Aquaculture
March 2009, Volume 288, Issues 1-2, Pages 98-105
http://dx.doi.org/10.1016/j.aquaculture.2008.11.010

The time course of fish oil wash-out follows a simple dilution model in gilthead sea bream (*Sparus aurata L.*) fed graded levels of vegetable oils

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Abstract:

The aim of the study was to determine whether changes in the tissue fatty acid (FA) profile follows a simple test dilution model after changing the dietary oil sources in gilthead sea bream. A 14-month trial was conducted with juvenile fish of 18 g initial body weight fed either a fish oil-based diet (FO diet) or vegetable oils replacing 33% (33VO) and 66% (66VO) of fish oil. The trial included 3 months feeding a fish oil finishing diet to follow the restoration of the FA profile with the FO diet. Fish oil replacement with/without a finishing phase of fish oil re-feeding did not affect growth and all groups reached 520–531 g body weight. Changes in body composition with weight gain did not modify the FA profile of fish continuously fed FO, 33VO or 66VO diets. Increased amounts of oleic acid (18:1n−9), linoleic acid (18:2n−6) and linolenic acid (18:3n−3), in combination with reduced proportions of n−3 long chain polyunsaturated FAs, were found with the partial replacement of fish oil. Hence, multivariate component analysis highlighted a gradient of fish oil load determined by the total intake of fish oil over the entire production cycle. The simple dilution model was a good descriptor of these tissue FA changes, and excellent correlations between observed and predicted values were found at the end of finishing period in fish grow out with either 33VO or 66VO diets.

Keywords: Fish; Growth; Flesh; Fatty acids; Plant proteins

1. Introduction

Fish oil supplies are finite (FAO, 2006) and the continuous increase in global aquaculture production has necessitated research on alternative lipid sources for fish feeds (Watanabe, 2002). Since fish oils are also highly susceptible to contamination with persistent organic pollutants, the use of vegetable oils can contribute toward a reduction in contaminant loadings in the tissue of farmed fish (Sargent et al., 1995; Bell et al., 2005). However, vegetable oils are devoid of n-3 long chain polyunsaturated fatty acids (LC-PUFA), and can adversely affect the tissue fatty acid (FA) composition if added at high inclusion levels (Sargent and Tacon, 1999; Torstensen et al., 2005). Thus, it may be desirable to use finishing diets formulated with uncontaminated or decontaminated fish oils to restore the wild tissue FA profile of farmed fish. For instance, southern hemisphere fish oils are cleaner than northern hemisphere fish oils and can deliver similar levels of n-3 LC-PUFA at lower dietary inclusion levels (Pratoomyot et al., 2008).

Lipid tailoring is, however, a fish-specific process and marine fish show extremely low capabilities for the bioconversion of C_{18} polyunsaturated FAs into C_{20} and C_{22} PUFA (Sargent et al., 2002). Despite this, the essential FA requirements of fast growing juvenile gilthead sea bream are met in practical diets by a 25% inclusion of marine ingredients (fish meal plus fish oil) (Benedito-Palos et al., 2007). Besides, eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are selectively incorporated into the polar lipid fraction resulting in a high membrane phospholipid robustness (Benedito-Palos et al., 2008). The same study pointed out the fact that muscle fat depots highly reflect the composition of the diet regardless of season. Earlier studies in gilthead sea bream have also monitored the effect of fish oil

re-feeding on the tissue FA profile (Izquierdo et al., 2005). In turbot and brown trout, a simple dilution model was proposed and validated by Robin et al. (2003) to follow the time-course of FA changes after shifting levels in dietary lipid sources. The same model was re-evaluated with different success in Atlantic salmon (Jobling, 2004a,b), red sea bream (Jobling, 2004a), Murray cod (Turchini et al., 2006) and Atlantic cod (Jobling et al., 2008). The rationale for the present study was to investigate whether dietary FAs are incorporated in the tissue of gilthead sea bream following similar patterns. Specifically, we monitored FA dynamics after fish oil re-feeding in fish previously fed plant protein-based diets at two levels of fish oil replacement. Economy of fish oil usage was analysed for the entire 14-month production cycle, including both grow-out and finishing periods.

2. Materials and methods

2.1. Diets

Three plant protein diets with pellet size increasing (2-5 mm) according to fish weight were coated with vegetable oils and fish oil from the southern hemisphere to contain 53% crude protein and 21% crude lipid on a dry weight basis (Table 1). Fish oil was the only lipid source in the reference/finishing diet (FO). The other two diets contained a blend of vegetable oils (17:58:25 of rapeseed oil: linseed oil: palm oil), replacing 33% (33VO) and 66% (66VO) of fish oil. The fatty acid composition of diets is reported in Table 2; reduction in fish oil levels decreased the proportion of n-3 LC-PUFA (predominantly EPA and DHA) from 19.4% in the FO diet to 6.6% in the 66VO

89 diet. All diets were manufactured using a twin-screw extruder (Clextral, BC 45) at the
90 INRA experimental research station of Donzacq (Landes, France), dried under hot air,
91 sealed and kept in air-tight bags until use.
92
93 *2.2. Grow-out trial*
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95 Juvenile gilthead sea bream (*Sparus aurata* L.) of Atlantic origin (Ferme Marine
96 de Douhet, Ile d'Oléron, France) were acclimated to laboratory conditions at the
97 Institute of Aquaculture Torre de la Sal (IATS) for 20 days before the start of the study.
98 Fish of 18 g initial mean body weight were distributed into 9 fibreglass tanks (3000 l) in
99 groups of 150 fish per tank. Water flow was 20 l/min, and oxygen content of outlet
100 water remained higher than 5 mg/l. The growth study was undertaken over 11 months
101 (July 11th 2006 – June 6th 2007), and day-length and water temperature (10-26ºC) varied
102 over the course of the trial following natural changes (40º 5'N; 0º 10'E).
103 Each diet was randomly allocated to triplicate groups of fish. Feed was offered
104 by hand to apparent visual satiety twice a day (0900 and 1400 h, 6-7 days per week)
105 from July 2006 to September 2006, and once a day (1200 h, 4-6 days per week) from
106 October 2006 to June 2007. At regular intervals, fish were counted and group-weighed
107 under moderate anaesthesia (3-aminobenzoic acid ethyl ester, MS 222, 100 μg/ml)
108 (Sigma-Aldrich, Madrid, Spain). Feed distributed and mortalities (< 5% during the
109 course of the whole 14-month period) were registered daily.
110

2.3. Fish oil finishing trial and sampling protocol

To follow the restoration of marine FA profile in fish fed vegetable oils, two of the three replicates of fish fed 33VO and 66VO diets were fed the FO diet once a day (6 days per week) from June 2007 to September 2007 (12 weeks). These duplicate groups became 33VO/FO and 66VO/FO, respectively. The remaining fish continued to be fed with the FO (3 tanks), 33VO (1 tank) and 66VO (1 tank) diets.

