Flesh Lipid and Carotenoid Composition of Scottish Farmed Atlantic Salmon (*Salmo salar*)

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Samples of steaks from market-size salmon (2.5−5 kg), produced under the requirements of the Product Certification Scheme for Scottish Quality Farmed Salmon, were obtained from five Scottish salmon producers on a weekly basis for over 2 years. Samples were assayed for total lipid content, lipid class composition, fatty acid composition, vitamin E content, and carotenoid pigment [astaxanthin (AX) and canthaxanthin (CX)] content. In addition, samples were obtained from the same producers to assess loss of carotenoid pigment in the period up to 120 h post-harvest and to measure lipid and pigment content of different body regions. The analyses showed a positive correlation between dietary lipid and deposition of lipid in flesh, although there was great variation in flesh lipid content within each dietary lipid level. The average lipid content of Scottish salmon was 10.1 ± 2.9% (n = 495). Salmon flesh was rich in the $n-3$ highly unsaturated fatty acids, 22:6$n-3$ and 20:5$n-3$, with average values of 11.3 and 5.4% of total fatty acids or 10.2 and 4.8 g/kg flesh, respectively. Vitamin E content of salmon flesh was around 30 mg/kg but was significantly higher in fish fed the lowest dietary lipid level. Studies investigating changes in carotenoid pigmentation up to 120 h post-harvest suggested that fish fed AX alone showed a loss of pigment over time that did not occur in fish fed either CX alone or a combination of AX and CX. Measurement of lipid content of different body regions showed that the highest levels were found in the region immediately in front of the dorsal fin and that the lowest lipid levels were in the tail region. Total lipid content of all five body zones was positively correlated with dietary lipid. Carotenoid pigment levels were the same across all body zones and were not affected by dietary lipid.

Keywords: *Lipids; carotenoid; salmon; n−3 PUFA; vitamin E*

INTRODUCTION

The lipid content, distribution between and within tissues, and fatty acid composition in fish vary from species to species and are further influenced by seasonal and dietary variations (Sidwell et al., 1974; Henderson and Tocher, 1987). The sites of lipid storage are different from species to species and may include liver, muscle, mesenteric, and subdermal depots (Ackman, 1980). In Atlantic salmon (*Salmo salar*), lipids are stored largely in the muscle (Polvi and Ackman, 1992), which is composed of parallel muscle fibers separated by layers of connective tissue called endomysium, perimysium, and epimesium. At the termination of muscle fibers, the connective tissue elements combine to form strong connective tissue sheets known as myosepta (Harder, 1975). It is within these myosepta that large numbers of adipocytes are located (Ackman and Zhou, 1994). Numerous studies have suggested that the level of fat deposition in fish flesh can influence both the nutritional and organoleptic qualities of the product to the consumer (Austreng and Krogdahl, 1987; Ackman, 1989) In recent years, Scottish salmon producers have tended to use higher levels of lipid in their dietary formulations, which minimize protein utilization as an energy source and, consequently, result in improved growth performance. However, the use of so called "high-energy" diets, containing up to 33% dietary lipid, has led to concern that the resulting product could be oily in texture, strongly flavored, poorly pigmented, and susceptible to rancidity.

Fish produced by intensive aquaculture are important sources of both protein and essential fatty acids, and salmon, which can accumulate high levels of lipid in their white muscle, are especially useful in delivering long-chain $n-3$ PUFA to consumers (Ackman, 1989). However, these PUFA, namely, eicosapentaenoic acid (20:5$n-3$; EPA) and docosahexaenoic acid (22:6$n-3$; DHA), being highly unsaturated, are particularly susceptible to autoxidative degradation; adequate antioxidant protection must be provided to prevent postmortem rancidity in salmon flesh (Hsieh and Kinsella, 1989). While cellular antioxidant protection involves a complex multilevel system of enzymes and accessory molecules, α-tocopherol (vitamin E) is recognized as the major chain-breaking lipid-soluble antioxidant and is widely used as a feed additive (Buettner, 1993).

Wild salmon derive their pink flesh coloration from astaxanthin (AX) found in wild prey organisms whereas farmed salmon obtain chemically-synthesized AX and/or canthaxanthin (CX) from formulated feeds supplemented with around 50−100 mg/kg synthetic carotenoid

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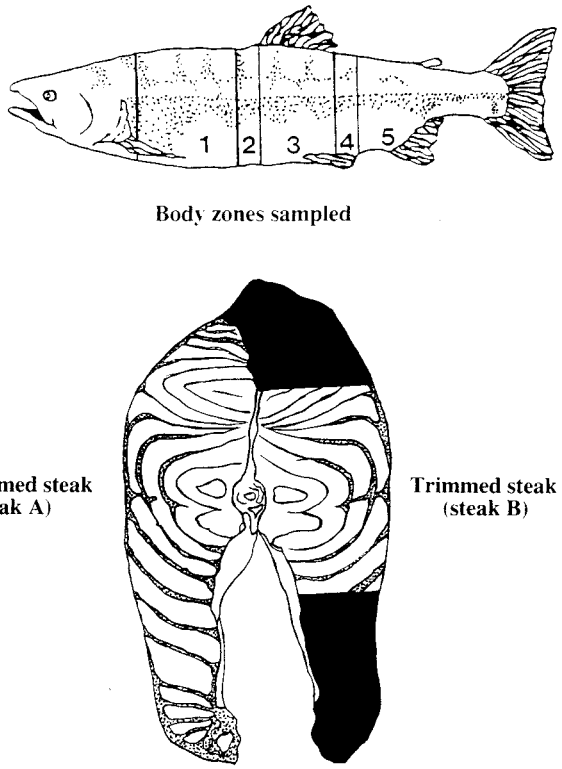

Figure 1. Analysis of lipid and pigment deposition in farmed Atlantic salmon. Figure shows dissection pattern for the five body zones and the areas of the dorsal and belly flap regions that were removed in the trimmed steaks (steak B).

(Smith et al., 1992). Synthetic pigments comprise around 15% of feed costs (Prendergast et al., 1994) with <20% of the ingested carotenoid being retained in the flesh (Storebakken and No, 1992). Anecdotal evidence from Scottish salmon producers suggests that feeding high-energy diets may compromise flesh pigment deposition and that feeding a mixture of AX and CX may give superior pigment retention as compared to feeding AX alone.

