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Atlantic salmon, Salmo salar, utilizes wax ester rich oil from *Calanus finmarchicus* effectively.

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Abstract

Against a background of decreasing availability of fish oils for use in aquaculture, the present study was undertaken to examine whether a wax ester – rich oil derived from the calanoid copepod *Calanus finmarchicus*, could be used effectively by Atlantic salmon when supplied in their diet. Individually tagged Atlantic salmon of initial weight around 500g were divided into replicate tanks of two dietary groups and fed either a fish oil supplemented diet, or an experimental diet coated with *Calanus* oil. Wax esters accounted for 37.5% of the lipids in the *Calanus* oil diet but were absent from the fish oil diet in which triacylglycerols (TAG) were the major lipid class. Over the feeding period (140 days) the salmon fed fish oil displayed a greater increase in length, but there was no significant difference between the two groups in weight gained. The specific growth rates (0.75) and the feed conversion ratio of fish fed the two diets were similar throughout the study.

No differences were observed in the apparent digestibility coefficients (ADC) of fish fed *Calanus* oil or fish oil. The ADC of fatty acids decreased with chain length and increased with unsaturation. Long-chain alcohol utilization showed a similar tendency although there was a notable difference in that saturated long-chain alcohols were utilized better than the comparable fatty acid homologue. In fecal lipid of fish fed *Calanus* oil, the content of 16:0 alcohol decreased in both the free long-chain alcohol and wax ester fractions, while the corresponding fatty acid increased in the feces of both dietary groups of fish. In contrast, the proportion of the 22:1n-11 alcohol increased in both fecal wax esters and free long-chain alcohol fractions whereas 22:1n-11 fatty acid displayed no accumulation. The observed patterns of fatty acid and long-chain alcohol compositions in fecal lipid compared to those of the initial dietary lipid are consistent with the digestive lipases of salmon preferentially hydrolyzing esters containing PUFA moieties. The wax esters of *Calanus* oil contained substantial amounts of the n-3 PUFA, 20:5n-3 and 22:6n-3, that were effectively deposited in muscle and liver tissues. No major differences were seen in either lipid content/lipid classes or in gross fatty acid composition of these tissues between the two dietary groups. It is concluded that that Atlantic salmon in seawater can effectively utilize diets in which a major lipid component is derived from zooplankton rich in wax ester without any detrimental change in growth or body lipid composition. This finding gives support to the use of lipid from zooplankton from high latitudes as an alternative or as a supplement to fish oil and a provider of long chain n-3 PUFA in diets for use in salmon aquaculture.

1. Introduction

The demand for feed in the salmonid aquaculture industry has shown a strong increase over recent years in parallel with increases in total production within the industry (Waagbø et al., 2001). This production relies heavily on fish meal and oil supplies (Waagbø et al., 2001). At present traditional marine biological resources are being exploited to the highest possible level, and in many seas beyond the sustainable level (FAO, 1997). Further growth in the carnivorous fish aquaculture industry is thus dependent on new feed resources becoming available.

The most sought alternatives at present are vegetable protein and oil sources. Most data suggest that these can be included into salmonid diets at relatively high amounts without compromising growth rate or nutrient utilization. Vegetable oils do not, however, contain the long chain n-3 polyunsaturated fatty acids (PUFA) traditionally found in fish oils. These fatty acids are known to play a beneficial role in several areas of human health and development including the reduction of coronary heart disease (Imazio et al., 2003).

A possible alternative to vegetable oils as a replacement for fish oils in salmonid aquaculture is the use of oils from unexploited marine resources. *Calanus finmarchicus,* the most abundant herbivore in the Nordic Seas, has an annual production of several hundred million tonnes (Melle et al., in press). Although rich in both lipid and n-3 PUFA, *Calanus* contains wax esters as the main lipid component rather than triacylglycerols, which comprise the bulk of fish oils. The acyl moieties of triacylglycerols consist only of fatty acids whereas wax esters are composed of a fatty acid esterified to a long-chain alcohol. Studies have shown that many species of fish have the capacity to digest wax esters (Patton and Benson, 1975; Patton et al., 1975; Mankura et al., 1984) but it appears that the hydrolytic activity in the gut is much lower towards wax esters than triacylglycerols (Patton et al., 1975; Tocher and Sargent, 1984; Olsen and Ringø). This has caused some concern that wax esters may not be effectively utilized, particularly in juvenile fish (Olsen et al., 1991).

The fact that *C. finmarchicus* is the main food of young salmon in the sea and also feature in the natural diet of older salmon feeding in the North Atlantic, suggests that this species can digest zooplankton with a high content of wax esters although the efficiency of this process is not known. The intention of this study was to evaluate the effect of feeding oil derived from *C. finmarchicus* on growth rate, feed utilization and composition of Atlantic salmon under controlled conditions with a view to the potential utilization of this source of oil in commercial aquaculture.

2. Materials and Methods

2.1 *Feed preparation*

Calanus finmarchicus was purchased block frozen from Norsk Medisinaldepot Ltd. Bergen, Norway. Lipid extraction and feed preparations were performed at the experimental plant of the Norwegian Institute of Fisheries and Aquaculture at Titlestad, Bergen, Norway. In brief, 40 kg of thawed *Calanus* was heated to 85 – 90°C in a steam heated vessel containing 10 l of water/press liquid. Most of the liquid phase was removed in a double screw press (Stord Bartz P13). The press liquid was then heated to at least 90°C in a scraped surface heat exchanger (Alfa Laval Contherm 4×6), and particulate matter removed in a 100 mm wet sieve before the oil was extracted using Westfalia SA1 separator. The diets were then prepared by extrusion using similar standards and additives as to commercial diets. After extrusion, the diets were coated with either fish oil or *Calanus* oil. The chemical compositions of the two diets used are shown in Table 1. All diets contained 0.01% yttrium oxide as digestibility marker.

2.2 *Fish*

One hundred Atlantic salmon, *Salmo salar* (NLA strain - Norwegian breeding programme, 500 g), were transferred into four 1.5 × 1.5 × 1 m^3 standard fiberglass tanks equipped with feed collectors, supplied with aerated seawater (10 ± 1.5 °C) and kept under a 12:12h daylight regime at the Matre Aquaculture Research Station, Norway. The fish were then individually tagged with T-Bar anchor tags (Floy Tag & MFG., Inc, Seattle, USA) and allowed to adapt to the new conditions for two weeks. One day prior to initiation of experiment, the fish were anaesthetized in 0,4% benzocaine and weight (to nearest 0.1 g) and length (to nearest 5 mm) determined. The fish were fed the two experimental diets in duplicate every day using disc feeders from 08.00 until 12.00 h.

