HGCA

PROJECT REPORT No. OS46

APPLICATIONS OF RAPE SEED OIL IN ONGROWING DIETS FOR ATLANTIC SALMON (*SALMO SALAR*)

OCTOBER 2000

Price £4.50

PROJECT REPORT No. OS46

APPLICATIONS OF RAPE SEED OIL IN ONGROWING DIETS FOR ALTANTIC SALMON (*SALMO SALAR*)

by

J G Bell

Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland

This is the final report of a one-year research project, which started in June 1999. The work was funded by the Home-Grown Cereals Authority (project 2125) with a grant for £18,739.

Contents

ABSTRACT

The overall aim of this project was to investigate the substitution of fish oil with rape seed oil in the diets of post-smolt (sea water) Atlantic salmon. Of primary importance was to investigate the effects of feeding rape seed oil on growth performance and flesh quality parameters important to retailers and consumers e.g. total oil content of the flesh, flesh fatty acid composition, particularly the content of n-3 highly unsaturated fatty acids (HUFA) and flesh colour. Duplicate groups of Atlantic salmon post-smolts were fed five practical-type diets in which the added lipid was either 100% fish oil [FO, **0% rape seed oil (RO**)], 90% FO, 10% RO (**10% RO**), 75% FO, 25% RO (**25% RO**), 50% FO, 50% RO (**50% RO**) or **100% RO** in two separate trials of 17 and 26 weeks. Most of the data described in this report refers to the former trial as growth rates were better compared to the second trial which used smaller fish and was performed when water temperatures were lower. There were no effects of diet on growth rate and feed conversion nor were any histopathological lesions found in liver, heart, muscle or kidney. Proximate analysis showed that the highest accumulation of muscle lipid was in fish fed 0% RO which correlated with significantly lower muscle protein in the same group, compared to all other treatments. Conversely, the highest lipid levels in liver were found in fish fed 100% RO. Fatty acid compositions of muscle were correlated with RO inclusion such that total monounsaturated fatty acids were increased, largely due to increased 18:1(n-9), along with 18:2(n-6) and 18:3(n-3). The concentration of muscle eicosapentenoic acid (20:5(n-3), EPA) and docosahexaenoic acid (22:6(n-3), DHA) were reduced, along with total saturated fatty acids, with increasing inclusion of dietary RO. Dietary-induced changes in liver fatty acid compositions were broadly similar. Hepatic fatty acid desaturation and elongation activity, i.e. the ability of the fish to synthesise long-chain HUFA from their 18-carbon dietary precursors, increased with addition of dietary RO and this appeared to compensate, at least in part, for the reduced levels of dietary EPA and DHA. In summary, RO can be used successfully as a substitute for fish oil in the culture of Atlantic salmon in sea water although, at levels of RO above 50% reductions in muscle EPA, DHA and (n-3)/(n-6) polyunsaturated fatty acid (PUFA) ratio will result which could reduce the availability of these fatty acids that are essential for human health.

SUMMARY

The overall aim of this project was to investigate the substitution of fish oil with rape seed oil in the diets of post-smolt (sea water) Atlantic salmon. Of primary importance was to investigate the effects of feeding rape seed oil on growth performance and flesh quality parameters important to retailers and consumers e.g. total oil content of the flesh, flesh fatty acid composition, particularly the content of n-3 highly unsaturated fatty acids (HUFA) and flesh colour.

Two separate dietary trials were performed using duplicate groups (tanks) of Atlantic salmon post-smolts in sea water. Five experimental diets were prepared by BioMar Ltd. at their pilot plant in Denmark. The ten tanks, initially stocked with 35 Atlantic salmon post-smolts were fed the five practical-type diets in which the added lipid was either 100% fish oil [FO, 0% rape seed oil **(0% RO**), 90% FO, 10% RO **(10% RO),** 75% FO, 25% RO **(25% RO),** 50% FO, 50% RO **(50% RO)** or **100% RO.** The commercial extruded diets were formulated (BioMar Ltd., Brande, Denmark) to provide 47% crude protein and 25% lipid and differed only in their content of rape seed oil. The diets were designed to fully satisfy the nutritional requirements of salmonid fish. The experimental diets contained the carotenoid astaxanthin ,at a concentration of 50 mg/kg.

The second trial was conducted because the first trial had to be terminated after 17 weeks (August-December) due to power supply reductions occurring at the trial site. The second trial, which ran from January to July, showed lower growth rates than the first, largely due to smaller fish being used that were less well adapted to sea water and because the experiment was conducted when water temperatures were low, particularly in the early experimental phase. However, in both trials there were no significant effects of diet on growth rate and feed conversion nor were any histopathological lesions found in liver, heart, muscle or kidney.

In the first trial 350 Atlantic salmon S1 (entering sea water after 1 year) smolts (intitial mean weight, ~80 g) were obtained from the F.R.S. Marine Research Unit, Aultbea, Wester Ross, Scotland. In the second trial 350 $S^1/_2$ (entering sea water in < 1 year due to production using enhanced photoperiod) smolts (initial weight, ~40 g) were obtained from Marine Harvest Ltd., Loch Garry, Scotland. The smolts were randomly distributed into 10 one metre diameter tanks of 500 L capacity supplied with non-recirculated sea water at 10 L/min at the F.R.S. Marine Research Unit. The tanks were subjected to a photoperiod regime of 12h light:12h dark. The diets were supplied by automatic feeders, which were activated for a few seconds every 15 min during daylight hours and adjusted to provide between 25 and 20g/kg biomass each day, depending on fish size and water temperature. Fish were weighed individually at the start and finish of the experiment and bulk weighed every 28 days and the ration adjusted accordingly.

An initial sample of six fish was taken at the start of the experiment to determine baseline levels of lipid and fatty acid composition in liver and white muscle, and carotenoid in muscle. A similar sample of 12 fish per dietary treatment was taken after 9 wk (first trial only) of the trial. At the termination of the trial each analytical measurement was performed on at least 10 fish, selected at random, from each tank. Fish were killed by a blow to the head and samples of liver, were dissected and immediately placed in liquid nitrogen. A muscle sample, representative of the edible portion, was obtained by cutting a steak from the "Norwegian Quality Cut" region, between the dorsal and ventral fins. This section was then skinned, de-boned and the muscle homogenised, after removing the dorsal fat body, in a Waring blendor. The homogenate was frozen in liquid nitrogen and all samples were stored at -40^{o}C prior to analysis.

.The nutrient composition of the five experimental diets and muscle samples was determined by proximate analysis. Moisture was determined by thermal drying to constant weight in an oven at 110^{o}C for 24 h. Sample weight was recorded before drying and following removal from the oven, after cooling in a dessicator. Moisture was expressed as a percentage of wet weight. Crude protein was determined by combustion. Crude lipid in diets was determined by acid hydrolysis followed by Soxhlet extraction and ash content (% of dry weight) was determined by dry ashing in porcelain crucibles in a muffle furnace at 600^{o}C overnight

Total lipid was extracted from liver and muscle by homogenising in 20 volumes of chloroform/methanol (2:1, v/v) in an Ultra-Turrax tissue disrupter and was used for measurement of tissue fatty acid compositions. Fatty acid methyl esters were separated and quantified by gas-liquid chromatography.

Total carotenoid extracted from salmon muscle and diets was measured spectrophotometrically. Measurement of astaxanthin (Ax) was carried out by high performance liquid chromatography. Total fatty acid desaturation and elongation activity [the conversion of 18-carbon fatty acids obtained in the diet to essential long chain highly unsaturated fatty acids (HUFA) was measured in suspensions of isolated liver cells (hepatocytes). Hepatocytes were incubated with a radiolabelled tracer fatty acid i.e. [1-^{14}C] 18:3(n-3) at 20 ^{o}C for 2 h. after which the radioactive products were extracted and measured by thin-layer chromatography and quantified by scintillation counting.

Samples of liver, heart, kidney and white muscle were fixed buffered formol saline and embedded in paraffin wax. Five μm sections were cut and stained with haematoxylin and eosin for histological analysis. Pathological assessment was carried out on coded, randomised slides to eliminate bias in interpretation. All organs were examined for general abnormalities such as tumours, infections inflammation, loss or replacement of tissues.

Significance of difference ($P < 0.05$) between dietary treatments was determined by one-way analysis of variance (ANOVA). Differences between means were determined by Tukey's test.

There were no significant differences between initial weights at the start of the trials. The final weights also were not significantly different in both trials, either between dietary treatments or, between replicate tanks from the same treatment. Mortalities over the experimental period were < 7% for all treatments, in both trials. All groups of fish increased in weight by around 4-fold and specific growth rates (SGR) varied between 1.20 and 1.27 across the dietary treatments in trial 1 and between 0.75 and 0.79 in trial 2. Feed conversion ratios (FCR) were very similar for all treatments ranging from 1.28 to 1.34 in the first trial and 1.84 and 2.11 in the second. However, during the last 8 weeks of the second trial SGR and FCR values improved to similar values to those recorded in the first trial. The lower SGR and FCR values in the second trial can be explained by the smaller smolts used in the second trial which took longer to acclimatise to sea water coupled with the low water temperatures at the start of the trial. Once the fish were properly adapted and water temperatures improved SGR and FCR values improved accordingly. Although not significantly different fish fed the 100% RO diet had the lowest final mean weights and SGR in trial 1. However, in trial 2 the lowest final mean weight and SGR values were found in the 0% RO (control) treatment. This latter result may be explained by a better digestible energy content due to a better balance between less digestible saturated fatty acids in fish oil and more digestible monounsaturated and polyunsaturated fatty acids in the rape seed oil. This effect is likely to be more apparent at lower water temperatures as was the case, particularly in the first 8 weeks, in the second trial. No histopathologies were evident in liver, heart, muscle or kidney samples taken from fish fed the five experimental diets.

