

The effect of vitamin E and oxidized fish oil on the nutrition of rainbow trout (*Salmo gairdneri*) grown at natural, varying water temperatures

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1. Groups of rainbow trout (*Salmo gairdneri*) of approximate mean initial weight 8 g were grown in outdoor tanks over a 14-week period at water temperatures between 12° (start) and 6° (end). Four diets were used. Two contained non-oxidized fish oil (120 g/kg) with or without supplementary DL- α tocopheryl acetate and two contained moderately oxidized fish oil again with or without DL- α -tocopheryl acetate. The measured selenium content of the diets was 0.10 mg/kg.

2. No significant differences occurred as a consequence of the use of moderately oxidized oil compared with the corresponding treatments using non-oxidized oil. Significant differences did occur between dietary treatments that contained supplementary DL- α -tocopheryl acetate and those that did not. These differences applied to weight gain, haematocrit, erythrocyte fragility, mortalities, liver and muscle tocopherol concentrations and lipid peroxidation of liver mitochondria *in vitro*. Liver glutathione peroxidase (EC 1.11.1.9) activity was unaffected by the dietary treatments used and the proportions of fatty acids in polar lipids of liver and muscle were little changed by the diets used. Severe muscle damage occurred in trout given diets lacking supplementary DL- α -tocopheryl acetate.

3. Previous experiments carried out on rainbow trout at a constant water temperature of 15° (Hung *et al.* 1981; Cowey *et al.* 1981, 1983), using diets lacking supplementary vitamin E, did not lead to differences in weight gain, pathological changes or mortalities.

4. Vitamin E requirement may increase as water temperature decreases; minimum dietary requirements for vitamin E measured at a constant water temperature of 15° may not be valid under practical conditions where water temperatures vary over the year.

Few pathologies have been observed in rainbow trout (*Salmo gairdneri*) given diets containing fresh (non-oxidized) oil but deficient in vitamin E. Using partially defined diets containing linolenic and palmitic acids as the lipid component, Cowey *et al.* (1981) did not detect pathologies in vitamin E-deficient fish other than an increase in erythrocyte fragility and a decrease in haematocrit. Subsequently (Cowey *et al.* 1983), similar results were obtained when the fatty acid component of the diet was changed to polyunsaturated fatty acids (100 g/kg) derived from white fish offal oil. Largely in agreement with this, Watanabe *et al.* (1981) failed to detect deficiency symptoms in trout given diets of low lipid content and, even when diets contained pollock liver oil or methyl esters of pollock liver oil (150 g/kg), deficiency symptoms (convulsions and high mortalities) were observed in only one of the two experiments. By contrast a number of pathological changes occur in carp (*Cyprinus carpio*) deprived of vitamin E for 90 d or more, the most characteristic of which is apparent muscular dystrophy (Watanabe *et al.* 1970; Watanabe & Takashima, 1977).

Hung *et al.* (1981) demonstrated that practical diets containing 75 g fresh herring oil/kg and with a basal vitamin E content of 24 mg/kg afforded rapid growth without the occurrence of deficiency symptoms such as an increase in erythrocyte fragility. They inferred that where these conditions were met no further supplementation of the diet with vitamin E or other anti-oxidant was necessary to prevent vitamin E deficiency in practical situations. Diets containing oxidized oil did, however, require supplementation with DL- α -tocopheryl acetate to prevent the occurrence of these deficiency symptoms.

Table 1. *Composition (g/kg dry diet) of the experimental diets*

Component	Diet			
	1	2	3	4
Casein	550	550	550	550
White dextrin	100	100	100	100
Maize starch	140	140	140	140
Vitamin premix*	20	20	20	20
Mineral premix†	40	40	40	40
Carboxymethyl cellulose	30	30	30	30
Fish oil, fresh	120	120	0	0
Fish oil, moderately oxidized	0	0	120	120
DL- α -Tocopheryl acetate	0.4	0	0.4	0
Measured tocopherol content (mg/kg)	395.2	18.4	388.4	7.4
Peroxide value of lipid at start (mmol/kg)	2.0	2.0	47.0	47.0
Peroxide value of lipid at end (mmol/kg)	7.8	117.0	210.1	333.3

* Supplied (mg/kg diet): thiamin hydrochloride, 50; riboflavin, 50; calcium pantothenate, 100; nicotinic acid, 200; pyridoxine hydrochloride, 40; biotin, 6; folic acid, 15; cyanocobalamin, 0.1; inositol, 2000; ascorbic acid, 1000; choline chloride, 4000; menadione, 40; *p*-amino benzoic acid, 50; vitamin A acetate, 2000 i.u.; vitamin D₃, 1000 i.u.