The fish were anaesthetized in 0.4 % benzocaine and measured for growth and feed conversion after 71 days of feeding and at termination of study at 140 days. At termination of the study feces were stripped from five fish per tank according to Ringø (1991). The resulting contents were then split into two equal portions.

2.3 *Analysis*

One portion of feces was heated at 105°C for 24 h to obtain dry weight of the samples. These were then processed further for yttrium oxide content as described previously (Otterå et al., 2002). Total lipid was extracted from the other portion using chloroform/methanol (2:1, by volume) according to Folch et al. (1957). Dilute HCl (30% of original feces weight) (3 M)

was added to the feces prior to the last extraction to dissolve any calcium soap present. After evaporation to dryness *in vacuo* at room temperature, total lipid was re-dissolved in chloroform/methanol (2:1, by vol.) and stored under nitrogen at –80ºC prior to analysis. Following feces collection, the fish were killed by a sharp blow to the head. Livers and sections of muscle (sampled as a vertical strip posterior to the dorsal fin) were taken and lipid extracted from them according to Folch et al. (1957). Lipid extracts were stored under nitrogen at –80ºC until analyzed further.

Lipid class composition was determined by double development high-performance thin layer chromatography (HPTLC) coupled with scanning densitometry, as described by Olsen and Henderson (1989). Methyl acetate / isopropanol / chloroform / methanol / 0.25% aqueous KCl (25:25:25:10:9 by vol) was employed as the first solvent system and isohexane / diethyl ether /glacial acetic acid (85:15:1 by vol) as the second developing solvent mixture. Developed chromatograms were scanned using a Camag 3 TLC Scanner (Camag, Muttenz, Switzerland) and winCATS software. The identities of individual lipid classes were confirmed by comparison with reference to the Rf values of authentic standards run alongside samples on HPTLC plates and developed in the above solvent systems.

To determine the fatty acid composition of total lipid an aliquot of known weight was subjected to acid-catalyzed transesterification using 1% (v/v) H_2SO_4 in methanol. A known amount of 17:0 was added to the sample prior to transesterification as internal standard. The resulting fatty acid methyl esters (FAME) were extracted and purified using thin layer chromatography (TLC) on 20*20 cm TLC plates as described previously (Tocher and Harvie, 1988). The total long-chain alcohols present in the lipid samples extracted from the *Calanus* oil diet and the feces of fish fed this diet were isolated during this purification step as a single component and recovered from the adsorbent by elution with chloroform / methanol (2: 1 by vol). A known amount of 17:0 alcohol was added as internal standard before the recovered fatty alcohols were subjected to acetylation as described below.

Wax esters in diet and fecal lipid samples were isolated by TLC on 20 * 20 glass plates coated with silica gel G using isohexane / diethyl ether / glacial acetic acid (90: 10: 1 by vol) as developing solvent. The separated classes were visualized by spraying the developed chromatograms with 0.01% (w/v) 2',7'–dichlorofluorescein in methanol and viewing under UV light. The wax ester band was recovered from the adsorbent by elution with isohexane / diethyl ether (1: 1 by vol). Similarly, free long-chain alcohols in fecal lipid samples were isolated by TLC as above but using isohexane / diethyl ether / glacial acetic acid (70: 30: 1 by vol) as developing solvent. Purified wax esters were subjected to acid-catalyzed

transesterification as above to generate the FAME of the component fatty acids and free long-chain alcohols. The FAME and free long-chain alcohols were purified by TLC and recovered from the adsorbent as described above. Prior to analysis by gas chromatography (GC), long-chain alcohols were converted to their acetate derivatives by reaction with acetic anhydride / pyridine (1: 2 by vol) as described previously (Farquhar, 1962) and purified by TLC as described for FAME.

FAME and long-chain alcohol acetates were separated and quantified by GC using a 30m * 0.32mm i.d. fused silica capillary column coated with ZB-Wax (Phenomenex Ltd., Macclesfield, UK) and a Thermo Finnigan Trace gas chromatograph. Hydrogen was used as carrier gas and temperature programming was from 50 to 150 ^{o}C at a rate of 40C^{o}/min, from 150^{o}C to 195^{o}C at 1.5C^{o}/min and then to a final temperature of 205^{o}C at 0.5C^{o}/min. Individual components were identified by comparison with known standards and a well-characterized fish oil. When required, the absolute amounts of individual fatty acid and long-chain alcohols present in lipids could be calculated by reference to the internal standard.

2.4 *Calculations and statistical treatment*

Specific growth rate (SGR) was estimated according to the formula:

$$SGR = 100 * (LN(\text{end wt}) - LN(\text{start wt}) / \Delta t$$

where end wt = end weight of fish, start wt = start weight of fish, Δ t = days of experiment in days.

Apparent digestibility was estimated according to the formulae:

$$ADC = 100\text{-}100*((Y_{feed}/Y_{feces})*(N_{feces}/N_{feed}))$$

where Y_{feed} = yttrium oxide in feed, Y_{feces} = yttrium in feces, N_{feces} = nutrient in feces, N_{feed} = nutrient in feed. All data were based on calculated dry weight of the samples. For fatty acid / long-chain alcohol digestibilities, data were based on the amount of the individual component in μg mg^{-1} of total lipid.

Kfactor was estimated according to:

$$Kfactor = Wt / Lth^3$$

where Wt is fish weight in grams, and Lth is fork length of the fish in cm.

Feed conversion was estimated as:

$$Fc = \Delta \text{ feed} / \Delta \text{ growth}$$

where Δ feed is the amount of feed in grams consumed by the fish in the tank between each weighing of the fish, and Δ growth is the increase of fish biomass during the same period.

All data are given as means ± S.D. of 10 fish unless otherwise stated. Feed conversion was obtained from both replicate tanks (n=2). The data presented are the average values from these two observations. All statistical analysis was performed using SPSS software for Windows (SPSS Inc., MI). Data were checked for homogeneity of variances by the Levene test. Whenever necessary, statistical analysis was carried out using arcsin-transformations on percentage data, or LN transformations for remaining samples. Data were subjected to General Linear model analysis where significance was accepted at $P < 0.05$.

