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Iron catalyzed oxidation of trout diets and its effect on the growth and physiological response of rainbow trout

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Abstract

Two experiments were conducted to determine 1) the effect of iron supplementation and the quality of fish oils on dietary lipid peroxidation and 2) the concurrent effects of diet rancidity and iron overload on the growth and physiological response of rainbow trout. Semi-purified diets supplemented with graded levels of iron (0–6250 mg/kg diet as ferrous sulphate) were fed to trout for 12–36 weeks. The malonaldehyde (MA) concentration of the test diets increased as the iron levels in the diets increased indicating that iron catalyzed lipid oxidation was occurring. However, when ethoxyquin was added to the oils, the increase in dietary MA level was significantly reduced. Fish oils with an initial high peroxide value were more susceptible to iron-catalyzed lipid oxidation. The concurrent effects of diet rancidity and iron overload (greater than 86 mg/kg) led to the development of unique histopathological signs, poor growth and high mortalities in the trout. In contrast, when diet rancidity was low (less than 10 μg MA/g diet), the toxic level of dietary iron was greater than 1380 mg/kg diet. The concentration of iron in trout tissues, and the hematocrit and hemoglobin concentrations increased as dietary iron levels increased and were not affected by the degree of diet rancidity.

Introduction

There have been few studies on the effect of iron supplementation on the growth and physiological response of rainbow trout. In addition, the NRC (1983) does not give a dietary recommended level of iron in fish. Despite this, commercial trout diets in Ontario, Canada are routinely supplemented with 60 mg iron/kg feed as ferrous sulphate (Hilton and Slinger 1981). Furthermore, commercial trout diets can contain levels of endogenous iron between 200 to 1000 mg iron/kg feed (Desjardins and Hilton, unpublished data) due to diet ingredients such as fish meal and blood meal which are rich in heme iron. Therefore, it is possible that supplementation of trout diets with ferrous sulphate increases the potential of iron overload in rainbow trout.

Salmonids rely heavily on lipids as a major source of dietary energy. Hence, many commercial trout diets contain approximately 12–16% lipid supplied as fish oils, many of which contain 20% or more of highly unsaturated fatty acids (Watanabe 1982). Because iron is a potent catalyst of lipid peroxidation (Lee *et al.* 1981; Dillard *et al.* 1982), it is possible that supplementation of trout diets with ferrous sulphate increases the potential of diet rancidity. As a consequence, the concurrent effects of diet rancidity and iron overload could lead to the development of unique pathological signs in the rainbow trout.

The objectives of this study were to determine 1) the effects of iron supplementation on dietary lipid

oxidation and 2) the effects of the interaction between iron and dietary lipid oxidation on the growth and physiological response of rainbow trout.

Materials and methods

Experiment 1

Care and feeding of the fish

Juvenile rainbow trout (*Salmo gairdneri*) were obtained from a commercial trout farm (Spring Valley Farms Ltd., New Dundee, Ontario, Canada). The fry were distributed randomly at a rate of 100 fish/tank (initial weight 1.5 g/fish) among 12 enamel-lined rectangular metal tanks with a length and width of 0.44 M and a depth of 0.28 M, and a volume of 50 l. Each tank was individually aerated with compressed air using an air stone and the incoming water flowed-through at a rate of 1.5–2 l/min. The water temperature in the tanks was thermostatically maintained at 15°C and measured daily. The pH and dissolved oxygen in the system were monitored bi-weekly and ranged between 7.3–7.7 and 7.1–7.9 mg/l, respectively. The water hardness was between 145–165 mg/l as $CaCO_3$ (APHA, 1980) and the waterborne iron was 28–40 μg/l. This aquatic system was housed in a windowless laboratory with a 12 h light: 12 h dark photoperiod as provided by overhead fluorescent tubes. The six test diets were fed to duplicate groups of juvenile trout (initial weight 3 g) 3–4 times daily as described by Hilton and Slinger (1981). Mortalities were monitored daily and the fish were weighed at the end of each 28-day period.

