

'Dioxin and dioxin-like polychlorinated biphenyls (PCBs) in Scottish farmed salmon (*Salmo salar*): effects of replacement of dietary marine fish oil with vegetable oils' by J. Gordon Bell, Fiona McGhee, James R. Dick and Douglas R. Tocher. *Aquaculture*, Volume 243, Issues 1-4, January 2005, pp. 305 – 314.

Published in *Aquaculture* by Elsevier. *Aquaculture*, Volume 243, Issues 1-4, January 2005, pp. 305 - 314.

This is the peer reviewed version of this article.

NOTICE: this is the author's version of a work that was accepted for publication in Aquaculture. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in Aquaculture, VOL 243, ISSUE 1-4, (January 2005). DOI 10.1016/j.aquaculture.2004.10.016.

1 **Abstract**

2 Duplicate groups of Atlantic salmon were fed one of four practical-type diets from first
3 feeding to harvest after 115 weeks. The four diets were low fish oil (16% w/w, LFO),
4 high fish oil (35% w/w, HFO), low vegetable oil (17%, linseed oil/rapeseed oil, 1:1 w/w;
5 LVO) and high vegetable oil (35%, linseed oil/rapeseed oil, 1:1 w/w; HVO). Following
6 sample collection of fish around 2 kg weight all groups were switched to the HFO diet for
7 a further 24 weeks. The dioxin concentration in diets was in order, HFO > LFO > LVO >
8 HVO with values ranging from 0.16-1.4 ng TEQ/kg. The dioxin-like PCB (DL-PCB)
9 concentrations were in the same order with values ranging from 0.62-3.68 ng TEQ/kg.
10 Concentrations of dioxins and DL-PCBs in flesh samples were correlated with feed
11 concentrations although values in flesh were always lower than in feed. Flesh dioxin
12 concentrations ranged from 0.10-0.53 ng TEQ/kg and DL-PCBs from 0.58-1.48 ng
13 TEQ/kg. After 24 weeks feeding a fish oil-containing finishing diet (HFO) the flesh
14 dioxin concentrations ranged from 0.20-0.54 ng TEQ/kg and the DL-PCBs from 0.66-
15 1.07 ng TEQ/kg. Feeding the HVO diet resulted in significant reductions in flesh
16 concentrations of 20:5n-3 (EPA) and 22:6n-3 (DHA) to around 25% of the values in fish
17 fed the HFO diet. However, feeding the HFO finishing diet to all groups for 24 weeks
18 resulted in restoration of flesh EPA and DHA concentrations to 80% of the values in fish
19 fed the HFO diet throughout. This study suggests that salmon cultured on diets based on
20 fish meal and oil (HFO) attain flesh dioxin concentrations that are <14% of the current
21 European Commission limit. However, by replacing marine fish oils with vegetable oils
22 for most of the production cycle dioxin and DL-PCB concentrations can be substantially
23 reduced.

1 *Keywords:* salmon, polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated
2 dibenzo-*p*-furans (PCDFs), polychlorinated biphenyls (PCBs), fish oil, vegetable oils.

3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24

1 **1. Introduction**

2 Polychlorinated dibenzodioxins and polychlorinated dibenzofurans (dioxins) can be
3 produced naturally, arising from, for example, forest fires and incomplete combustion
4 of organic matter, as well as from industrial chemical processes. Dioxin-like
5 polychlorinated biphenyls (DL-PCBs) arise from man made products such as
6 electrical transformers, heat exchange fluids, hydraulic oils and plastic manufacture.
7 Although production of these chemicals is now banned they have been deposited in
8 the oceans, due to industrial activity over the last century, where they are distributed
9 across the marine environmental biota (North Sea Task Force, 1993). Dioxins and
10 DL-PCBs are highly lipophilic and have biological half-lives measured in decades
11 which means they tend to accumulate in predators at the top of the food chain
12 (Froescheis et al. 2000).

13 The use of fishmeals and oils in aquafeeds, to supply essential amino acids, fatty
14 acids and dietary energy, has been scientifically and economically sound. However,
15 the feed grade fisheries that supply fish meal and oil have reached sustainable limits
16 (Sargent & Tacon, 1999) and, if aquaculture production is to expand, alternative raw
17 materials must be investigated and introduced. Replacement of significant amounts of
18 either fish oil or meal can be achieved without loss of growth performance or affects
19 on fish health (Bell et al. 2002, 2003; Torstensen et al 2004; Watanabe et al. 1999;
20 Kaushik et al. 2004). However, when significant inclusion of vegetable oils occurs
21 there is a marked reduction in the n-3 highly unsaturated fatty acid (HUFA) content
22 of the flesh (Bell et al. 2002, 2003; Torstensen et al 2004). The reduction in n-3
23 HUFA, principally eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid

1 (22:6n-3; DHA), could potentially be offset by reduced levels of dioxins and DL-
2 PCBs in salmon flesh as vegetable oils generally contain lower levels of these
3 pollutants than most marine fish oils (Ministry of Agriculture, Fisheries and Food,
4 1995; European Commission (EC), 2000a; Jacobs et al. 2002a & b). Atlantic salmon
5 currently produced in Scotland, fed on diets containing only marine fish oil, are of
6 high nutritional quality and are rich in EPA and DHA (Bell et al. 1998; Aursand et al.
7 2000). However, recent literature has suggested that farmed salmon from Northern
8 Europe contains elevated concentrations of dioxins and DL-PCBs due to dietary input
9 from fish oils and meals (Jacobs et al 2002a & b, Hites et al. 2004). The values
10 quoted (Hites et al. 2004) of ~3 ng /kg World Health Organisation Toxic Equivalent
11 values (WHO TEQ) for dioxins + DL-PCBs are well within the EU permitted values
12 for salmon of 4.0 ng TEQ/kg, for dioxins alone, which were implemented in July
13 2002.

14 In the present study, Atlantic salmon were fed one of four diets from first feeding
15 through to harvest. The diets were low fish oil (LVO), high fish oil (HFO), low
16 vegetable oil (LVO) and high vegetable oil (HVO) where the vegetable oil was a
17 mixture of rapeseed and linseed oil (1:1 w/w). The fish were sampled at ~2kg and
18 thereafter all fish were switched to a HFO finishing diet for 24 weeks. The aim was to
19 investigate the influence of feeding dietary VO on flesh dioxin and DL-PCB
20 concentrations as well as the effects of feeding a FO finishing diet on flesh
21 dioxin/DL-PCB and n-3 HUFA concentrations.

