosapentaenoic acid) and C22:6 n-3 (docosahexaenoic acid) were high and stable throughout the experiment. Small

Table 1. Chemical composition of the diets (as fed).

			Diets				
			1	2	3	4	Com-mer-cial
Ingredients:							
Fish meal (LT-quality)		g/kg	587	557	535	478	
Spray-dried blood (BLOSOL)[2]		g/kg	0	24	48	95	
Spray-dried solubles		g/kg	47	47	47	47	
Wheat starch		g/kg	136	130	136	136	
Wheat		g/kg	0	10	10	21	
Fish oil		g/kg	217	219	211	210	
Sodium ascorbate		g/kg	10	10	10	10	
Rovimix Stay-C		g/kg	3	3	3	3	
Analytical data:							
Dry matter		g/kg	923	922	923	927	901
Protein (N x 6.25)		g/kg	491	495	498	493	394
Crude fat		g/kg	300	310	290	300	280
Ash		g/kg	51	51	50	48	59
Crude fiber		g/kg	<2	<2	<2	<2	11
NFE[1]		g/kg	81	66	85	86	157
Total iron		mg/kg	114	168	210	307	87
Protein-bound iron		mg/kg	72	118	148	229	71
Ascorbic acid[2]		g/kg	4	4	4	4	0
Vitamin A		IU/g	19.1	19.4	17.1	18.7	16.8
Vitamin E		mg/kg	36	34	25	31	207
Pyridoxine:	week 0	mg/kg	0.4	0.3	0.5	0.5	19
	coated	mg/kg	20	18	19	19	
Ethoxyquin		mg/kg	185	140	163	134	63
C20:5 n-3+C22:6 n-3[3]	week 0	mg/kg	46	47	44	43	56
	week 30	mg/kg	44	40	40	38	56
	coated	mg/kg	45	45	43	42	60
EAA-index[4]			1.02	1.03	1.04	1.05	0.97
ME[5]		mJ/kg	19.2	19.4	19.0	19.3	18.0

[1] NFE (Nitrogen Free Extract) determined by difference.
[2] The contribution from Rovimix Stay-C™ not included in the ascorbic acid figures.
[3] C20:5 n-3 (eicosapentaenoic acid). C22:6 n-3 (docosahexaenoic acid).
[4] Essential amino acid index (EAA-index) according to Steffens (1989) and using Atlantic salmon whole body tissue according to Wilson and Cowey (1985).
[5] ME according to Phillips and Brockway (1959) and Singh and Nose (1967).

variations in the levels of crude fat might have caused the minor variations in the levels of these fatty acids. The peroxide values, not shown in the table, were low, between 3.6 and 8.9 meq/kg extracted fat, indicating a slowly proceeding oxidation. Ethoxyquin and ascorbic acid were the main antioxidants in the test diets, and vitamin E and astaxanthin (27.5 mg/kg diet) in the commercial diet. The levels of the potentially toxic, histamine, putrescine and cadaverine (150 mg cadaverine/kg feed, the highest value observed) were far below critical levels. The EAA-indexes showed a very similar and high protein quality.

Table 2 shows the fish weights per diet group (means ± SD of means in three parallels). At week 30 the commercial diet group weighed significantly less than the test diet groups, and the test diet group no. 4 significantly less than the other test diet groups. No statistical difference was detected between test diet group no. 1 and 2. As the feed strength was changed during the experiment, and the fish were fed in excess, the feed quotients (FQ, weight of feed given/ increase in wet weight) are of minor interest and not shown in the table. The growth rates of the test diet group no. 1 and 2 were in accordance with the standard feeding tables for Atlantic salmon (Austreng *et al.*, 1987).

Table 2. Weight (g/fish). Means ± SD. (n = 3).

Diet group	Research week 0	6	12	18	23	30
1	125±0	271±14	577± 7	815± 3	952±22	1147±16
2	125±0	267± 0	549±15	807±24	951±15	1151±18
3	125±0	270±10	553±19	794±11	932±41	1118±18
4	125±0	64± 8	552±17	743±14	895±26	1059±15
Commercial	125±0	255± 5	492±10	681±23	804±28	967±37

Table 3 shows the levels of fish blood haemoglobin and stored liver iron. As a diet, similar to the commercial diet, was fed to all salmon before the start of the experiment, the low haemoglobin levels in the commercial diet group (6.6 g hb/dl blood) might have been due to the iron content of that diet. After 12 weeks the haemoglobin level of the commercial diet group was significantly lower than those of the other diet groups. No significant differences were detected between the test diet groups. After 30 weeks the haemoglobin level of the commercial diet group was significantly lower than the level of diet group no. 3. In diet group no. 4 the reduction in haemoglobin from week 12 to 30 was significant. The correlation between the dietary total iron levels and the haemoglobin levels was significant after 12 weeks, but not after 30 weeks.

Table 3. Iron status indicators (Means ± SD). Haemoglobin (g hb/dl blood). Stored liver iron (mg iron/kg wet weight of liver). (n = 3).

