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Restoration of Fillet n-3 Long-Chain Polyunsaturated Fatty Acid Is Improved by a Modified Fish Oil Finishing Diet Strategy for Atlantic Salmon (*Salmo salar* L.) Smolts Fed Palm Fatty Acid Distillate

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ABSTRACT: Reducing the lipid content in fish prior to feeding a fish oil finishing diet (FOFD) has the potential to improve n-3 long-chain ($\geq C_{20}$) polyunsaturated fatty acid (LC-PUFA) restoration. This study had two main objectives: (1) determine whether feeding Atlantic salmon smolt a 75% palm fatty acid distillate diet (75PFAD) improves the apparent digestibility (AD) of saturated fatty acids (SFA) and (2) examine whether a food deprivation period after growth on 75PFAD leads to higher n-3 LC-PUFA restoration in the fillet when applying a FOFD. The AD of SFA was higher for 75PFAD compared to that of a fish oil (FO) diet. The relative level (as % total fatty acids (FA)) of n-3 LC-PUFA was higher in unfed fish compared to that in continuously fed fish after 21 and 28 day FOFD periods, respectively. Our results suggest that a food deprivation period prior to feeding a FOFD improves the efficiency of n-3 LC-PUFA restoration in the fillet of Atlantic salmon smolt.

KEYWORDS: *fish oil finishing diet, palm fatty acid distillate, apparent digestibility, food deprivation, Atlantic salmon*

INTRODUCTION

Substitution of fish oil (FO) in aquafeeds for salmonids has become inevitable due to the limited global supply of FO.[1,2] Vegetable oils (VO) are common substitutes, but the main shortcoming with their use is the absence of n-3 long-chain ($\geq C_{20}$) polyunsaturated fatty acids (LC-PUFA). Consequently, Atlantic salmon fed VO-based diets have lower n-3 LC-PUFA content compared to fish fed exclusively on FO-based diets.[3−5] The low content of n-3 LC-PUFA in fish fed the VO diet is generally not detrimental to fish growth and health.[2,6] The human health promoting benefits of consuming seafood-derived n-3 LC-PUFA have been increasingly acknowledged,[8] and farmed Atlantic salmon has been recognized as a major source of these fatty acids (FA) in the human diet.[6] Since feeding fish a VO based diet reduces the n-3 LC-PUFA content in fish, decreased associated human health benefits can occur when consuming farmed fish fed a VO-containing diet.[7]

Feeding farmed fish a fish oil finishing diet (FOFD) for a period prior to harvest is an efficient way to restore n-3 LC-PUFA.[2] It is generally accepted that restoration of n-3 LC-PUFA is via the dilution of existing carcass FA by feeding an n-3 LC-PUFA rich FOFD.[9] Feeding the FOFD for several months is generally required to restore n-3 LC-PUFA, particularly the two major health beneficial FA, eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), in Atlantic salmon.[4,10−12] Considerable amounts of FO are still required in aquafeeds to fully restore n-3 LC-PUFA content in fish when using the FOFD strategy.[2,11,13] Preferential FA metabolism is another key process which can contribute to restoring n-3 LC-PUFA content when using a FOFD.[14] In this respect, a diet rich in saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) during the grow-out period has been proposed to be better suited because these FA groups are the preferred substrates for β-oxidation, thus sparing n-3 LC-PUFA when feeding the FOFD.[2] Palm fatty acid distillate (PFAD), a byproduct of refining crude palm oil, is rich in free FA, particularly SFA and MUFA;[15] hence, it has potential for use in a FOFD strategy. A major concern with high dietary SFA and MUFA is their lower apparent digestibility (AD) especially for cold water species such as Atlantic salmon.[2] However, PFAD predominantly contains free fatty acids (~80%) which have been shown to improve SFA digestibility in rainbow trout grown at optimal (15 °C) and elevated (20 °C) temperatures.[16]

To date, there are no reports of the use of PFAD in diets for Atlantic salmon. Therefore, it is of interest to examine the growth performance of Atlantic salmon fed a PFAD-based diet and to also assess the restoration of n-3 LC-PUFA using a FOFD strategy. Furthermore, since the restoration of n-3 LC-PUFA by the FOFD is mainly a dilution of existing FA stores, it was proposed that reducing the initial fillet lipid content by short-term feed deprivation prior to feeding the FOFD may lead to higher n-3 LC-PUFA restoration.[33] For large Atlantic salmon approaching harvest size, several months of feed deprivation is needed to significantly reduce the fillet lipid content;[17−19] therefore, to verify this concept for application after use of a PFAD-based diet, we used Atlantic salmon smolt of ~70 g. Hence, this experiment was designed to reduce the starvation period (7 days) by using small fish, and consequently, the FOFD period after starvation was only 21−28 days, and the growth period on the PFAD diet was 77 days.

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This study can be useful as a model for the application to market size Atlantic salmon under commercial conditions.

MATERIALS AND METHODS

Experimental Diets. Two isonitrogenous and isolipidic diets were formulated in which the added lipid source varied; one diet contained only FO, and the other diet contained a ratio of 75% PFAD/25% FO (75PFAD) (Table 1). PFAD was melted in a water bath and thoroughly mixed with FO before the mixture was blended with dry ingredients. Diets were manufactured into 3 mm diameter pellets using a California Pellet Mill (CL-2, San Francisco, CA, USA), dried, and stored at −5 °C until use.[20] Yttrium oxide was added to the diets (1.0 g/kg) as an inert marker for the measurement of the apparent digestibility (AD) of fatty acids.[21]

Table 1. Ingredient and Chemical Composition of Experimental Diets

	diet	
	FO	75PFAD
Ingredient Composition (g/kg)		
fishmeal[a]	300	300
casein[b]	50	50
wheat gluten[c]	100	100
soybean meal[d]	139	139
fish oil[a]	200	50
palm fatty acid distillate[e]	0	150
Pregel starch[f]	127	127
vitamin mix[g]	7	7
mineral mix[h]	7	7
Stay-C[I]	6	6
choline chloride[j]	2	2
Sipernat[k]	40	40
CMC[j]	10	10
monobasic calcium phosphate[j]	20	20
yttrium oxide[j]	1	1
Chemical Composition (g/kg DM)		
dry matter (g/kg)	916.6	919.9
crude protein	356.2	354.3
total lipid	235.1	233.6
ash	106.2	104.1
energy (MJ/kg)	19.8	19.7

[a]Skretting Australia, Cambridge, Tasmania, Australia. [b]MP Biomedicals Australasia Pty. Ltd., Seven Hills NSW, Australia. [c]Starch Australasia, Lane Cove, NSW, Australia. [d]Hamlet Protein A/S, Horstens, Denmark. [e]Wilmar Edible Oils Ltd., Penang, Malaysia. [f]Penford Limited, Lane Cove, NSW, Australia. [g]Vitamin mix (ASV4).[21] [h]Mineral mix (TMV4).[21] [I]L-Ascorbyl-2-polyphosphate (Roche Vitamins Australia, Frenchs Forest, NSW, Australia). [j]Sigma-Aldrich, Castle Hill, NSW, Australia. [k]Degussa, Frankfurt, Germany. FO; fish oil diet; 75PFAD, 75% palm fatty acid diet.