In the present study, samples of salmon steaks from market-size salmon, produced under the requirements of the Product Certification Scheme for Scottish Quality Farmed Salmon, were obtained from five Scottish salmon producers on a weekly basis for over 2 years. Samples were assayed for total lipid content, lipid class composition, fatty acid composition, vitamin E content, and carotenoid content. In addition, samples were obtained from the same producers to assess the loss of carotenoid pigment in the period up to 120 h post-harvest and to measure lipid and pigment content of different body regions.

MATERIALS AND METHODS

Experimental Material. Samples of salmon steaks were obtained from five salmon producers on a weekly basis. Steaks of 2−3 cm were cut from the region immediately anterior to the dorsal fin (zone 2, Figure 1) and thereafter held on ice or in a refrigerator at <4 °C until 72 h post-harvest. A period of 72 h was chosen to represent the time between harvesting and availability to the consumer. After 72 h, the Roche color score was recorded in natural daylight; the skin, bone, and dorsal fat body were removed; and the flesh was homogenized in a Waring blender. This method of dissection is hereafter referred to as steak A. One-gram portions of homogenized flesh were then stored at −40 °C and used for a range of biochemical measurements. For comparative purposes, samples of wild salmon obtained from fish caught by rod and line on the rivers Dee and Deveron, Aberdeenshire, Scotland, were also subjected to the same analyses.

Three fish were sampled from each of the five producers to assess pigment degradation up to 120 h post-harvest. Five thin steaks of around 1 cm thickness were cut from each fish immediately in front of the dorsal fin. Each steak was dissected as described above (steak A). One steak was homogenized as described above within 2 h post-harvest, and the carotenoid pigments were extracted immediately, as described below, on site at that time. This sample was regarded as the zero time point. The remaining steaks were transported on ice to the laboratory where they were stored at <4 °C and processed (steak A) and extracted at 24, 48, 72, and 120 h post-harvest.

Three fish were obtained from each of the five producers and dissected into five body zones as indicated in Figure 1. Zones 1−4 were subdivided as indicated (Figure 1) with steak A being homogenized after removing the dorsal fat body and steak B homogenized after further trimming of the dorsal and belly flap regions as indicated. Total lipid and carotenoid content were determined in each sample homogenate.

Lipid Extraction and Analysis. Samples of salmon flesh were homogenized in 10 vol of chloroform/methanol (2:1 v/v) using an Ultra-Turrax tissue disruptor, and total lipid was determined gravimetrically according to the method of Folch et al. (1957). The quanitification of lipid classes was performed using double-development high-performance thin-layer chromatography (HPTLC) and scanning densitometry as described previously (Bell et al., 1993). Ten micrograms of total lipid was applied to a 10 × 10 cm HPTLC plate that had been prerun in hexane/diethyl ether (1:1 v/v) and activated at 110 °C for 30 min. The plates were developed to 6 cm in methyl acetate/2-propanol/chloroform/methanol/0.25% (w/v) aqueous KCl (25:25:25:10:9 by volume) to separate polar lipid classes, with neutral lipids running at the solvent front (Vitiello and Zanetta, 1978). After drying, the plates were developed fully in hexane/diethyl ether/acetic acid (80:20:2 v/v/v) to separate neutral lipids and cholesterol. Lipid classes were visualized by charring at 160 °C for 15 min after spraying with 3% copper acetate (w/v) in 8% (v/v) phosphoric acid and identified by comparison with commercially available standards. Lipid classes were quantified by scanning densitometry using a Shimadzu CS-9000 dual-wavelength TLC scanner and a DR-13 recording integrator. Fatty acid methyl esters were prepared by acid-catalyzed transesterification of total lipids according to Christie (1982). Fatty acid methyl esters were separated and quantified by gas−liquid chromatography (Carlo Erba Vega 6000, Fisons Ltd. Crawley, U.K.) using a 30 m × 0.32 mm capillary column (CP Wax 52 CB, Chrompak Ltd., Millharbour, London, U.K.). Hydrogen was used as carrier gas, and temperature programming was from to 50 to 150 °C at 40 °C/min and then to 230 °C at 2 °C/min. Individual methyl esters were identified by comparison with known standards and by reference to published data (Ackman, 1980).

Vitamin E Measurement. Vitamin E in salmon flesh was saponified and extracted by the method modified from that described by Cowey et al. (1981). Flesh samples (up to 1 g) were homogenized in 4 mL of 2% ethanolic pyrogallol (w/v) and incubated for 5 min at 70 °C in 10-mL stoppered test tubes. One milliliter of 60% KOH (w/v) was added, and the tubes were flushed with nitrogen and returned to the water bath for 20 min, with shaking every 5 min. The tubes were then cooled on ice, the volume was made up to 10 mL with distilled water, and the vitamin E was extracted into 4 mL of hexane containing 0.02% BHT. Three milliliters of the hexane layer was removed, dried under nitrogen, and taken up in 0.5 mL prior to analysis using HPLC.

Separation and quantitation of α-tocopherol was performed using a 5-μm Hypersil ODS column (4.6 mm × 25 cm, Capital HPLC, Broxburn, Scotland). The chromatographic system was equipped with a Waters Model 501 pump, and α-tocopherol was detected by UV detection at 293 nm using a Waters 490E multiwavelength UV/vis detector (Millipore (U.K.) Ltd., Watford). An isocratic solvent system was used containing 98%

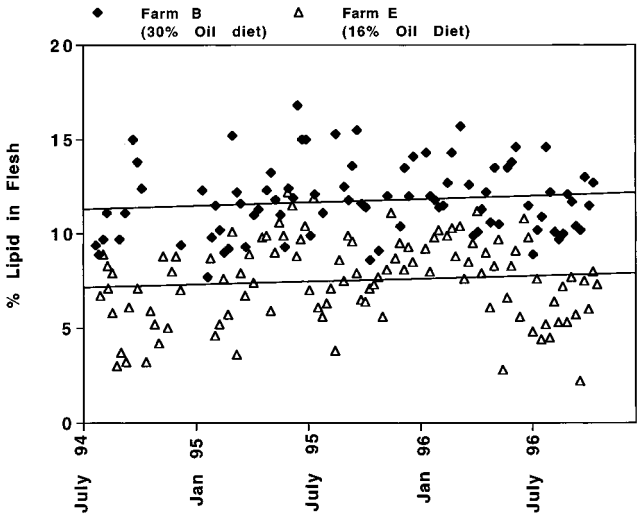

Figure 2. Relationship between % flesh lipid (steak A) and % dietary lipid for salmon fed diets containing either 16% (△) or 30% oil (◆) in the period from July 1994 to November 1996. The regression lines are represented by the equations $y = 0.001x + 11.316$ and $y = 0.001x + 7.162$ for fish fed 30% and 16% oil, respectively.

methanol (v/v) at a flow rate of 1.1 mL/min. Sample concentrations were calculated using an external standard of D-α-tocopherol (1 mg/mL).