At regular intervals after the start of the finishing diet period (zero time, 27, 55 and 88 days), 8 randomly selected fish per dietary treatment were killed by a blow on the head. The right-hand side whole fillet (denuded from skin and bone) was excised, vacuum packed and stored at -80 ºC until analyses. All procedures were carried out according to institutional regulations (Consejo Superior de Investigaciones Científicas, IATS Review Board) and the current European Union legislation on handling experimental animals.

2.4. Proximate analyses

The proximate composition of diets and fillets was analysed by standard procedures (AOAC, 1990). Moisture content was determined by drying in an oven at 105º C for 24 h. Subsequently, diets and fillets were freeze-dried and blended for protein, lipid and ash determinations. Crude protein content (N x 6.25) was determined in 75-100 mg samples using the automated Kjeldahl method (Kjeldahl Auto 4002430 Analyser, Selecta, Barcelona, Spain). Samples (0.5 g) for lipid analyses were desiccated (105º C for 3 h) in porous recipients before Soxhlet extraction with 50 ml diethyl ether

135 at 120 ºC (Soxhlet 4001046 Auto extraction apparatus; Selecta, Barcelona, Spain). Ash
136 content was determined after heating at 600 ºC in a muffle furnace for 2 h.
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138 *2.5. FA analyses*
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140 Total lipids for FA analyses were extracted by the method of Folch et al. (1957),
141 using chloroform:methanol (2:1) containing 0.01% butylated hydroxytoluene (BHT) as
142 antioxidant. After the addition of nonadecanoic FA (19:0) as internal standard, total
143 lipids (TL) were subjected to acid-catalysed transmethylation for 16 h at 50 ºC using 1
144 ml toluene and 2 ml of 1% (v/v) sulphuric acid in methanol (Christie, 1982). FA methyl
145 esters (FAME) were extracted with hexane:diethyl ether (1:1), and purified by thin layer
146 chromatography (Silica gel G 60, 20 x 20 cm glass plates, Merck, Darmstadt, Germany)
147 using hexane:diethyl-ether:acetic acid (85:15:1.5) as a solvent system. FAME were then
148 analyzed with a Fisons Instruments GC 8000 Series (Rodano, Italy) gas chromatograph,
149 equipped with a fused silica 30 m x 0.25 mm open tubular column (Tracer, TR-WAX;
150 film thickness: 0.25 μm, Teknokroma, Spain) and a cold on-column injection system.
151 Helium was used as a carrier gas, and temperature programming was from 50 to 180 ºC
152 at 40 ºC/min and then to 220 ºC at 3 ºC/min. Peaks were recorded in a personal
153 computer using the Azur software package (version 4.0.2.0. Datalys, France). Individual
154 FAME were identified by reference to well characterized fish oil standards, and the
155 relative amount of each FA was expressed as a percentage of the total amount of FA in
156 the analysed sample.
157 BHT and internal standard (19:0) were obtained from Sigma-Aldrich (Madrid,
158 Spain). All solvents in lipid extraction and FA analyses were HPLC grade and were
159 obtained from Merck (Darmstadt, Germany).

160 *2.6. Dilution model*

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162 Changes in the tissue FA profile as a result of fish oil re-feeding were described
163 according to Robin et al. (2003) by the following equation:

164 $$P_T = P_{RT} + [(P_0 - P_{RT}) / (Q_T / Q_0)]$$

165 where P_T is the percentage at time T of a given FA, P_0 is the FA percentage at the start
166 of the finishing period, and P_{RT} is the FA percentage at time T in fish continuously fed
167 the reference/finishing diet. Q_0 and Q_T represent the initial and final (at time T) tissue
168 lipid content, respectively.

169 In the present study, P_T is the predicted FA percentage at a given time T in
170 finishing groups (33VO/FO, 66VO/FO), P_0 is the FA percentage of a given FA at the
171 start of the finishing period in 33VO and 66VO groups, P_{RT} represents at time T that of
172 the reference group always fed the finishing FO diet. Q_0 and Q_T are the initial and final
173 tissue lipid content in the respective group. The adequacy of the dilution model was
174 evaluated by direct comparisons of model predictions with the observed values.

175

176 *2.8. Statistical analysis*

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178 Growth parameters (fish average values per tank) and the relative amount of FA
179 were checked for normal distribution and homogeneity of variances, and when
180 necessary arcsin transformation was performed. Data were analysed by one-way
181 ANOVA followed by Student-Newman-Keuls (SNK) test at a significance level of 5%.
182 The percentages of each FA were chemometrically analysed by multivariate principal
183 components analysis (MPCA). All analyses were made using the SPSS package version
184 14.0 (SPSS Inc, Chicago, USA).

3. Results

3.1. Growth performance

Growth, feed intake and feed conversion ratios were not affected by the dietary treatment over the course of the feeding trial. Hence, at each sampling point, all data on body weight and feed intake were pooled and represented in the fitting plot as the mean of the 9 experimental tanks (Fig. 1B). Overall, fish grew during the 11-month grow-out period from 18 g to 284 - 294 g with a feed efficiency (wet wt gain/dry feed intake) of 0.82 - 0.86 over this period. The subsequent trial (3-month period) was conducted over the course of summer, and the cumulative feed intake (g/fish) was of the same order of magnitude as that of the initial period (324 *vs* 307 g). At the end of the finishing diet period, mean body weight of fish among tanks varied between 520 and 531 g with a feed efficiency for the finishing period of 0.73 – 0.79.

In absolute terms, fish oil usage (g feed intake x ingredient percentage of fish oil in the diet) in fish always fed FO, 33VO and 66VO was concordant with the percentage of replacement (Fig. 2). At the end of the finishing diet period, fish oil usage in fish fed 33VO became equal to that found in the 66VO/FO group. In the 33VO/FO group, fish oil usage was reduced by 15% in comparison to fish always fed the FO diet.

205 *3.2. Lipid content and tissue FA profile*

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207 Fillet yield and lipid content of skinned fillets was not altered by dietary
208 intervention. However, lipid deposition increased by a 20–30 % over the course of the
209 finishing period regardless of dietary treatment (Tables 3-5).