3. Results

The group of salmon fed *Calanus* oil grew from 525g to 1553g after 140 days (Fig. 1A). The corresponding data for the control fish oil group was 559 and 1641g, respectively. The average weights of the control group were marginally higher at the beginning of the study, but these differences were evened out after 71 days and at the termination of the study although there was a general but nonsignificant (P=0.199) tendency of this group to maintain its weight advantage. Interestingly, the control group appeared to have marginally better length growth (Fig. 1B) compared to the *Calanus*-oil fed group, being significant at all measurements following initiation of study. These differences also became apparent with the higher condition index in the fish fed *Calanus* oil being significant at termination (Fig. 1C), indicating that length increase but not weight growth was higher in the fish oil group.

The specific growth rate (SGR) of the fish fed the two diets was not significantly different throughout the study being an average of 0.7 for fish up to 1000g and 0.8 for fish between 1000 to 1500g (Fig. 1D). This gave an average SGR of 0.75 for both groups during the experiment. The tendency of *Calanus* oil fed fish to have a higher growth rate during the first 71 days, and lower SGR from 71 to 140 days was noticeable however.

The feed conversion was also similar between the two groups increasing from 0.9 in both groups from start to 71 days, to 0.95 for fish fed fish oil and 1.02 for the group fed *Calanus* oil at termination of the study (Fig. 1 E).

The lipid class composition of the *Calanus* oil diet differed from that of the control diet, particularly in terms of the neutral lipid components. The major lipid class in the control diet was triacylglycerols (TAG), which accounted for more than 56% of the total lipid with free fatty acids (FFA) and cholesterol (C) making up most of the remainder of the neutral lipids. The main polar lipid classes present in the control diet were PE (5.6%) and PC (6.0%) with only very small amounts of PI, SM and LPC being found (Table 2). The lipid of the diet supplemented with *Calanus* oil, on the other hand, contained 37.5% wax esters and TAG

accounted for only 24% of the dietary lipid. The remaining lipid classes were to a large extent similar to that of the control diet being mainly FFA and cholesterol with PE and PC as the main phospholipids. Only traces amounts of free (non-esterified) long-chain alcohols were present.

The feces had a moisture content of 82% for both diets, with lipid constituting 8-9% of the dry matter. Fecal lipids of the control group of fish contained lowered levels of total polar lipids and TAG and increased levels of FFA when compared to the original dietary lipid.

The wax ester content of the fecal lipid of the fish fed *Calanus* oil was only 12% compared to 37.5% in the lipid of the diet whereas the TAG level was similar in lipid from both feces and diet at around 24%. The level of FFA in the fecal lipid was not much higher than that present in the original *Calanus* oil diet but free long-chain alcohols, which were only present at trace levels in the *Calanus* oil, accounted for 16.2% of the fecal lipid of fish fed diet containing this oil. There were also significant amounts of substances in the fecal lipid from both dietary groups whose identities were not certain but which were pigmented before staining and others which had similar Rf values to glycolipids.

Table 3 shows the fatty acid and long-chain alcohol compositions of the two diets and the feces from the fish consuming the diets. The control diet had a total of 886.5 μg fatty acids mg lipid $^{-1}$ compared to 558.7 μg mg lipid^{-1} in the *Calanus* oil diet. However, the latter diet contained in addition 198.3 μg long-chain alcohols mg lipid^{-1} so that the overall acyl moiety content of the *Calanus* oil diet was 757 μg mg^{-1} lipid compared to 886.5 μg mg^{-1} lipid in the control diet.

The total PUFA content of the two dietary lipids was fairly similar being 233.9 and 207.8 μg mg^{-1} lipid in the control and *Calanus* diet, respectively. Overall, the fatty acid composition of the two diets were quite similar in relative amounts except for a notable higher level of monounsaturated fatty acids, mainly 22:1n-11 and to a lesser extent 20:1n-9 and 18:1n-9 in the control diet, and a higher proportion of 18:4n-3 in the *Calanus* diet. The main long-chain alcohols present in the *Calanus* oil diet were 20:1n-9 and 22:1n-11 which together comprised almost 70% of the total alcohol composition with most of the remainder being the 16:0 alcohol.

In the lipid of the feces from both dietary groups, there was a notable increase in the proportion of saturated fatty acids, and a reduction in that of PUFA, compared to the corresponding dietary lipid. The level of monounsaturated fatty acids in fecal lipid was similar to that of the diet in both groups of fish. For the long-chain alcohols however, there was a reduction in the proportions of both PUFA and saturates with a notable increase in the

content of the 22:1n-11 alcohol increasing from 40% in the diet to 56% in feces of fish fed *Calanus* oil.

To examine whether the high content of 22:1n-11 alcohol in fecal lipid was due to wax esters containing this acyl moiety being resistant to digestion, the compositions of undigested wax ester and free long-chain alcohols in the feces were determined (Table 4). Compared to those of the diet the wax esters in feces contained notably lower levels of polyunsaturated (1.8 vs. 5.6 %) and saturated (5.1 vs. 14.9%) long-chain alcohols. The level of monounsaturated long-chain alcohols rose from 79.5% in the dietary wax esters to 93.0% in those of the feces. This was almost entirely due to an increase in the proportion of 22:1n-11.

The composition of the free long-chain alcohol fraction in the fecal lipid was intermediate to those of the dietary and fecal wax esters particularly with regard to the proportions of saturated and polyunsaturated long-chain alcohols, but was still dominated by 22:1n-11 (55.6%). In comparison with those present in the wax esters of the diet the fatty acids of the fecal wax esters were enriched in saturated fatty acids. This was due mainly to a decrease in the proportion of polyunsaturated fatty acids, which fell from 37.5% in the dietary wax esters to 7.6% in those of the feces. The content of monounsaturated fatty acids was similar in the wax esters of the diet and feces.

Lipid digestibility (91-92%) was somewhat lower than that of dry matter, which was around 95% regardless of dietary origin. The digestibilities of both the fatty acid and long-chain alcohol components of the *Calanus* oil were in general comparable to those of the fatty acids in the fish oil diet (Table 5) being in the range 92-95%. No difference was found between the two dietary groups. There was a clear tendency for digestibility to increase with increasing degree of unsaturation for fatty acids of similar chain length. For example, the utilization of 18:0 fatty acid was 88%, increasing to 95-96% for 18:1 and 98-99% for 18:3/18:4. Likewise, the digestibility of 20:1n-9 was lower than that of 20:5n-3 (95.7 vs. 98.6). There was also a general reduction in digestibility with an increase in chain length, particularly for saturated and monounsaturated fatty acids. A similar trend was observed for the long-chain alcohols, particularly for 18:1n-9, 20:1n-9 and 24:1n-9.

