

# **Effects of dietary lipid level and vegetable oil on fatty acid metabolism in Atlantic salmon (*Salmo salar* L.) over the whole production cycle**

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## **Abstract**

Changes in fatty acid metabolism in Atlantic salmon (*Salmo salar*) induced by vegetable oil (VO) replacement of fish oil (FO) and high dietary oil in aquaculture diets can have negative impacts on the nutritional quality of the product for the human consumer, including altered flesh fatty acid composition and lipid content. A dietary trial was designed to investigate the twin problems of FO replacement and high energy diets in salmon throughout the entire production cycle. Salmon were grown from first feeding to around 2 kg on diets in which FO was completely replaced by a 1:1 blend of linseed and rapeseed oils at low (14-17%) and high (25-35%) dietary oil levels. This paper reports specifically on the influence of diet on various aspects of fatty acid metabolism. Fatty acid compositions of liver, intestinal tissue and gill were altered by the diets with increased proportions of C<sub>18</sub> polyunsaturated fatty acids and decreased proportions of n-3 highly unsaturated fatty acids (HUFA) in fish fed VO compared to fish fed FO. HUFA synthesis in hepatocytes and enterocytes was significantly higher in fish fed VO, whereas  $\beta$ -oxidation was unaltered by either dietary oil content or type. Over the entire production cycle, HUFA synthesis in hepatocytes showed a decreasing trend with age interrupted by a large peak in activity at seawater transfer. Gill cell prostaglandin (PG) production showed a possible seasonal trend, with peak activities in winter and low activities in summer and at seawater transfer. PG production in seawater was lower in fish fed the high oil diets with the lowest PG production generally observed in fish fed high VO. The changes in fatty acid metabolism induced by high dietary oil and VO replacement contribute to altered flesh lipid content and fatty acid compositions, and so merit continued investigation to minimize any negative impacts that sustainable, environmentally-friendly and cost-effective aquaculture diets could have in the future.

*Abbreviations:* FO, fish oil; HUFA, highly unsaturated fatty acids (carbon chain length  $\geq$  C<sub>20</sub> with  $\geq$  3 double bonds); LO, linseed oil; RO, rapeseed oil; VO, vegetable oil.

## Introduction

Salmonid and marine fish culture presently relies heavily on available supplies of fish meal and fish oil which, because of increased exploitation pressure on strictly limited global feed grade fisheries, as well as the increasing ecological and ethical objections to exploiting a non-sustainable resource, places the industry in a vulnerable position (Sargent and Tacon 1999; Barlow 2000; Tidwell and Allan 2002). However, anadromous fish like Atlantic salmon (*Salmo salar*) have limited ability to convert  $\alpha$ -linolenic (18:3n-3) and linoleic (18:2n-6) acids, which are abundant in many vegetable oils, to their long-chain highly unsaturated fatty acid (HUFA) products which are essential physiological components of all cell membranes and organs (Tocher 2003). Therefore, replacement of dietary fish oil with high quality, n-3 polyunsaturated fatty acid (PUFA)-rich vegetable oils may have potential in salmonids culture (Sargent et al. 2002). The hypothesis which we wish to test is that salmon can be grown on diets containing appropriate vegetable oils without deleterious effects on fish physiology or health or its value as an important nutritious food. However, in addition, the use of high-energy feeds containing high percentages of oil, which have drastically reduced production times in the salmon industry, can have undesirable side effects including increased oil deposition in the flesh (adiposity), reduced pigment visualisation and reduced smoking performance, leading to processor and retailer rejection and consumer dissatisfaction (Sheehan et al 1996; Johansen and Jobling 1998). Replacement of fish oil with vegetable oils may exacerbate this problem through the deposition of triglycerides as a result of feeding high levels of fatty acids not readily utilised by salmon, particularly in seawater (Bell 1998).

Several previous trials have investigated aspects of fish oil replacement in diets for Atlantic salmon. There have been trials investigating the replacement of fish oil with vegetable oil in the diets of salmon parr in freshwater (Bell et al. 1997; Tocher et al. 2000), and other trials that have looked at the effects of feeding vegetable oils to smolts in seawater (Bell et al. 2001a, 2002; Rosenlund et al.

2001; Torstensen et al. 2000; Tocher et al. 2002). A variety of different oils such as soybean (Hardy et al. 1987; Lie et al. 1993), sunflower (Bell et al. 1991, 1993), borage (Tocher et al. 1997), rapeseed (Tocher et al. 2000; Bell et al. 2001a), linseed (Tocher et al. 2000, 2002) and palm oil (Torstensen et al. 2000; Bell et al. 2002) have been investigated as well as oil blends (Bell et al. 2003a; Rosenlund et al. 2001; Jobling et al. 2002a,b; Tocher et al. 2003). The effects of dietary oil level have been investigated for a number of years (see Cowey 1993; Jobling 2001) with more recent studies including levels of oil that are routine in the salmon farming industry today (Jobling et al. 2002c). There has also been a trial looking at the effect of dietary oil level and vegetable oil replacement (Jobling et al. 2002a,b). However, most of the above trials have been of relatively short duration and none of the previous trials have been run throughout the entire 2 year production cycle of the salmon from first-feeding to harvest.

The present dietary trial was designed to investigate the twin problems of replacement of fish oil with alternative oils and high energy (oil) diets in Atlantic salmon culture. Salmon were grown throughout the entire production cycle, from first feeding to harvest size, on diets in which fish oil was replaced by a vegetable oil blend at a high and low dietary oil level. This paper specifically reports on the effects of the experimental diets on various aspects of fatty acid metabolism. Thus, the effects of feeding the diets for an entire two year growth cycle on fatty acid compositions of liver and intestinal tissue, and fatty acid desaturation/elongation and  $\beta$ -oxidation in hepatocytes and intestinal enterocytes were determined in fish of harvest size. In addition, the time course of hepatocyte fatty acid desaturation and elongation, and the production of prostaglandin in gills, throughout the trial, including the effects of seawater transfer, were determined.

## **Materials and methods**

### *Animals and diets*

In March 2000, Atlantic salmon fry were distributed randomly into 8 tanks (3m x 3m, depth 0.5m) at a stocking level of 3000/tank, and weaned onto extruded feeds containing either fish oil (FO) or vegetable oil (VO), a 1:1 blend of rapeseed and linseed oils. Each oil was fed to duplicate tanks of fry/parr at either 14% (L) or 25% total oil (H), resulting in four dietary treatments in total, LFO (low fish oil), HFO (high fish oil), LVO (low vegetable oil) and HVO (high vegetable oil). Fish were fed the diets described above until sea water transfer in April 2001, at which point fish (average weight ~ 40g) were transferred into 5m x 5m net pens at 600 fish/pen. The fish were fed the same diet in seawater as in freshwater although the dietary oil levels were increased to 17% in the low oil diets (LFO and LVO) and 30% (3mm pellet) rising to 37% (6 and 9mm pellets) in the high oil diets (HFO and HVO) in the seawater phase. The diets aimed to be practical and therefore were formulated according to current practices in the salmon feed industry and were manufactured by the major salmon feed producers (BioMar A/S, Brande, Denmark; Ewos Technology Centre, Livingston, Scotland; Skretting, Stavanger, Norway). All diets were formulated to satisfy the nutritional requirements of salmonid fish (U.S. National Research Council 1993). Diet formulations and proximate compositions are given in Table 1 and fatty acid compositions in Table 2. Fish were ongrown until June 2002 at which time they had reached around 2 kg. Final weights in kg were  $1.44 \pm 0.37$  (LFO),  $1.88 \pm 0.57$  (HFO),  $1.71 \pm 0.36$  (LVO) and  $2.02 \pm 0.57$  (HVO) (all n = 80). Two-way ANOVA showed that growth was significantly affected by both oil type ( $F = 14.68$ ,  $P = 0.0002$ ) and oil level ( $F = 49.11$ ,  $P < 0.0001$ ) in an independent manner (interaction  $F = 1.48$ ,  $P = 0.2754$ ), with higher growth in fish fed high oil level and vegetable oil. Water temperature throughout the trial averaged  $10.5 \pm 2.2$  °C (measured at 2 m), varying by season with lowest temperatures recorded in February (5.9 °C) and highest in August (14.4 °C).

At the end of the 2 year trial, HUFA synthesis and  $\beta$ -oxidation was determined in isolated hepatocytes and intestinal caecal enterocytes, and samples of liver and pyloric caecae collected for

lipid and fatty acid analyses. In addition though, samples were collected throughout the time course of the trial at predetermined time intervals including immediately pre- and post- seawater transfer. At all these points, livers and gills were collected for fatty acid analyses and determination of hepatocyte HUFA synthesis and gill cell prostaglandin F production.