22 23 **2. Materials and methods**

1 *2.1 Fish, culture conditions and diets*

2 In March 2000, 24,000 Atlantic salmon fry were distributed randomly into 8 square
3 fibreglass tanks (3m x 3m, depth 0.5m) at Kinlochmoidart smolt unit (Marine Harvest
4 (Scotland) Ltd., Highland, Scotland) and weaned onto the experimental extruded
5 diets. Each diet was fed to duplicate tanks of fry/parr at either 14% (L) or 25% total
6 oil (H), resulting in four dietary treatments in total, LFO (low fish oil), HFO (high
7 fish oil), LVO (low vegetable oil) and HVO (high vegetable oil). Fish were fed the
8 experimental diets until sea water transfer in April 2001, at which point fish (average
9 weight ~ 40g) were transferred into 5m x 5m net pens (600 fish/pen) at Loch Duich,
10 Lochalsh, Scotland. The fish were fed the same diets in seawater as in freshwater
11 although the dietary oil levels were increased to 17% in the low oil diets (LFO and
12 LVO) and 30% (3mm pellet) with the latter increasing to 35% (6 and 9mm pellets) in
13 HFO and HVO diets. The diets were formulated and manufactured by the major
14 salmon feed producers; Ewos (freshwater phase; Ewos Technology Centre,
15 Livingston, Scotland), BioMar (sea water on-growing to ~1 kg; BioMar A/S, Brande,
16 Denmark) and Skretting (seawater phase to 2kg and finishing diet; ARC Nutreco,
17 Stavanger, Norway). All diets were formulated to satisfy the nutritional requirements
18 of salmonid fish (National Research Council 1993). Diet formulations and proximate
19 compositions of the 6 & 9 mm diets are shown in Table 1. Fish were on-grown until
20 June 2002 at which time they had reached ~2 kg. Thereafter, all treatments were
21 switched to the HFO feed for 24 weeks when they had reached ~4.5kg.

22 *2.2 Sample collection*

1 Samples of 6 mm and 9 mm feed, from each of the four dietary treatments, analysed
2 for dioxin and DL-PCB were collected from the centre of a newly opened feed bag
3 and placed in a glass jar. Equal quantities of each feed size were mixed before
4 grinding. The 9 mm finishing diet was the same as the HFO diet and a sample of this
5 feed was collected as described above. Dioxin and DL-PCB concentrations were
6 measured in single analyses of each 6 and 9 mm composite or 9 mm finishing diet.
7 In June 2002, when fish had reached an average weight of ~2 kg, 12 fish per
8 treatment were sampled for dioxin and DL-PCB analysis and a further 12 were
9 sampled for flesh fatty acid analysis. The fish were killed by a blow to the head and a
10 steak cut from the Norwegian Quality cut region. Each individual steak for dioxin
11 analysis was immediately wrapped in aluminium foil and frozen on dry ice. The
12 steaks were pooled to provide three samples of flesh from 4 individual fish for each
13 dietary treatment. Before analysis, the steaks were blended in a food processor, after
14 removal of skin, bones and the dorsal fat body. Similar samples of pooled flesh were
15 processed in the same way and the homogenised flesh frozen prior to lipid extraction
16 and fatty acid analysis.

17 *2.3 Analysis of dioxins and DL-PCBs*

18 All analytical procedures for dioxin and DL-PCB analysis were sub-contracted to the
19 United Kingdom Accreditation Service (UKAS) accredited laboratories of Scientific
20 Analytical Laboratories Ltd. (SAL), Medlock House, New Elm Road, Manchester M3
21 4JH. All analyses were conducted in accordance with SAL Standard Operating
22 Procedures.

23 *2.3.1 Sample extraction and clean up procedures*

1 For feed samples a 50 g aliquot was ground and placed in a pre-extracted Soxhlet
2 thimble, spiked with ¹³C-labelled internal standards and extracted with 300 ml toluene
3 for 16h in an automated soxhlet extraction apparatus. For flesh samples a 50 g aliquot
4 was spiked with internal standards as for feed samples, and digested in concentrated
5 HCl overnight. The solution was then back extracted with 200 ml of
6 hexane/dichloromethane (1:1 v/v) for 16h. A method blank was processed in the same
7 manner. The extracts were then purified by acid washing followed by elution through
8 a column combining sulphuric acid impregnated silica, potassium hydroxide
9 impregnated silica and anhydrous sodium sulphate. The eluate from this column was
10 further purified on a column containing activated Florasil and then concentrated to
11 near dryness prior to analysis on a gas chromatograph/mass spectrometer (GC/MS)
12 apparatus. Immediately prior to GC/MS analysis the sample, dissolved in nonane was
13 spiked with recovery standard, ¹³C₆-1,2,3,4-tetrachlorodibenzo-*p*-dioxin (TCDD).

14 *2.3.2 GC/MS methodology*

15 The dioxin and DL-PCB analyses were carried out using a HP 5890A GC coupled to
16 a VG Autospec Ultima mass spectrometer. Samples were injected using an HP7673B
17 autosampler and data was collected and processed using the VG OPUS package. The
18 GC was equipped with a DB-5ms capillary column (60m x 0.25mm i.d, 0.25 μ m film
19 thickness) and helium was used as carrier gas with a head pressure of 30 p.s.i. The
20 oven temperature program was 140°C for 4 minutes then to 220°C at 15°C/min then to
21 240°C at 1.5°C/min, hold for 2 min then to 310°C at 4°C/min and held for 10 min.
22 Injection was in splitless mode at 300°C. The MS source was set at 30eV, the trap
23 current was 700 μ A and the source temperature was 250°C. The samples were

1 analysed for the 17 PCDDs/PCDFs which have toxic equivalency to 2,3,7,8-TCDD
2 and the 12 co-planar PCBs 77, 81,105, 114, 118, 123, 126, 156, 157, 167, 169 & 189.
3 EC/WHO toxic equivalent factors (TEF) were used to convert dioxin/PCB values in
4 ng/kg to WHO toxic equivalent values (TEQ).

5 *2.4 Lipid extraction and fatty acid analysis*

6 Total lipid contents of salmon flesh were determined gravimetrically after extraction
7 by homogenization in chloroform/methanol (2:1, v/v) containing 0.01 % butylated
8 hydroxytoluene (BHT) as antioxidant, basically according to Folch et al. (1957). Fatty
9 acid methyl esters (FAME) were prepared from total lipids by acid-catalyzed
10 transesterification in 2 ml of 1% H₂SO₄ in methanol plus 1 ml toluene as described by
11 Christie (1982) and FAME extracted and purified as described previously (Ghioni et
12 al. 1996). FAME were separated and quantified by gas-liquid chromatography (Carlo
13 Erba Vega 8160, Milan, Italy) using a 30m x 0.32 mm capillary column (CP wax
14 52CB; Chrompak Ltd., London, U.K). Hydrogen was used as carrier gas and
15 temperature programming was from 50°C to 150°C at 40°C/min and then to 230°C at
16 2°C/min. Individual methyl esters were identified by comparison to known standards
17 and by reference to published data (Ackman 1980). Data were collected and processed
18 using the Chromcard for Windows (version 1.19) computer package (Thermoquest
19 Italia S.p.A., Milan, Italy).

