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ORIGINAL ARTICLE

Fish Oil Finishing Diet Maintains Optimal n-3 Long-Chain Fatty Acid Content in European Whitefish (*Coregonus lavaretus*)

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Abstract This study examined the effect of substituting vegetable oil for fish oil in feed, with subsequent re-introduction of fish oil-rich feed (finishing feeding) in late stages of growth, on the fatty acids of cultivated European whitefish (*Coregonus lavaretus*). Restorative finishing feeding with fish oil-rich feed for 15 and 25 weeks was sufficient to change the total content of nutritionally valuable long-chain n-3 fatty acids, eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3), to correspond to that of fish fed the fish oil-rich feed throughout their lifespan. Under natural conditions, 15 and 25 weeks correspond to weight gains of 75% and 100% (i.e. doubling), respectively. Also, the fatty acid profile of the fish was restored after finishing periods of 15 and 25 weeks. Limiting the use of fish oil by lowering the overall fat content of the feed (no vegetable oil added) resulted in a decrease in the long-chain n-3 fatty acids. Based on the results, after receiving a vegetable oil-rich diet, restorative fish oil-rich feeding in the last stages of growth in European whitefish is nutritionally justified in order to balance nutritional gain for consumers with sustainable use of finite marine oils. The results encourage commercial efforts to further utilize and optimize finishing feeding practices.

Keywords Aquaculture · *Coregonus lavaretus* · Fatty acids · Feed · Rapeseed

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Abbreviations

CHD	Coronary heart disease
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FAME	Fatty acid methyl ester
FOF	Fish oil-rich high-fat feed
GC-FID	Gas chromatography-flame ionization detection
LFF	Fish oil-rich low-fat feed
PUFA	Polyunsaturated fatty acid
VOF	Vegetable oil-rich high-fat feed

Introduction

European whitefish (*Coregonus lavaretus*) is a high-value salmonid fish commercially available as both farmed and wild varieties in the Nordic countries, especially Finland. Sustaining the high quality of commercial European whitefish is of great importance, since the fish and fish products need to be distinguished from cheaper, imported white-fleshed fish species such as lake whitefish (*Coregonus clupeaformis*) and basa fish (*Pangasius bocourti*).

In our previous study [1], we showed that farmed European whitefish is rich in vitamin D and has considerably higher fat content than the wild-grown fish. High content of the nutritionally valuable fatty acids is an important quality criterion of fatty fish. The health benefits of the n-3 PUFAs in fish have been well documented [2, 3]. Nonetheless, in recent years, due to availability and price constraints, the proportion of vegetable oil relative to fish oil in fish feed has increased [4, 5], which has implications for the fatty acid composition of the product. When fish oil is replaced with vegetable oils in the feed, the proportion of nutritionally valuable very-long-chain n-3 fatty acids decreases [6–8]. As fish consumption recommendations are not yet widely met,

this will make it even more difficult to gain the aforementioned nutritional benefits.

For juvenile fish, fish oil intake is regarded as beneficial or even essential, whereas during later stages, it can be replaced with vegetable oil without major effects on the growth and welfare of the fish [9, 10]. Feeding a vegetable oil-rich diet to fish spares the use of finite marine materials. To restore nutritionally beneficial fatty acid composition in fish fillets, a finishing diet with high fish oil content can be used in the late stage of fish growth [11, 12].

The present study examined the effect of timing of the restorative finishing feeding on the fatty acid composition of tissues, and hence the nutritional quality, of farmed European whitefish. In addition, the study evaluated the effect of the fat content of feed on fatty acid composition.

Materials and Methods

Chemicals and Reagents

Triheptadecanoin and 1,2-dinonadecanoyl-*sn*-glycero-3-phosphocholine were purchased from Larodan Fine Chemicals AB (Malmö, Sweden). Potassium chloride (p.a. grade) was purchased from Merck KGaA (Darmstadt, Germany) and boron trifluoride (~10% in methanol, p.a.) from Fluka (Buchs, Switzerland). Fatty acid methyl ester mixture (68D) was obtained from Nu-Chek Prep, Inc. All other reagents and solvents were of chromatography or analytical grade and were purchased from local suppliers.