† Supplied (g/kg diet): CaHPO₄ · 2H₂O, 29; MgSO₄ · 7H₂O, 5.1; KCl, 2.0; NaCl, 2.4; FeSO₄ · 7H₂O, 1.0; ZnSO₄ · 7H₂O, 0.22; CuSO₄ · 5H₂O, 0.03; MnSO₄ · 4H₂O, 0.105; CoSO₄ · 7H₂O, 0.019; CaIO₃ · 6H₂O, 0.012; CrCl₂, 0.0051.

The experiments of Watanabe *et al.* (1981) were carried out at temperatures of 16–22°, those of Hung *et al.* (1981) and Cowey *et al.* (1981, 1983) at a constant 15°. As trout inhabit waters at considerably lower temperatures than 15°, both in nature and in commercial practice, the experiment described in this paper was carried out in outdoor tanks at water temperatures (12–6°) occurring in North Britain in late summer and autumn. The object was to determine the necessity, or otherwise, of supplementation with vitamin E of diets containing either fresh or oxidized oil under typical rearing conditions. The results demonstrate that under these environmental conditions pathologies, including severe muscle damage, occur in trout given diets containing fresh as well as moderately oxidized oil unless supplemental vitamin E is supplied.

MATERIALS AND METHODS

Diets and fish

Four experimental diets were used and their compositions are shown in Table 1. Two kg of each diet was prepared in dry (powder) form; 750 ml water were added to and mixed with this powder and pellets were formed from the resulting paste, they had a moisture content in the range 30–32% for the four diets. All diets were stored at –20° until fed. The oil used was a non-stabilized (i.e. containing no added anti-oxidants) fish body oil (Stearine; Marfleet Refinery, Hull) with an iodine value of 98.8 and a peroxide value of 2.0 mmol/kg. The oil was moderately oxidized by heating at 100° for 7 h with constant aeration (compressed air) and continuing the aeration for a further 14 h without heat. Although this procedure led to an increase in peroxide value (Table 2) the extent of the oxidation was limited as shown by the presence of appreciable amounts of tocopherol; also the iodine value was unchanged (Table 2). In line with this, the fatty acid compositions of the fresh and oxidized oils were sensibly the same (Table 3). During the course of the 14-week experiment the peroxide value of lipid in diets 2, 3 and 4 increased appreciably (Table 1).

Table 2. Some characteristics of the fish oil used in the experimental diets

	Fresh	Moderately oxidized
Tocopherol (mg/kg)	107	44
Peroxide value (mmol/kg)	2	47
Iodine value	99	97

Table 3. Proportions of fatty acids (g/kg) in fish oil used in experimental diets

Fatty acid	Fresh	Moderately oxidized
14:0	55.3	57.6
16:0	157.0	154.0
16:1 (<i>n</i> -9)	87.9	90.5
16:1 (<i>n</i> -7)	3.1	3.1
18:0	33.4	26.2
18:1 (<i>n</i> -9)	255.5	253.6
18:1 (<i>n</i> -7)	46.5	51.4
18:2 (<i>n</i> -6)	23.2	18.8
18:3 (<i>n</i> -3)	9.3	9.0
20:0	1.3	1.3
20:1 (<i>n</i> -9)	72.4	75.9
20:2	2.7	2.7
20:3	3.9	4.0
20:4 (<i>n</i> -6)	1.7	1.8
20:5 (<i>n</i> -3)	42.8	43.1
22:1 (<i>n</i> -11)	28.4	31.2
22:1 (<i>n</i> -9)	5.3	5.8
22:6 (<i>n</i> -3)	63.0	65.1

The measured selenium contents of these diets varied between 0.092 and 0.108 mg/kg with a mean value of 0.10 mg/kg.

Rainbow trout of mean weight 7–9 g were obtained from Howietoun Fisheries, Bannockburn, Scotland, and weaned from a commercial diet to a purified diet (diet 1, Table 1), a procedure that required approximately 1 week. The fish were then randomly distributed at the rate of forty fish/tank among eight circular glass-fibre rearing tanks and the four diets were fed to duplicate groups (tanks) of fish. Each tank was of 40 l capacity and was continuously supplied with artificially aerated (Venturi aspirator) tap water at a rate of 2 l/min; the tanks were situated out of doors at the University of Stirling. Over the course of the experiment, which lasted 14 weeks (August–November), water temperature decreased gradually from 12° to 6° and fish were subject to a natural photoperiod.

Fish were given a fixed ration, usually at two feeds daily; no food was given on Sundays. On each occasion, food was given to the fish only for as long as they continued actively to seek and ingest it. Feeding rates were (g/kg biomass per d) weeks 0–2, 75; 3–6, 50; 7–8, 40; 9–14, 30. Fish were weighed individually at weekly intervals as described previously (Cowey *et al.* 1981).