Measurement of Carotenoids. Total carotenoid was extracted from salmon flesh largely by the method described by Barua et al. (1993). One-gram portions of homogenized salmon flesh were homogenized in 5 mL of absolute ethanol and 5 mL of ethyl acetate using an Ultra-Turrax tissue disrupter. The homogenate was centrifuged (1000*g*, 5 min) and the supernatant removed to a stoppered tube. The pellet was rehomogenized in 5 mL of ethyl acetate and recentrifuged, and the supernatant was combined with the first supernatant. Finally, the pellet was rehomogenized in 5 mL of hexane and recentrifuged, and the supernatant was 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 0.02% BHT. Total carotenoid was measured spectrophotometrically at 470 nm using the $E_{1\%}$ (w/v) of 2100. Measurement of AX and CX was carried out using the HPLC column, pump, and detector described above. An isocratic solvent system was used containing ethyl acetate/90% methanol (v/v) (1:4 v/v) at a flow rate of 1.0 mL/min. AX and CX were detected at 470 nm and quantified using external standards of AX and CX obtained from Roche (Welwyn Garden City, U.K.).

Statistical Analysis. Significance of difference ($P < 0.05$) between samples was determined by analysis of variance (ANOVA) or Student's *t*-test. Analyses were performed using a Statgraphic (System 3.0) computer package. Data that were identified as nonhomogeneous (using Bartlett's test) were subjected to either arcsine square root or log transformation before analysis. Differences between means were determined by Tukey's test.

Materials. TLC plates (20 × 20 cm × 0.25 mm) and HPTLC plates (10 × 10 cm × 0.25 mm), precoated with silica gel 60 were obtained from Merck (Darmstadt, Germany). All solvents were of HPLC grade and were purchased from Fisher Scientific U.K. Ltd. (Loughborough, England).

RESULTS AND DISCUSSION

Figure 2 shows the relationship between percent lipid in flesh (steak A) versus time (from July 1994 to October 1996) from two different producers respectively feeding diets containing either 16 or 30% oil (see Table 1 for % dietary lipid). The regression analysis clearly shows

Table 1. Lipid Content of Salmon Steaks (Steak A) from Five Scottish Salmon Producers

producer	dietary lipid (%)	mean % flesh lipid[a]	fish weight (kg)[b]	*n*
A	25−33	10.9 ± 2.7	3.89 ± 1.14	97
B	30	11.8 ± 2.0	3.06 ± 0.47	85
C	28	8.6 ± 2.2	2.34 ± 0.42	99
D	21−31	11.0 ± 2.8	2.98 ± 0.62	106
E	16	7.7 ± 2.2	2.89 ± 0.76	104
all producers	16−33	10.1 ± 2.9	3.03 ± 0.87	491

[a] Values are lipid percent of wet flesh ± SD. [b] Values are mean ungutted weight ± SD.

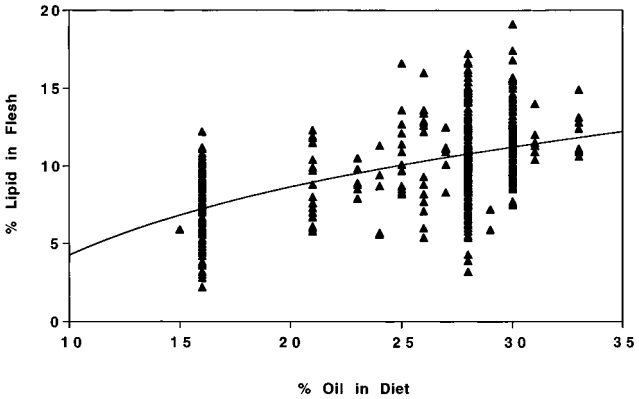

Figure 3. Relationship between % flesh lipid and % dietary oil. Individual values for each measurement at each dietary oil level are shown. The equation representing the line through the points is $y = 14.577 \log(x) - 10.309$.

a correlation between dietary lipid and lipid deposited in the flesh. The average values for flesh lipid content in fish fed either 16 or 30% dietary lipid were 7.7 and 11.8%, respectively. These values are in agreement with the value of 9.6% quoted by Aursand et al. (1994) in Norwegian salmon fed 21% dietary oil and with the 10.82% lipid in Canadian salmon (Zhou et al., 1996). The values of percent muscle lipid in fish fed 16% dietary oil show seasonal variation with highest values occurring in spring and early summer and lowest values occurring in late summer and autumn. This probably reflects the mobilization and redistribution of body lipids into the ovary during oogenesis that occurs during sexual maturation. Many fish species can store high levels of lipid in their adipose tissue when seasonal food supplies are plentiful, which can subsequently be mobilized both for metabolic energy during spawing migration and for deposition in the roe (Sargent, 1995). The high flesh lipid recorded during spring and early summer reflects the high proportion of grilse, which are marketed at that time and which tend to store higher levels of lipid in the flesh as compared to immature fish. The same peaks and troughs in flesh lipid were not so apparent in fish fed 30% dietary lipid, possibly since the flesh lipid levels in these fish may be effectively saturated and thus any effects of mobilization and redistribution tend to be obscured.

Figure 3 shows the relationship between percent lipid in the flesh and percent of oil in the diets actually fed. The figure shows a positive correlation between dietary lipid and deposition of lipid in muscle, although the flesh lipid levels appear to be reaching a plateau at dietary oil levels in excess of 25%. Literature values for flesh lipid content of Atlantic salmon fed high-energy diets are not available, but the values of Polvi and Ackman (1992) for fish fed diets containing 14% oil (4.4−6.6%) and of Aursand et al. (1994) feeding 21% oil (9.6 ± 1.2%)

are consistent with the present study. It is interesting to note the variation in flesh lipid levels within fish fed the same level of dietary lipid, e.g., the percent lipid in muscle of fish fed 28% oil varied from 3% up to 17%. Clearly, certain individuals can utilize high-energy feeds but deposit little lipid in their flesh while others show a tendency toward increased adiposity. While the reasons for this variation are likely to be multifactorial, a genetic predisposition toward low adiposity seems likely, and thus, further studies utilizing a program of selective breeding could prove beneficial.