Fish Feeds

European whitefish were fed either fish oil-rich low-fat feed (LFF), vegetable oil-rich high-fat feed (VOF), or fish oil-rich high-fat feed (FOF) (Table 1). LFF, used as the starting material for the other two feeds, was extruded, oil uncoated "Royal Silver" fish feed (Raisioagro Ltd, Raisio, Finland). In LFF, the fat originated primarily from fish meal. The oil content of the other two experimental feeds was increased either with a rapeseed oil/camelina oil mixture (1:1) to final fat content (VOF) or with pure fish oil to final fat content (FOF) (Table 2). Feed pellet size was adjusted depending on fish size according to the feed manufacturer's recommendations. Furthermore, the commercial formulation and feed composition complied with the requirements of the fish—for example, fishmeal content and protein composition was increased with decreasing fish size. The fatty acid composition of the feeds are presented in Fig. 1.

Experimental Fish and Study Design

The experiment started in mid-June 2009, when fish with an average weight of 30 g (commercial strain, Savon Taimen Ltd.) were transferred from a freshwater farm to the fisheries research station (Game and Fisheries Research Institute, Rymättylä, Finland). The three experimental diets were fed to triplicate groups of fish (310 in each VO cage, 180 fish

Table 1 Formulation of "Royal Silver" fish feed representing final pellet size of 5.0 mm used in the study

Feed ingredients	wt %
Fishmeal	32
Wheat	22
Soy protein concentrate	14
Wheat gluten	6
Premixes	3
Supplementary fish oil or vegetable oil mixture[a]	23

As the major oil ingredients, fish oil (FOF), vegetable oil mixture (VOF), or neither (LFF) were added to the feed

[a]Not added to the low-fat feed (LFF)

Table 2 Final fat content of the experimental feeds according to pellet size

Group Pellet size	FOF	VOF	LFF
1.7	22.2	22.2	13.8
2.5	23.7	23.5	15.4
3.5	21.4	21.7	12.9
5.0	24.3	24.1	11.3

Results expressed as wt%

FOF fish oil-rich high-fat feed, *VOF* vegetable oil-rich high-fat feed, *LFF* fish oil-rich low-fat feed

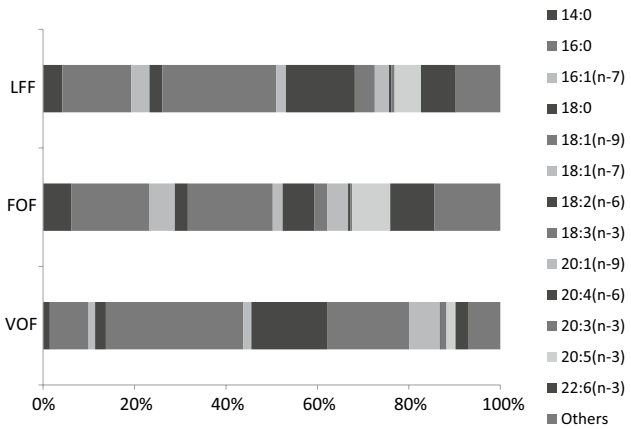

Fig. 1 Fatty acid composition of the feeds used for different groups of fish. See Table 2 for abbreviations

in others) according to the feeding tables by Raisioagro in brackish sea water in experimental net cages (4 × 4 × 4 m) under natural light and temperature conditions. The procedure followed typical farming conditions for European whitefish.

During the first growth period (June 2009–2010), fish reached an average weight of 300 g. Thereafter, fish doubled (LFF group) or more than doubled (other groups) their weight and were sampled three times during the experiment: at the beginning, middle and end of the second growth period (Fig. 2). At the time of harvest, the weight of energy-constrained fish (LFF) was less than 100 g lower than the fish in the other two groups. Nonetheless, all fish reached a size acceptable for price category II (400–800 g), and 17% reached a size for price category I (>800 g), which is the most valued and thus most desired target size in farming, above price categories II, III and IV.

