

# Feed and Sustainability Trial (FAST) Final report

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## Executive Summary

This study looked at the effects of partial replacement of dietary fish oil with vegetable oil in Atlantic salmon feeds on growth, flesh quality, sensory parameters, flesh fatty acid composition and pollutant levels. Three diets were trialled in triplicate seawater pens at a commercial salmon farming site in Shetland, Scotland over a 154 day trial period. Two new feeds were formulated for the trial; a vegetable oil based feed (VO), 58% vegetable oil and 42% fish oil, with a similar cost of production to the control feed. The second, a northern hemisphere based fish oil feed (FO) which contained higher quality ingredients and had a higher cost of production compared to the control feed. An existing feed currently used on site (CT) was trialled against the two new formulations and acted as a control in the experiment.

In this study growth performance and feed efficiency was not affected by feed treatment. All feeds performed well in terms of basic quality and flesh quality parameters with only minor differences seen between the three treatment groups. In the taste tests, panellists showed no clear preference for fish fed a particular feed with all samples being well received. Fatty acid profiles of the flesh reflected those of the feeds with fish fed the VO feed showing reductions in concentrations of important n-3 fatty acids and increases in concentrations of n-6 fatty acids. There was a 27% reduction in n-3 fatty acids, eicosapentanoic acid (EPA) and 33% reduction in docosahexanoic acid (DHA) in the VO fish compared with concentrations found in the CT fish. There was a 4.5 times greater concentration of n-6 fatty acid, linoleic acid (LA) found in the VO fish compared with the fish fed the FO feed. All fish tested were found to have levels of persistent organic pollutants well below the EU maximum permitted limit with 50% of the pollutants tested showing lower levels of pollutants in the flesh of the fish fed the VO feed compared with fish fed the two fish oil based feeds. The FO feed was formulated to be of superior quality to the other feeds however it showed very little difference in performance compared with the control feed therefore it would not be cost effective to implement this feed on site. Vegetable oil however proved it could be a cost effective substitute for fish oil in farmed Atlantic salmon feeds in Shetland waters.

## 1. Introduction

Since 1970 the aquaculture industry has grown more rapidly than any other animal food producing sector with an estimated average annual growth rate of 8.8% (FAO 2007). The fishmeal and fish oil industry is of global importance to the fish farming industry as it supplies the main components used in aquaculture feeds. With the continued growth in the aquaculture industry and the competing demands for fishmeal and fish oil from other industries there are doubts regarding the future supplies of these resources used to feed cultured species such as salmon (FAO 2007). Feed costs account for 35-60% of the total operational costs of an aquaculture company and continues to dominate aquaculture needs. Over exploitation of wild fish stocks used to produce fishmeal and fish oil and the influence of the El Nino phenomenon in the South East Pacific, which sees landings of anchovies, the main fishery used in the production of these ingredients, dramatically decline, has caused uncertain supply and variable cost of these resources. There are now calls for more cautious and closely controlled development and management of the world's oceans to allow for recovery of fish stocks (FAO 2007). The aquaculture industry has the potential to meet growing global demand for nutritious food fish, as global consumption of fish produced through aquaculture has increased from 9% in 1980 to 43% in 2005 (FAO 2007). Since 1985 global production of fishmeal and fish oil has remained relatively stagnant at 6-7 million tonnes and 1 million tonnes respectively (FAO 2007). Under a situation of apparently limited supply of fishmeal and fish oil, assuming little or no improvement in efficiency of use, the expansion of some types of aquaculture may be constrained if not stopped completely and prices of these resources will continue to rise (FAO 2007). Fishmeal and fish oil are still relatively available and their use will continue until availability becomes seriously constrained. Rising prices of these marine resources is driving research in the feed industry towards finding suitable substitutes (FAO 2007).

Vegetable oils show potential as cost effective substitutes for fish oils with Norway and Chile already successfully implementing vegetable oils in aquaculture feeds. The global production of vegetable oils and fats in 2006 was 115 million tonnes, approximately 100 times greater than the production of fish oils (USDA 2006). Alternative oils for use in aquaculture feeds of salmonids have been obtained from a variety of plant sources including soybean, (Hardy *et al.*, 1987; Thomassen & Rosjo 1989; Grisdale-Helland *et al.*, 2002; Rora *et al.*, 2005; Pratoomyot *et al.*, 2008) rapeseed, (Bell *et al.*, 2001(1) & 2003; Regost *et al.*, 2004; Torstensen *et al.*, 2004 & 2005) linseed (Greene & Selivonchick 1990; Menoyo *et al.*, 2005, 2006 & 2007; Drew *et al.*, 2007) and palm oil (Bell *et al.*, 2001(2); Caballero *et al.*, 2002; Nanton *et al.*, 2007; Oo *et al.*, 2007). Most studies carried out have shown that replacing or

partially substituting fish oil with vegetable oil has no effect on growth or feed efficiency but it does have a marked effect on lipid composition of the flesh. Replacing fish oil with vegetable oil has been shown to increase levels of n-6 fatty acids and generally lower levels of n-3 fatty acids in tissues of Atlantic salmon and rainbow trout (Grisdale-Helland *et al.*, 2002).

Fish consumption is recommended because of the beneficial effects of the long chain n-3 fatty acids, namely eicosapentaenoic acid (EPA 20:5n-3) and docosahexaenoic acid (DHA 22:6n-3), in a range of human disorders including cardiovascular and inflammatory conditions (Sargent *et al.*, 2001). Fish oil has a huge advantage in being an unrivalled source in supplying these important n-3 fatty acids in their purest form as both salmonids and humans have limited capacity for elongation and desaturation of 18:3n-3,  $\alpha$ -linolenic acid (ALA), into EPA and DHA (Miller *et al.*, 2007). Feeds high in vegetable oils may reduce the health benefits associated with eating salmon fed a fish oil based feed as long chain n-3 fatty acids which are characteristic of marine oils are absent in vegetable oils. Producers and consumers of salmon will want to minimize any perceived reduction in quality arising from the inclusion of vegetable oils in feeds, both in terms of growth and health of the fish, and in the healthy image of salmon as part of the human diet.

While marine oils may be superior in their fatty acid composition they also contain a variety of persistent organic pollutants (POPs) including dioxins and polychlorinated biphenyls (PCBs) (Drew *et al.*, 2007). With farmed fish, potential exposure hazards of POPs are associated with leaching of agricultural and industrial chemicals into surface waters which are taken up by the wild fish which are subsequently used to produce fishmeal and fish oil for aquafeeds. Long term exposure, at high levels to these toxic compounds can interfere with fundamental biological systems of humans and have been linked to learning difficulties, general malformations and immunotoxic effects (FIN 2007). While POP levels in farmed salmon fall well below the maximum permitted EU and UK limits, with increasing consumption of farmed fish worldwide, further reductions in POP would be desirable. It has been reported that vegetable oils contain lower levels of POPs than fish oils so utilizing vegetable oils for use in aquafeeds could lead to reduced exposure for consumers (Bell *et al.*, 2005; Oo *et al.*, 2007).

In the present study, three feeds were trialled. Two were new formulations for use on commercial marine salmon farming sites in Shetland; the third was a feed currently used on site and acted as a control. One of the experimental feeds had partial substitution of fish oil for vegetable oil while the other contained higher quality ingredients. The effects of the new feeds on growth, flesh quality, fatty acid composition and levels of persistent organic pollutants found in the flesh of the farmed Atlantic salmon (*Salmo salar*) were examined.

## 2. Materials and methods

### 2.1 Fish and facilities

Atlantic salmon smolts (*Salmo salar*) were transferred into seawater pens at Hjaltland Seafarms Ltd., Setterness Bomlo site, located on the East coast of Shetland, Scotland (60° 25,418N, 001° 07,664W). The site comprised of 20 steel cages (20x20x20m) and had a consented production capacity of 3,500 tonnes. Two strains of smolts were supplied by The Lakeland Group from Mowi eggs stripped on 23<sup>rd</sup> and 24<sup>th</sup> December 2004 from 10kg, 2 sea winter broodstocks at Coutts Mill, Unst, Shetland. The third strain was supplied by Landcatch Ltd. from eggs produced in Ormsary, Argyll. The first strain of Lakeland smolts were transferred into allocated cages on the 7<sup>th</sup> April 2006 at an average weight of 85g per fish with the other Lakeland and Landcatch strain entering allocated cages on the 25<sup>th</sup> April 2006 with average weights per fish of 75g and 69g respectively (Figure 1). The Lakeland smolts were given the Birnagen Forte vaccine whereas the Landcatch smolts were given the Furogen2/ FNM+ vaccine. Nine trial cages were allocated to the experiment, with fish stocked in triplicates within the group of 20 steel cages, with each triplicate being allocated a treatment. The smolt strains were allocated one cage from each triplicate group.

Water temperature and oxygen concentration were recorded daily using the Oxymeter YSI incorporated 550A and clarity of the water in metres was assessed using a Secchi disk. The temperature over the trial period ranged from 7 to 11.3°C, generally increasing as the trial progressed. The number of mortalities in each trial cage were recorded every few days with the cause of death also noted, these included failed smolts, physical damage, predator damage, sea lice and other diseases, or unknown causes. The net feed intake was recorded daily for each trial cage.

Landcatch 69g	
	Lakeland 75g
Lakeland 85g	
Lakeland 85g	
Landcatch 69g	Lakeland 75g
Lakeland 85g	Lakeland 75g
Landcatch 69g	



Figure 1. Layout of the trial cages within the cage block at the Bomlo site, indicating which feed type was used and the origin and average start weight of the smolts in each trial cage.

2.2 Trial feeds

Two new feeds were formulated for the trial, a vegetable oil based feed (58% vegetable oil and 42% fish oil) which was designed to provide greater digestibility at lower temperatures (designated VO) and had a similar cost of production to the control feed. The second feed, a northern hemisphere based fish oil feed (designated FO) which contained higher quality ingredients designed to support optimal growth and lower the feed conversion ratio (FCR), which had a higher cost of

production compared to the other two feeds. Feeds were made under commercial conditions (20 ton batches) at Biomar's Grangemouth factory, Scotland. The two new formulations were trialled against the existing feed, a Biomar standard production on-growing feed (designated CT) currently used at Hjaltland Seafarms salmon farming sites.

The feeds were introduced on the 1<sup>st</sup> February 2007, once the salmon had reached an average weight of 1300g. The feed trial lasted 154 days ending on the 5<sup>th</sup> July 2007. The feeds were switched from a 9mm pellet to a 12mm pellet on 24<sup>th</sup> April 2007, day 82 of the trial, when fish in all cages had reached a minimum average weight of 2200g. A sample of the 9mm and 12mm pellets of the three trial feeds were analysed by Biomar for composition and nutritional values (Table 1). The fatty acid compositions of the three experimental feeds are shown in Table 2.

Table 1. Composition and proximate analysis of the experimental feeds for the 9mm and 12mm pellet size.

Raw Materials	Units	CT		FO		VO	
		9mm	12mm	9mm	12mm	9mm	12mm
Fishmeal	%	25	25	30	30	25.0	25.0
Fish oil	%	30.25	33.4	30.3	30.8	12.6	12.6
Vegetable oil	%					17.5	19.3
Wheat	%		6.5	14.7	15.2	6.5	6.5
Vegetable inclusions	%	36.5	24.5	13.9	12.1	28.0	25.2
Soya products	%	9.7	10.6	12.0	13.0	11.0	12.0
Vitamins and Minerals	%	0.6	1.5	0.5	0.4	0.6	0.4
Moisture	%	-2.26	-1.7	-1.40	-1.4	-1.20	-1.00
<u>Nutritional Values, as fed basis</u>							
Oil	%	35.1	34.7	34.1	34.2	33.1	34.10
Protein	%	33.4	35.4	34.3	34.1	33.5	33.70
Moisture	%	6.7	3.5	4.9	4.3	4.7	4.90
Ash	%	3.5	6.7	6.9	6.6	6.5	6.50
Starch	%	21.3	19.70	19.8	20.80	22.2	20.80
DE	MJ/Kg	22.4	22.4	22.0	22.1	21.7	22.0
DP	%	28.7	30.4	29.5	29.3	28.8	29.0
DP/DE	g / MJ	12.8	13.6	13.4	13.3	13.3	13.2

DP, digestible protein, DE, digestible energy

Table 2. Fatty acid composition (g/100g fat) of the three experimental feeds for the 9mm and 12mm pellet size.