		Research week		
	Diet group	0	12	30
Haemoglobin	1	6.6±0.6	8.5±0.7	8.8±0.3
	2	6.6±0.6	8.6±0.3	9.0±1.2
	3	6.6±0.6	8.8±1.0	9.3±1.6
	4	6.6±0.6	9.2±0.2	8.3±0.2
	Commercial	6.6±0.6	7.0±0.6	7.5±0.6
Liver iron	1	10±1	31± 4	138± 9
	2	10±1	64±14	180±10
	3	10±1	81±18	204±14
	4	10±1	81±13	219± 7
	Commercial	10±1	11± 2	50± 5

In the first 12 weeks with four times weight increase (Table 2) the levels of stored liver iron were lower than in the following 18 weeks with two times weight increase. At week 12 the level of stored liver iron in the control diet group was significantly lower than the levels in the test diet groups. No significant difference between the test diet groups no. 2, 3 and 4 was detected. After 30 weeks the level of stored liver iron was significantly lower in the control diet group than in diet group no. 1, the level in no. 1 significantly lower than in no. 2 and the level in no. 2 significantly lower than in no. 3 and 4. No significant difference was detected between the levels in diet group no. 3 and 4.

Lice infestation is shown in Table 4. The numbers of lice/fish/diet group were low and no relation was found between the lice numbers and the dietary

Table 4. Salmon lice (*Lepeoptheirus salmonis* Krøyer and *Caligus elongatus* Nordmann). Means ± SD of number of lice/fish/diet group. (n = 3).

	Research week				
	*6	12	*18	23	30
Diet group					
1	2.9±0.1	4.3±0.7	1.3±0.2	1.7±0.2	2.7±0.2
2	3.5±1.2	5.2±0.6	1.4±0.2	2.4±0.4	3.7±0.7
3	3.3±0.3	4.6±0.3	1.5±0.1	2.3±0.3	3.2±0.6
4	3.2±0.7	4.5±0.9	1.6±0.4	2.1±0.4	3.1±0.6
Commercial	4.1±1.2	5.4±0.8	1.4±0.4	2.3±0.6	3.8±1.2

* De-lousing on July 29th (week 3) and October 23th (week 15).

iron levels. The number (mean ± SD) of lice/fish thoughout the experiment was found to be 3.0 ± 0.5.

The number of fish with winter-ulcers was quite small, as shown in Table 5. The decreasing water temperature from week 13 to 18 might have increased the prevalence of this disease. The mortality deserves notice as only 43 of 4.050 salmon died during the experiment.

Table 5. Winter-ulcers and mortality. (Total number and % of ulcered and dead fish/diet group).

	Research week					
Diet group	6	12	18	23	30	Total
Winter-ulcers						
1 (%)	0	2 (0.3)	1 (0.1)	0	0	3 (0.4)
2 (%)	0	3 (0.4)	0	0	0	3 (0.4)
3 (%)	0	1 (0.1)	2 (0.3)	0	0	3 (0.4)
4 (%)	0	2 (0.3)	3 (0.4)	0	0	5 (0.6)
Commercial (%)	0	2 (0.3)	0	0	0	2 (0.3)
Total (%)	0	10 (0.3)	6 (0.2)	0	0	16 (0.4)
Mortality						
1 (%)	0	1 (0.1)	3 (0.4)	0	1 (0.1)	5 (0.6)
2 (%)	0	2 (0.2)	6 (0.7)	0	0	8 (1.0)
3 (%)	1 (0.1)	4 (0.5)	7 (0.9)	2 (0.3)	0	14 (1.7)
4 (%)	0	4 (0.5)	4 (0.5)	0	1 (0.1)	9 (1.1)
Commercial (%)	3 (0.4)	3 (0.4)	1 (0.1)	0	0	7 (0.9)
Total (%)	4 (0.1)	14 (0.4)	21 (0.5)	2 (0.1)	2 (0.1)	43 (1.1)

DISCUSSION

Salmon lice and winter-ulcers are serious problems for the farming of Atlantic salmon in Norway. Skin damage, osmotic shock and infections from the lice feeding on mucus, skin and blood of their hosts result in loss of production and may result in high mortalities (Tully, 1989). Although the mortalities in Atlantic salmon caused by the winter-ulcers appear to be low (Lunder and Håstein, 1991), the winter-ulcers reduce the market value of the fish.

Rørvik (1990) suggested thrombosis, due to excess iron clotting the erythrocytes, the clots then damaging the walls of the capillaries in the skin causing a prolonged haemorrhage in cold water, as the aetiology of winter-ulcers. Rørvik *et al.* (1991a) fed 120, 220, 295 and 435 mg iron/kg diet to Atlantic salmon in the sea and found a significantly lower frequency of salmon lice and

winter-ulcers at 120 mg/kg. Rørvik *et al.* (1991b) suggested a poorly regulated iron metabolism in Atlantic salmon, in contrast to that in mammals as reviewed by Bernat (1983), as the causal relation between the dietary iron levels and the levels of absorbed iron exceeding the iron binding capacity of the blood.