The mean flesh lipid content in all samples measured during this study was 10.1 ± 2.9% (n = 491) although the range of measured values was from 2 to 19%. The lipid content of steaks received from the five producers are shown in Table 1. Statistical comparison of the data from farms B and E (Students t-test) show that flesh lipid (steak A) is significantly different ($P < 0.001$) between fish fed the diet containing 16 and 30% lipid. The mean values for flesh lipid from farm C were lower than all other producers except farm E feeding 16% dietary lipid. This may be explained by the lower average size of fish obtained from farm C or a result of differences in genetic stock or husbandry techniques utilized by this producer. Producers A and D utilize different dietary lipid levels at different times of the year and at different geographical locations.

During the course of our analyses, fish were encountered that were difficult to handle and process because of free oil exuding from the flesh. The appearance of these oily fish occurred primarily at specific periods in the calendar year, one being at the end of September until mid-November and the second from the end of March until the end of April. The rise in flesh lipid in the autumn may reflect a reduction in lipid metabolism with falling water temperatures, while the spring increase in muscle lipid may be due to producers increasing feeding rates before water temperatures have risen sufficiently to permit increased lipid utilization by the fish. Alternatively, the increased oiliness may reflect changes in flesh lipid deposition that precede or accompany maturation in adult salmon. In general, the appearance of very oily fish represented <5% of the total sample and was not apparent in flesh containing <12% lipid. Free oil, i.e., oil exuding from the steak on contact or after homogenization, and presumably arising from ruptured adipocytes, was only found in fish fed diets containing >28% lipid.

The distribution of flesh lipid between triacylglycerol (TAG; storage lipid) and polar (membrane) lipid is shown in Figure 4. The graph shows that increased dietary lipid results in more TAG deposited relative to polar lipid. The square symbols represent two wild fish caught from rivers in the northeast of Scotland and show reduced TAG, which is utilized as an energy source during spawning migration. The levels of TAG found in the present study are similar to those recorded in farmed salmon in previous studies, 87−93% (Polvi et al., 1991; Polvi and Ackman, 1992; Aursand et al., 1994).

The lipid class and selected fatty acid compositions of salmon steaks with the average lipid content (see above and Table 1) from all five producers as well as a comparison with two wild fish are shown in Table 2. The farmed salmon from the five different producers show similar levels of TAG and total phospholipid (PL) (average 89.2 and 7.6, respectively), as would be expected since they all have a total flesh lipid of 10.0 ± 0.02%. Total saturated and monounsaturated fatty acids are broadly similar in the farmed salmon, ranging from 20.7 to 23.2% for saturates and from 43.6 to 45.3% for monounsaturates. The saturated fatty acids were slightly higher and the monounsaturates were slightly lower in farmed fish as compared to the wild fish. These values in Scottish farmed salmon are broadly similar to those found by Ackman (1989) while the saturates are higher and the monoenes are lower than the values of Aursand et al. (1994) and Hardy et al. (1987). Clearly, the content of saturated and, more especially, of monounsaturated fatty acids reflects the origin and fatty acid composition of the oils that are added to salmon diets. The high levels of 20:1n−9 and 22:1n−11, found in both farmed and wild salmon, reflect the high input of these monoenoic fatty acids from the marine food web of northern latitudes. These monoenoic fatty acids are derived from fatty alcohols synthesized by calanoid copepods (Pascall and Ackman, 1976), which are in turn consumed by zooplankonivorous fish like herring, capelin, mackerel, and sand eels and which are subsequently utilized for fish meal and oil production (Sargent et al., 1995).

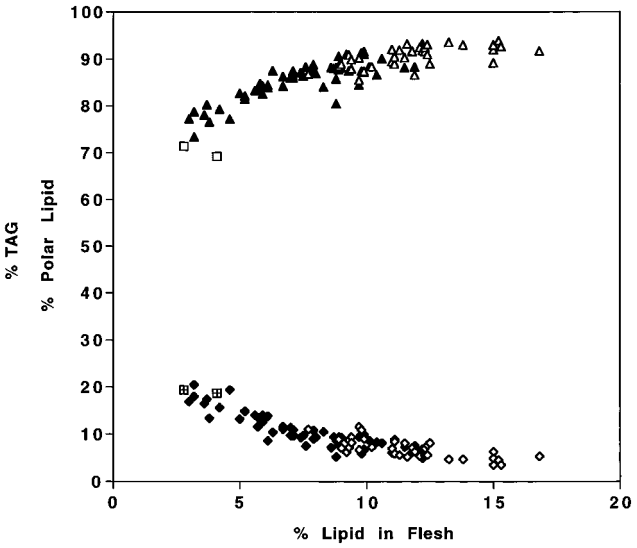

Figure 4. Relationship between flesh TAG and polar lipid (PL), expressed as a percentage of total flesh lipid, and % flesh lipid in fish fed diets containing either 16 or 30% oil (n = 40). TAG values are indicated by (▲) for 16% oil and (△) for 30% oil. PL values are indicated by (◆) for 16% oil and (◇) for 30% oil. TAG (□) and PL (box with cross) values in two wild salmon are also indicated.

The linoleic acid (18:2n−6) content of Scottish farmed salmon flesh ranges from 2.8 to 6.7%, higher than the value of 1.2% in the two wild fish analyzed. The higher and variable value for 18:2n−6 in farmed salmon reflects the content of plant-derived oils arising from inclusion of varying levels of plant meals, largely from soya and wheat, in commercial feed preparations. The 18:2n−6 content of Scottish farmed salmon is similar to values quoted by other researchers (Hardy et al., 1987; Hardy and King, 1989; Aursand et al., 1994). Linoleic acid was the major n−6 PUFA in farmed salmon flesh and was, therefore, largely responsible for the n−3/n−6 PUFA ratios of 3.2−6.9 that were lower than the value of 10.3 found in the two wild salmon with lower 18:2n−6 contents. Eicosapentaenoic acid (20:5n−3; EPA) in farmed Scottish salmon flesh varied from 5.0 to 5.8% of total flesh fatty acids and was similar to wild fish and to literature values (Hardy et al., 1987; Ack-