Analytical methods

Tocopherol was extracted from diets either as described previously (Cowey *et al.* 1981) or as described by McMurray *et al.* (1980); both methods involved saponification prior to solvent extraction and gave similar values for all diets. Tocopherol in fish tissues was

extracted as described previously (Covey *et al.* 1981) and in both diet and tissue extracts tocopherol was resolved and measured by high performance liquid chromatography (Hung *et al.* 1980). Peroxide values and iodine values were measured in the oil by prescribed methods (Association of Official Analytical Chemists, 1975). The selenium content of the diets was measured as described by Hasunuma *et al.* (1982).

Lipids were extracted from liver and muscle of fish as described by Folch *et al.* (1957) and lipid classes separated by thin-layer chromatography on silica gel G plates (0.25 mm thickness) using hexane:diethyl ether:formic acid (140:60:1, by vol.) as the developing solvent. Methyl esters were prepared by sulphuric acid catalyzed methylation (Christie, 1973) of polar lipids, and the fatty acid methyl esters were separated and quantified by gas-liquid chromatography (Fractovap 4160, Erba Science Ltd, Swindon). A 50 m capillary column coated with free fatty acid phase (nitroerephthalic acid ester of carbowax 20 M) was used, hydrogen being the carrier gas. Temperature programming was from 150°, at 5°/min, to 200° (20 min) and then from 200°, at 2°/min, to 210° (25 min). Individual fatty acid methyl esters were identified by comparison with known standards and by reference to the results of Ackman & Eaton (1978).

Erythrocyte fragility and ascorbic acid-stimulated lipid peroxidation of liver mitochondria were carried out as described previously (Covey *et al.* 1981). Glutathione peroxidase (EC 1.11.1.9) activity was measured in whole blood (after lysing by addition of an equal volume of water and centrifuging at 5000 g for 15 min at 4°) and in liver cytosol as previously described (Covey *et al.* 1981).

At the end of the experiment, five fish were removed at random from each tank. They were transported live to Aberdeen where measurements of lipid peroxidation in liver mitochondria and glutathione peroxidase activity were made on six individuals from each treatment (three each from duplicate tanks); fatty acid analysis of polar lipids from liver and muscle were made on three individuals (two fish from one of the duplicate tanks, one fish from the other). Erythrocyte fragility measurements were carried out at Stirling on three individuals randomly removed from each tank (six individuals per treatment). Tissues from another six individuals, chosen in similar random manner from each treatment, were immediately deep frozen before being transported in solid carbon dioxide to Aberdeen for tocopherol measurements. Finally liver, gill, spleen, muscle and brain were dissected from five fish from each treatment (three fish from one of the duplicate tanks and two fish from the other) and tissue slices were fixed in 4% (w/v) buffered formalin for histological examination as described by Tacon & Cooke (1980).

Statistical comparisons were made using the analysis of variance. Mean differences were obtained using Duncan's multiple range test (Duncan, 1955). Standard errors were calculated to identify the range of the means.

RESULTS

Mean final weights of trout, given diets 1 and 3 supplemented with vitamin E, were significantly greater ($P < 0.05$) than those of trout given diets 2 and 4 (Table 4), the highest specific growth rate occurring in trout given diet 3 and the lowest in trout given diet 4. These values are reflected in the food conversion ratios and indicate that the main factor affecting growth rate in this experiment was the presence or absence of supplementary vitamin E in the diet. In addition, mortalities occurred only in those groups of fish given diets lacking supplementary vitamin E. The moderately oxidized oil was as effective in sustaining growth rate as the fresh oil. This conclusion is borne out by the haematocrit values and the results of the erythrocyte fragility measurements, also shown in Table 4.

Concentrations of vitamin E in some of the trout tissues at the end of the experiment are shown in Table 5. The very marked differences evident in liver and muscle levels reflect

Table 4. Growth results, haematocrit and erythrocyte fragility of rainbow trout given the experimental diets

	Diet				SEM*
	1	2	3	4	
Mean initial weight (g)	9.36 ^a	7.30 ^a	7.59 ^a	7.82 ^a	1.02
Mean final weight (g)	51.14 ^b	37.55 ^a	48.29 ^b	35.33 ^a	3.65
Specific growth rate† (%/d)	1.73	1.67	1.89	1.54	—
Mean food intake (g/fish per d)	0.43	0.37	0.43	0.39	—
Food conversion ratio (feed: gain)	1.05	1.20	1.05	1.38	—
Mortalities	0	3	0	6	—
Haematocrit (%)	45.5 ^a	13.2 ^a	39.9 ^c	20.3 ^b	1.78
Erythrocyte fragility (%)	50.8 ^a	94.9 ^b	57.7 ^a	73.9 ^{ab}	7.83

^{a, b, c} Values in the same line with different superscripts were significantly different ($P < 0.05$).