Diet formulation, processing and analysis

Six semi-purified test diets were prepared as described by Cho *et al.* (1974) with a few alterations (Table 1). The diets were supplemented with $FeSO_4 \cdot 7H_2O$ in concentrations ranging from 0 to 6250 mg iron/kg (Table 2). No ethoxyquin was added to the oil prior to mixing. The diets were stored at −20°C until required for feeding. After processing, the iron concentration of the diets was measured by flame atomic absorption spectrophotometry using a Perkin-Elmer Model 372. Similarly, after processing the malonaldehyde content of the diets was determined by high performance liquid chromatography by the method of Bird *et al.* (1983) using a solvent delivery system Model M6000A, an automatic injection Wisp Model 701B, an absorbance detector Model 440 and a data module equipped with a computing integrator (Waters Associates Inc., Milford, MA).

Table 1. The composition of the semi-purified test diet

Ingredients	%
Casein	40
Gelatin	5
Amino acid premix[1]	2
Starch	10
Dextrin	10
Alpha-floc (cellulose)	4
Vitamin premix[2]	2
Mineral premix[3]	8
Iron premix[4]	4
Fish oil	15

[1]Amino acid premix (g/100 kg): arginine 500, DL-methionine 1300, starch 200; [2]Vitamin premix (g/100 kg): thiamin 4, riboflavin 6, niacin 30, pyridoxine HCl 4, D-Ca-pantothenate 20, folic acid 2, biotin (1%) 5, cobalamin (2%) 4, inositol 50, choline 550, ascorbic acid 100, butylated hydroxy toluene 2.5, vitamin A (retinyl palmitate 500,000 IU/g) 700, vitamin D (cholecalciferol 500,000 IU/g) 300, vitamin E (DL-α-tocopheryl acetate 50%) 200, vitamin K (sodium menadione) 0.5, α-cellulose 1180; [3]Mineral premix (g/100 kg): calcium phosphate dihydrate 300, calcium carbonate 300, sodium chloride 1500, magnesium sulphate 1000, manganous sulphate hydrate (33% Mn) 30, zinc sulphate hydrate (41% Zn) 55, copper sulphate pentahydrate (29% Cu) 16, cobalt chloride hexahydrate (25% Co) 2.6, potassium iodide (76% I) 1.5, α-cellulose 2094.9; [4]Iron premix: ferrous sulphate heptahydrate (20% Fe) and α-cellulose made up to 4 g iron premix/100 kg diet.

Biochemical analysis

Iron concentration in trout livers, kidneys and carcasses were measured by flame atomic absorption spectrophotometry. The concentration of the metal in these tissues was determined by reference to a standard curve prepared from Fisher Certified atomic absorption Standard Reference Solution (Fisher Scientific Co., Fairlawn, N.J., U.S.A.). Blood samples were taken by amputation of the caudal peduncle for hemoglobin determination by

Table 2. Iron supplement and iron and malonaldehyde concentrations in the semi-purified test diets – Experiment 1

Experiment number		Diet number					
		1	2	3	4	5	6
	(mg/kg)						
1	Iron supplement[1]	0	10	50	250	1250	6250
	Iron conc.[2]	25	42	86	265	1334	5934
	Malonaldehyde[3]	73[a4]	68[a]	81[a]	88[a]	160[b]	139[b]

		OCO			FHO			OHO		
		Diet number								
		1	4	7	2	5	8	3	6	9
2	Iron supplement[1]	50	250	1250	50	250	1250	50	250	1250
	Iron conc.[2]	87	315	1291	98	300	1384	90	303	1340

[1]as $FeSO_4 \cdot 7H_2O$; [2]Results based upon the mean of six samples from each test diet, as fed basis; [3]Results based upon the mean of five samples from each test diet, as fed basis; [4]Values that do not share a common superscript are significantly different: $P < 0.05$.

the cyanmethemoglobin method (Unopette test 5857, Becton Dickinson Co., Ltd., Mississauga, Ontario, Canada). Hematocrits were measured by the heparinized microhematocrit method described by Hesser (1960) and plasma protein levels determined by the method of Lowry *et al.* (1951). Plasma samples were diluted 1:9 with 1% HNO_3 (v/v) and analysed for iron concentration by graphite furnace atomic absorption spectrophotometry. Fish tissues (liver, kidney, spleen, gill, heart, and pseudobranch) from 5 fish were fixed in 10% buffered formalin for subsequent histopathological examination.

Significant differences between means were determined at the 5% level using analysis of variance and Tukey's honestly significant difference method as described in Steel and Torrie (1980).