Growth Experiment. The experiment was conducted at the University of Tasmania (Launceston, Tasmania, Australia) in accordance with the University of Tasmania Animal Ethics guidelines (Investigation A0009731). Atlantic salmon (*Salmo salar* L.) smolts, of average weight of ∼70 g obtained from Wayatinah Salmon Hatchery (SALTAS, Tasmania, Australia), were acclimated in seawater for a period of 14 days before the experiment. Fish were fed a commercial diet (Skretting, Tasmania, Australia) during the acclimation period. After acclimation, fish were randomly distributed in six 300 L fibreglass tanks at a stocking density of 35 fish/tank. Rearing tanks were connected to a partial recirculating system equipped with a protein skimmer and physical, UV, and biological filters.[22] At the start of the experiment, fish were anesthetized (50 mg/L, benzocaine), weighed, and fork length measured. Six fish were euthanized (100 mg/L), and fillets were dissected and stored at −20 °C for measurement of initial lipid content and FA composition. Fish were fed one of the two experimental diets (3 replicate tanks/diet) at a fixed ration of 1.5% body weight/d (BW/d) in two equal rations, and water temperature was kept constant at 15 °C. Every 14 days, fish were bulk weighed to readjust the feed ration.

After 42 days, fish from each tank were randomly removed for fecal collection until there were 20 fish remaining in each tank. Three hours after the last feeding, fish from each tank were anesthetized (50 mg/L, benzocaine) and fecal samples collected from the hind gut region by gently squeezing the ventral abdominal area.[23] Fecal samples were pooled by tank and stored at −20 °C prior to the analysis of FA composition and yttrium oxide. After fecal stripping, fish were euthanized (100 mg/L, benzocaine). Remaining fish were grown for a further 35 days on the two experimental diets. At the end of the 77 day growth period, fish were bulk weighed. Two fish per tank were weighed, their fork length measured, and viscera and fillet were dissected and stored at −20 °C prior to the analysis of lipid content and FA composition. Remaining fish per tank (∼16) were pooled per treatment (∼49), and fish fed on 75PFAD diet were randomly allocated to four tanks (12 fish/tank). Fish fed on the FO diet were randomly allocated to two tanks (12 fish/tank). All fish were then fed at 1.5% BW on the FO diet except for fish in two tanks previously fed the 75PFAD diet, which were unfed for 7 days. After 7 days of food deprivation, six fish per treatment [fish fed FO throughout (FO), fish fed 75PFAD then FO (75PFAD/FO), and fish fed 75PFAD then unfed (75PFAD/UF)] were dissected for viscera and fillet and stored at −20 °C prior to analysis of total lipid content and FA composition. All treatments were then fed to satiation on the FO diet for a further 21 days. At the end of the FOFD period, six fish for each of the three treatments [(a) FO, (b) 75PFAD/FO, and (c) fish fed 75PFAD then unfed then FO (75PFAD/UF/FO)] were dissected to obtain viscera and fillet and stored at −20 °C prior to the analysis of lipid content and FA composition. An illustration of the experimental design is presented in Figure 1.

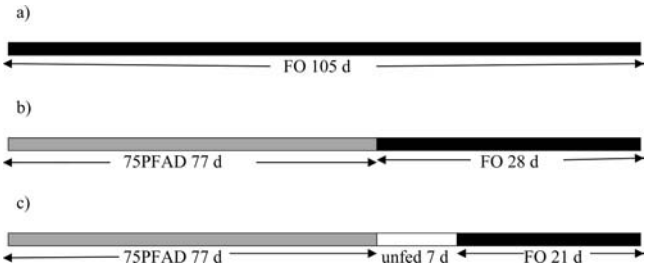

Figure 1. Schematic illustration of treatments. d denotes period in days. (a) Fish fed for 105 days with fish oil diet throughout (FO). (b) Fish fed for 77 days with 75PFAD, followed by 28 days with FOFD (75PFAD/FO). (c) Fish fed for 77 days with 75PFAD, followed by 7 days of food deprivation, then fed with FOFD for 21 days (75% PFAD/UF/FO).

Apparent Digestibility. Fecal samples were freeze-dried prior to chemical analysis. AD was calculated using the formula AD (%) = 100 − [100 (Y_{diet}/Y_{feces}) × (FA_{feces}/FA_{diet})], where Y is the % of yttrium oxide and FA is the % of particular fatty acids.[24]

Chemical Analysis. Standard methods were used to determine dry matter (DM) (freeze-dry to constant weight then drying at 135 °C for 2 h)[25] of experimental diets; total lipid;[26] nitrogen (Kjeldahl using selenium catalyst; crude protein was calculated as N × 6.25); energy (bomb calorimeter, Gallenkamp Autobomb, calibrated with benzoic acid); and ash by combustion at 600 °C for 2 h.[25] Apart from DM, freeze-dried samples were used for all analyses and corrected for DM.

Lipid Extraction, Lipid Class, and Fatty Acid Analyses. Diets, fillet, viscera, and fecal samples were freeze-dried and extracted overnight using a modified Bligh and Dyer protocol.[26] This involved a single phase extraction using $CHCL_3$/MeOH/H_2O (1:2:0.8, v/v/v), followed by phase separation to yield a total lipid extract (TLE).

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Table 2. Growth, Feed Efficiency, and Body Indices of Atlantic Salmon Fed Either FO or 75PFAD during the Grow-out Period for 77 Days Followed by Either 28 Days of FOFD or 7 Days Food Deprivation Followed by 21 Days of FOFD[a]

	initial weight (g)	final weight (g)	SGR (%W)	FER (g/g)	K	fillet weight (g)	viscera weight (g)	VSI (%)
grow-out (day 77)								
FO	71.5 ± 1.9	147.9 ± 4.1	0.97 ± 0.0	1.01 ± 0.0	1.30 ± 0.0	48.3 ± 6.3	17.3 ± 1.6	9.7 ± 0.1
75PFAD	73.5 ± 1.0	152.8 ± 3.3	0.98 ± 0.0	1.02 ± 0.0	1.32 ± 0.0	40.8 ± 6.9	15.4 ± 2.2	9.9 ± 0.3
finishing (day 84)								
FO					1.34 ± 0.0 a	41.1 ± 3.3	16.6 ± 0.9 a	10.6 ± 0.4 a
75 PFAD/FO					1.38 ± 0.0 a	40.8 ± 3.5	16.5 ± 0.9 a	10.8 ± 0.5 a
75PFAD/UF					1.21 ± 0.0 b*	38.4 ± 3.8	12.5 ± 1.0 b*	8.7 ± 0.3 b*
finishing (day 105)								
FO	154.3 ± 2.4	202.8 ± 2.3	0.98 ± 0.0	1.02 ± 0.0	1.33 ± 0.0	54.2 ± 4.2	21.0 ± 1.0	10.8 ± 0.4 ab
75PFAD/FO	157.6 ± 4.3	208.0 ± 6.4	0.99 ± 0.0	1.01 ± 0.0	1.36 ± 0.0	54.3 ± 3.8	20.2 ± 0.7	10.0 ± 0.3 b
75PFAD/UF/FO	154.4 ± 4.1	193.5 ± 10.1	1.07 ± 0.1	1.00 ± 0.0	1.37 ± 0.0	57.2 ± 4.6	23.0 ± 1.8	11.3 ± 0.3 a

[a]Values are the means ± SEM, $n = 3$ for initial weight, final weight, SGR, and FER, and $n = 6$ for fillet weight, viscera weight, K, and VSI. Means in a column belonging to different feeding periods sharing different letters were significantly different ($P < 0.05$). An (*) represents significant difference ($P < 0.05$) in K and VSI between fish fed 75PFAD and fish fed 75PFAD then unfed for 7 days. FO, fish fed FO; 75PFAD, fish fed 75PFAD; 75PFAD/FO, fish fed 75PFAD then fed FOFD; 75PFAD/UF, fish fed 75PFAD then unfed for 7 days; 75PFAD/UF/FO, fish fed 75PFAD then unfed for 7 days then fed FOFD for 21 days.