The proximate composition of flesh showed no significant differences in moisture content but lipid content was significantly greater in fish fed %0 RO (fish oil) compared to those fed 50% RO. The protein content of muscle was significantly lower in fish fed 0% RO compared to all other treatments while ash content was greatest in fish fed 10% and 50% RO followed by 0% and 25% RO and lowest in fish fed 100% RO. Liver lipid content was significantly higher in fish fed 100% RO compared to fish fed 0%, 10% and 25% RO. There was no significant difference in total carotenoid pigment and astaxanthin deposition in muscle from fish fed the different experimental diets.

The highest level of total saturated fatty acids in flesh was in fish fed 10% RO followed by those fed 0% RO and was reduced with increasing RO inclusion above 10%.

The concentrations of 16:1(n-7), 20:1(n-9) and 22:1(n-11), monounsaturated fatty acids abundant in fish oils, were highest in fish fed 0% RO and were reduced with increasing inclusion

of RO. Conversely, the concentration of 18:1(n-9) was lowest in fish fed 0% RO and was significantly increased with increasing inclusion of RO. Total monounsaturated fatty acids were highest in fish fed 100% RO and were reduced with decreasing levels of RO. The concentration of 18:2(n-6) was highest in fish fed 100% RO and was reduced with decreasing levels of RO inclusion. Arachidonic acid (20:4(n-6); ARA) concentration was significantly higher in the 0% RO muscle compared to all other treatments. As with 18:2(n-6), total n-6 PUFA were highest in fish fed 100% RO and were reduced with decreasing inclusion of RO.

The concentration of 18:3(n-3) in muscle was highest in fish fed 100% RO and were reduced with decreasing inclusion of RO. The opposite effect was observed for EPA and DHA, which are characteristically high in fish oils, where highest concentrations were found in fish fed 0% RO and lowest concentrations in fish fed 100% RO. However, in fish fed diets containing 10, 25 and 50% RO the levels of these three HUFA were not significantly different. Total PUFA were highest in fish fed 50 and 100% RO followed by those fed 0 and 25% RO with lowest levels in fish fed 10% RO. The (n-3)/(n-6) PUFA ratio was highest in fish fed 0% RO and was reduced significantly with increasing inclusion of RO.

Hepatocyte fatty acid desaturation and elongation activity was significantly affected by dietary treatment. The recovery of total (summed) desaturation products of [1-^{14}C] 18:3(n-3) was lowest in hepatocytes from fish fed 0% RO, was significantly increased in those fed 10 and 25% RO and was significantly increased again in fish fed 50% RO, with the highest concentrations in fish fed 100% RO. This result suggests that fish increase the activities of enzymes responsible for converting 18-carbon PUFA to their longer chain products in response to feeding rape seed oil.

In summary, the present study suggests that rape seed oil is an effective substitute for fish oil in Atlantic salmon, both in terms of permitting similar growth rates and feed efficiency, while having no apparent effects on fish health. However, inclusion of RO at levels in excess of 50% of supplementary lipid will result in a significant decrease in muscle (n-3)/(n-6) PUFA ratio and EPA and DHA concentrations such that the nutritional benefits to the human consumer would be considerably reduced. This does not preclude the use of higher levels of rape seed oil in dietary formulations for Atlantic salmon since such diets could be used for the majority of the growth cycle providing, at an appropriate time before marketing, the fish were returned to a fish oil-containing diet thereby restoring the 18:2(n-6), EPA and DHA concentrations to their "normal" values. i.e similar to values found in farmed salmon grown on diets containing fish oil or in wild-caught salmon. These, and other aspects of fish oil substitution, are the subject of ongoing research activity. Currently, the use of fish oil in salmon feeds in the UK accounts for more than 30 kmt per annum and that in Europe as a whole is around 4-5 fold greater. Given that annual

salmon harvests are projected to increase by around 10%/annum, and production figures for trout will probably rise by a similar percentage, the potential market for UK-grown rape seed oil is considerable. However, one of the main benefits of rape seed oil, over other vegetable oils which might be candidates for fish oil substitution, is it's relatively high concentration of 18:3(n-3). Therefore, strains of rape seed having an 18:3(n-3) content in excess of 10% of total fatty acids would be regarded as the most desirable for inclusion in fish diets. There have been attempts to reduce 18:3(n-3) in new strains of rape seed, due to the higher potential for oxidation in PUFA-rich oils. The essential nature of 18:3(n-3) in fish means that any such reduction in oils used for aquaculture would be viewed as undesirable.

INTRODUCTION

The benefits of fish consumption by humans, particularly of oily fish like salmon, herring and mackerel, due to their high content of (n-3) highly unsaturated fatty acids (HUFA), especially eicosapentaenoic acid (20:5(n-3); EPA) and docosahexaenoic acid (22:6(n-3); DHA), has been well documented (British Nutrition Foundation, 1992, 1993; International Society for the Study of Fatty Acids and Lipids (ISSFAL), 1994a & b). However, Global capture fisheries are a finite resource which, due to over fishing and subsequently tighter regulation, means that future demand for wild-caught fisheries products is likely to exceed supply (Sargent & Tacon, 1999).

Fish produced by aquaculture currently represents the fastest growing food sector and is projected to increase at around 10% per annum for at least the next ten years (Hempel, 1993). Aquaculture has traditionally utilised products from industrial fisheries, namely fish meal and oil, to convert cheap protein and oil into high value products which is sound both scientifically and commercially. However, of the global fish oil production in 1996 of 1.4 million metric tonnes (mmt), 576 kilo tonnes (kt) was used for salmon and trout production while the predicted use of fish oil for aquaculture is estimated to rise to around 85% by 2010 (Pike, 1996; Anon., 1998). In addition, competition for fish oil for inclusion in human nutritional supplements and other agriculture sectors will soon make fish meals and oils highly prized commodities. Therefore, if aquaculture is to continue to expand alternatives to the current dependence on fish oils must be investigated.

Many freshwater fish, including salmonids, can convert 18:3(n-3) to EPA and thence to DHA (Castell et al. 1972; Sargent et al. 1995, Buzzi at al 1997) and, by the same enzymatic pathways of desaturation and elongation, can convert 18:2(n-6) to 20:4(n-6). In freshwater, Atlantic salmon parr would consume invertebrates which contain an abundance of 18:2(n-6) and18:3(n-3) with lower amounts of EPA and almost no DHA (Bell et al. 1994). For this reason it is not surprising that salmon parr have been grown successfully to smoltification (sea water transfer) on diets containing rape seed and linseed oil (Bell et al. 1997; Tocher at al. 2000). Following smoltification salmon would enter the marine environment where zooplankton, crustacea and piscine prey organisms would contain an abundance of EPA and DHA. Therefore, it might be expected that the genes encoding the desaturase and elongase enzynes, responsible for the conversion of 18:3(n-3) to DHA, may be switched off, possibly irreversibly, soon after migration to sea. However, a number of studies have suggested that salmonids can utilise vegetable oils in seawater provided the diets contain enough 18:3(n-3) to satisfy essential fatty acid (EFA) requirements (Polvi & Ackman, 1992, Bell et al 1993, Guillou et al. 1995). More recent evidence suggests that there is no switch off of fatty acid metabolising enzymes in salmon post-smolts and

that fish fed vegetable oils showed increased conversion of 18:3(n-3) to DHA [and 18:2(n-6) to 20:4(n-6)] compared to those fed fish oil (Tocher et al. 1997).

At the present time the product of the salmon aquaculture industry is of high quality containing an abundance of (n-3) HUFA and a high (n-3)/(n-6) polyunsaturated fatty acid (PUFA) ratio (Bell et al. 1998). Clearly, producers and consumers of salmon will want to minimise any perceived reduction in quality, both in terms of growth and health of the fish and in the healthy image of salmon as part of the human diet, arising from the inclusion of vegetable oils. The high growth rates of cultured salmon are due to the use of high energy (oil) diets which are rich in the C_{20} and C_{22} monoenoic fatty acids characteristic of northern hemisphere marine oils. In summary, any substitute for fish oil should avoid excessive deposition of 18:2(n-6), while enhancing conversion of 18:3(n-3) to EPA and DHA, and should provide sufficient energy in the form of monoenoic fatty acids to maintain high growth rates. In this regard rape seed oil (RO) is a potential candidate having moderate levels of 18:2(n-6) and 18:3(n-3) while containing an abundance of 18:1(n-9). In this study duplicate groups of Atlantic salmon post-smolts were fed diets containing RO at 10, 25, 50 and 100% of their added oil, in comparison to fish oil (0% RO), for 17 weeks. Growth and feed utilisation were measured along with muscle and liver total lipid and fatty acid composition, muscle proximate composition, muscle carotenoid content (astaxanthin) and hepatic fatty acid desaturation and elongation activities.

MATERIALS AND METHODS

Fish, husbandry and experimental diets Three hundred and fifty Atlantic salmon S1 smolts (intitial mean weight, ~80 g) were obtained from the F.R.S. Marine Research Unit, Aultbea, Wester Ross, Scotland. The smolts were randomly distributed into 10 one metre diameter tanks of 500 L capacity supplied with non-recirculated sea water at 10 L/min at the F.R.S. Marine Research Unit. The tanks were subjected to a photoperiod regime of 12h light:12h dark and the temperature over the experimental period (August-December 1999) ranged from 7.9-14.2^{o}C with an average temperatutre of 11.7 ± 1.5^{o}C. The diets were supplied by automatic feeders, which were activated for a few seconds every 15 min during daylight hours and adjusted to provide 20g/kg biomass each day. Fish were weighed individually at the start and finish of the experiment and bulk weighed every 28 days and the ration adjusted accordingly. An initial sample was taken at the start of the trial, after 9 weeks, and final sampling was performed after 17 weeks of the feeding experiment. The experiment was conducted in accordance with the British Home Office guidelines regarding research on experimental animals.