Experiment 2

Rainbow trout from Shamrock Springs Trout Farm, Erin, Ontario, Canada weighing approximately 20 g were distributed randomly at a rate of 60 fish/tank (initial weight 3.5 g/fish) and acclimatized in a 27-tank aquatic flow-through system to the conditions described in Experiment 1. The trout were fed to satiation 3 times daily for three 28-day periods.

Diet formulation and processing

Nine semi-purified test diets were prepared using the same basal diet previously described (Table 1). Three levels of ferrous sulphate (50, 250, 1250 mg iron/kg diet) and three different 'qualities' of fish oils were added to the basal diet in a 3 × 3 factorial design experiment (Table 2). A 'poor' quality fish oil was defined as having a moderate to high peroxide value and a rancid smell. The fish oils consisted of good quality fresh herring oil (FHO), artificially oxidized poor quality herring oil (OHO) and the same capelin oil (age of oil ~18 months) which had been used in Experiment 1 (OCO). Artificially oxidized herring oil (OHO) was obtained by heating and bubbling 95% compressed oxygen into fresh herring oil for six hours at which time the peroxide value was similar to that of the moderately autoxidized capelin oil (OCO). The peroxide values were determined by the method described in AOAC (1980). The OHO, FHO, and OCO were then stabilized by adding ethoxyquin at the level of 0.05% (w/w). Fatty acid composition of the three fish oils was determined by gas liquid chromatography using a Hewlett-Packard 5170 gas chromatograph equipped with a spectrophysics SP4100 computing integrator with plotter. The oils were methylated according to the method described by Luddy *et al.* (1968). A 30 M fused silica capillary column, 0.25 mm in diameter packed with SP-2330, 90% biscy-

anopropyl/10% phenyl cyanopropyl polysiloxane (Supelco Canada Ltd., Oakville, Ontario, Canada) was used. Identification of individual peaks was made by comparing retention times with known standards (Nu Chek Prep., Elysian, MN). The fatty acid analysis indicated that the total level of ω3-type fatty acids was 8.23% of the total fatty acids in the OCO to 12.11% in the FHO. This would indicate that none of the test diets would be deficient in ω3-type fatty acids.

Biochemical analysis

After processing, the test diets were stored at −20°C until required for analysis. The malonaldehyde and iron concentrations of the test diets were then determined as described in Experiment 1. Biochemical and histopathological analysis of the fish tissues (liver, kidney, spleen, gill, pseudobranch, heart, pyloric caeca, stomach, intestine, skin, red and white muscle) were conducted as described in Experiment 1.

Statistical analysis

Significant differences between treatment means were determined at the 5% level using analysis of variance and Tukey's honestly significant difference test as described in Steel and Torrie (1980) in Experiment 1. In Experiment 2, the data were subjected to analysis of variance for a two-factor factorial in a completely randomized design. If the interaction between the iron and quality of the fish oil was not significant ($P > 0.05$), the means of the trout fed the same iron supplementation level and the means of the trout fed the same fish oils were combined and analysed as two independent experiments.

Results

Experiment 1

It was observed during processing that the test diets containing in excess of 86 mg iron/kg changed colour. The diets were progressively darker brown as the ferrous sulphate supplementation level increased in contrast to the usual yellow colour of the control diets. Iron-catalyzed peroxidation of the fish oil in the test diets was believed to be cause of this change in colour. Malonaldehyde concentrations of the diets were measured in order to quantify this chemical process. Diets containing levels greater than 265 mg iron/kg diet were found to have significantly higher malonaldehyde levels than the other test diets (Table 2).

After a 20-week feeding trial, the weight gain of the rainbow trout reared on diets 1, 2 and 3 (25, 42,86 mg iron/kg diet) were significantly higher ($P < 0.05$) than those reared on diets 4, 5 and 6 (265, 1334, 5934 mg iron/kg diet) (Table 3). The feed:gain ratios of trout reared on diets 1 to 3 were also significantly higher and liver:body weight ratios were significantly lower. Trout reared on diets 1 to 3 also had significantly fewer mortalities than trout reared on the other test diets (Table 3). During the third week of the feeding trial, feed refusal was observed in trout reared on diets containing in excess of 86 mg iron/kg (diets 4 to 6) and feed intake by trout reared on diet 6 was extremely low (data not shown). Feed refusal was exemplified by fish taking pellets into the buccal cavity and subsequently expelling them back into the water or by swimming around the pellets but not taking them into the buccal cavity. Also, trout reared on diets 4 to 6 (265 to 5934 mg iron/kg) appeared to have diarrhea characterized by a high level of fecal excretion.