However, iron is an essential element, which is bound to protein in the body, partly as haeme compounds, such as haemoglobin, partly as non haeme compounds, such as ferritin (Lall, 1989). The minimum dietary iron requirement of Atlantic salmon has been found to be between 60 and 100 mg/kg (Lall and Hines, 1987; Lall, 1989; Andersen *et al.* 1992; Bjørnevik, 1992). Dietary iron levels at 1250 mg/kg diet was fed to rainbow trout by Desjardins *et al.* (1987) with no untoward effects on growth or health. The same lack of effect was observed by Andersen *et al.* (1993 and *pers. comm.*), when 70 g Atlantic salmon was fed 500 mg haeme iron/kg diet.

The growth (Table 2) was taken as an indirect parameter of the health status of the fish. After thirty weeks all the test diet groups weighed significantly more than the commercial diet group. The significantly lower growth of the test diet group fed the highest dietary iron level remains unexplained.

Blood haemoglobin levels at 7.3 g/dl blood was observed by Conroy (1972) in wild Atlantic salmon smolts and at 11.0 g/dl blood in wild, adult Atlantic salmon. In farmed, adult Atlantic salmon Sandnes *et al.* (1988) found haemoglobin levels between 8.9 and 10.4 g/dl blood, and in Atlantic salmon parr fed increasing dietary iron levels Bjørnevik and Maage (1993) found haemoglobin levels between 9.0 and 10.3 g/dl blood. Relative to this, the levels found with the commercial diet group indicate that this group reached normal haemoglobin levels somewhat late in the experiment (Table 2), levels reached by the test diet groups during the first 12 weeks. The, almost anaemic, condition might have influenced the growth of the commercial diet group (Gatlin and Wilson, 1986) together with the difference of about 10% in the protein level (Table 1) as all groups were fed in excess.

There are few studies on iron metabolism in fish and the poorly regulated iron metabolism in Atlantic salmon, suggested by Rørvik *et al.* (1991b), is not fully supported by the observed, almost identical, levels of stored liver iron in test diet group no. 3 and 4 (Table 3) in spite of the difference in the dietary iron levels (Table 1). This observation points to some kind of regulation of the absorption and/or excretion of iron.

The levels of hepatic iron observed were similar to the levels between 157 and 326 mg iron/kg wet weight observed in wild Atlantic salmon by Maage *et al.* (1991), and these levels may probably be considered as normal. The method used by Maage *et al.* measured total liver iron, and the method used in the present study claims to measure stored liver iron. However, as the concentration of iron in the liver has been found to be as low as 17 mg/kg wet weight (Andersen

and Maage *pers. comm.*) the difference in hepatic iron levels measured by the two methods may be negligible.

The dietary levels of vitamin E in the test diets between 25 and 36 mg/kg (Table 1) were close to the requirement of 30 mg/kg (Hamre and Lie, 1993). Vitamin E deficiency was found to interfere with iron absorption, causing iron deficiency symptoms (Hilton, 1989). The haemoglobin levels in the test diet groups were normal after 12 weeks, and the haemoglobin levels of the commercial diet group were low in spite of a high dietary vitamin E level of 207 mg/kg. The high dietary levels of vitamin C in the test diets might have protected the vitamin E levels.

An experiment with Atlantic salmon fry indicated a minimum dietary pyridoxine requirement for growth of 2-3 mg/kg feed (Albrektsen *et al.*, 1993). In the present study the fish grew well in spite of the analyzed pyridoxine levels of the test diets between 0.3 and 0.5 mg/kg (Table 1) in the first 12 weeks.

No relation was found between the dietary iron levels and the frequencies of salmon lice and winter ulcers. As Nylund *et al.* (1992a) observed between 4 and 8 lice/fish in wild and Johannessen (1990) between 400 and 2.000 lice/fish in farmed populations of Atlantic salmon, the lice numbers observed (Table 4) with a mean number of lice/fish/diet group on 3.0 must be considered as rather low.

Lunder and Håstein (1991) found winter-ulcers to be a transmissible disease with haemolytic *Vibrio spp.*, different from *Vibrio anguillarum*, playing an important role. In contrast to the suggestions by Rørvik (1990) of thrombosis as the cause of winter-ulcers, Lunder and Håstein (1991) found skin lesions to take part in the development of the ulcers. In the present experiment the frequencies of winter-ulcers were very low in spite of the handling of 100 fish/pen every six weeks and all fish at the start and the end of the experiment. The observation by Rørvik *et al.* (1991a) of a positive correlation between the dietary iron levels and the occurrence of winter-ulcers was not supported by the observations in this experiment.

As salmon lice have been found to spread infectious diseases (Nylund *et al.*, 1992b), infectious agents might have been present in the waters of the experimental site. Moreover, in a challenge test with *Vibrio anguillarum* Rørvik *et al.* (1991a) found the mortality of Atlantic salmon to increase linearly with increased dietary iron levels. In spite of high dietary iron levels and the presence of lice no diseases were observed, and the mortalities were very low (Table 5).