Five practical-type commercial extruded diets were formulated (BioMar Ltd., Brande, Denmark) to provide 47% crude protein and 25% lipid and differed only in their content of rape

seed oil being added at 0, 10, 25, 50 and 100% of added oil**(Table 1)**. The diets were designed to satisfy the nutritional requirements of salmonid fish (National Research Council,1993). The proximate composition of the experimental diets including total carotenoid and astaxanthin content is shown in **Table 2.**

Sampling procedure. An initial sample of six fish was taken at the start of the experiment to determine baseline levels of lipid and fatty acid composition in liver and white muscle, and carotenoid in muscle. A similar sample of 12 fish per dietary treatment was taken after 9 wk of the trial. After 17 weeks each analytical measurement was performed on at least 10 fish, selected at random, from each tank. Fish were killed by a blow to the head and samples of liver, were dissected and immediately placed in liquid nitrogen. A muscle sample, representative of the edible portion, was obtained by cutting a steak from the "Norwegian Quality Cut" region, between the dorsal and ventral fins. This section was then skinned, de-boned and the muscle homogenised, after removing the dorsal fat body, in a Waring blendor. The homogenate was frozen in liquid nitrogen and all samples were stored at -40^{o}C prior to analysis.

Proximate analysis.

The nutrient composition of the five experimental diets and muscle samples was determined by proximate analysis. Moisture was determined by thermal drying to constant weight in an oven at 110^{o}C for 24 h. Sample weight was recorded before drying and following removal from the oven, after cooling in a dessicator. Moisture was expressed as a percentage of wet weight. Crude protein was determined by combustion using the Dumas process (AOAC, 1990). Crude lipid in diets was determined by acid hydrolysis followed by Soxhlet extraction (Woyewoda et al. 1986). Ash content (% of dry weight) was determined by dry ashing in porcelain crucibles in a muffle furnace at 600^{o}C overnight (Woyewoda et al. 1986).

Lipid extraction and fatty acid analysis.

Total lipid was extracted from liver and muscle by homogenising in 20 volumes of chloroform/methanol (2:1, v/v) in an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough,U.K.). Total lipid was prepared and measured gravimetrically according to the method of Folch et al. (1957). Fatty acid methyl esters were prepared by acid-catalysed transesterification of total lipid according to the method of Christie (1982). Extraction and purification of fatty acid methyl esters was performed as described by Ghioni et al. (1996). Fatty acid methyl esters were separated and quantified by gas-liquid chromatography (Carlo Erba Vega 6000, Milan, Italy) using a 30m x 0.32 mm i.d. capillary column (CP Wax 52CB, Chrompak, London, U.K.). Hydrogen was used as carrier gas and temperature programming was from 50^{o}C

to 150°C at 40°C/min and then to 230°C at 2.0°C/min. Individual methyl esters were identified by comparison with known standards and by reference to published data (Ackman, 1980).

Astaxanthin measurement. Total carotenoid was extracted from salmon muscle largely by the method of Barua et al. (1993). Tissue samples were homogenised in 5 ml of absolute ethanol and 5 ml of ethyl acetate using an Ultra-Turrax tissue disrupter. The homogenate was centrifuged (1000 x g, 5 min) and the supernatant removed to a stoppered glass tube. The pellet was rehomogenised in 5 ml of of ethyl acetate and recentrifuged, and the supernatant was combined with the first supernatant. Finally, the pellet was rehomogenised in 10 ml of hexane and recentrifuged, and the supernatant combined with the pooled supernatant. The pooled supernatant was dried under a stream of nitrogen and vacuum desiccated for 2 h before redissolving the residue in 2 ml of hexane containing 0.2g/L butylated hydroxy toluene (BHT). Total carotenoid was measured spectrophotometrically at 470 nm using the $E_{1\%}$ (w/v) of 2100. Measurement of astaxanthin (Ax) was carried out using the HPLC column, pump and detector described above. An isocratic solvent system was used containing ethyl acetate/methanol/water (20:72:8 v/v/v) at a flow rate of 1 ml/min. Ax was detected at 470 nm and quantified using an external standard of Ax obtained from Roche (Heanor, England). Ax in diets was extracted and measured similarly after an enzymatic digestion of the ground extruded pellets with Maxatase® (International Biosynthetics, Rijswijk, Netherlands). One gram portions of ground diet were mixed with 10 ml water and 10 mg of Maxatase in a 50 ml stoppered glass tube followed by incubation in a water bath at 50°C for 30 min.

Protein determination. Protein concentration in hepatocytes was determined according to the method of Lowry et al. (1951) after incubation with 0.25 mL of 2.5 g/L SDS/1mol/L NaOH for 45 min at 60°C.

Preparation of isolated hepatocytes. The gall bladder and main blood vessels were carefully dissected from the liver. The liver was perfused via the hepatic vein with solution A [calcium-and magnesium-free Hank's balanced salt solution (HBSS) + 10 mmol/L HEPES + 1mmol/L EDTA] to clear blood from the tissue. The liver was chopped finely with scissors and incubated with 20 mL of solution A containing 0.1% (w/v) collagenase in a 25 mL 'Reacti-flask' in a shaking water bath at 20°C for 45 min. The digested liver was filtered through 100 µm nylon gauze and the cells collected by centrifugation at 1000 x g for 5 min. The cell pellet was washed with 20 mL of solution A containing fatty acid-free bovine serum albumin (10 g/L, FAF-BSA) and recentrifuged. The hepatocytes were resuspended in 10 ml of Medium 199 containing 10 mmol/L HEPES, 2 mmol/L glutamine, 100U/mL penicillin and 0.1 mg/mL streptomycin. One hundred µL of cell suspension was mixed with 400 µL of trypan blue, and hepatocytes were counted and their

viabiltity assessed using a hemocytometer. One hundred μL of the cell suspension was retained for protein determination as described above.

Assay of hepatocyte fatty acyl desaturation/elongation activities. Five mL of each hepatocyte suspension were dispensed into two 25 cm^2 tissue culture flasks. Hepatocytes were incubated with 0.25 μCi of [1-^{14}C] 18:3(n-3) or [1-^{14}C], added as a complexe with FAF-BSA. Briefly, 25 μCi of fatty acid (0.5 μmol) in ethanol were placed in a reaction vial, solvent evaporated under a stream of nitrogen and 100 μL of 0.1mol/L KOH added. The mixture was stirred for 10 min at room temperature before 5 mL of 50 mg/mL FAF-BSA in HBSS containing 10 mmol/L HEPES buffer were added and the mixture stirred for 45 min at 20 ^{o}C. After addition of isotope the flasks were incubated at 20 ^{o}C for 2 h. After incubation, the cell layer was dislodged by gentle rocking, the cell suspension transferred to glass conical test tubes and the flasks washed with 1 mL of ice-cold HBSS containing 10 g/L FAF-BSA. The cell suspensions were centrifuged at 300 g for 2 min, the supernatant discarded and the cell pellets washed with 5 mL of ice-cold HBSS/FAF-BSA and re-centrifuged. The supernatant was again discarded and the tubes placed upside down on paper towels to blot for a 15 sec before extraction of total lipid using ice-cold chloroform/methanol (2:1, v/v) containing 0.1g/L BHT essentially as described by Folch et al. (1957) and as described in detail previously (Tocher et al. 1988).

Total lipid was transmethylated and fatty acid methyl esters prepared as described by Tocher et al. (1988). The methyl esters were redissolved in 100 μL hexane containing 0.1 g/L BHT and applied as 2.5 cm streaks to TLC plates impregnated by spraying with 2 g silver nitrate in 20 mL acetonitrile and pre-activated at 110 ^{o}C for 30 min. Plates were fully developed in toluene/acetonitrile (95:5, v/v) (Wilson and Sargent, 1992). Autoradiography was performed with Konica A2 film (Anachem Ltd. Luton, U.K.) for 4-7 days at room temperature. Silica corresponding to individual PUFA was scraped into scintillation mini-vials containing 2.5 mL of scintillation fluid (Ecoscint A. National diagnostics, Atlanta, Georgia) and radioactivity determined in a TRI-CARB 2000CA scintillation counter (United Technologies Packard, Pangbourne, England). Results were corrected for counting efficiency and quenching of ^{14}C under exactly these conditions

Histology. Samples of liver, heart, kidney and white muscle were fixed in 200 mL/L buffered formol saline and embedded in paraffin wax. Five μm sections were cut and stained with hematoxylin and eosin for histological analysis. Pathological assessment was carried out on coded, randomised slides to eliminate bias in interpretation. Examination of slides was performed using a Reichert (Microstar IV) compound microscope at an objective magnification of x 40 or x

100. All organs were examined for general abnormalities such as tumours, infections inflammation, loss or replacement of tissues.

Statistical analysis. Significance of difference ($P < 0.05$) between dietary treatments was determined by one-way analysis of variance (ANOVA). Differences between means were determined by Tukey's test. Data identified as nonhomogeneous, using Bartlett's test, were subjected to log or arcsin square root transformation before applying the ANOVA. ANOVA was performed using a Graphpad Prism ™ (version 2.0) statistical package (Graphpad Software, San Diego, CA).

RESULTS

There were no significant differences between initial weights at the start of the trials **(Tables 4 & 5)**. The final weights and lengths also were not significantly different, either between dietary treatments or, between replicate tanks from the same treatment. Mortalities over the experimental period were < 3% for all treatments in the first trial and < 7% in the second. The higher mortalities in the second trial were due to loss of small fish which did not adapt to sea wayer transfer. All groups of fish increased in weight by around 4-fold and specific growth rates (SGR) varied between 1.20 and 1.27 in the first trial and 0.75 and 0.79 in the second, across the dietary treatments. Feed conversion ratios (FCR) were very similar for all treatments ranging from 1.28 to 1.34 in the first trial and 1.84 and 2.11 in the second. However, during the last 8 weeks of the second trial SGR and FCR values improved to similar values to those recorded in the first trial. Although not significantly different fish fed the 100% RO diet had the lowest final mean weights and SGR in trial 1. However, in trial 2 the lowest final mean weight and SGR values were found in the 0% RO (control) treatment. No histopathologies were evident in liver, heart, muscle or kidney samples taken from fish fed the five experimental diets.