#### *Lipid extraction and fatty acid analyses*

Fish were not extensively starved prior to sampling and so were fed the day before but not on the day of sampling giving a time of sampling around 12-15 hours after last feeding. Livers, gills (at all time points) and pyloric caecae (at trial end) were dissected from 4 fish per dietary treatment and immediately frozen in liquid nitrogen. Total lipid contents of salmon tissues were determined gravimetrically after extraction by homogenization in chloroform/methanol (2:1, v/v) containing 0.01 % butylated hydroxytoluene (BHT) as antioxidant, basically according to Folch et al. (1957). Samples of total lipid (2 mg) were applied as 2 cm streaks to thin-layer chromatography plates, the plates developed fully with isohexane/diethyl ether/acetic acid (90:10:1, by vol.) as developing solvent, and the origin area corresponding to total polar lipids were scraped into stoppered glass test tubes. Fatty acid methyl esters (FAME) were prepared from total polar lipids by acid-catalyzed transesterification directly on the silica using 2 ml of 1% H<sub>2</sub>SO<sub>4</sub> in methanol plus 1 ml toluene as described by Christie (1982) and FAME extracted and purified as described previously (Tocher and Harvie 1988). FAME were separated and quantified by gas-liquid chromatography (Fisons GC8600, Fisons Ltd., Crawley, U.K.) using a 30m x 0.32 mm capillary column (CP wax 52CB; Chrompak Ltd., London, U.K). Hydrogen was used as carrier gas and temperature programming was from 50°C to 180°C at 40°C/min and then to 225°C at 2°C/min. Individual methyl esters were identified by comparison to known standards and by reference to published data (Ackman 1980).

### *Preparation of isolated hepatocytes, caecal enterocytes and gill cells*

Fish were killed by a blow to the head and livers, intestinal tract and gills dissected immediately. The gall bladder was removed carefully from the liver and the main blood vessels trimmed. The liver was perfused via the hepatic vein with solution A (calcium and magnesium-free Hanks balanced salt solution (HBSS) + 10 mM HEPES + 1 mM EDTA) to clear blood from the tissue. The pyloric caecae were cleaned of adhering adipose tissue before being dissected from the intestinal tract and luminal contents rinsed away with solution A. The gills were rinsed in solution A and blotted on tissue to remove excess blood and medium. Essentially the same method based on collagenase digestion and sieving has been utilized successfully to isolate gill cell, hepatocyte- and enterocyte-enriched preparations (Tocher et al. 2000, 2002, 2003). Briefly, the tissues were chopped finely with scissors and about 0.5 g of chopped tissue was incubated with 20 ml of solution A containing 0.1% (w/v) collagenase at 20°C for 45 min. The digested tissues were filtered through 100 µm nylon gauze and the cells collected by centrifugation at 300 x g for 2 min. The cell pellets were washed with 20 ml of solution A containing 1% w/v fatty acid-free bovine serum albumin (FAF-BSA) and re-centrifuged. The washing was repeated with a further 20 ml of solution A without FAF-BSA. Hepatocytes and enterocytes were resuspended in 10 ml of Medium 199 containing 10 mM HEPES and 2 mM glutamine, whereas gill cells were resuspended in 1 ml of HBSS containing 2mM CaCl<sub>2</sub>. One hundred µl of cell suspensions were mixed with 400 µl of Trypan Blue, cells counted and their viability assessed using a haemocytometer. The viability at isolation was >97% and >94%, for hepatocytes and enterocytes respectively, and in both cell types only decreased by between 5 and 10% over the period of the incubation. One hundred µl of cell suspensions were retained for protein determination according to the method of Lowry et al. (1951) after incubation with 0.4 ml of 0.25% (w/v) SDS/1M NaOH for 45 min at 60°C.

### *Incubation of hepatocyte and enterocyte preparations with [1-<sup>14</sup>C]18:3n-3*

Six ml of each hepatocyte or enterocyte suspension were dispensed into a 25 cm<sup>2</sup> tissue culture flask. Hepatocytes and enterocytes were incubated with 0.3 μCi (~ 1 μM) [1-<sup>14</sup>C] 18:3n-3, added as a complex with FAF-BSA in HBSS (albumin:fatty acid ratio of 7-8:1) prepared as described previously (Ghioni et al. 1997). After addition of isotope, the flasks were incubated at 20 °C for 2 h. After incubation, the cell layer was dislodged by gentle rocking and the cell suspension transferred to glass conical test tubes and 1 ml of each suspension withdrawn into a 2 ml microcentrifuge tube for β-oxidation assay as described below. The cell suspensions remaining in the glass conical centrifuge tubes were used for the desaturation/elongation assay as described below.

### *Assay of hepatocyte and enterocyte fatty acyl desaturation/elongation activities*

The cell suspensions were centrifuged at 500 x g for 2 min, the supernatant discarded and the hepatocyte or enterocyte cell pellets washed with 5 ml of ice-cold HBSS/FAF-BSA. The supernatant was discarded and the tubes placed upside down on paper towels to blot for 15 sec before extraction of total lipid using ice-cold chloroform/methanol (2:1, v/v) containing 0.01% (w/v) BHT essentially as described by Folch et al. (1957) and as described in detail previously (Tocher et al. 1988). Total lipid was transmethylated and FAME prepared as described above. The methyl esters were redissolved in 100 μl isohexane containing 0.01% 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 °C for 30 min. Plates were fully developed in toluene/acetonitrile (95:5, v/v) (Wilson and Sargent 1992). Autoradiography was performed with Kodak MR2 film for 6 days at room temperature. Areas of silica containing individual PUFA were 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, U.K.). Results were corrected for counting efficiency and quenching of  $^{14}\text{C}$  under exactly these conditions.

#### *Assay of hepatocyte and enterocyte fatty acyl oxidation activities*

Measurement of fatty acid oxidation by estimation of acid-soluble radioactivity after incubating intact cells with labelled fatty acids has been described previously (Frøyland et al. 1996, 2000; Madsen et al. 1998; Torstensen et al. 2000; Tocher et al. 2002). Briefly, after incubation with  $[1-^{14}\text{C}]18:3\text{n-3}$ , 1 ml of hepatocyte or enterocyte suspension was homogenized with a hand-held tissue disrupter (Ultra-Turrax T8/S8N-5G probe, IKA-Werke GmbH & Co., Slauchen, Germany) and centrifuged at 10000 x g for 10 min. Five hundred  $\mu\text{l}$  of the supernatant was taken into a clean 2 ml microcentrifuge tube and 100  $\mu\text{l}$  of ice-cold 6% FAF-BSA solution in water was added. After mixing thoroughly, the protein was precipitated by the addition of 1.0 ml of ice-cold 4M perchloric acid ( $\text{HClO}_4$ ), and the tubes centrifuged at 10000 x g for 10 min. Five hundred  $\mu\text{l}$  of the supernatant was transferred to a scintillation vial, 4 ml of scintillant added and radioactivity in the acid-soluble fraction determined as described above for desaturation/elongation assay.

#### *Assay of gill cell prostaglandin F production*

Gill cell suspensions were incubated for 10 min at 20 °C in a shaking water bath before the addition of A23187 to a final concentration of 10  $\mu\text{M}$ . Incubation was continued for 15 min after which the cells were removed by centrifugation (12000 x g, 2 min) and the supernatant transferred to tubes containing 150  $\mu\text{l}$  ethanol and 50  $\mu\text{l}$  2 M formic acid. The supernatants were centrifuged (12000 x g, 2 min) to remove any precipitate before being extracted using octadecyl silica (C18) 'Sep-Pak' minicolumns (Millipore (UK), Watford) as described in detail by Bell et al. (1994). The final extract was dissolved in

0.1 ml methanol and stored at  $-20\text{ }^{\circ}\text{C}$  prior to analysis by enzyme immunoassay. Samples were dried under nitrogen and redissolved in immunoassay buffer. Measurement of total PGF was performed using an enzyme immunoassay kit for  $\text{PGF}_{2\alpha}$  according to the manufacturer's protocols (SPI-Bio, Massy, France).

### *Materials*

$[1\text{-}^{14}\text{C}]18:3\text{n-}3$  (50-55 mCi/mmol) was obtained from NEN (DuPont (U.K.) Ltd., Stevenage, U.K.). HBSS, Medium 199, HEPES buffer, glutamine, collagenase (type IV), FAF-BSA, BHT, A23187, silver nitrate and perchloric acid were obtained from Sigma Chemical Co. (Poole, U.K.). Thin-layer chromatography (TLC) plates, precoated with silica gel 60 (without fluorescent indicator) were obtained from Merck (Darmstadt, Germany). All solvents were HPLC grade and were obtained from Fisher Scientific UK, Loughborough, England.

### *Statistical analysis*

All the data are presented as means  $\pm$  SD ( $n=3$  or  $4$ ). The effects of dietary treatment on HUFA synthesis and  $\beta$ -oxidation in hepatocytes and enterocytes, and liver and pyloric caecal fatty acid compositions at the end of the 2 year trial were determined by one-way analysis of variance (ANOVA) followed, where appropriate, by Tukey's comparison test. The effects of dietary oil type (FO v. VO) and oil level (H v. L) on liver and gill fatty acid compositions and hepatocyte HUFA synthesis and gill cell PG production at selected points in the time course were determined by two-way ANOVA. Percentage data and data which were identified as non-homogeneous (Bartlett's test) were subjected to

either arcsine transformation before analysis. Differences were regarded as significant when  $P < 0.05$  (Zar 1984).

## **Results**

### *Dietary fatty acid compositions*

The diets containing VO were characterized by having increased proportions of 18:1n-9, 18:2n-6 and 18:3n-3 and reduced proportions of saturated fatty acids, long chain monoenes (20:1 and 22:1) and n-3 HUFA (20:5n-3 and 22:6n-3) compared to diets containing FO (Table 2). These differences were slightly more prominent in the diets with higher oil contents due to the reduction in the relative contribution of fatty acids from the non-oil components of the diet, the fish and plant meals. The diets aimed to reflect current feeding practices and so the precise level of long-chain monoenes and n-3HUFA in the diets containing FO varied depending on which fish oil (herring or capelin) was utilized by the individual feed company at the time of diet production.