	CT		FO		VO	
	9mm	12mm	9mm	12mm	9mm	12mm
14:0	5.9	6.4	5.6	6.7	3.1	3.1
16:0	14.6	15.7	14.6	16	13.8	14
18:0	3.7	4.1	3.8	4.1	4.4	4.9
20:0	0.2	0.2	0.2	0.2	0.3	0.3
22:0	0.1	0.1	0.1	0.1	0.3	0.3
Total Saturated	24.5	26.5	24.3	27.1	21.8	22.7
16:1 n-7	4.8	5.5	4.6	6.1	3	3.1
18:1 (n-9)+(n-7)+(n-5)	12.2	13.7	13.5	15.1	18.3	19.6
20:1 (n-9)+(n-7)	4.8	4.6	5.4	4.8	1	0.8
22:1 (n-11)+(n-9)+(n-7)	6.8	6.6	7	7.1	0.1	0.5
24:1 n-9	0.5	0.5	0.5	0.5	0.2	0.1
Total Monounsaturated	29.2	30.9	31	33.6	22.7	24.2
18:2 n-6 (Linoleic acid)	4.7	4	4.3	4.4	28.4	29.6
18:3 n-6	0.1	0.2	0.1	0.2	0.1	0.1
20:2 n-6	0.2	0.2	0.2	0.2	0.1	<0.1
20:3 n-6	0.1	0.1	0.1	0.1	<0.1	0.1
20:4 n-6	0.6	0.7	0.6	0.7	0.9	0.5
22:4 n-6	<0.1	0.1	<0.1	0.1	<0.1	<0.1
Total n-6	5.7	5.3	5.4	5.7	29.5	30.2
18:3 n-3	0.9	0.8	1	0.9	3.1	3.3
18:4 n-3	2.4	2.4	2.5	2.3	1	0.9
20:3 n-3	0.1	0.1	0.1	0.1	0.3	<0.1
20:4 n-3	0.7	0.3	0.9	0.3	0.3	0.3
20:5 n-3 (EPA)	9.3	12.2	8.7	13	6.7	7.7
21:5 n-3	0.4	0.5	0.4	0.6	0.2	0.3
22:5 n-3	1.3	1.6	1.6	1.6	0.7	0.9
22:6 n-3 (DHA)	9.9	8.1	10.5	8.5	4.2	3.3
Total n-3	25	26.1	25.7	27.2	16.5	16.7
16:2 n-4	0.5	0.8	0.5	0.8	0.4	0.5
16:3 n-4	0.5	1	0.4	1	0.5	0.7
16:4 n-1	0.9	0.3	0.7	0.3	1	0.2
Total Polyunsaturated	32.6	33.4	32.7	35.2	47.9	48.4
n3:n6	4.39	4.92	4.76	4.77	0.56	0.55

### 2.3 Feeding

All cages were fed automatically using a GMT pneumatic air blown centralised feed delivery system consisting of four hoppers feeding into two separate delivery systems. Each delivery system delivered feed to ten cages (10 south cages and 10 north cages, Figure 1). The software system which controlled the feeding system was the Pneumo Autofeeder 3 V1.22. Feeds were delivered daily from 6.30-9.00 and 12.20-15.00 with the fish receiving 60% of their daily ration in the morning and 40% in the afternoon. Following routine site practice, the site manager used surface

response to determine the feeding rates of the fish in each cage and fed the fish to appetite.

#### *2.4 Growth performance*

Assessments of fish growth were made throughout the trial period with three methods used to measure fish weight, these were hand weighing, Aqua-DRUM acoustic technology and the Vaki Biomass Counter. Three hand weighs were undertaken in March, May and July 2007. At the first hand weigh 100 fish were collected with a sweep net and removed individually using a hand net, anaesthetised with MS222 (Tricaine Methane Sulphonate) and weighed using POLS marine scales, P-15/S-182 model and finally returned to the cage. Due to reports from the site manager of high numbers of mortalities in cages after the first hand weigh the decision was made to slaughter the fish at subsequent hand weighs. The fish were netted, killed by a blow to the head and weighed, followed by cutting of the gill arches to bleed.

Aqua-DRUMS produced by Guigne Aquaculture Products Ltd. used a non invasive method of sonar acoustic technology to accurately provide fish size data. The system consists of an underwater sensor coupled with a topsides electronic module. The sensor is mounted outside the cage and can record the size of hundreds of individual fish in a matter of minutes. The Aqua-DRUM was used twice in this experiment, in January and June 2007.

The Vaki Submersible Biomass Counter produced by Vaki Aquaculture Systems Ltd. gathered data on average weight, size distribution and condition factor of farmed fish providing. The Biomass counter consists of a scanner frame and a display unit. The frame is submerged in the cage and as the fish swim through the frame they interrupt a net of infrared light beams which capture a silhouette image of the fish which is used to calculate the size of the fish. The frame is left submerged for between 12-24 hours and can measure up to 5000 fish at any one time (Vaki Brochure). As the fish are measured, data on average weight, fish number and condition factor appear on the display unit, which is mounted outside the cage. The data can then be downloaded from the display unit onto a computer and can be investigated further using the Biomest software programme. The Vaki counters were suspended in the cages at a depth of three metres, nine metres out from the side of the net, in a North-South direction, and one metre out from the side netting, in an East-West direction. The Vaki frame was always placed in the corner nearest the feed delivery pipe to maximise the number of fish swimming through the frame. The frames were moved between trial cages over the trial period to give on average eight Vaki readings per cage during the experiment. The Vaki data gained during the trial was investigated and found to have large periods of missing data and unusual results compared with the hand and Aqua-DRUM weights, therefore the data was deemed to be unreliable and a decision was made to omit all Vaki data from assessments of fish growth.

The population growth was represented using the hand weights and Aqua-DRUM measurements of average fish weight with Specific Growth Rates (SGR, % live weight gain day<sup>-1</sup>) and Thermal Growth Coefficient (TGC, Cho 1992) being calculated for each treatment group. As there was no facility for feed waste quantification an economic and not a biological feed conversion ratio (FCR, g feed fed/g live weight gain) was determined for each treatment group.

- Specific Growth Rate:

$$\text{SGR} = ((\ln W_b - \ln W_a) / (T_b - T_a)) * 100$$

where  $W_a$  and  $W_b$  are the weights(g) at the start and end of the trial at times  $T_a$  and  $T_b$ (days).

- Thermal Growth Coefficient (Cho, 1992):

$$\text{TGC} = ((W_b^{1/3} - W_a^{1/3}) / (\sum T)^{-1}) * 1000$$

where  $W_a$  and  $W_b$  are the weights(g) at the start and end of the trial and  $\sum T$  is the sum of day degrees (°C).

- Economic Feed Conversion Ratio:

$$\text{FCR} = (\text{kg feed fed} / B_b - B_a)$$

where  $B_b$  is the final biomass and  $B_a$  is the initial biomass in kg.

### *2.5 Sample collection and assessment of fish composition and quality*

Samples for assessment of flesh quality attributes were collected at four separate dates throughout the trial period, with an initial random sample of twenty-four fish (n=8 per treatment) being taken on day 0 of the trial on 1st February 2007. Thereafter ten fish per pen (n=30 per treatment), representative of the mean fish weight in each pen were removed for assessment at sample dates in May, June and July 2007. Fish were selected for assessment if they were within  $\pm 20\%$  of the mean weight of the population expected for that particular day of the trial, calculated using weight data already gathered for the trial and plotted on a linear regression. Fish were removed from the cage using a hand net and anaesthetised before being weighed to ensure the fish were of the correct sample weight, then killed by a blow to the head and bled by cutting the gill arches. All fish were tagged for identification purposes, taken ashore and immediately transported in bins of ice slurry back to the laboratory for composition and quality assessment.

Assessments of colour, texture, fillet fat content and flesh fatty acid composition were carried out on the Scottish Quality Cut (SQC) fillet portion of the salmon. The SQC is the fillet area directly below the dorsal fin and is used as it is a recognised standard sampling point which is considered to be the most consistent indicator of whole fillet fat content (Einen *et al.*, 1998).

#### 2.5.1. Basic quality analysis

Round weight (whole fish weight after bleeding), fork length, gutted weight, condition factor (on a round and gutted weight basis), carcass yield, hepatosomatic index (HSI), gonadosomatic index (GSI), and caecal fat were assessed as described by Morris *et al.*, (2003). Caecal fat scoring, a subjective measure of visceral fat deposition, estimates the amount of fat around the pyloric caecae and between individual caeca. Caecal fat was scored using an index ranging from 1-4:

No fat = 1

Fat less than the width of a caeca = 2

Fat equal to the width of the caeca = 3

Fat obscuring the caecae = 4

#### 2.5.2. Colour analysis

Colour was assessed on the SQC's taken from the right side of the fish (n=30 per treatment) on the fillet surface directly below the dorsal fin and above the lateral line using a Roche SalmoFan<sup>TM</sup> Lineal, scale 20-34. Colour scoring was conducted under simulated daylight conditions using a Colour Assessment Cabinet 60-5 (VeriVide, UK) illuminated with the D65 fluorescent discharge light. The SQC fillet portions were then individually sealed in plastic bags and frozen at -18°C prior to analysis for flesh fat content.

#### 2.5.3. Texture measurements

SQC's were taken from the left side of the fish, to determine flesh texture in the June and July samples only (n=30 per treatment). Each SQC was individually sealed in a plastic bag and stored on ice for 24 hours prior to analysis. Maximum force ( $F_{max}$  newtons (N)) was measured at four evenly spaced points, horizontally across the fillet surface just below the dorsal fin and above the lateral line, using the TA-HDi Texture Analyser (Stable Micro Systems Ltd., Surrey, England). The analyser was calibrated with a 10kg load cell and  $F_{max}$  was recorded at a penetration height of 20% using a 12.5mm diameter flat ended probe. The mean maximum force was calculated by taking the mean of the four compression tests for each fillet.

#### 2.6 Sensory analysis

Consumer sensory triangle taste tests were carried out on nine fish (n=3 per treatment) by an untrained panel of twenty four, mixed sex and age, at the Food Innovation Institute (FII), Edinburgh, Scotland. The salmon sides were prepared by cutting into steaks, foiled wrapped and boiled for 13 minutes to ensure a core

temperature of 75°C. The experimental design used was the forced choice triangle method where panellists are presented with batches of three samples, two matching and one different. They each had to choose the 'odd one out' and rank their confidence of detecting the "odd one out" on a linear scale from 0-10 with 0 being 'not at all confident' and 10 being 'extremely confident'. Three different pairings were compared: CT and VO, CT and FO and FO and VO. Samples were given random three digit code numbers and were presented in a balanced order with all six possible orders being used an equal number of times. Panellists were also asked to select their preferred sample in each taste test and asked to give brief comments on the samples tested. Panellists had the option to choose 'no preference' meaning they did not favour either sample. At the end of the session the numbers of correctly identified cases were calculated. It was then determined whether the results were statistically significant at a given probability level, for a given number of panellists. The data gathered was analysed by FII using FIZZ and Microsoft Excel computer packages.

## 2.7 Chemical analysis

### 2.7.1. Fat Content

Fillet fat content was assessed in the SQC fillet portions, taken from the right side of the fish collected in February (n=8 per treatment), May, June and July (n=15 per treatment). Following storage at -18 °C in sealed labelled plastic bags, the SQC's were defrosted at room temperature, individually homogenized in a domestic food processor and prepared for Soxhlet analysis. Fat content was then determined using the Soxtec System HT 1043 extraction unit (Tecator AB, Sweden). The method used can be found attached as Appendix 1.

### 2.7.2. Fatty acid analysis

Fatty acid profiles were assessed on fish sampled at the start (n=4 per treatment) and end of the trial (n=15 per treatment) by the Nutrition Analytical Services, Institute of Aquaculture, University of Stirling, Scotland. The lipid extraction and fatty acid analysis procedure were performed as described by Bell et al. (2003).

### 2.7.3. Analysis of Persistent Organic Pollutants (POPs)

Tests for trace elements, Polycyclic aromatic hydrocarbons (PAHs), dioxins, non *ortho* and *ortho*-Polychlorinated biphenyls (PCBs) and Polybrominated biphenyl ethers (PBDE) were carried out by the Central Scientific Laboratory, York, England on nine fish (n=3 per treatment), taken from the trial cages at the end of the experiment.

For the trace elements each test sample was digested in nitric acid using quartz high pressure closed vessels and microwave heating prior to quantification by inductively coupled plasma mass spectrometry (ICP-MS).

The PAH samples were fortified with appropriate  $^{13}\text{C}$  internal standards and subjected to saponification followed by liquid-liquid extraction. Clean-up was by DMF/cyclohexane partition followed by elution through a silica gel column. Analysis was by HRGC-LRMS. The results were expressed on a whole weight basis.

The remaining dioxin, PCB and PBDE samples were homogenised, freeze dried and fortified with known amounts of surrogate ( $^{13}\text{C}_{12}$ -labelled) analogues of target analytes and exhaustively extracted using mixed organic solvents. The extract was cleaned up using adsorption chromatography. *Ortho*-PCBs, non *ortho*-PCBs, Polychlorinated dibenzodioxins (PCDDs)/Polychlorinated dibenzofurans (PCDFs) and PBDE were segregated into two separate fractions. Each fraction was concentrated and further cleaned up before inclusion of additional surrogate standards. Final determination was by high resolution gas chromatography with either low resolution mass spectrometric detection (*ortho*-PCBs) or high resolution mass spectrometric detection (non *ortho*-PCBs, PCDDs/PCDFs and PBDEs). The results were expressed on a whole and fat weight basis, as TEQ upper and lower bound concentrations, where appropriate and as summed TEQ values.