210 As shown in Table 3, no consistent changes in the FA profile were found over
211 the course of the finishing period in fish continuously fed the same diet (FO, 33VO and
212 66VO groups), and only a few FAs (16:1 n-7, 17:0, 18:2 n-6) showed significant
213 differences (less than 5-30% of variation) in one or two of the three experimental
214 groups. Regarding the effect of dietary treatment, fish fed the FO diet contained 29%
215 saturates (mainly 16:0 and 14:0), almost 32% monoenes (over half of which were
216 18:1n-9), 1% n-6 LC-PUFA, and 17% n-3 LC-PUFA (predominantly EPA and DHA).
217 Increased amounts of 18:1n-9, 18:2n-6 and 18:3n-3, in combination with reduced
218 proportions of n-3 LC-PUFA and saturated FAs were found with the progressive
219 replacement of fish oil by vegetable oils.

220 The time course of changes through the finishing period on the tissue FA profile
221 of fish previously fed vegetable oils are shown in Tables 4 and 5. Both in 33VO/FO and
222 66VO/FO groups, the finishing diet caused a progressive increase in the FAs present in
223 higher amounts in fish oil (i.e. 14:0, 16:1n-7, 20:1n-9, 22:1n-11, EPA and DHA), while
224 those characteristic of vegetable oils (i.e. 18:1n-9, 18:2n-6 and 18:3n-3) decreased in
225 proportion to the degree of fish oil replacement in the diet.

226 The MPCA analysis of fillet FA profiles before, during and at the end of the
227 finishing period revealed that the two first components accounted for 62% of the total
228 variation, with 52.5% of the variation being explained by component 1 itself (Fig. 3A).
229 Some of the most characteristic variables of marine versus vegetable oils had the

highest loadings on function 1 and were located at the extremes. The results of the score plot are represented only for the first component since it accounted for the majority of the variation (Fig. 3B). The plot revealed that the three invariable groups (FO, 33VO and 66VO) were well separated from each other, with 66VO and FO at the extremes. The finishing 33VO/FO and 66VO/FO groups were also clearly separated from each other on a time- FO intake-manner (Fig. 3B). Thus, a gradient of fish oil load caused either by the amount of this ingredient in the diet, or by the total intake per unit of body weight, could be easily distinguishable. At the end of the finishing period, the resulting FA profile of the 66VO/FO became equal to that of fish always fed the 33VO diet, and intermediate values between 33VO and FO groups were found for the 33VO/FO group.

Regardless of nutritional background (33VO and 66VO diets), the concordance between the observed FA values (x-axis) and those predicted by the dilution model (y-axis) was extremely high at the end of the finishing period (Fig. 4). This gave a regression line with a slope very close to 1 (0.96-0.95) when 32 FAs were considered in the models derived from both 33VO/FO and 66VO/FO fish. Similar slopes (1.04-1.05) were obtained when calculations were repeated for 14 selected FAs having a high weight in the MPCA.

4. Discussion

Data reported here, along with those of Benedito-Palos et al. (2008) over an 8-month feeding trial, convincingly demonstrate that dietary fish oils of northern and southern origin can be replaced by up to 66% without negative effects on the growth performance of gilthead sea bream. These data are novel in a stenohaline marine teleost maximizing the simultaneous replacement of fish meal and fish oil by alternative plant

255 ingredients without histopathological signs of liver and intestine damage (Benedito-
256 Palos et al., 2008). Other metabolic effects are complex, interconnected and, to date, not
257 fully understood. However, it must be noted that growth-compensatory mechanisms are
258 orchestrated at the local tissue level (skeletal muscle) by the somatotropic axis when
259 rapidly growing gilthead sea bream juveniles are fed high levels of vegetable oils
260 (Benedito-Palos et al., 2007). Besides, n-3 LC-PUFA are selectively incorporated into
261 polar lipids, and the stability of muscle phospholipid FA composition is a useful and
262 complementary criterion to assess the suitability of the replacement strategy in fish
263 feeds with low levels of marine derived ingredients (Benedito-Palos et al., 2008).
264 In fish and higher vertebrate species, neutral lipids are less conservative than
265 phospholipids (Tocher, 2003; Skalli and Robin, 2004; Schulz et al., 2005). This is
266 because they are the fat storage form and its FA profile highly reflects that of the diet. In
267 the present study, the muscle lipid content was greater than 10% on a wet matter basis,
268 and the FA profile of total lipids and thereby that of triacylglycerols (TAG) remained
269 mostly unchanged through the finishing period in fish always fed either FO, 33VO or
270 66VO diets. The result of these temporal series agrees with data on a previous seasonal
271 study (Benedito-Palos et al., 2008), and reinforces the idea that accelerated growth of
272 farmed fish might override most of the changes in the flesh FA profile (Grigorakis,
273 2007). However, it should be born in mind that the tissue-specific FA profile varies in
274 salmonids with the size and age of fish (Bell et al., 2002, 2003). Fish oil replacement by
275 alternative lipid sources has also a pronounced effect on the tissue FA profile of fish,
276 and we found in fish always fed 33VO and 66VO diets a 22-36 % increase of 18:1n-9
277 and 18:2n-6 with a concurrent 20-65 % reduction in EPA and DHA. Similar results
278 have been reported in gilthead sea bream (Izquierdo et al., 2005) and a wide variety of
279 fish species, including Atlantic salmon (Bell et al., 2002 2003b; Bransden et al., 2003;

Bell, 2004; Torstensen et al., 2004; Nanton et al., 2007), rainbow trout (Drew et al., 2007), turbot (Regost et al., 2003), European sea bass (Montero et al., 2005; Mourente and Bell, 2006), Murray cod (Francis et al., 2007a, b), red sea bream (Huang et al., 2007; Piedecausa et al., 2007) and black sea bream (Peng et al., 2008). Since this feature can compromise the beneficial effects of seafood (Din et al., 2004; Psota et al., 2006) as the main source of EPA and DHA in the human diet, there is increased interest in finfish aquaculture for modelling the time-course of FA changes during fish oil re-feeding.