An aliquot of the TLE (50 μL) was trans-methylated in methanol/chloroform/hydrochloric acid (10:1:1, v/v/v; 3 mL) for 2 h at 100 °C. After the addition of Milli-Q water (1 mL), the mixture was extracted with hexane/chloroform (4:1, v/v, 3×) to obtain fatty acid methyl esters (FAME). Samples with an internal injection standard (19:0 FAME) added were analyzed by gas chromatography (GC) using an Agilent Technologies 7890B GC (Palo Alto, California, USA) equipped with an Equity-1 fused silica capillary column (15 m × 0.1 mm i.d., 0.1 μm film thickness), a flame ionization detector (FID), a split/splitless injector and an Agilent Technologies 7683 B Series auto sampler. Helium was the carrier gas. Samples were injected in splitless mode at an oven temperature of 120 °C. After injection, the oven temperature was raised to 270 at 10 °C/min and finally to 310 at 5 °C/min. Peaks were quantified with Agilent Technologies ChemStation software (Palo Alto, California, USA). GC results are typically subject to an error of up to ±5% of individual component area.

Individual components were identified by mass spectral data and by comparing retention time data with authentic and laboratory standards. GC-mass spectrometric (GC-MS) analyses were performed on a Finnigan Thermoquest GCQ GC-MS fitted with an on-column injector and using Thermoquest Xcalibur software (Austin, Texas, USA). The GC was equipped with an HP-5 cross-linked methyl silicone fused silica capillary column (50 m × 0.32 mm i.d.). Helium was used as the carrier gas, with the operating conditions previously described.[27]

Biometrics and Statistical Analysis. Specific growth rate (SGR) was calculated as SGR (%/d) = 100 × (ln W_f/ln W_i)/d, where W_f and W_i are the final and initial weights (g) and d is the number of days of the experiment. Feed consumption (FC) was calculated as the total average amount of dry feed (g) consumed per tank over the number of days of the experiment. The feed efficiency ratio was calculated as FER (g/g) = total weight gain (g)/FC (g). Condition factor (K) was calculated as K (%) = 100 × (W/FL^3), where FL is the fork length (cm). The viscera somatic index (VSI) was calculated as VSI = 100 × (weight of viscera/W). Values are reported as the mean ± standard error of the mean (SEM). Normality and homogeneity of variance were confirmed, and percentage data were arcsine transformed prior to analysis. Samples from individual fish ($n = 6$) were compared between treatment means for FA composition, and growth performance comparison was by independent samples t test and one way ANOVA followed by multiple comparisons using Tukey–Kramer HSD wherever applicable. Significance was accepted at probabilities $P < 0.05$.

The dilution model is expressed as follows:[28]

$$P_t = P_r + (P_i - P_r)/(Q_t - Q_i)$$

where P_t is defined as the percentage of FA in the fillet and viscera of fish fed 75% PFAD/FO at time t, P_i is the initial percentage of FA in the fillet of fish previously fed 75% PFAD, and P_r is the percentage of FA in the fillet and viscera of fish fed FO throughout at time t. Q_i is the initial total amount of lipid present, and Q_t is the amount present at time t in the fillet and viscera of fish fed 75% PFAD/FO. A regression analysis between predicted (from the dilution model) and observed FA values was performed, and individual regression lines were compared to the line of equity.[14] Analysis of covariance (ANCOVA) was used to compare slopes and intercept of regression lines to the line of equity. Statistical analysis was performed using SPSS for Windows, version 16.0.

RESULTS

Growth. There were no significant differences in SGR, FER, and body indices between FO and 75PFAD fish during the grow-out period (Table 2). Unfed fish (75PAFD/UF) had lower viscera weight, K, and VSI than those of FO and 75PFAD/FO fish after the first 7 days of the FOFD period. Similarly, 75PAFD/UF fish had lower viscera weight, K, and VSI when compared to those of 75PFAD fish. There was no significant difference for all treatments in SGR, FER, and body indices except for lower VSI in 75PFAD/FO fish at the end of the 28 days of the FOFD period.

Fatty Acid Analyses: Diet. Total SFA and total MUFA were higher in 75PFAD than in the FO diet due to 2-fold higher 16:0 and 3-fold higher 18:1n-9c (Table 3). There was higher PUFA in the FO diet compared to that in the 75PFAD, particularly total n-3 and total n-3 LC-PUFA. The n-3:n-6 ratio was higher in the FO diet compared to that in the 75PFAD.

Apparent Digestibility (AD). AD generally decreased with increasing FA chain length and increased with increasing degree of FA unsaturation (Table 4). AD of total PUFA was highest followed by total MUFA and was lowest for total SFA. AD of all SFA measured was higher for 75PFAD compared to the FO diet. AD of total MUFA and total PUFA was lower for 75PFAD than that for the FO diet.

Lipid and Fatty Acid Analyses: Fillet. Fish fed the FO diet throughout generally had the highest relative levels in fillet (as % of total FA) of EPA, DHA, total n-3, n-3 LC-PUFA, and n-3:n-6 ratio at all three sampling points. One exception was for the 75PFAD/UF fish where DHA was not significantly different from that of the FO fish (Table 5). Total SFA (as

Table 3. Fatty Acid Composition (% Total Fatty Acids) of Experimental Diets[a]

FA	diet	
	FO	75PFAD
14:0	3.3 ± 0.1 a	1.5 ± 0.1 b
16:0	19.9 ± 0.0 b	38.2 ± 0.1 a
17:0	0.5 ± 0.0	0.3 ± 0.0
18:0	4.1 ± 0.0	4.5 ± 0.0
other SFA[b]	1.3 ± 0.0 a	0.4 ± 0.1 b
16:1n-7c	9.2 ± 0.0 a	2.6 ± 0.0 b
18:1n-7c	4.0 ± 0.0 a	1.9 ± 0.0 b
18:1n-9c	11.0 ± 0.1 b	29.4 ± 0.1 a
20:1n-7c	0.4 ± 0.0 a	0.1 ± 0.0 b
20:1n-9	0.9 ± 0.0 a	0.4 ± 0.0 b
22:1n-11c	0.6 ± 0.0 a	0.3 ± 0.0 b
24:1n-9c	0.4 ± 0.0 a	0.1 ± 0.0 b
other MUFA[c]	1.2 ± 0.0 a	0.3 ± 0.2 b
18:2n-6	3.6 ± 0.0 b	8.8 ± 0.0 a
20:4n-6	0.8 ± 0.0 a	0.3 ± 0.0 b
other n-6	0.6 ± 0.0 a	0.2 ± 0.0 b
18:3n-3	0.7 ± 0.0 a	0.4 ± 0.0 b
18:4n-3	2.6 ± 0.0 a	0.6 ± 0.0 b
20:5n-3	18.0 ± 0.0 a	4.8 ± 0.1 b
22:5n-3	2.1 ± 0.0 a	0.5 ± 0.0 b
22:6n-3	9.0 ± 0.0 a	2.8 ± 0.0 b
other n-3	1.7 ± 0.0 a	0.5 ± 0.0 b
other PUFA[c]	4.2 ± 0.0 a	1.1 ± 0.0 b
total SFA	29.1 ± 0.1 b	45.0 ± 0.0 a
total MUFA	27.7 ± 0.1 b	35.1 ± 0.1 a
total PUFA	43.2 ± 0.0 a	19.9 ± 0.1 b
total n-3	34.0 ± 0.0 a	9.5 ± 0.2 b
total n-3 LC-PUFA	30.8 ± 0.0 a	8.6 ± 0.0 b
total n-6	5.1 ± 0.0 b	9.2 ± 0.1 a
n-3:n-6	6.7 ± 0.1 a	1.0 ± 0.1 b

[a]Values are the means ± SEM, $n = 3$. Means in a row sharing different letters were significantly different ($P < 0.05$). FO, fish oil diet; 75PFAD, 75% palm fatty acid diet. [b]Saturated fatty acids. [c]Monounsaturated fatty acids. [c]Polyunsaturated fatty acids.