20 *2.5 Statistical analysis*

21 Significance of difference (P < 0.05) between dietary treatments was determined by
22 one-way ANOVA. Differences between means were determined by Tukey's test.
23 Data identified as non-homogeneous, using Bartlett's test, were subjected to log or

1 arcsin transformation before applying the ANOVA. ANOVA was performed using a
2 GraphPad Prism (version 3.0) statistical package (GraphPad Software, San Deigo,
3 CA, USA).

4 **3. Results**

5 Dioxin concentrations in the four experimental diets were in order HFO > LFO >
6 LVO > HVO with the concentrations ranging from 1.40 to 0.16 ng TEQ/kg (Fig.1A).
7 The finishing feed had a slightly higher concentration, than the combined 6 mm & 9
8 mm HFO feeds, of 1.7 ng TEQ/kg (Fig. 1A). DL-PCB concentrations in the four
9 experimental diets were in the same rank order as for dioxins with concentrations
10 ranging from 3.68 to 0.62 ng TEQ/kg although the concentration of DL-PCB in the
11 finishing feed was considerably lower than that in the HFO experimental feeds at 1.31
12 ng TEQ/kg (Fig. 1B).

13 Dioxin concentrations in salmon flesh broadly followed the concentrations in the
14 feeds with the highest value in fish fed the HFO diet (0.53 ng TEQ/kg) and the lowest
15 in fish fed the LFO diet (0.10 ng TEQ/kg) although there were no significant
16 differences between the dioxin concentrations in fish fed the LFO, LVO and HVO
17 diets (Fig. 2A). DL-PCB concentrations in salmon flesh also followed the pattern in
18 diets with the highest values in fish fed the HFO diet (1.48 ng TEQ/kg) with the
19 lowest values in fish fed the LFO diet (0.58 ng TEQ/kg) although, as for dioxins,
20 there were no significant differences between the DL-PCB concentrations in fish fed
21 the LFO, LVO and HVO diets (Fig. 2B).

22 After the initial sampling of fish fed the four experimental diets the remaining fish
23 were switched to a FO-containing finishing diet (the HFO 9 mm diet) for a further 24

1 weeks. Following the 24 week finishing diet period, fish were sampled again for flesh
2 dioxin and DL-PCB analysis. Dioxin concentrations, after 24 weeks on the finishing
3 diet, were still significantly higher in fish fed the HFO diet throughout (0.54 ng
4 TEQ/kg) compared to fish previously fed the other three diets. The lowest values
5 were in fish previously fed the HVO diet (0.20 ng TEQ/kg) and they were
6 significantly lower than fish previously fed both the LFO and HFO diets (Fig. 3A).
7 Apart from fish previously fed the HFO diet fish from all other groups had
8 significantly higher flesh dioxin concentrations after the finishing diet period
9 compared to the concentrations after the experimental diet phase 24 weeks earlier
10 (Fig. 3A). DL-PCB concentrations, after the 24 weeks on the finishing diet, were
11 significantly higher in fish fed the HFO diet throughout (1.07 ng TEQ/kg) compared
12 to fish previously fed the LVO and HVO diets (Fig. 3A). Fish previously fed the LFO
13 and HVO diets had significantly higher flesh DL-PCB concentrations after the
14 finishing diet period compared to the concentrations after the experimental diet phase
15 24 weeks earlier (Fig. 3B).

16 Flesh EPA concentrations were significantly different for each of the treatment
17 groups with the highest values in fish fed HFO (7.0%) and the lowest in fish fed HVO
18 (1.4%) (Fig. 4A). After feeding the FO finishing diet for 24 weeks flesh EPA
19 concentrations in fish previously fed HVO were still significantly lower than fish
20 previously fed HFO or LVO but had been restored to 80% of the value in the HFO
21 fish (Fig. 4A). Flesh DHA concentrations were significantly different for each of the
22 treatment groups with highest values in fish fed HFO (18.5%) and the lowest in fish
23 fed HVO (4.9%) (Fig. 4B). After feeding the FO finishing diet for 24 weeks flesh

1 DHA concentrations in fish previously fed HVO were still significantly lower than
2 fish previously fed HFO or LFO but had been restored to 80% of the value in the
3 HFO fish (Fig. 4B).

4 **4. Discussion**

5 The dioxin and DL-PCB concentrations in the experimental diets followed a
6 predictable pattern based on the FO content of the diets with the highest values in the
7 HFO diet and lowest values in the HVO diet. The 9 mm finishing diet had a higher
8 dioxin concentration, but a lower DL-PCB concentration, compared to HFO diet
9 which was a composite of the 6 and 9 mm feeds. The FO in the finishing diet was
10 capelin oil while that used in the 6 mm diet was herring oil. The concentration of
11 dioxins and DL-PCB in capelin is generally lower than that in herring although the
12 values can vary seasonally and geographically for both species (Rappe et al. 1989;
13 Lundebye-Haldorsen and Lie, 1999; EC 2000a). All diets used in this study were
14 considerably lower than the European Union (EU) permitted value for dioxins in fish
15 feeds of 2.25 ng TEQ/kg (EC 2000a). The highest dioxin concentration in the feed
16 used was 1.7 ng TEQ/kg for the 9 mm feed containing capelin oil followed by the 6
17 mm/9 mm composite at 1.4 ng TEQ/kg where the 6 mm feed contained herring oil.
18 The lowest dioxin concentration was in the HVO feed being 0.16 ng TEQ/kg or 9%
19 of the value in the finishing feed and 7% of the EU recommended limit (EC 2000a).
20 In the recent publication by Hites et al. (2004) dioxin concentrations in Scottish feed
21 samples ranged from 3-7 ng TEQ/kg with lowest values ~0.5 TEQ/kg being found in
22 feeds from British Columbia, Canada. However, the dioxin values quoted by Hites et
23 al (2004) were based on dioxins plus DL-PCBs. In the feeds from the present study

1 the values for dioxins plus DL-PCBs ranged from 5.1 (HFO) to 0.78 ng TEQ/kg
2 (HVO).