Table 2. Lipid Class and Selected Fatty Acid Compositions of Salmon Flesh from Five Scottish Producers and Wild Rod Caught Fish[a]

	product					
lipid component	A	B	C	D	E	wild
% lipid	10.1 ± 0.5	10.0 ± 0.6	10.0 ± 0.4	10.1 ± 0.5	10.0 ± 0.2	3.5 ± 0.9
TAG %	89.2 ± 1.1	89.3 ± 1.6	89.8 ± 1.7	89.0 ± 1.6	88.8 ± 2.1	72.5 ± 1.5
total PL%	7.9 ± 1.3	8.0 ± 1.4	6.5 ± 2.2	7.3 ± 1.3	8.2 ± 1.5	19.7 ± 0.4
total saturates	22.5 ± 1.3	20.8 ± 1.3	20.7 ± 0.8	20.8 ± 1.5	23.2 ± 1.8	19.3 ± 1.5
total monoenes	43.6 ± 1.5	45.3 ± 1.0	43.6 ± 2.1	43.7 ± 2.0	43.9 ± 2.1	49.8 ± 0.4
18:2*n*−6	3.5 ± 0.6	2.8 ± 0.2	6.7 ± 0.8	5.7 ± 1.3	5.6 ± 0.8	1.2 ± 0.1
20:5*n*−3	5.8 ± 0.5	5.7 ± 0.4	5.0 ± 0.6	5.3 ± 0.6	5.0 ± 0.3	5.9 ± 1.5
22:6*n*−3	11.6 ± 1.2	12.4 ± 1.0	11.3 ± 1.3	11.0 ± 0.9	10.4 ± 1.0	12.9 ± 3.8
total *n*−6	4.5 ± 0.5	3.8 ± 0.3	7.9 ± 0.8	6.8 ± 1.3	6.9± 0.7	2.5 ± 0.5
total *n*−3	25.6 ± 2.1	26.5 ± 1.7	24.3 ± 2.5	24.2 ± 1.8	22.4 ± 1.8	25.2 ± 1.6
total PUFA	30.1 ± 2.3	30.3 ± 1.8	32.2 ± 1.9	31.0 ± 1.9	29.3 ± 1.7	27.7 ± 2.1
n−3/*n*−6	5.8 ± 0.8	6.9 ± 0.7	3.2 ± 0.8	3.6 ± 0.9	3.3 ± 0.5	10.3 ± 1.4
20:5*n*−3 (g/kg)	5.3	5.2	4.5	4.8	4.5	2.0
22:6*n*−3 (g/kg)	10.6	11.2	10.2	10.0	9.3	4.5

[a] Values are derived from 10 individuals from each producer having the average lipid content (see Table 1). Values from wild fish are from 2 individuals. Total lipid is expressed as percent of wet weight tissue. Triacylglycerol (TAG) and polar lipid (PL) are % wt of total lipid, and individual fatty acids are % wt of total fatty acids. Absolute values for 20:5*n*−3 and 22:6*n*−3 are expressed as g/kg flesh.

man, 1989; Hardy and King, 1989; Aursand et al., 1994). Docosahexaenoic acid (22:6*n*−3; DHA) levels in farmed Scottish salmon flesh varied from 10.4 to 12.4% of total fatty acids, which was slightly lower than the level in wild fish. The phospholipid fraction is known to contain more PUFA as compared to the TAG fraction (Polvi and Ackman, 1992) explaining the slightly elevated DHA level in wild salmon flesh, which have less TAG than the farmed fish. However, the absolute concentration of EPA and DHA in grams per kilogram of flesh show that farmed salmon provide more than twice as much of each long-chain *n*−3 PUFA as wild salmon. Clearly, consumption of farmed salmon can be beneficial in providing a high level of these essential fatty acids to the human consumer.

While the nutritional importance of long-chain *n*−3 polyunsaturated fatty acids PUFA) as anti-inflammatory substances was recognized by the early 1980s, serious interest in this area followed the conference on Health Effects of Polyunsaturated Fatty Acids in Seafoods in 1985 (Simopoulos et al., 1986). Since then, research activity in long-chain *n*−3 PUFA, as attenuators of a wide range of inflammatory conditions and modulators of cellular function, has increased dramatically (Salem et al., 1996) such that the importance of fish and, more specifically, of long-chain *n*−3 PUFA as essential elements of the human diet are now widely accepted (Anonymous, 1992, 1994; Salem et al., 1996). Evidence suggests that the beneficial effects of EPA are at least partly due to this fatty acid inhibiting the production and efficacy of eicosanoids derived from arachidonic acid (AA; 20:4*n*−6). Many of the beneficial effects of increased intake of fish oils in attenuating a number of atherothrombotic, autoimmune, and inflammatory conditions are due to the modulation of arachidonic acid-derived eicosanoids by both EPA and DHA (Weber, 1990). EPA can compete with AA at the cyclooxygenase or lipoxygenase binding site and, thereby, reduce production and efficacy of AA-derived eicosanoids (Crawford, 1983; Lee et al., 1984). In addition, inadequate supplies of DHA, particularly in early development, can lead to cognitive and visual impairment (Neuringer et al., 1988; Bell et al., 1995). A dietary deficiency of DHA in premature human infants is now known to cause subnormal visual and cognitive performance that is not apparently rectified in later life, and thus, it has been recommended that formula feeds for human infants by supplemented with DHA (Anonymous, 1992). This scientific evidence suggest that fish consumption, especially of an oil-rich fish like salmon, during pregnancy should be encouraged (Gibson et al., 1996).

Levels of vitamin E found in Scottish salmon steaks from three producers in the period from July 1994 to November 1996 are shown in Figure 5. The average values from producers A, C, and E were 31.2 ± 9.0, 28.6 ± 6.7, and 35.9 ± 15.0 μg of vitamin E/g of flesh, respectively (n = 97, 99, and 104, respectively). All producers used feed supplemented with 250 mg of vitamin E/kg. These values in feed are in excess of 5-fold greater than the value recommended by the U.S. National Research Council (1993) for salmonid fish. The values in flesh are over 10-fold greater than those found in previous studies with vitamin E-deficient rainbow trout (Cowey et al., 1984) and salmon (Raynard et al., 1991). Clearly, the vitamin E levels found in the salmon steaks examined in this study, even after 72 h post-cull, are such that the potential for autoxidation of flesh lipids appears minimal. The flesh vitamin E levels in salmon from producer E appeared to increase after July 1995, and although there was no apparent increase in the dietary supplementation of vitamin E, this producer's product did have a significantly higher vitamin E content as compared to producers A and C ($P < 0.01$). Since producer E used diets with a lower oil content (16%) as compared to the other two (25−33%), the significantly higher vitamin E value in the flesh from the former might reflect less demand for vitamin E in fish provided with a lower level of dietary oil and consequently less PUFA in tissues. Vitamin E or its most abundant isomer, α-tocopherol, is the primary lipid-soluble antioxidant in vertebrates and is capable of quenching both initiators of lipid peroxidation, like superoxide and hydroxyl radicals, as well as the propagating species like lipid peroxy and alkoxy radicals (Buettner, 1973; Yu, 1994). Left unchecked, lipid peroxidative damage can lead to accumulation of oxidative breakdown products, which result in rancidity and off-flavor (Allen and Hamilton, 1989). The high levels of EPA and DHA in fish tissues make them very susceptible to lipid peroxidation as it has been long established that the rate of autoxidation increases as the number of double bonds in fatty acids increase (Hsieh and Kinsella, 1989). Consequently, adequate levels of vita-