* SEM, Standard error of mean from error mean square.

† The percentage increase in body-weight (W) per unit time (T), determined as $[(\ln W_2 - \ln W_1) \times 100] / (T_2 - T_1)$.

Table 5. Concentrations of tocopherol in liver, muscle and whole blood from rainbow trout given the experimental diets

(Mean values with standard errors of mean from error mean square)

Diet	Liver (mg/kg)		Muscle (mg/kg)		Whole blood (mg/l)	
	Mean	SEM	Mean	SEM	Mean	SEM
1	166.2 ^a	16.20	8.06 ^a	0.48	21.55 ^a	4.41
2	6.4 ^b	0.68	2.48 ^b	0.15	16.71 ^a	3.76
3	109.8 ^a	6.11	6.08 ^a	0.97	12.15 ^a	1.45
4	6.7 ^a	1.33	2.35 ^a	0.27	10.79 ^a	5.10

^{a, b} Values in the same column with different superscripts were significantly different ($P < 0.01$).

the dietary intake; no significant differences occurred in blood levels of vitamin E irrespective of intake. This contrasts with earlier results (Cowey *et al.* 1981) when there was a marked reduction in erythrocyte vitamin E levels in trout given diets lacking supplementary vitamin E.

Liver glutathione peroxidase activity was not significantly different ($P > 0.1$) in any of the experimental treatments (Table 6). Activity in the blood of trout given diet 4 containing moderately oxidized oil without supplementary vitamin E was significantly greater than that of trout given diet 3 containing added vitamin E ($P < 0.05$).

Little ascorbic acid-Fe³⁺ stimulated malondialdehyde was formed when mitochondria from livers of trout given diets 1 and 3 were incubated *in vitro*. Much more malondialdehyde was formed when hepatic mitochondria from treatments 2 and 4 were incubated. This result is probably a reflection of the vitamin E contents of livers for the different treatments (Table 5, Kornbrust & Mavis, 1980).

The fatty acid compositions of polar lipids from liver and muscle of trout given the four experimental treatments are shown in Tables 7 and 8. The most striking feature of the results from liver is the reduction in docosahexaenoic acid (22:6, $n-3$) in trout given diet 4 which is almost wholly compensated by an increase in oleic acid (18:1, $n-9$). No parallel changes

Table 6. *Glutathione peroxidase activity in liver and blood and ascorbic acid-Fe³⁺ stimulated malondialdehyde formation in liver mitochondria from rainbow trout given the experimental diets*

(Mean values with standard errors of mean from error mean square)

	Diet							
	1		2		3		4	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
GSH px in liver ($\mu\text{mol}/\text{min}$ per g protein)	10.59	1.12	11.79	0.33	8.28	0.68	10.27	0.53
GSH px in blood ($\mu\text{mol}/\text{min}$ per g protein)	9.71 ^{ab}	1.33	L		7.89 ^a	0.19	13.67 ^b	1.04
Malondialdehyde formed ($\mu\text{mol}/\text{mg}$ protein in 60 min at 15°)	0.24 ^a	0.008	14.96 ^b	3.31	0.25 ^a	0.88	6.55 ^{ab}	4.23

GSH px, glutathione peroxidase (EC 1.11.1.9)

L, no sample available.

^{a, b} Values in the same line with different superscripts were significantly different ($P < 0.01$).

Table 7. *Proportions of principal fatty acids (g/kg) in polar lipids from livers of rainbow trout given the experimental diets*

(Mean values with standard errors of mean from error mean square)

Diet treatment...	1		2		3		4	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Fatty acid								
14:0	11.8	0.7	8.8	0.1	11.7	1.0	11.9	0.7
16:0	152.9	2.5	138.2	1.3	141.1	8.1	140.9	3.6
16:1 ($n=9$)	12.3	0.9	11.0	0.2	13.7	0.7	13.7	0.6
16:1 ($n=7$)	34.0	6.1	30.7	0.5	30.3	1.6	39.1	0.8
18:0	71.8	3.0	72.3	3.5	69.4	2.7	81.0	3.4
18:1 ($n=9$)	210.0	20.5	203.4	2.2	181.0	10.5	276.3	31.4
18:1 ($n=7$)	33.7	2.2	36.7	4.1	42.9	1.5	47.3	5.3
20:1 ($n=9$)	24.7	2.4	25.6	9.2	26.0	3.4	20.6	0.2
20:2	38.0	4.1	43.1	3.1	63.3	3.0	73.8	8.9
20:3	17.5	1.7	17.3	3.1	27.1	3.5	22.0	1.4
20:5 ($n=3$)	14.7	1.8	15.3	1.3	14.9	0.9	9.4	1.2
22:6 ($n=3$)	306.3	27.0	302.0	22.0	292.6	16.6	172.1	19.8

were found in polar lipids from muscle, relative amounts of fatty acids in this fraction from the four treatments being very similar. This is a little surprising as pathologies were very obvious in muscle of treatments 2 and 4 but much less so in liver.