On June 6 and September 30, fish from each VOF diet were marked by fin cutting and transferred to the FOF diet to study how efficiently and quickly the fatty acid composition of fish can be changed. Hence, the length of the restorative finishing feeding was either 25 weeks (from June to December; VOF/FOF1a), 15 weeks (from June to September; VOF/FOF1b) or 10 weeks (from September to December; VOF–FOF2).

Upon sampling, as part of customary commercial procedure, fish were given a percussive blow to the head, bled, gutted, and transferred on ice to a filleting plant where they were processed. One to 2 days after the slaughter, all fish were filleted. Five individual fish from the same cage were pooled together as one sample. Thereafter, the samples were transferred on ice for storage at −80 °C and subsequent analysis of triplicate samples of each fish group.

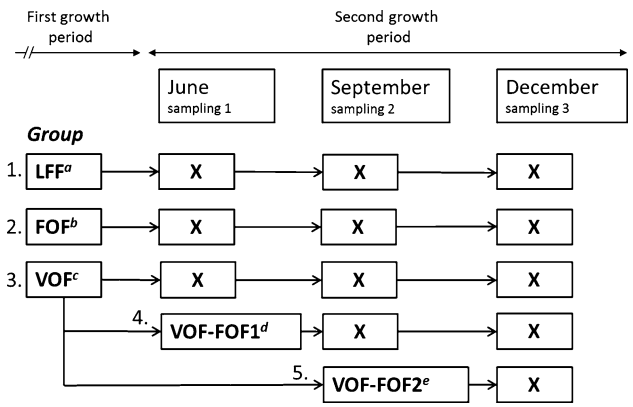

Fig. 2 Time of sampling (marked with *cross symbol*) and introduction of finishing feeding. Fish groups receiving *a* fish oil-rich low-fat (13%) feed; *b* fish oil-rich high-fat feed (23% fat); *c* vegetable oil-rich high-fat feed (23% fat); *d* fish oil-rich high-fat (23% fat) feed from June to December after initial vegetable oil-rich feeding; *e* fish oil-rich (23% fat) feed from September to December after initial vegetable oil-rich feeding

Lipid Extraction

Lipids were extracted from homogenized skinned fillets and from visceral fat deposits, i.e. storage fat, with chloroform/methanol (2:1, v/v) following Folch's procedure [13]. The sample treatment was carried out protected from light. The lipids were extracted from 2.5–3.5 g of skinned fillet sample or to 0.3–0.5 g of storage fat sample, and 0.88% (w/v) potassium chloride solution was used to wash the lipid extracts. The fat extracts were weighed and dissolved in 2 mL of chloroform. The extracts of duplicate samples were combined.

An aliquot of the combined lipid extract corresponding to 0.3 mg of lipids was transferred into separate glass tubes (duplicate samples). A 50 µL volume of each internal standard (triheptadecanoin in chloroform, 1 mg/mL and 1,2-dinonadecanoyl-*sn*-glycero-3-phosphocholine in chloroform, 0.1 mg/mL) was added.

Fatty Acid Methylation and Analysis

Fatty acid methyl esters (FAME) were prepared at 90–95 °C by boron trifluoride-catalyzed transesterification of the samples without a separate purification step [14, 15]. FAMEs (dissolved in hexane) were analyzed by gas chromatography with flame ionization detection (GC-FID) (PerkinElmer AutoSystem, Norwalk, CT) using a DB-23 column (60 m × 0.25 mm i.d., 0.25 µm film thickness; Agilent Technologies, Palo Alto, CA, USA). The FAMEs were identified with the help of 68D FAME mixture (Nu-Chek-Prep, Inc.).

Statistical Methods

IBM® SPSS® Statistics version 22 (Chicago, IL) was used for analysis of the data. Normal distribution of the chemical data was tested with the Shapiro–Wilk test. Homogeneity of variances was tested with the Levene's test, and the statistical differences between different groups were studied using the one-way analysis of variance (ANOVA) or the Brown–Forsythe test, depending on the homogeneity of variances. Tukey's honest significant difference (HSD) test and Tamhane's T2 tests were used as *post hoc* tests depending on the homogeneity of variances. When the chemical data were not normally distributed, the Kruskal–Wallis test and Mann–Whitney *U*-test with Bonferroni corrections were used. When only two groups were compared, either a *t* test or the Mann–Whitney *U*-test was used, depending on the distribution of the data.