The observations of this study indicate, that in diets to post-smolt Atlantic salmon low temperature processed (LT-) fish meal in amounts of between 1.2 and 1.3 kg may be substituted with 1 kg spray-dried blood at inclusion rates up to 50 g/kg without any adverse effect on growth or health.

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DETERMINATION OF VITAMIN D_3 IN FISH MEALS BY HPLC.

by

OLE HORVLI and ØYVIND LIE

Institute of Nutrition Directorate of Fisheries, P.O. Box 1900 Nordnes N-5024 Bergen Norway

ABSTRACT

An HPLC method for the separate analysis of vitamin D_2 or vitamin D_3, using the alternate form as an internal standard and UV-detection, is described. The method involves saponification and extraction of small samples (0.25 g) in small tubes (10 mL), sample clean-up on a silica column, and analysis on a C18-column, both by HPLC. The limit of detection was 1.3 ng, or 5.2ng/g sample. The intra-assay precision (CV) was 5.6% (n=8), and the accuracy was ±17% deviation from a standard. Fifteen fish meals were analysed for vitamin D_3 by the method described, the samples were also determined by a chick bioassay method.

INTRODUCTION

Vitamin D refers to any substance showing antirachitic activity in mammals or birds, and includes a number of different forms of which vitamin D_2 (ercalciol; ergocalciferol) and vitamin D_3 (calciol; cholecalciferol) are the most abundant. Vitamin D_3 is produced in the skin of birds and mammals, and is activated to a hormone ($1,25(OH)_2D_3$; calcitriol) involved in the regulation of bone and mineral metabolism (DeLuca, 1979). The discoveries of the wide distribution of the vitamin D-receptor in cells and tissues, together with the demonstrations of the regulatory effects of calcitriol on cell growth and differentiation, suggest however a more extended physiological role of vitamin D (Walters, 1992).

Vitamin D_2, which is mainly a synthetic product, can be activated to $1,25(OH)D_2$; or ercalcitriol, which shows equal potency to calcitriol in mammals. Vitamin D_2 is therefore used in fortification of certain foods. While calcitriol is active in both mammals and birds, ercalcitriol shows only minor activity in birds.

Vitamin D is lipid-soluble, and it is with few exceptions found in extremely low concentrations. The vitamin is often associated with high concentrations of

other structurally related sterols (e.g. cholesterol). Because of the low concentrations of vitamin D in foods and feeds, it has been necessary to determine the contents in assays with rats or chicks as the experimental animals. The introduction of HPLC in the analysis of the vitamin revolutionized not only the time required, but also the precision and the reproducibility of the methods compared to the bioassays. However, the bioassays are sensitive and well established, and the high sensitivity is due to the high potency of the active hormone.

A number of methods based on HPLC are described for the analysis of vitamin D in different matrices such as foods and feeds (Kobayashi *et al.* 1986; Johnsson and Hessel 1987 ; Hung 1988; Johnsson *et al.* 1989; Agarwal 1992; Bravand and Walter 1992; Homberg 1993a; Thompson and Plouffe 1993), formulas and mixtures (Agarwal 1989; Sliva *et al.* 1992; Hart *et al.* 1992; Hasegawa 1992), fish-liver and fish tissues (Egaas and Lambertsen 1979; Pask-Hughes and Calam 1982; Stancher and Zonta 1983; Takeuchi *et al.* 1984a; Monard *et al.* 1986; Takeuchi *et al.* 1987; Suzuki *et al.* 187; Homberg 1993b) and human serum (Aksnes 1980a,b; Chen *et al.* 1990; Aksnes 1992; Vreeken *et al.* 1993; Zamarreno *et al.* 1993). Most of the methods include a step of saponification of the sample before extraction, and one or more purification steps on HPLC before final HPLC-analysis.

The aim of the present study was to develop a simple HPLC-method for vitamin D-determinations in small biological samples, so that the pretreatment of the samples (saponification and extraction) could be performed in in small volumes (10 mL).

MATERIALS AND METHODS

Fish meals

Fifteen fish meals were provided by the International Fishmeal & Oil Manufacturers Association, U.K., through the Norwegian Herring Oil and Meal Research Institute

Reagents

Crystalline vitamin D_2 and vitamin D_3 (Fluka) were dissolved and stored in n-hexane at - 20°C. Standards (internal or external) were prepared by taking an appropriate volume of the standard solution to dryness by evaporation under vacuum, and dissolve it (about 0.1 mg or 4000 I.U.) in 5 mL of absolute ethanol. The exact concentration of the vitamin was determined spectrophotometrically,

and aliquots were taken for further dilution to desired concentrations. At 265 nm, the extinction coefficients of vitamin D_2 and vitamin D_3 are 19.4 and 18.3 $mL^*\mu mol^{-1}*cm^{-1}$, respectively, and readings of extinction against concentration (μg/mL) followed Beer's law. Acetonitrile, n-hexane, methanol, chloroform and tetrahydrofurane (Fison) were of HPLC-grade. Potassium hydroxide, ascorbic acid and pyrogallol (Merck), and absolute and 96% ethanol were of p.a. quality.