Hemoglobin concentrations and plasma iron and protein levels increased but not significantly, as the iron supplementation levels increased (Table 4). Similarly, the hematocrit values increased with dietary iron levels but were significantly higher in trout reared on diets 4 and 5 (265 and 1334 mg iron/kg) than trout reared on diet 1 (25 mg iron/kg). Plasma protein and iron concentrations of trout reared on diet 6 were not determined because not enough blood could be collected due to the small size of the fish.

Carcass iron concentrations increased as the level of ferrous sulphate supplementation increased and was significantly higher in fish reared on diets 5 and 6 (1334 and 5934 mg iron/kg) (Table 5). Liver and kidney iron concentrations also increased as dietary iron levels increased but was only significantly higher in trout fed diet 6 (5934 mg iron/kg).

Histopathological analysis indicated that trout

Table 3. Growth, feed efficiency and mortalities of rainbow trout – Experiment 1 and 2

Experiment number		Diet number						
		1	2	3	4	5	6	SEM
1	Weight gain[1] (g/fish)	35[a3]	34[a]	31[a]	15[b]	13[b]	10[b]	1
	Mortalities[1] (death/10000 fishdays)	11.4[a]	8.9[a]	9.2[a]	15.0[b]	14.9[b]	23.1[b]	1.6
	Liver/body wt[2]	1.8[a]	1.8[a]	2.0[b]	2.9[cd]	3.0[d]	2.8[c]	0
	Feed/gain	1.5[a]	1.5[a]	1.5[a]	2.1[b]	1.9[b]	2.3[b]	0.1
		Iron concentration (mg/kg)			Type of oil			
		50	250	1250	FHO	OHO	OCO	SEM
2	Weight gain[1] (g/fish)	57	58	58	60[a3]	56[b]	56[b]	1
	Mortalities[1] (deaths/10000 fishdays)	15.0[a]	10.2[ab]	16.8[a]	16.8[a]	8.2[b]	9.2[b]	2.4
	Liver/body wt[2]	1.2	1.2	1.2	1.2	1.2	1.2	0.1
	Feed/gain	1.0	1.0	1.1	1.1	1.1	1.1	0

[1]Results based upon the means of 2 tanks – Experiment 1 and 3 tanks – Experiment 2; [2]Results based upon the means of 2 pooled samples (20 fish/sample) Experiment 1 and 3 pooled samples (10 fish/sample) Experiment 2; [3]Values that do not share a common superscript are significantly different: $P < 0.05$.

Table 4. Blood and plasma analysis of rainbow trout – Experiment 1 and 2

Experiment number		Diet number						
		1	2	3	4	5	6	SEM
1	Hemoglobin[1] (g/dl)	5.53	5.80	5.84	6.73	7.88	6.54	0.43
	Hematocrit[2] (%)	29[a4]	30[ab]	33[abc]	39[bc]	40[c]	37[abc]	2
	Plasma protein[3] (g/dl)	2.79	2.29	2.87	3.01	3.60	N.D.[5]	5.31
	Plasma iron[1] (μg/ml)	0.51	0.71	0.76	0.80	0.90	N.D.	0.14
		Iron concentration (mg/kg)			Type of oil			
		50	250	1250	FHO	OHO	OCO	SEM
2	Hemoglobin conc.[1] (g/dl)	5.35[a]	5.67[a]	6.61[b]	5.64	6.05	5.93	0.24
	Hematocrit[2] (%)	32[a]	33[a]	36[b]	32[z]	34[yz]	35[y]	1

[1]Results based upon the means of 2 tanks, 6 samples per tank in Experiment 1 and 3 tanks, 5 samples per tank in Experiment 2; [2]Results based upon the means of 2 tanks, 20 samples per tank in Experiment 1 and 3 tanks, 10 samples per tank in Experiment 2; [3]Results based upon the means of 2 tanks, 5 samples per tank; [4]Values that do not share a common superscript are significantly different: $P < 0.05$; [5]Not determined.