## 2.8 Statistical analysis

Differences between treatments in growth, feed efficiency, quality, texture, fatty acid composition and pollutant concentrations were analysed using analysis of variance (ANOVA), with differences between treatments identified using Tukey's HSD post hoc test. Prior to running ANOVAs, data involving percent terms were arcsine transformed and homogeneity of variance was assured using Levene's test. The non-parametric Kruskal Wallis test was used to test parameters when variances were shown to be non parametric through the Levene's test.

Fillet fat content was analysed using general linear model factorial analysis of covariance (GLM factorial ANCOVA) with gutted weight as a covariate, to identify whether gutted weight or treatment was having a significant effect on fat content. To permit post hoc comparisons gutted weight corrected residuals were calculated and analysed using a GLM factorial ANOVA and a post hoc Tukey HSD test.

Caecal fat scores and Roche colour scores were analysed using the non parametric Kruskal Wallis test as this data was scored on a scale and therefore medians were calculated rather than means.

As directed sampling had been based on the population growth graph including the Vaki weight data, t-tests were carried out to identify whether the average weights of the directed sampled fish were still a representative sample of the total population at a treatment level with the Vaki data removed from the dataset.

Due to the small sample size ( $n=3$  per treatment) of the pollutant data set no statistical tests were carried out on this data. The data was summarised as simple mean values to enable interpretation by the reader.

All statistical analysis was carried out using SPSS 15.0 (SPSS Inc., USA).

### 3. Results

#### 3.1 Growth and FCR

Over the 154 day trial period the fish significantly increased their weight ( $P < 0.05$ ) from between 1570g and 1633g to between 3166g and 3456g (Figure 2) with cumulative SGRs ranging from 0.45 to 0.51% day<sup>-1</sup> and TGCs ranging from 2.36 to 2.72. The FCRs were variable across the trial, ranging between 0.96 and 3.32. There were no significant differences ( $P > 0.05$ ) found between the treatments for any of the growth or feed efficiency data (Table 3).

Table 3. Growth and feed efficiency of salmon fed the three experimental feeds.

	CT	Feed FO	VO	ANOVA*	
				S.E.M.	P-value
<i>Mean weight(g)</i>					
February 2007	1633	1570	1570	50.87	0.876
March 2007	2267	2147	2167	86.27	0.867
May 2007	3100	3080	2960	115.23	0.896
July 2007	3433	3456	3166	143.18	0.715
<i>Specific Growth Rate (% day<sup>-1</sup>)</i>					
February-March	0.59	0.58	0.58	0.02	0.943
March-May	0.5	0.56	0.49	0.018	0.252
May-July	0.29	0.32	0.18	0.035	0.434
Cumulative	0.48	0.51	0.45	0.016	0.379
<i>Thermal Growth Coefficient</i>					
February-March	3.36	3.2	3.22	0.1416	0.914
March-May	2.66	2.97	2.58	0.0985	0.261
May-July	1.36	1.53	0.86	0.1705	0.279
Cumulative	2.52	2.72	2.36	0.1051	0.437
<i>Feed Conversion Ratio (economic)</i>					
February-March	1.01	1.08	0.96	0.03	0.299
March-May	1.23	1	1.1	0.0549	0.283
May-July	1.91	2.06	3.32	0.3485	0.202
Cumulative	1.26	1.3	1.28	0.024	0.834

n=3 for each feed type.

\*Results from factorial analysis of variance (ANOVA) where S.E.M. is the pooled error of means and P-value is the significance level.

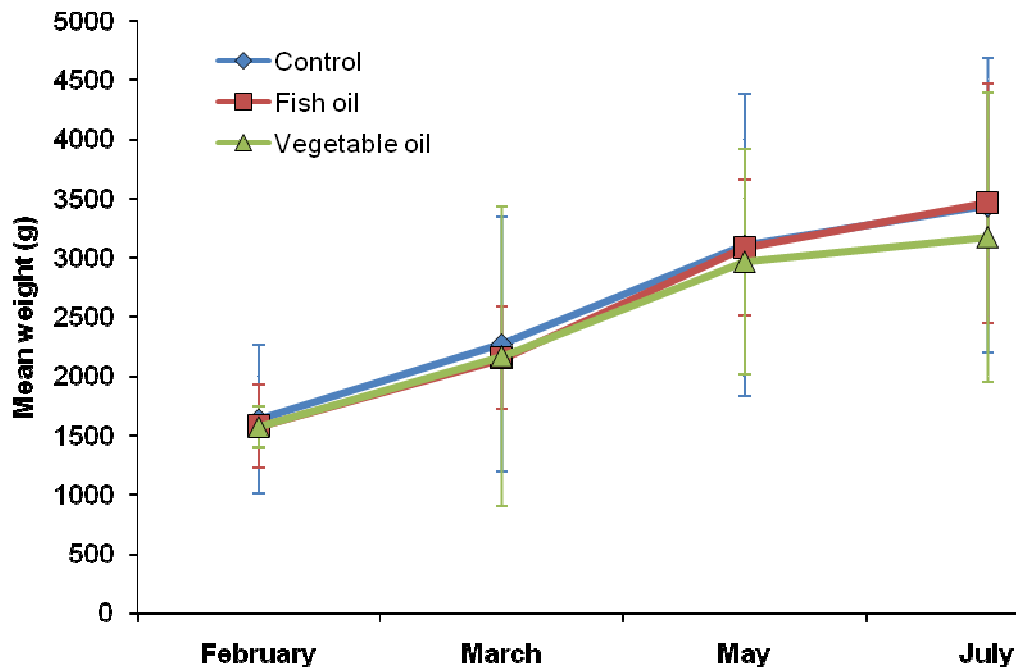


Figure 2. Mean population weights (g) of salmon at different points during the feed trial fed the three experimental feeds. Error bars, 95% confidence intervals.

### 3.2 Fish Quality Analysis

The population growth graph which was used to estimate the weights for the directed sampling contained weights from the Vaki biomass counters, which were then subsequently removed from the data set at the end of the trial as they were found to be unreliable. The directed sampling weights and the population weights from hand weighs carried out around the same time as the directed sampling were compared and found to show no significant differences ( $P > 0.05$ ) between the directed sampled fish weights and population fish weights for the three feeds at sampling points in February, June and July, therefore the directed sampled fish were found to be a representative sample of the population. A comparison for the second directed sampling point in May had no corresponding population growth weight sampling point for comparison as the closest hand weigh was in March, therefore significant differences ( $P < 0.001$  for all treatments) were found between the average weights of the population and the directed sampled fish, which would be expected as the two sampling points were a month apart. It can therefore only be assumed that the sampled fish in May are a representative sample of the population from what can be seen from the data compared.

A number of quality parameters were assessed on the ninety fish removed through directed sampling, at quarterly intervals throughout the trial period, except those taken on 1<sup>st</sup> February where twenty four fish were randomly sampled. The

round weight, gutted weight and fork length of the fish increased significantly ( $P < 0.001$ ) over the whole trial period with significant differences ( $P < 0.05$  for all) observed between the fish fed the three feeds. Round weight and gutted weight showed no significant differences at the first sampling point in February ( $P > 0.05$ ) however significant differences were found at the subsequent sample dates ( $P < 0.05$  on each occasion) (Table 4). In May the CT fish had significantly greater round weights ( $P < 0.05$ ) than the VO fish and at the June and July sampling dates both the CT and FO fish had significantly greater round weights than the VO fish ( $P < 0.01$ ). A similar trend was seen in relation to fork length however no significant difference was seen in February and May, with CT fish and FO fish having significantly greater fork lengths ( $P < 0.01$ ) in comparison with the VO fish at both the June and July sampling dates.

A significant decrease in condition factor on both a gutted weight and round weight basis was observed in the FO and VO fish ( $P < 0.05$ ) over the 154 day trial period however there was no significant differences ( $P > 0.05$ ) over the trial period for the CT fish in relation to condition factor. There was no significant difference between the three feeds for condition factor on a round weight basis at any of the sampling points ( $P > 0.05$ ) (Table 4). Condition factor on a gutted weight basis did however show a significant difference at the first sampling point in February with the FO fish having significantly greater condition factor than the VO fish (Table 4).

Carcass yield was not significantly influenced by feeds fed up until July, the final sampling point, where a significant difference was observed ( $P < 0.05$ ) with the CT and FO fish having significantly greater carcass yields than the VO fish. All three of the feeds encountered their poorest carcass yields in May which were found to be significantly lower ( $P < 0.01$ ) than the July carcass yields for the fish fed the CT and FO feeds and was significantly lower ( $P < 0.01$ ) than the June carcass yields for those fish fed the VO feed.

Hepatosomatic index (HSI) between the three feeds showed no significant differences at the start and end sampling dates ( $P > 0.05$ ) but did show significant differences at the May and June sampling dates ( $P < 0.05$ ). In May the CT fish had significantly greater HSI than the VO fish and in June the FO fish showed significantly greater HSI than the VO fish (Table 4).

Table 4. Changes in basic quality, condition, yield and hepatosomatic index (HSI) of salmon fed the three experimental feeds

	CT	Feed		ANOVA*	
		FO	VO	S.E.M.	P-value
<i>Round weight after bleeding (g)</i>					
1 <sup>st</sup> February (start weight)	1596	1803	1626	60.03	0.326
1st May	2744 <sup>a</sup>	2513 <sup>ab</sup>	2448 <sup>b</sup>	43.1	0.011
4 <sup>th</sup> June	3084 <sup>a</sup>	3035 <sup>a</sup>	2696 <sup>b</sup>	51.35	0.003
9 <sup>th</sup> July	3451 <sup>a</sup>	3462 <sup>a</sup>	3106 <sup>b</sup>	52.08	0.005
<i>Fork length (cm)</i>					
1 <sup>st</sup> February	49.8	50.6	49.6	0.623	0.797
1st May	60.1	58	58.5	0.306	0.053 KW
4 <sup>th</sup> June	62.6 <sup>a</sup>	62.2 <sup>a</sup>	60 <sup>b</sup>	0.316	0.001
9 <sup>th</sup> July	66.1 <sup>a</sup>	65.9 <sup>a</sup>	63.6 <sup>b</sup>	0.359	0.006
<i>Gutted weight (g)</i>					
1 <sup>st</sup> February	1377	1575	1426	54.18	0.312
1st May	2359 <sup>a</sup>	2169 <sup>ab</sup>	2120 <sup>b</sup>	36.13	0.015
4 <sup>th</sup> June	2693 <sup>a</sup>	2645 <sup>a</sup>	2377 <sup>b</sup>	43.14	0.004
9 <sup>th</sup> July	3056 <sup>a</sup>	3054 <sup>a</sup>	2708 <sup>b</sup>	47.1	0.002
<i>Condition factor on round weight basis (%)</i>					
1 <sup>st</sup> February	1.29	1.39	1.32	0.021	0.136
1st May	1.26	1.29	1.22	0.013	0.135
4 <sup>th</sup> June	1.26	1.26	1.24	0.014	0.845
9 <sup>th</sup> July	1.19	1.21	1.2	0.009	0.753
<i>Condition factor on gutted weight basis (%)</i>					
1 <sup>st</sup> February	1.11 <sup>b</sup>	1.21 <sup>a</sup>	1.15 <sup>ab</sup>	0.017	0.042
1st May	1.08	1.11	1.06	0.011	0.148
4 <sup>th</sup> June	1.1	1.1	1.09	0.011	0.998
9 <sup>th</sup> July	1.05	1.06	1.05	0.008	0.626
<i>Carcass yield (%)</i>					
1 <sup>st</sup> February	86.3	87.3	87.7	0.752	0.808
1st May	86.1	86.3	86.6	0.223	0.649
4 <sup>th</sup> June	87.4	87.3	88.3	0.201	0.072
9 <sup>th</sup> July	88.6 <sup>a</sup>	88.2 <sup>a</sup>	87.2 <sup>b</sup>	0.17	0.001
<i>HSI on gutted weight basis (%)</i>					
1 <sup>st</sup> February	1.04	1.01	1.01	0.03	0.921
1st May	1.31 <sup>a</sup>	1.27 <sup>ab</sup>	1.21 <sup>b</sup>	0.016	0.045
4 <sup>th</sup> June	1.18 <sup>ab</sup>	1.28 <sup>a</sup>	1.11 <sup>b</sup>	0.021	0.001
9 <sup>th</sup> July	1.07	1.1	1.1	0.02	0.703

n=90 for each sample date, n=24 for samples taken on the 1<sup>st</sup> February

\*Results from analysis of variance (ANOVA) where S.E.M. is the pooled error of means and P-value is the significance level. Mean values on each row ascribed a common superscript were found to be not significantly different from each other using the Tukey test (P>0.05)

KW indicates that the non-parametric Kruskal Wallis test was carried out on the data when the Levenes test showed the variances were non-homogenous.