The HPLC-method

Sample treatment

Approximately 0.25 g of finely ground fish meal was transferred to a 10 mL's tube (e.g. Sovirel) equipped with a teflon sealed screw cap. Three mL of 96% ethanol, 0.2 mL 60% KOH, a spatula-tip each of pyrogallol and ascorbic acid, and 100 μl of internal standard (vitamin D_2, 0.25 μg/mL) were added. The tube was sealed, and the sample saponified by heating to 70°C for 20 minutes. Unsaponifiable matter was extracted by adding 1 mL of destilled water and 3 mL of n-hexane; the sample was whirl-mixed and spun at 1000 g for a few seconds. The top layer was collected, and another extraction, this time without the addition of water, was performed. The combined hexane phase was washed by adding 2 mL of destilled water, and the sample was whirl-mixed and spun at 1000 g for a few seconds. The water phase was discarded. To the washed hexane portion was added 1 ml of iso-propanol, and the mixture was evaporated under a flow of N_2, and resuspended redissolved in 0.3 mL of n-hexane.

High-performance liquid chromatography

The HPLC-equipment consisted of a Spectra Physics P1000 isocratic pump, a Shimadzu SPD 6AV UV-detector, a Shimadzu C-3A integrator, a Rheodyne 7125 injector with 200 μl loop, a Rheodyne 7000 switching valve, a 4.6 mm * 25 cm Brownlee silika column, and a 4.6 mm * 25 cm Supelco C_{18} column. Both columns had particle size of 5 μm.

The mobile phase in the preparative clean-up step was tetrahydrofurane:n-hexane (12.5:87.5 v/v), and in the analytical step chloroform:methanol:acetonitrile (6:12:82 v/v). The flow rates were 1 mL/min. The fraction containing vitamin D in the preparative step (identified by its retention-time of 11 min.) was evaporated under a flow of N_2, and resuspended in 0.3 mL methanol. Vitamin D_2 and vitamin D_3 were separated in the analytical step, and identified by their retention times of 14 and 15 minutes, respectively, and detected on-line by the UV-detector at 265 nm.

Calculation

The amount of vitamin D_3 was determined by the formula:
$\mu g/g\ D_3 = (a_1 * CF * i) / (a_2 * w)$
where:

a_1 = peak area of vitamin D_3
a_2 = peak area of vitamin D_2
CF = correction factor
(the quotient of the peak areas of vitamin D_3 and vitamin D_2, measured at identical concentrations of the vitamin forms.)
i = amount of internal standard added
(expressed in grams)
w = weight of sample
(expressed in grams)

Table I: Composition of the chick bioassy feed.

Coarsely ground maize	5.700 kg
Ground whole wheat	2.800 kg
Casein	1.200 kg
$Ca_3(PO4)_2$	0.200 kg
NaCI	0.100 kg
$Mnso_4*4H_2O$	0.002 kg
Vitamin mix.	1%

To 6.93 kg of this mixture was added 70 g oil (soy bean) with or without D3standard.

Composition of the vitamin mix.:

Vitamin A	2.8 g	
Vitamin D_3	Only in standards - see text	
Vitamin E (50%)	10.0 g	
Vitamin K (50%)	1.6 g	
Thiamine (mononitrate)	0.2 g	
Riboflavin (Roviflav)	0.8 g	
Niacin	4.5 g	
Pantothenic acid	1.2 g	
Pyridoxine (hydrochloride)	0.6 g	
Vitamin B_{12} (1%)	0.35 g	
Folic acid	0.1 g	
Biotin (0.2%)	1.0 g	
Choline (hydrogentartrate)	60.0 g	
	Sum	83.15 g
	+ Maize	916.85 g

The chick bioassay method

The chick bioassay (British Standards Institution 1940;) used, was the same as that used at this institute for control purposes for many years. The method is prophylactic, which means that the chicks are given graded levels of vitamin D_3 from the beginning of the experimental period. The dose levels are chosen so that a graded rachitic response is obtained. One day old chicks were first given a vitamin D-free feed for one week (table 1). They were then divided into groups of 15 animals each. Standard vitamin D_3 and the sample fish meals were mixed into the diet at three levels. The standard levels were 125, 208 and 347 ng vitamin D_3 per 100 g diet. The levels of the fish meals in the diets were calculated from the amount of vitamin D_3 assumed to be present in them. The experimental diets were fed for three weeks. The chicks tibia were then x-rayed, the cartilage-thickness between tarsus and metatarsus (t.m.t.-distance) was measured by using a magnifying glass with a built-in mm-scale. The degree of calcification in the intertarsal joint is a measure of the amount of vitamin D_3 supplied in the feed, and the vitamin D_3-content in the samples were calculated as described in the British Standard leaflet.