Table 3 Amount of total lipids in the skinned whitefish fillets

Time point[a]	June	September	December
Group[c]			
FOF	7.6 ± 0.9	8.1 ± 1.5	9.4 ± 2.3
LFF	6.7 ± 0.5	6.0 ± 0.4[b]	6.8 ± 0.9
VOF	7.4 ± 0.8	8.5 ± 0.3	12.0 ± 1.6
VOF–FOF1	–	8.7 ± 1.3	9.1. ± 3.0
VOF–FOF2	–	–	10.3 ± 1.5

Results expressed as g/100 g (mean ± SD, $n = 3$)

[a]Time points: 1, June (sampling 1); 2, September (sampling 2); 3, December (sampling 3)

[b]$n = 2$

[c]See Fig. 2 for abbreviations

Results

The total lipid content of the pooled samples of skinned fish fillets is presented in Table 3. The lipid content of the fillets of fish fed a vegetable oil-rich diet until the end (group VOF) and the fish transferred to the finishing diet only in September (group VOF/FOF2) seemed to be higher in December compared with other groups, although the differences were not statistically significant (difference between groups LFF and VOF was close to significance, $p = 0.059$). Similarly, the lipid content of group LFF seemed to be lower than other groups in December. Moreover, fish in the LFF group remained behind in growth due to lower energy content in their feed compared to other treatments.

Differences in the total amounts of EPA and DHA per fresh weight of skinned fillets (g/100 g) between fish receiving different types of feed were evident already at the beginning of the second growth period (June 2010) (Fig. 3a). In September, the EPA and DHA contents of VOF–FOF1 were already at the same level as those of group FOF (Fig. 3b); the restorative diet with high-fish oil feed had at that point lasted for 15 weeks. However, after a 10-week restorative diet (September to December), the fatty acid composition of group VOF–FOF2 still seemed to be similar to that of group VOF (Fig. 3c). At each sampling time point, the amount of EPA and DHA had decreased considerably due to vegetable oil-rich high-fat and fish oil-based low-fat feeding.

Similarly, differences in the relative proportions of different fatty acids between skinned fillets of different fish groups were noted as early as June (Table 4). At each of the three sampling time points (June, September, December), the fatty acid composition somewhat reflected that of the oils used in the feeds (Tables 4, 5, 6; Fig. 1). Due to the solely fish-based fat in the feeds, the proportions of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) were higher in groups FOF and LFF than in group VOF. Correspondingly, the proportion of α-linolenic acid