Gonadosomatic index (GSI) was variable throughout the trial for males and females with no significant effects of feed on this parameter (P>0.05). GSI varied considerably over the trial with no set trends observed (Table 5).

Table 5. Gonadosomatic index (GSI) for male and female salmon fed the three experimental feeds.

	n	CT	n	Feed		ANOVA*		
				FO	n	VO	S.E.M.	P-value
<i>GSI – Males on gutted weight basis (%)</i>								
1 <sup>st</sup> February	6	0.056	6	0.063	2	0.046	0.007	0.717
1st May	18	0.041	15	0.128	16	0.037	0.062	0.739
4 <sup>th</sup> June	18	0.043	14	0.072	14	0.044	0.022	0.621
9 <sup>th</sup> July	20	0.04	18	0.035	12	0.038	0.004	0.427
<i>GSI – Females on gutted weight basis (%)</i>								
1 <sup>st</sup> February	2	0.117	2	0.144	6	0.112	0.011	0.592
1st May	12	0.089	15	0.083	14	0.098	0.009	0.375
4 <sup>th</sup> June	12	0.094	16	0.1	16	0.1	0.009	0.746
9 <sup>th</sup> July	10	0.085	12	0.102	18	0.105	0.011	0.281

\*Results from analysis of variance (ANOVA) where S.E.M. is the pooled error of means and P-value is the significance level. Mean values on each row ascribed a common superscript were found to be not significantly different from each other using the Tukey test ( $P > 0.05$ )

KW indicates that the non-parametric Kruskal Wallis test was carried out on the data when the Levenes test showed the variances were non-homogenous.

Perceived colour scores showed only a significant difference at the end sampling point in July ( $P < 0.001$ ), where the fish fed the FO feed were showing a significantly darker colour compared with the fish fed the CT and VO feeds. Again the scores varied across the trial with no set trends being shown (Table 6).

Caecal fat scores showed significant differences at the start and end sampling points with the fat scores being significantly greater in the VO fish in February and significantly lower at the July sampling point ( $P < 0.05$ ) compared with the CT and FO fish. The caecal fat scores of the CT and FO fish were quite consistent throughout the trial with the VO fish showing some variation as the trial progressed (Table 6).

Table 6. Roche colour scores and caecal fat scores of salmon fed the three experimental feeds.

	CT	Feed		Kruskal Wallis*		
		FO	VO	$X^2$	df	P-value
<i>Roche colour score</i>						
1 <sup>st</sup> February	24	24	24	0.39	2	0.823
1st May	26	26	26	1.304	2	0.521
4 <sup>th</sup> June	25.5	25.5	25	2.392	2	0.302
9 <sup>th</sup> July	24.5	26	24	17.893	2	<0.001
<i>Caecal fat score</i>						
1 <sup>st</sup> February	2	3	3.25	6.068	2	0.048
1 <sup>st</sup> May	3	2.75	3	2.116	2	0.347
4 <sup>th</sup> June	3	3	3	0.348	2	0.84
9 <sup>th</sup> July	3	3	2	6.702	2	0.035

n=90 for each sample date, n=24 for samples taken on the 1<sup>st</sup> February.

\*Results from the Kruskal Wallis where  $X^2$ =Chi-Square, df=degrees of freedom and P-value is the significance level.

Texture measured as maximum force (N) was only carried out on samples from the June and July sampling dates with no significant differences found between

the June samples but highly significant difference ( $P<0.01$ ) found between samples taken in July with significantly greater force being required to penetrate the flesh of the FO samples compared with the VO samples (Table 7).

Table 7. Instrumentally measured texture of flesh of salmon fed the three experimental feeds

	Feed			ANOVA*	
	CT	FO	VO	S.E.M.	<i>P</i> -value
<i>SQC texture (N)</i>					
4 <sup>th</sup> June	1.72	1.67	1.66	0.053	0.872
9 <sup>th</sup> July	1.59 <sup>ab</sup>	1.78 <sup>a</sup>	1.35 <sup>b</sup>	0.049	0.001

n=90 for each sample point

\*Results from analysis of variance (ANOVA) where S.E.M. is the pooled error of means and *P*-value is the significance level. Mean values on each row ascribed a common superscript were found to be not significantly different from each other using the Tukey test ( $P>0.05$ )

### 3.3 Sensory analysis

In test pair 1, comparing the CT fed fish and the FO fed fish, the majority of the panellists (79%) could not tell the difference between the two samples. Confidence of the panellists in detecting the 'odd one out' was scored on average, 6 (scale 0-10). In test pair 2, comparing the CT samples and VO samples the opposite was true with 79% of panellists able to successfully differentiate between the two samples, with the confidence score averaging 6.4. Finally in test pair 3, comparing the FO fed fish and the VO fed fish, again a much lower percentage (38%) of the panellists could distinguish between the two samples, however the degree of confidence in identifying the different sample in this test was the highest of the three tests at 7.7. A statistically significant difference was only found in test 2 ( $P<0.01$ ), between the CT fish and VO fish, indicating that the samples were perceived as very different. In comparison, analysis indicated that the CT versus the FO samples and the FO versus the VO samples were perceived to be similar (Table 8).

Consumers were also asked to select their preferred sample in the triangle tests. Test 1, CT versus FO fish showed the majority, 71% of the consumers, favouring the FO fed fish. In the second pairing of CT versus VO samples, the VO fed fish were preferred over the CT fed fish but with less of a margin at 58% to 42%. In pairing 3, comparing FO and VO samples, 50% preferred the FO fish, 46% favoured the VO fish and 4% showed no preference. The most preferred sample over the three taste tests was calculated using a combined total from the three tests which found the fish fed the FO feed to be the most preferred at 40% followed by the fish fed the VO feed, which 35% of consumers favoured and then the fish fed the CT feed which were least preferred with only 24% consumers favouring these samples, 1% of consumers showed no preference for any samples (Table 9).

Table 8. Consumer sensory triangle tests results and degree of confidence

Test pairs	Samples	Correctly identified (% out of 24)	Wrongly identified (% out of 24)	Mean score of confidence when correct	Significant or not significant	Level of significance
Pair 1	CT vs. FO	21	79	6.0	NS	0.9406
Pair 2	CT vs. VO	79	21	6.4	***	0.0001
Pair 3	FO vs. VO	38	62	7.7	NS	0.4055

NS: Indicates that differences were not statistically significant

\*\*\* Indicates that differences were statistically highly significant at  $P < 0.01$

Table 9. Consumer preference taste test

Test pairs	Samples	Preferred CT (%)	Preferred FO (%)	Preferred VO (%)	No preference (%)
Pair 1	CT vs. FO	29	71		0
Pair 2	CT vs. VO	42		58	0
Pair 3	FO vs. VO		50	46	4
Over three tests		24	40	35	1

For Pairs 1-3 n=24, over the three tests n=72

No preference meant the consumer did not like any of the samples

### 3.4 Fillet Fat content

It is well recognized that fat content of salmonids is influenced by fish size therefore gutted weight was included as a covariate in the analysis of covariance test (ANCOVA). The fat content of the SQC fillet portion was not affected by treatment ( $P > 0.05$ ) (Table 10) however we can see there were significant differences in relation to gutted weight at the May and July sample dates ( $P < 0.05$ ). Gutted weight was having more of an effect on fat content at all of the sampling points excluding June where the  $P$ -value of the feed effect was lower than that of the weight effect however this was not significant (Table 10).

After calculating residuals corrected for gutted weight, an ANOVA was performed to investigate the effect of the feeds on fat content. No significant differences were found between the three feeds at any of the sampling points ( $P > 0.05$ ) (Table 10).

Table 10. Fillet fat content analysed using gutted weight as a covariate and gutted weight corrected fillet fat residuals for the three experimental feeds.

	Feed			S.E.M.	P-value	
	CT	FO	VO		GLM univariate <sup>1</sup>	
						Diet
<i>SQC fillet fat content (%)</i>						
1 <sup>st</sup> February 2007	13.4	15	13.5	0.558	0.804	0.053
1 <sup>st</sup> May 2007	13.9	14.6	14.2	0.485	0.207	<0.001
4 <sup>th</sup> June 2007	15	14.6	12.8	0.394	0.129	0.459
9 <sup>th</sup> July 2007	15.2	15.2	15	0.574	0.825	0.017
<i>SQC fat residuals</i>						
1 <sup>st</sup> February 2007	-0.184	0.465	-0.281	0.49363	0.812	ANOVA <sup>2</sup>
1 <sup>st</sup> May 2007	-0.995	0.707	0.288	0.41163	0.216	
4 <sup>th</sup> June 2007	0.689	0.328	-1.017	0.38351	0.161	
9 <sup>th</sup> July 2007	-0.34	-0.096	0.436	0.537	0.974 KW	

n=45 for each sample point, n=24 for 1<sup>st</sup> February samples.

<sup>1</sup> Results from General Linear Model Univariate Analysis of variance using gutted weight as a covariate

<sup>2</sup> Results from factorial analysis of variance (ANOVA) where S.E.M. is the pooled error of means and P-value is the significance level.

KW indicates that the non-parametric Kruskal Wallis test was carried out on the data when the Levene's test showed the variances were non-homogenous

### 3.5 Fatty acid composition

From Table 11 we can see that the majority of the fatty acids tested showed significant differences in concentrations between fish fed the three experimental feeds over the 154 day trial period. Looking at the main fatty acid groups we can see the VO fish have significantly less saturated fatty acids (SFA) than the CT and FO fish ( $P < 0.001$ ). Monounsaturated fatty acids (MUFA) showed very highly significant differences between all three feeds ( $P < 0.001$ ) with total monounsaturated fatty acids for the FO fish, 36.62, CT fish, 35.73 and the VO fish showing the lowest concentration, 29.7. In the polyunsaturated fatty acid (PUFA) group the VO fish showed significantly higher levels than the FO and CT fish ( $P < 0.001$ ).

Table 11. Fatty acid composition (% of total FA) of flesh of salmon, at the end of the trial period, after being fed the three experimental feeds.

Fatty acid	Feed			ANOVA*	
	CT	FO	VO	S.E.M.	P-value
14:0	5.54	4.3	3.57	0.1415	<0.001KW
15:0	0.38	0.36	0.25	0.0092	<0.001KW
16:0	14.75 <sup>a</sup>	14.93 <sup>a</sup>	13.74 <sup>b</sup>	0.1238	<0.001
18:0	3.07 <sup>b</sup>	3.5 <sup>a</sup>	3.7 <sup>a</sup>	0.0685	<0.001
20:0	0.14 <sup>b</sup>	0.15 <sup>b</sup>	0.19 <sup>a</sup>	0.0042	<0.001
22:0	0.06	0.07	0.13	0.0076	<0.001KW
Total Saturated	23.91 <sup>a</sup>	23.27 <sup>a</sup>	21.63 <sup>b</sup>	0.2156	<0.001
16:1n-9	0.21 <sup>a</sup>	0.21 <sup>a</sup>	0.14 <sup>b</sup>	0.009	0.006
16:1n-7	6.74	6.33	4.76	0.1402	<0.001KW
18:1n-9	13.77	14.16	17.14	0.2443	<0.001KW
18:1n-7	3.11 <sup>b</sup>	3.3 <sup>a</sup>	2.65 <sup>c</sup>	0.044	<0.001
20:1n-11	0.58	0.66	0.28	0.0507	<0.001
20:1n-9	5.04 <sup>a</sup>	5.1 <sup>a</sup>	2.32 <sup>b</sup>	0.2022	<0.001
20:1n-7	0.32 <sup>b</sup>	0.34 <sup>a</sup>	0.23 <sup>c</sup>	0.008	<0.001
22:1n-11	5.48	5.7	1.97	0.2643	<0.001KW
22:1n-9	0.48 <sup>a</sup>	0.51 <sup>a</sup>	0.19 <sup>b</sup>	0.0235	<0.001
24:1n-9	0.66 <sup>a</sup>	0.74 <sup>a</sup>	0.42 <sup>b</sup>	0.0254	<0.001
Total Monounsaturated	35.73 <sup>b</sup>	36.62 <sup>a</sup>	29.7 <sup>c</sup>	0.4835	<0.001
18:2n-6 (Linoleic acid)	5.48	4.83	21.55	1.2017	<0.001KW
18:3n-6	0.13	0.14	0.14	0.0065	0.869
20:2n-6	0.4	0.42	1.19	0.06	<0.001KW
20:3n-6	0.19	0.19	0.19	0.0044	0.935
20:4n-6	0.73	0.76	0.54	0.0162	<0.001KW
22:5n-6	0.25 <sup>a</sup>	0.27 <sup>a</sup>	0.19 <sup>b</sup>	0.0072	<0.001
Total n-6	7.19	6.62	23.59	1.2287	<0.001KW
18:3n-3	0.97	0.99	2.25	0.098	<0.001KW
18:4n-3	1.92	1.82	1.07	0.0611	<0.001KW
20:3n-3	0.13	0.15	0.19	0.0046	<0.001KW
20:4n-3	1.64 <sup>b</sup>	1.75 <sup>a</sup>	1 <sup>c</sup>	0.0518	<0.001
20:5n-3 (EPA)	10.71	10.53	7.81	0.2315	<0.001KW
22:5n-3	3.98 <sup>a</sup>	4.2 <sup>a</sup>	3.09 <sup>b</sup>	0.0849	<0.001
22:6n-3 (DHA)	12.19	12.58	8.22	0.3316	<0.001KW
Total n-3	31.55 <sup>a</sup>	32.03 <sup>a</sup>	23.81 <sup>b</sup>	0.6433	<0.001
16:2	0.25 <sup>b</sup>	0.27 <sup>a</sup>	0.14 <sup>c</sup>	0.009	<0.001
16:3	0.6 <sup>a</sup>	0.52 <sup>b</sup>	0.49 <sup>b</sup>	0.0099	<0.001
16:4	0.77 <sup>a</sup>	0.68 <sup>b</sup>	0.61 <sup>c</sup>	0.0144	<0.001
Total Polyunsaturated	40.36 <sup>b</sup>	40.12 <sup>b</sup>	48.67 <sup>a</sup>	0.6524	<0.001
n-3/n-6	4.39 <sup>b</sup>	4.84 <sup>a</sup>	1.05 <sup>c</sup>	0.2585	<0.001

n=15 per treatment

\*Results from analysis of variance (ANOVA) where S.E.M. is the pooled error of means and *P*-value is the significance level. Mean values on each row ascribed a common superscript were found to be not significantly different from each other using the Tukey test ( $P>0.05$ )