Gilthead sea bream shows, in our latitude, a pronounced growth seasonality (Mingarro et al., 2002), and for the most effective restoration of EPA and DHA, the finishing window should take place in the broadly active feeding period of May-October. Thus, after the growth stop of winter, one month was spent before the start of the finishing diet period that then was continued through the summer (June-September). Several variables, including among others the growth and lipid deposition rates, need to be considered when analysing the effectiveness of fish oil wash-out. Therefore, one must be cautious before drawing a definitive conclusion, but the literature is prolific on studies in which a complete restoration of the FA profile was not fully achieved after fish oil re-feeding: 32 days in red sea bream (Glencross et al., 2003), 8 weeks in turbot (Regost et al., 2003); 8 weeks in brown trout (Robin et al., 2003), 12-25 weeks in Atlantic salmon (Bell et al., 2003a, b; Bell, 2004; Torstensen et al., 2004); 3 months in gilthead sea bream (Izquierdo et al., 2005); 4 months in Murray cod (Turchini et al., 2006); and 5 months in European sea bass (Montero et al., 2005). Most evidence points toward a dilution model, which was proven in turbot, brown trout and Murray cod. Using original data and those derived from red sea bream (Glencross et al., 2003) and Atlantic salmon (Bell et al., 2003a) studies, Jobling (2004a, b) concluded that a dilution process also plays a key role in governing the muscle FA profile of these fish species.

Our work in gilthead sea bream points clearly toward the same direction, and gradual changes in the FA profiles of the 33VO/FO and 66VO/FO groups were found during the finishing period, making them increasingly similar to the FO group. This is particularly highlighted by the results of the MPCA that shows the gradient of fish oil load along the ordinate axis.

Therefore, changes in the FA profile arise because the existing stores become diluted as fish grow and deposit increasing amounts of dietary-derived FAs. In other words, nutritional background in fish with no apparent signs of FA deficiencies has a marginal role on FA turnover and tissue FA profiles, although age- and nutritional condition affect the expression pattern of cytokines and key limiting enzymes on tissue FA uptake and mobilization (Saera-Vila et al., 2005, 2007). Thus, using the simple dilution model, a reliable FA prediction was found herein at the end of the fish oil finishing period regardless of the level of fish oil replacement. The model is in fact a good general descriptor of FAs, and regression curves (predicted *vs* observed values) give slopes nearby to the line of equality when either selected or almost all FAs were considered in the model. In Atlantic salmon, Jobling (2004b) tested three FAs (18:1 isomers, 18:2 n-6 and 18:3 n-3) and confirmed closely the predictions made with the dilution model. Jobling (2004a) again evaluated the dilution model with data from red sea bream studies (Glencross et al., 2003), and a high degree of concordance was found between the predicted and observed values. However, lipid retention is a tissue and fish-specific process, and probably the concordance with the dilution model will be higher in species with high lipid tissues, which may explain why the predictions are better in salmonids and sparid fish than in Murray cod (Turchini et al., 2006) or Atlantic cod (Jobling et al., 2008). Thus, our gilthead sea bream study highlights that fish grown-out with the 33VO diet need more than 12 weeks to revert back the FA composition toward

330 the normal variability of fish fed fish oil-based diets. Besides, low and intermediate
331 levels of fish oil replacement can produce equally acceptable fillets when the latter is
332 accompanied by a fish oil finishing phase. This is because temporal changes on fish oil
333 intake gave a minor effect on the muscle FA profile if the absolute amount becomes
334 equal at the end of the trial. In our experimental model, this was the case for the 33VO
335 and 66VO/FO groups, and the tissue FA profile at the end of the 3-month finishing
336 period was very close in both groups.

337 Savings on fish oil resources are therefore limited to a simple dilution, and new
338 approaches are required to improve any mobilisation or turnover of pre-existing FAs.
339 Intake of conjugate linoleic acid complex (CLA) has a lipid-lowering effect in gilthead
340 sea bream juveniles, promotes the diversion of dietary-derived TAG from muscle and
341 adipose tissue to liver, and increase hepatic peroxisomal β oxidation (Diez et al., 2007).
342 At the same time, however, LC-PUFA biosynthesis is reduced and changes in the
343 muscle FA profile indicate that the inclusion of CLA in aquaculture diets would be of
344 little benefit in gilthead sea bream. Nevertheless, the use of more specific
345 agonists/antagonists of peroxisome proliferator-activated receptors (PPARs) cannot be
346 excluded to improve the retention of n-3 LC-PUFA. Attention also needs to be focused
347 on the transfer from fish oil of PCBs, dioxins and other harmful lipophilic organic
348 chemicals that are now ubiquitous contaminants in the marine ecosystems (Sargent et
349 al., 1995; Jacobs et al., 2002; Bell et al., 2005; Domingo, 2007). The effects of feeding
350 strategies on toxic-kinetics will be reported separately to have a more complete
351 framework of nutritional fish tailoring, and to gain public acceptance for the fish fed
352 with alternative and sustainable diets.

Acknowledgements

This research was funded by EU (FOOD-CT-2006-16249: Sustainable Aquafeeds to Maximise the Health Benefits of Farmed Fish for Consumers, AQUAMAX) and Spanish (AGL2004-06319-CO2) projects.

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515 L**egends**

516

517 Figure 1. (A) Seasonal changes on temperature (solid line) and day length (dashed line).
518 White and black boxes at the top of figure refer to summer and winter period,
519 respectively. (B) Body weight representation as mean ± SEM of all experimental groups
520 in the experimental design. Arrows indicate the start of the study and finishing periods.
521 Cumulative feed intake is indicated at the top of figure for each period.

522

523 Figure 2. Effects of diet composition and feeding protocol on fish oil intake (g feed
524 intake x ingredient percentage of fish oil in the diet) per fish through grow-out (330
525 days, black bars) and finishing (88 days, grey bars) periods.

526

527 Figure 3. Component plot (A) and factor score plot (B) of the MPCA for the fillet FA
528 profile through the 3-month finishing period (June-September). Temporal series (July,
529 August and September) derived from 33VO/FO and 66VO/FO fish are represented in
530 factor score 1 as mean ± SEM (n = 8). No temporal changes were found for FO, 33VO
531 and 66VO groups and data from June and September (initial and final steps of finishing
532 period) are represented as one point for each group.

533

534 Figure 4. Plot prediction (dilution model) in 33VO/FO (A) and 66VO/FO (B) groups of
535 the tissue FA profile at the end of the finishing period. Observed values are the mean ±
536 SEM of 8 fish per treatment. The solid line is the plotted regression. The equations were
537 calculated considering both the selected and all (square brackets) the identified FAs.

538

539 **Table 1.** Ingredients and chemical composition of the experimental diets.