Table 5. Carcass, liver and kidney concentrations of iron in rainbow trout – Experiment 1 and 2

Experiment number		Diet number						
		1	2	3	4	5	6	SEM
1	Carcass[1]	24^{a2}	31^{ab}	30^{ab}	45^{b}	79^{c}	153^{d}	3
	Liver	69^{a}	69^{a}	85^{a}	120^{a}	116^{a}	536^{b}	13
	Kidney	138^{a}	184^{a}	181^{a}	233^{a}	198^{a}	493^{b}	39
		Dietary iron concentration (mg/kg)			Type of oil in diet			
		50	250	1250	FHO	OHO	OCO	SEM
2	Carcass[1]	49^{a2}	53^{a}	73^{b}	58	58	60	4
	Liver	118^{a}	144^{a}	256^{b}	169	166	183	28
	Kidney	168^{a}	198^{b}	220^{c}	191	199	195	11

[1]Values are shown as μg iron/g tissue; results based upon the means of 2 tanks, 5 samples per tank in Experiment 1 and 3 tanks, 6 samples per tank in Experiment 2; results expressed on a dry weight basis; [2]Values that do not share a common superscript are significantly different: $P < 0.05$.

reared on test diets containing 265 and 1334 mg iron/kg feed (diets 4 and 5) had lesions in the liver (Fig. 1a). Trout fed diet 6 (5934 mg iron/kg) were not examined. The hepatocytes were vacuolated and the nuclei were extremely pleomorphic. There were large intranuclear inclusions in the nuclei with peripheral clumping of the chromatin. In addition, the hepatocytes were very vacuolated and appeared to contain large amounts of fat and reduced levels of glucogen.

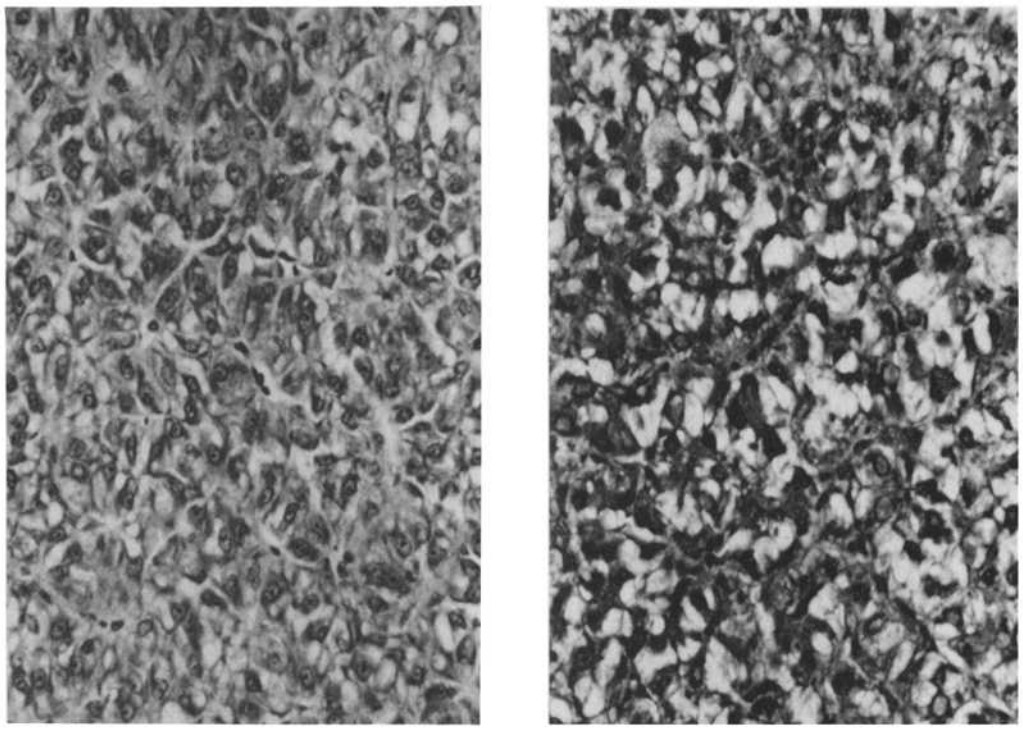
Experiment 2

The interaction between ferrous sulphate and the quality of the fish oil was significant ($P < 0.05$) in relation to malonaldehyde production. Increasing the level of ferrous sulphate led to a significant increase in the malonaldehyde content of the test diets (Fig. 3). Diets containing 'poor' quality fish oils had higher peroxide values (OCO 58.3 mEq/kg and OHO 56.9 mEq/kg) and higher levels of malonaldehyde (Fig. 3) than the FHO-based diets (Peroxide Value 4.3 mEq/kg). The diets containing FHO appeared to be stabilized even when 1250 mg iron as ferrous sulphate/kg diet was included. In contrast, the OCO- and OHO-based diets had increasing malonaldehyde concentrations in relation to increased ferrous sulphate supplementation.