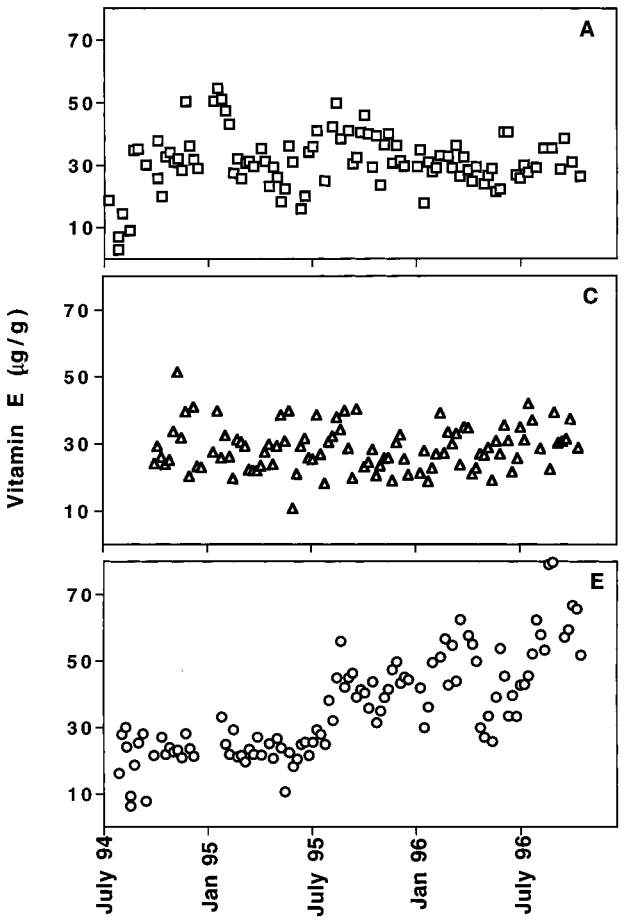

Figure 5. Measured flesh vitamin E content (μg/g flesh) in salmon steaks supplied by three Scottish salmon producers in the period from July 1994 to November 1996. All three producers used feed supplemented with 250 mg/kg vitamin E. Sample sizes were n = 97, 99, and 104 for producers A, C, and E, respectively.

min E must be present in salmon flesh to prevent excessive autoxidation, both in vivo and in vitro.

The characteristic pink coloration of wild salmon flesh derives from carotenoid pigments, largely AX, from ingested crustaceans. Scottish farmed salmon producers currently use synthetic AX or CX, either alone or in combination, to achieve the desired pigmentation. Both AX and CX are naturally occurring compounds in marine organisms. The mean carotenoid pigment content of Atlantic salmon steaks from three producers (using either AX, CX, or a combination of the two) were 6.7 ± 2.2, 8.2 ± 2.6, and 9.0 ± 2.4, respectively. The "target" value for carotenoid deposition in salmon flesh is 7 mg/kg, and while the latter two producers are in excess of this value, evidence suggests that less than 20% of ingested carotenoid is retained in the flesh (Storebakken and No, 1992). Considerable anecdotal evidence exist that suggests that using a combination of AX and CX produces a better flesh pigmentation as compared to using AX alone. The present data support this observation, although it should be noted that the producer feeding AX alone was providing 50 mg/kg carotenoid as compared to 75 and 85 mg/kg, respectively, for those feeding CX or a combination of AX and CX. Experimental evidence with rainbow trout suggests that, while there is a more efficient uptake and deposition of AX as compared to CX, a higher total flesh carotenoid level is achieved when AX and CX are used

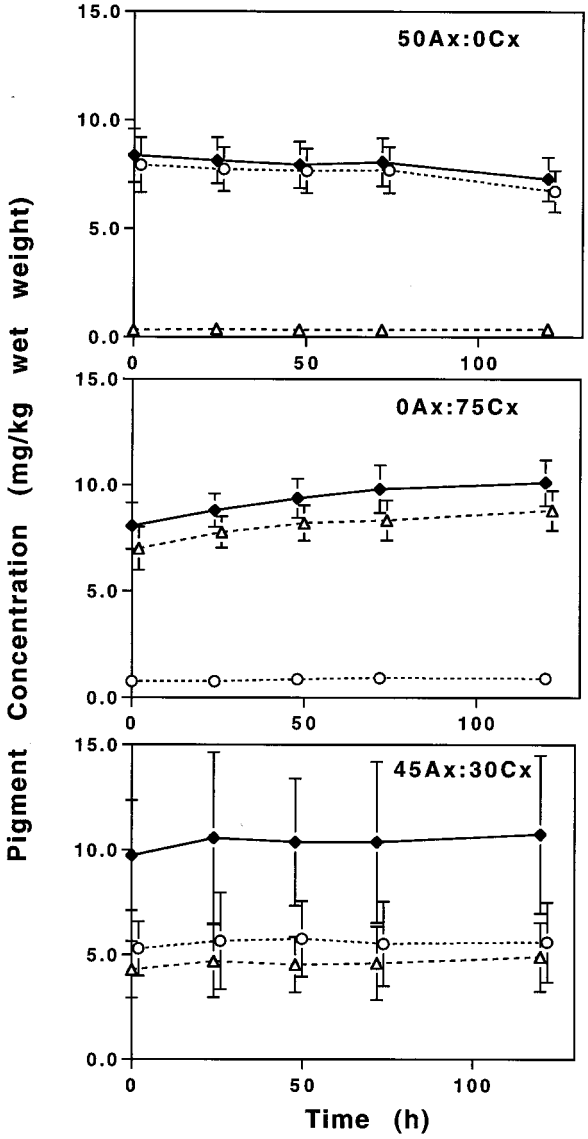

Figure 6. Carotenoid pigment concentration versus time after cull in salmon fed diets containing 50 mg/kg AX, 75 mg/kg CX, or 45 mg/kg AX and 30 mg/kg CX. Total carotenoid (measured spectrophotometrically at 470 nm (◆), AX (○), and CX (△) are indicated. Each point represents the mean ± SD from three fish per dietary treatment.