### *Effects of diet on fatty acid compositions of liver and pyloric caecae*

The differences in the fatty acid compositions of the diets were reflected in the fatty acid compositions of the tissues of the fish at the end of the two year dietary trial. Thus, the fatty acid compositions of total polar lipids of livers from salmon fed the LVO and HVO diets were characterized by increased proportions of 18:1n-9, 18:2n-6 and 18:3n-3 and reduced proportions of 16:0 and total saturated fatty acids, long chain monoenes (20:1 and 22:1), 20:4n-6, 20:5n-3 and 22:6n-3 compared to fish fed diets containing FO (Table 3). Again, the differences tended to be greatest between fish fed the high oil contents, HFO and HVO. Increased percentages of elongated and  $\Delta 6$  desaturated products of 18:2n-6 and 18:3n-3, that is 20:2n-6 and 20:3n-6, and 18:4n-3, 20:3n-3 and 20:4n-3, respectively, were

observed in fish fed the VO diets (Table 3). Essentially the same pattern was observed in the fatty acid composition of the pyloric caecae although the differences tended to be more pronounced both between FO and VO diets and also between low and high oil contents (Table 4).

*Effects of diet on HUFA synthesis and fatty acid oxidation in isolated hepatocytes and caecal enterocytes at the end of trial*

HUFA synthesis, as measured by the conversion of [1-<sup>14</sup>C]18:3n-3 to desaturated and elongated products, was greater in both isolated hepatocytes and caecal enterocytes in fish fed VO (Fig. 1A). Rates of conversion were generally higher in hepatocytes compared to enterocytes, except for fish fed diet HFO, which showed the lowest HUFA synthesis activity in hepatocytes, whereas the lowest activity in enterocytes was obtained with fish fed the LFO diet (Fig.1A). Oxidation of [1-<sup>14</sup>C]18:3n-3, as measured by the recovery of acid-soluble radioactivity, was 3-4-fold higher than conversion by the desaturation/elongation pathway in both hepatocytes and enterocytes but, in contrast to that pathway, fatty acid oxidation was not affected by diet in either cell type (Fig.1B).

*Time course of hepatocyte HUFA synthesis and liver polar lipid fatty acid compositions*

In general, the activity of the fatty acid desaturation/elongation pathway tended to decrease throughout the two year cycle, with the activities when first determined in parr being 2-3 fold higher than the activities measured at the end of the trial (Fig.2). However, this pattern was interrupted around seawater transfer at which point there was a marked peak of activity, before the pattern of declining activity was re-established. Although there was some variability in the freshwater phase, the activities in fish fed the VO diets were higher than those in fish fed the FO diets with the lowest activities consistently obtained in fish fed the HFO diet (Fig.2). HUFA synthesis was significantly affected by oil

type and also often by oil level as determined by two-way ANOVA (Table 5). Irrespective of diet and sampling point, the main products of the pathway were the  $\Delta 6$  desaturated products 18:4n-3 and 20:4n-3, with smaller amounts of  $\Delta 5$  desaturated products and 22:6n-3 (Fig.3). The fatty acid composition of liver polar lipids at selected time points showed C<sub>18</sub> PUFA, 18:2n-6 and 18:3n-3, increasing, and 20:5n-3 and 22:6n-3, decreasing, in fish fed the LVO and HVO diets over the time-course of the dietary trial in comparison with fish fed the FO diets (Fig.4). As with hepatocyte HUFA synthesis, both oil type and oil level significantly affected fatty acid composition of liver throughout the dietary trial (Table 5).

#### *Time course of gill prostaglandin F (PGF) production and gill polar lipid fatty acid compositions*

In general, there was a trend for the capacity of PGF production by isolated gill cells to be greatest at mid-winter samplings in December, with lower activities recorded at the mid-summer samplings in June (Fig.5). However, the most striking feature overall was the very low levels of PGF production capacity measured around the period of parr-smolt transformation and seawater transfer. The effects of dietary treatment were also less pronounced at the summer samplings and, especially, seawater transfer (Fig. 5). However, the most prominent dietary effect was that PGF production capacity was generally lower in fish fed the VO diets, at least in the seawater phase, with the lowest level of PG production generally observed in fish fed the HVO diet (Fig.5). Gill PGF production was affected by dietary oil level between seawater transfer and final sampling with levels of PGF lower in fish fed the diets with higher oil content (Fig.5. & Table 5). In contrast, dietary oil level generally did not significantly affect the levels of the PG precursor fatty acids, 20:4n-6 and 20:5n-3 and the 20:5n-3/20:4n-6 ratio at selected time points (winter peaks, seawater transfer and end point) during the trial (Fig.6. & Table 5). The level of gill 20:4n-6 was not significantly affected by oil type at the earlier time points but was significantly lower in fish fed the VO diets in the seawater phase (Fig.6. & Table 5). The level of gill 20:5n-3 increased in all the dietary treatments during the trial, although levels were generally

significantly higher in fish fed the diets containing FO (Fig.6 & Table 5). Gill 20:5n-3/20:4n-6 ratio was generally not significantly affected by dietary treatment from seawater transfer onwards (Table 5).

## **Discussion**

Several studies have shown that both dietary oil type and oil content can have significant effects on fatty acid metabolism in Atlantic salmon (Bell et al. 1997; Tocher et al. 2000, 2002, 2003; Torstensen et al. 2000; Rosenlund et al. 2001; Jobling et al. 2002a,b,c). Major consequences of these effects can be altered lipid content and fatty acid composition of the flesh, both of which can compromise the nutritional quality of the fish as food for the human consumer (Sheehan et al 1996; Johansen and Jobling 1998; Bell et al. 2001a, 2002; Torstensen et al. 2000; Rosenlund et al. 2001). The present study aimed to investigate the effects of dietary oil content and composition on key pathways of fatty acid biochemistry throughout a two year growth cycle. Two important pathways of lipid and fatty acid homeostasis, whose relative activities contribute to final tissue fatty acid compositions, are HUFA synthesis through desaturation and elongation, and fatty acid oxidation through the  $\beta$ -oxidation pathway. It has been reported that a major fate of dietary 18:3n-3 in salmonids was oxidation for energy (Bell et al. 2001b). Thus, 18:3n-3 not only serves as a substrate for the synthesis of the important long-chain n-3 HUFA, but also as an energy source. Therefore, we investigated the effects of the diets on the balance between these two pathways, using [1- $^{14}$ C]18:3n-3 as fatty acid substrate, in two important organs, liver (hepatocytes) as the major lipid metabolising organ, and intestine (enterocytes), as it is the first organ to encounter dietary fatty acids and has been shown to be a site of significant HUFA synthesis (Bell et al. 2003b). Irrespective of diet, the primary fate of [1- $^{14}$ C]18:3n-3 was  $\beta$ -oxidation rather than HUFA synthesis in both hepatocytes and enterocytes. Dietary oil type had no significant effect on  $\beta$ -oxidation in hepatocytes or enterocytes, as there was no increased oxidation of 18:3n-3 in cells from fish fed VO compared to fish fed FO. Perhaps surprisingly though, dietary oil

level also had no significant effect on  $\beta$ -oxidation activity when measured using  $[1-^{14}\text{C}]18:3n-3$  as substrate in either hepatocytes or enterocytes. Thus, the effect of the dietary treatments was that the balance between the pathways competing for dietary  $18:3n-3$  was moved in the favour of HUFA synthesis in both liver and intestine in fish fed VO. However, as has been observed previously, the shift in the balance towards HUFA synthesis is not sufficient to maintain  $n-3$ HUFA levels or prevent the deposition of excess dietary  $\text{C}_{18}$  PUFA in the tissues (Bell et al. 2001a, 2002, 2003a; Tocher et al. 2002, 2003).

In the present study, HUFA synthesis in hepatocytes showed a generally decreasing trend over the two year time-course of the trial, interrupted with a peak in activity around seawater transfer, and that the activity was greater throughout in fish fed diets containing VO. The peak in activity had been observed in previous trials, although it's precise timing in relation to seawater transfer can vary (Bell et al. 1997; Tocher et al. 2000). The timing of the peak in the present trial, virtually at transfer, was also observed in the first of our earlier trials (Bell et al. 1997) and supports our hypothesis that the peak in activity coincides with the completion of the parr-smolt transformation and that variation in its temporal relationship to seawater transfer is because transfer is an artificially imposed time point (Tocher et al. 2000). In the earlier parr-smolt trials, hepatic HUFA synthesis declined during the seawater phase, but this was not unexpected in those trials as all fish were shifted to diets containing FO after seawater transfer. In the present trial, the VO diets continued to be fed throughout the seawater phase, but hepatocyte HUFA synthesis activity still declined, albeit the activity was always higher in fish fed the VO diets than in fish fed the diets containing FO. Therefore, once in seawater and as the fish ages the HUFA synthetic capacity appears to decline. In addition, activities were relatively high in the fish when first sampled ( $\sim 3$  months post-hatch) and much higher than the activities in 2 year-old fish at the end of the trial. This general decline in HUFA synthesis capacity in hepatocytes with age of the fish was not apparent in the earlier trials which only looked at fish during a

window around 20 weeks before and after seawater transfer (Bell et al. 1997; Tocher et al. 2000). Decreasing fatty acid desaturation capacity with age and development has been noted previously in mammals. Specifically, the activity of hepatic  $\Delta 6$  desaturase has been reported to decrease with aging in rats (Hrelia et al. 1989; Bourre and Piciotti 1992).