Histological examination of tissues from the four treatments revealed that the most severe changes occurred in the skeletal muscle of those fish receiving diets 2 and 4 lacking supplementary vitamin E. The changes (Plates 1 and 2) consisted in an alteration of fibre size with the fibres being more rounded, partly swollen and partly shrunken. The degeneration of fibres was clear in both treatments 2 and 4 (Plates 1*b* and 2*b*) but was much more marked in treatment 4 where there were zonal necrotic areas showing typical hyaline

Table 8. Proportions of principal fatty acids (g/kg) in polar lipids from muscle of rainbow trout given the experimental diets

(Mean values with standard errors of mean from error mean square)

Diet treatment...	1		2		3		4	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
14:0	21.5	3.4	20.1	1.9	24.0	1.3	13.3	4.2
16:0	161.7	10.3	169.4	4.0	146.9	4.5	146.9	9.8
16:1 (<i>n</i> -9)	10.5	0.5	9.8	1.2	12.6	2.1	11.5	0.4
16:1 (<i>n</i> -7)	71.6	4.9	50.1	8.7	67.6	1.8	53.3	2.9
18:0	42.1	1.8	43.4	1.0	38.8	0.8	43.6	2.4
18:1 (<i>n</i> -9)	229.2	0.9	206.4	13.8	235.0	10.7	233.3	2.6
18:1 (<i>n</i> -7)	34.8	0.8	33.0	0.6	37.7	1.1	36.0	0.6
20:1 (<i>n</i> -9)	21.1	1.5	16.8	0.7	23.6	1.1	27.8	7.4
20:2	13.0	2.4	14.0	1.1	15.9	0.4	18.4	0.8
20:3	9.9	0.7	10.7	0.6	9.9	0.1	11.5	0.7
20:5 (<i>n</i> -3)	26.6	1.3	28.9	2.9	25.1	0.5	21.9	1.6
22:6 (<i>n</i> -3)	280.6	20.5	303.7	17.0	261.3	6.2	263.3	17.5

degeneration with distinct eosinophilia. Both treatments also showed a cellular infiltration in the sub-pericardial area, some pycnotic nuclei were present and there was some vacuolization of the cytoplasm.

Some changes also occurred in the anterior region of the kidney from treatments 2 and 4 and this amounted to a generalized loss of haemopoietic tissue.

Differences between treatments were consistent in the duplicated tanks.

DISCUSSION

The substitution of a moderately oxidized oil for fresh oil in diets containing supplementary vitamin E (400 mg DL- α -tocopheryl acetate/kg) did not affect the performance of the trout (growth rate, haematocrit, erythrocyte fragility, absence of pathology, etc.) in this experiment. As stated earlier, the extent of oxidation of the oil was limited (see pages 444, 445) and in some respects resembled the moderately oxidized oil used by Hung *et al.* (1980) in a practical diet. The oil they used was aerated at room temperature for 265 h during which time the peroxide value rose from 6.0 to 50.5 mmol/kg and the vitamin E content fell from 125 to 50 mg/kg. Its inclusion, at a level of 75 g/kg, in practical diets supplemented with 33–99 mg DL- α -tocopheryl acetate/kg, did not lead to any loss of performance nor to any pathologies in experiments carried out at 15°. Even when a more highly oxidized oil (aerated 1348 h at room temperature, peroxide value 60 mmol/kg, vitamin E content zero) was used in the practical diet (Hung *et al.* 1981) there was no significant difference in weight gain, food conversion ratio or carcass composition from controls although there was an increase in mortality during weeks 9–24 and in erythrocyte fragility; these symptoms were eliminated when this diet was supplemented with α -tocopheryl acetate (33 mg/kg). When fresh, non-oxidized fish oil was used in these practical diets no vitamin E deficiency symptoms occurred even in the absence of supplementation with α -tocopheryl acetate. The measured level of tocopherol in this diet was 25 mg/kg.