Ingredient (%)	FO	33VO	66VO
Fish meal (CP 70%) [1]	15	15	15
CPSP 90 [2]	5	5	5
Corn gluten	40	40	40
Soybean meal	14.3	14.3	14.3
Extruded wheat	4	4	4
Fish oil [3]	15.15	10.15	5.15
Rapeseed oil	0	0.85	1.7
Linseed oil	0	2.9	5.8
Palm oil	0	1.25	2.5
Soya lecithin	1	1	1
Binder	1	1	1
Mineral premix [4]	1	1	1
Vitamin premix [5]	1	1	1
$CaHPO_4.2H_2O$ (18%P)	2	2	2
L-Lys	0.55	0.55	0.55
Proximate composition			
Dry matter (DM, %)	93.13	92.9	92.77
Crude protein (% DM)	53.2	52.81	52.62
Crude fat (% DM)	21.09	21	20.99
Ash (% DM)	6.52	6.69	6.57

540
541 [1]Fish meal (Scandinavian LT)
542 [2]Fish soluble protein concentrate (Sopropêche, France)
543 [3]Fish oil (Sopropêche, France)
544 [4]Supplied the following (mg/kg diet, except as noted): calcium
545 carbonate (40% Ca) 2.15 g, magnesium hydroxide (60% Mg)
546 1.24 g, potassium chloride 0.9 g, ferric citrate 0.2 g, potassium
547 iodine 4 mg, sodium chloride 0.4 g, calcium hydrogen phosphate
548 50 g, copper sulphate 0.3, zinc sulphate 40, cobalt sulphate 2,
549 manganese sulphate 30, sodium selenite 0.3.
550 [5]Supplied the following (mg/kg diet): retinyl acetate 2.58, DL-
551 cholecalciferol 0.037, DL-α tocopheryl acetate 30, menadione
552 sodium bisulphite 2.5, thiamin 7.5, riboflavin 15, pyridoxine 7.5,
553 nicotinic acid 87.5, folic acid 2.5, calcium pantothenate 2.5,
554 vitamin B_{12} 0.025, ascorbic acid 250, inositol 500, biotin 1.25
555 and choline chloride 500.
556

557 **Table 2**. FA composition of the experimental diets (% of total FAME).
558

FA %	FO	33VO	66VO
14 :0	7.18	5	2.7
15:0	0.12	0.09	tr
16:0	22.26	20.30	18.48
16:1n-7	7.06	4.85	2.62
16:2	0.47	0.31	0.15
16:3	1.66	1.09	0.46
16:3n-3	0.11	0.07	0.03
16:4	1.8	1.1	0.47
17:0	0.96	0.64	0.32
18:0	4.27	3.92	3.55
18:1n-9	12.49	20.39	24.59
18:1n-7	2.97	0.23	tr
18:2n-6	10.35	14.03	17.48
18:3 n-6	0.34	0.21	0.09
18:3n-3	0.81	9.16	17.33
18:4n-3	1.8	1.17	0.62
20:0	0.07	0.07	0.06
20:1n-9	0.92	0.97	1.03
20:2n-6	0.6	0.16	0.19
20:3 n-6	0.07	0.11	tr
20:4n-6	0.69	0.43	0.18
20:4n-3	0.3	0.21	0.15
20:5n-3	13.57	8.84	4.38
22:1n-11	0.97	0.82	0.76
22:5n-3	0.81	0.56	0.23
22:6n-3	4.78	3.3	1.88
Total	97.83	98.25	97.81
Saturates	34.86	30.02	25.11
Monoenes	24.41	27.26	29
n-3 LC-PUFA[1]	19.46	12.91	6.64
n-6 LC-PUFA[2]	1.36	0.7	0.37

559 tr = trace values
560 [1]Calculated excluding 16 C and 18 C.
561 [2]Calculated excluding 18 C.

562 Table 3. Fillet weight, wet lipid content and FA profile (% of total FAME) in fish always fed
563 FO, 33VO and 66VO diets. Fish were sampled at the beginning (June 2007) and at the end of
564 the finishing period (September 2007). Mean values and standard deviations of individual fish
565 are presented (n = 8). Mean values within dietary groups with unlike superscript letters are
566 significantly different (P<0.05).

	FO				33VO				66VO			
	June		September		June		September		June		September	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fillet (g)	61.50[a]	12.5	114.7[b]	14.4	59.52[a]	5.73	113.1[b]	20.5	58.23[a]	13.2	124.2[b]	14.9
Lipid (%)	9.19[a]	2.01	11.36[b]	1.21	10.97[a]	3.20	12.84[b]	3.24	9.33[a]	2.02	11.40[b]	0.88
Σ FAs (mg/g)	53.99	6.61	61.67	10.8	62.46	18.8	66.78	16.5	59.76	14.2	60.54	7.86
FA (%)												
14:0	5.34	0.47	5.72	0.51	4.24	0.52	4.21	0.43	2.78	0.32	2.92	0.42
15:0	0.20	0.09	0.17	0.05	0.27	0.14	0.19	0.08	0.27	0.22	0.21	0.18
16:0	17.78	1.37	19.34	0.51	17.62	0.70	18.65	0.99	16.58	0.36	18.00	0.68
16:1n-7	7.96	0.64	8.36	0.25	5.96	0.47	5.94	0.18	3.85[a]	0.14	4.08[b]	0.17
16:2	1.03	0.20	0.90	0.29	0.60	0.10	0.69	0.15	0.27	0.09	0.35	0.08
16:3	1.21	0.21	1.35	0.06	0.82	0.08	0.91	0.17	0.43	0.23	0.55	0.14
16:4	1.09	0.30	0.95	0.08	0.52	0.06	0.53	0.07	0.24	0.02	0.26	0.02
17:0	0.66[a]	0.31	0.45[b]	0.37	0.24	0.02	0.25	0.01	0.25	0.09	0.25	0.14
18:0	3.70	0.38	3.71	0.30	3.76	0.17	3.90	0.19	3.85	0.12	3.84	0.12
18:1 n-9	18.13	2.24	18.11	0.77	23.21	1.05	23.27	0.59	27.06	0.71	26.97	0.59
18:1 n-7	3.23	0.25	3.30	0.14	2.56	0.12	2.52	0.10	2.00	0.12	1.98	0.19
18:2 n-6	9.39[a]	0.83	8.95[b]	0.31	12.32[a]	0.75	11.96[b]	0.55	15.02	0.31	14.34	0.31
18:3 n-6	0.38	0.06	0.39	0.02	0.25	0.02	0.25	0.01	0.14	0.01	0.15	0.03
18:3 n-3	1.16	1.20	0.71	0.04	7.39	0.93	7.11	0.26	12.94	0.33	10.82	4.33
18:4 n-3	1.16	0.14	1.23	0.07	0.82	0.02	0.82	0.05	0.60	0.06	2.03	4.07
20:0	0.20	0.02	0.20	0.01	0.19	0.01	0.22	0.06	0.18	0.02	0.19	0.01
20:1 n-7	0.21	0.02	0.22	0.01	0.15	0.01	0.16	0.01	0.11	0.01	0.11	0.01
20:1 n-9	1.14	0.08	1.21	0.02	1.02	0.05	1.08	0.03	0.96	0.03	1.02	0.03
20:1 n-11	0.24	0.02	0.24	0.02	0.20	0.02	0.20	0.02	0.17	0.02	0.17	0.01
20:2 n-6	0.25	0.08	0.26	0.04	0.28	0.06	0.29	0.05	0.32	0.07	0.32	0.07
20:3 n-6	0.26	0.07	0.28	0.03	0.23	0.04	0.25	0.07	0.24	0.11	0.21	0.06
20:3 n-3	0.16	0.20	0.23	0.25	0.23	0.09	0.26	0.09	0.34	0.04	0.36	0.05
20:4 n-6	0.57	0.18	0.53	0.19	0.40	0.12	0.36	0.09	0.23	0.03	0.24	0.03
20:4 n-3	0.61	0.06	0.62	0.03	0.51	0.03	0.51	0.03	0.42	0.02	0.40	0.03
20:5 n-3	8.49	0.89	8.84	0.36	5.57	0.39	5.25	0.38	2.88	0.18	2.98	0.31
22:0	0.17	0.06	0.16	0.06	0.16	0.06	0.15	0.05	0.14	0.03	0.15	0.03
22:1 n-9	0.32	0.02	0.32	0.03	0.31	0.02	0.30	0.02	0.29	0.01	0.28	0.02
22:1 n-11	0.89	0.06	0.95	0.03	0.72	0.05	0.73	0.05	0.58	0.01	0.59	0.02
22:5 n-3	2.66	0.33	2.63	0.15	1.92	0.12	1.94	0.19	1.19	0.04	1.20	0.17
22:6 n-3	5.48	0.90	5.16	0.20	3.92	0.21	3.58	0.27	2.57	0.21	2.29	0.18
24:1 n-9	0.35	0.06	0.28	0.11	0.31	0.03	0.26	0.11	0.31	0.04	0.30	0.03
Saturates	28.04	1.92	29.75	0.74	26.47	1.64	27.26	1.29	24.04	0.67	25.56	0.90
Monoenes	32.53	2.58	33.22	0.84	34.45	1.81	34.51	0.64	35.18	0.84	35.32	0.73
n-3 LC-PUFA[1]	17.40	2.06	17.47	0.60	12.15	0.84	11.54	0.76	7.40	0.22	7.23	0.62
n-6 LC-PUFA[2]	1.09	0.21	1.07	0.18	0.92	0.22	0.90	0.09	0.79	0.06	0.78	0.06

