FEEDING THREE LEVELS OF N-3 POLYUNSATURATED FATTY ACIDS AT TWO LEVELS OF VITAMIN E TO ATLANTIC SALMON *(SALMO SALAR)*. GROWTH AND CHEMICAL COMPOSITION.

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ABSTRACT

Duplicate groups of Atlantic salmon *(Salmo salar)* fed three dietary levels of n-3 polyunsaturated fatty acids (PUFA), (1.0, 2.5 and 5.0 % of the diets at 17 % feed lipid level), each with two vitamin E contents (30 and 300 mg α-tocopherol/Kg diet), showed no significant differences in growth and mortality during a feeding study from March 1988 to September 1989.

Increased levels of dietary n-3 PUFA resulted in a reduction of the total lipid content in the liver, and significantly affected the fatty acid composition of this organ. The fatty acid composition of the fillet reflected the dietary fatty acids, which points to the possibility of modifying the fillet lipid composition according to consumer's preferences.

The levels of vitamin E in fillet and liver reflected the feed levels, and were further influenced by dietary n-3 PUFA.

INTRODUCTION

Fish oils are often used as the lipid source in feeds in fish farming. These oils contain large proportions of n-3 polyunsaturated fatty acids (PUFA) (Lambertsen, 1978; Sargent *et al.*, 1989; Pike, 1990). The lipid content as well as the lipid composition of Atlantic salmon *(Salmo salar)* fillet are affected by the dietary lipids (Lie *et al.*, 1988). This is presently of nutritional interest, as recent studies have shown that n-3 fatty acids, which are present in farmed Atlantic salmon in considerable amounts, may prevent or reduce the risk of ischaemic heart diseases and other systemic diseases in man (Herold and Kinsella, 1986).

The overall lipid metabolism in salmonids and other fish species was recently reviewed by Sargent *et al.* (1989). The dietary requirement of essential fatty acids (EFA) for fish was discussed by Castell (1979), Cowey and

Sargent (1979), Watanabe (1982) and Bell *et al.* (1986). Rainbow trout *(Oncorhynchus mykiss)* requires approximately 1 % 18:3 n-3 in the diet (w/w) for optimum growth (Castell *et al.*,1972; Watanabe *et al.*,1974). The exact requirement for Atlantic salmon has not been established, but it is suggested that 1 % of the diet as n-3 fatty acids is sufficient for optimal growth. However, further studies are needed to define the dietary requirement for n-3 PUFA in farmed Atlantic salmon at different stages of the life cycle, as well as under different environmental conditions. Further, the role of fatty acids of the n-6 series as EFA remains to be elucidated.

Unsaturated fatty acids are easily oxidized in feeds, and oxidized fatty acids may cause lipoid liver degeneration in fish (Roberts, 1989). To prevent oxidation, synthetic antioxidants are usually added to fish feeds. *In vivo*, vitamin E, is of importance in protecting against lipid oxidation and scavenging toxic radicals (Bell and Cowey, 1985). Vitamin E is the main preventor of PUFA oxidation in biomembranes, and in fish an increased requirement of E has been demonstrated when the level of dietary unsaturated lipids is increased (Watanabe *et al.* 1981; Cowey *et al.* 1984; Roem *et al.*, 1990). Increased PUFA concentration in cell membranes of rainbow trout induced by low water temperature also increased the vitamin E requirement (Cowey *et al.*, 1984).

The aim of this study was to examine the long term influence of three levels of dietary n-3 PUFA using different natural lipid sources at the same feed level, each fed with and without added vitamin E. The present paper is one in a series of communications on the impact of dietary n-3 PUFA and vitamin E on lipid metabolism and physiological functions in Atlantic salmon during different stages of the life cycle. Further reports comprise fillet quality including sensoric evaluation, studies on fish health, osmoregulation, mineral interactions (Maage and Waagbø, 1990), organ glycerophospholipid fatty acid compositions, lipid transport, lipid retention, oocyte lipid accumulation and hatcing performance of eggs.