Another possible consequence of feeding vegetable oils is disturbance of eicosanoid metabolism. Vegetable oils contain no 20:4n-6, the predominant eicosanoid precursor fatty acid, whereas fish oils usually contain around 1-1.5% of this fatty acid (Ackman 1980; Padley et al. 1994). Therefore, this potential 20:4n-6 deficiency in VO diets must be met by conversion of 18:2n-6 to 20:4n-6. Previously, we had shown that PGF production by gill cells in response to calcium ionophore A23187 was low around the period of seawater transfer with peaks of activity before and after transfer (Bell et al. 1997). This pattern was explained in terms of it being a relatively acute response to changes in the physiology of the fish, and the gills in particular. That is, the peak in activity prior to seawater challenge being due to physiological changes in the gills as an integral part of the preadaptive parr-smolt transformation, with the increased PG production after transfer being a response to increased salinity once the fish were in seawater (Bell et al. 1997; Tocher et al. 2000). However, the present trial, over a much longer time course, has shown that the pattern may in fact be seasonal, in that the peaks of PGF production capacity appear to be around midwinter, with low PGF production around midsummer and, especially, seawater transfer. Immune responses of ectothermic animals such as salmon are known to vary seasonally, but generally parameters tend to be suppressed in winter and highest in summer (Slater and Schreck 1998). The very low capacity for PGF production around seawater transfer may have consequences for fish health, suggesting that the fish could be more susceptible to infection or disease at that time. This is known to be a period of increased stress in salmon with increases in cortisol, glucose and IGF-1 recorded around this time, all of which could also contribute to an immuno-suppressive effect (Weyts et al. 1999). Other studies have shown changes in serum proteins, IgM levels and leucocyte



populations, that may affect cellular and humoral immunity, take place around parr-smolt transformation in salmon (Maule et al. 1989; Melingen and Wergeland 2000; Melingen et al. 2002). In addition, some diseases, such as infectious pancreatic necrosis virus (IPNV) (Bowden et al. 2002), tend to strike in the period immediately after seawater transfer, whereas fish still in freshwater or successfully transferred are highly resistant, consistent with fish being more susceptible to infection immediately post-seawater transfer. However, the interactions between endocrine, immune and physiological changes at smoltification are complex (Weyts et al. 1999).

In the present study, the dual effects of dietary oil content and composition on the capacity of gill cells to produce PG gave variable results, but a generally consistent feature was that PGF production in response to A23187 was lowest in fish fed the HVO diet. Previously, PGF<sub>2 $\alpha$</sub>  and PGF<sub>3 $\alpha$</sub>  production were both reduced in fish fed FO in comparison to fish fed a 1:1 blend of RO and LO at dietary oil levels that were closer to the low lipid diets used in the present trial, being 19% and 24% pre- and post-transfer, respectively (Bell et al. 1997). In the earlier trial, the 20:5n-3/20:4n-6 ratio in gill polar lipids was generally higher in fish fed the FO diets, consistent with the PGF<sub>2 $\alpha$</sub>  data, but not the PGF<sub>3 $\alpha$</sub>  data. PGF<sub>2 $\alpha$</sub>  production exceeded PGF<sub>3 $\alpha$</sub>  production by up to 4-fold (Bell et al. 1997), and so total PGF production, as determined in the present trial, will reflect PGF<sub>2 $\alpha$</sub>  production, but there was no strong relationship between PGF production and 20:5n-3/20:4n-6 ratio, or gill cell 20:4n-6 and 20:5n-3 levels in the present study. Relatively poor correlation between gill polar lipid 20:4n-6 and 20:5n-3 levels and gill cell PGF production was noted in another earlier study (Tocher et al. 2000). This is further supported by the effects of dietary oil content on gill PGF production. During the seawater phase there was a clear relationship between dietary oil content and gill PGF production, with lower production in fish fed the high oil diets compared to fish fed the equivalent oil at lower level. This relationship was very significant at the mid-winter sampling (11 Dec 2001) but dietary oil had no significant effect on gill 20:4n-6 or 20:5n-3 levels or the 20:5n-3/20:4n-6 ratio. The conclusion must

be that other factors are also important in determining the level of PG production. Among these could be direct effects of other specific fatty acids, such as 20:3n-6 and 20:4n-3, that can compete for binding to the eicosanoid generating enzymes, or more indirect effects of fatty acids on general membrane composition leading to altered fluidity or membrane microenvironments. In addition, modulation of gene expression via changes in oxidant stress, nuclear receptor activation or covalent modification of specific transcription factors could affect eicosanoid metabolism (Jump et al. 1999).

In summary, the present trial has shown that the combination of feeding high energy (oil) diets, and diets in which vegetable oils replace fish oil, throughout the growth cycle of Atlantic salmon have significant effects on various pathways of fatty acid metabolism. Based on these data and the overall performance of the fish during the trial, we can draw three main conclusions. Firstly, although feeding dietary vegetable oil throughout the production cycle significantly altered fatty acid biochemistry, it did not lead to any metabolic effects that were obviously detrimental to general fish health and physiology. Secondly, the combination of high dietary oil content and 100% replacement of FO with VO oil did not significantly exacerbate the effects of feeding salmon VO. Thirdly, there are temporal and/or seasonal patterns of fatty acid metabolism throughout the salmon growth cycle that, although affected by feeding VO, are not greatly, or apparently adversely, altered. Despite this, the changes in fatty acid metabolism induced by high dietary oil and vegetable oil replacement do contribute to possible detrimental effects for the human consumer, such as altered flesh lipid content and fatty acid compositions, and so this area merits continued investigation to minimize any negative impacts that sustainable, environmentally-friendly and cost-effective aquaculture diets could have in the future.

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## Legends to Figures:

FIG. 1. Metabolism of  $[1-^{14}\text{C}]18:3n-3$  in isolated hepatocytes and caecal enterocytes at the end of the dietary trial. HUFA synthesis (A) was determined by measuring total fatty acid desaturation/elongation activity and represents the rate of conversion ( $\text{pmol}\cdot\text{h}^{-1}\cdot\text{mg protein}^{-1}$ ) of  $[1-^{14}\text{C}]18:3n-3$  to all desaturated products (sum of radioactivity recovered as 18:4n-3, 20:4n-3, 20:5n-3, 22:5n-3 and 22:6n-3). Fatty acid  $\beta$ -oxidation activity (B) was determined by the recovery ( $\text{pmol}\cdot\text{h}^{-1}\cdot\text{mg protein}^{-1}$ ) of radioactivity from  $[1-^{14}\text{C}]18:3n-3$  as acid soluble products. All results are means  $\pm$  S.D. ( $n = 3$ ). Columns for a specific activity and tissue with different superscript letters are significantly different as determined by one-way ANOVA followed by the Tukey post test ( $P < 0.05$ ). HFO, high fish oil; HVO, high vegetable oil; LFO, low fish oil; LVO, low vegetable oil.

FIG. 2. Evolution of HUFA synthetic capacity in isolated hepatocytes throughout the dietary trial. Total HUFA synthetic capacity was determined as described in legend to Fig.1. and results presented as means  $\pm$  S.D. ( $n = 3$ ). The vertical dotted line denotes the point of seawater transfer. HFO, high fish oil; HVO, high vegetable oil; LFO, low fish oil; LVO, low vegetable oil.

FIG.3. Individual fatty acid products of the desaturation and elongation of  $[1-^{14}\text{C}]18:3n-3$  in hepatocytes immediately after seawater transfer (peak activity, 25 April 2001). Results are means  $\pm$  S.D. ( $n = 3$ ) and represent the rate production ( $\text{pmol}\cdot\text{h}^{-1}\cdot\text{mg protein}^{-1}$ ) of individual fatty acids as determined by the recovery of radioactivity in each fatty acid fraction. Columns within a specific fatty acid with different superscript letters are significantly different as determined by one-way ANOVA followed by the Tukey post test ( $P < 0.05$ ). HFO, high fish oil; HVO, high vegetable oil; LFO, low fish oil; LVO, low vegetable oil.



FIG.4. Content of C<sub>18</sub> PUFA (18:2n-6 and 18:3n-3), and 20:5n-3 and 22:6n-3 in liver polar lipids at selected time points during the dietary trial. Results are presented as percentage of total fatty acids and are means  $\pm$  S.D. (n = 3). HFO, high fish oil; HVO, high vegetable oil; LFO, low fish oil; LVO, low vegetable oil.

FIG.5. Evolution of prostaglandin F (PGF) synthesis capacity of isolated gill cells throughout the dietary trial. PGF production (pg/mg protein) was determined after stimulation of gill cells with the calcium ionophore, A23187. Results presented as means  $\pm$  S.D. (n = 3). The vertical dotted line denotes the point of seawater transfer. HFO, high fish oil; HVO, high vegetable oil; LFO, low fish oil; LVO, low vegetable oil.

FIG.6. Content of 20:4n-6, 20:5n-3 and the 20:5n-3/20:4n-6 ratio in gill polar lipids at selected time points during the dietary trial. Results are presented as percentage of total fatty acids and are means  $\pm$  S.D. (n = 3). HFO, high fish oil; HVO, high vegetable oil; LFO, low fish oil; LVO, low vegetable oil.