RESULTS AND DISCUSSION

The vitamin D_3-contents of the fish meals analysed by the proposed HPLC-method and the chick bioassay, are listed in table 2. The meals were found to contain from 5.5 to 178.9 ng/g of vitamin D_3 analysed by the HPLC-method, and from 10.3 to 546 ng/g analysed by the chick bioassay.

On the basis of a few preliminary analyses of fish meals by the HPLC-method it was assumed that the meals under test contained about 50 ng vitamin D per gram. There were available only small amounts of most of the fish meals so that repetitions of the chick assays were not possible, except for one meal (No. 3) which contained appreciably more vitamin D than the others. It was possible to calculate with some confidence the vitamin D content of 10 of the meals although the true fiducial limits were wide in some cases. However, the results obtained are presented for these meals and also results estimated for the other 5 in Table 2.

The differences between values for some of the individual fish meals were considerable: In five of the fifteen meals, the chemically determined values were less than 50% of the values determined biologically. This may, at least in part, reflect the greater experimental error of the bioassay, for which reason the chick bioassay results should more be considered *indicative* of the vitamin D_3-levels in the fish meals rather than absolute. However, the fact that all values except one were higher when determined in the bioassay than when they were

determined chemically may indicate that the latter method does not detect all vitamin D active compounds. Further studies to elucidate this point are necessary.

The HPLC-methods commonly used for vitamin D-determinations involve saponification of the materials. The saponification step, which is included in the proposed method as well as in most other methods cited, is a first purification step in that the bulk of neutral lipids is removed. Saponification is also generally required because vitamin D may be present in ester form. In cod-liver oil, only 43-53% of the total vitamin D-content was free vitamin D (Monard *et al.* 1986). They appear rather tedious in the sample pretreatment step. This is due to the handling of the relatively large sample weights (5-25 g), which requires large solvent volumes for efficient saponification and extraction.The saponification and extraction steps are normally performed in 250 mL's or 500 mL's saponification flasks. When sample numbers are high, these steps are laborious, rate-limiting and costly, due to the handling of large flasks and large volumes of extracting solvents. The rationale for applying the high sample quantities, is both to secure a representative sample of a potentially non-homogenous material, and to secure an appropriate signal output in the final analysis, especially when the vitamin D-contents of the materials are low. This is illustrated in some of the methods proposed for vitamin D-determination in foods, premixes, feeds and fish-oils, in which sample quantities of 5-25 g are processed (e.g. Thompson

Table 2: The vitamin D_3-content of the fish meals, expressed in nanograms per gram, analysed by the propose HPLC-method and the chick bioassay method.

Fish meal no.	Vitamin D3-content HPLC (ng/g)	Vitamin D3-content Chick bioassay (ng/g)
1	9.9	10.3
2	70.1	76.2
3	178.9	546.0
4	51.4	278.0
5	33.2	31.7
6	5.5	12.3
7	44.1	88.7
8	11.6	16.1
9	32.4	71.5
10	37.8	49.1
11	17.1	31.4
12	23.1	39.4
13	22.5	40.2
14	56.3	196.0
15	17.4	17.4

et al. 1982; Beckhof and van den Bedem 1988; Chiang *et al.* 1990). Other methods are described for processing smaller sample quantities (0.1-5 g), but still applying large solvent volumes (e.g. Okano *et al.* 1981; Takeuchi *et al.* 1984b, 1986). Micromethods for determination of multivitamin preparations (Hart *et al.* 1992), and human serum (Chen *et al.* 1990; Aksnes 1992) are described, but these methods do not involve saponfication of the samples.

The choice of a two-column system, in which the sample is purified by HPLC prior to analysis, is inevitable when measuring vitamin D in biological samples. The usaponifiable matter consists of relatively large amounts sterols (e.g. cholesterol) which would severely interfere in the detection of vitamin D in the analytical step. The system applied in this method is two-dimensional, with a silica normal phase column for purification, and a C_{18}-column for analysis. This is the most preferred configuration, probably because interfering substances are more easily eluted from a silica column than from a reverse-phase column (Thompson and Plouffe 1993). Other configurations involve the use of two similar columns for both purification and analysis (Sertl and Molitor 1985), or reverse-phase columns for purfication and silica columns for analysis (Kobayashi *et al.* 1986; Takeuchi *et al.* 1984b). However, in the last two studies, an initial step of purification on a Sep-Pak silica clean up column was performed.

The chromatographic conditions chosen in this method are in part based on earlier experiences at this laboratory. The eluting solvent in the first chromatographic step; 87.5:12.5 n-hexane:tetrahydrofurane, is a modification of a method previously developed in this laboratory (Sandvin 1988). The retention time for both vitamin D forms were approximately 12 minutes, determined by injection of standards. Chromatographic profiles are shown in Figure 1. Some other examples of normal phased eluting solvents for vitamin D appearing in the literature, are 1:2:3:94 iso-butanol:tetrahydrofuran: chloroform: iso-octane (Johnsson *et al.* 1989), 99:1 n-hexane:iso-propanol (Aksnes 1980a) and 98.4:1.6 n-hexane:amyl alcohol (Monard *et al.* 1986).