In contrast, the absence of supplementary vitamin E from the diets used in the present study (diets 2 and 4) had profound effects on the trout. Growth rate, food conversion ratio and fatty acid composition of hepatic polar lipids were all affected and, in particular, overt pathologies occurred in all the fish examined. By contrast with these results, trout given

partially defined diets lacking supplementary vitamin E in earlier experiments (Cowe *et al.* 1981, 1983) did not differ in any of these characteristics from control trout. Effects on erythrocyte fragility and ascorbic acid stimulated peroxidation of biomembranes from trout deficient in vitamin E were common to all experiments.

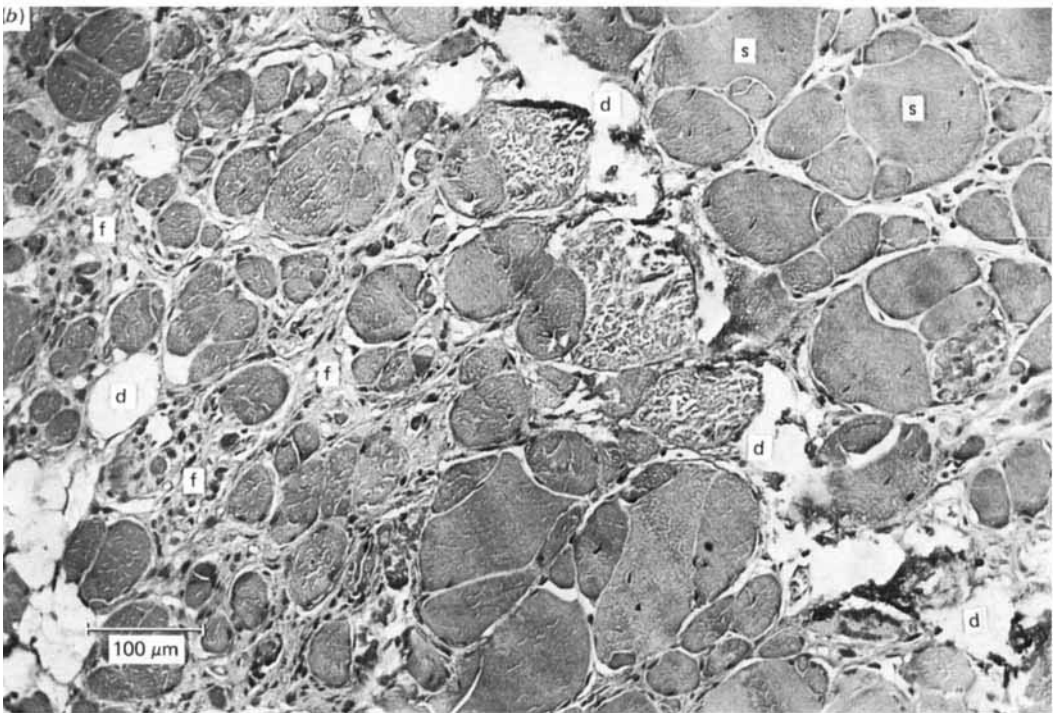
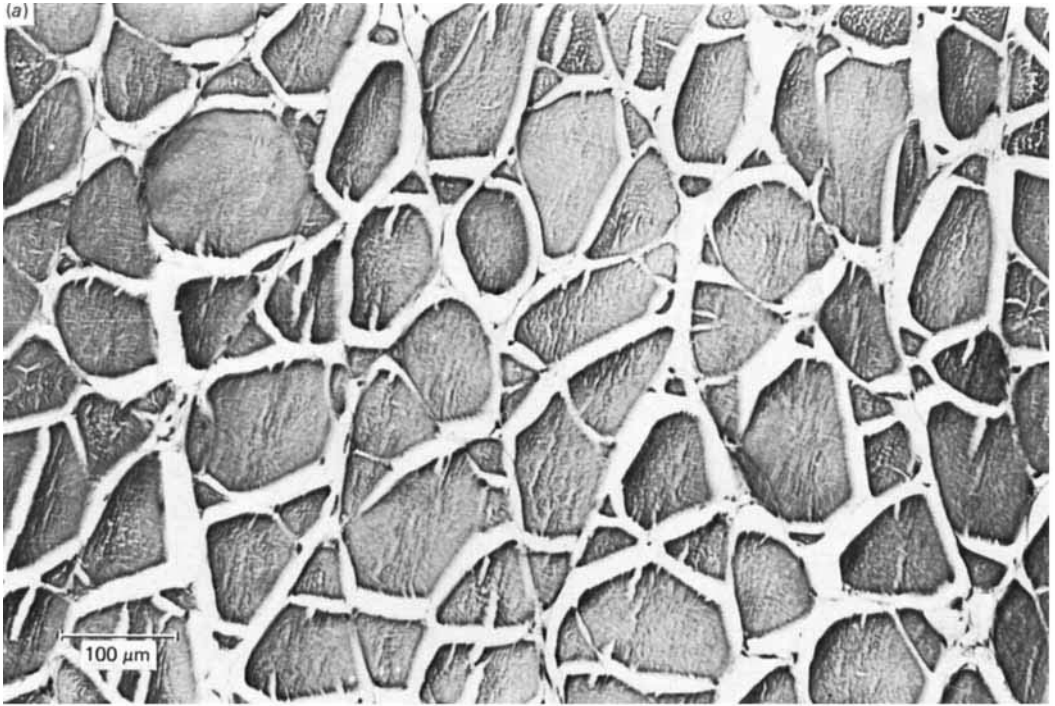
In these earlier experiments basal (adventitious) levels of vitamin E in the deficient diets were zero (when the diet contained 10 g linolenic acid/kg as essential fatty acid component) and 20 mg/kg (when the diet contained 100 g fish oil fatty acids/kg as essential fatty acid component). The latter value was similar to the vitamin E concentration in diet 2 in the present experiment, the former value lower than that. In line with this, tissue concentrations of vitamin E-deficient and control fish in the present experiment were very similar to those found in analogous treatments in previous experiments (for vitamin E-deficient fish 5.6 µg/g liver and 2.4 µg/g muscle, Cowe *et al.* 1981; 8.7 µg/g liver and 3.1 µg/g muscle, Cowe *et al.* 1983). Consequently the major differences — histopathology and other characteristics — between vitamin E-deficient fish in the present and in previous experiments seem to be related to other factors in addition to a low vitamin E intake.