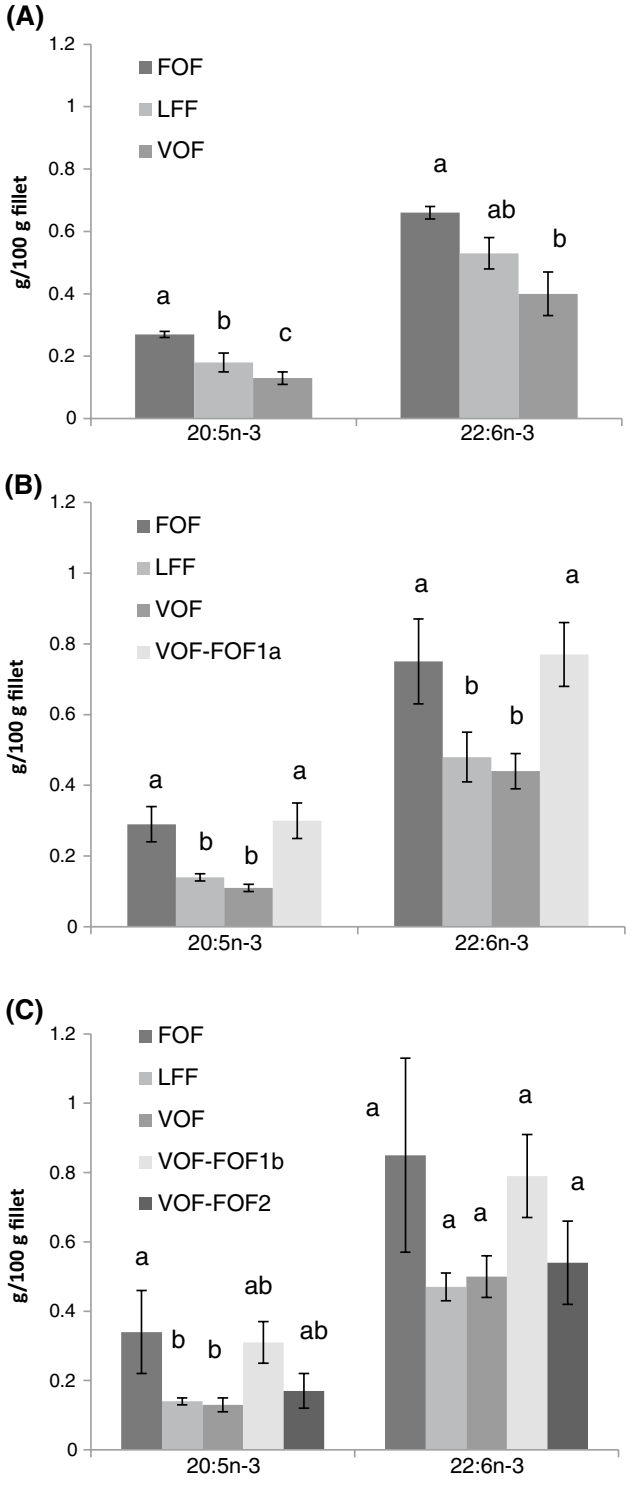

Fig. 3 Content of eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) in the skinned whitefish fillets; **a** June, **b** September, **c** December. Results expressed as g/100 g (mean ± SD, $n = 3$; except group LFF in September, $n = 2$). *Different letters* indicate significant differences between the groups ($P < 0.05$). See Fig. 2 for abbreviations

Table 4 Fatty acid composition of the skinned whitefish fillets, June

Group[A]	FOF	LFF	VOF
Fatty acid			
14:0	5.3 ± 0.1[a]	4.5 ± 0.1[b]	3.1 ± 0.1[c]
16:0	18.6 ± 0.4[a]	19.1 ± 0.5[a]	13.6 ± 0.4[b]
16:1n-7	8.6 ± 0.2[a]	9.4 ± 0.4[b]	5.3 ± 0.1[c]
18:0	2.4 ± 0.0[a]	2.7 ± 0.1[a]	2.3 ± 0.1[a]
18:1n-9	26.7 ± 1.2[a]	30.0 ± 0.3[b]	32.8 ± 0.4[c]
18:1n-7	2.9 ± 0.0[a]	3.0 ± 0.1[a]	2.5 ± 0.1[b]
18:2n-6	7.0 ± 0.2[a]	8.7 ± 0.1[b]	13.3 ± 0.2[c]
18:3n-3	3.0 ± 0.1[a]	3.1 ± 0.1[a]	9.1 ± 0.1[b]
20:1n-9	3.1 ± 0.1[a]	3.2 ± 0.0[a]	4.9 ± 0.1[b]
20:4n-6	0.2 ± 0.4[a]	0.0 ± 0.0[a]	0.1 ± 0.2[a]
20:5n-3	5.4 ± 0.1[a]	3.5 ± 0.1[b]	2.3 ± 0.1[c]
22:6n-3	12.1 ± 0.4[a]	9.5 ± 0.5[b]	6.7 ± 0.2[c]
Others	4.8 ± 0.8[a]	3.2 ± 1.2[a]	4.0 ± 0.3[a]

Results expressed as mole percentages (%) of the total fatty acids (mean ± SD; $n = 3$). Different letters in a row indicate significant differences between the groups ($P < 0.05$)