The proximate composition of white muscle **(Table 6)** showed no significant differences in moisture content but lipid content was significantly greater in fish fed %0 RO (fish oil) compared to those fed 50% RO. The protein content of muscle was significantly lower in fish fed 0% RO compared to all other treatments while ash content was greatest in fish fed 10% and 50% RO followed by 0% and 25% RO and lowest in fish fed 100% RO. Liver lipid content **(Table 6)** was significantly higher in fish fed 100% RO compared to fish fed 0%, 10% and 25% RO. There was no significant difference in total carotenoid pigment and astaxanthin deposition in muscle from fish fed the different experimental diets.

The fatty acid compositions of muscle total lipid are shown in **Table 7**. The concentration of 14:0 was similar in fish fed 0 and 10% RO but decreased significantly with increasing dietary

inclusion of RO. The concentration of 16:0 was significantly higher in muscle of fish fed 10% RO, compared to those fed 0% RO, but was significantly reduced with increasing RO inclusion above 10%. The highest level of total saturated fatty acids was in fish fed 10% RO followed by those fed 0% RO and was significantly reduced with increasing RO inclusion above 10%.

The concentrations of 16:1(n-7) and 20:1(n-9) were highest in fish fed 0% RO and were significantly reduced with increasing inclusion of RO. Conversely, the concentration of 18:1(n-9) was lowest in fish fed 0% RO and was significantly increased with increasing inclusion of RO. The concentration of 18:1(n-7) was significantly greater in fish fed 100% RO compared to fish fed 0 and 25% RO. The concentration of 22:1(n-11) was highest in fish fed 0% RO and was reduced with increasing inclusion of RO although the values for fish fed 25 and 50% RO were not significantly different. The highest concentration of 24:1 was found in fish fed 50% RO followed by those fed 0 and 25% RO with the lowest levels in fish fed 10% and 100% RO. Total monounsaturated fatty acids were highest in fish fed 100% RO and were reduced with decreasing levels of RO inclusion although in fish fed 10% and 25% RO the values were not significantly different.

The concentration of 18:2(n-6) was highest in fish fed 100% RO and was reduced significantly with decreasing levels of RO inclusion. A similar trend was observed for 20:2(n-6) and 20:3(n-6) although the concentration of 20:2(n-6) was not significantly different between the 0 and 10% RP treatments while the values for 20:3(n-6) in the 10, 25 and 50% RO treatments were not significantly different. ARA concentration was significantly higher in the 0% RO muscle compared to all other treatments. As with 18:2(n-6), total n-6 PUFA were highest in fish fed 100% RO and were reduced significantly with decreasing inclusion of RO.

The concentration of 18:3(n-3) in muscle was highest in fish fed 100% RO and were reduced significantly with decreasing inclusion of RO. The opposite effect was observed for 18:4(n-3) with the highest concentration in fish fed 0% RO and the lowest in those fed 100% RO. Similar trends were observed for 20:4(n-3), EPA and DHA where highest concentrations were found in fish fed 0% RO and lowest concentrations in fish fed 100% RO. However, in fish fed diets containing 10, 25 and 50% RO the levels of these three HUFA were not significantly different. The concentrations of 22:5(n-3) and total (n-3) PUFA were highest in fish fed 0% RO followed by those fed 25 and 50% RO and then fish fed 10% RO with the lowest levels in fish fed 100% RO. Total PUFA were highest in fish fed 50 and 100% RO followed by those fed 0 and 25% RO with lowest levels in fish fed 10% RO. The (n-3)/(n-6) PUFA ratio was highest in fish fed 0% RO and was reduced significantly with increasing inclusion of RO.

The fatty acid compositions of liver total lipid are shown in **Table 8**. In general terms the effects of the experimental diets on fatty acid compositions of liver are very similar to those which have been described in muscle. However, a number of important differences were observed particularly with respect to monounsaturated fatty acids and HUFA. While the dietary induced changes in liver monounsaturated fatty acids are similar to those found in muscle to levels of monounsaturated fatty acids are much lower in liver while PUFA, particularly the (n-3) HUFA, are increased. For both EPA and DHA the highest concentrations were found in fish fed 0% RO, but only small reductions were seen in fish fed 10 and 25% RO, with more substantial reductions in these HUFA seen in fish fed 50 and 100% RO.

Hepatocyte fatty acid desaturation and elongation activity was significantly affected by dietary treatment **(Figs.1 & 2)**. The recovery of total (summed) desaturation products of [1-^{14}C] 18:3(n-3) **(Fig. 1)** was lowest in hepatocytes from fish fed 0% RO, was significantly increased in those fed 10 and 25% RO and was significantly increased again in fish fed 50% RO, with the highest concentrations in fish fed 100% RO. The effect of diet on individual desaturation and elongation products of [1-^{14}C] 18:3(n-3) is shown in **Fig. 2.** The product of Δ6-desaturation, 18:4(n-3), was increased significantly, in stepwise fashion, in hepatocytes from fish fed diets containing 25, 50 and 100% RO, compared to fish fed 0% RO. The product of Δ6-desaturation and elongation, 20:4(n-3), was the most abundant metabolite of radiolabelled 18:3(n-3) recovered from the isolated hepatocytes and was increased significantly with increasing dietary RO inclusion in a similar way to 18:4(n-3). Although much lower concentrations of DHA were produced by isolated hepatocytes incubated with radiolabelled 18:3(n-3), in comparison to products of Δ6- and Δ5-desaturation, the highest concentrations were found in fish fed 50 and 100% RO followed by 25% RO with lowest levels in fish fed 10 and 0% RO.

DISCUSSION

Salmon production worldwide in 1996 was 0.64 mmt and production for 2000 is expected to be around 0.8 mmt with a 1 mmt harvest predicted around 2002. With global fish oil supplies static, or even in decline, the expansion of aquaculture production in general, and salmon production in particular, will only occur if other oil sources are utilised. A number of studies have investigated the suitability of different vegetable oils as replacements for fish oil in a range of fish species including, rainbow trout (*Oncorhynchus mykiss)* (Cho et al. 1974; Greene and Selivonchick, 1990), brown trout (*Salmo trutta)* (Arzel et al. 1994), arctic charr (*Salvelinus alpinus)* (Olsen and Henderson, 1997), brook charr (*Salvelinus fontinalis)* (Guillou et al. 1995), Atlantic salmon (Hardy et al. 1987; Polvi and Ackman 1992; Bell et al. 1993) and chinook

salmon (*Oncohynchus tshawytscha)* (Dosanjh et al. 1988). However, while these studies considered effects of diet on fish growth, lipid absorption and fish health they did not consider the implications for the human consumer nor did they use oil-rich diet formulations similar to the high energy feeds currently favoured for salmonid culture. Given present concerns about the imbalance of (n-6) and (n-3) fatty acids in the diets of developed nations and the encouragement to consume oily fish, such as mackerel, sardines, salmon and trout, it is important that cultured salmon maintains a high level of essential (n-3) HUFA in the edible flesh (ISSFAL, 1994a & b). Therefore, successful substitution of fish oil with vegetable oils should maximise desaturation and elongation of 18:3(n-3) to EPA and DHA while minimising deposition of 18:2(n-6) and, at the same time, permit equivalent growth rates to those presently achieved with fish oils. The present study has investigated the potential of rapeseed oil, fed at different inclusion levels, to satisfy the above criteria in Atlantic salmon post-smolts in sea water.

In the present study feeding diets containing RO, in the range 10-100% of added oil, had no significant effect on growth rate and feed conversion ratio, compared to fish fed marine fish oil (0% RO). However, although not significantly different from the other treatments fish fed 100% RO had lower mean final weights, and hence lower SGR, than the other four treatments. In a longer feeding trial this effect may have been significant in fish fed 100% RO. However, in the second dietary trial the fish fed 0% RO had the lowest mean final weights and the lowest SGR of all the treatments. This latter result may be explained by a better digestible energy content due to a better balance between less digestible saturated fatty acids in fish oil and more digestible monounsaturated and polyunsaturated fatty acids in the rape seed oil. This effect is likely to be more apparent at lower water temperatures as was the case, particularly in the first 8 weeks of the second trial. In previous studies using rape seed oil as lipid source, (canola) brook charr, chinook salmon and Atlantic salmon in freshwater (Guillou et al. 1995; Dosanjh et al. 1988; Tocher et al. 2000), and Atlantic salmon in seawater (Polvi et al1992) showed no reduction in growth rate and FCR although dietary lipid contents were lower (14-19%) than in the present study.

The percentage of lipid in the muscle was significantly greater in fish fed 0% RO compared to fish fed 50% RO although the differences between the dietary treatments were small. Conversely, the muscle protein content was significantly lower in fish fed 0% RO, compared to all other treatments, and the second highest muscle lipid level, found in fish fed 100% RO, was correlated with the second lowest protein content. This correlation between muscle protein and lipid content has been observed in previous studies with salmonids (Reinitz and Hitzel, 1980; Jokumsen and Alsted, 1990). Interestingly, liver lipid concentration was significantly higher in fish fed 100% RO compared to the other four treatments. This result, coupled with the second highest value for

muscle lipid content, suggests that high levels of RO inclusion might lead to increased adiposity, particularly if these diets were fed for a longer period. In other studies where rape seed oil was fed to salmonids no significant differences in muscle proximate compositions were observed, although these studies either used diets with lower lipid content and smaller fish (Dosanjh et al. 1988), or used fish which had only doubled in weight and only three fish per treatment were analysed (Dosanjh et al. 1998). Variation in the type and quantity of dietary PUFA can influence uptake and deposition of carotenoid pigments in salmon flesh (Christiansen et al. 1993) although in the present study there were no significant differences in total muscle carotenoid and astaxanthin concentration between the five dietary treatments. Flesh pigmentation is an important factor in perception of flesh quality in salmonids (Bell et al. 1998, Refsgaard et al 1998) and a previous study observed reduced pigment deposition in salmon when fish oil was replaced with soybean oil or beef tallow (Hardy et al. 1987).