All saturated long-chain alcohols were better utilized than the corresponding fatty acids in both diets. Although the 16:1,18:1 and 20:1 alcohols were digested as well as the corresponding fatty acids it was notable that 22:1n-11 alcohol was the only alcohol digested less efficiently than its corresponding fatty acid in both diets groups. Thus, the digestibility of 22:1n-11 alcohol was 92%, compared to 93,9% for the fatty acid in the *Calanus* diet, and 95,6% in the control diet. The PUFA utilization was similar in both diets.

The liver and muscle lipid content and class compositions of fish in the two dietary groups are presented in Table 6. Livers contained around 4% lipid and muscle 12-13% with no difference between groups. Lipid extracted from liver contained just less than 25% TAG and 13-14% cholesterol. PC was the major polar lipid (19%) followed by PE. The type of lipid supplied in the diet appeared to have no effect on the lipid class composition. The TAG content of muscle lipid, around 78%, was much higher than that extracted from liver. In general, diets appeared to have no major influence on the lipid class composition of muscle, except for a small decrease in the proportion of cholesterol and a small increase in the level of free fatty acids in the fish fed *Calanus* oil.

Although feeding *Calanu*s oil to fish did not cause massive changes in muscle fatty acid composition, some differences were observed (Table 7). Most notably, with the exception of a marginal increase in 22:1n-11, the proportion of monounsaturated fatty acids showed a general decrease following feeding with the *Calanus* diet, which was accompanied by an increase of similar magnitude in the proportion of PUFA due mainly to increased levels of 18:3n-3 and 18:4n-3. The overall level of saturated fatty acids in muscle lipid (around 22%) was, however, not influenced by dietary *Calanus* oil. In contrast, a small but significant increase in the level of saturated fatty acids was the main effect of feeding this oil observed on the liver fatty acid composition. This increase was largely attributable to an increase in the proportion of 16:0. For the remaining fatty acids, changes were rather small and followed much of the same tendency as muscle. Thus, the proportions of 18:3n-3 and 18:4n-3 almost doubled but overall, there was no difference between the control and *Calanus* oil groups in terms of total PUFA or monounsaturated fatty acid levels in liver lipid.

4. Discussion

The acyl moieties of triacylglycerols consist only of fatty acids whereas wax esters are composed of a fatty acid esterified to a long-chain alcohol. Such arrangements are generally considered to be a poorly digested food source, especially in mammals (Place, 1992). However, many fish species including Atlantic salmon feed on several species of crustaceans like Euphausiids, Hyperiids and copepods (Jacobsen and Hansen, 1996) that will contain substantial amounts of wax esters in their depot lipids (Sargent et al., 1976; Falk-Petersen et al., 1982). Especially, feeding on *Calanus finmarchicus* by the young salmon will introduce a high fraction of wax esters in the diet. Consequently, some capacity to utilize these energy sources would be expected. Indeed, studies have shown that many species of fish do have the capacity to digest wax esters (Patton and Benson, 1975; Patton et al., 1975; Mankura et al.,

1984). However, it also appears that the hydrolytic activity in the gut is much lower towards wax esters than triacylglycerols (Patton et al., 1975; Tocher and Sargent, 1984; Olsen and Ringø, 1997). If this also lowers the utilization of wax esters, this may limit their usefulness in aquaculture feeds.

Under the present experimental conditions, substituting fish oil with oil extracted from the zooplankton *C. finmarchicus* neither influenced fish growth nor feed conversion of Atlantic salmon. Furthermore, the apparent digestibility coefficients (ADC) of dietary dry matter and lipid were similar. This clearly shows that Atlantic salmon is capable of assimilating lipids from wax esters at comparable rates as triacylglycerols (TAG). Whether the rate of hydrolysis of wax esters is lower than TAG remains to be determined and may be of importance if the level of wax ester in dietary lipid increases to levels higher than in the present experiment. It is also possible that the hydrolytic capacity exceeds the rate necessary for lipid utilization so that possible differences are of biological insignificance in absolute terms.

The ADC of the individual fatty acids of fish fed both the fish oil and the *Calanus* oil diet followed much of the same tendency as that observed in most fish species (Olsen and Ringø, 1997), decreasing with chain length and increasing with unsaturation. The absolute variation between the different fatty acids was however less than expected from literature (Olsen and Ringø, 1997). In Atlantic salmon, for example, Sigurgisladottir et al. (1992), using TAG and ethyl esters, reported ADC of 64% for 16:0, 85% for 18:1n-9 and 96% for 20:5n-3. In the present study, the corresponding values were 91, 96 and 98%, respectively. The reason for these differences is at present uncertain, but it cannot be excluded that the use of internal standard to calculate actual fatty acid/alcohol content of the lipid on an absolute basis may increase the value obtained for ADC and give more accurate digestibility values than those calculated from the relative fatty acid composition of total lipid.

The decreased digestibility of long-chain alcohols with increasing chain length followed the same tendency as that of fatty acids showing that the utilization of dietary long-chain alcohols in general is comparable to fatty acids in this respect. There were, however, some other notable differences. In particular, it seemed saturated long-chain alcohols were better utilized than the corresponding fatty acids. This was confirmed by detailed analysis of fecal lipids from fish fed the *Calanus* oil where the predominant saturated fatty acid 16:0 accumulated in the remaining fecal lipids, whereas the opposite was true for 16:0 alcohol whose relative amount was reduced both in the remaining wax ester and free the long-chain alcohol fractions. This observation that the well-established poor intestinal digestion and

absorption of long chain-saturated fatty acids (Sigurgisladottir et al., 1992; Olsen et al., 1998) does not hold for their long-chain alcohol homologs may result from the substrate specificity of the digestive lipases. In cod, *Gadus morhua*, Lie and Lambertsen (1985) found that saturated fatty acids were resistant to lipolysis and accumulated in the undigested fractions of both TAG and wax ester substrates when subjected to hydrolysis by intestinal juice.