KW indicates that the non-parametric Kruskal Wallis test was carried out on the data when the Levene's test showed the variances were non-homogenous.

Comparing the flesh fatty acid composition of the fish on day 0 of the trial and day 154 we can see there were no significant differences ( $P>0.05$ ) found between

the three treatment groups on day 0 for total n-3 PUFAs, EPA and DHA, total n-6 PUFAs and linoleic acid (Figure 3). On day 154 of the experiment, n-3 PUFAs for the CT fish and the FO fish were significantly higher than those seen in the VO fish ( $P<0.001$ ) (Figure 4). In comparison extremely high concentrations of n-6 PUFAs were seen in the VO fish at the end of the trial with a concentration of 23.59 compared with the FO and CT fish with concentrations of 6.62 and 7.19 respectively. This was mainly due to the huge increase in linoleic acid (LA), which is abundant in many vegetable oils, with the VO fish having a concentration of 21.55 and the CT and FO fish having much lower concentrations at 5.48 and 4.83. A very highly significant difference ( $P<0.001$ ), was seen in the EPA and DHA concentrations of the flesh between the three treatments with the fish fed the CT feed showing the highest concentrations of EPA (10.71) and the FO fish having the highest concentrations of flesh DHA (12.58). Both EPA (7.81) and DHA (8.22) were found in the lowest concentrations in fish fed the VO feed. The n-3/n-6 ratio was clearly affected by the dietary oil source as higher ratios were observed in the fish fed the FO and CT feeds compared with the fish fed the VO feed with very highly significant differences found between all three feeds ( $P<0.001$ ) (Table 11).

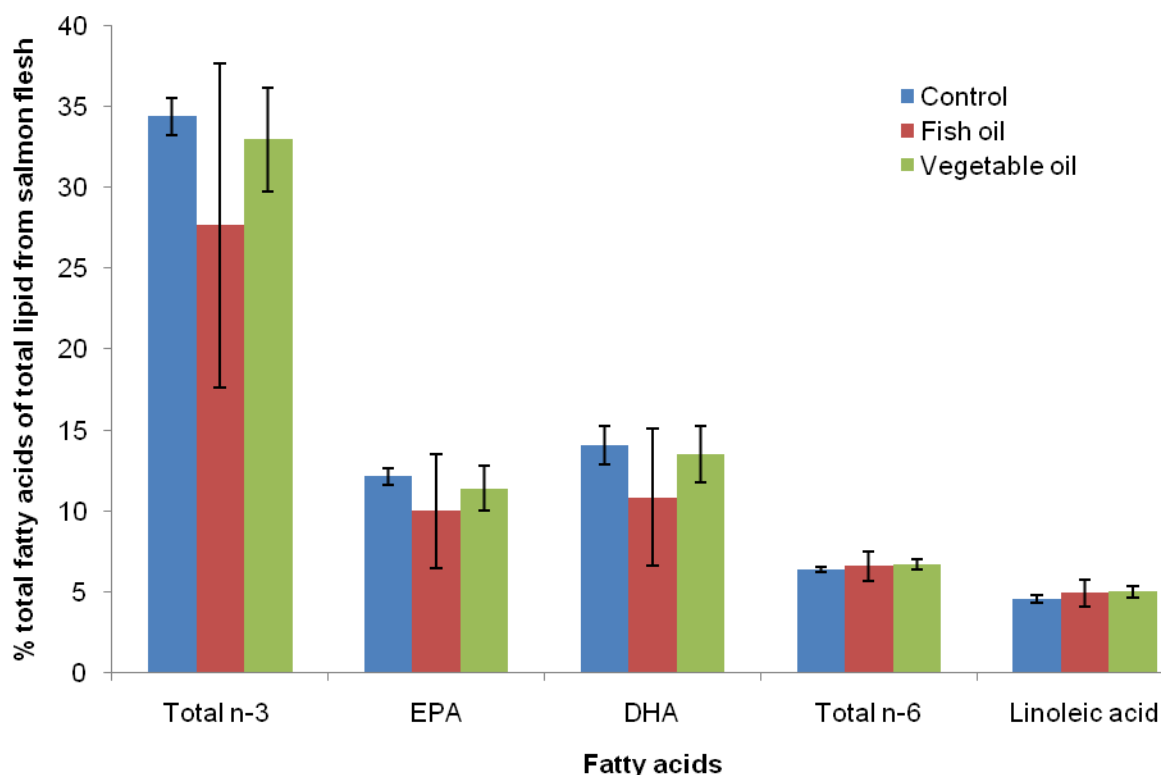


Figure 3. Concentrations (% total fatty acids in total lipid from flesh) of fatty acids in the flesh of salmon at day 0 of the trial, before trial feeds were administered. Error bars, 95% confidence intervals.

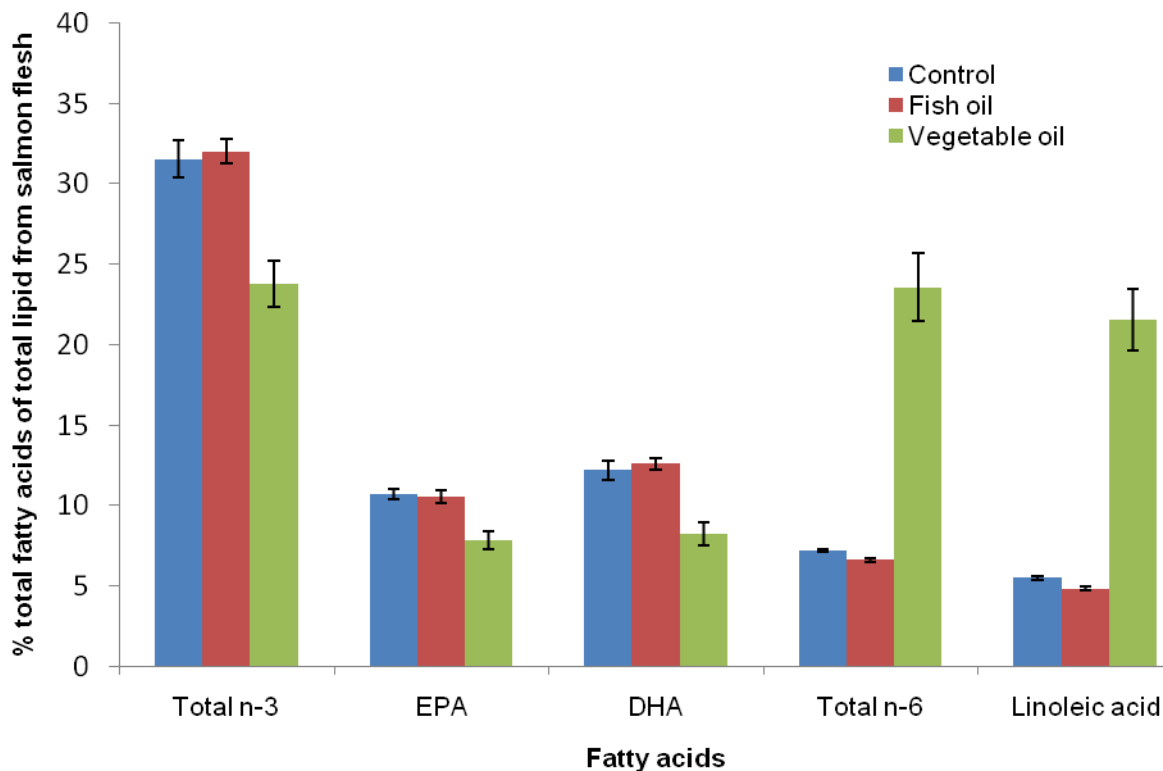


Figure 4. Concentrations (% total fatty acids in total lipid from flesh) of fatty acids in the flesh of salmon fed the three experimental feeds on day 154, the end of the feed trial. Error bars, 95% confidence intervals.

### 3.6 Pollutants

The presence of a total of 88 persistent organic pollutants (POPs) were tested for in flesh of fish fed the three different feeds (Table 12) with 44 of the pollutants tested showing lower concentrations in the fish fed the VO feed.

Of the heavy metals tested, there were no differences in the concentrations of cadmium and lead found in fish fed the three feeds. Concentrations of arsenic and mercury found in the flesh of the salmon were lowest in the VO fish with concentrations of copper in the fish showing very little difference with the lowest concentration found in the FO fish.

Feed type had very little effect on concentrations of PAHs in the salmon flesh as 21 of the 28 PAHs tested for were found to have the same concentrations in fish from all three treatments. 6 of the PAHs showed higher concentrations in the VO fish with only 1 showing lower concentrations in the VO fish.

Of the 18 *ortho*-PCBs tested, all showed lower concentrations in the flesh of the VO fish. All four of non *ortho*-PCBs, had lower concentrations in the flesh of the VO fish compared with the other feeds. Of the 17 dioxins tested, 9 showed lower concentrations in the flesh of the fish fed the VO feed, with 7 having the same concentration for all treatments and 1 showing a higher concentration in the VO fish. Of the 16 PBDEs tested, 9 showed lower concentrations in the flesh of fish fed the

VO feeds with the other 7 showing the same concentrations in the fish from all three groups.

Looking at three key pollutant groups - dioxins, *ortho*-PCBs and non *ortho*-PCBs, the total TEQ's for each group showed lower concentrations in the fish fed the VO feed compared with the other two feeds, with the sum of the concentrations of the three pollutant groups being well below the EU maximum permitted level, 4ppt, for fish deemed for human consumption for all three treatment groups (Figure 5).

Table 12. Total pollutants in the flesh of salmon (on a whole weight basis), at the end of the feed trial, after being fed the three experimental feeds.