MATERIALS AND METHODS

Fish and diets

Atlantic salmon *(Salmo salar)*, with an initial weight of 30 g (March 1988) were distributed into 12 tanks (1.5 m x 1.5 m) with 750 fish in each tank. They were fed for 5 months on the experimental diets. Medio September 1988 they were transferred to net pens in the sea (2.75 m x 5.5 m x 6.0 m) and the number of fish per unit was reduced to 450. The feeding regime was continued for further 12 months. At the end of the experiment reported here the fish were again restocked, and 180 fish in each pen were fed until maturation (Fig. 1).
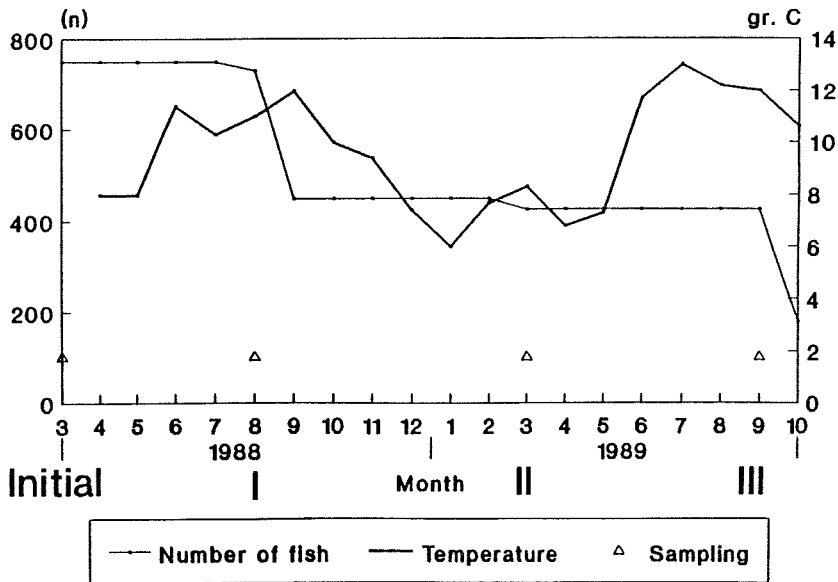
Fig. 1. The number of Atlantic salmon, sampling of fish and the water temperature during the experimental period.

The amount of feed given by means of automatic feeders during the indoor period was adjusted according to standard tables (Austreng *et al.*, 1987). The fish were fed *ad lib* in the sea pens. The water temperature (Fig. 1) and salinity of the filtered sea water supplying the indoor tanks at Matre Aquaculture Station were recorded regularly. The salinity was adjusted to 20 g/L, and the water flow was regulated according to fish density. After transfer to net pens, the sea water temperature was recorded regularly (Fig. 1).

The basal test diet was extruded dry pellets composed of fish meal (Norseamink LT, Nordsildmel, Norway) and soyabean protein concentrate (Danpro A, Aarhus Oliefabrik A/S, Denmark) as protein sources, and extruded wheat and a vitamin and mineral mix (Table 1). Different levels of polyunsaturated fatty acids (PUFA) were obtained by coating the pellets with 16 % of either soyabean oil (refined soyabean oil, A/S Denofa & Lilleborg Fabrikker, Norway), winter capelin oil (Norsalmoil containing Ethoxyquin, Nordsildmel, Norway) or sardine oil (Pescomar, J.C. Martens & Co, Norway, to which Ethoxyquin (0.2 g/l oil) was added. Vitamin E as 300 mg α-tocopheryl acetate (Rovimix 50, Roche) per Kg feed was added (+E) or omitted (-E) in each of the n-3 PUFA groups. The diets with soyabean oil,

Table 1. Feed composition and proximate analyses of the diets used.