Table 1. Dietary formulations and proximate compositions

Component	LFO	LVO	HFO	HVO	LFO	LVO	HFO	HVO	LFO	LVO	HFO	HVO
Pellet size (mm)	2				3				6			
LT Fishmeal	50.0		66.0		65.0		50.0		35.2		43.5	
Wheat	15.0		13.0		14.0		12.0		22.0		13.5	
Soybean meal	20.0		0.0		10.0		11.5		12.0		6.5	
Other plant products <sup>1</sup>	5.5		0.0		4.8		2.5		11.8		7.2	
Premixes etc. <sup>2</sup>	2.0		2.0		1.0		1.0		1.0		1.0	
Fish oil <sup>3</sup>	7.5	-	19.0	-	10.0	-	25.4	-	14.6	-	29.2	-
Linseed oil	-	3.8	-	9.5	-	5.0	-	12.7	-	7.3	-	14.6
Rapeseed oil	-	3.8	-	9.5	-	5.0	-	12.7	-	7.3	-	14.6
Protein (%)	53.7	52.0	50.8	51.5	51.5	51.4	48.4	47.9	38.0	37.2	39.7	37.6
Fat (%)	14.1	13.6	26.3	24.4	16.2	17.9	28.9	28.8	16.9	16.9	36.9	36.5
Moisture (%)	4.4	5.7	3.9	4.8	8.0	7.7	3.2	3.1	7.9	8.7	1.8	5.8

<sup>1</sup>Rapeseed meal, wheat flour, wheat and corn glutens.

<sup>2</sup>Vitamin and mineral pre-mixes, Finnstim, pigments according to feed company specifications.

<sup>3</sup>Capelin or herring oils

Table 2. Fatty acid compositions (percentage of weight) of diets used in freshwater (FW) and seawater (M6, 6mm pellet and M9, 9 mm pellet).

	LFO			HFO			LVO			HVO		
	FW	M6	M9	FW	M6	M9	FW	M6	M9	FW	M6	M9
14:0	5.1	4.8	5.7	5.6	5.6	6.3	3.0	0.9	1.4	1.5	0.6	0.7
16:0	14.4	16.6	12.2	14.7	16.9	11.9	11.0	8.0	8.1	8.0	7.0	6.6
18:0	2.0	2.9	1.3	2.3	2.6	1.1	2.2	3.1	2.5	2.4	2.9	3.0
Total saturates <sup>1</sup>	22.6	25.7	20.1	23.6	26.5	20.2	17.0	12.7	13.1	12.7	11.3	11.0
16:1n-7 <sup>2</sup>	4.3	5.5	7.6	4.9	6.3	8.5	2.2	1.1	1.7	1.2	0.7	0.8
18:1n-9	15.2	16.2	11.2	14.0	15.0	11.1	24.2	28.5	30.4	30.5	32.6	33.6
18:1n-7	2.5	2.1	2.9	2.5	2.4	3.2	2.9	1.8	2.2	1.8	2.1	1.9
20:1n-9 <sup>3</sup>	8.9	4.1	16.6	9.8	5.8	18.4	5.4	0.9	3.9	3.1	1.2	2.0
22:1n-11 <sup>4</sup>	12.6	5.9	14.6	13.5	8.0	15.8	7.3	0.3	3.9	3.7	0.7	1.8
24:1	1.2	0.9	0.9	0.9	1.1	0.8	0.8	0.2	0.4	0.4	0.3	0.3
Total monoenes	44.6	34.8	53.8	45.6	38.7	57.7	42.8	32.8	42.4	40.8	37.6	40.3
18:2n-6	5.4	10.4	6.2	3.2	2.9	3.1	11.3	22.9	17.8	13.4	19.1	17.0
20:4n-6	0.4	0.5	0.3	0.5	0.6	0.3	0.1	0.2	0.1	0.1	0.1	0.1
Total n-6PUFA <sup>5</sup>	7.0	11.7	7.2	5.0	4.3	4.0	12.0	23.2	18.1	14.0	19.3	17.1
18:3n-3	2.6	1.8	1.0	1.4	1.7	0.8	16.7	24.1	18.2	26.3	27.5	26.9
18:4n-3	2.7	2.3	2.8	3.2	2.7	3.0	1.3	0.4	0.5	0.7	0.2	0.4
20:4n-3	0.8	0.6	0.4	0.9	0.6	0.4	0.3	0.1	0.1	0.1	0.1	0.1
20:5n-3	6.2	8.6	6.7	6.7	9.6	6.5	3.0	2.1	3.0	1.6	1.4	1.6
22:5n-3	1.0	1.0	0.5	0.9	1.0	0.5	0.3	0.2	0.2	0.1	0.2	0.1
22:6n-3	11.3	11.9	6.0	11.3	13.1	5.4	6.0	3.5	3.7	3.3	2.3	2.1
Total n-3PUFA <sup>6</sup>	24.6	26.2	17.5	24.5	28.8	16.6	27.6	30.5	25.8	32.2	31.6	31.2
Total PUFA <sup>7</sup>	32.4	39.5	26.1	30.4	34.8	22.1	40.0	54.5	44.5	46.4	51.2	48.7
(n-3)/(n-6)	3.5	2.2	2.4	4.9	6.8	4.1	2.3	1.3	1.4	2.3	1.6	1.8

Results are means of two determinations. <sup>1</sup>, totals include 15:0, 17:0, 20:0 and 22:0, present at up to 0.5%; <sup>2</sup>also contains n-9 isomer; <sup>3</sup>also contains n-11 and n-7 isomers; <sup>4</sup>also contains n-9 and n-7 isomers; <sup>5</sup>totals include 18:3n-6, 20:2n-6, 20:3n-6 and 22:5n-6 present at up to 0.5%; <sup>6</sup>totals include 20:3n-3 present at up to 0.2%; <sup>7</sup>totals include C<sub>16</sub>PUFA present at up to 1.5% in FO diets and up to 0.4% in VO diets; HFO, high fish oil; HVO, high vegetable oil; LFO, low fish oil; LVO, low vegetable oil; PUFA, polyunsaturated fatty acids.

Table 3. Total lipid content (percentage of wet weight) and fatty acid composition (percentage of weight) of total polar lipid of liver at the end of the dietary trial (final time point).

	LFO	HFO	LVO	HVO
<u>Lipid content</u>	3.0 ± 0.6 <sup>c</sup>	3.8 ± 0.6 <sup>bc</sup>	4.7 ± 0.7 <sup>ab</sup>	5.2 ± 0.8 <sup>a</sup>
<u>Fatty acid composition</u>				
14:0	1.6 ± 0.1 <sup>b</sup>	2.1 ± 0.2 <sup>a</sup>	0.6 ± 0.0 <sup>c</sup>	0.4 ± 0.0 <sup>c</sup>
16:0	18.8 ± 0.5 <sup>a</sup>	17.7 ± 0.8 <sup>a</sup>	13.8 ± 1.2 <sup>b</sup>	12.6 ± 0.7 <sup>b</sup>
18:0	5.0 ± 0.2 <sup>b</sup>	4.7 ± 0.2 <sup>c</sup>	5.9 ± 0.2 <sup>a</sup>	5.6 ± 0.4 <sup>ab</sup>
Total saturated <sup>1</sup>	26.0 ± 0.6 <sup>a</sup>	25.2 ± 0.8 <sup>a</sup>	20.8 ± 1.3 <sup>b</sup>	19.1 ± 0.8 <sup>b</sup>
16:1n-7 <sup>2</sup>	1.9 ± 0.1 <sup>b</sup>	2.5 ± 0.2 <sup>a</sup>	0.9 ± 0.0 <sup>c</sup>	0.7 ± 0.0 <sup>c</sup>
18:1n-9	9.6 ± 0.2 <sup>b</sup>	8.7 ± 0.6 <sup>b</sup>	17.8 ± 1.5 <sup>a</sup>	18.1 ± 0.7 <sup>a</sup>
18:1n-7	2.0 ± 0.1 <sup>b</sup>	2.4 ± 0.1 <sup>a</sup>	1.4 ± 0.0 <sup>c</sup>	1.4 ± 0.1 <sup>c</sup>
20:1n-9 <sup>3</sup>	3.5 ± 0.7 <sup>a</sup>	4.6 ± 0.5 <sup>a</sup>	1.2 ± 0.2 <sup>b</sup>	1.1 ± 0.2 <sup>b</sup>
22:1n-11 <sup>4</sup>	0.6 ± 0.1 <sup>b</sup>	0.9 ± 0.1 <sup>a</sup>	0.3 ± 0 <sup>c</sup>	0.3 ± 0 <sup>c</sup>
24:1n-9	1.5 ± 0.1 <sup>a</sup>	1.4 ± 0.1 <sup>a</sup>	1.1 ± 0.1 <sup>b</sup>	1.1 ± 0.1 <sup>b</sup>
Total monoenes	19.1 ± 1.2 <sup>b</sup>	20.7 ± 1.2 <sup>ab</sup>	22.7 ± 1.7 <sup>a</sup>	22.7 ± 1.3 <sup>a</sup>
18:2n-6	2.9 ± 0.2 <sup>c</sup>	1.4 ± 0.1 <sup>d</sup>	8.8 ± 0.3 <sup>b</sup>	9.6 ± 0.3 <sup>a</sup>
20:2n-6	0.5 ± 0.1 <sup>b</sup>	0.3 ± 0.0 <sup>b</sup>	1.2 ± 0.1 <sup>a</sup>	1.1 ± 0.2 <sup>a</sup>
20:3n-6	0.4 ± 0.1 <sup>b</sup>	0.2 ± 0.0 <sup>b</sup>	1.0 ± 0.1 <sup>a</sup>	0.9 ± 0.1 <sup>a</sup>
20:4n-6	2.5 ± 0.1 <sup>a</sup>	2.5 ± 0.2 <sup>a</sup>	1.7 ± 0.2 <sup>b</sup>	1.2 ± 0.1 <sup>c</sup>
Total n-6 PUFA <sup>5</sup>	6.8 ± 0.2 <sup>b</sup>	4.8 ± 0.2 <sup>c</sup>	13.2 ± 0.3 <sup>a</sup>	13.1 ± 0.3 <sup>a</sup>
18:3n-3	0.4 ± 0.1 <sup>c</sup>	0.4 ± 0.0 <sup>c</sup>	6.4 ± 0.4 <sup>b</sup>	11.1 ± 0.9 <sup>a</sup>
18:4n-3	0.2 ± 0.0 <sup>b</sup>	0.3 ± 0.0 <sup>b</sup>	0.7 ± 0.2 <sup>a</sup>	0.9 ± 0.2 <sup>a</sup>
20:3n-3	0.1 ± 0.0 <sup>c</sup>	0.1 ± 0.0 <sup>c</sup>	0.8 ± 0.1 <sup>b</sup>	1.2 ± 0.1 <sup>a</sup>
20:4n-3	0.8 ± 0.0 <sup>c</sup>	1.1 ± 0.1 <sup>bc</sup>	1.2 ± 0.2 <sup>b</sup>	1.8 ± 0.1 <sup>a</sup>
20:5n-3	9.4 ± 0.9 <sup>ab</sup>	10.6 ± 0.7 <sup>a</sup>	8.0 ± 0.2 <sup>b</sup>	8.7 ± 0.8 <sup>b</sup>
22:5n-3	3.3 ± 0.3 <sup>a</sup>	3.6 ± 0.1 <sup>a</sup>	2.5 ± 0.1 <sup>b</sup>	2.1 ± 0.1 <sup>b</sup>
22:6n-3	32.8 ± 1.7 <sup>a</sup>	32.1 ± 1.3 <sup>a</sup>	23.1 ± 0.9 <sup>b</sup>	18.5 ± 0.6 <sup>c</sup>
Total n-3 PUFA	46.9 ± 1.1 <sup>a</sup>	48.2 ± 0.9 <sup>a</sup>	42.7 ± 0.8 <sup>b</sup>	44.2 ± 0.6 <sup>b</sup>
Total DMA	0.3 ± 0.1 <sup>a</sup>	0.3 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>b</sup>	0.1 ± 0.0 <sup>b</sup>
Total PUFA <sup>6</sup>	54.5 ± 1.2 <sup>bc</sup>	53.8 ± 1.0 <sup>c</sup>	56.3 ± 0.5 <sup>b</sup>	57.5 ± 0.9 <sup>a</sup>