Pollutant	CT	Feed FO	VO
<u><i>ortho</i>-PCBs (ug/kg)</u>			
PCB 18	0.1	0.1	0.05
PCB28	0.31	0.29	0.11
PCB31	0.21	0.2	0.07
PCB52	0.79	0.73	0.25
PCB99	0.92	0.89	0.28
PCB101	1.73	1.62	0.58
PCB105	0.31	0.29	0.11
PCB114	0.03	0.03	0.01
PCB118	1.3	1.21	0.42
PCB123	0.05	0.05	0.02
PCB128	0.3	0.29	0.1
PCB138	3.42	3.28	1.09
PCB153	3.56	3.43	1.1
PCB156	0.14	0.14	0.05
PCB157	0.05	0.05	0.02
PCB167	0.09	0.09	0.03
PCB180	0.99	0.96	0.36
PCB189	0.02	0.02	0.01
Total TEQ <i>ortho</i> -PCBs (ng/kg)	0.28	0.27	0.1
<u>non <i>ortho</i>-PCBs (ng/kg)</u>			
PCB77	29.46	27.89	13.67
PCB81	1.01	1.01	0.33
PCB126	10.32	9.52	4.05
PCB169	1.38	1.18	0.36
Total TEQ non <i>ortho</i> -PCBs (ng/kg)	1.05	0.97	0.41
<u>Dioxins (ng/kg)</u>			
2378-TCDD	0.03	0.03	0.01
12378-PeCDD	0.06	0.06	0.02
123478-HxCDD	0.01	0.01	0.01
123678-HxCDD	0.03	0.03	0.01
123789-HxCDD	0.01	0.01	0.01
1234678-HpCDD	0.01	0.01	0.01
OCDD	0.04	0.04	0.05
2378-TCDF	0.96	0.82	0.22
12378-PeCDF	0.1	0.09	0.02
23478-PeCDF	0.24	0.2	0.06
123478-HxCDF	0.02	0.01	0.01
123678-HxCDF	0.02	0.01	0.01
123789-HxCDF	0.01	0.01	0.01
234678-HxCDF	0.03	0.02	0.01
123678-HpCDF	0.01	0.01	0.01

1234789-HpCDF	0.01	0.01	0.01
OCDF	0.01	0.01	0.01
Total TEQ Dioxins (ng/kg)	0.33	0.28	0.09

PBDEs (ug/kg)

BDE17	0.01	0.01	0.01
BDE28	0.06	0.06	0.02
BDE47	1.08	1.06	0.31
BDE49	0.29	0.28	0.08
BDE66	0.07	0.07	0.02
BDE71	0.01	0.01	0.01
BDE77	0.01	0.01	0.01
BDE85	0.01	0.01	0.01
BDE99	0.27	0.26	0.1
BDE100	0.29	0.29	0.08
BDE119	0.02	0.02	0.01
BDE126	0.01	0.01	0.01
BDE153	0.04	0.04	0.01
BDE138	0.01	0.01	0.01
BDE154	0.13	0.09	0.03
BDE183	0.01	0.01	0.01

PAHs (ug/kg)

acenaphthylene	1.16	1.53	0.77
acenaphthene	0.35	0.35	0.36
fluorene	0.48	0.52	0.77
phenanthrene	0.7	0.71	1.97
anthracene	0.3	0.33	0.34
fluoranthene	0.33	0.33	0.33
benzo[c]fluorene	0.02	0.02	0.02
pyrene	0.26	0.26	0.27
benzo[ghi]fluoranthene	0.04	0.04	0.04
benz(a)anthracene	0.03	0.03	0.03
benzo[b]naphtho[2,1-d]thiophene	0.05	0.05	0.05
cyclopenta[c,d]pyrene	0.01	0.01	0.01
chrysene	0.17	0.17	0.17
5-methylchrysene	0.01	0.01	0.01
benzo[b]fluoranthene	0.08	0.08	0.08
benzo[j]fluoranthene	0.02	0.02	0.02
benzo[k]fluoranthene	0.02	0.02	0.02
benzo[e]pyrene	0.08	0.08	0.08
benzo[a]pyrene	0.09	0.09	0.09
indeno[1,2,3-cd]pyrene	0.05	0.04	0.05
dibenz[ah]anthracene	0.03	0.03	0.03
benzo-[g,h,i]perylene	0.04	0.04	0.04
anthanthrene	0.1	0.1	0.1
dibenzo[a,l]pyrene	0.1	0.1	0.1
dibenzo[a,e]pyrene	0.17	0.17	0.17
dibenzo[a,i]pyrene	0.1	0.1	0.1
dibenzo[a,h]pyrene	0.1	0.1	0.1
coronene	0.1	0.1	0.1

Trace Elements (mg/kg)

Copper	0.37	0.43	0.4
Arsenic	0.97	0.72	0.46
Cadmium	0.005	0.005	0.005
Mercury	0.021	0.017	0.012
Lead	0.005	0.005	0.005

n=3 per treatment

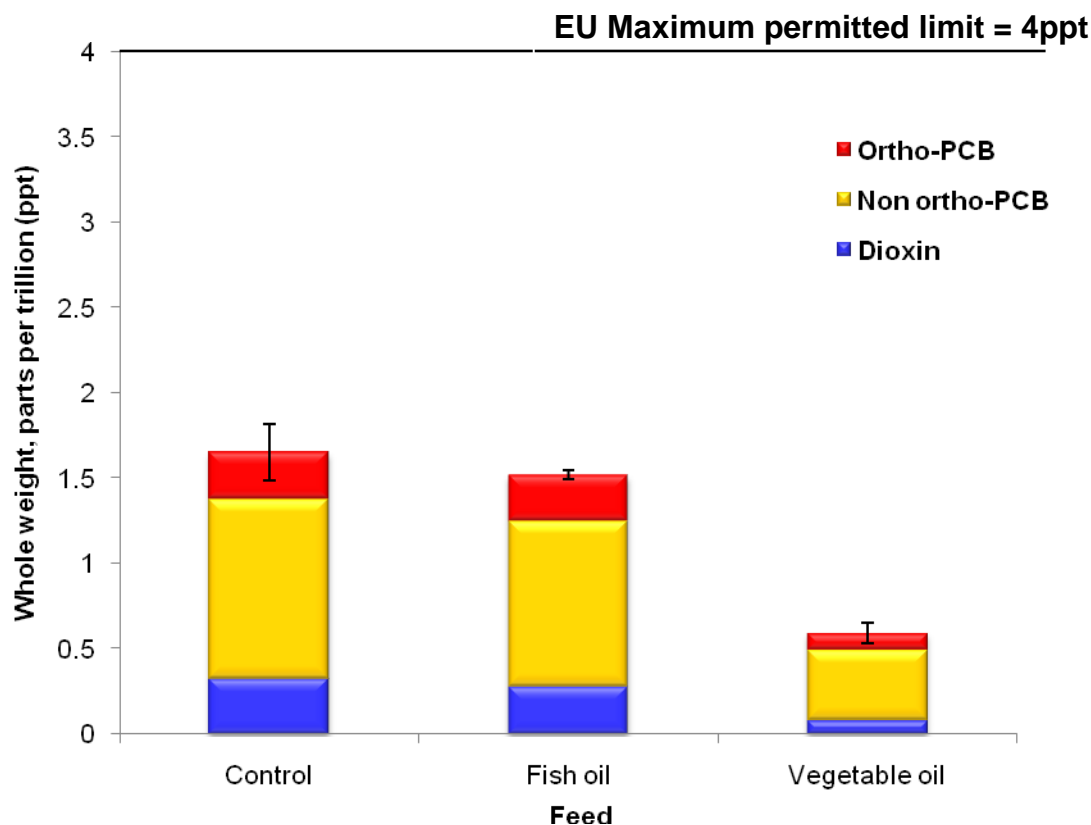


Figure 5. Sum Dioxin, non ortho-PCB and ortho-PCB concentrations(ppt) in salmon, whole weight, after 154 day feed trial on three experimental feeds; error bars, standard error of the mean of sum of the three pollutant groups (1ppt=1ng/kg).

## 4. Discussion

### 4.1 Growth and FCR

In this study substituting fish oil with vegetable oil in feeds of Atlantic salmon had no significant effect on growth or feed efficiency. Similar results were reported in previous studies where the inclusion of vegetable oil at differing substitution levels in Atlantic salmon feeds have been investigated (Thomassen & Rosjo, 1989; Bell *et al.*, 2001 & 2003; Bransden *et al.*, 2003; Torstensen *et al.*, 2004; Rora *et al.*, 2005; Menoyo *et al.*, 2007; Miller *et al.*, 2007; Pratoomyot *et al.*, 2008). It must be noted however that most of these studies were carried out on Atlantic salmon of a much smaller size (final weights 50-500g) on a much smaller scale making it difficult to compare with this experiment as it was carried out on a commercial scale with fish with final weights of over 3000g. Bell *et al.*, (2001) found that feeds with 10-100% inclusion of rapeseed or palm oil had no effect on growth or feed conversion ratio (FCR) of Atlantic salmon. Bransden *et al.*, (2003) found the inclusion of sunflower oil in Atlantic salmon feeds did not significantly affect the growth or FCR. Also other marine species have shown successful substitution of fish oil with vegetable oil, as Izquierdo *et al.*, (2005) reported substitution of 60% fish oil with soybean oil in feeds of gilthead seabream showing no negative effect on growth or feed utilisation.

In the present study there were no significant differences in SGR, TGC and FCR between treatments. Pratoomyot et al., (2008) conducted a study over a 10 week period, which is a shorter trial than the one seen in this experiment, with salmon reaching final weights of around 2190g and found no significant differences in final weights, SGR and TGC between fish fed fish oil based feeds and those fed vegetable oil based feeds but the SGR and TGC seen in Pratoomyot's experiment were considerably greater than those seen in this experiment. A study carried out by Menoyo et al., (2003) over a 24 week period which looked at salmon of a similar size (final weights between 3311-3825g) and had similar SGRs to this experiment, between 0.29-0.4, observed fish fed lower levels of n-3 fatty acids (15%) growing significantly better than those fed a higher level of n-3 fatty acids at 29% which is in contrast to this study. The FCRs found in this experiment were reasonable for commercial production on this scale. Due to a large proportion of the production costs devoted to feeds, even a slight reduction of 0.01 in the FCR can make huge savings for aquaculture companies. It is very difficult to compare growth rates and feed efficiency between different studies as the majority of studies are not carried out on a commercial scale, like this study, and use Atlantic salmon of a much smaller size. Fish tend to grow at different rates at different periods of their lifecycle making it more difficult to compare between studies which are looking at different sized fish.

For the majority of trials carried out on marine net pens, it is not possible to quantify genuine feed consumption and wastage. To calculate the economic impacts of feeding the different feeds and thereby estimate the price premium required for the fish fed each of the different feeds, it would be necessary to obtain an accurate estimate of FCR. This would require a relatively short trial in facilities with waste feed collection to accurately evaluate feed intake and wastage (Morris *et al.*, 2003)

#### *4.2 Fish quality*

After comparing the means of the directed sampled fish weights and population weights at particular points through the trial, the directed sampling weights were still found to be representative of the population even after the Vaki data had been removed. Significant differences were however observed for the directed sampling fish between fish fed the VO feeds and the fish fed the CT and FO feeds for round weight and gutted weight for the May, June and July sampling dates and for fork length for the June and July sampling points which differs to that of the population weights for the three feeds where no significant differences were observed. The differences in weights seen between the CT and FO fish and the VO fish for the directed sampling (350g) was greater than those found between the population weights (275g). This could be due to the variability and possible inaccuracy of the Vaki data which was included in the growth graph which was used to estimate the directed sampling weights for the trial cages at each sampling point, this could have amplified the differences in weights of the fish fed the three feeds

resulting in significant differences. The number of individual fish weights used in the analysis of the data for the directed sampling was thirty per treatment whereas for the population growth it was only three per treatment. Individually tagged fish were not used in this experiment to measure growth therefore only averages from the three cages in the replicates could be used in the growth analysis for the population. This would mean more variation would be seen in the directed sampled weights allowing more chance of there being a significant difference.

Studies carried out have shown little effect of dietary lipid source on quality parameters in farmed fish (Caballero *et al.*, 2002; Grisdale-Helland *et al.*, 2002; Rora *et al.*, 2005; Morkore *et al.*, 2007). Condition factor in this study was not influenced by treatment; this was irrespective of the differences seen between the feeds in relation to weights and fork length. Condition factor relates to the combined effect of somatic growth (weight gain) and vertebral growth (length growth) and often shows seasonal variations (Young *et al.*, 2006). Regost *et al.*, (2004) found no significant differences in round weight, gutted weight or length between treatments for Atlantic salmon but found condition factor to be higher in those fish fed capelin and rapeseed oil feeds compared with those fed Peruvian fish oil and soybean oil feeds. Grisdale-Helland *et al.*, 2002 reported that condition factor was not affected by inclusion of soybean oil in the feeds of Atlantic salmon but was affected by temperature, as fish raised at 5°C showed significantly higher condition factors than those fish raised at 12°C. In this study the temperature increased over the 154 day trial period from 7°C in February to 11.3°C in July and a significant decrease in condition factor was present over the 154 day trial period for the FO and VO fish but not for the CT fish. Similar to the findings of Grisdale-Helland *et al.*, (2002), in this study feed had no effect on condition factor however temperature may have been the cause of the significant decrease in condition factor seen in the FO and VO fish over the trial period. It is unclear why the fish fed the CT did not experience a significant decrease in condition factor in line with the FO and VO feed fish. The condition factor of the fish does indicate that the fish are broader and fatter rather than leaner and thinner. Salmon which have a higher fat content and higher condition factor are preferred for the use in smoked salmon products (Rora *et al.*, 1998). The type of fish produced in this experiment would be good candidates for smoked salmon products as they have a broader surface area to work with and have relatively high fat contents.