Table 4. Fatty Acid Apparent Digestibility (%) of Experimental Diets Fed to Atlantic Salmon[a]

FA	diet	
	FO	75PFAD
14:0	83.6 ± 1.3 b	92.5 ± 1.7 a
16:0	67.5 ± 1.7 b	78.5 ± 1.3 a
17:0	60.0 ± 1.6 b	81.2 ± 1.8 a
18:0	52.5 ± 2.2 b	75.1 ± 1.4 a
16:1n-7c	96.9 ± 0.1	96.5 ± 0.4
18:1n-7c	91.5 ± 0.2	91.1 ± 0.6
18:1n-9c	93.4 ± 0.2 a	90.6 ± 0.6 b
20:1n-7c	82.1 ± 0.5	92.3 ± 3.8
20:1n-9	89.8 ± 0.2	91.8 ± 1.3
22:1n-11c	86.0 ± 0.9	90.0 ± 2.7
24:1n-9c	64.8 ± 1.0 b	75.3 ± 2.8 a
18:2n-6	91.8 ± 0.1	93.2 ± 0.5
20:4n-6	98.9 ± 1.1	100.0 ± 0.0
18:3n-3	96.6 ± 2.0	96.7 ± 1.7
18:4n-3	99.1 ± 0.1	100.0 ± 0.0
20:5n-3	98.7 ± 0.1	97.7 ± 0.4
22:5n-3	97.5 ± 0.0	97.4 ± 1.5
22:6n-3	96.0 ± 0.1	95.0 ± 0.5
total SFA	67.3 ± 1.7 b	78.5 ± 1.3 a
total MUFA	93.2 ± 0.2 a	90.9 ± 0.6 b
total PUFA	97.6 ± 0.1 a	95.6 ± 0.4 b

[a]Values are the means ± SEM, $n = 3$. Means in a row sharing different letters were significantly different ($P < 0.05$). FO, fish oil diet; 75PFAD, 75% palm fatty acid diet.

% of total FA) was not significantly different between treatments after the 77 day grow-out period. Total MUFA level was higher for 75PFAD fish compared to that of FO fish, and total PUFA of FO fish was higher than that of 75PFAD fish after the grow-out period. EPA, DHA, and n-3 LC-PUFA of 75PFAD fish after grow-out were 51%, 25%, and 36%, respectively, lower than that of FO fish. After 7 days of feeding the FOFD, 75PFAD/FO fish had lower relative levels of DHA, total n-3, and n-3 LC PUFA than 75PFAD/UF fish (unfed for 7 days). Similarly, compared to 75 PFAD/UF fish, 75PFAD fish (grow-out) had lower relative levels of DHA, total n-3, and n-3 LC-PUFA. The lipid content (DM basis) of 75PFAD/UF fish was around 10% lower (78.5 mg/g) than for that of 75PFAD fish (89.9 mg/g) (Table 5).

At the end of the 21 day and 28 day FOFD periods for 75PFAD/UF/FO fish and 75PFAD/FO fish, respectively, 75PFAD/UF/FO fish had higher relative levels of EPA, total PUFA, total n-3, and n-3 LC-PUFA than 75PFAD/FO fish. The relative levels of EPA, DHA, and n-3 LC-PUFA in the fillet of PFAD/FO fish were restored to 62%, 80%, and 72%, respectively, of that of FO fish after 28 days of the FOFD period. Restoration of EPA, DHA, and n-3 LC-PUFA expressed as % composition in the fillet of 75PFAD/UF/FO fish was greater, reaching 75%, 86%, and 81%, respectively, of that of FO fish after a 21 day FOFD period.

FO fish had higher absolute amounts of n-3 LC-PUFA after a 77 day growth period compared to that of 75PFAD fish (Figure 2). In contrast to the relative percentage FA levels, there was no significant difference in absolute amounts of n-3 LC-PUFA between 75PFAD/UF fish and initial fish (75PFAD). Similarly, there was no difference in absolute amounts of n-3 LC-PUFA between 75PFAD/UF fish and 75PFAD/FO fish after 7 days of food deprivation and 7 days of FOFD, respectively. FO fish had higher amounts of n-3 LC-PUFA at the end of 28 days of FOFD compared to those of both the 75PFAD/FO fish and 75PFAD/UF/FO fish. We noted that the 75PFAD/UF/FO fish showed higher content (19.0 ± 0.3 mg/g) of n-3 LC-PUFA than that observed for 75PFAD/FO fish (17.0 ± 0.2 mg/g), although this difference was not significant (Figure 2). Similar to results comparing the relative levels of FA, absolute amounts of n-3 LC-PUFA in 75PFAD fish after grow-out were 37% lower than that of FO fish, and restoration of n-3 LC-PUFA content in 75PFAD/FO fish and 75PFAD/UF/FO fish after 28 days and 21 days of FOFD was 71% and 80%, respectively.

Lipid and Fatty Acid Analyses: Viscera. Fish fed FO throughout had the highest relative levels in the viscera of EPA, DHA, total n-3, n-3 LC-PUFA, and n-3:n-6 ratio at all three sampling points (Table 6). Total SFA was not different between treatments after the grow-out period. Total MUFA levels were higher for 75PFAD fish compared to those of FO fish, and total PUFA of FO fish was higher than that of 75PFAD fish after the grow-out period. EPA, DHA, and n-3 LC-PUFA of 75PFAD fish after grow-out were 62%, 34%, and 47% lower, respectively, than that of FO fish. After 7 days of feeding the FOFD, 75PFAD/FO fish had higher relative levels of DHA, total n-3, and n-3 LC PUFA than 75PFAD/UF fish

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Table 5. Fatty Acid Composition (% of Total FA) and Lipid Content (mg/g) of the Fillet of Atlantic Salmon Fed Either FO or 75PFAD during the Grow-out Period Followed by Either FOFD Periods of 7 Days and 28 Days or 7 Days of Food Deprivation and 21 Days of FOFD periods[a]