3 In general, the flesh dioxin and DL-PCB concentration varied in accordance with the
4 concentration in the diet although flesh values were always considerably lower than
5 those in the diet fed. However, fish fed the LFO diet had the lowest mean flesh dioxin
6 concentration, but the second highest diet dioxin concentration, although the value
7 was not significantly different from fish fed LVO and HVO. The lower dioxin
8 concentration in LFO flesh may have been due to these fish having a significantly
9 lower mean weight at harvest (1.44 ± 0.37 kg) compared to fish fed the LVO ($1.71 \pm$
10 0.36), HVO (2.02 ± 0.57) or HFO (1.88 ± 0.57) diets (Tocher et al. 2004). Salmon
11 generally deposit more flesh lipid as a function of growth rate, feed intake and dietary
12 lipid concentration (Lie, 2001).

13 In the period from 1995-1999 the EU undertook a comprehensive analysis of dioxin
14 and PCB levels in foods for human consumption across the EU and EFTA members
15 states (EC 2000b). In farmed salmon they found dioxin values in the range 0.43-1.04
16 ng TEQ/kg wet weight and in farmed trout the range was 0.24-0.81 ng TEQ/kg. The
17 values in the present study, for fish fed HFO, fall within the lower end of this range
18 (0.53) while values for fish grown on VO diets were below this range both at 2kg
19 harvest weight (~ 0.15) and in HVO fish fed the HFO finishing diet for 24 weeks (0.2
20 ng TEQ/kg). Values quoted for fatty fish in the EC (2000b) report, including
21 anchovy, herring, mackerel, sardine, tuna and wild salmon, ranged from 0.01-7.04 ng
22 TEQ/kg, with lowest concentrations in tuna and highest concentrations in Baltic

1 salmon. When the tuna values, along with those for fish caught in the Baltic, were
2 removed the range was 0.29-2.10 ng TEQ/kg (EC 2000b).

3 Some studies describing dioxin and DL-PCBs in foodstuffs present data as pg
4 TEQ/g lipid. When presented in this way the dioxin concentrations in fish from this
5 study had values ranging from 1.87 pg TEQ/g lipid, in fish fed the HVO diet, to 7.69
6 in fish fed the HFO diet. In two studies by Jacobs et al. (2000, 2002b), the range for
7 samples of Scottish and Norwegian farmed salmon was 4.7-11.5 pg TEQ/g lipid. Our
8 own value for Scottish salmon grown on FO for the whole production cycle is
9 intermediate within this range while fish grown on the HVO diet have a considerably
10 reduced flesh dioxin concentration compared to any of the farmed fish sampled by
11 Jacobs et al. (2000, 2002b). Interestingly, the highest value for flesh dioxin found by
12 Jacobs et al. (2000, 2002b) was 18.21 pg TEQ/g lipid from a wild Scottish salmon
13 caught in the River Deveron.

14 In the present study, the DL-PCB concentration (12 congeners) in salmon flesh was
15 in the range 0.58-1.48 ng TEQ/kg wet weight. These values are lower than those
16 quoted in a recent report by the RIVO Fisheries Institute of Holland (2000) which
17 found values up to 4 ng TEQ/kg in farmed salmon from Norway and Scotland. When
18 the DL-PCB values from the present study are quoted as pg TEQ/g lipid the range is
19 from 8.7 pg TEQ/g lipid, in fish fed the HVO diet, to 22.84 in fish fed the LFO diet.
20 By comparison, the range of Jacobs et al. (2000, 2002a) was 9.05-24.82 (7 congeners)
21 in farmed Scottish and Norwegian salmon, ~20 pg TEQ/g lipid found by the RIVO
22 Fisheries Institute of Holland (2000), 15.4-43.7 in Scottish salmon (Jacobs et al.
23 2002b). A study of one Atlantic and three Pacific farmed salmon from Canada found

1 slightly lower concentrations of DL-PCBs (12 congeners) ranging from 3.81-10.54 pg
2 TEQ/g lipid (Easton et al. 2002). Values for wild Pacific salmon were considerably
3 lower in the range 0.26-1.11 pg TEQ/g lipid (Easton et al. 2002) although those for
4 wild Atlantic salmon were similar to farmed salmon being in the range 17.02-23.99
5 pg TEQ/g lipid (Jacobs et al. 2000, 2002 a & b).

6 The dioxin concentration in salmon flesh samples quoted by Hites et al. (2004),
7 which quoted dioxins to include dioxins plus DL-PCBs, was ~3 ng TEQ/kg for
8 Scottish salmon. In the present study, the values for flesh dioxins plus DL-PCBs
9 ranged from 2.00 ng TEQ/kg (HFO) to 0.73 ng TEQ/kg (HVO) with lowest in LFO
10 flesh (0.68 ng TEQ/kg). The values in the latter two groups were found in wild
11 Pacific salmon (Hites et al 2004). The combined values for dioxins and DL-PCBs are
12 relevant to current EU advice for human consumption which recommends a
13 maximum combined intake of 14 pg TEQ/kg body weight/week (SCF 2000). For a 75
14 kg adult this equates to ~1000 pg TEQ/week. Based on the values from the present
15 study this would allow around two 200g portions of Scottish salmon per week when
16 grown on FO diets or around six 200g portions if salmon grown on the HVO diet
17 were consumed. However, it is important to consider that the majority of dioxins
18 consumed in Europe comes from other foodstuffs, particularly dairy products, and not
19 principally from seafood. Taking this into account, the present recommendation of the
20 UK Food Standards Agency that we consume one portion of oily fish per week then
21 the values for dioxin plus DL-PCBs in Scottish salmon would still leave consumers
22 well within the EU recommended consumption limit.

1 Feeding diets containing 100% of added oil as LO and RO (1:1 w/w) from first
2 feeding to harvest at 2 kg resulted in flesh EPA and DHA concentrations being
3 reduced to around 25% of the concentrations found in fish fed the HFO diet over the
4 same period. However, after feeding the HFO finishing diet for 24 weeks flesh EPA
5 and DHA concentrations in fish previously fed HVO were still significantly lower
6 than fish previously fed HFO but were restored to 80% of the values in the fish fed
7 HFO throughout. This result supports a number of recent studies in salmon post-
8 smolts fed diets containing either RO or LO where restoration of flesh concentrations
9 of EPA and DHA was achieved by feeding a FO finishing diet for periods between 16
10 and 24 weeks (Bell et al. 2003, 2004 Torstensen et al. 2004). These data have been
11 further supported by modelling studies demonstrating the application of a dilution
12 model to predict tissue fatty acid changes following alteration of dietary oils in
13 Atlantic salmon and other species (Jobling, 2004, Robin et al. 2003).