A. Composition of the diets	(g/100 g)
Fish meal	33
Danpro A Soy concentrate	29
Extruded wheat	16
Vitamin/mineral mix[1]	6
Lipid source[2]	16

B. Proximate analyses of the diets (Mean ± S. D., n = 5x6)		
Dry matter	93.4	(3.5)
Protein	42.8	(3.2)
Lipid	16.9	(1.3)
Ash	6.4	(0.1)

[1] T. Skretting A/S commercial vitamin and mineral mix exclusive vitamin C, vitamin E and pigments.
[2] Either of soyabean oil, capelin oil or sardine oil, with addition of vitamin C (E.C., Roche), vitamin E (α-tocopheryl acetate, Rovimix 50 E, Roche) and astaxanthin (Carophyll pink 5%, Roche) corresponding to 500 mg Kg^{-1}, 300 mg Kg^{-1} (+ E groups only) and 50 mg Kg^{-1}, respectively.

capelin oil and sardine oil are referred to as Low n-3 ± E, Medium n-3 ± E and High n-3 ± E, respectively. Each diet was given to duplicate groups.

Sampling procedure

Feed samples were taken from each feed batch prepared. Proximate analyses, fatty acid composition and vitamin E content in the feeds were determined regularly.

One hundred fish, including twenty fish taken for analyses, were weighed per unit (tank/net pen) initially (March 1988) and after 5 months (Sample I, August 1988), 12 months (Sample II, March 1989) and 18 months (Sample III, September 1989). The fish were fasted for 24 hours prior to sampling and were anaesthetized in water with a dose of saturated benzocain-ethanol solution, and weighed before blood samples were withdrawn with a syringe from the caudal vein. Blood samples from individual fish were collected in heparinized tubes for haematological analyses and in standard tubes for blood serum analyses after centrifugation (3 000 g, 10 min.). Liver, spleen, heart and

fillet from ten fish per tank/pen were dissected, the organs were weighed and organ somatic indexes calculated (% of body weight). The samples were stored at -80 °C until analyses of proximate composition, vitamin E and fatty acid composition in total lipid. Only the liver and fillet analyses and the hepato-somatic indexes (HSI) from sample III are reported here.

Analytical methods

Two pooled samples (n=5) from each tank/net pen of carcass, liver and fillet were ground and homogenized. Samples of the experimental feeds, liver, fillet and carcass were analysed for dry matter, protein, lipid (ethyl acetate extraction), ash content and fatty acid composition of total lipids according to Lie *et al.* (1988).

Vitamin E (total α-tocopherol) levels in feeds and pooled samples of liver and fillets were assayed according to Lambertsen (1983) and Hvidsten and Lambertsen (1987).

RESULTS AND DISCUSSION

The diets used in this experiment were of a conventional composition used for salmonids but with part of the fish meal replaced by soyabean protein concentrate to reduce the contribution of n-3 PUFA from the fish meal. Table 2 details the fatty acid composition of the feeds. The n-3/n-6 ratios 0.3, 3.1 and 5.6, respectively, were found in the diets containing soyabean oil, capelin oil and sardine oil. The total n-3 PUFA content in gram per 100 g feed were 1.9 (18:3 n-3 accounted for approximately 50 %) in Low (n-3), 3.5 in Medium n-3 and 6.0 in High n-3. The sums of 20:5 n-3 (EPA) and 22:6 n-3 (DHA) were 0.8, 2.5 and 4.9 g per 100 g feed, respectively.

Feed analyses of vitamin E given in Table 8 indicate a basal level in the (-E) diets in the range of 45 to 70 mg α-tocopherol Kg^{-1}.

Final weight, daily growth rate, feed conversion factor, protein efficiency ratio (PER), protein productive value (PPV), mortality and final hepatosomatic index (HSI) are presented in Table 3. The PER and PPV values were calculated from weight gain based on the mean weights (n=200) and feed supplied. None of these parameters showed variations which could be attributed to the feed composition. Increased growth and less effective utilization of the feed in the Low (n-3, E) groups were most probably due to an accidental loss of fish occurring in both replicates. The mortality in this group during the net-pen period in the sea is approximated (Table 3).