The mobile phase for separation of the two vitamin forms in the reverse phased step, was initially 90:10 acetonitrile:methanol. This gave retention times for vitamin D_2 and D_3 at 18 and 19 minutes, respectively. By introducing chloroform (Johnsson *et al.* 1989) into 82:12:6 acetonitrile: methanol: chloroform, the retention times were shortened to 14 and 15 minutes of the two forms, respectively, and with no negative effect on the baseline separation. Figure 1 shows the chromatographic profile of the baseline separation in this step. There is also some variation in the choice of eluting solvent in the reverse phase step, with no clear effects on the baseline separation. Some examples are 95:5 methanol:water (Egaas and Lambertsen 1979), 85:10:5 methanol:iso-propanol:water (Aksnes 1980b) and 91:3:6 acetonitrile:methanol:chloroform (Homberg 1993). None of the eluting solvents appearing in the literature, in

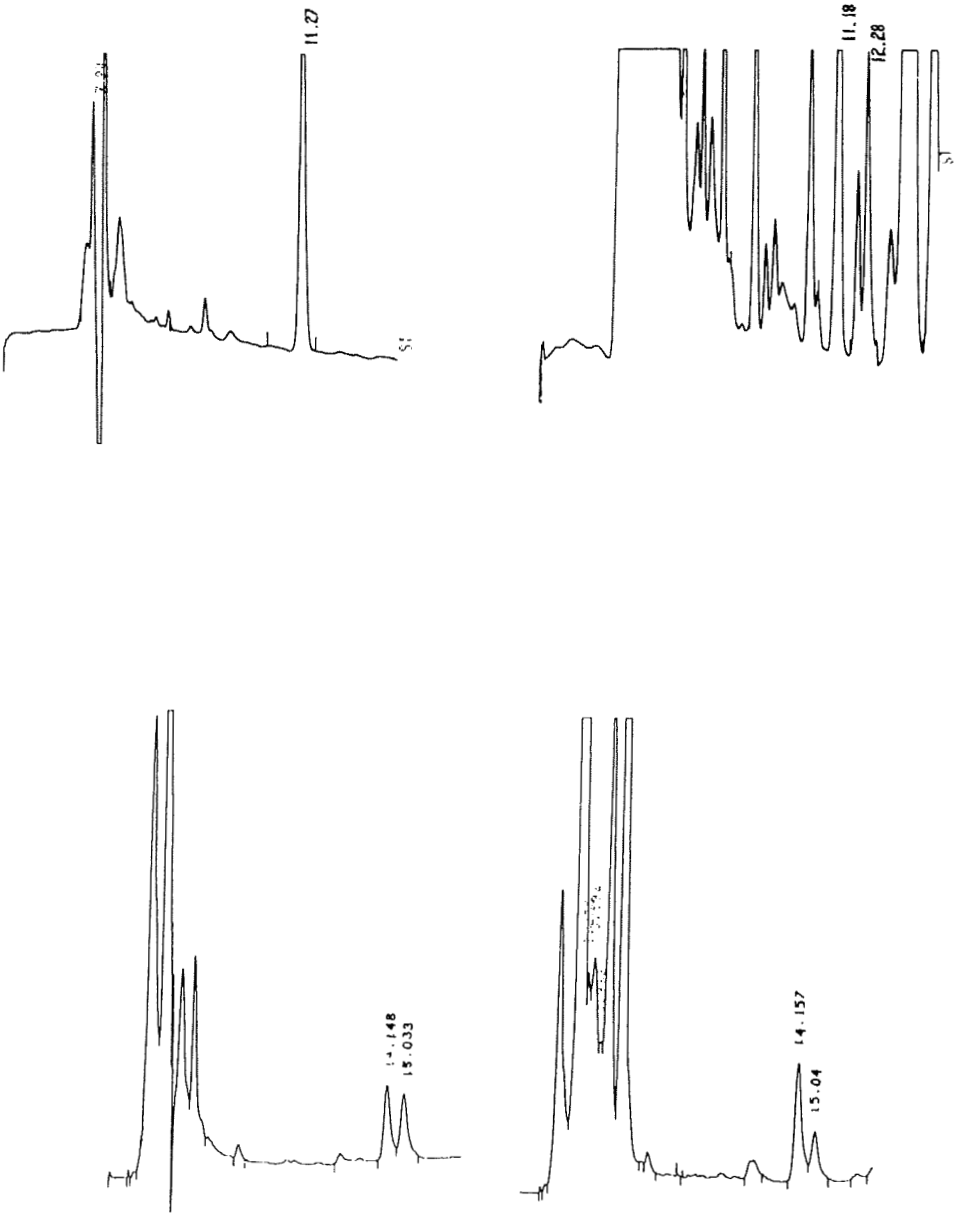

Figure 1: Chromatogram of standards (left) and a sample of the normal phase purification step (A) and the reverse phase analytical step (B D_2 first and then D_3).

the purification step and in the analytical step, seem to have any obvious advantages over the others. The choice of solvents therefore largely can be based on personally preferred laboratory practices.