Analyses of the wax ester in *C. finmarchicus* have shown that the long-chain monounsaturated fatty alcohols 20:1 and 22:1 are esterified predominantly to shorter-chain fatty acids such as 14:0 whereas the medium-chain alcohol 16:0 is esterified mostly to PUFA, particularly 18:4n-3 (Sargent and Henderson, 1986). It follows that a preference of the salmon digestive lipase for ester linkages of unsaturated fatty acids would result in a decrease in the proportions of 18:4n-3 and 16:0 alcohol in fecal lipid as observed in the present study. At the same time, molecular species of wax esters containing 14:0 fatty acid esterified with 20:1 or 22:1 alcohols would be poor substrates for the lipase and would be concentrated in the fecal material. In keeping with this there was a substantial increase in the proportions of 14:0 fatty acid and 22:1n-11 alcohol in the lipids of the feces relative to the diet. To confirm the apparent substrate specificity in future studies the individual molecular species of dietary and fecal wax esters should be isolated and compared.

It was also interesting to note that 22:1n-11 alcohol accumulated to a similar extent in both the wax ester and the free long-chain alcohol fractions indicating that both the hydrolysis and absorption across the enterocyte barrier may be rate limiting for its utilization. Elevated levels of 22:1n-11 alcohol in digesta has been observed in both rainbow trout (Sargent et al., 1979) and cod (Lie and Lambertsen, 1991) fed *C. finmarchicus* wax esters indicating some similarities in the resistance towards the absorption of this alcohol. Interestingly, Lie and Lambertsen (1985) failed to show lower activity of digestive juices against wax esters containing 22:1n-11 alcohol compared to other alcohols thus indicating that the limiting step in cod may be the absorption across the intestinal barrier and not lipase specific as it appears to be in salmonids

Despite their abundance in the diet of salmon fed *Calanus* oil, wax esters had no influence on the lipid content or lipid class composition of liver and muscle. Furthermore, no wax esters were found in either tissue. This shows the existence of an efficient long-chain alcohol dehydrogenase similar to that demonstrated in rainbow trout (Bauermeister and Sargent, 1978) efficiently oxidizing long chain fatty alcohols into their corresponding fatty acids before being transported and deposited in various tissues.

Of particular importance to the nutritional quality of salmon is the level of long chain (C20 + C22) n-3 PUFA that are required in human diets playing a role in reducing the prevalence of human coronary heart diseases (Imazio et al., 2003). Although the *Calanus* oil diet contained a level of long chain n-3 PUFA about two-thirds of that found in the fish oil diet, this had relatively little impact on the fatty acid composition of liver and muscle in the fish. Thus the level of long chain n-3 PUFA in the flesh of salmon fed the *Calanus* oil diet was almost 94% of the value in fish fed the fish oil diet. Most of the differences that could be observed, such as lower 18:1n-9 and higher 18:4n-3 in fish fed the *Calanus* oil diet could be attributed to differences in the composition of the dietary lipids.

In conclusion, the present study demonstrates that Atlantic salmon in seawater can effectively utilize diets in which a major lipid component is derived from zooplankton rich in wax esters without any detrimental effects on growth or body lipid composition. This may in part to be linked to the prevalence of the bile stimulated lipase in fish rather than the pancreatic lipase that dominates in mammalian systems (Olsen and Ringø, 1997). As the pancreatic lipase is only poorly active towards wax esters, the bile-stimulated lipase hydrolyzes wax esters rather efficiently (Place 1992). An interesting notion is also that some seabirds that feed on wax ester containing fish seem to have higher bile-stimulated lipase activity and accordingly wax ester utilization than birds that do not (Place 1992). The lipolytic enzyme in fish do however to contain certain specificities with regard to chain length and saturation of the long-chain alcohols and fatty acids. In particular, saturated fatty acids are poorly utilized while saturated long-chain alcohols are not. Furthermore, the long chain monoenoic alcohol 22:1n-11 seems to be poorer substrate for digestive lipases and intestinal absorption compared to its fatty acid homologue. These results need further examination. Overall, digestibility and feed efficiency of diets containing calanoid copepod oil is not significantly different to that in fish maintained on diets containing fish oil.

This finding gives support to the use of lipid from zooplankton from high latitudes as an alternative or as a supplement to fish oil in the formulation of diets for use in salmon aquaculture thus providing substantial amounts of the long chain n-3 PUFA, 20:5n-3 and 22:6n-3. An additional benefit associated with *Calanus* oil is the fact that it originates from a lower tropic level than fish oil, and can therefore be expected to have a lower content of PCBs and dioxins, compounds that are undesirable in food for human consumption and are of current concern in farmed salmon (Hites et al., 2004). Further studies to examine whether this zooplankton oil is equally effective at all developmental stages of the salmon and with very high lipid levels should be undertaken to maximize the potential of this marine oil.

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Legend to figure

Figure 1. Growth and feed data of Atlantic salmon fed diets containing either fish oil or oil derived from *Calanus finmarchicus*. A: Weight development, B: length development and C: k factor of fish at beginning of the study, and after 71 and 140 days of feeding. D: Specific growth rate (SGR) at 71 and 140 days of feeding. Total SGR represents overall growth from start to 140 days. E: feed conversion at 71 and 140 days of feeding. Values are means of two replicate tanks for each treatment. *: $P<0.05$. **: $P<0.01$

Table 1. Composition of the experimental diets used in the experiment.

	Ingredients (g/100g)		Chemical composition (by calculation)	
	Fish oil diet	*Calanus* diet		
Fish meal 52/02	59.4	59.4	Protein	47.0
Maize suprex	16.9	16.9	Lipid	29.5
Vitamin mixture*	1.0	1.0	Carbohydrate	14.0
Mineral mixture**	0.4	0.4	Ash	8.7
Inositol	0.03	0.03		
Betain	0.1	0.1		
Soybean lecithin	0.5	0.5		
Y_3O	0.01	0.01		
Carophyll pink	0.08	0.08		
Fish oil	21.6	0		
Calanus oil	0	21.6		

* will supply diets with the following vitamins pr kg diet: vitamin D3, 3000 I.E; vitamin E (Rovimix, 50%), 160 mg; thiamin, 20 mg; riboflavin, 30 mg; pyridoxine-HCl, 25 mg; vitamin C (Rovimix Stay C 35%), 200mg; calcium pantothenate, 60 mg; biotine, 1 mg; folic acid, 10 mg; niacin, 200 mg; vitamin B 12, 0,05 mg; menadione bisulphite, 20 mg.
** will supply the diet with the following minerals pr kg diet: magnesium, 500 mg; potassium, 400 mg; zinc 80 mg; iron, 50 mg; manganese, 10 mg; copper, 5 mg.