14 **5. Conclusion**

15 This study has shown that Atlantic salmon can be cultured, over the whole production
16 cycle, using diets in which 100% of added dietary fish oil (FO) can be replaced by a
17 blend of rapeseed (RO) and linseed oils (LO) without detriment to growth
18 performance. While flesh concentrations of EPA and DHA were significantly
19 reduced in fish fed vegetable oils (VO) these values could be restored, to 80% of the
20 values in fish fed FO, by feeding a FO-containing finishing diet for 24 weeks.
21 Feeding the 100% VO diet reduced flesh dioxin and dioxin-like PCB concentrations
22 by 75% and 64%, respectively, compared to fish fed FO. After feeding the FO
23 finishing diet for 24 weeks the flesh dioxin and dioxin-like PCB concentrations were

1 still 60% and 47% lower than in salmon fed FO throughout. While Scottish salmon
2 cultured using diets based on fish meal and oil have flesh dioxin concentrations that
3 are < 14% of current EU limits these values can be substantially reduced by the use of
4 vegetable oils in feed formulations.

5 **Acknowledgements**

6 This work was funded by an award from the NERC-LINK Aquaculture initiative
7 (project no. SAL 038) with industrial support from BioMar Ltd., Ewos Innovation,
8 Highlands and Islands Enterprise, Marine Harvest (Scotland) Ltd., Skretting, The
9 Highland Council and Uniq Prepared Foods (Annan) Ltd. We would like to thank Bill
10 Gordon and Iain Martin for their assistance with fish husbandry.

11 **References**

- 12 Ackman, R.G. (1980) Fish lipids, part 1. In: Advances in Fish Sciences and
13 Technology (Connell, J.J. ed.), pp. 86-103. Fishing News Books Ltd., Farnham,
14 U.K.
- 15 Aursand, M., Mabon, F., Martin, G.J. 2000. Characterization of farmed and wild
16 salmon (*Salmo salar*) by a combined use of compositional and isotopic analyses.
17 J.A.O.C.S. 77, 659-666.
- 18 Bell, J.G., Henderson, R.J., Tocher, D.R., McGhee, F., Dick, J.R., Porter, A.,
19 Smullen, R., Sargent, J.R., 2002. Substituting fish oil with crude palm oil in the
20 diet of Atlantic salmon (*Salmo salar*) affects tissue fatty acid compositions and
21 hepatic fatty acid metabolism. J. Nutr.132, 222-230.
- 22 Bell, J.G., Henderson, R.J., Tocher, D.R., Sargent, J.R. 2004. Replacement of dietary
23 fish oil with increasing levels of linseed oil: modification of flesh fatty acid
24 compositions in Atlantic salmon (*Salmo salar*) using a fish oil finishing diet.
25 Lipids "in press".

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

4 Bell, J. G., Tocher, D. R., Henderson, R. J., Dick, J. R., Crampton, V.O. 2003.
5 Altered fatty acid compositions in Atlantic salmon (*Salmo salar*) fed diets
6 containing linseed and rapessed oils can be partially restored by a subsequent fish
7 oil finishing diet. J. Nutr. 133, 2793-2801.

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

10 Easton, M.D.L., Luszniak, D., Von der Geest, E. 2002. Preliminary examination of
11 contaminant loadings in farmed salmon, wild salmon and commercial salmon
12 feed. Chemosphere 46, 1053-1074.

13 European Commission Health and Consumer Protection Directorate-General.
14 Opinion of the Scientific Committee on Animal Nutrition. Dioxin contamination
15 of feeding stuffs and their contribution to the contamination of food of animal
16 origin; 6th November 2000, 105 pages.
17 http://europa.eu.int/comm/food/fs/sc/scf/index_en.html

18 European Commission, Health and Consumer Protection Directorate-General.
19 Assessment of dietary intake of dioxin and related PCBs by the population of EU
20 member states. Report on tasks for scientific cooperation, Report of experts
21 participating in Task 3.2.5, 7th June 2000. 141 pages.
22 http://europa.eu.int/comm/food/fs/sc/scf/index_en.html

23 Folch, J., Lees, M., Sloane-Stanley, G.H. 1957. A simple method for the isolation
24 and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497-509.
25
26

1 Froescheis, O., Looser, R., Cailliet, G.M., Jarman, W.M. Ballschmiter, K. 2000. The
2 deep-sea as a final global sink of semivolatile persistent organic pollutants? Part
3 1: PCBs in surface and deep-sea dwelling fish of North and South Atlantic and the
4 Monterey Bay Canyon (California). *Chemosphere* 40, 651-660.

5 Ghioni, C., Bell, J.G., Sargent, J.R. 1996. Polyunsaturated fatty acids in neutral lipids
6 and phospholipids of some freshwater insects. *Comp. Biochem. Physiol.* 114B,
7 161-170.

8 Hites, R.A., Foran, J.A., Carpenter, D.O., Hamilton, M.C., Knuth, B.A., Schwager,
9 S.J. 2004. Global assessment of organic contaminants in farmed salmon. *Science*
10 303, 226-229.

11 Jacobs, M.N., Covaci, A., Schepens, P. 2002b. Investigation of selected persistent
12 organic pollutants in farmed Atlantic salmon (*Salmo salar*), salmon aquaculture
13 feed, and fish oil components of the feed. *Environ. Sci. Technol.* 36, 2797-2805.

14 Jacobs, M.N., Ferrario, J., Byrne, C. 2000. Investigation of PCDDS, PCDFS and
15 selected coplanar PCBS in Scottish farmed salmon (*Salmo salar*) Organohalogen
16 Compounds 47, 338-341.

17 Jacobs, M.N., Ferrario, J., Byrne, C. 2002a. Investigation of polychlorinated dibenzo-
18 *p*-dioxins dibenzo-*p*-furans and selected coplanar biphenyls in Scottish farmed
19 Atlantic salmon (*Salmo salar*). *Chemosphere* 47, 183-191.

20 Jobling, M. 2004. Are modifications in tissue fatty acid profiles following a change in
21 diet the result of dilution? Test of a simple dilution model. *Aquaculture* 232, 551-
22 562.

1 Kaushik, S.J., Coves, D., Dutto, G., Blanc, D. 2004. Almost total replacement of fish
2 meal by plant protein sources in the diet of a marine teleost, the European seabass,
3 *Dicentrarchus labrax*. Aquaculture 230, 391-404.

4 Lie, Ø. 2001. Flesh quality-the role of nutrition. Aquacul. Res. 32, 341-348.

5 Lundebye-Haldorsen, A.-K., Lie, Ø. 1999. An overview of dioxins in Norwegian fish
6 and marine products. Norwegian Agricultural Inspection Service, Oslo, Norway.