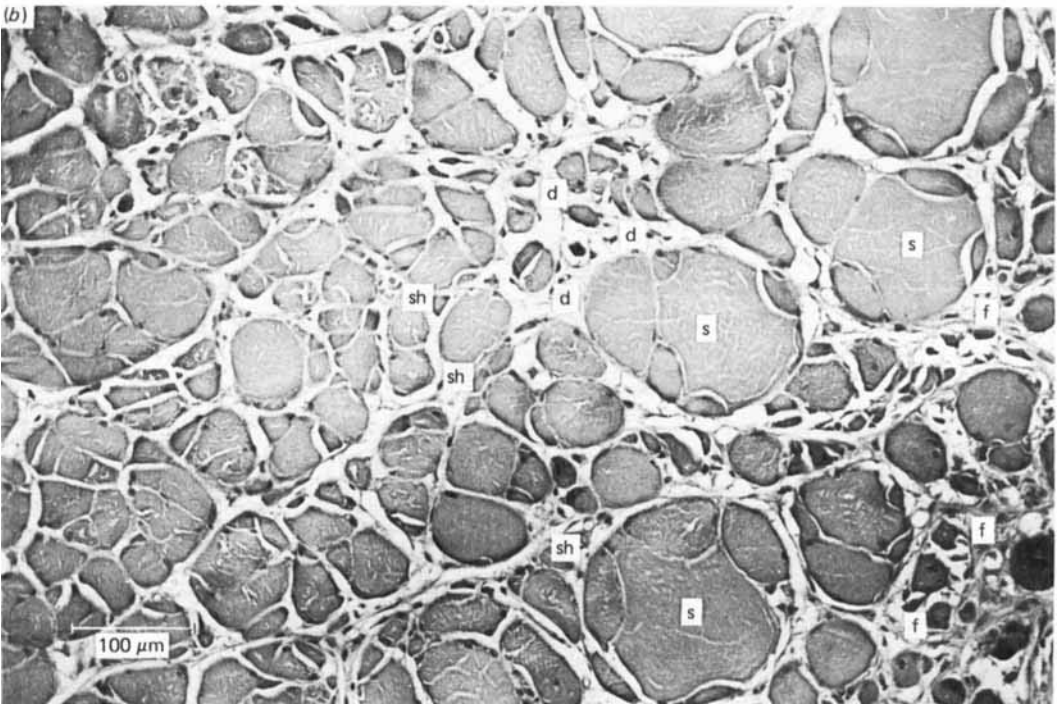
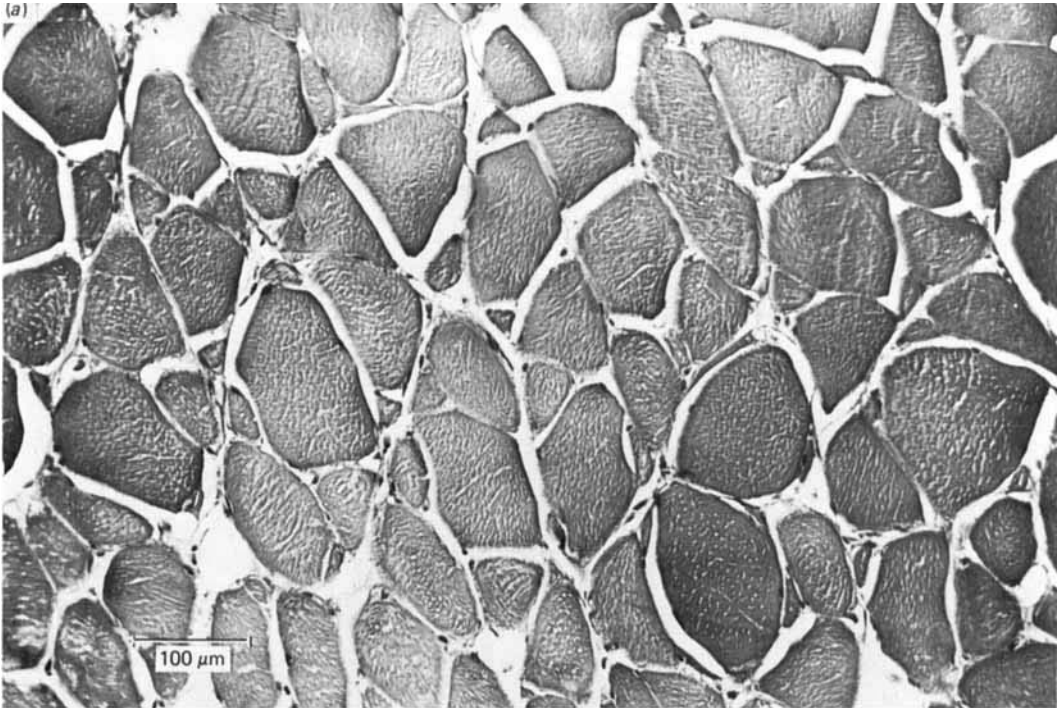
One possible explanation of the results is that the severe myopathy observed was associated with a low level of dietary selenium, that is a combined deficiency of vitamin E and Se led to myopathies while a sufficiency of dietary vitamin E with low Se prevented them. No supplementary Se was added to the purified diets used in this experiment and measured dietary Se concentrations were only 0.10 mg/kg. In this connection it may be observed that Poston *et al.* (1976) found a supplemental Se level of 0.10 mg/kg to be adequate for Atlantic salmon (*Salmo salar*) of 0.9 g mean initial weight (calculated Se level in the basal diet 0.03 mg/kg). Later Hilton *et al.* (1980) showed that a dietary Se level of 0.07 mg/kg (their lowest level) was sufficient to prevent Se deficiency symptoms in rainbow trout (dietary vitamin E level about 0.4 mg, water-borne Se level 0.4 µg/l). While these observations do not rule out the possibility that myopathies in the present experiment were associated with a low dietary Se level they render it unlikely, especially as glutathione peroxidase activities did not differ between treatments and were of a similar or greater magnitude than those previously reported in salmonids (Poston *et al.* 1976; Hilton *et al.* 1980).