There was no effect of the interaction between ferrous sulphate and the quality of the fish oils on the growth and physiological response of rainbow trout ($P > 0.05$). Therefore, the iron treatments and fish oil treatments were statistically analyzed independently. The feed:gain and liver:body weight ratios were not significantly different ($P > 0.05$) (Table 3). Similarly, there was no difference in the weight gain of trout reared on the different iron supplementation levels ($P > 0.05$). However, trout reared on FHO-based diets gained significantly more weight than did the trout reared on the OHO- and OCO- based diets ($P < 0.05$). There was a significant difference in the number of mortalities but it is not believed to be diet related. Two weeks before the end of the experiment, the line to the air stones was severed and caused some fish to die of anoxia.

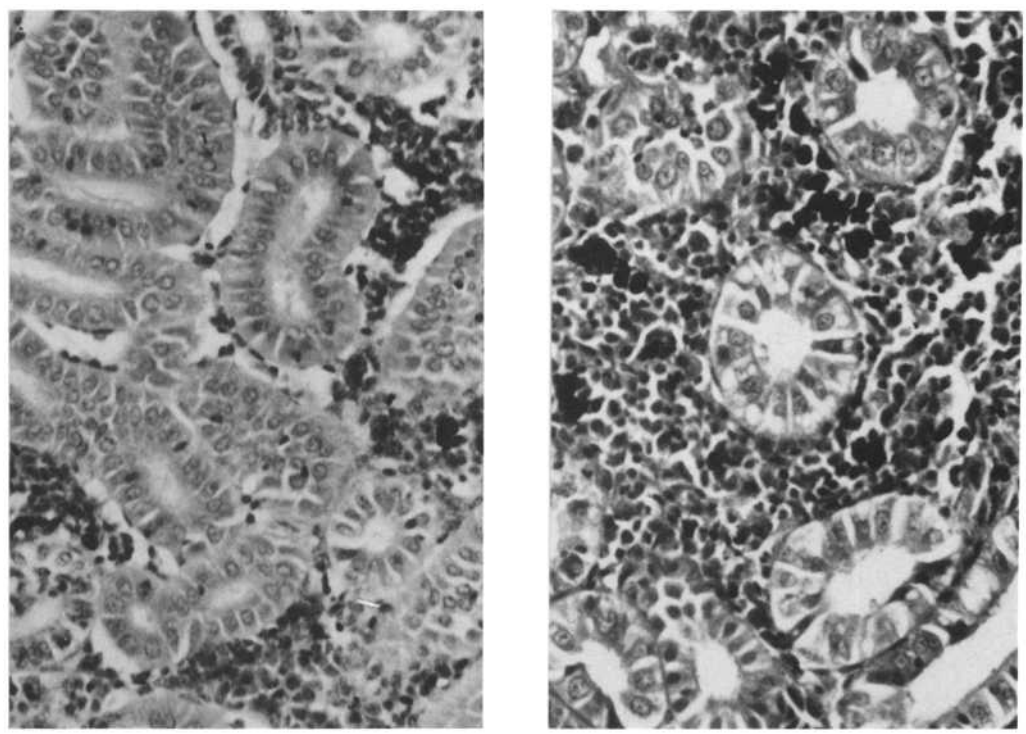
Hemoglobin concentrations and hematocrit values increased as dietary iron levels increased and were significantly higher in trout reared on diets containing 1250 mg iron/kg as ferrous sulphate ($P < 0.05$) (Table 4). Quality of the fish oil did not have any effect on the blood parameters with the exception of trout reared on the OCO-based diets which had significantly higher hematocrit values ($P < 0.05$).