Table 2. Lipid class and composition of dietary and fecal lipids.

	Fish oil diet		*Calanus* oil diet	
Lipid class	Diet	Feces	Diet	Feces
SE	2.5	0.1 ±0.2	-	-
WE	-	-	37.5	12.0 ±5.8
TAG	56.1	30.6 ±3.7	24.0	23.4 ±6.2
UNL	-	2.8 ±0.5	-	1.4 ±0.5
FFA	13.5	26.7 ±4.5	14.9	17.9 ±5.5
FFAI	-	-	-	16.2 ±3.6
C*	10.4	8.2 ±1.3	7.8	7.7 ±0.6
Pig**	2.1	14.3 ±1.2	2.9	9.1 ±2.1
UPL	-	10.3 ±2.3	-	5.1 ±1.8
PE	5.6	4.0 ±1.7	4.6	4.9 ±1.7
PI + PS	2.3	-	2.4	-
PC	6.0	2.3 ±0.6	4.8	1.8 ±0.2
SM	0.9	-	0.7	-
LPC	0.7	0.7 ±0.2	0.6	0.4 ±0.1
Moisture %	5.5	82.0 ±0.7	5.8	82.0 ±0.9
Lipid (% of dry weight)	25.6	8.1 ±1.8	26.2	9.0 ±1.3

SE, sterol esters; WE, wax esters, TAG, triacylglycerols; UNL, unknown neutral lipid; FFA, free fatty acids; FFAI, free fatty alcohols; C, cholesterol; Pig, pigmented material; UPL, unknown polar lipid; PE, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; SM, sphingomyelin; LPC, lysophosphatidylcholine

- less than 0,5% of total lipid
- *, may contain some diacylglycerols **, may contain some monoacylglycerols.

Table 3. Total fatty acid and long-chain alcohol content (μg mg^{-1}) and composition in feed and feces of Atlantic salmon fed diets supplemented with fish oil or *Calanus* oil.

	Content of dietary lipid in μg mg^{-1}			Composition (wt %) of fatty acids and long-chain alcohols in diet and feces					
	Fish oil	*Calanus* oil		Fish oil		*Calanus* oil			
				Fatty acid		Fatty acid		Long-chain alcohol	
	FA	FA	FAI	Diet	Feces	Diet	Feces	Diet	Feces
14:0	59.9	59.0	3.3	6.8	9.7±1.0	10.6	14.1±3.6	1.7	0.5±0.1
16:0	118.7	80.9	29.0	13.4	25.5±1.3	14.5	25.1±2.6	14.6	5.7±1.0
18:0	14.0	9.2	1.8	1.6	4.1±0.4	1.7	3.8±0.6	0.9	0.7±0.1
16:1n-7	40.5	23.8	3.2	4.6	1.8±0.9	4.3	2.8±0.5	1.6	0.7±0.1
18:1n-9	100.4	49.2	7.6	11.3	8.2±0.7	8.8	8.7±1.9	3.8	1.7±0.2
18:1n-7	15.9	8.6	4.0	1.8	1.7±0.5	1.5	2.0±0.5	2.0	2.0±0.5
20:1n-9	104.2	39.0	57.9	11.8	10.7±0.6	7.0	8.0±1.0	29.2	27.8±1.0
22:1n-11	160.2	59.0	79.9	18.1	17.1±0.8	10.6	11.5±1.8	40.3	56.4±2.3
22:1n-9	10.6	4.5	3.1	1.2	1.8±0.2	0.8	1.9±0.7	1.6	0.8±0.3
24:1n-9	8.7	4.7	2.5	1.0	2.3±0.1	0.9	1.5±0.3	1.3	2.6±0.9
18:2n-6	21.8	18.2	2.9	2.6	2.4±0.3	3.2	3.0±0.6	1.5	0.6±0.8
20:4n-6	2.9	2.2	-	0.3	0.3±0.1	0.4	0.2±0.1	-	-
18:3n-3	10.2	14.0	2.6	1.1	0.5±0.1	2.5	1.1±0.3	1.3	0.6±0.1
18:4n-3	31.5	61.3	-	3.5	0.7±0.2	10.9	2.2±0.9	-	-
20:4n-3	5.3	6.1	-	0.6	0.3±0.1	1.1	0.8±0.3	-	-
20:5n-3	60.7	42.6	-	6.8	2.2±0.5	7.6	2.6±1.1	-	-
22:5n-3	5.3	3.5	-	0.6	0.4±0.1	0.6	0.5±0.2	-	-
22:6n-3	81.2	50.3	-	9.1	5.8±1.3	8.9	6.2±2.6	-	-
SAT	199.8	154.2	34.1	22.6	40.8±2.0	27.7	44.2±5.0	17.2	6.9±1.2
MONO	452.7	196.7	158.8	51.1	45.5±0.7	35.3	37.6±4.3	80.1	91.9±1.2
n-3 PUFA	195.2	178.5	2.6	21.9	10.0±2.1	31.8	13.5±5.1	1.3	0.6±0.1
n-6 PUFA	29.5	23.6	2.9	3.3	3.1±0.4	4.2	3.6±0.7	1.5	0.6±0.8
PUFA*	233.9	207.8	5.5	26.3	13.4±2.5	37.0	17.4±5.6	2.8	1.2±0.5
Total	886.5	558.7	198.3						

FA; fatty acids, FAI; long chain alcohols; - not detected
* total PUFA also includes some C_{16} PUFA not identified as n-6 or n-3.

Table 4. Fatty acid and long chain alcohol composition (wt %) of wax esters in *Calanus* oil diet and feces of salmon fed the diet along with the free long chain alcohols of feces.