During the indoor feeding period two outbreaks of vibriosis *(Vibrio anguilla-*

Table 2. Fatty acid composition of total lipid of the experimental diets (Mean ± SEM, n=8).

	Low n-3	Medium n-3	High n-3
14:0	1.3 ± 0.1	5.7 ± 0.2	6.5 ± 0.1
16:0	12.0 ± 0.2	12.8 ± 0.1	5.7 ± 0.2
18:0	3.6 ± 0.0	1.4 ± 0.0	2.5 ± 0.0
Σ Saturated	17.8 ± 0.3	20.4 ± 0.1	25.2 ± 0.2
16:1 n-9	1.0 ± 0.1	6.1 ± 0.2	6.7 ± 0.1
18:1 n-9	20.2 ± 0.1	11.9 ± 0.2	12.0 ± 0.1
20:1 n-9	1.8 ± 0.1	13.3 ± 0.2	4.1 ± 0.1
22:1 n-11	2.7 ± 0.1	18.2 ± 0.3	4.4 ± 0.1
Σ Monenes	25.8 ± 0.2	50.2 ± 0.6	28.1 ± 0.4
18:2 n-6	44.4 ± 0.6	6.1 ± 0.6	4.9 ± 0.2
20:4 n-6	0.1 ± 0.0	0.2 ± 0.0	1.3 ± 0.0
20:5 n-3	1.6 ± 0.1	7.2 ± 0.1	15.5 ± 0.3
22:6 n-3	2.9 ± 0.1	7.6 ± 0.1	13.2 ± 0.1
Σ n-6	44.6 ± 0.6	6.5 ± 0.6	6.3 ± 0.2
Σ n-3	11.2 ± 0.2	20.3 ± 0.2	35.3 ± 0.5
n-3/n-6	0.25	3.12	5.60

Table 3: Final weight (g), hepatosomatic index (HSI), daily growth rate (% weight day^{-1}), feed conversion factor, % mortality of Atlantic salmon fed experimental diets varying in n-3 PUFA and vitamin E.

	Low (n-3)		Medium (n-3)		High (n-3)	
	–E	+E	–E	+E	–E	+E
Mean final weight [1]	1929	1657	1693	1549	1697	1728
± SEM	29	27	27	27	27	24
HSI	1.11	1.14	1.13	1.16	1.10	1.05
Daily growth rate	0.74	0.72	0.73	0.71	0.72	0.74
Feed conversion factor [2]	1.61	0.98	1.03	1.12	0.95	1.03
Protein efficiency ratio [3]	1.45	2.38	2.27	2.09	2.46	2.27
Protein productive value [4]	0.26	0.43	0.39	0.36	0.42	0.39
Mortality in tanks	3.3	4.4	7.4	13.8	0.9	0.4
Mortality in sea net pens	15.6	15.0	22.3	19.4	13.6	16.3

[1] Mean initial weight ± SEM: 31.0 ± 0.2, n=1200; Mean final weight, n=200

[2] Feed supplied (g)/live weight gain (g)

[3] Weight gain (g)/protein fed (g)

[4] Protein gain (g)/protein fed (g)

rum) were the main cause of the mortality. Overall mortality was somewhat higher than expected, but the interpretation of the data is complicated by the fact that the mortality within each group differed significantly between replicates, making it less reliable to correlate mortality to dietary treatment.

In experiments in which menhaden oil or soyabean oil were substituted for herring oil at the level of 37 % of the total dietary lipid (18.7 %), Hardy *et al.* (1987) did not find any differences in growth of Atlantic salmon. Similarly, Thomassen and Røsjø (1989) also working with Atlantic salmon did not find a difference when soyabean oil and rapeseed oil were used as the lipid sources. The present results confirm these results, and suggest that the requirement for essential fatty acids (EFA) and vitamin E were covered at the lowest levels of our two variables.