The effects of increased inclusion of RO in diet formulations is reflected in increased levels of the fatty acids predominant in RO, namely 18:1(n-9), 18:2(n-6) and 18:3(n-3), in the diet formulations. Consequently, the predominant fatty acids in fish oils namely 20:1(n-9), 22:1(n-11), 20:5(n-3) and 22:6(n-3) are reduced with increasing inclusion of RO in diet formulations. The accumulation of total saturated fatty acids, total monounsaturated fatty acids and 18:1(n-9) in the muscle closely reflected the composition of these fatty acids in the diet. A similar accumulation of these fatty acids is seen in liver although values are generally less well correlated to diet values than is the case with muscle. This reflects the predominance of storage triacylglycerols in salmon muscle whereas liver contains predominantly more phospholipids whose composition is not so closely related to dietary input (Henderson and Tocher, 1987; Bell et al. 1998). The concentration of 18:2(n-6) and 18:3(n-3) in muscle and liver, although correlated with increasing levels in the diet, were always present at lower levels than in the diet, particularly in the liver. EPA and DHA concentrations were significantly reduced in muscle and liver with increasing rape seed oil inclusion although the reduction in fish fed 10, 25 and 50% RO was relatively small and less than the reduction of the same fatty acids in the diets. This suggests that either there is a preferential incorporation of EPA and DHA into these tissues or it reflects increased desaturation and elongation of 18:3(n-3) to EPA and DHA. The latter effect is supported by the increased hepatic desaturation and elongation activities measured in the present study **(Figs. 1 & 2)**. Similar increases in hepatic desaturation and elongation activities have been observed in previous studies where salmon have been fed vegetable oils, or vegetable oil blends (Bell et al 1997; Tocher et al. 1997, 2000). However, despite hepatocytes from fish fed 100% RO having the highest fatty acid desaturation and elongation activities these fish are unable to synthesise enough EPA and DHA,

from dietary 18:3(n-3), to maintain normal levels of these HUFA in the muscle. It does appear, however, that in salmon fed diets containing RO up to 50% the endogenous production of EPA and DHA from 18:3(n-3) can maintain the levels of these HUFA in the fish tissues at levels on slightly lower than found in fish fed fish oil (0% RO).

In previous studies with Atlantic salmon fed vegetable oils, including corn, sunflower, grape seed and safflower oils, there was a significant increase in concentrations of arachidonic acid [20:4(n-6)] in fish tissues, resulting in increased eicosanoid production, and the appearance of cardiac histopathologies (Bell et al. 1989, 1991, 1992, 1993). All the oils mentioned above contain high levels of 18:2(n-6) and only trace levels of 18:3(n-3). The presence of significant levels of 18:3(n-3) in rape seed oil, despite an excess of 18:2(n-6), appears to prevent significant conversion of the latter to 20:4(n-6) in the present study. The desaturation and elongation of radiolabelled 18:2(n-6) to 20:4(n-6) has been shown be significantly lower than the conversion of 18:3(n-3) to EPA and DHA in a previous study with Atlantic salmon (Bell et al. 1997). The reason for this can be explained by the relative affinity of the two C_{18} precursors for the Δ6-desaturase enzyme where linolenic acid [18:3(n-3)] is preferred over 18:2(n-6) for metabolic conversion (Brenner , 1981; Christiansen et al. 1991). Therefore, while an excess of (n-6) PUFA may inhibit desaturation and elongation of 18:3(n-3) in general the (n-3) PUFA are more efficient in inhibiting the metabolism of 18:2(n-6) than vice versa (Horrobin, 1991).

In summary, the present study suggests that rape seed oil is an effective substitute for fish oil in Atlantic salmon, both in terms of permitting similar growth rates and feed efficiency, while having no apparent effects on fish health. However, inclusion of RO at levels in excess of 50% of supplementary lipid will result in a significant decrease in muscle (n-3)/(n-6) PUFA ratio and EPA and DHA concentrations such that the nutritional benefits to the human consumer would be considerably reduced. This does not preclude the use of higher levels of rape seed oil in dietary formulations for Atlantic salmon since such diets could be used for the majority of the growth cycle providing, at an appropriate time before marketing, the fish were returned to a fish oil-containing diet thereby restoring the 18:2(n-6), EPA and DHA concentrations to their “normal” values. These, and other aspects of fish oil substitution, are the subject of ongoing research activity.

ACKNOWLEDGMENTS

We would like to thank Philip MacGlaughlin and the staff of the Fisheries Research Services Marine Research Unit for their assistance with fish husbandry.

LITERATURE CITED

Ackman, R.G. (1980) Fish lipids, part 1. In: Advances in Fish Sciences and Technology (Connell, J.J. ed.), pp. 86-103. Fishing News Books Ltd., Farnham, U.K.

Anon. (1998) World fish meal production plunging to 15 year low. Oil World 41: 108.

Association of Official Analytical Chemists (1990) Official Methods of Analysis Volume 1. 15th Edition. pp. 71-72.

Arzel, J., Martinez-Lopez, F.X., Metallier, R., Stephan, G., Viau, M., Gandemer, G. and Guillaume, J. (1994) Effect of dietary lipid on growth performance and body composition of brown trout (*Salmo trutta)* raised in seawater. Aquaculture 123: 361-375.

Barua, A.B., Kostic, D. & Olson, J.A. (1993) New simplified procedures for the extraction and simultaneous high-performance liquid chromatographic analysis of retinol, tocopherols and carotenoids in human serum. J. Chromatogr. 617: 257-264.

Bell, J.G., Dick, J.R., McVicar, A.H., Sargent, J.R. & Thompson, K.D. (1993) Dietary sunflower, linseed and fish oils affect phospholipid fatty acid composition, development of cardiac lesions, phospholipase activity and eicosanoid production in Atlantic salmon, *Salmo salar*. Prostaglandins, Leukotienes & Essent. Fatty Acids 49: 665-673.

Bell, J.G., Dick, J.R., Sargent, J.R. & McVicar, A.H. (1992) Dietary linoleic acid affects phospholipid fatty acid composition in heart and eicosanoid production by cardiomyocytes from Atlantic salmon (*Salmo salar).* Comp. Biochem. Physiol. 103A: 337-342.

Bell, J.G., Ghioni, C. & Sargent, J.R. (1994) Fatty acid compositions of ten freshwater invertebrates which are natural food organisms of Atlantic salmon parr (*Salmo salar)*; a comparison with commercial diets. Aquaculture 128: 301-313.

Bell, J.G., McEvoy, J., Webster, J.L., McGhee, F., Millar, R.M. & Sargent, J.R. (1998) Flesh lipid and carotenoid composition of Scottish farmed Atlantic salmon (*Salmo salar).* J.Agric. Food Chem. 46: 119-127.

Bell, J.G., McVicar, A.H., Park, M.T. & Sargent, J.R. (1991) High dietary linoleic acid affects the fatty acid compositions of individual phospholipids from tissues of Atlantic salmon (*Salmo salar)*: Association with stress susceptibility and cardiac lesion. J. Nutr. 121: 1163-1172.

Bell, J.G., Tocher, D.R., Farndale, B.M., Cox, D.I., McKinney, R.W. & Sargent, J.R. (1997) The effect of dietary lipid on polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar)* undergoing parr-smolt transformation. Lipids 32: 515-525.

Bell, J.G., Youngson, A., Mitchell, A.I. & cowey, C.B. (1989) The effect of enhanced intake of linoleic acid on the fatty acid composition of tissue polar lipids of post-smolt Atlantic salmon (*Salmo salar).* Lipids 24: 240-242.

Brenner, R.R. (1981) Nutritional and hormonal factors influencing desaturation of essential fatty acids. Prog. Lipid Res. 20: 41-47.

British Nutrition Foundation Task Force (1992) Unsaturated Fatty Acids. Nutritional and Physiological Significance. The Report of the British Nutritional Foundation Task Force. Chapman & Hall, London 211pp.

British Nutrition Foundation (1993) Nutritional Aspects of Fish. British Nutrition Foundation Briefing Paper No. 10. 18pp. British Nutrition Foundation, London.

Buzzi, M., Henderson, R.J. & Sargent, J.R. (1997) Biosynthesis of docosahexaenoic acid proceeds via 24-carbon intermediates. Comp. Biochem. Physiol. 116B: 263-267.

Castell, J.D., Lee, D.J. & Sinnhuber, R.O. (1972) Essential fatty acids in the diet of rainbow trout: lipid metabolism and fatty acid composition. J.Nutr. 102: 93-99.

Cho, C.Y., Bayley, H.S. & Slinger, S.J. (1974) Partial replacement of herring meal with soybean meal and other changes in a diet for rainbow trout (*Salmo gairdneri).* J. Fish. Res. Bd. Can. 31: 1523-1528.

Christie, W.W. (1982) Lipid Analyses, 2nd ed., pp. 52-56. Pergamon Press, Oxford, England.

Christiansen, E.N., Lund, J.S., Rørtveit, T. & Rustan, A.C. (1991) Effect of dietary n-3 and n-6 fatty acids on fatty acid desaturation in rat liver. Biochim. Biophys. Acta 1082: 57-62.