in combination, in comparison to feeding either AX or CX alone (Torrissen, 1989). In addition to measuring the pigment concentration in steaks at 72 h post-harvest, the flesh pigment concentration was measured in the period from 0 h (harvest) to 120 h post-harvest. Changes in flesh pigment concentration with time after harvest (in fish fed either AX, CX, or a combination of the two) are shown in Figure 6. In fish fed AX alone, there was a gradual reduction in flesh pigment over the experimental period, while fish fed both carotenoids showed no change, and those fed CX alone showed a slight rise in flesh pigment over time. These results suggest that AX in salmon flesh is subject to degradation during storage at 4 °C, whereas the presence of CX inhibits degradation. Considerable evidence exists to suggest a significant antioxidant role for both AX and CX in free radical-initiated lipid peroxidation (Palozza and Krinsky, 1992) and as quenchers of other species of activated oxygen, such as singlet oxygen (Hirayama et al., 1994). In addition, Andersen et al. (1990) showed that rainbow trout with flesh AX of 9.1 mg/kg had lower

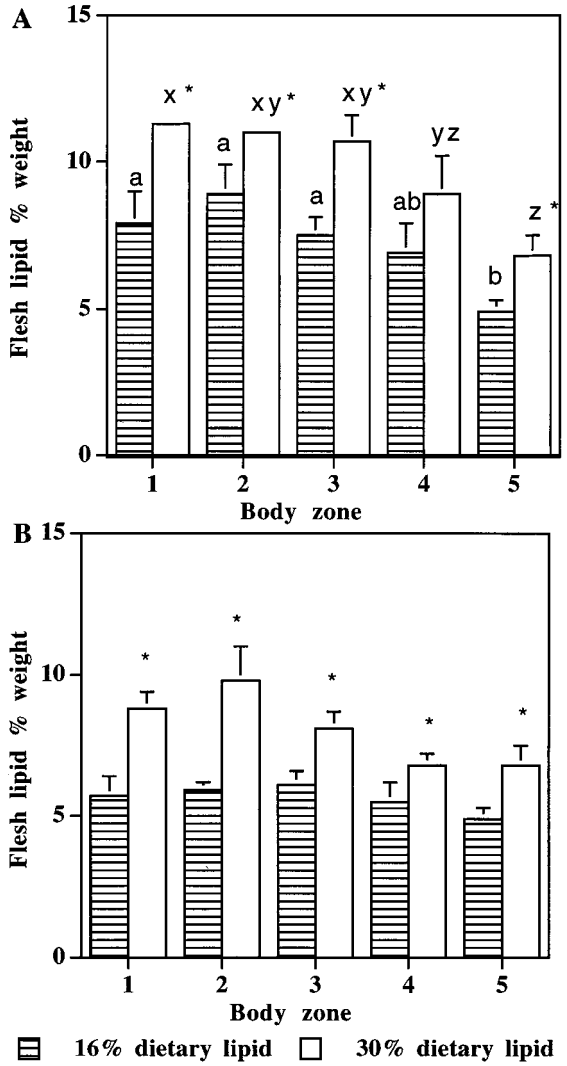

Figure 7. Body zone lipid content vs dietary lipid. Figure shows lipid content (weight %) of five body zones from untrimmed (A) and trimmed (B) steaks (described in Figure 1) from salmon fed either 16 or 30% lipid. Columns representing fish fed the same dietary lipid level having different superscript letters are significantly different. Columns with an asterisk are significantly different between diets, within the same body zone. Values are mean ± SD for three fish per dietary treatment.

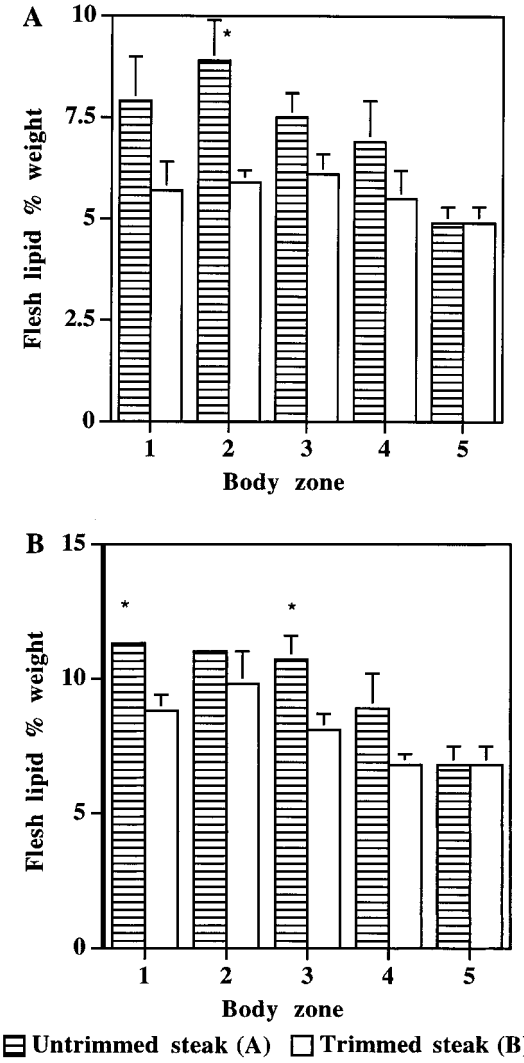

Figure 8. Body zone lipid content vs dietary lipid. Figure shows lipid content (% weight) of five body zones, (described in Figure 1) before and after trimming (steaks A and B), in salmon fed either 16% (A) or 30% (B) lipid. Columns with an asterisk are significantly different, within each body zone. Values are mean ± SD for three fish per dietary treatment.

thiobarbituric acid-reactive substances (TBARS) as compared to trout with 4.9 mg/kg AX, although the antioxidative efficacy is also influenced by other compounds, most notably vitamin E. Interestingly, producer E increased the level of CX in feed from 50 to 75 mg/kg in August 1995, and this corresponded with an increase in flesh vitamin E levels (see Figure 5), despite dietary vitamin E levels being unchanged. This observation suggests there may be a synergistic antioxidant relationship between CX and vitamin E such that increasing dietary CX has a sparing effect on flesh vitamin E.