[A]See Fig. 2 for abbreviations

Table 5 Fatty acid composition of the skinned whitefish fillets, September

Group[A]	FOF	LFF[B]	VOF	VOF–FOF1
Fatty acids				
14:0	5.7 ± 0.3[a]	4.1 ± 0.0[ab]	2.4 ± 0.1[b]	5.3 ± 0.1[ab]
16:0	19.0 ± 0.3[a]	19.0 ± 0.1[a]	12.6 ± 0.3[b]	18.0 ± 0.7[a]
16:1n-7	8.2 ± 0.6[a]	8.8 ± 0.5[a]	4.7 ± 0.3[b]	7.6 ± 0.4[a]
18:0	2.6 ± 0.0[ab]	3.0 ± 0.1[a]	2.3 ± 0.0[b]	2.5 ± 0.1[ab]
18:1n-9	27.3 ± 0.9[a]	33.7 ± 0.3[b]	35.3 ± 0.4[b]	29.1 ± 0.5[c]
18:1n-7	2.8 ± 0.1[a]	2.9 ± 0.0[a]	2.3 ± 0.0[b]	2.8 ± 0.1[a]
18:2n-6	6.8 ± 0.6[a]	8.4 ± 0.3[b]	13.7 ± 0.2[c]	8.3 ± 0.5[b]
18:3n-3	2.9 ± 0.3[a]	2.7 ± 0.0[a]	10.2 ± 0.3[b]	4.0 ± 0.3[c]
20:1n-9	3.7 ± 0.3[a]	3.0 ± 0.1[b]	4.8 ± 0.1[c]	4.1 ± 0.2[a]
20:4n-6	0.4 ± 0.3[a]	0.0 ± 0.0[a]	0.1 ± 0.1[a]	0.3 ± 0.3[a]
20:5n-3	4.8 ± 0.2[a]	2.9 ± 0.1[b]	1.6 ± 0.0[c]	4.3 ± 0.2[d]
22:6n-3	11.4 ± 0.4[a]	9.1 ± 0.4[b]	5.6 ± 0.1[c]	10.3 ± 0.2[d]
Others	4.2 ± 1.4[a]	2.5 ± 0.0[a]	4.3 ± 0.2[a]	3.4 ± 0.4[a]

Results expressed as mole percentages (%) of the total fatty acids (mean ± SD, $n = 3$). Different letters in a row indicate significant differences between the groups ($P < 0.05$)

[A]See Fig. 2 for abbreviations

[B]$n = 2$

(18:3n-3) was highest in group VOF due to the high proportion of the fatty acid in rapeseed, and especially camelina oils, that were incorporated into the feed. Also, compared with the fish in group VOF, the proportions of fatty acids 16:0, 16:1n-7 were higher and those of fatty acids 18:1n-9 and 18:2n-6 lower in the fish of groups FOF and LFF.

In September, the relative fatty acid proportions of group VOF–FOF1 were already very close to those of group FOF (Table 5). Unlike this 15-week period of restorative diet with high-fish oil feed, after a 10-week period (September–December) of restorative diet (group VOF–FOF2), the fatty acid composition of the fish was still fairly similar to that of group VOF (Table 6).

The fatty acid composition of the visceral fat of final samples (December) is presented in Table 7. When comparing different tissues, the summed relative proportion of EPA and DHA of total fatty acids in muscle (skinned fillets) was 18% in group FOF and 7% in group VOF, and 14 and 4% in visceral fat, respectively.

Discussion

A meta-analysis by He *et al*. [16] examining fish consumption and coronary heart disease (CHD) in 13 cohort studies revealed an inverse relationship between fish consumption and both CHD and sudden cardiac death. According to the study, each increase of 20 g/day in fish consumption was associated with a 7% lower risk of fatal CHD.