Christiansen, R., Waagbø, R. & Torissen, O.J. (1993) Effects of polyunsaturated fatty acids and vitamin E on flesh pigmentation in Atlantic salmon, *Salmo salar.* In: Fish Nutrition in Practice (Kaushik, S.J. & Luquet, P. eds.) Proceedings of the 4th International Symposium on Fish Nutrition and Feeding, Biarritz, France. INRA Editions, Paris. pp. 339-343.

Dosanjh, B.S., Higgs, D.A., McKenzie, D.J., Randall, D.J., Eales, J.G., Rowshandeli, N., Rowshandeli, M. & Deacon, G. (1998) Influence of dietary blends of menhaden oil and canola oil on growth, muscle lipid composition, and thyroidal staus of Atlantic salmon (*Salmo salar)* in sea water. Fish Physiol. Biochem. 19: 123-134.

Dosanjh, B.S., Higgs, D.A., Plotnikoff, M.D., Markert, J.R. & Buckley, J.T., (1988) Preliminary evaluation of canola oil, pork lard and marine lipid singly and in combination as supplemental dietary lipid sources for juvenile fall chinook salmon (*Oncorhynchus tshawytscha).* Aquaculture 68: 325-343.

Folch, J., Lees, M. & Sloane-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497-509.

Ghioni, C., Bell, J.G. & Sargent, J.R. (1996) Polyunsaturated fatty acids in neutral lipids and phospholipids of some freshwater insects. Comp. Biochem. Physiol. 114B: 161-170.

Greene, D.H.S. & Selivonchick, D.P. (1990) Effects of dietary vegetable, animal and marine lipids on muscle lipid and hematology of rainbow trout (*Oncorhynchus mykiss).* Aquaculture 89: 165-182.

Guillou, A., Soucy, P., Khalil, M. and Adambounou, L. (1995) Effects of dietary vegetable and marine lipid on growth, muscle fatty acid composition and organoleptic quality of flesh of brook charr (*Salvelinus fontinalis)* Aquaculture 136: 351-362.

Hardy, R.W., Scott, T.M. & Harrell, L.W. (1987) Replacement of herring oil with menhaden oil, soybean oil, or tallow in the diets of Atlantic salmon raised in marine net-pens. Aquaculture 65: 267-277.

Henderson, R.J. & Tocher, D.R. (1987) The lipid composition and biochemistry of freshwater fish. Prog. Lipid Res. 26: 281-347.

Hempel, E. (1993) Constraints and possibilities for developing aquaculture. Aquac. Int. 1: 2-19.

Horrobin, D.F. (1991) Interactions between n-3 and n-6 essential fatty acids (EFAs) in the regulation of cardiovascular disorders and inflammation. Prostaglandins, Leukotrienes Essent. Fatty Acids 44: 127-131.

International Society for the Study of Fatty Acids and Lipids (1994a) Health message statement. International Society for the Study of Fatty Acids and Lipids News Letter. 1: 3-4.

International Society for the Study of Fatty Acids and Lipids (1994b) Recommendations for the essential fatty acid requirements for infant formulae. International Society for the Study of Fatty Acids and Lipids News Letter. 1: 4-5.

Jokumsen, A. & Alsted, N. (1990) Ørredvaekst I relation til forholdet mellem protein og fedt I foderet. Meddelesler fra Forsøgsdambruget, 80: 1-15.

Lowry, O.H., Roseborough, N.J., Farr, A.L. & Randall, R.J. (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265-275.

National Research Council (1993). Nutrient Requirements of Fish. National Academy Press, Washington DC. ISBN 0-309-04891-5.

Olsen, R.E. & Henderson, R.J. (1997) Muscle fatty acid composition and oxidative stress indices in Arctic charr, *Salvelinus alpinus* (L.), in relation to dietary polyunsaturated fatty acid levels and temperature. Aquac. Nutr. 3: 227-238.

Pike, I. (1996) Future role of fish meal and oil in salmon diets. Salmon News 4/5: 3-5.

Polvi, S.M. & Ackman, R.G. (1992) Atlantic salmon (*Salmo salar)* muscle lipids and their response to alternative fatty acid sources. J. Agric. Food Chem. 40: 1001-1007.

Refsgaard, H.H.F., Brockhoff, P.B. & Jensen, B. (1998) Biological variation of lipid constituents and distribution of tocopherols and astaxanthin in farmed Atlantic salmon (*Salmo salar).* J. Agric. Food Chem. 46: 808-812.

Reinitz, G. & Hitzel, F. (1980) Formulation of practical diets for rainbow trout based on desired performance and body composition. Aquaculture 19: 243-252.

Sargent, J.R., Bell, J.G., Bell, M.V., Henderson, R.J. & Tocher, D.R. (1995) Requirement criteria for essential fatty acids. J. Appl. Ichthyol. 11: 183-198.

Sargent, J.R., & Tacon, A.G.J. (1999) Development of farmed fish: a nutritionally necessary alternative to meat. Proc. Nutr. Soc. 58: 377-383.

Tocher, D.R., Bell, J.G., Dick, J.R., Henderson, R.J., McGhee, F., Mitchell, D.F. & Morris, P.C. (2000) Polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar)* undergoing parr-smolt transformation and the effects of dietary linseed and rapeseed oils. Fish Physiol. Biochem. "in press".

Tocher, D.R., Bell, J.G., Dick, J.R. & Sargent, J.R. (1997) Fatty acyl desaturation in isolated hepatocytes from Atlantic salmon (*Salmo salar)* : stimulation by dietary borage oil containing □-linolenic acid. Lipids 32: 1237-1247.

Tocher, D.R., Sargent, J.R. & Freriches, G.N. (1988). The fatty acid compositions of established fish cell lines after long-term culture in mammalian sera. Fish Physiol. Biochem. 5: 219-227.

Wilson, R. & Sargent, J.R. (1992). High-resolution separation of polyunsaturated fatty acids by argentation thin-layer chromatography. J. Chromatogr. 623: 403-407.

Woyewoda, A.D., Shaw, S.J., Ke, P.J. & Burns, B.G. (1986) Recommended Laboratory Methods for Assessment of Fish Quality. Canadian Technical Report of Fisheries and Aquatic Sciences No. 1448.

Figure legends;

FIGURE 1 Total amount of [1-^{14}C] 18:3(n-3) desaturated and elongated /hour per mg protein by isolated hepatocytes from Atlantic salmon post-smolts fed diets containing 0, 10, 25, 50 and 100% of supplementary dietary lipid as rape seed oil (RO). Each column represents the mean ± SD for four replicate samples. Columns assigned a different letter are significantly different between dietary treatment ($P < 0.05$) as determined by ANOVA and Tukey's test as described in Materials and Methods.

FIGURE 2 Amount of [1-^{14}C] 18:3(n-3) desaturated and elongated to18:4(n-3), 20:4(n-3), 20:5(n-3) and 22:6(n-3) by isolated hepatocytes from Atlantic salmon post-smolts fed diets containing 0, 10, 25, 50 and 100% of supplementary dietary lipid as rape seed oil (RO). Each column represents the mean ± SD for four replicate samples. Columns representing each fatty acid assigned a different letter are significantly different between dietary treatment ($P < 0.05$) as determined by ANOVA and Tukey's test as described in Materials and Methods.

TABLE 1

Feed components (% of dry weight)

Component	0% RO	10% RO	25% RO	50% RO	100% RO
Fish meal[1]	60	60	60	60	60
Corn gluten[2]	2.96	2.96	2.96	2.96	2.96
Wheat[3]	15.17	15.17	15.17	15.17	15.17
Fish oil[4]	19.0	17.1	14.25	9.5	0
Rapeseed oil[5]	0	1.9	4.75	9.5	19.0
Methionine	0.3	0.3	0.3	0.3	0.3
Micronutrients[6]	2.57	2.57	2.57	2.57	2.57

[5]Croda Universal Ltd., Hull, U.K.

[6]Vitamins, minerals and astaxanthin (carophyll pink®)

TABLE 2

Proximate composition of experimental diets including carotenoid pigment content.

Analysis	0% RO	10% RO	25% RO	50% RO	100% RO
			g/100g		
Protein	46.7	46.3	44.8	45.6	46.9
Oil	27.7	26.4	26.3	25.7	25.6
Ash	9.8	9.6	9.6	9.7	9.7
Moisture	7.8	8.1	8.2	7.5	6.8
Total carotenoid[1]	50.6	48.8	49.5	53.1	50.6
Astaxanthin[1]	48.3	47.6	48.4	53.3	50.1

[1]Values are in mg/kg diet.

TABLE 3

Fatty acid composition of the five experimental diets.

Parameter/Diet	0% RO	10% RO	25% RO	50% RO	100% RO
			g/100g total fatty acids		
Total saturates	23.9	21.4	19.4	17.4	11.9
Total monoenes	42.5	45.9	47.3	50.7	56.4
18:1(n-9)	12.3	18.8	23.3	29.6	43.9
18:2(n-6)	2.4	5.1	7.1	9.4	15.4
20:4(n-6)	0.6	0.4	0.4	0.3	0.2
Total (n-6)	4.6	7.2	9.1	11.0	16.7
18:3(n-3)	1.6	2.9	3.8	4.9	8.1
20:5(n-3)	8.5	6.8	6.5	5.2	2.6
22:6(n-3)	11.9	10.1	8.7	6.8	2.8
Total (n-3)	27.4	24.4	22.8	19.8	14.5
(n-3)/(n-6) ratio	6.1	3.4	2.5	1.8	0.9

TABLE 4

Growth and performance parameters of Atlantic salmon post-smolts fed experimental diets for 17 weeks.