[1]Calculated excluding 18 C. [2]Calculated excluding 18 C.

567 Table 4. Effects of finishing diet on fillet weight, wet lipid content and FA profile (% of total
568 FAME) in fish fed 33VO diet and then FO diet (33VO/FO group). Fish were sequentially
569 sampled through the finishing period (June, +0; July, +27; August, +55; September, +88).
570 Mean values and standard deviations of individual fish are presented (n = 8). Raw values with
571 unlike superscript letters are significantly different over sampling time (P<0.05).

	Jun (+0)		Jul (+27)		Aug (+55)		Sep (+88)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fillet (g)	59.52^{a}	5.73	74.58^{b}	14.8	98.66^{c}	8.59	126.1^{d}	12.5
Lipid (%)	10.97	3.20	11.44	1.82	13.19	1.73	12.36	1.62
Σ FAs (mg/g)	62.46	18.8	68.26	23.5	69.64	12.6	63.84	19.9
FA (%)								
14 :0	4.24^{a}	0.52	4.39^{ab}	0.36	4.55^{ab}	0.35	4.98^{b}	0.32
15:0	0.27	0.14	0.17	0.08	0.17	0.07	0.15	0.08
16:0	17.62^{a}	0.70	18.29^{b}	0.40	18.77^{b}	0.52	19.30^{b}	0.36
16:1n-7	5.96^{a}	0.47	6.48^{ab}	0.20	6.97^{b}	0.32	7.15^{b}	0.12
16:2	0.60^{a}	0.10	0.78^{b}	0.14	0.74^{b}	0.25	0.72^{b}	0.22
16:3	0.82^{a}	0.08	0.94^{ab}	0.07	1.01^{b}	0.06	1.08^{b}	0.10
16:4	0.52^{a}	0.06	0.55^{ab}	0.24	0.62^{ab}	0.21	0.71^{b}	0.12
17:0	0.24	0.02	0.26	0.03	0.40	0.28	0.30	0.05
18:0	3.76	0.17	3.89	0.08	3.70	0.18	3.93	0.10
18:1 n-9	23.21^{a}	1.05	22.61^{ab}	0.98	21.84^{ab}	0.88	21.58^{b}	0.78
18:1 n-7	2.56^{a}	0.12	2.75^{b}	0.12	2.89^{bc}	0.14	2.94^{c}	0.12
18:2 n-6	12.32^{a}	0.75	11.61^{b}	0.60	10.77^{c}	0.29	10.16^{d}	0.17
18:3 n-6	0.25^{a}	0.02	0.29^{b}	0.01	0.31^{c}	0.01	0.33^{d}	0.01
18:3 n-3	7.39^{a}	0.93	5.78^{ab}	0.45	4.74^{bc}	0.12	3.61^{c}	0.13
18:4 n-3	0.82	0.02	0.76	0.30	0.97	0.07	0.90	0.34
20:0	0.19	0.01	0.19	0.01	0.18	0.01	0.20	0.02
20:1 n-7	0.15^{a}	0.01	0.17^{ab}	0.01	0.18^{b}	0.01	0.19^{b}	0.01
20:1 n-9	1.02^{a}	0.05	1.11^{b}	0.09	1.14^{b}	0.05	1.11^{b}	0.04
20:1 n-11	0.20	0.02	0.21	0.01	0.22	0.03	0.22	0.02
20:2 n-6	0.28	0.06	0.32	0.03	0.29	0.04	0.25	0.07
20:3 n-6	0.23	0.04	0.24	0.03	0.24	0.03	0.24	0.03
20:3 n-3	0.23^{a}	0.09	0.22^{a}	0.13	0.15^{b}	0.01	0.17^{b}	0.13
20:4 n-6	0.40	0.12	0.43	0.11	0.47	0.03	0.46	0.11
20:4 n-3	0.51	0.03	0.55	0.03	0.58	0.03	0.55	0.02
20:5 n-3	5.57^{a}	0.39	6.06^{a}	0.52	6.83^{b}	0.31	6.91^{b}	0.22
22:0	0.16	0.06	0.14	0.03	0.13	0.05	0.17	0.07
22:1 n-9	0.31	0.02	0.30	0.01	0.31	0.03	0.30	0.01
22:1 n-11	0.72^{a}	0.05	0.77^{ab}	0.03	0.80^{b}	0.05	0.80^{b}	0.02
22:5 n-3	1.92	0.12	2.10	0.09	2.20	0.20	2.16	0.11
22:6 n-3	3.92	0.21	4.08	0.41	4.08	0.28	4.19	0.21
24:1 n-9	0.31	0.03	0.33	0.03	0.32	0.04	0.30	0.02
Saturates	26.47^{a}	1.64	27.34^{a}	0.19	27.89^{ab}	0.88	29.03^{b}	0.46
Monoenes	34.45	1.81	34.72	1.11	34.71	0.79	34.67	0.86
n-3 LC-PUFA[1]	12.15^{a}	0.84	13.00^{ab}	0.83	13.85^{ab}	0.65	13.97^{b}	0.46
n-6 LC-PUFA[2]	0.92	0.22	0.99	0.10	1.01	0.05	0.95	0.11