7 Ministry of Agriculture, Fisheries and Food. 1995. Dioxins in food-UK dietary
8 intakes. Food Safety Information Sheet 71, MAFF, London, UK.

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

11 North Sea Task Force. 1993. North Sea Quality Status Report. Oslo and Paris
12 Commissions/International Council for Exploration of the Sea, London.

13 Rappe, C., Bergqvist, P.-A., Kjeller, L.O. 1989. Levels, trends and patterns of PCDDs
14 and PCDFs in Scandinavian environmental samples. Chemosphere 18 (1-6), 651-
15 658.

16 RIVO Fisheries Institute of Holland. 2000. Report number C034/00.

17 Robin, J.H., Regost, C., Arzel, J., Kaushik, S.J. 2003. Fatty acid profile of fish
18 following a change in dietary fatty acid source: model of fatty acid composition
19 with a dilution hypothesis. Aquaculture 225, 283-293.

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

22

23

- 1 Tocher, D.R., Bell, J.G., McGhee, F., Dick, J.R., Fonseca-Madrigal, J. 2004. Effects
2 of dietary lipid level and vegetable oil on fatty acid metabolism in Atlantic salmon
3 (*Salmo salar* L.) over the whole production cycle. Fish Physiol. Biochem.
4 Submitted for publication.
- 5 Torstensen, B.E., Frøyland, L., Ørnsrud, R., Lie, Ø. 2004. Tailoring of a
6 cardioprotective muscle fatty acid composition of Atlantic salmon (*Salmo salar*)
7 fed vegetable oils. Food Chem. “in press”.
- 8 Watanabe, T., Aoki, H., Shimamoto, K., Hadzuma, M., Maita, M., Yamagata, Y.,
9 Viswanath, K., Satoh, S. 1999. A trial to culture yellowtail with non-fishmeal
10 diets. Fish. Sci. 64, 505-512.
- 11

1 Figure legends

2 Fig. 1. Concentrations of dioxin (A) and dioxin-like PCBs (B) in diets (ng TEQ/kg)
3 used during the trial.

4 Fig. 2. Concentrations of dioxin (A) and dioxin-like PCBs (B) in flesh (ng TEQ/kg)
5 samples collected after 115 weeks. Columns assigned a different superscript letter
6 are significantly different ($P < 0.05$).

7 Fig. 3. Concentrations of dioxin (A) and dioxin-like PCBs (B) in flesh (ng TEQ/kg)
8 samples collected after 115 weeks and after feeding the HFO finishing diet for 24
9 weeks. Columns assigned a different letter, within each feeding period, are
10 significantly different ($P < 0.05$). Columns assigned an asterisk in the post-finishing
11 diet group are significantly different from the same dietary group in the pre-finishing
12 diet sample.

13 Fig. 4. Concentrations (g/100g total fatty acids) of flesh eicosapentaenoic (20:5n-3;
14 EPA) and docosahexaenoic acids (22:6n-3; DHA) before (0 weeks) and after (24
15 weeks) feeding the HFO finishing diet. Columns assigned a different letter, within
16 each time point, are significantly different ($P < 0.05$).

17

18

19

20

21

22

23

1 Table 1

2 Diet formulations and proximate compositions (g/100g).

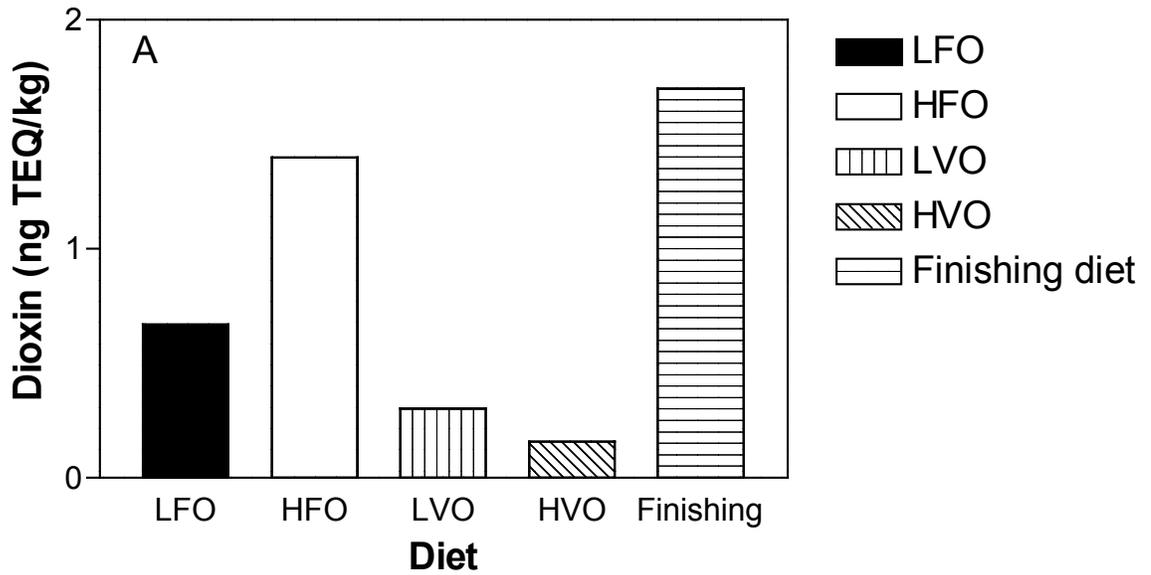
3	Component	LFO	LVO	HFO	HVO	LFO	LVO	HFO	HVO
4	Pellet size	6 mm ¹				9 mm ²			
5	LT Fishmeal	35.2		43.5		32.9		44.5	
6	Wheat	22.0		13.5		33.2		14.8	
7	Soybean meal	12.0		6.5		11.4		0	
8	Other plant meals ³	11.8		7.2		10.2		8.5	
9	Micronutrients ⁴	1.0		1.0		0.4		0.4	
10	Fish oil ⁵	14.6		29.2		12.0		31.8	
11	Rapeseed oil		7.3		14.6		6.0		15.9
12	Linseed oil		7.3		14.6		6.0		15.9
13									
14	Protein (%)	38.0	37.2	39.7	37.6	39.2	39.4	38.9	39.5
15	Oil (%)	16.9	16.9	36.9	36.5	16.5	16.8	33.6	34.2
16	Moisture (%)	7.9	8.7	1.8	5.8	5.6	5.9	5.7	5.7
17	Ash (%)	7.6	7.6	8.8	8.7	6.4	6.6	6.5	6.6
18									