While it is well documented that salmon tend to deposit more lipid in the belly flap region as compared to the dorsal area (Zhou et al., 1995; Aursand et al., 1994), little information is available on the lateral distribution of lipid in this species. In the present study, whole salmon were dissected into five lateral body zones (Figure 1), and regions 1−4 were dissected longitudinally to produce an untrimmed (steak A) and a trimmed steak (steak B), with the latter having the belly flap and dorsal regions removed as indicated (Figure 1). Total lipid and carotenoid were extracted and measured in each zone from three fish fed diets containing either 16% or 30% lipid. Fish fed 16% dietary lipid received 75 mg/kg CX while those fed 30% dietary lipid received 75 mg/kg carotenoid in the ratio 25 AX:50 CX initially but received 70 mg/kg AX only in the 6-month period preceding sampling. Consequently, flesh carotenoid in the group fed 30% dietary oil contained around 20% of total pigment as CX at the time of sampling. The distribution of lipid between the various body zones in fish fed either 16 or 30% lipid is shown in Figures 7 and 8. Figure 7A shows the body zone lipid content in untrimmed steaks from the two dietary treatments. In both dietary treatments there is significantly less lipid in the tail muscle (zone 5) as compared to zones 1−3, with the highest mean values found in zone 2, the steak routinely studied in the previous part of this study. Interestingly, zone 4, the region quoted by the Norwegian salmon industry ("The Norwegian Quality Cut") to assign flesh lipid content, contains a lower mean lipid content compared to zone 2 used in this study. In zones 1, 2, 3, and 5, the lipid content was significant greater in untrimmed steaks (A) from fish fed 30% lipid as

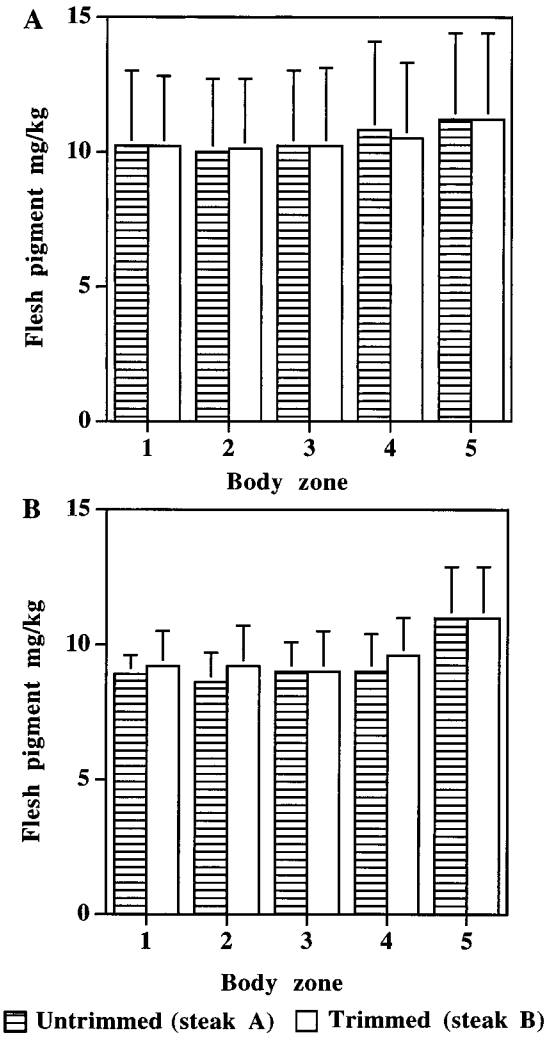

Figure 9. Body zone pigment content vs dietary lipid. Figure shows carotenoid pigment content (mg/kg) of five body zones (described in Figure 1), before and after trimming, in salmon fed either 16% (A) or 30% (B) dietary lipid. Values are mean ± SD for three fish per dietary treatment.

compared to fish fed 16% lipid. Figure 7B shows the effect of trimming the steaks, as shown in Figure 1 (steak B), on lipid content of steaks from fish fed 16 or 30% lipid. Trimming the adipocyte-rich zones from the steaks eliminated any significant differences between the different body zones in fish fed each dietary lipid level. However, in all five body zones the level of flesh lipid was significantly different between different dietary treatments within the same body zone. Figure 8A shows the lipid levels in trimmed (steak B) and untrimmed steaks (A) from each body zone in fish fed 16% dietary lipid.

Only in zone 2 was there a significant difference between trimmed (steak B) and untrimmed steaks (A). Figure 8B shows the lipid levels in trimmed (steak B) and untrimmed steaks (A) from each body zone of fish fed 30% dietary lipid. In body zones 1 and 3, lipid content was significantly greater in the untrimmed steaks compared to trimmed steaks. The total carotenoid pigment content of each body zone, in trimmed and untrimmed steaks, from fish fed 16 or 30% dietary lipid is shown in Figure 9. The data indicated that there was no significant effect of dietary lipid on pigment deposition, nor was there a significant difference between trimmed (steak B) and untrimmed steaks (A) from either dietary treatment. These observations suggest that there is no correlation between lipid content of the tissue and pigment deposition and support the finding that AX and CX are primarily protein-bound (Henmi et al., 1989). Although not statistically different, there is an increase in mean carotenoid accumulation in the tail region (zone 5), particularly of fish fed 30% dietary lipid as compared to the other body zones. The preferential accumulation of carotenoid pigment (up to 40% more) in the tail region has been observed previously in other salmonid species (Christiansen and Wallace, 1988; No and Storebakken, 1991).

The present study provides valuable information on the flesh quality of Atlantic salmon produced by the Scottish salmon industry and particularly highlights the consequences of feeding diets with varying lipid content. We conclude from our studies that the salmon produced by the Scottish aquaculture industry is of a high standard, showing no evidence of lipid degradation, and representing a rich source of nutritionally valuable 20:5$n-3$ and 22:6$n-3$. Some concern exists regarding the use of dietary lipid levels in excess of 30% since such diets can induce excessive oiliness in some individual fish, which may prove unpalatable to consumers and present difficulties to the smoking and processing sectors. However, our study indicates that some salmon can consume high lipid diets without excessive deposition of oil in the flesh. Further investigations into the regulation of adiposity together with selective breeding to eliminate very oily fish remain a challenging priority.

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