The efficacy of soyabean protein in fish feeds has been a topic of debate for several years. Dabrowski *et al.* (1989) reported significant negative effects in growth rate and food conversion when 50 % of soyabean protein was substituted for fish meal protein in feeds to small rainbow trout (1 g). Other studies have shown the possibility to replace as much as 75 % of the fish meal by extracted soyabean meal in older rainbow trout (Tacon *et al.*, 1983; Alexis *et al.*, 1985). Compared to other feeding experiments with Atlantic salmon in net pens (Lie *et al.*, 1988), the present results do not indicate negative effects of 45 % soyabean protein concentrate included in the diets.

As the proximate composition and the fatty acid composition in total lipid of pooled carcass, liver and fillet of fish from the +E and -E dietary groups did not differ, the results are presented in Tables 4-7 according to dietary n-3 PUFA level only. There were no significant effects of the dietary lipids on carcass proximate composition (data not shown).

Table 4. Proximate composition (g 100g^{-1} wet weight)[1])of pooled liver samples (5 livers) from Atlantic salmon fed three levels of n-3 fatty acids in the diets.

Feeding group		Low n-3			Medium n-3			High n-3		
Sample[2])	Initial	I	II	III	I	II	III	I	II	III
Dry matter	26.5	24.8	24.3	26.3	24.7	24.0	26.1	24.1	23.8	25.3
Protein	16.7	17.0	13.4	16.1	16.3	13.2	16.2	16.3	13.3	16.1
Lipid	1.4	1.3	2.2	5.1	1.0	1.6	5.3	0.8	1.4	3.3
Ash	n.d.[3])	1.4	1.2	1.2	1.4	1.2	1.2	1.3	1.2	1.3

[1]) Mean values; n=2 samples initially; n=8 for sample I and II; n=4 for sample III.
[2]) Initial sample: March; Sample I: August; Sample II: March; Sample III: September
[3]) n.d.: not determined.

The liver lipid content generally decreased with increasing dietary n-3 PUFA (Table 4). The protein content in the liver did not differ significantly between diets, but was reduced in all groups during the winter (sample II). The lipid content in the fillets increased approximately tenfold in the course of the experiment, and reached a level of 11 g lipid per 100 g of fillet (Table 5). This is in accordance with findings in adult salmon as reported by Lie *et al.* (1988). A cocomitant increase was seen in the dry matter content, while the protein content remained stable. However, the proximate composition of the fillet was not affected by any of the dietary factors. The proximate analyses of liver and fillet show that the composition is affected mainly by fish size. This is in accordance with previous studies at this institute with adult Atlantic salmon (Lie *et al.*, 1988). Of the dietary variables studied, only liver lipid content differed, showing decreased values with increasing n-3 fatty acids in the diets. The liver plays an important role in the redistribution of lipids between tissues (Sargent *et al.*, 1989). Abnormal accumulation of lipid in the liver of salmonids has been reported as a result of imbalanced diets, toxic conditions or diseases (Roberts, 1989), but no such effects were seen in the present study.

With an increasing total lipid content in the liver (Table 4) there was a marked increase in the liver monoene fatty acids, primarily 18:1, in all groups at the end of the experiment (Table 6). The increased liver lipid level in sample III may explain the change in fatty acid composition at this stage. All feed lipids contained high amounts of 22:1, especially the Medium n-3 diet (capelin oil). The low level of 22:1 found in the liver points to a specific catabolism of this fatty acid as reported by several authors (Henderson *et al.*, 1982; Lie *et al.*, 1986; Lie and Lambertsen, 1990a, b. The n-3 and n-6 levels in the liver partly reflected the dietary differences, but an increased total lipid content in sample III reduced the relative proportions of PUFA in all groups.

The decline in total PUFA from sample II to sample III corresponds to the increased lipid content, and suggests combined effects due to increased water temperature and increased levels of neutral lipids (TAG).