19 _

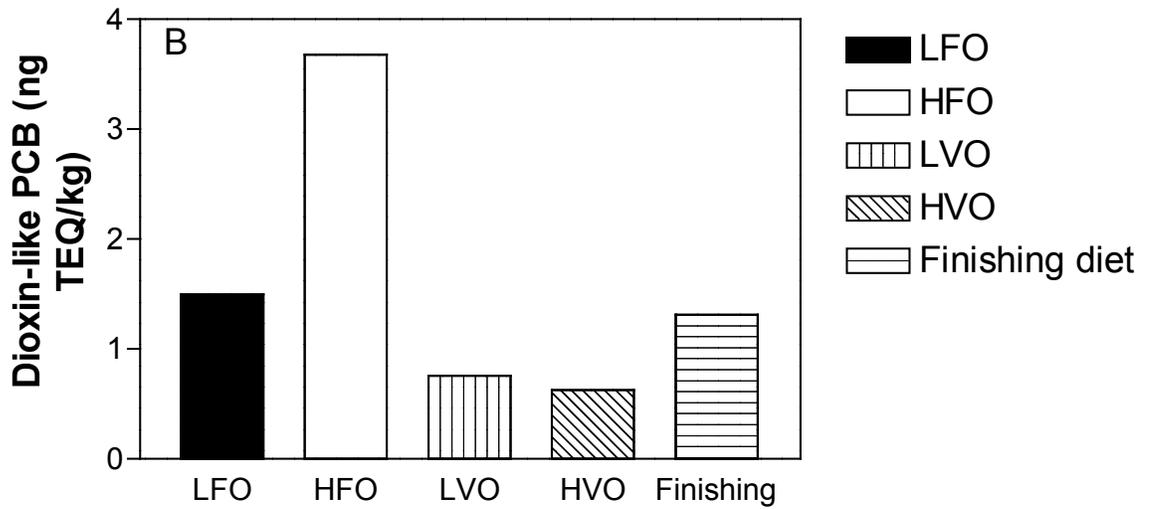
20 ¹Diets formulated and prepared by BioMar A/S, Brande, Denmark. ²Diets formulated
 21 and prepared by Nutreco ARC, Stavanger, Norway. ³Wheat flour, wheat and maize
 22 glutens. ⁴Vitamin and mineral premixes prepared according to individual company

1 specifications. Finnstim and Carophyll pink® to provide 60/mg astaxanthin. ⁵Herring
2 oil for 6 mm pellet and capelin oil for 9 mm pellet.

3 Fig.1.



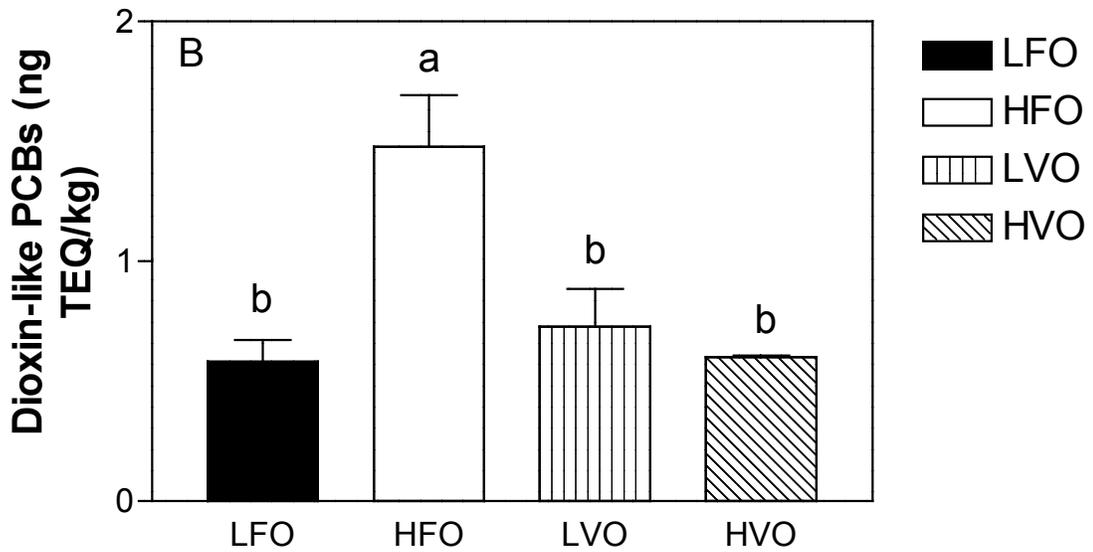
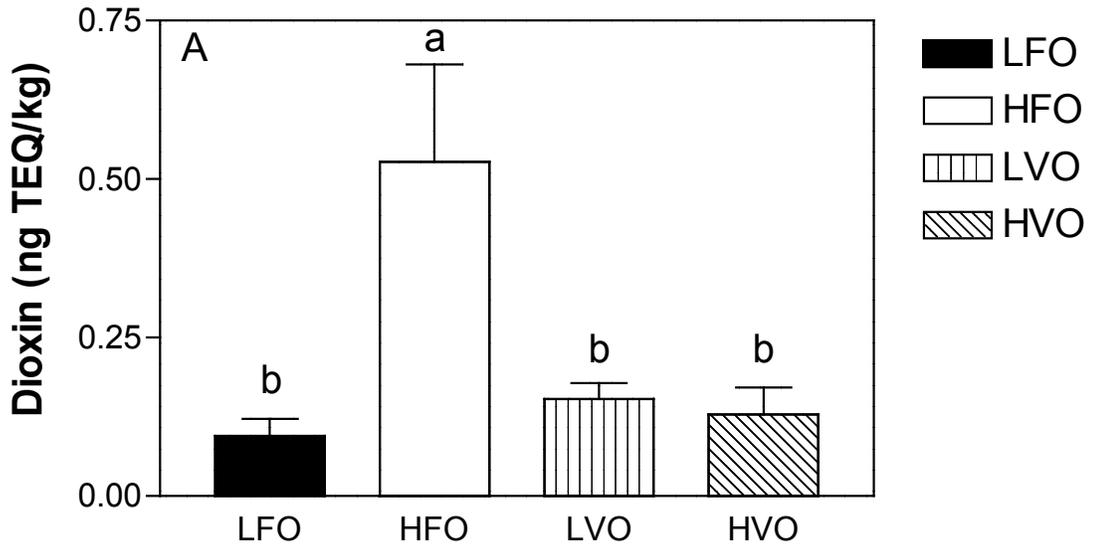
4