Results are means ± S.D. (n = 3). <sup>1</sup>, totals include 15:0, 17:0 and 20:0, present at up to 0.3%; <sup>2</sup>also contains n-9 isomer; <sup>3</sup>also contains n-11 and n-7 isomers; <sup>4</sup>also contains n-9 and n-7 isomers; <sup>5</sup>totals include 18:3n-6, 22:4n-6 and 22:5n-6 present at up to 0.3%; <sup>6</sup>totals include C<sub>16</sub>PUFA present at up to 0.6%; DMA, dimethylacetals; HFO, high fish oil; HVO, high vegetable oil; LFO, low fish oil; LVO, low vegetable oil; PUFA, polyunsaturated fatty acids.

Table 4. Total lipid content (percentage of wet weight) and fatty acid composition (percentage of weight) of total polar lipid of pyloric caecae at the end of the dietary trial (final time point).

	LFO	HFO	LVO	HVO
<u>Lipid content</u>	2.9 ± 0.6 <sup>b</sup>	5.2 ± 1.5 <sup>a</sup>	2.5 ± 0.4 <sup>b</sup>	3.9 ± 0.8 <sup>ab</sup>
<u>Fatty acid composition</u>				
14:0	2.7 ± 0.2 <sup>b</sup>	5.5 ± 0.3 <sup>a</sup>	1.6 ± 0.1 <sup>c</sup>	1.0 ± 0.1 <sup>d</sup>
16:0	19.0 ± 0.6 <sup>a</sup>	18.1 ± 0.9 <sup>a</sup>	14.5 ± 1.1 <sup>b</sup>	9.8 ± 0.7 <sup>c</sup>
18:0	5.6 ± 0.2 <sup>ab</sup>	4.2 ± 0.4 <sup>c</sup>	6.2 ± 0.7 <sup>a</sup>	4.5 ± 0.2 <sup>bc</sup>
Total saturated <sup>1</sup>	27.6 ± 0.8 <sup>a</sup>	28.4 ± 1.2 <sup>a</sup>	22.6 ± 1.8 <sup>b</sup>	15.5 ± 1.0 <sup>c</sup>
16:1n-7 <sup>2</sup>	2.6 ± 0.4 <sup>b</sup>	5.7 ± 0.7 <sup>a</sup>	1.2 ± 0.3 <sup>c</sup>	0.8 ± 0.0 <sup>c</sup>
18:1n-9	9.2 ± 0.3 <sup>c</sup>	11.0 ± 0.7 <sup>c</sup>	19.0 ± 3.1 <sup>b</sup>	31.0 ± 0.5 <sup>a</sup>
18:1n-7	2.6 ± 0.1 <sup>b</sup>	3.0 ± 0.1 <sup>a</sup>	2.1 ± 0.1 <sup>c</sup>	2.1 ± 0.1 <sup>c</sup>
20:1n-9 <sup>3</sup>	4.4 ± 0.5 <sup>b</sup>	7.2 ± 0.9 <sup>a</sup>	2.4 ± 0.2 <sup>c</sup>	2.4 ± 0.2 <sup>c</sup>
22:1n-11 <sup>4</sup>	3.5 ± 0.6 <sup>b</sup>	7.5 ± 1.4 <sup>a</sup>	1.7 ± 0.1 <sup>c</sup>	1.6 ± 0.1 <sup>c</sup>
24:1n-9	1.0 ± 0.8	1.0 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Total monoenes	23.3 ± 0.4 <sup>b</sup>	35.5 ± 0.9 <sup>a</sup>	27.0 ± 2.9 <sup>b</sup>	38.5 ± 0.3 <sup>a</sup>
18:2n-6	2.1 ± 0.1 <sup>c</sup>	2.5 ± 0.5 <sup>c</sup>	7.5 ± 1.3 <sup>b</sup>	13.4 ± 0.2 <sup>a</sup>
20:2n-6	0.4 ± 0.0 <sup>b</sup>	0.3 ± 0.0 <sup>b</sup>	0.8 ± 0.0 <sup>a</sup>	0.8 ± 0.0 <sup>a</sup>
20:3n-6	0.2 ± 0.0 <sup>b</sup>	0.2 ± 0.0 <sup>b</sup>	0.4 ± 0.1 <sup>a</sup>	0.5 ± 0.1 <sup>a</sup>
20:4n-6	2.1 ± 0.1 <sup>a</sup>	1.2 ± 0.2 <sup>b</sup>	1.6 ± 0.1 <sup>b</sup>	0.5 ± 0.0 <sup>c</sup>
Total n-6 PUFA <sup>5</sup>	5.4 ± 0.2 <sup>c</sup>	4.7 ± 0.4 <sup>c</sup>	10.6 ± 1.4 <sup>b</sup>	15.5 ± 0.1 <sup>a</sup>
18:3n-3	0.6 ± 0.0 <sup>c</sup>	1.0 ± 0.2 <sup>c</sup>	8.1 ± 1.1 <sup>b</sup>	15.8 ± 0.4 <sup>a</sup>
18:4n-3	0.8 ± 0.1 <sup>bc</sup>	1.8 ± 0.4 <sup>a</sup>	0.5 ± 0.1 <sup>c</sup>	1.3 ± 0.1 <sup>ab</sup>
20:3n-3	0.1 ± 0.0 <sup>b</sup>	0.1 ± 0.0 <sup>b</sup>	0.8 ± 0.0 <sup>a</sup>	1.0 ± 0.1 <sup>a</sup>
20:4n-3	0.7 ± 0.1	1.0 ± 0.3	0.6 ± 0.0	1.0 ± 0.2
20:5n-3	8.8 ± 0.6 <sup>a</sup>	8.2 ± 0.9 <sup>a</sup>	5.9 ± 1.2 <sup>b</sup>	2.2 ± 0.3 <sup>c</sup>
22:5n-3	2.2 ± 0.2 <sup>a</sup>	2.1 ± 0.6 <sup>a</sup>	1.4 ± 0.2 <sup>ab</sup>	0.7 ± 0.1 <sup>b</sup>
22:6n-3	30.1 ± 0.7 <sup>a</sup>	16.5 ± 1.3 <sup>b</sup>	18.9 ± 2.3 <sup>b</sup>	8.4 ± 0.2 <sup>c</sup>
Total n-3 PUFA	43.4 ± 0.7 <sup>a</sup>	30.8 ± 1.1 <sup>bc</sup>	36.3 ± 3.9 <sup>b</sup>	30.4 ± 1.1 <sup>c</sup>
Total DMA	0.4 ± 0.2	0.7 ± 0.1	0.6 ± 0.4	0.2 ± 0.1
Total PUFA	48.7 ± 0.9 <sup>a</sup>	35.5 ± 1.4 <sup>b</sup>	46.9 ± 3.9 <sup>a</sup>	45.9 ± 1.2 <sup>a</sup>

Results are means ± SD (n = 3). <sup>1</sup>totals include 15:0 present at up to 0.5%; <sup>2</sup>also contains n-9 isomer; <sup>3</sup>also contains n-11 and n-7 isomers; <sup>4</sup>also contains n-9 and n-7 isomers; <sup>5</sup>totals include 18:3n-6 and 22:5n-6 present at up to 0.4%; DMA, dimethylacetals; HFO, high fish oil; HVO, high vegetable oil; LFO, low fish oil; LVO, low vegetable oil; PUFA, polyunsaturated fatty acid.