	Wax ester				Free long-chain alcohol
	Long-chain alcohol		Fatty acid		
	Diet	Feces	Diet	Feces	Feces
14:0	1.6	0.4 ±0.1	20.2	36.6 ±6.2	0.5 ±0.1
16:0	12.6	4.2 ±0.7	13.2	24.8 ±1.7	6.1 ±0.7
18:0	0.7	0.6 ±0.1	0.6	1.7 ±0.5	0.5 ±0.1
16:1n-7	1.5	0.7 ±0.1	4.1	1.8 ±0.6	0.1 ±0.1
18:1n-9	3.6	1.3 ±0.3	6.0	5.2 ±1.4	1.0 ±0.3
18:1n-7	2.0	1.1 ±0.2	0.7	0.9 ±0.3	1.2 ±0.2
20:1n-9	28.2	27.0 ±0.5	6.0	6.0 ±1.3	28.4 ±1.0
22:1n-11	40.6	61.4 ±2.0	8.9	11.4 ±2.0	55.6 ±3.0
24:1n-9	3.2	1.6 ±0.5	0.3	0.9 ±0.2	2.6 ±0.5
18:2n-6	2.7	0.9 ±0.2	2.1	1.2 ±0.7	1.1 ±0.3
18:3n-3	2.9	0.9 ±0.2	3.5	0.5 ±0.2	1.2 ±0.3
18:4n-3	-	-	18.5	1.9 ±1.3	-
20:5n-3	-	-	6.1	1.1 ±0.6	-
22:6n-3	-	-	3.8	1.8 ±0.9	-
SAT	14.9	5.1 ±0.8	35.0	63.4 ±6.8	7.1 ±0.8
MONO	79.5	93.0 ±1.1	27.5	28.1 ±5.5	90.5 ±1.1
n-3 PUFA	2.7	0.9 ±0.2	34.0	5.6 ±2.7	1.2 ±0.3
n-6 PUFA	2.9	0.9 ±0.2	2.4	1.4 ±1.0	1.2 ±0.3
PUFA*	5.6	1.8 ±0.4	37.5	7.6 ±2.9	2.4 ±0.6

* total PUFA also includes some C_{16} PUFA not identified as n-6 or n-3.

Table 5. Digestibility of dry matter, total lipid, fatty acids and long-chain alcohols in Atlantic salmon fed diets supplemented with fish oil and *Calanus* oil.

	Fish oil diet	*Calanus* oil diet	
		Fatty acid	Long-chain alcohol
Dry matter	95.02 ± 0.17	94.95± 0.29	
Total lipid	92.06 ± 2.12	91.03±1.35	
14:0	93.45±2.66[a]	92.43±2.08[a]	98.35±0.40[b]
16:0	91.36±3.06[a]	90.27±1.41[a]	97.86±0.68[b]
18:0	88.38±3.36[a]	87.31±1.83[a]	95.81±0.69[b]
16:1n-7	98.27±1.18[a]	96.38±0.69[b]	97.39±0.69[ab]
18:1n-9	96.79±0.94[a]	94.46±1.12[a]	97.53±0.72[b]
18:1n-7	95.85±1.73[a]	92.70±1.56[b]	96.70±0.72[a]
20:1n-9	95.83±1.62[a]	93.61±0.59[b]	94.56±1.06[ab]
22:1n-11	95.66±1.64[a]	93.94±0.75[b]	92.00±1.30[c]
22:1n-9	93.07±2.89[a]	86.89±4.31[b]	95.63±2.71[a]
24:1n-9	89.41±3.55	89.70±2.54	89.71±3.44
18:2n-6	95.69±1.13[a]	94.82±1.48[a]	97.57±0.43[b]
20:4n-6	96.50±1.06	96.48±1.43	-
22:5n-6	95.94±2.39	94.07±3.32	-
18:3n-3	98.18±0.55	97.42±0.92	97.49±0.51
18:4n-3	99.14±0.30	98.87±0.49	-
20:5n-3	98.56±0.58	97.98±0.98	-
22:5n-3	96.94±1.21	95.22±2.53	-
22:6n-3	97.15±1.17	95.94±2.07	-
SAT	91.80±2.94[a]	90.99±1.54[a]	97.80±0.65[b]
MONO	95.97±1.42[a]	94.04±0.56[b]	93.48±1.12[b]
n-6 PUFA	95.97±1.15[a]	95.10±1.37[a]	97.57±0.43[b]
n-3 PUFA	97.98±0.80	97.53±1.13	97.49±0.51
PUFA*	97.75±0.80	97.28±1.12	97.53±0.45
TOTAL	95.48±1.57	94.36±0.84	94.33±1.00

* total PUFA also includes some C_{16} PUFA not identified as n-6 or n-3.

Table 6. Liver and muscle lipid class composition of Atlantic salmon fed fish oil and *Calanus* oil supplemented diets.