The fatty acid compositions of the fillet lipids reflected those of the dietary lipids (Table 7). Based on the research on the benefical health effects for humans of the n-3 fatty acids, a higher dietary intake of these fatty acids is recommended in many western countries. Farmed Atlantic salmon given capelin oil in the feed is an excellent n-3 source, giving approximately 2.5 g of n-3 PUFA per 100 g of fillet. The present results show that it is possible to increase the n-3 content in the fillet of Atlantic salmon to about 4 g n-3 per 100 g of fillet by means of the feed lipid composition. Increased fillet total lipid content, seems also to affect the fillet fatty acid profiles.

Table 5. Proximate composition (g 100g $^{-1}$ wet weight)[1]) of pooled fillet samples (5 fish) of Atlantic salmon fed three levels of n-3 fatty acids in the diets.

Feeding group		Low n-3			Medium n-3			High n-3		
Sample[2])	Initial	I	II	III	I	II	III	I	II	III
Dry matter	24.0	23.5	26.0	32.4	23.4	25.7	33.1	23.2	26.0	32.6
Protein	19.5	17.0	18.8	18.4	16.3	18.8	18.4	16.3	19.0	18.5
Lipid	1.1	0.7	3.4	10.6	0.8	3.3	11.2	0.7	3.1	10.7
Ash	1.6	1.4	1.7	1.2	1.4	1.6	1.2	1.3	1.6	1.1

[1]) Mean values; n = 4 samples initially and for sample III; n = 8 for sample I and II.
[2]) Initial sample; March; Sample I: August; Sample II; March; Sample III; September

Table 6. Fatty acid composition of total lipid of pooled liver samples (5 livers) from Atlantic salmon fed three levels of n-3 fatty acids in the diets.

		Low n-3			Medium n-3			High n-3		
	Initial	I	II	III	I	II	III	I	II	III
14:0	2.0	0.7	0.7	1.3	2.2	2.6	2.3	2.1	2.6	2.1
16:0	18.1	13.6	14.2	11.3	16.6	16.9	11.6	18.7	17.3	13.2
18:0	3.6	5.8	5.3	6.1	3.2	3.0	4.2	4.4	4.5	6.0
Σ Saturated	23.6	20.2	20.8	19.3	22.0	23.5	18.8	25.3	25.7	22.0
16:1[1]	2.9	1.0	0.7	2.6	3.3	3.0	4.5	2.3	2.7	3.0
18:1[1]	11.7	16.4	13.7	24.5	14.2	12.3	22.9	9.0	9.3	17.2
20:1[1]	4.6	1.4	1.3	4.1	5.8	5.9	11.0	1.7	2.0	4.3
22:1[1]	2.0	0.5	0.2	1.3	2.6	2.2	5.0	0.5	0.4	0.9
Σ Monenes	21.2	19.4	15.9	32.7	26.2	23.4	43.8	13.8	14.3	25.6
18:2 n-6	2.4	20.4	23.5	18.1	2.8	2.6	2.1	2.2	2.4	1.8
20:4 n-6	2.1	4.1	3.4	1.7	1.8	1.9	1.1	3.9	4.3	3.6
20:5 n-3	6.5	3.1	3.8	4.8	8.0	9.5	9.2	9.8	10.9	11.7
22:6 n-3	37.4	19.5	21.2	13.8	31.8	32.4	18.2	37.3	34.3	26.5
Σ n-6	4.5	24.5	33.0	25.3	4.6	5.3	3.9	6.2	7.2	6.1
Σ n-3	46.7	26.0	29.1	22.1	43.5	45.9	32.0	51.5	50.9	44.2

[1]) Sum of isomers.

Table 7.   Fatty acid composition of total lipid of pooled samples of fillet (5 fish) from Atlantic salmon fed three levels of n-3 fatty acids in the diets.