The carcass iron concentrations increased as the



a b

Fig. 1a. Normal rainbow trout liver H and E × 250. *b.* The hepatocytes are very vacuolated and the nuclei are pleomorphic. There is peripheral clumping of the nuclear chromatin. H and E × 250. (Reduced 85%)



a b

Fig. 2a. Normal tubular nephron of the rainbow trout. *b.* Large lipid droplets (clear vacuoles) are scattered throughout the epithelial cells of the second proximal segment of the nephron. H and E × 250. (Reduced 85%)

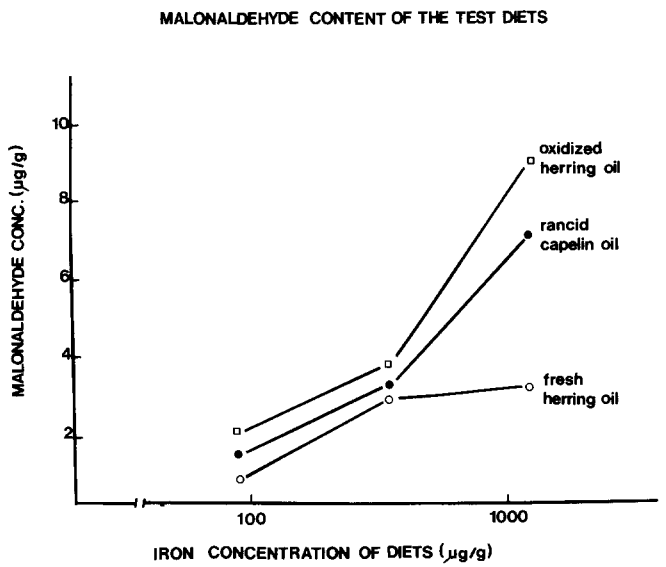


Fig. 3. Malonaldehyde[1] concentration of semi-purified test diets in Experiment 2.

dietary iron levels increased and were significantly higher in trout fed diets containing 1250 mg iron/kg ($P < 0.05$) (Table 5). Iron concentrations in livers and kidneys of trout also increased with a concomitant increase in the ferrous sulphate supplementation levels ($P < 0.05$). The quality of the fish oil did not have any effect on the concentration of iron in trout tissues.

Tissues of fish from each of nine test diets were examined. A lesion in the proximal segment of the nephron was observed in trout fed the high ferrous sulphate supplemented diets (1250 mg iron/kg, Fig. 2a, b). The epithelial cells lining the second proximal segment of the nephron contained variable quantities and sizes of lipid droplets. The nuclei of these epithelial cells were moderately to markedly pleomorphic and some nuclei appeared to be fragmented or multiple. This lesion appeared to be sublethal and unlikely to result in any signs of chronic disease in the fish.

Discussion

As the supplementary iron level increased, the malonaldehyde (MA) content, dark coloration and rancid smell of the test diets also increased (Table 2, Fig. 3) indicating that increasing the level of ferrous sulphate supplementation increases the level of dietary lipid oxidation. This is consistent with the fact that iron can be a potent catalyst of lipid oxidation (Chvapil *et al.* 1974; Lee *et al.* 1981). However, the quality of the fish oil added to the diet also significantly affected the MA content of the diets (Fig. 3). Both the OCO and OHO oils which had much higher peroxide values than the FHO, produced significantly higher dietary MA levels than the FHO-based diet when the same level of ferrous sulphate was included. There was a significant interaction ($P < 0.05$) between the iron supplementation level and the quality of the fish oil in terms of dietary lipid oxidation.

[1]The interaction between iron supplementation and the malonaldehyde concentration of the diets was significant: $P < 0.05$.

A great difference in the MA content of the diets in Experiments 1 and 2 was observed even though the level of ferrous sulphate added to the diets overlapped (Table 2, Fig. 3). Why such differences occurred cannot be explained simply on the basis of differences in the quality of the oil. The OCO oil used in Experiment 2 was also used in Experiment 1; however, the effect of increasing the supplementary iron level on dietary MA content in Experiment 2 was much less than observed in Experiment 1. The only difference in the procedure employed was that in Experiment 2, the different oils were stabilized with ethoxyquin while in Experiment 1, the OCO was not stabilized with the antioxidant prior to processing. The only other antioxidants added to the diets in either experiment were butylated hydroxytoluene at a level of 2.5 g/100 kg diet and DL-α-tocopheryl acetate at the level of 200 g/100 kg of diet in the vitamin premix (Table 1). Therefore, both the level and the type of antioxidants added to the fish oil and diet can have a significant effect on the stability of the dietary lipids during processing. The deletion of ethoxyquin in the OCO prior to the mixing and processing of the test diets in Experiment 1 obviously resulted in excessive oxidation of the dietary lipid. Support for this conclusion comes from the study of Murai and Andrews (1974) on the effect of ethoxyquin to either fresh or oxidized menhaden oil on the dietary MA content of catfish diets. When ethoxyquin was added to the oxidized menhaden oil diet, the levels of dietary MA remained low (2–4 μg/g); however, upon deletion of ethoxyquin, dietary MA levels increased dramatically (94–250 μg/g diet) when none or 2.5 g/100 kg DL-α-tocopherol was added to the diets.