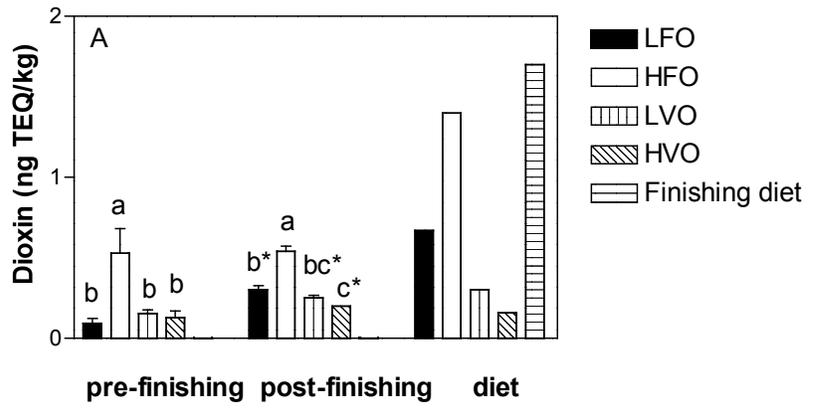
5

6

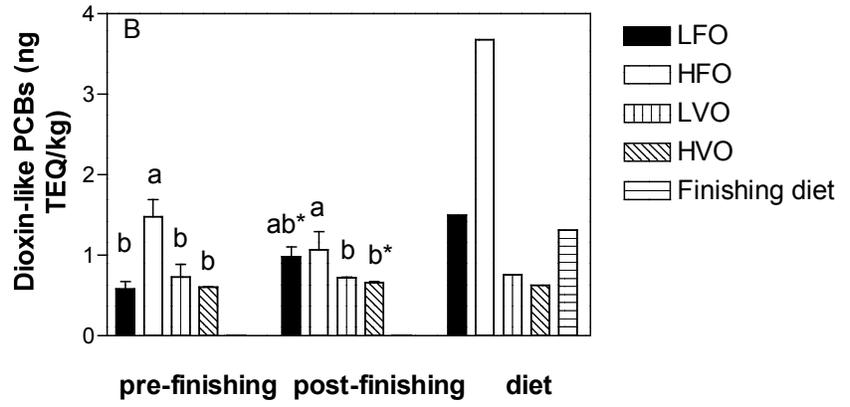
1 Fig. 2



1 Fig. 3



2



3

4

5

6

7

8

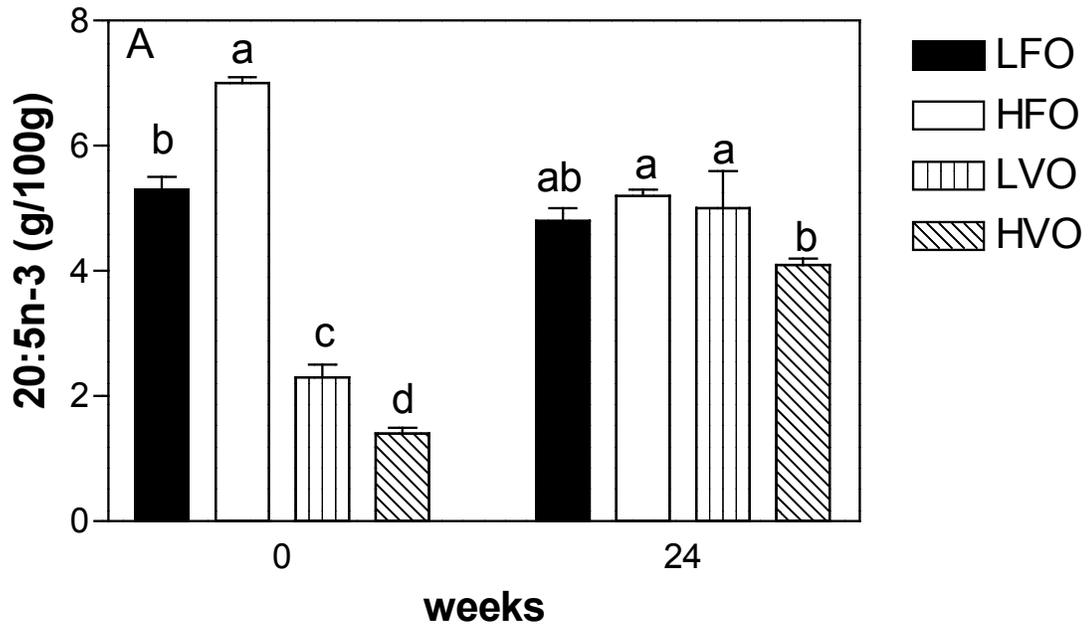
9

10

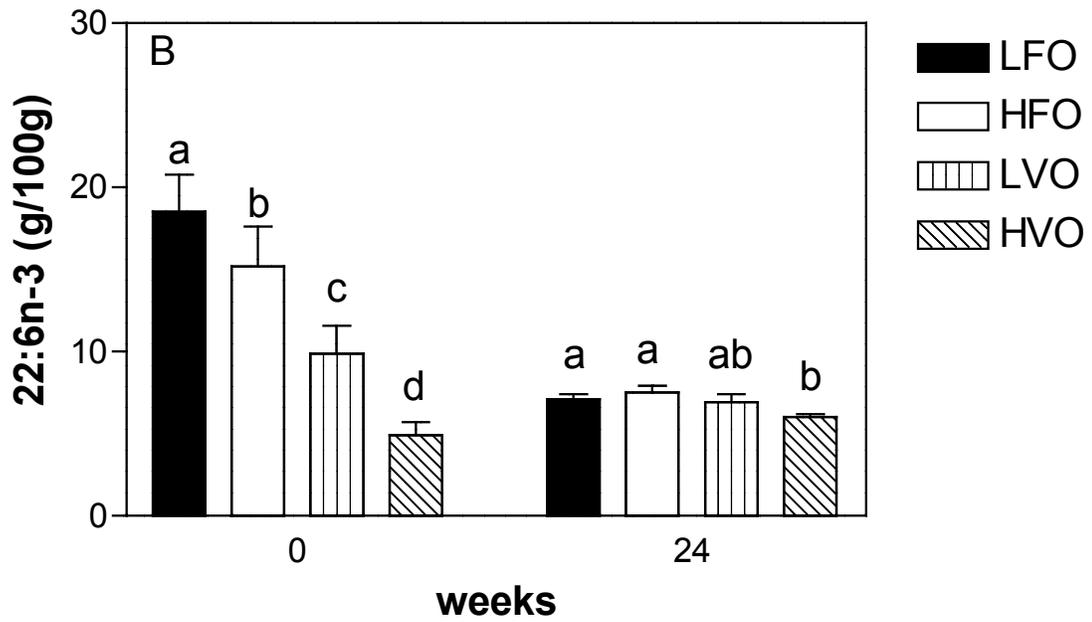
11

12

1 Fig. 4.



2



3

4