Because of their likely contribution to the above-described benefits and their general nutritional value, it was important to consider the total amounts of EPA and DHA in the edible part of the fish in addition to their relative proportions [17] (Fig. 3). The gain in EPA in the fillets of fish receiving the feed with moderate substitution of vegetable oil for fish oil (group VOF) seems to eventually decrease by almost two-thirds and that of DHA by almost half when compared with the high-fish oil group. However, the amounts of EPA and DHA (g/100 g skinned fillets) in group VOF–FOF1 were similar to those of group FOF as early as September, after only 15 weeks of restorative feeding. After both 15 and 25 weeks of restorative feeding, the combined content of EPA and DHA was close to that reported for typical farmed salmon (*Salmo salar*) [8], and was clearly higher than non-salmonid farmed fish species such as carp, tilapia, and catfish [18]. Due to the controlled, higher fat content of the feeds, wild fish usually contain less of the fatty acids than their farmed counterparts [1, 19, 20].

Since the relative fatty acid proportions of group VOF–FOF2 were still fairly similar to those of group VOF in December (Table 6), it can be concluded that the 10-week period (September–December) of restorative diet—unlike the 15-week period (June–September; Table 2b)—was not sufficient to change the fatty acid composition of the fillet samples of European whitefish to correspond to that of the fish of group FOF. This underscores the importance of taking into account the temperature during the finishing period, as temperature determines the feed intake in poikilothermic animals. The average weight gain of fish in the FOF

Table 6 Fatty acid composition of the skinned whitefish fillets, December

Group[A]	FOF	LFF	VOF	VOF–FOF1	VOF–FOF2
Fatty acids					
14:0	5.7 ± 0.1^{a}	4.0 ± 0.1^{b}	2.3 ± 0.0^{c}	5.3 ± 0.0^{ad}	3.1 ± 0.3^{bcd}
16:0	18.5 ± 1.0^{ab}	18.3 ± 0.2^{a}	12.5 ± 0.2^{bc}	17.6 ± 0.5^{a}	13.7 ± 0.3^{bc}
16:1n-7	8.5 ± 0.8^{a}	8.8 ± 0.4^{a}	5.4 ± 0.4^{b}	7.9 ± 0.9^{a}	5.9 ± 0.5^{b}
18:0	2.5 ± 0.1^{a}	3.0 ± 0.2^{a}	2.2 ± 0.1^{a}	2.4 ± 0.0^{a}	2.3 ± 0.0^{a}
18:1n-9	28.0 ± 0.5^{a}	33.8 ± 0.8^{b}	36.5 ± 0.3^{c}	28.4 ± 0.6^{a}	34.2 ± 0.5^{b}
18:1n-7	2.9 ± 0.1^{a}	3.0 ± 0.1^{a}	2.4 ± 0.0^{b}	2.9 ± 0.1^{a}	2.5 ± 0.1^{b}
18:2n-6	7.2 ± 1.0^{a}	8.4 ± 0.2^{a}	13.3 ± 0.3^{b}	8.0 ± 0.7^{a}	12.2 ± 0.8^{b}
18:3n-3	3.2 ± 0.9^{ab}	2.7 ± 0.1^{bc}	10.2 ± 0.1^{ad}	3.9 ± 0.3^{bc}	8.5 ± 0.8^{d}
20:1n-9	4.0 ± 0.3^{abc}	3.1 ± 0.1^{b}	5.0 ± 0.1^{ac}	4.1 ± 0.2^{abc}	4.8 ± 0.0^{ac}
20:4n-6	0.5 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.4 ± 0.3^{a}	0.1 ± 0.2^{a}
20:5n-3	4.7 ± 0.4^{a}	2.7 ± 0.2^{b}	1.3 ± 0.1^{c}	4.5 ± 0.2^{a}	2.2 ± 0.2^{b}
22:6n-3	11.1 ± 0.6^{a}	8.6 ± 0.5^{b}	4.7 ± 0.3^{c}	10.7 ± 0.8^{a}	6.6 ± 0.2^{d}
Others	3.3 ± 0.4^{a}	3.6 ± 0.4^{a}	4.2 ± 0.2^{a}	4.0 ± 0.2^{a}	4.0 ± 0.5^{a}

Results expressed as mole percentages (%) of the total fatty acids (mean ± SD, $n = 3$). Different letters in a row indicate significant differences between the groups ($P < 0.05$)