Table 5. Significance of effects of dietary oil type and oil content on HUFA synthesis in hepatocytes and PG synthesis in gill cells and fatty acid compositions of liver and gill at specific times during the two year cycle.

Fatty acid	Dietary oil type		Dietary oil level		Interaction	
	F value	significance	F value	significance	F value	significance
<u>LIVER</u>						
<u>6-Dec-00</u>						
C <sub>18</sub> PUFA	1140.0	<0.0001	92.5	<0.0001	162.5	<0.0001
20:5n-3	1.7	0.2268	1.7	0.2268	6.9	0.0307
22:6n-3	109.9	<0.0001	21.4	0.0017	6.9	0.0306
HUFA synthesis	26.9	0.0008	1.6	0.2472	44.7	0.0002
<u>25-Apr-01</u>						
C <sub>18</sub> PUFA	608.4	<0.0001	36.7	0.0003	69.2	<0.0001
20:5n-3	27.3	0.0008	20.5	0.0019	12.1	0.0083
22:6n-3	408.8	<0.0001	69.1	<0.0001	11.2	0.0101
HUFA synthesis	73.4	<0.0001	139.7	<0.0001	56.6	<0.0001
<u>11-Dec-01</u>						
C <sub>18</sub> PUFA	718.8	<0.0001	32.3	0.0005	103.4	<0.0001
20:5n-3	5.6	0.0450	3.4	0.102	1.7	0.2237
22:6n-3	239.5	<0.0001	15.4	0.0044	5.0	0.0560
HUFA synthesis	65.3	<0.0001	25.0	0.001	0.0	1.0000
<u>18-Jun-02</u>						
C <sub>18</sub> PUFA	2772.0	<0.0001	49.4	0.0001	140.0	<0.0001
20:5n-3	16.5	0.0036	5.5	0.0475	0.4	0.5554
22:6n-3	285.7	<0.0001	14.1	0.0056	8.4	0.0202
HUFA synthesis	138.6	<0.0001	10.1	0.0132	16.3	0.0038
<u>GILL</u>						
<u>6-Dec-00</u>						
Gill 20:4n-6	0.0	0.6938	4.2	0.0755	4.2	0.0755
Gill 20:5n-3	8.1	0.0216	0.1	0.7599	2.5	0.1525
Gill EPA/AA	37.5	0.0003	13.5	0.0063	1.5	0.2555
Gill PGF	25.3	0.0010	4.4	0.0687	70.1	<0.0001
<u>25-Apr-01</u>						
Gill 20:4n-6	0.3	0.6290	0.3	0.6290	0.7	0.4268
Gill 20:5n-3	8.9	0.0175	0.1	0.8099	0.6	0.4769
Gill EPA/AA	0.8	0.3972	7.2	0.0278	0.0	1.0000
Gill PGF	17.3	0.0031	21.7	0.0016	6.0	0.0400
<u>11-Dec-01</u>						
Gill 20:4n-6	138.9	<0.0001	1.7	0.2268	42.9	0.0002
Gill 20:5n-3	6.2	0.0380	0.6	0.4604	1.2	0.3093
Gill EPA/AA	0.1	0.7831	2.0	0.1923	0.1	0.7831
Gill PGF	62.8	<0.0001	47.0	0.0001	0.6	0.4629
<u>18-Jun-02</u>						
Gill 20:4n-6	30.0	0.0006	1.2	0.3052	30.0	0.0006
Gill 20:5n-3	1.9	0.2048	1.2	0.3143	0.6	0.4651
Gill EPA/AA	1.1	0.3216	4.5	0.0675	4.5	0.0675
Gill PGF	5.8	0.0423	0.0	0.9661	3.9	0.0838

Data from Figs. 2,4,5 and 6 at specific time points were subjected to two-way ANOVA as described in the Methods section. AA, arachidonic acid; EPA, eicosapentaenoic acid; PGF, prostaglandin F; HUFA, highly unsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Fig.1