Parameter	0% RO	10% RO	25% RO	50% RO	100% RO
Initial weight (g)[1]	80.3 ± 3.9	83.8 ± 4.6	83.6 ± 5.1	85.3 ± 5.8	80.8 ± 3.9
Final weight (g)[2]	343.3 ± 20.1	352.9 ± 21.5	350.8 ± 19.2	364.1 ± 19.9	320.3 ± 16.5
Final length (cm)[2]	30.1 ± 0.4	30.4 ± 0.4	30.3 ± 0.4	30.9 ± 0.4	29.5 ± 0.4
Mortalities	1	2	1	1	0
SGR[3]	1.25	1.26	1.25	1.27	1.20
FCR[4]	1.32	1.34	1.30	1.28	1.31

[1] Values are mean ± SEM (n = 70).

[2] Values are mean ± SEM (n = 60-64).

[3] SGR = specific growth rate (%/day) = $(e^{Gw} - 1) \times 100$ where $Gw = \ln W_1 - \ln W_0/T$. Where W_1 = final weight, W_0 = initial weight and T = time in days.

[4]FCR = Feed conversion ratio = Feed consumed/weight gain.

TABLE 5

Growth and performance parameters of Atlantic salmon post-smolts fed experimental diets for 26 weeks.

Parameter	0% RO	10% RO	25% RO	50% RO	100% RO
Initial weight (g)[1]	42.0 ± 1.8	42.0 ± 1.8	43.8 ± 1.6	44.1 ± 1.7	42.9 ± 1.4
Final weight (g)[2]	163.9 ± 8.5	175.3 ± 6.5	175.6 ± 5.2	185.8 ± 7.8	177.1 ± 6.0
Mortalities	4	4	3	5	5
SGR[3]	0.75	0.79	0.76	0.79	0.78
FCR[4]	2.11	1.84	1.87	1.86	1.99
SGR (final 8 weeks)	1.06	1.15	1.17	1.11	1.22
FCR (final 8 weeks)	1.47	1.28	1.25	1.24	1.34

[1] Values are mean ± SEM (n = 70).

[2] Values are mean ± SEM (n = 60-64).

[3] SGR = specific growth rate (%/day) = $(e^{Gw} - 1) \times 100$ where $Gw = \ln W_1 - \ln W_0/T$. Where W_1 = final weight, W_0 = initial weight and T = time in days.

[4]FCR = Feed conversion ratio = Feed consumed/weight gain.

TABLE 6

Proximate composition of muscle, lipid content of liver and carotenoid pigment content of muscle in salmon fed diets containing increasing levels of rape seed oil.

Parameter	0% RO	10% RO	25% RO	50% RO	100% RO
Moisture (%)[1]	74.7 ± 3.4	75.1 ± 2.8	75.2 ± 3.2	75.2 ± 3.1	74.2 ± 2.9
Lipid (%)[1]	5.37 ± 0.72[a]	4.75 ± 0.95[ab]	4.64 ± 0.78[ab]	4.50 ± 0.71[b]	5.10 ± 0.61[ab]
Protein (%)[2]	73.0 ± 3.0[b]	75.9 ± 2.8[a]	76.9 ± 3.2[a]	76.9 ± 3.1[a]	75.5 ± 2.4[a]
Ash (%)[2]	5.75 ± 0.40[b]	6.74 ± 0.41[a]	5.76 ± 0.36[b]	6.79 ± 0.45[a]	5.32 ± 0.31[c]
Liver lipid (%)[1]	3.59 ± 0.33[b]	3.61 ± 0.28[b]	3.64 ± 0.37[b]	3.96 ± 0.66[ab]	4.40 ± 0.53[a]
Total pigment[3]	2.45 ± 0.36	2.54 ± 0.47	2.41 ± 0.40	2.60 ± 0.38	2.56 ± 0.35
Astaxanthin[3]	2.19 ± 0.42	2.31 ± 0.59	2.07 ± 0.53	2.21 ± 0.39	2.38 ± 0.41

[1]Values are % of wet weight.

[2]Values are % of dry weight.

[3]Total carotenoid pigment and astaxanthin (mg/kg).

All values are mean ± SD, n = 20.

Values within each row assigned a different superscript letter are significantly different.

TABLE 7

Fatty acid compositions of muscle total lipid in Atlantic salmon post-smolts fed diets containing increasing levels of rape seed oil

Fatty acid	0% RO	10% RO	25% RO	50% RO	100% RO
			g/100g total fatty acids		
14:0	3.9 ± 0.6^{a}	4.0 ± 0.5^{a}	3.4 ± 0.2^{b}	2.6 ± 0.2^{c}	1.4 ± 0.1^{d}
16:0	17.3 ± 1.5^{b}	18.3 ± 1.2^{a}	15.5 ± 0.4^{c}	12.7 ± 0.4^{d}	10.3 ± 0.4^{e}
18:0	2.7 ± 0.4	2.7 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	2.7 ± 0.1
Total saturates[1]	24.5 ± 2.1^{b}	25.7 ± 1.5^{a}	22.3 ± 0.4^{c}	18.7 ± 0.5^{d}	14.8 ± 0.5^{e}
16:1(n-7)	5.0 ± 0.4^{a}	4.5 ± 0.2^{b}	3.9 ± 0.2^{c}	3.1 ± 0.2^{d}	1.8 ± 0.1^{e}
18:1(n-9)	17.1 ± 1.3^{e}	23.4 ± 1.0^{d}	26.0 ± 0.9^{c}	28.5 ± 0.6^{b}	39.1 ± 0.8^{a}
18:1(n-7)	2.6 ± 0.2^{b}	2.9 ± 0.9^{ab}	2.6 ± 0.5^{b}	3.0 ± 0.1^{ab}	3.2 ± 0.3^{a}
20:1(n-9)	7.6 ± 0.4^{a}	6.3 ± 0.2^{b}	5.7 ± 0.1^{c}	5.5 ± 0.2^{d}	4.1 ± 0.1^{e}
22:1(n-11)	8.9 ± 0.7^{a}	6.7 ± 0.7^{b}	6.0 ± 0.2^{c}	5.6 ± 0.2^{c}	2.6 ± 0.1^{d}
24:1	0.8 ± 0.1^{b}	0.7 ± 0.1^{c}	0.8 ± 0.1^{b}	0.9 ± 0.1^{a}	0.7 ± 0.1^{c}
Total monoenes[2]	42.3 ± 1.3^{d}	44.7 ± 1.4^{c}	45.2 ± 0.8^{c}	46.9 ± 0.9^{b}	51.8 ± 0.7^{a}
18:2(n-6)	2.5 ± 0.2^{e}	4.8 ± 0.2^{d}	6.2 ± 0.2^{c}	8.1 ± 0.2^{b}	12.3 ± 0.3^{a}
20:2(n-6)	0.4 ± 0.0^{d}	0.4 ± 0.0^{d}	0.6 ± 0.1^{c}	0.8 ± 0.0^{b}	1.2 ± 0.1^{a}
20:3(n-6)	0.1 ± 0.0^{c}	0.2 ± 0.1^{b}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.5 ± 0.1^{a}
20:4(n-6)	0.5 ± 0.1^{a}	0.4 ± 0.1^{b}	0.4 ± 0.1^{b}	0.4 ± 0.0^{b}	0.4 ± 0.1^{b}
Total (n-6)[3]	3.7 ± 0.2^{e}	5.8 ± 0.3^{d}	7.5 ± 0.1^{c}	9.6 ± 0.2^{b}	14.6 ± 0.2^{a}
18:3(n-3)	1.4 ± 0.1^{e}	2.1 ± 0.1^{d}	2.9 ± 0.1^{c}	4.0 ± 0.1^{b}	5.6 ± 0.2^{a}
18:4(n-3)	2.0 ± 0.1^{a}	1.5 ± 0.1^{b}	1.3 ± 0.1^{c}	1.2 ± 0.1^{d}	0.7 ± 0.1^{e}
20:4(n-3)	1.5 ± 0.2^{a}	1.1 ± 0.2^{b}	1.2 ± 0.1^{b}	1.1 ± 0.1^{b}	0.7 ± 0.1^{c}
20:5(n-3)	5.8 ± 0.9^{a}	4.2 ± 0.5^{b}	4.6 ± 0.3^{b}	4.3 ± 0.3^{b}	2.8 ± 0.2^{c}
22:5(n-3)	2.1 ± 0.3^{a}	1.4 ± 0.2^{c}	1.7 ± 0.1^{b}	1.7 ± 0.1^{b}	1.1 ± 0.1^{d}
22:6(n-3)	14.8 ± 1.9^{a}	11.6 ± 1.4^{b}	11.6 ± 0.7^{b}	11.2 ± 0.7^{b}	7.2 ± 0.7^{c}
Total (n-3)	27.7 ± 2.8^{a}	22.0 ± 2.0^{c}	23.3 ± 0.8^{bc}	23.9 ± 0.9^{b}	18.4 ± 0.8^{d}
Total PUFA	32.3 ± 2.8^{b}	28.3 ± 2.2^{c}	31.6 ± 0.7^{b}	34.2 ± 0.9^{a}	33.3 ± 0.7^{a}
(n-3)/(n-6)	7.5 ± 0.7^{a}	3.8 ± 0.3^{b}	3.1 ± 0.1^{c}	2.5 ± 0.1^{d}	1.3 ± 0.1^{e}

[1]Includes 15:0, 17:0, 20:0 and 22:0. [2]Includes 20:1(n-11), 20:1(n-7), and 22:1(n-9). [3]Includes 18:3(n-6) and 22:5(n-6). Values are mean ± SD, n = 20. Values in the same row assigned a different superscript letter are significantly different.