Carcass yield showed a significant difference at the last sample point in July with the VO fish having a lower yield compared with the other two feeds. This could indicate that the growth of the VO fish was slowing down. The weights of the fish on the VO feed were lower compared to the other two feeds at the July sampling point therefore a slight reduction in carcass yield may have been expected as you will be getting less final product as the fish are smaller. It has been reported that higher body masses contribute to higher fillet yields due to the lower proportion of head, bones and fins (Young *et al.*, 2006). Overall there was an increase in carcass yield as the trial progressed which would agree with the statement made above, however

the highest carcass yields in the February, May and June sampled fish were observed in the VO feed contradicting this statement as they had the significantly lower average weights at the May, June and July sampling points compared to the CT and FO feed fish. Previous research on the effect of feed on yield suggests that there may be differences between species with Regost *et al.*, (2004) finding yield to be higher in salmon fed a soybean oil feed compared with those fed fish oil feeds while Morkore *et al.*, (2007) found no significant variation in yield for cod fed soybean oil feeds or fish oil feeds.

Hepatosomatic index (HSI) can be an indicator of disease or nutritional imbalance in fish as feeds deficient in essential fatty acids can cause abnormal deposition of lipid resulting in larger livers and increased HSI (Bransden *et al.*, 2003). In this study significant differences were found at the May and June sampling points with fish fed the VO feed showing a significantly lower HSI, compared with the other two feeds. This is in contrast to what may have been expected which is not in agreement with the above statement due to the lower n-3 PUFA concentrations found in the fish fed the VO feed and given the lower HSI. The liver is a secondary energy reserve and a key glycogen store. These differences could indicate that the VO fish are not storing any excess energy, with all being used up by the fish, or there may be a possibility that vegetable oils are less transferable into glycogen stores. Most studies have shown vegetable oil replacements in fish oil feeds have had no effect on HSI on a number of marine fish species. Miller *et al.*, (2007) found different dietary oil sources for use in feeds of Atlantic salmon to have no effect on HSI. Similarly Drew *et al.*, (2007) observed no significant differences in HSI for rainbow trout between feeds with fish oil and those with canola and linseed oil and Morkore *et al.*, (2007) found no significant variation in HSI for cod due to treatment. It is difficult to pinpoint a reason for variation in HSI as it can vary in relation to a number of factors including sex, season, age, physical condition, feeding, maturation and stress.

Gonadosomatic index (GSI) of the fish fed the three feeds was variable with low levels of maturation (GSI<0.2%) observed throughout the trial. There were no significant effects of feed on the GSI of male or female salmon indicating that none of the feeds had any effect on maturation.

Visual appearance, especially colour is the most important characteristic of foods in determining their selection prior to actual consumption, and buying decisions are frequently based on the appearance of a product (Nickell & Springate, 2001). Visually assessed colour in this study showed no significant differences between feed types until the final sampling point when the fish fed the VO feed appeared to have significantly lighter coloured fillets than those fed the FO feed. This is unusual as flesh colour does not usually vary in relation to seasons. However Regost *et al.*, (2004) also reported higher colour scores in fish fed a fish oil feed compared with those fed soybean oil and rapeseed oil feeds. In contrast to the aforementioned visual assessments, comments from the sensory analysis taste test panellists indicated that the VO samples had the darkest colour however these

assessments were made on cooked salmon fillets whereas the colour score using the Roche colour scale was made on raw salmon fillets. As visually assessed colour is a subjective measurement, with the individual matching the colour of the sample to that on the colour card, an instrumentally measured colour may have proved to be a more accurate assessment of colour. There may be some issues with customer acceptance of the final product if the colour is too light as most consumers expect salmon to be an orange/red colour, similar to that found in wild salmon. Due to the importance of colour in consumer acceptance it would be recommended to monitor this parameter over a longer period of time to ensure there are no long term effects of the VO feed on the colour of the final product.

The pyloric caeca act as a storage organ for caecal fat with excess fat laid down in the viscera and it could be an indication of how efficient a feed is. The caecal fat scores showed significant differences at the start and the end of the experiment with the fish fed the VO feed starting with the highest caecal fat score and finishing with the lowest of the three feeds in July. This indicates that the VO fish at the end of the experiment do not have as much excess energy to lay down in the viscera which are secondary fillet stores used to store excess energy. This indicates that the VO fish are using up all the energy taken in through the feed, leaving no excess to be stored in the viscera. This could be due to the smaller overall size of the fish fed the VO feed or it may indicate that the VO feed is more efficient than the other two feeds.

Texture of the flesh is also a very important flesh quality property of fish. Factors such as fat content and distribution and fatty acid profile may influence the texture of the flesh. Reduction in fillet hardness of fish fed feeds with soybean inclusion and, particularly, with higher levels of fish oil substitution could be related to lower saturated and higher total polyunsaturated fatty acids contents in muscle (Izquierdo *et al.*, 2005). Studies on the effect of vegetable oils on texture in fish are inconclusive. In this study a significant difference was found between the FO feed fish and the VO feed fish at the July sampling point and indicated that the flesh of the FO feed group was firmer than the flesh of the fish fed the VO feed. Flesh texture can be seasonal however this trial is not long enough to see if there is any long term effect from using the VO feed on the fillet texture. Gullou *et al.*, (1995) reported significantly softer flesh in brook charr fed soybean oil feeds compared to canola oil, similarly Izquierdo *et al.*, (2005) found a reduction in fillet hardness in gilthead seabream fed soybean oil feeds compared to those fish fed fish oil feeds. Several authors have however reported no significant differences in texture caused by dietary lipid source including Morkore *et al.*, (2007) in a study on farmed cod, and Rora *et al.*, (2003) and Regost *et al.*, (2004) in studies on Atlantic salmon. Comments made in relation to the texture of the fish during the sensory analysis taste tests included the CT feed samples having a moister but tougher texture compared to the other feeds; the FO feed samples having a drier but softer texture, which was preferred to the CT feed samples; the VO feed samples with a drier but pleasant texture, and one comment that the VO sample was firmer than the CT feed

fish. Again these comments were based of cooked salmon fillets whereas the instrumental texture analysis was carried out on raw fillets. It is difficult to draw any firm conclusions from the comments made on the texture in the taste test as comments were often contradictory. It does however have to be stressed that, in order to represent consumers, the panellists were untrained and therefore used a much wider vocabulary and meanings than expected from trained panellists. The differences recorded in the July samples didn't seem to negatively affect the consumers' acceptance of the VO samples, as very little difference was shown in preference to samples from all three feed types. Again as texture is a very important factor in terms of consumer acceptance it would be recommended to monitor this parameter over a longer trial period to ensure the VO feed had no negative effects on texture.

#### *4.3 Sensory analysis*

Taste test panels are important in ensuring customers acceptance of products. In this study very little difference was detected in sensory attributes between the salmon fed the three feeds with all samples being well received by the panellists. In the sensory analysis triangle taste tests the only significant difference that was found was between the CT feed samples and the VO feed samples when the majority of the panellists correctly identified the 'odd' sample indicating that the samples were perceived as different. Due to the larger differences in feed composition, it was expected that if any differences were detected, they would have been between the FO feed samples and the VO feed samples, but a significant number of panellists did not correctly identify the 'odd' sample on that comparison, even though their confidences in choices were high, averaging 7.7 on the 10 point scale. Differences were not expected between samples from the CT feed and FO feed as these feeds had only minor differences in their composition, and samples were therefore expected to be perceived as similar, which they were.

In the preference tests the FO sample was preferred by the majority of panellists (71%) over the CT sample whereas less of a majority (58%) was shown between the VO samples and the CT feed samples and FO (51%) and VO feed samples. These results are in contrast to the abilities of the panellists to identify the 'odd' samples and are therefore unexpected. Vegetable oils are not used in the majority of feeds of salmon in the UK but can be found routinely in feeds used to produce salmon in Norway and Chile. The inclusion of vegetable oils in feeds could potentially have affected the flesh taste or texture, so it was expected that if any, either positive or negative effects were present, they would have been detected in the taste tests. However the results indicate that there was very little difference in organoleptic qualities between the feeds and some evidence of the CT feed samples, the feed currently in commercial use, producing the least favoured samples. Comments made by panellists on the CT feed samples included the texture being watery and tougher than the other samples, the samples being oilier

and fattier and having little flavour. Comments made on the FO feed samples included strong aroma, best texture and most liked flavour while the VO samples had the darkest colour, a drier texture and a succulent flavour. However it must be noted that the panellists were untrained and often contradictory comments were given on each feed type. It must also be stressed that the flavour of all three samples were liked and comments such as 'all samples tasted good' indicated that all samples were well received by panellists and no sample was particularly disliked. From these results we can see some differences were detected between samples however there didn't seem to be a strong preference for fish fed a particular feed. If, in addition to the taste test design undertaken, samples from all three feeds were tested together an overall preferred sample group may have been evident.

Results of studies investigating the effect of inclusion of vegetable oils in feeds on sensory quality parameters are varied. Hardy *et al.*, (1987) reported no major differences in the flavour and texture of the Atlantic salmon fed feeds of soybean oil compared with those fed fish oil. However, Thomassen and Rosjo, (1989) did report significant differences in colour, odour and taste when Atlantic salmon were fed feeds containing vegetable oils. In other species, Morkore *et al.*, (2007) found consumers able to differentiate between the flavours of cod, fed feeds containing either soybean oil or fish oil but found no preference to fish from one particular feed type. Izquierdo *et al.*, (2005) found that samples with inclusion of different types of vegetable oils with varying levels of substitution in the feeds of gilthead seabream had very little effect on consumer perception as all samples were well received by the consumer panel. Torstensen *et al.*, (2005) reported marginal differences between flesh sensory characteristics of Atlantic salmon fed feeds with 100% fish oil and 100% vegetable oil, but these differences became undetectable by a trained taste panel after feeding of a fish oil based finishing feed. This was also reported by Regost *et al.*, (2003) as they found that soybean and linseed oil influenced the organoleptic properties of flesh of turbot, particularly the odour and texture, but after the fish were put on a fish oil based feed for 8 weeks these differences were no longer apparent. It is difficult to compare organoleptic properties reported in different studies because a number of parameters vary including the level of inclusion of vegetable oils, methods of cooking, storage, the time interval between slaughter and analyses and different age or species of fish (Regost *et al.*, 2003).

#### *4.4 Fillet fat content*

The majority of published studies have reported that fat content is not significantly affected by dietary oil source (Hardy *et al.*, 1987; Regost *et al.*, 2004; Nanton *et al.*, 2007). With gutted weight used as a covariate it was found that feed had no significant effect on fat content and this is in concurrence with the above studies.

#### 4.5 Fatty acid composition

It is generally accepted that the fatty acid composition of fish tissues reflects that of the dietary fatty acid composition (Hardy *et al.*, 1987; Nanton *et al.*, 2007; Drew *et al.*, 2007). This was true of the findings in this study with comparisons of tissue composition between the three feed groups at both the start and end of the 154 day trial period indicating significant changes in the VO group during the trial period. With inclusion of vegetable oils in the feeds of farmed fish it has been reported that concentrations in the flesh of important n-3 fatty acids are often reduced and n-6 fatty acids are increased (Nanton *et al.*, 2007; Rora *et al.*, 2005; Morkore *et al.*, 2007; Pratoomyot *et al.*, 2008). Results of our study are in concurrence with previous findings. Fillet flesh of the salmon fed the CT and FO feeds were mainly characterised by higher levels of monounsaturated fatty acids (MUFA) and n-3 fatty acids especially 20:5n-3, EPA and 22:6n-3, DHA while flesh from fish fed the VO feed were characterised by high levels of 18:1n-9, oleic acid (OA) and n-6 fatty acids mainly caused by a large increase in 18:2n-6, linoleic acid (LA). The flesh of the salmon fed the VO feed had a 3.5 fold higher total amount of n-6 PUFA than the salmon fed the FO feed, which was mainly due to the increase in LA which was 4.5 times that found in the fish fed the FO feed. Bell *et al.*, (2003) reported flesh percentages of LA in fish fed 100% rapeseed oil to be almost 5-fold higher than those fed 0% rapeseed oil.

In our study the salmon fed the VO feed, showed a 27% reduction in EPA and a 33% reduction in DHA compared to the CT feed fish. This is less than the 60% reduction in EPA and DHA reported by Thomassen and Rosjo, (1989) for Atlantic salmon fed soybean and rapeseed oil feeds compared to those fed capelin oil feeds. Results of our study are in agreement with Bell *et al.*, (2001) who reported that 50% of dietary fish oil can be replaced with vegetable oil resulting in only a modest decrease in flesh DHA but replacement above 50% may cause significant reductions in n-3 fatty acids and increased deposits of LA such that the nutritional benefits to consumers may be reduced. In this study the substitution level of fish oil with vegetable oil was 58% which did result in considerable reductions in EPA and DHA concentrations in the fish fed VO feeds. Bransden *et al.*, (2003) reported only 40% of sunflower oil could be incorporated in Atlantic salmon feeds before significant reductions n-3 PUFA were observed in the muscle.