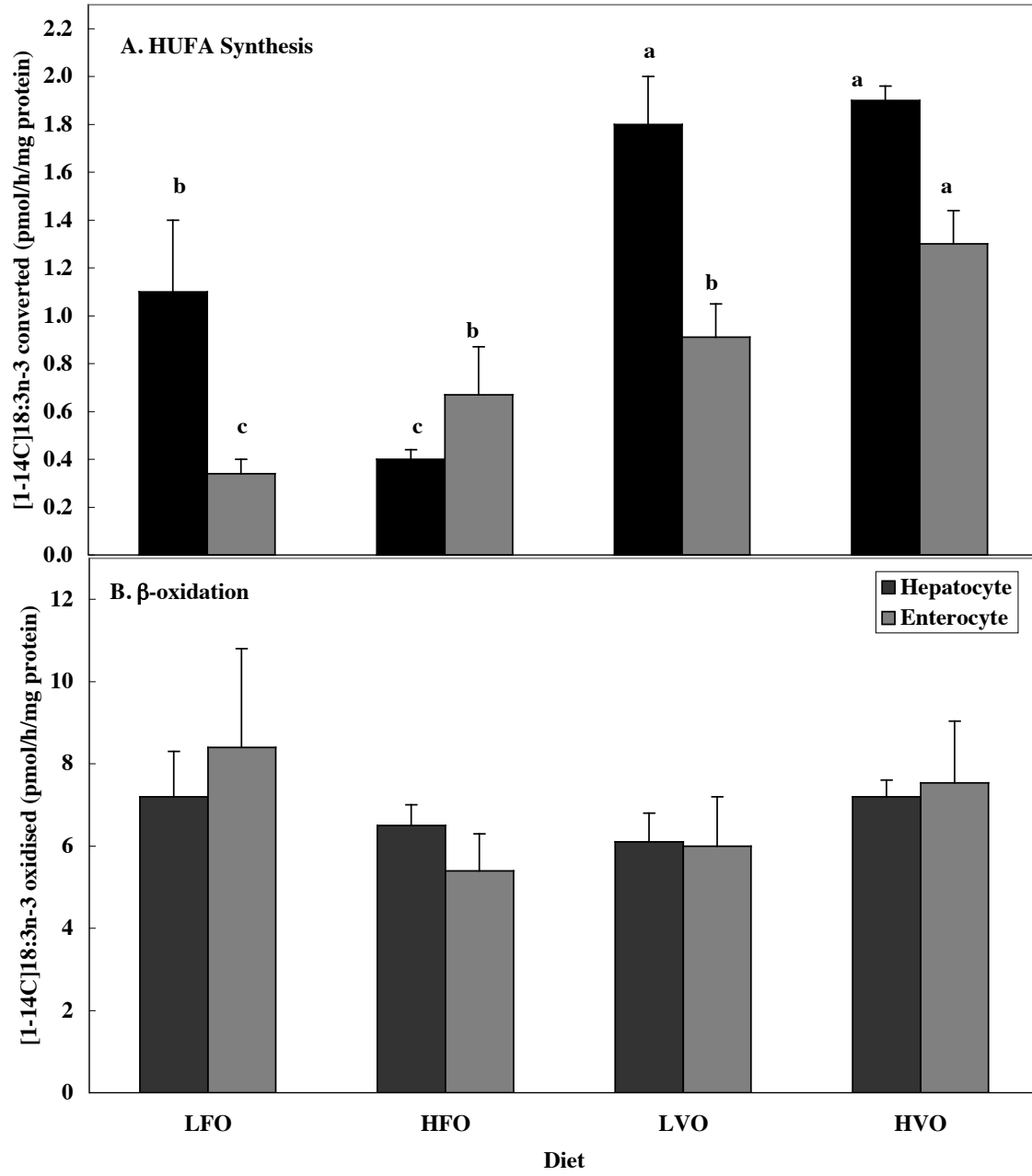


Fig.2

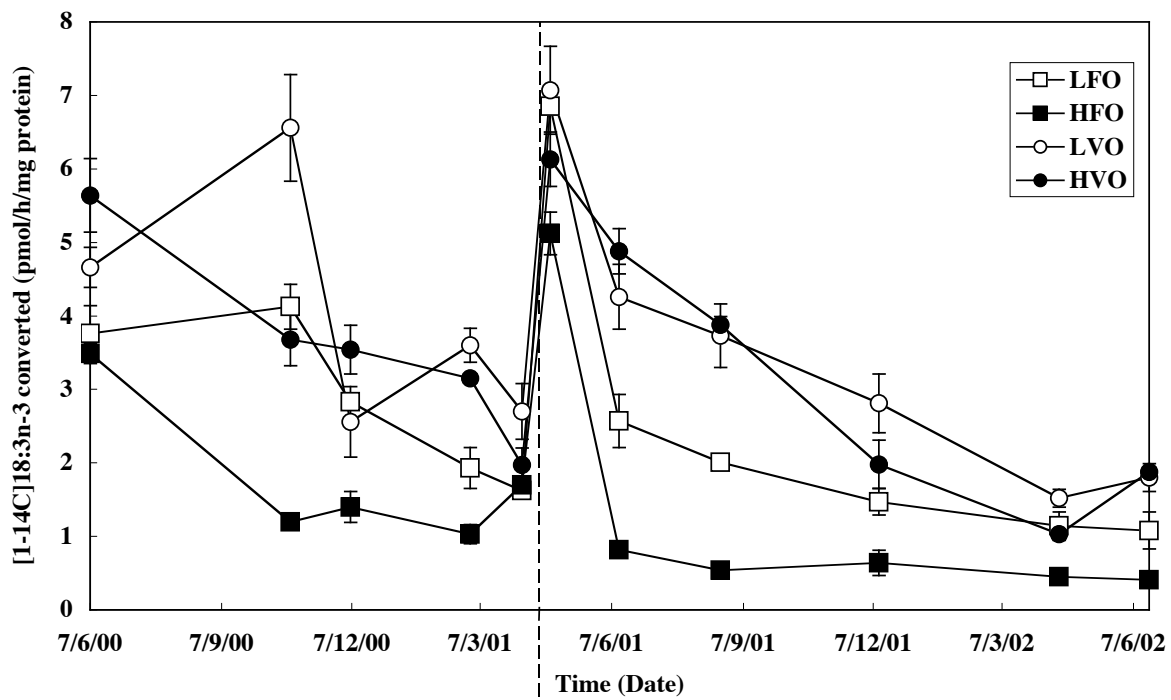




Fig.3

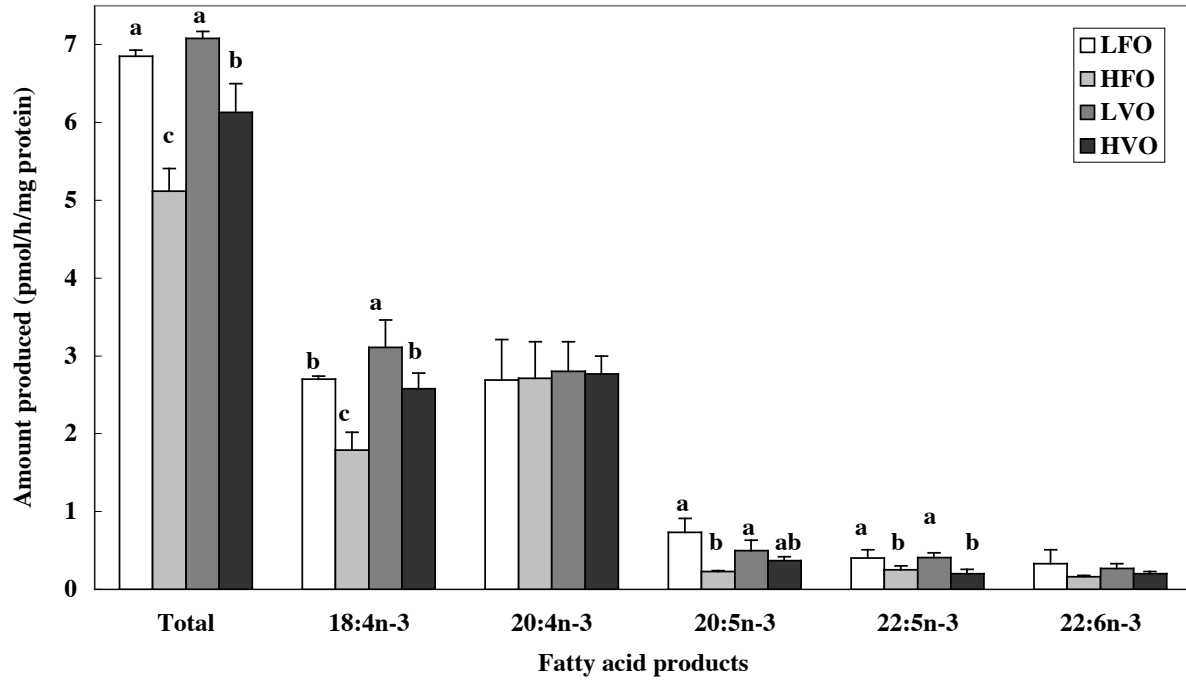


Fig.4

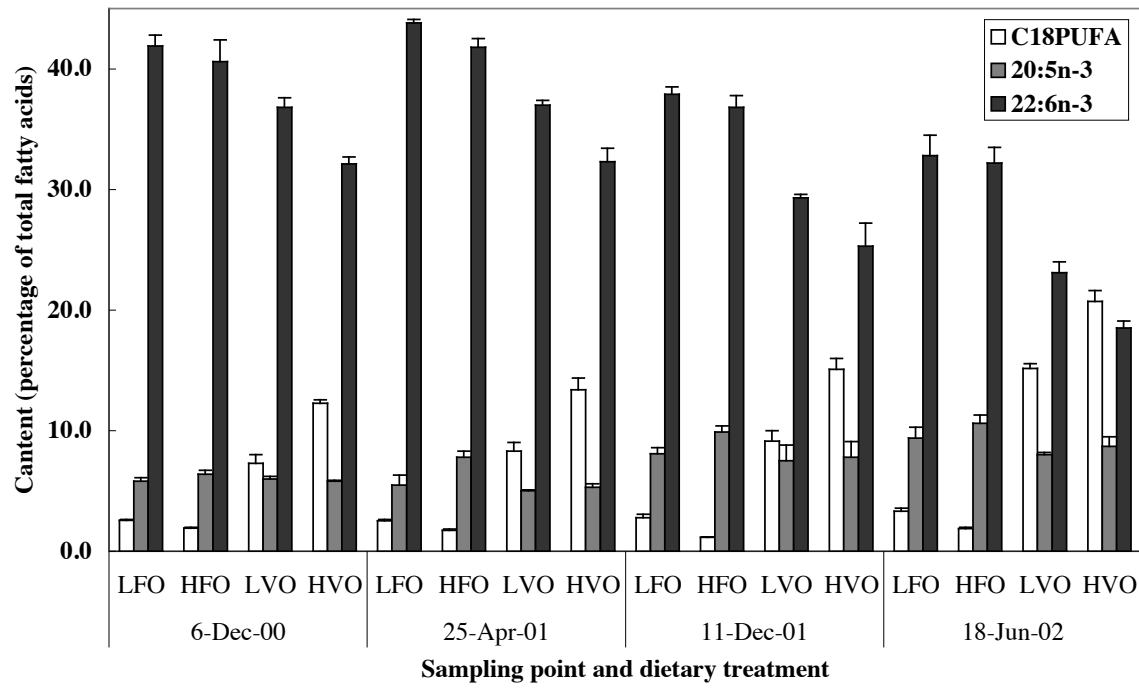


Fig.5

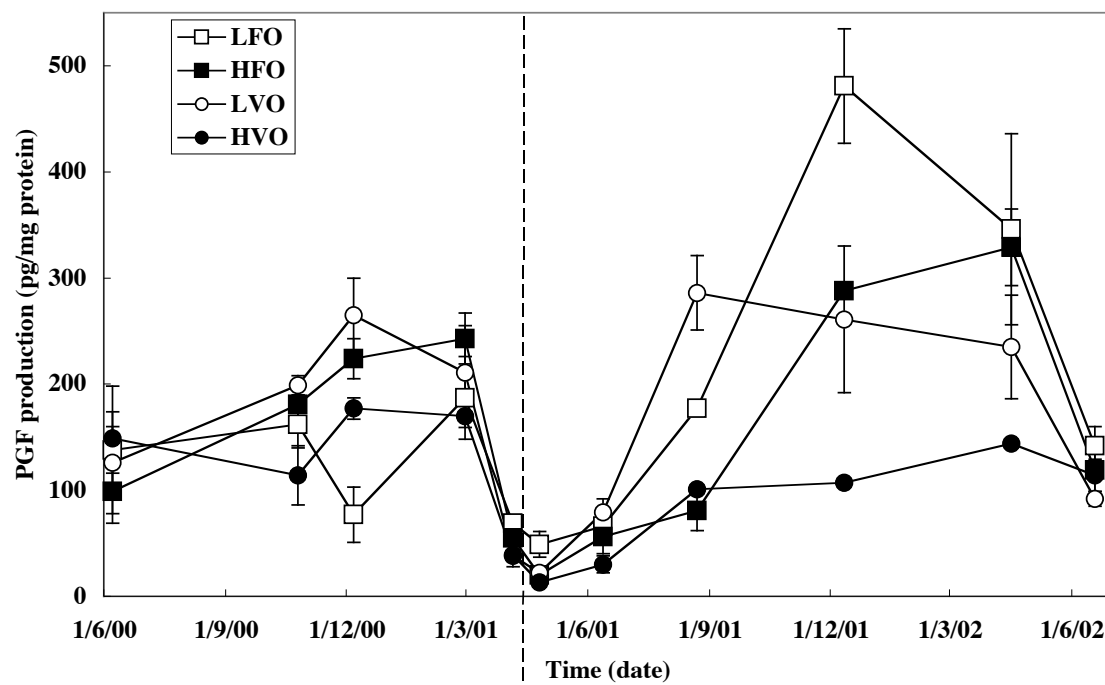


Fig.6