	grow-out (day 77)		FOFD or unfed (day 84)			FOFD (day 105)		
FA	FO	75PFAD	FO	75PFAD/FO	75PFAD/UF	FO	75PFAD/FO	75PFAD/UF/FO
14:0	2.6 ± 0.2 a	1.6 ± 0.2 b	1.8 ± 0.1	1.7 ± 0.1	1.5 ± 0.1	2.7 ± 0.2 a	2.0 ± 0.1 b	1.5 ± 0.0 b
16:0	18.1 ± 0.3 b	19.6 ± 0.4 a	18.4 ± 0.1	19.2 ± 0.3	19.5 ± 0.4	17.2 ± 0.6 b	19.1 ± 0.4 a	17.9 ± 0.0 ab
17:0	0.4 ± 0.0 a	0.3 ± 0.0 b	0.4 ± 0.0	0.2 ± 0.1	0.3 ± 0.0	0.4 ± 0.0 a	0.3 ± 0.0 b	0.3 ± 0.0 b
18:0	4.5 ± 0.1	4.5 ± 0.1	4.8 ± 0.0 a	4.6 ± 0.0 b	4.6 ± 0.1 b	4.5 ± 0.2	4.5 ± 0.0	4.4 ± 0.0
16:1n-7c	7.8 ± 0.2 a	4.6 ± 0.1 b*	7.5 ± 0.0 a	5.3 ± 0.1 b	4.1 ± 0.1 c	7.9 ± 0.4 a	5.9 ± 0.2 b	5.7 ± 0.2 b
18:1n-7c	4.4 ± 0.0 a	3.2 ± 0.0 b	4.4 ± 0.0 a	3.4 ± 0.0 b	3.0 ± 0.1 c	4.4 ± 0.0 a	3.7 ± 0.1 b	3.7 ± 0.1 b
18:1n-9c	13.6 ± 0.2 b	28.2 ± 1.0 a	13.9 ± 0.2 b	26.8 ± 0.2 a	25.8 ± 0.6 a	13.5 ± 0.6 b	24.0 ± 0.8 a	22.9 ± 0.1 a
20:1n-7c	0.3 ± 0.0 a	0.2 ± 0.0 b	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
20:1n-9	1.6 ± 0.1 b	1.9 ± 0.1 a	1.5 ± 0.0 b	1.9 ± 0.1 a	1.8 ± 0.1 ab	1.7 ± 0.1	1.8 ± 0.1	1.8 ± 0.1
22:1n-11c	0.6 ± 0.1	0.6 ± 0.0	0.8 ± 0.0 a	0.6 ± 0.1 ab	0.5 ± 0.0 b	0.6 ± 0.0	0.5 ± 0.0	0.6 ± 0.1
24:1n-9c	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
18:2n-6	3.7 ± 0.2 b	7.1 ± 0.2 a	3.6 ± 0.0 b	6.6 ± 0.0 a	6.6 ± 0.3 a	4.2 ± 0.6 b	6.5 ± 0.4 a	6.4 ± 0.1 a
20:4n-6	0.8 ± 0.0 a	0.6 ± 0.0 b*	0.8 ± 0.0 a	0.6 ± 0.0 b	0.8 ± 0.0 a	0.7 ± 0.0	0.6 ± 0.0	0.7 ± 0.1
18:3n-3	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.0 a	0.4 ± 0.0 b	0.4 ± 0.0 b	0.8 ± 0.2	0.5 ± 0.1	0.5 ± 0.0
18:4n-3	2.3 ± 0.0 a	1.2 ± 0.0 b	2.2 ± 0.1 a	1.3 ± 0.2 b	1.1 ± 0.0 b	2.2 ± 0.1 a	1.7 ± 0.1 b	1.7 ± 0.1 b
20:5n-3	10.8 ± 0.2 a	5.3 ± 0.3 b	10.8 ± 0.2 a	5.8 ± 0.0	6.1 ± 0.2	10.6 ± 0.4 a	6.6 ± 0.1 c	7.9 ± 0.2 b
22:5n-3	4.1 ± 0.1 a	2.4 ± 0.1 b	4.0 ± 0.0 a	2.6 ± 0.0 b	2.5 ± 0.0 b	4.0 ± 0.3 a	2.8 ± 0.1 b	3.1 ± 0.1 b
22:6n-3	15.9 ± 0.7 a	11.9 ± 0.7 b*	16.5 ± 0.1 a	12.3 ± 0.1 b	15.3 ± 0.7 a	15.9 ± 0.4 a	12.7 ± 0.3 b	13.7 ± 0.5 b
total SFA	26.4 ± 0.6	26.7 ± 0.5	26.3 ± 0.1	26.4 ± 0.2	26.4 ± 0.6	25.9 ± 0.7	26.7 ± 0.8	25.1 ± 0.1
total MUFA	29.7 ± 0.4 b	39.9 ± 0.8 a	29.8 ± 0.3 c	39.4 ± 0.2 a	36.7 ± 1.0 b	29.5 ± 0.5 b	37.1 ± 0.7 a	36.0 ± 0.5 a
total PUFA	43.9 ± 0.8 a	33.4 ± 1.3 b	43.9 ± 0.2 a	34.2 ± 0.3 c	36.8 ± 0.4 b	44.5 ± 0.3 a	36.2 ± 0.1 c	38.9 ± 0.6 b
total n-3	36.2 ± 0.9 a	22.8 ± 1.3 b*	36.5 ± 0.1 a	24.1 ± 0.2 c	26.7 ± 0.7 b	36.1 ± 0.4 a	26.1 ± 0.3 c	28.9 ± 0.5 b
total n-6	5.4 ± 0.3 b	9.4 ± 0.2 a	5.2 ± 0.0 b	8.6 ± 0.1 a	8.9 ± 0.3 a	5.9 ± 0.7 b	8.4 ± 0.4 a	8.3 ± 0.2 a
n-3 LC-PUFA	33.2 ± 1.0 a	21.1 ± 1.2 b*	33.7 ± 0.2 a	22.4 ± 0.1 c	25.2 ± 0.8 b	33.0 ± 0.5 a	23.9 ± 0.3 c	26.6 ± 0.6 b
n-3: n-6	6.8 ± 0.4 a	2.4 ± 0.2 b	7.0 ± 0.0 a	2.8 ± 0.1 b	3.0 ± 0.1 b	6.2 ± 0.7 a	3.1 ± 0.2 b	3.5 ± 0.0 b
			Lipid Content (mg/g)					
dry	90.4 ± 9.2	90.1 ± 1.4*	88.8 ± 6.7	89.9 ± 2.2	78.5 ± 2.8	105.2 ± 3.6	102.7 ± 4.9	106.7 ± 1.8
Wet	28.1 ± 3.4	25.3 ± 0.5	25.7 ± 1.4	26.5 ± 1.0	22.0 ± 1.1	31.2 ± 1.1	31.9 ± 1.1	31.9 ± 1.1

[a]Values are the means ± SEM, $n = 6$. Means in a row belonging to different feeding periods sharing different letters were significantly different ($P < 0.05$). An (*) represents significant difference ($P < 0.05$) in % FA and lipid content between fish fed 75 PFAD and fish fed 75PFAD then unfed for 7 days. FO, fish fed FO; 75PFAD, fish fed 75PFAD; 75PFAD/FO, fish fed 75PFAD then fed FOFD; 75PFAD/UF, fish fed 75PFAD then unfed for 7 days; 75PFAD/UF/FO, fish fed 75PFAD then unfed for 7 days then fed FOFD for 21 days.

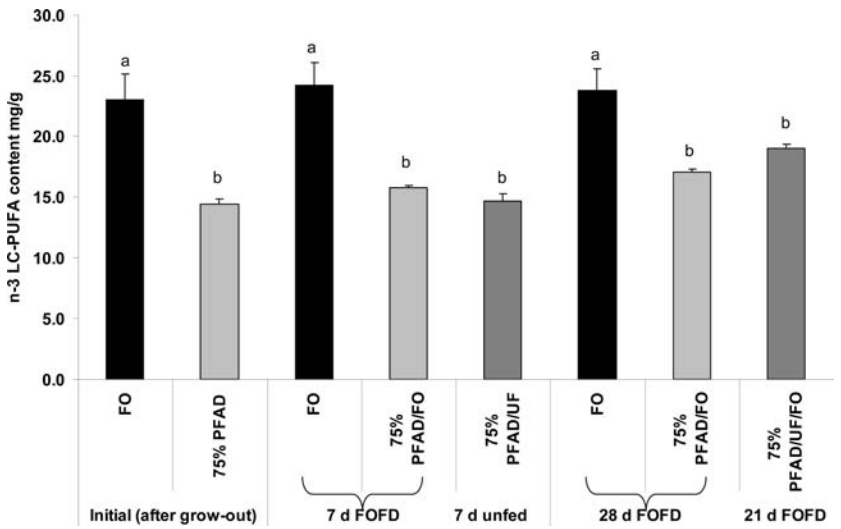

Figure 2. n-3 LC-PUFA content (mg/g) in the fillet of Atlantic salmon fed the 75PFAD diet and FO diet for 77 days followed by either 28 days of feeding on FOFD (75PFAD/FO fish) or 7 days of food deprivation and 21 days of feeding on FOFD (75PFAD/UF/FO). d denotes periods in days. Values are the means ± SEM, $n = 6$. Different letters represent significant differences ($P < 0.05$) between treatments at the same sampling periods.