	Initial	Low n-3			Medium n-3			High n-3		
		I	II	III	I	II	III	I	II	III
14:0	3.7	1.6	1.1	1.7	5.8	4.4	5.0	6.5	4.3	5.6
16:0	15.0	17.0	11.1	12.8	19.5	12.7	13.5	25.0	14.2	15.9
18:0	2.8	5.3	3.8	3.9	3.1	2.1	2.2	4.7	2.9	3.2
Σ Saturated	21.5	24.2	16.6	19.4	28.4	19.9	21.2	36.2	22.4	25.5
16:1[1]	4.6	1.1	1.0	2.0	5.1	6.1	6.5	4.2	5.5	6.6
18:1[1]	14.2	15.5	18.9	20.8	13.6	16.0	15.0	10.9	13.5	15.0
20:1[1]	10.5	2.4	2.3	3.1	9.7	11.9	13.5	3.7	4.6	4.8
22:1[1]	10.3	2.4	2.4	3.2	10.0	12.2	15.0	3.1	3.4	4.6
Σ Monenes	39.6	21.6	24.8	29.2	38.8	46.8	50.4	22.1	27.5	31.6
18:2 n-6	4.4	27.0	36.7	34.3	4.0	4.9	4.4	3.6	4.7	4.2
20:4 n-6	0.4	0.9	0.8	0.3	0.6	0.3	0.2	1.1	1.2	1.1
20:5 n-3	4.5	2.0	1.8	1.6	4.9	4.8	5.3	7.8	9.9	10.2
22:6 n-3	20.2	12.4	8.7	4.6	14.7	15.3	8.6	18.0	21.5	14.3
Σ n-6	4.8	28.0	41.1	38.2	4.6	5.8	5.2	4.7	6.3	5.8
Σ n-3	30.7	19.8	16.3	11.9	24.2	25.3	20.1	32.2	39.9	32.6

[1]) Sum of isomers.

Table 8.   Mean α-tocopherol (mg/kg) in the feeds ($\pm$SEM, n=8) and pooled samples (2 x 5) of liver and fillet in Atlantic salmon fed three levels of n-3 fatty acids and two levels of vitamin E.

	Sample	Low n-3		Medium n-3		High n-3	
		-E	+E	-E	+E	-E	+E
Diet (SEM)	I-III	45(8)	276(11)	56(7)	241(31)	69(12)	300(14)
Liver	I	164	1860	78	777	19	765
Fillet	I	8	15	4	15	4	13
Liver	III	117	1405	61	1380	329	1556
Fillet	III	21	44	8	35	15	43

Table 8 presents the vitamin E content (total α-tocopherol) in the diets, livers and fillets. The livers and fillets at sampling I were clearly influenced by the content of dietary vitamin E and also the n-3 PUFA, the latter suggesting increased requirement for vitamin E with increasing levels of n-3 PUFA in the diet. The content in liver and fillet in sample III showed the same tendency, obscured by elevated levels in all High n-3 groups due to an accidental supplementation of α-tocopherol in the sardine oil used. This feed was given for two months prior to sampling III and led to an increase in the vitamin E content of all tissues examined. The feed levels in the High n-3 -E and +E groups were in this period 125 mg and 375 mg Kg $^{-1}$ feed, respectively, and corresponded well with the vitamin E increase in the livers from the High n-3 -E and the +E groups. All the supplemented groups (+E) showed liver vitamin E concentrations at least 10 times higher than the levels found in the corresponding -E groups in samples I and III, except for the High n-3 groups at sample III. No significant effects of dietary vitamin E on tissue lipid levels or compositions were observed in this experiment. The results demonstrate that the vitamin E content of the feed is reflected in the liver and the liver content may be used as an indicator of the dietary intake.

The requirement of vitamin E may vary due to interactions with nutrients in the feed and to environmental conditions and age of the fish (Cowey *et al.* 1984; Bell *et al.*, 1985; Gatlin *et al.*, 1986; Lie *et al.*, 1986). Based upon the inverse relationship observed between the contents of vitamin E and PUFA in fish tissues, several reports stress the necessity to increase the dietary vitamin E supplementation with increased contents of PUFA in the feeds (Watanabe *et al.*, 1981; Cowey *et al.*, 1984; Roem *et al.*, 1990).

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