The principal differences between our earlier experiments and the one now reported are the physical conditions employed, especially the low temperatures pertaining toward the end of the experiment. The maintenance of fluidity in biomembranes is known to be more demanding, in terms of polyunsaturated fatty acids, at low than at high temperatures (Hazel, 1979) and it now appears that trout may be more susceptible to vitamin E deficiency at low water temperatures. Hung *et al.* (1981) laid emphasis on commercial situations in evaluating vitamin E requirement, having previously observed that there can be no single vitamin E requirement for rainbow trout under all practical situations (Hung *et al.* 1980). The present results indicate that water temperature warrants consideration as a factor in arriving at a vitamin E requirement for certain of these practical situations.

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EXPLANATION OF PLATES

Plate 1. (a), Skeletal muscle from trout given diet 1; normal appearance of tissue with fibre integrity maintained. Absence of fibroblasts and macrophages from inter-fibre space. (b), Skeletal muscle from trout given diet 2; fibres are all more rounded and some partly swollen (s). Some areas show hyaline degeneration (d) of muscle fibres and a proliferation of fibroblasts (f) in the interstitial spaces. For details of diets, see Table 1.

Plate 2. (a), Skeletal muscle from trout given diet 3, appearance typical of normal muscle. (b), Skeletal muscle from trout given diet 4; fibres have a rounded shape and some are partly swollen (s). Other fibres appear partly shrunken (sh). Zonal necrotic areas show eosinophilic hyaline degeneration (d). No macrophages are visible but in the interstitial spaces a proliferation of fibroblasts (f) has occurred. For details of diets see Table 1.