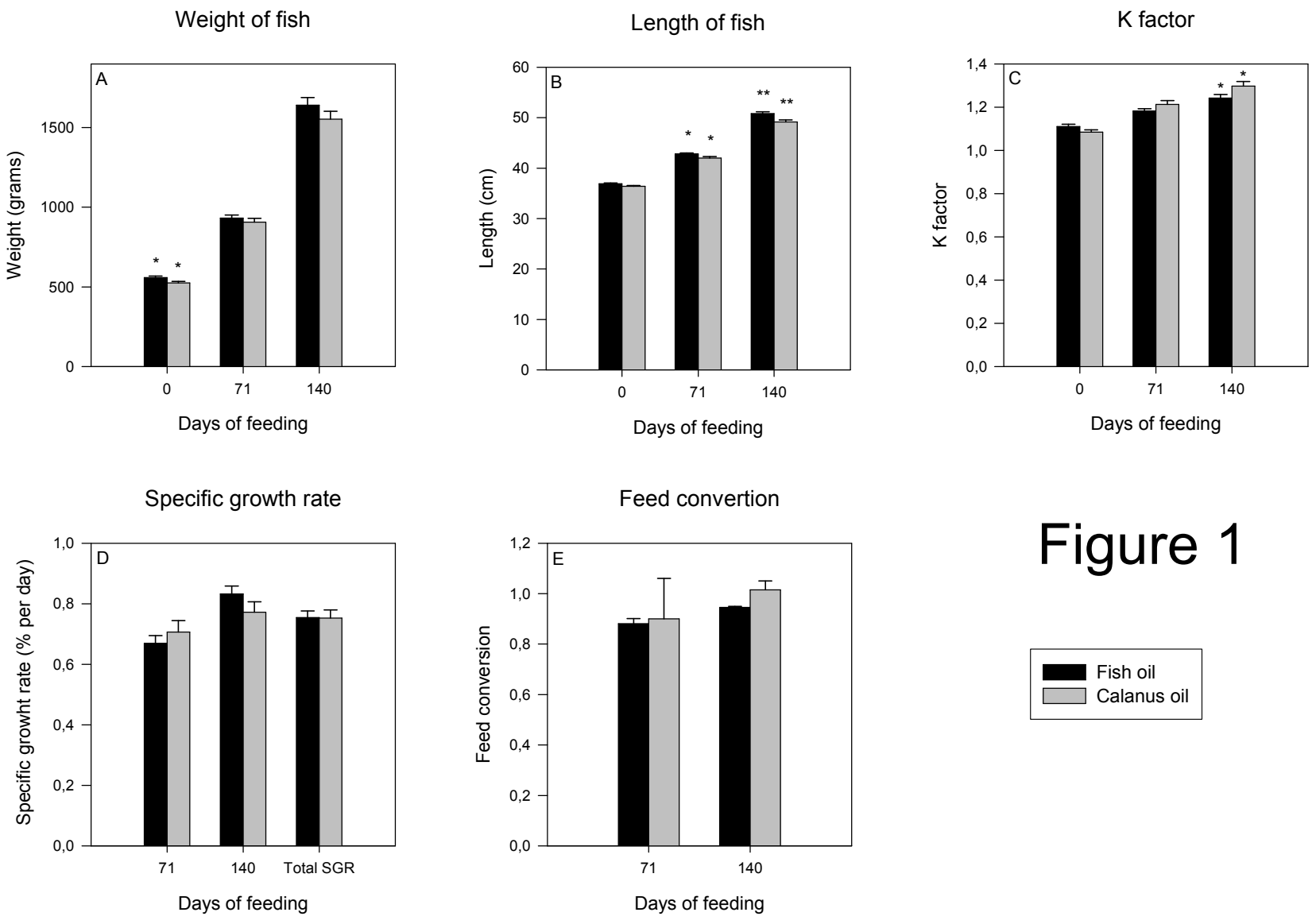
	Liver		Muscle	
	Fish oil	*Calanus* oil	Fish oil	*Calanus* oil
SE	9.6 ±4.1	6.5 ±3.8	-	-
TAG	24.8 ±5.2	23.8 ±4.8	78.5 ±3.8	77.7 ±3.9
FFA	2.0 ±0.9	3.9 ±2.8	3.5 ±2.4*	6.3 ±1.9*
C	13.4 ±1.2	14.4 ±1.6	5.4 ±1.0*	4.5 ±0.6*
UPL	3.5 ±0.5	3.0 ±1.0	-	-
PE	11.6 ±0.5	12.6 ±1.3	3.5 ±1.2	3.1 ±1.7
PG	2.5 ±1.0	3.0 ±1.2	tr	tr
PI	6.5 ±0.7	6.7 ±0.6	2.1 ±0.4	1.9 ±0.6
PS	3.8 ±0.9	3.9 ±0.9	tr	tr
PC	19.2 ±2.7	19.1 ±3.2	6.4 ±1.5	6.1 ±1.5
SM	3.0 ±0.5	3.3 ±0.6	0.6 ±0.2	0.5 ±0.3
Lipid of wet weight (%)	4.2 ±0.5	3.7 ±0.4	11.6 ±2.7	13.6 ±4.0

Abbreviations as in Table 3.
* P< 0,05
tr less than 0.4%

Table 7. Total lipid fatty acid composition of liver and muscle of Atlantic salmon fed diets supplemented with fish oil or *Calanus* oil.

	Muscle		Liver	
	Fish oil	*Calanus* oil	Fish oil	*Calanus* oil
14:0	5.6 ±0.1	5.5 ±0.3	2.7 ±0.3	3.1 ±0.6
16:0	14.0 ±0.6	14.1 ±0.3	15.9 ±1.3**	17.7 ±0.8**
18:0	2.3 ±0.3	2.2 ±0.1	4.9 ±0.4	5.0 ±0.7
16:1n-7	4.7 ±0.2***	4.2 ±0.3***	2.6 ±0.4	2.3 ±0.5
18:1n-9	14.5 ±0.9***	12.3 ±1.1***	13.3 ±2.2*	11.2 ±1.9*
18:1n-7	2.6 ±0.2***	2.1 ±0.2***	2.1 ±0.2***	1.7 ±0.1***
20:1n-9	11.0 ±0.2**	11.6 ±0.4**	6.1 ±0.8	6.2 ±0.9
20:1n-7	0.3 ±0.1***	0.2 ±0.0***	0.2 ±0.0**	0.1 ±0.1**
22:1n-11	13.2 ±0.3*	13.7 ±0.4*	3.8 ±0.7	4.3 ±1.4
22:1n-9	1.4 ±0.2**	1.1 ±0.1**	0.4 ±0.1	0.4 ±0.1
24:1n-9	0.9 ±0.1***	0.7 ±0.1***	1.1 ±0.1	1.1 ±0.2
18:2n-6	2.7 ±0.2***	3.2 ±0.2***	1.5 ±0.2**	1.9 ±0.2**
20:2n-6	0.3 ±0.0***	0.4 ±0.0***	0.4 ±0.1**	0.4 ±0.0**
20:4n-6	0.4 ±0.1**	0.3 ±0.1**	2.2 ±0.3	1.9 ±0.4
18:3n-3	1.0 ±0.1***	2.3 ±0.4***	0.6 ±0.2***	1.1 ±0.2***
18:4n-3	2.5 ±0.2***	5.3 ±1.0***	0.8 ±0.3**	1.5 ±0.5**
20:4n-3	1.7 ±0.1***	2.5 ±0.3***	1.6 ±0.4***	2.5 ±0.2***
20:5n-3	5.5 ±0.4***	4.7 ±0.4***	8.8 ±0.3	8.9 ±1.1
22:5n-3	1.8 ±0.1**	1.6 ±0.2**	2.4 ±0.2**	2.1 ±0.2**
22:6n-3	10.2 ±0.9*	9.2 ±1.0*	25.5 ±2.9	23.8 ±3.4
Total				
SAT	22.7 ±0.9	22.5 ±0.7	24.1 ±1.2***	26.3 ±0.8***
MONO	49.7 ±1.3***	46.9 ±1.4***	30.8 ±3.7	28.5 ±4.3
n-3 PUFA	23.1 ±1.6**	25.7 ±1.8**	40.0 ±2.9	40.0 ±3.8
n-6 PUFA	3.8 ±0.2**	4.3 ±0.3**	4.7 ±0.3	4.8 ±0.4
PUFA[a)]	27.6 ±1.7**	30.7 ±2.0**	45.1 ±3.1	45.2 ±4.1

* P < 0,05; ** P < 0,01; *** P < 0,001; [a)] total PUFA also includes some C_{16} PUFA not identified as n-6 or n-3.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Days of feeding
Fish oil
Calanus oil

Figure 1