(unfed for 7 days). In contrast to the fillet, there was no significant difference for the viscera in the relative levels of any FA inclusive of total n-3, total PUFA, and n-3 LC-PUFA between 75PFAD fish (after grow-out) and 7 day unfed fish

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Table 6. Fatty Acid Composition (% of Total FA) and Lipid Content (mg/g) of the Viscera of Atlantic Salmon Fed Either FO or 75PFAD during the Grow-out Period Followed by Either FOFD Periods of 7 Days and 28 Days or 7 Days of Food Deprivation and 21 Days of FOFD Periods[a]

	grow-out (day 77)		FOFD or unfed (day 84)			FOFD (day 105)		
FA	FO	75PFAD	FO	75PFAD/FO	75PFAD/UF	FO	75PFAD/FO	75%PFAD/UF/FO
14:0	2.9 ± 0.1 a	1.1 ± 0.1 b	2.8 ± 0.2 a	1.1 ± 0.1 b	1.3 ± 0.0 b	2.3 ± 0.0	1.9 ± 0.1	2.2 ± 0.2
16:0	16.1 ± 0.1 b	17.6 ± 0.3 a	16.6 ± 0.1	16.7 ± 0.4	17.7 ± 0.4	16.3 ± 0.1	17.4 ± 0.2	17.5 ± 0.4
17:0	0.4 ± 0.0 a	0.3 ± 0.0 b	0.4 ± 0.0 a	0.3 ± 0.0 b	0.3 ± 0.0 b	0.4 ± 0.0 a	0.3 ± 0.0 b	0.3 ± 0.0 b
18:0	4.0 ± 0.0	4.4 ± 0.1	4.1 ± 0.0 b	4.5 ± 0.1 a	4.3 ± 0.0 ab	4.3 ± 0.0	4.3 ± 0.0	4.2 ± 0.1
16:1n-7c	8.9 ± 0.0 a	4.7 ± 0.1 b	9.3 ± 0.0 a	5.2 ± 0.1 b	4.8 ± 0.1 b	8.9 ± 0.0 a	6.0 ± 0.2 b	6.4 ± 0.1 b
18:1n-7c	4.6 ± 0.1 a	3.3 ± 0.0 b	4.5 ± 0.0 a	3.5 ± 0.1 c	3.4 ± 0.0 b	4.7 ± 0.1 a	3.7 ± 0.0 b	3.7 ± 0.1 b
18:1n-9c	14.2 ± 0.2 b	33.8 ± 0.4 a	14.9 ± 0.1 c	30.9 ± 0.6 b	33.8 ± 0.2 a	15.4 ± 0.2 b	28.4 ± 1.0 a	28.1 ± 0.6 a
20:1n-7c	0.4 ± 0.0 a	0.2 ± 0.0 b	0.4 ± 0.0 a	0.3 ± 0.0 b	0.2 ± 0.0 b	0.4 ± 0.0 a	0.3 ± 0.0 b	0.3 ± 0.0 b
20:1n-9	1.7 ± 0.0 b	2.2 ± 0.0 a	1.9 ± 0.1 b	2.4 ± 0.1 a	2.2 ± 0.0 a	1.8 ± 0.0 c	2.0 ± 0.0 a	1.9 ± 0.0 b
22:1n-11c	0.8 ± 0.1	0.6 ± 0.1	0.9 ± 0.0	0.8 ± 0.1	0.7 ± 0.0	0.8 ± 0.0 a	0.6 ± 0.0 b	0.6 ± 0.0 b
24:1n-9c	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
18:2n-6	4.0 ± 0.0 b	8.5 ± 0.3 a	4.2 ± 0.0 c	7.6 ± 0.2 b	8.7 ± 0.1 a	4.1 ± 0.0 b	7.1 ± 0.2 a	7.3 ± 0.4 a
20:4n-6	0.8 ± 0.0 b	0.5 ± 0.0 a	0.8 ± 0.0 a	0.5 ± 0.0 b	0.4 ± 0.0 b	0.7 ± 0.0 a	0.5 ± 0.0 b	0.6 ± 0.0 b
18:3n-3	0.7 ± 0.0	0.6 ± 0.1	0.7 ± 0.0 a	0.6 ± 0.1 ab	0.4 ± 0.0 b	0.7 ± 0.0 a	0.7 ± 0.0 a	0.6 ± 0.1 b
18:4n-3	2.7 ± 0.0 a	1.3 ± 0.0 b	2.7 ± 0.0 a	1.6 ± 0.1 b	1.3 ± 0.0 c	2.7 ± 0.1 a	1.6 ± 0.2 b	1.7 ± 0.1 b
20:5n-3	11.7 ± 0.3 a	4.4 ± 0.1 b	10.9 ± 0.1 a	5.4 ± 0.3 b	4.5 ± 0.1 b	11.3 ± 0.1 a	6.4 ± 0.2 b	6.6 ± 0.3 b
22:5n-3	4.2 ± 0.1 a	2.4 ± 0.1 b	4.2 ± 0.0 a	2.8 ± 0.1 b	2.5 ± 0.1 c	4.3 ± 0.0 a	2.9 ± 0.1 b	2.9 ± 0.1 b
22:6n-3	11.9 ± 0.1 a	7.8 ± 0.0 b	11.5 ± 0.1 a	9.0 ± 0.1 b	7.7 ± 0.1 c	11.9 ± 0.0 a	8.7 ± 0.2 b	8.3 ± 0.2 b
total SFA	24.8 ± 0.1	24.0 ± 0.5	25.2 ± 0.2 a	23.2 ± 0.3 b	24.2 ± 0.4 ab	24.5 ± 0.2	24.9 ± 0.2	25.0 ± 0.3
total MUFA	32.0 ± 0.3 b	45.8 ± 0.1 a	33.3 ± 0.2 c	44.1 ± 0.4 b	46.0 ± 0.1 a	33.2 ± 0.1 b	42.1 ± 0.7 a	41.7 ± 0.3 a
total PUFA	43.2 ± 0.4 a	30.2 ± 0.5 b	41.5 ± 0.1 a	32.7 ± 0.7 b	29.9 ± 0.3 c	42.3 ± 0.3 a	33.0 ± 0.6 b	33.3 ± 0.4 b
total n-3	34.2 ± 0.4 a	18.2 ± 0.4 b	32.6 ± 0.1 a	21.3 ± 0.7 b	17.9 ± 0.3 c	33.8 ± 0.2 a	22.1 ± 0.8 b	22.1 ± 0.6 b
total n-6	6.1 ± 0.1 b	10.7 ± 0.3 a	5.9 ± 0.1 c	9.8 ± 0.1 b	10.6 ± 0.1 a	5.8 ± 0.0 b	9.2 ± 0.3 b	9.4 ± 0.4 b
n-3 LC-PUFA	30.8 ± 0.4 a	16.3 ± 0.2 b	29.3 ± 0.1 a	19.1 ± 0.6 b	16.1 ± 0.3 c	30.3 ± 0.2 a	19.9 ± 0.6 b	19.8 ± 0.5 b
n-3: n-6	5.6 ± 0.1 a	1.7 ± 0.1 b	5.5 ± 0.1 a	2.2 ± 0.1 b	1.7 ± 0.0 c	5.9 ± 0.0 a	2.4 ± 0.2 b	2.4 ± 0.2 b
				Lipid Content (mg/g)				
dry	757.5 ± 20.7	729.2 ± 18.6	756.1 ± 20.8	732.6 ± 38.6	776.7 ± 41.7	779.1 ± 25.1	742.1 ± 5.6	751.1 ± 14.5
wet	380.6 ± 29.1	369.8 ± 12.8	399.6 ± 24.2	370.2 ± 20.7	391.4 ± 10.7	404.2 ± 4.4	393.3 ± 9.2	411.7 ± 10.5