[A]See Fig. 2 for abbreviations

Table 7 Fatty acid composition of visceral fat samples, December

Group[a]	FOF	VOF	VOF–FOF1b	VOF–FOF2
Fatty acids				
14:0	6.2 ± 0.2	2.3 ± 0.0	6.0 ± 0.2	3.3 ± 0.4
16:0	18.0 ± 0.2	12.0 ± 0.3	17.5 ± 0.7	12.8 ± 0.5
16:1n-7	9.8 ± 1.3	5.9 ± 0.1	9.4 ± 1.8	6.4 ± 1.0
18:0	2.3 ± 0.0	2.2 ± 0.0	2.3 ± 0.0	2.2 ± 0.1
18:1n-9	31.3 ± 1.6	38.6 ± 0.2	30.7 ± 1.4	36.7 ± 0.8
18:1n-7	3.0 ± 0.0	2.5 ± 0.1	3.0 ± 0.0	2.6 ± 0.0
18:2n-6	7.2 ± 0.7	13.2 ± 0.2	7.8 ± 0.6	12.3 ± 0.7
18:3n-3	3.0 ± 0.5	10.1 ± 0.1	3.4 ± 0.3	8.4 ± 1.0
20:1n-9	4.1 ± 0.4	5.1 ± 0.0	4.3 ± 0.4	5.0 ± 0.2
20:4n-6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
20:5n-3	4.3 ± 0.2	1.0 ± 0.0	4.4 ± 0.6	1.9 ± 0.4
22:6n-3	7.9 ± 0.4	2.5 ± 0.0	7.7 ± 0.9	4.0 ± 0.7
Others	2.9 ± 0.7	4.6 ± 0.0	3.5 ± 0.8	4.4 ± 0.1

Results expressed as mole percentages (%) of the total fatty acids (mean ± SD, $n = 2$). No statistical analysis performed due to limited number of samples

[a]See Fig. 2 for abbreviations

and VOF groups between September and December was less than half (44%) of the gain observed between June and September.

In the case of visceral fat, there were only two parallel samples, which precluded statistical comparison between different feed treatments (Table 7). However, in terms of the effects of feeding patterns, similar conclusions can be drawn from these results as from the fatty acid composition of fillets. A notable difference was that the summed proportions of EPA and DHA in different feed treatments differed more in visceral fat samples than in skinned fillets. This indicates that long-chain n-3 fatty acids are actively accumulated in muscle, whereas in visceral storage fat, their relative proportion declines when their availability is restricted.

Health-related factors can be expected to become increasingly important in terms of competitiveness in aquaculture. At the same time, it is highly likely that fish feeds will contain ever higher proportions of plant-based oils. To date, there is no consensus among health and scientific organizations on dietary reference levels of EPA and DHA intake; there is, however, general agreement that the general population should be consuming at least two portions of fish per week, one of which should be oily [8]. In 2016, the Global Organization for EPA and DHA Omega-3s published its recommendation for daily intake of EPA and DHA of 500 mg in healthy individuals [21]. In contrast, and from the perspective of cardiovascular health, Mozaffarian *et al*. [22] estimated that 250 mg/day constituted a sufficient intake level. Based on our results, restorative feeding of European whitefish with fish oil-rich diet is justified in order to improve the nutritional quality of the farmed fish. The daily amount of EPA and DHA could be obtained by consuming approximately 25–50 g of the fillets.

Aside from the use of fish oil-enriched finishing diets to restore unique levels of LC-PUFA, there are no options that are yet feasible or accepted by consumers. Microalgal meals containing these fatty acids have gradually entered the market, but are not yet economically feasible or are devoid of EPA [23, 24]. Furthermore, genetically modified *Camelina sativa* oil rich in EPA is devoid of DHA, and as a genetically

modified organism (GMO) it is still rejected by the European market [25, 26].

The findings of the present study provide support for practical steps to be taken during utilization of finishing feeding in European whitefish, a species with a short history of cultivation compared with other salmonids. By restoring fish-oil based feeding in the last stages of growth, the nutritional quality of the fish can be enhanced in both an economic and ecological manner.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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