In this study the n-3/n-6 ratio was greatly reduced in the VO samples with a ratio of 1.05 compared to the CT feed ratio of 4.39 and the FO feed ratio of 4.84, which is a reduction of 78% in the n-3/n-6 ratio observed between the VO feed fish and the FO feed fish. Bell *et al.*, (2003) similarly reported a 66% reduction in the n-3/n-6 ratio in Atlantic salmon that had been fed rapeseed oil feeds compared to fish fed fish oil feeds. A reduction in n-3 PUFA in the fillet, especially EPA and DHA, can reduce the potential health benefits for the consumer. Essential fatty acids both n-3 and n-6 are required by humans to play important roles in many metabolic processes however there is evidence to suggest that low levels of these essential fatty acids or

the wrong balance of types may be a factor in a number of illnesses. The ratio of n-6/n-3 in the human feeds needs to be consumed in a balanced proportion, with healthy ratios ranging from 1:1 to 4:1. Excessive amounts of n-6 PUFAs and a high n-6/n-3 ratio, which can be commonly found in Western diets today, with ratios of 10:1 up to 30:1, promote the pathogenesis of many diseases including cardiovascular disease, cancer, inflammatory and autoimmune disease, whereas increased levels of n-3 PUFAs exert suppressive effects (Simopoulos, 2002). In this study the fish fed the VO feed provided a ratio of n-6/n-3 of approximately 1:1, which is the optimal ratio for these fatty acids to be consumed however due to the ratio in Western diets currently being skewed towards n-6 PUFAs, many retailers would still be looking at fish as being a unique source which can provide increased levels of n-3 PUFAs to balance out the ratio.

It is possible to restore n-3 PUFA levels in fish fed vegetable oil feeds by feeding a fish oil based feed for a limited time, prior to harvest, known as a finishing diet. Many studies have looked at the use of fish oil finishing diets to restore the levels of beneficial n-3 fatty acids such as EPA and DHA (Bell *et al.*, 2001; Bell *et al.*, 2003; Regost *et al.*, 2003; Torstensen *et al.*, 2005; Morkore *et al.*, 2007). Most studies found that even after implementing a finishing diet the influence of previous feeds were still present with levels of EPA and DHA recovering more rapidly compared to those of LA and OA. Bell *et al.*, (2001) reported that EPA and DHA levels could be restored to 80% of those found in fish originally fed fish oil feeds. These finishing diets have a drawback as in addition to adding back valuable and highly desirable n-3 PUFA they are also increasing the levels of pollutants in a previously low contaminant product (Bell *et al.*, 2005).

The blend of fish oils used in farmed fish feeds is also key to the nutritional quality of the fish. Northern hemisphere (NH) fish oil is lower in saturated fatty acids compared with southern hemisphere (SH) fish oil, however SH fish oil is higher in EPA and DHA content. At lower inclusion levels the impact of vegetable oils is reduced on the flesh fatty acid composition therefore an alternative to the finishing feed would be to use lower levels of substitution particularly if the oil blends used are carefully chosen to limit reductions in n-3 essential fatty acids.

An important requirement for fish oil replacements is that they retain the health promoting properties of the end product for the consumer, which means keeping the levels of EPA and DHA at relatively high levels and limiting the concentration of linoleic acid. The focus is no longer just on promoting good growth but also on producing fish of a good nutritional quality. Producers trying to implement vegetable oils in place of fish oils in feeds have encountered opposition from UK retailers, with many still against any inclusion of vegetable oils in feeds used to produce fish. Although producers are investigating the inclusion of vegetable oil they understand the benefits of eating salmon and desire to keep EPA and DHA at a set level. The importance of fatty acids, such as EPA and DHA and a high n-3/n-6 ratio, in the promotion of human health obviously needs to be considered when replacing fish oil with vegetable oils.

#### 4.6 Pollutants

Whilst feeds based on marine fish oils are currently used by the Scottish aquaculture industry, it is likely that these oils are contributing to the contamination of farmed salmon through high levels of persistent organic pollutants (POPs) such as PCBs and dioxins. Dioxins are unwanted by-products of combustion and incineration processes and PCBs are pollutants now banned from use but can still be found in the environment as they do not degrade easily. PCBs could be found in a number of products including coolants, insulating fluids, lubricating oils, coatings for wiring and electrical components, flame retardants, sealants and adhesives. In this study all the pollutants tested for in the flesh of the salmon fed the three experimental feeds were well within maximum limits set by the European Union (EU) in terms of fish products deemed for human consumption. Concentrations for whole weight ranged from 0.07-0.37ng/kg for dioxins, 0.33-1.16ng/kg for non *ortho*-PCBs and 0.09-0.31ng/kg for *ortho*-PCBs, showing non *ortho*-PCBs to be the most problematic in terms of contamination. The EU's maximum permitted levels in fish and fish products for dioxins and PCBs is 4pg/g and for animal feeds is 1.25ng/kg for dioxins and 3.25ng/kg for PCBs. An analysis of dioxin and PCBs levels in foods for human consumption in EU found that farmed salmon contained dioxin levels in the range 0.43-1.04ng TEQ/kg wet weight. Bell *et al.*, (2005) found values of dioxins in fish fed high fish oil feeds at the lower end of this range, at 0.53ng TEQ/kg, and fish fed vegetable oil feeds were below the range even after 24 weeks on a fish oil based finishing diet. Bell found dioxin like-PCB (dl-PCB) concentrations in the range of 0.58-1.48ng TEQ/kg wet weight. Although concentrations of pollutants in farmed fish including salmon have been shown to be below all national and international limits their presence in farmed fish especially salmon has received some negative reporting.

The dominant contaminants from the PCBs were *ortho*-PCBs 153 and 138 which ranged in concentrations in the flesh from 1.1-3.56ug/kg and 1.09-3.42ug/kg. Non *ortho*-PCBs, PCB 77 and 126 were also found in quite high concentrations compared to other non *ortho*-PCBs with ranges of 13.67-29.44ng/kg and 4.05-10.32ng/kg respectively. Bell *et al.*, (2005) found that dioxin and dl-PCB concentrations were reduced as vegetable oil inclusion increased. They found that feeding a 100% vegetable oil feed reduced dioxin and PCBs concentration by 75% and 64% but after the fish oil finishing diet a reduction of only 60% and 47% respectively was present. Also evident were heavy metals such as copper and arsenic which ranged in concentrations from 0.37-0.43mg/kg and 0.46-0.97mg/kg. An adult's maximum threshold for daily dietary intake of copper is 11mg with a minimum daily dietary intake of 1mg being recommended. The EU maximum levels for arsenic in seafood for human consumption is 4mg/kg. Both the copper and arsenic concentrations found in the flesh of the salmon tested in this study were well below any maximum levels which have been set.

Known benefits of eating fish and fish products outweigh any possible risks associated with pollutants, with the Food Standards Agency advising people to consume at least two portions of fish per week with one portion being an oily fish. It is also important to consider that the majority of dioxins consumed in Europe come from other foodstuffs, particularly dairy products, and not principally seafood, since seafood only contributes as a minor component to the overall diet for the majority of Europeans. Including vegetable oils in feeds of farmed fish for a considerable duration could remove dioxins and PCBs and maintain low pollutant levels in fish body. Some manufacturers have invested in techniques such as active carbon to reduce contamination levels in fish meal and fish oil which removes 80-95% of dioxins and 20-30% of PCBs from fish oil, but obviously this would be very costly for producers for use in feeds and doesn't reduce the increasing pressure placed on depleted wild fish stocks used in production of feeds (FIN 2007).

## 5. Conclusions

- No significant differences were detected in growth or feed efficiency between the three feeds trialled.
- Minor differences reported in terms of carcass measurements and flesh quality of the fish between treatment groups. Texture and colour of the flesh should be monitored over a longer period of time to ensure no negative effects on these parameters from use of vegetable oil in feeds of salmon.
- All samples were well received by the taste test panel with no strong preference for samples fed one particular feed.
- Increased levels of n-6 fatty acids and reduced levels of n-3 fatty acids were observed in the fish fed the vegetable oil feed which could affect the nutritional benefits of the final product to the consumer.
- Use of a fish oil based finishing diet prior to harvest could be used to restore the levels of essential n-3 fatty acids which may be reduced by use of vegetable oils in feeds, although this would consequently increase pollutant levels in a low contaminant product.
- Ensure the optimum blend of southern hemisphere and northern hemisphere fish oils are being used in the feeds, with a lower inclusion of vegetable oil to ensure the n-3 fatty acids are not compromised when partially replacing fish oil for vegetable oils in feeds.
- Lower levels of persistent organic pollutants were found in the flesh of the salmon fed the vegetable oil feed compared with the other two feeds, producing a safer product for consumers to eat.
- The fish oil feed which contained higher quality ingredients and had a greater cost of production, showed little difference in performance compared with the control feed therefore it would not be cost effective to implement this feed on site.

- This study indicates that vegetable oil could be used as a successful substitute for fish oil in feeds for Atlantic salmon in Shetland waters and it would be financially attractive as implementing the vegetable oil diet would make savings in terms of production costs for the company.
- The main challenges faced by the industry in terms of vegetable oil inclusion in salmon feeds are in relation to retailer and consumer preconceptions and education, trying to prove vegetable oils are not inferior to fish oils when used in aquaculture feeds.
- The main issue of vegetable oil inclusion in salmon feeds is the differences seen in the fatty acid profiles. If a decision could be reached in terms of EPA and DHA concentrations and n3:n6 ratios between producers and retailers, vegetable oil could be considered for use in salmon feeds.

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## **Appendix 1**

### **1 DETERMINATION OF TOTAL FAT IN FISH TISSUES II.**

#### **Soxhlet extraction using the FOSS Techator**

#### **SOP No. 5.2**

##### **1.1 Scope**

The level of fat (lipid) in fish tissues is an important parameter in the assessment of fish quality. The method can be used to determine fat levels in all fish tissues. There are many methods currently in use, which have been developed over recent decades. The method chosen is the long-established Soxhlet extraction procedure, in this case employing the use of FOSS Techator solvent extraction equipment.

A hot organic solvent is used to extract the lipid from a known weight of fish flesh. The amount of lipid extracted is determined gravimetrically following evaporation of the solvent and drying to constant weight. The method is based upon the directions given in the Techator's Operating Manual.

##### **1.2 References**

The method employed is based on:

##### **1.2 Apparatus**

- i) Balance 3 d.p. (Sartorius MC1)
- ii) Freezer at  $-10^{\circ}\text{C}$  or below.
- iii) Drying oven at  $100^{\circ}\text{C}$  (Mettler)
- iv) Food Processor (Magimix)
- v) Techator Soxtec HT extraction system plus accessories (FOSS).
- vi) Standard items of laboratory equipment (spatulas, measuring cylinders, etc)

##### **1.3 Materials**

Petroleum spirit ( $40-60^{\circ}\text{C}$ ) GPR (VWR International)

Extraction thimbles (FOSS)

Non-absorbent cotton wool (Fisher)

Anhydrous sodium sulphate (VWR International)

##### **1.4 Preparation of samples.**

- a. The sample (a skinless and deboned salmon fillet) is homogenized in a food processor using 2 x 1 minute bursts with mixing between bursts with

- a spatula. Further homogenization follows, if necessary, to ensure homogeneity.
- b. 7g of homogenate are accurately weighed (3 d.p.) into a labelled weighing boat containing approximately 11.5g of anhydrous sodium sulphate.
  - c. The sample is left overnight in a fume hood to desiccate.

## 1.5 Procedure

- a. The desiccated sample and standard are transferred to an extraction thimble and gently packed down using the end of a 10ml measuring cylinder. The sample is sealed with a cap of anhydrous sodium sulphate and then the thimble is plugged with non absorbent cotton wool to prevent sample loss.
- b. The packed thimble is placed in the oven at 100 °C ( $\pm$  1 °C) for approximately one hour to dry.
- c. After drying, the thimble is placed into the Techator which is set to 115 °C. The pre-weighed extraction cup, containing 60ml of petroleum spirit is placed into the unit and the unit is sealed. Pull the extraction lever down to seal. Ensure all 6 outlets have a cup underneath. The coolant water is turned on and the unit set to boil.
- d. After one hour boiling the sample, the unit is set to rinse for 2 hours.
- e. After rinsing, the stopcocks are closed so that the rinsing condensate is collected in the condensers.
- f. After 30-40 minutes, the unit is set to evaporate for approximately 1 hour by pushing the evaporator lever up the air is switched on keeping the extraction lever down.
- g. The extraction cup is then removed from the Techator and placed in the oven for one hour to dry.
- h. The extracted fat is weighed to 3 d.p.

## 1.6 Calculation and Expression of Results

The fat content is expressed as a percentage of the wet weight of the fish tissue and is calculated as follows:

$$F = 100 \times (W_f - W_s)/W$$

Where:

F = % Fat content

W = Weight of sample

$W_s$  = Weight of empty extraction cup

$W_f$  = Weight of extraction cup plus extracted fat

## 1.7 Health and Safety

COSHH regulations apply to the procedures undertaken. All analysts must read and sign Risk Assessments and COSHH forms associated with this procedure.