[a]Values are the means ± SEM, $n = 6$. Means in a row belonging to different feeding periods sharing different letters were significantly different ($P < 0.05$). FO, fish fed FO; 75PFAD, fish fed 75PFAD; 75PFAD/FO, fish fed 75PFAD then fed FOFD; 75PFAD/UF, fish fed 75PFAD then unfed for 7 days; 75PFAD/UF/FO, fish fed 75PFAD then unfed for 7 days then fed FOFD for 21 days.

(75PFAD/UF). There was also no significant difference in lipid content of viscera between 75PFAD/FO fish and 75PFAD/UF fish. These findings indicate that 7 days of food deprivation did not affect the FA profile and lipid content of the viscera. At the end of the 28 day and 21 day FOFD period for 75PFAD/FO fish and 75PFAD/UF/FO fish, respectively, there was no significant difference in the FA profile of the viscera. Relative (% of total FA) levels of EPA, DHA, and n-3 LC-PUFA in the viscera of 75PFAD/FO fish and 75PFAD/UF/FO fish after 28 days and 21 days of FOFD, respectively, were restored to 56%, 71%, and 66% of that of FO fish.

Regression Analyses. The regression line between predicted (from dilution model) and observed %FA values for fillet and viscera were highly significant ($R^2 = 0.99$, $P < 0.001$) (Figure 3). The regression line had a strong degree of similarity with the line of equity; the line crossed at the origin and slope was close to 1. The regression equations for fillet and viscera are given, respectively, as predicted = 0.95 and observed = +0.215; and predicted = 1.00 and observed = −0.003.

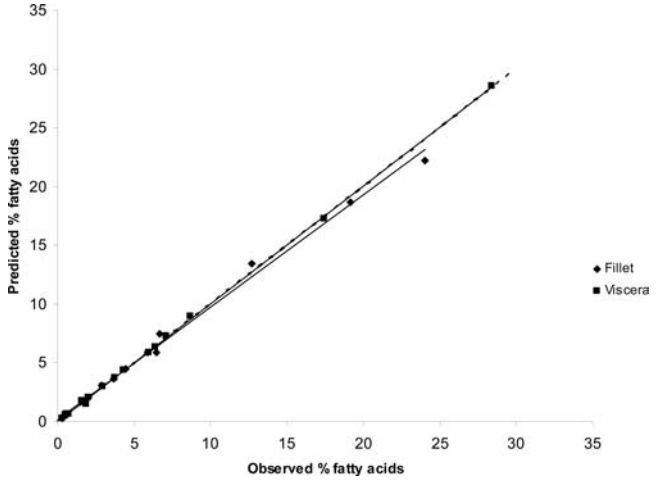

Figure 3. Total observed and predicted (from dilution model) fatty acid percentages (14:0, 16:0, 17:0, 18:0, 16:1n-7c, 18:1n-7c, 18:1n-9c, 20:1n-7c, 20:1n-9, 22:1n-11c, 24:1n-9c, 18:2n-6, 20:4n-6, 18:3n-3, 18:4n-3, 20:5n-3, 22:5n-3, and 24:6n-3) in the fillet and viscera of Atlantic salmon after dietary shift from 75PFAD for 77 days to FO for 28 days (75PFAD/FO).

DISCUSSION

Growth and FA Apparent Digestibility. The evaluation of alternate oils to replace FO in aquafeeds for Atlantic salmon has been the subject of a range of studies.[2−6] Suitable candidate oils should be less expensive than FO and should not compromise fish health and growth. Various oils of vegetable and in some cases animal origin have thus become popular

substitutes for FO in aquafeeds. Palm-based oils are alternative oils characterized by high amounts of SFA. However, palm-based oils are presently not preferred FO substitutes in aquafeeds for cold water species such as Atlantic salmon due to the reduction in energy availability associated with the low digestibility of SFA.[29] PFAD, a byproduct of production of crude palm oil, is rich in free fatty acids (~80%); the use of PFAD has been recently shown to markedly improve the AD of SFA in rainbow trout fed a diet where FO was substituted by 50% and 75% PFAD at 15 and 20 °C.[16] Similarly, in the present study we observed that a 75PFAD diet improved the AD of SFA in Atlantic salmon, especially that of palmitic acid (16:0), which is the most abundant FA in PFAD. The high levels of free fatty acids in PFAD was identified as the reason for the high SFA digestibility by enhancing digestion and absorption of free FA as opposed to SFA in the form of triacylglycerols (TAG).[16] In the present study, growth was not impaired by replacing 75% of FO with PFAD in the diet. The improvement in AD of SFA, in particular that of palmitic acid, in PFAD clearly increased energy availability. Surprisingly though, the AD of MUFA and PUFA by Atlantic salmon in our study was negatively affected by PFAD, which is in contrast to a FA digestibility study for rainbow trout.[16] Although the AD of MUFA and PUFA was significantly lower, albeit by only a small amount (~2%), the relatively large improvement in AD of SFA (~11%) compensated for the small reduction in AD of MUFA and PUFA from the 75PFAD. It could be argued that since dietary SFA was high for 75PFAD and that the AD of SFA was markedly increased hence increasing energy availability, that the growth of 75%PFAD fish should be higher than FO fish. However, the FO diet had relatively high PUFA content with an AD of >90% which compensates for the energy discrepancy viz. SFA digestible energy from 75PFAD. A simple computation of total apparent digestible FA intake per fish during the grow-out period (average total food consumption × dietary FA composition × AD of individual FA) gives a total of 13.1 g FA per fish for FO and 13.7 g FA per fish for 75% PFAD. The AD of FA was highest for PUFA followed by MUFA and was lowest for SFA, which agrees with other FA digestibility studies for salmonids.[16,30] Furthermore, AD generally decreased with increasing FA chain length and increased with increasing degree of unsaturation.[16,29,31] These findings suggest that high dietary SFA from PFAD does not pose any restriction for Atlantic salmon smolt due to the improved SFA digestibility. It is important to note that our experiment was performed at 15 °C; this is typical of Tasmanian water temperatures and increasingly typical of temperatures experienced globally in Atlantic salmon aquaculture.[6,27] It would be of interest to compare the digestibility and growth of Atlantic salmon fed a PFAD-based diet at lower temperatures.

FA Profile: Grow-Out Period. A common observation in all studies on FO substitution in aquafeeds is that the FA profile of fish generally mirrors the FA profile of the diet. In contrast, it was observed that the relative levels of SFA in Atlantic salmon fillet and viscera were not different between FO and 75PFAD fish despite a 1.5-fold higher dietary SFA in 75PFAD. It has been suggested that suitable alternate oils should contain set proportions of SFA and MUFA (typically around 33% each) and particularly of palmitic acid and oleic acid (18:1n-9) because these FA are preferentially used for energy production by fish.[2] Palmitic acid was approximately 2-fold higher in the 75PFAD diet but was only marginally higher in both fillet and viscera of 75PFAD fish. Since the net intake of SFA was higher for 75PFAD fish and SFA were not largely accumulated relative to FO fish, SFA, particularly palmitic acid, were most probably the preferred FA for β-oxidation. While there is a preferential order for FA β-oxidation, this is subservient to dietary FA being present in excess;[5] hence, it may be argued that excess dietary n-3 LC-PUFA in FO fish may have been extensively used for energy production, thus resulting in relative SFA accumulation in FO fish. Therefore, both preferential FA β-oxidation and differential lipogenic activity may have contributed to the observed SFA composition between 75PFAD and FO fish.