TABLE 8

Fatty acid compositions of liver total lipid in Atlantic salmon post-smolts fed diets containing increasing levels of rape seed oil

Fatty acid	0% RO	10% RO	25% RO	50% RO	100% RO
			g/100g total fatty acids		
14:0	2.0 ± 0.3^{a}	1.8 ± 0.2^{b}	1.3 ± 0.2^{d}	1.5 ± 0.2^{c}	0.8 ± 0.1^{e}
16:0	18.0 ± 0.9^{a}	17.0 ± 0.8^{b}	16.5 ± 1.0^{b}	15.0 ± 1.5^{c}	12.3 ± 1.2^{d}
18:0	5.2 ± 0.5^{ab}	5.2 ± 0.5^{ab}	5.5 ± 0.6^{a}	5.0 ± 0.4^{b}	5.3 ± 0.5^{ab}
Total saturates[1]	25.8 ± 1.1^{a}	24.6 ± 0.8^{b}	24.0 ± 1.3^{b}	22.0 ± 1.5^{c}	18.6 ± 1.4^{d}
16:1(n-7)	2.7 ± 0.4^{a}	2.4 ± 0.3^{b}	2.1 ± 0.2^{c}	2.1 ± 0.3^{c}	1.3 ± 0.3^{d}
18:1(n-9)	12.5 ± 1.0^{d}	15.1 ± 1.8^{c}	17.2 ± 1.6^{c}	21.5 ± 3.3^{b}	30.0 ± 4.1^{a}
18:1(n-7)	2.0 ± 0.1^{d}	2.1 ± 0.1^{cd}	2.2 ± 0.2^{c}	2.5 ± 0.2^{b}	2.7 ± 0.3 ^{a}
20:1(n-9)	4.4 ± 0.6^{a}	4.1 ± 0.5^{a}	4.0 ± 0.4^{a}	4.1 ± 0.4^{a}	3.3 ± 0.5^{b}
22:1(n-11)	2.8 ± 0.8^{a}	2.5 ± 0.5^{a}	2.0 ± 0.3^{b}	1.9 ± 0.4^{b}	0.9 ± 0.2^{c}
24:1	1.1 ± 0.2^{b}	1.1 ± 0.1^{b}	1.3 ± 0.1^{a}	1.0 ± 0.1^{b}	0.8 ± 0.1^{c}
Total monoenes[2]	25.6 ± 2.2^{d}	27.4 ± 2.8^{cd}	28.8 ± 2.2^{c}	33.1 ± 4.1^{b}	40.4 ± 5.0^{a}
18:2(n-6)	1.3 ± 0.2^{e}	2.5 ± 0.3^{d}	3.2 ± 0.3^{c}	4.8 ± 0.6^{b}	8.0 ± 0.6^{a}
20:2(n-6)	0.4 ± 0.0^{e}	0.6 ± 0.1^{d}	0.8 ± 0.1^{c}	1.0 ± 0.1^{b}	1.6 ± 0.2^{a}
20:3(n-6)	0.2 ± 0.0^{d}	0.3 ± 0.0^{c}	0.4 ± 0.1^{b}	0.4 ± 0.1^{b}	1.4 ± 0.2^{a}
20:4(n-6)	2.3 ± 0.3^{a}	2.2 ± 0.2^{a}	2.1 ± 0.2^{a}	1.8 ± 0.3^{b}	1.7 ± 0.3^{b}
Total (n-6)[3]	4.6 ± 0.2^{e}	6.0 ± 0.3^{d}	6.7 ± 0.3^{c}	8.4 ± 0.5^{b}	13.0 ± 0.9^{a}
18:3(n-3)	0.6 ± 0.1^{d}	1.1 ± 0.1^{c}	1.2 ± 0.2^{c}	1.9 ± 0.3^{b}	2.6 ± 0.3^{a}
18:4(n-3)	0.6 ± 0.2^{a}	0.5 ± 0.1^{ac}	0.4 ± 0.1^{bc}	0.4 ± 0.1^{bc}	0.3 ± 0.1^{c}
20:4(n-3)	1.2 ± 0.2^{a}	1.2 ± 0.1^{a}	1.1 ± 0.1^{ab}	1.0 ± 0.1^{b}	0.8 ± 0.1^{c}
20:5(n-3)	9.7 ± 0.7^{a}	8.5 ± 0.8^{b}	8.6 ± 0.7^{b}	7.6 ± 0.9^{c}	5.3 ± 0.8^{d}
22:5(n-3)	2.7 ± 0.3^{a}	2.4 ± 0.3^{bc}	2.5 ± 0.2^{ab}	2.2 ± 0.2^{c}	1.7 ± 0.3^{d}
22:6(n-3)	28.2 ± 1.7^{a}	27.0 ± 2.1^{ab}	25.6 ± 1.6^{b}	21.9 ± 2.8^{c}	16.4 ± 2.9^{d}
Total (n-3)	43.0 ± 1.8^{a}	40.8 ± 2.4^{ab}	39.5 ± 1.8^{b}	35.3 ± 3.3^{c}	27.5 ± 3.8^{d}
Total PUFA	48.2 ± 1.9^{a}	47.0 ± 2.4^{a}	46.4 ± 1.8^{a}	43.8 ± 3.0^{b}	40.5 ± 4.3^{c}
(n-3)/(n-6)	9.5 ± 0.4^{a}	6.9 ± 0.4^{b}	5.9 ± 0.4^{c}	4.3 ± 0.5^{d}	2.2 ± 0.3^{e}

[1]Includes 15:0, 17:0, 20:0 and 22:0. [2]Includes 20:1(n-11), 20:1(n-7), and 22:1(n-9). [3]Includes 18:3(n-6) and 22:5(n-6). Values are mean ± SD, n = 20. Values in the same row assigned a different superscript letter are significantly different.

Fig.1

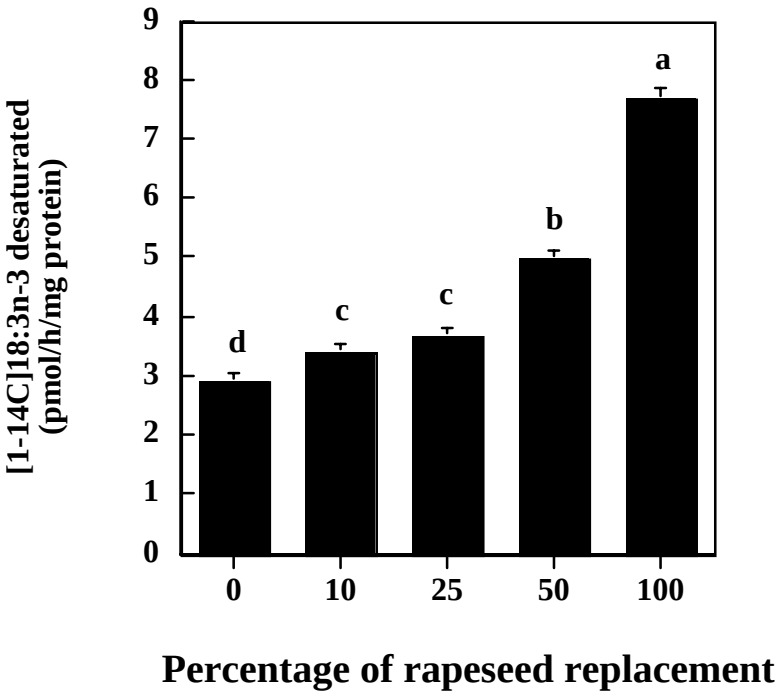
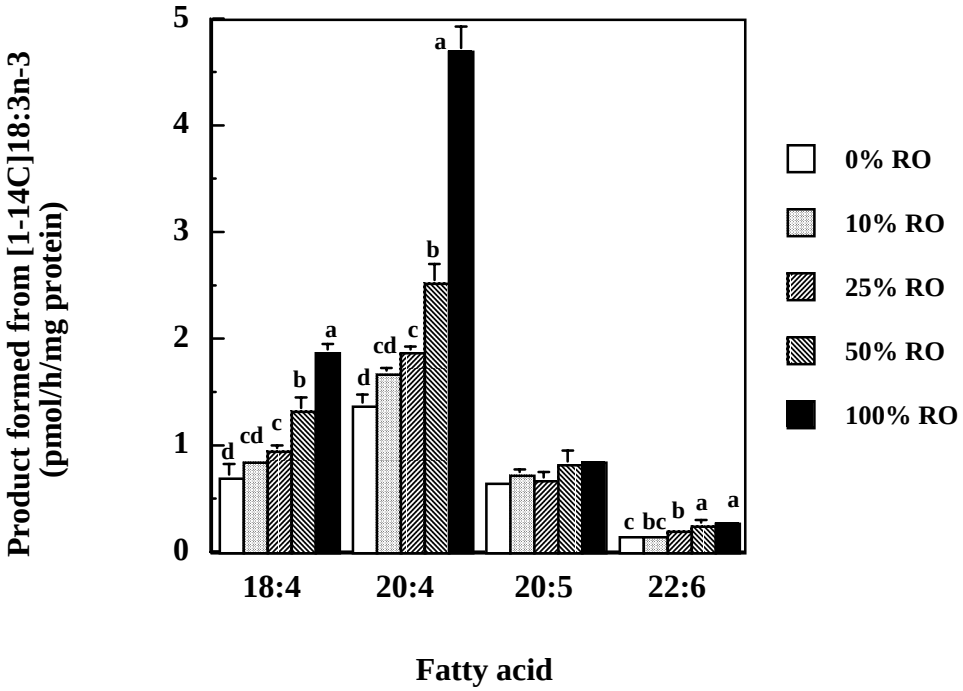

[1-14C]18:3n-3 desaturated
(pmol/h/mg protein)
9
8
7
6
5
4
3
2
1
0
d
c
c
b
a
0
10
25
50
100
Percentage of rapeseed replacement

Fig.2


Product formed from [1-14C]18:3n-3
(pmol/h/mg protein)
5
4
3
2
1
0
d
cd
c
b
a
d
cd
c
b
a
c
bc
b
a
a
18:4
20:4
20:5
22:6
Fatty acid
0% RO
10% RO
25% RO
50% RO
100% RO