The separation of the two forms on the reverse phase column is essential to the method. The first successful separation of vitamin D_2 from vitamin D_3 (Osadca and Araujo 1977; Wiggins 1977) opened for the use of one form of the vitamin as an internal standard in the quantitation of the other. This required that the sample did not contain the form applied as the internal standard. It was assumed that the vitamin D forms would react equally to the conditions of saponification and extraction used. The first application of this technique was reported with the analysis of vitamin D_3 in fish products (Egaas and Lambertsen 1979), and was later applied in other methods for determinations in e.g. fish oils, feeds and foods. It was later shown that the thermal isomerization of vitamin D into the pre-vitamin D form that occurs under the high temperature-conditions of saponification applied in the proposed method, could be neglected, as the isomerization rates of both the internal standard and vitamin D-form of interest were found to be practically equal (Johnsson and Hessel 1987).

The characteristic UV-absorption spectrum of vitamin D has made analysis of the vitamin by UV-detection a simple and preferred method. This detection mode is, however, not selective, and it requires the sample to be relatively pure and free of contaminating substances absorbing at this particular wavelength prior to assay. Recently, more selective and sensitive detection modes for vitamin D based on electro-chemical detection or mass spectrometry have been reported (Hasegawa 1992; Hart *et al.* 1992; Vreeken *et al.* 1993; Zamarreno *et al.* 1993).

The limit of detection, as defined by three times the background signal, was 1.3 ng, or 5.2 ng/g of sample. The limit of determination, as defined by 10 times the background signal, was 4.3 ng, or 17.2 ng/g of sample. (It should be noted here from table II that fish meals number 1,6,8 and 11 analysed by the proposed HPLC-method, are presented with a vitamin D-content in the range below the limit of determination.) The values were obtained with the Shimadzu SPD detector at 265 nm. The sensitivity can, however, be increased by using a fixed wavelength detector at 254 nm by a low pressure mercury lamp, which display a sensitivity of about five times higher than the one applied in this method (Kobayashi *et al.* 1986). The literature seem not to prescribe any standards on how to determine or to express these values, and some examples of detection limits, are 0.4-1.3 µg/g (Muniz *et al.* 1982; Wickroski and McLean 1984), 2.5 µg/l (Sandvin 1988), 2.5 ng/g (Kobayashi *et al.* 1986), 25 ng/g (Agarwal 1992) and 250 pg (Johnsson and Hessel 1987). The latter detection limit was defined as five times the background signal.

The intra-assay precision, as expressed by the coefficient of variation (CV),

was 5.6% (n=8). This value reflects not only the method and the matrix variation, but also the laboratory worker (Takeuchi *et al.* 1984b). In the literature values from 1.6% to almost 20% (e.g. Egaas and Lambertsen 1979; Thompson *et al.* 1982; Stancher and Zonta 1983; Takeuchi *et al.* 1984b; Thompson and Plouffe 1993) are reported.

The recovery was determined by comparing the peak area of the internal standard added to the sample, to that of the same internal standard injected directly to the analytical column, and it was 58.9% (±2.9, n=10). This is in accordance with previous studies using the same extracting solvent, in which the recoveries for vitamin D_2 and D_3 were 60.6% and 56.9% (Stancherer and Zonta 1983), and 66.9% and 50.5% (Suzuki *et al.* 1987), respectively. Another study using hexane as extracting solvent (Johnsson *et al.* 1989), gave recoveries in the range of 40-80%.

The rather low recovery obtained with the method may be due to the use of n-hexane as extracting solvent for the fish meals with high fat contents. n-Hexane is non-polar, and may not be the ideal choice for the extraction of vitamin D, which possess some polarity through its hydroxy-group in the 3-position. Attempts were made on using the more polar diethyl-ether as extracting solvent, but these were unsuccessful, probably due to volume restrictions of the extracting procedure. Other studies show recovery-values for vitamin D, when employing diethyl-ether, in the range well above 90% (Egaas and Lambertsen 1979; Bravand and Walter 1992). However, recoveries of the vitamin in the range above 90% in fatty matrices have been obtained also with hexane as extracting solvent (Thompson *et al.* 1982; Agarwal 1992; Thompson and Plouffe 1993). Other extracting solvents for vitamin D appearing in the literature include benzene, and a study using this solvent found the recovery of vitamin D in fishmeal to be 94.3% (Takeuchi *et al.* 1984b).

In conclusion, the presented HPLC-method has demonstrated high sensitivity and precision and is through its low requirement of sample quantity, together with its simpleness and speed of performance in the initial steps, especially suited for determinations of vitamin D in large numbers of small biological samples.

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