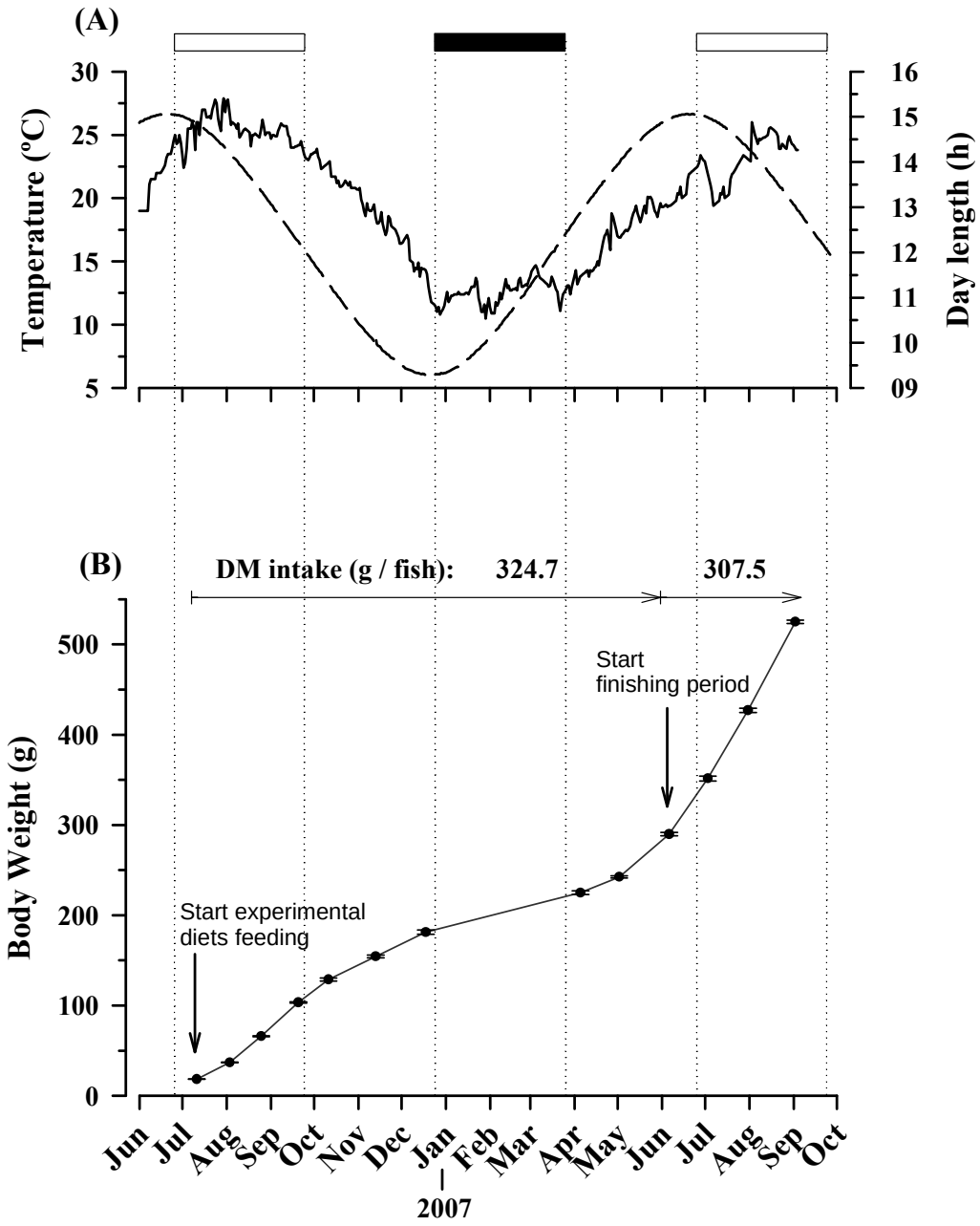
[1]Calculated excluding 18 C. [2]Calculated excluding 18 C.

572 Table 5. Effects of finishing diet on fillet weight, wet lipid content and FA profile (% of total
573 FAME) in fish fed 66VO diet and then FO diet (66VO/FO group). Fish were sequentially
574 sampled through the finishing period (June, +0; July, +27; August, +55; September, +88).
575 Mean values and standard deviations of individual fish are presented (n = 8). Raw values with
576 unlike superscript letters are significantly different over sampling time (P<0.05).
577