FA Profile: Fish Oil Finishing Diet Period. Similar to all studies involving FO substitution in aquafeeds, the main drawback with the use of PFAD remains the reduced levels of n-3 LC-PUFA in the fillet after growing on this VO-based diet. Feeding a FOFD for a time before harvest is a suitable way to restore n-3 LC-PUFA levels in fish.[32] In the present study, 28 days of feeding of the FOFD restored n-3 LC-PUFA levels in the fillet and viscera of 75PFAD fish to 72% and 66% of that of FO fish. The dynamics of n-3 LC-PUFA restoration in the fillet and viscera followed principally the dilution of existing FA stores, and there was no evidence of preferential FA metabolism occurring. In general, small fish such as those used in this present study, respond more quickly to dietary FA changes, and thus, for market sized Atlantic salmon a longer FOFD period would be required for n-3 LC-PUFA restoration. Thus, to achieve complete restoration of n-3 LC-PUFA, longer growth on the FOFD would be required, which could undermine the very purpose of FO substitution in aquafeeds.[2]

FA Profile: Short-Term Food Deprivation Followed by a FOFD Period. Another strategy to improve the efficiency of n-3 LC-PUFA restoration, when applying a FOFD, is to reduce the initial lipid content in fish after growth on the alternate oil diet prior to commencing feeding on the FOFD.[33] Food deprivation is one way to reduce the lipid content in fish. The way fish lose lipid seems to be species-specific, and this has to be considered before applying this strategy.[33] When deprived of food, Murray cod uses principally protein and hepatic lipid stores as their energy source.[33,34] In a study with Murray cod, fish deprived of food for 5, 10, and 15 days did not lose lipid from the fillet, and the hypothesis that lowering lipid content prior to feeding the FOFD would improve restoration of n-3 LC-PUFA could not be verified for this freshwater species.[33]

In Atlantic salmon, the fillet lipid content decreased most, followed by that of the viscera and the liver after food deprivation.[18] Consistent with these findings for Atlantic salmon in the present study, there was a significant reduction in fillet lipid content (dry weight basis) during starvation. The reduced K values in unfed fish further supports this result. There was also a reduction in VSI of unfed fish, which indicated a loss in visceral fat. However, there was no difference in visceral lipid content between fed and unfed fish. The probable reason for the "apparent" reduction in VSI in unfed fish was because fed fish might still contain residual feed in their gastrointestinal tract at sampling compared to unfed fish. For the same reason, the lipid content in the viscera of large Atlantic salmon (5 kg) unfed for 7−86 days was even higher compared to the fed group.[18] The effect of lipid loss from the fillet of Atlantic salmon in the present study was reflected by higher relative levels of n-3 LC-PUFA, specifically that of DHA in unfed fish compared to that in fed fish (albeit fish fed FO). Upon feed deprivation, fish used TAG rich lipid stores in the fillet for energy production, thus resulting in an increase in polar lipid (PL) relative to TAG. An increase in PL will lead to

an increase in relative levels of n-3 LC-PUFA, particularly that of DHA, which is abundant in PL. Subsequently, feeding a FOFD after a food deprivation period improved the efficiency of n-3 LC-PUFA restoration. However, in terms of the absolute amounts of n-3 LC-PUFA, there was no significant difference between fish fed for 28 days with FOFD and fish unfed for 7 days then fed for 21 days with FOFD. During the FOFD period, fish were fed to satiation to compensate for growth and to restore total lipid content in the fish that were unfed for 7 days. Feed consumption (g/fish) was lower for 75PFAD/UF/FO compared to 75PFAD/FO and FO fish (37.1 ± 10.0 b, 49.9 ± 2.1 a, 47.6 ± 0.1 a), though final weights, SGR, and FER were not different. This was probably due to large variations in fish size and the relative lipid contact between small and large fish especially for the refeeding of unfed fish after 7 days. In comparison to the fillet, the FA composition in viscera of unfed fish was similar to the composition in the viscera of fish fed 75% PFAD after grow-out (75% PFAD). In harvest size Atlantic salmon after food deprivation periods ranging from 7 to 86 days, the lipid level in the viscera was relatively constant.[18] In the present study, there were higher relative levels of n-3 LC-PUFA inclusive of DHA in the viscera of fish fed for 7 days of FOFD (PFAD/FO) compared to 7 days without feeding (PFAD/UF). This suggests that depletion of FA stores in early stages of food deprivation did not occur in the viscera and that the deposition of new FA stores occurred in the viscera immediately after the switch to a new diet. When fed to satiation on the FOFD for 21 days after 7 days of food deprivation, the relative levels of n-3 LC-PUFA were similar to that of the fish fed for 28 days.

As highlighted earlier, a key element in the restoration of n-3 LC-PUFA in Atlantic salmon is the reduction of initial lipid content in the fillet, which can be achieved by a period of food deprivation. Since the energy requirement in unfed fish depends on water temperature and body weight,[35] the loss of lipid in the fillet of fish will increase with increasing temperature and decrease with increasing body weight. In this study, Atlantic salmon were ∼150 g, and the temperature was 15 °C. Both the fish weight and temperature were favorable for significant lipid loss in the fillet after 7 days of food deprivation. In larger Atlantic salmon of ∼5 kg weight and at a temperature averaging 4.1 °C, there was a small but significant reduction (1.4%) in lipid content of the muscle after 58 and 86 days of food deprivation.[18] In Atlantic salmon of 3.5 kg weight at temperatures ranging from 3.2 to 4.9 °C, there was a reduction of fillet fat content (2−3%) after 110 days of food deprivation.[19] In Atlantic salmon of 2 kg weight, there was a more pronounced reduction in fillet fat (2−4%) after 35 and 78 days of food deprivation.[17] Since the harvest size of Atlantic salmon is generally around 4 kg and feeding with a FOFD should occur a few months prior to harvest, it is very likely that several months of food deprivation would be needed to significantly reduce the lipid content in fish fillet. However, as temperature will affect the reduction in lipid content, a higher temperature, such as that in this present study (15 °C) and as now commonly occurs in Tasmanian waters in the summer period, may shorten the food deprivation period. In light of the above observations, the next steps to attempt to improve the efficiency of n-3 LC-PUFA restoration in harvest size Atlantic salmon by using a FOFD can include targeting summer months for a food deprivation period. These should also include an evaluation of the viability of a relatively longer food deprivation period in a commercial production system with market size fish.

A 75% FO substitution by PFAD in the diet of Atlantic salmon smolt did not impair fish growth and the AD of SFA was markedly improved using 75PFAD. Feeding with 75PFAD for 77 days resulted in lower relative levels and absolute amounts of n-3 LC-PUFA in the fillet. Subsequent feeding on a FOFD for 28 days restored n-3 LC-PUFA relative levels and absolute amounts to 72% and 71%, respectively, of that of fish fed FO throughout. It was also shown that a short-term food deprivation period of 7 days prior to feeding a FOFD for 21 days improved n-3 LC-PUFA restoration (to 81% and 80% for relative levels and absolute amounts, respectively) in the fillet of fish previously fed on 75 PFAD.

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