On the basis of the growth parameters, mortalities and histopathological analysis of the fish in Experiment 1, the toxic level of dietary iron appears to be in the range of 86 to 265 mg iron/kg diet. However, the levels of dietary MA indicate that the test diets in Experiment 1 were highly oxidized (Hung *et al.* 1980, 1983). Studies by Hung *et al.* (1980) and Cowey *et al.* (1984) have shown that feeding moderately oxidized diets to rainbow trout has no effect on their growth or physiological response as long as there are adequate levels of vitamin E present. Trout reared on test diets 1 to 3 (25 to 86 mg iron/kg diet) in Experiment 1 were not affected by the highly oxidized diets but when excess supplementary iron was present, adverse effects on the fish were observed despite the high level of tocopheryl acetate in the diets. The histopathological analysis of the adversely affected fish in Experiment 1 are not specifically indicative of liver lipoid disease resulting from feeding rancid diets to trout (Roberts 1978) nor a condition of iron overload as occurs in terrestrial animals (Fairbanks *et al.* 1971; Halliday and Powell 1982). It is difficult to determine whether the lesions observed in Experiment 1 are due to iron toxicity, diet rancidity or both.

There is a possibility that dietary iron toxicity occurs at a much lower level in the trout when the diet is rancid due to the additive effect of iron and MA. In support of this, the results in Experiment 2 indicate that the toxic level of dietary iron in the trout is in excess of 1380 mg iron/kg diet when the diet is not rancid (MA content less than 10 μg/g diet). Histopathological examination of the trout in Experiment 2 showed that trout reared on the highest dietary iron level (1380 mg iron/kg diet) had a specific kidney lesion (Fig. 2a, b). However, because growth and other physiological parameters in the trout were not adversely affected, the significance of this lesion remains to be determined. It is interesting to note that none of the trout examined in Experiment 1 had this lesion. Furthermore, considering the higher iron levels in the trout kidney in comparison to the liver, the occurrence of this lesion may be the first uncomplicated sigh of dietary iron toxicity in the trout. This warrants further study.

It was noted that hemoglobin and hematocrit levels in the trout generally increased in relation to increased supplementary iron in Experiment 1 and 2 (Table 4). Despite the increase, the hemoglobin and hematocrit levels are considered to be in the normal range for rainbow trout (Roberts, 1978). In addition, the plasma protein (2.3–3.6 g/dl), copper (0.5–0.8 μg/ml), iron (0.5–0.9 μg/ml) and zinc (15–20 μg/ml) levels were not affected by the different dietary treatments. It would appear that the trout is able to effectively regulate plasma iron, copper and zinc levels over a wide range of dietary iron levels, from 25 to 5934 mg iron/kg diet. In

contrast, Knox *et al.* (1982) and Lanno *et al.* (1985) both observed that plasma copper levels were responsive to the copper content of the diet in rainbow trout.

Carcass, kidney and liver iron concentrations all tended to increase as the supplementary iron levels increased in Experiments 1 and 2 (Table 5); however, the iron levels of these tissues were not affected by the quality of the supplementary oil. Similarly, the zinc levels of these tissues were not affected by the different dietary treatments (data not shown). Carcass copper levels were also unaffected by dietary treatment; however, liver copper levels significantly declined from 204 to 111 μg/g tissue in the trout of Experiment 1 receiving in excess of 86 mg iron/kg diet. This may indicate that dietary iron toxicity in combination with rancid diets affects either the uptake, storage and perhaps metabolism of copper in the trout. It was also noted that kidney iron levels in the trout were always significantly higher than those of the liver. This probably reflects the fact that the kidney is the site of red blood cell synthesis and is also the most important site of the reticuloendothelial system for red blood cell breakdown in the trout (Roberts 1978).

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