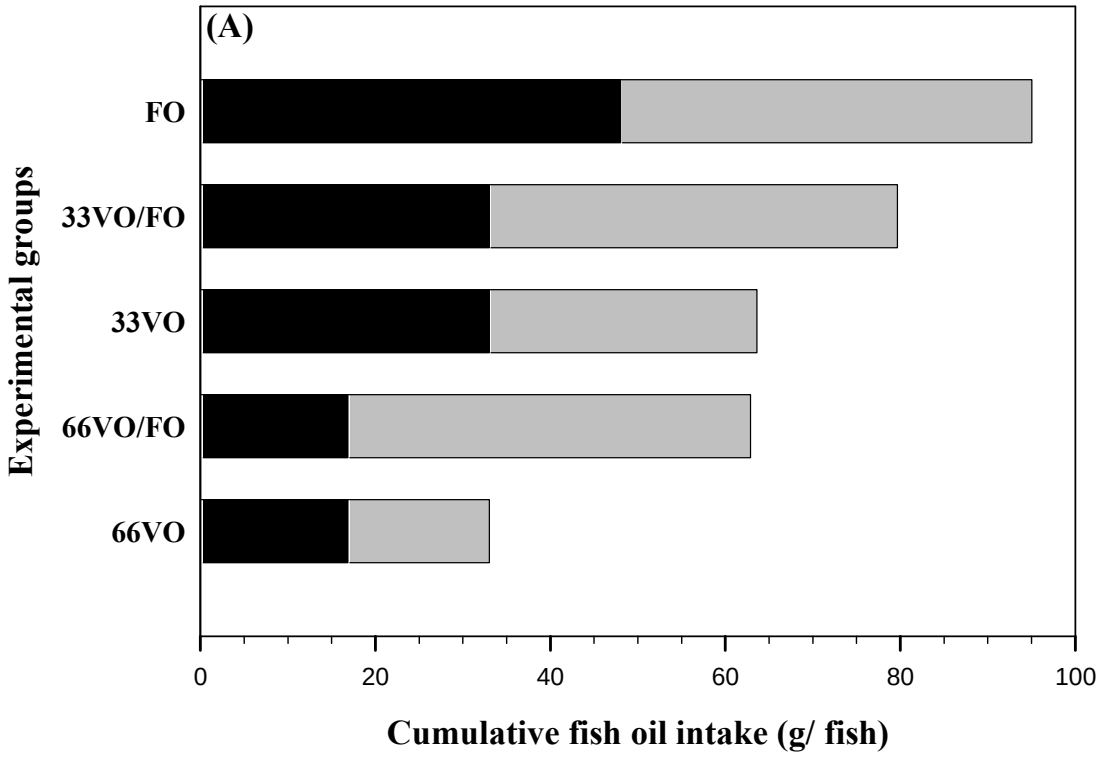
	Jun (+0)		Jul (+27)		Aug (+55)		Sep (+88)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fillet (g)	58.23^{a}	13.2	71.61^{b}	11.1	97.65^{c}	9.33	116.2^{d}	12.9
Lipid (%)	9.33	2.02	10.30	3.27	11.81	2.51	11.15	1.11
Σ FAs (mg/g)	59.76	14.2	56.27	20.6	67.46	17.6	55.14	7.23
FA (%)								
14 :0	2.78^{a}	0.32	3.27^{a}	0.34	3.68^{ab}	0.27	4.38^{b}	0.39
15:0	0.27	0.22	0.10	0.06	0.18	0.09	0.16	0.09
16:0	16.58^{a}	0.36	17.63^{b}	0.62	18.14^{b}	0.54	18.81^{c}	0.61
16:1n-7	3.85^{a}	0.14	4.81^{ab}	0.31	5.60^{bc}	0.19	6.20^{c}	0.13
16:2	0.27^{a}	0.09	0.39^{ab}	0.11	0.55^{b}	0.20	0.59^{b}	0.19
16:3	0.43^{a}	0.23	0.63^{a}	0.09	0.70^{ab}	0.21	0.91^{b}	0.07
16:4	0.24^{a}	0.02	0.31^{ab}	0.15	0.50^{bc}	0.05	0.60^{c}	0.08
17:0	0.25	0.09	0.28	0.17	0.28	0.12	0.34	0.20
18:0	3.85	0.12	3.86	0.18	3.81	0.13	3.85	0.21
18:1 n-9	27.06^{a}	0.71	25.89^{b}	1.43	24.62^{c}	0.58	22.80^{d}	0.90
18:1 n-7	2.00^{a}	0.12	2.31^{b}	0.11	2.46^{c}	0.16	2.66^{d}	0.09
18:2 n-6	15.02^{a}	0.31	13.81^{b}	0.48	12.67^{c}	0.31	11.69^{d}	0.34
18:3 n-6	0.14^{a}	0.01	0.20^{ab}	0.02	0.23^{bc}	0.01	0.27^{c}	0.02
18:3 n-3	12.94^{a}	0.33	10.35^{b}	0.47	8.36^{c}	0.45	6.31^{d}	0.50
18:4 n-3	0.60^{a}	0.06	0.61^{ab}	0.23	0.83^{b}	0.03	0.92^{c}	0.07
20:0	0.18	0.02	0.19	0.02	0.18	0.00	0.20	0.02
20:1 n-7	0.11^{a}	0.01	0.13^{bc}	0.01	0.14^{c}	0.01	0.17^{d}	0.01
20:1 n-9	0.96^{a}	0.03	1.00^{ab}	0.06	1.05^{bc}	0.03	1.08^{c}	0.06
20:1 n-11	0.17	0.02	0.20	0.02	0.18	0.02	0.20	0.02
20:2 n-6	0.32	0.07	0.30	0.13	0.24	0.09	0.30	0.04
20:3 n-6	0.24	0.11	0.25	0.06	0.22	0.04	0.24	0.05
20:3 n-3	0.34^{a}	0.04	0.30^{ab}	0.03	0.26^{ab}	0.05	0.24^{b}	0.09
20:4 n-6	0.23^{a}	0.03	0.32^{ab}	0.10	0.33^{ab}	0.06	0.39^{b}	0.08
20:4 n-3	0.42^{a}	0.02	0.47^{b}	0.06	0.49^{bc}	0.02	0.52^{c}	0.02
20:5 n-3	2.88^{a}	0.18	3.90^{b}	0.61	5.01^{c}	0.22	5.86^{d}	0.31
22:0	0.14	0.03	0.17	0.06	0.13	0.04	0.18	0.04
22:1 n-9	0.29	0.01	0.30	0.02	0.29	0.01	0.30	0.02
22:1 n-11	0.58^{a}	0.01	0.65^{b}	0.03	0.69^{c}	0.03	0.76^{d}	0.03
22:5 n-3	1.19^{a}	0.04	1.40^{b}	0.09	1.61^{c}	0.06	1.87^{d}	0.11
22:6 n-3	2.57^{a}	0.21	3.14^{b}	0.38	3.28^{b}	0.26	3.65^{c}	0.42
24:1 n-9	0.31	0.04	0.33	0.03	0.32	0.04	0.32	0.03
Saturates	24.04^{a}	0.67	25.50^{b}	1.10	26.39^{b}	0.74	27.92^{b}	0.92
Monoenes	35.18	0.84	35.50	1.66	35.29	0.46	34.47	0.86
n-3 LC-PUFA[1]	7.40^{a}	0.22	9.21^{b}	0.65	10.65^{c}	0.47	12.13^{d}	0.75
n-6 LC-PUFA[2]	0.79	0.06	0.86	0.18	0.80	0.09	0.93	0.09

[1]Calculated excluding 18 C. [2]Calculated excluding 18 C.

578 Figure 1

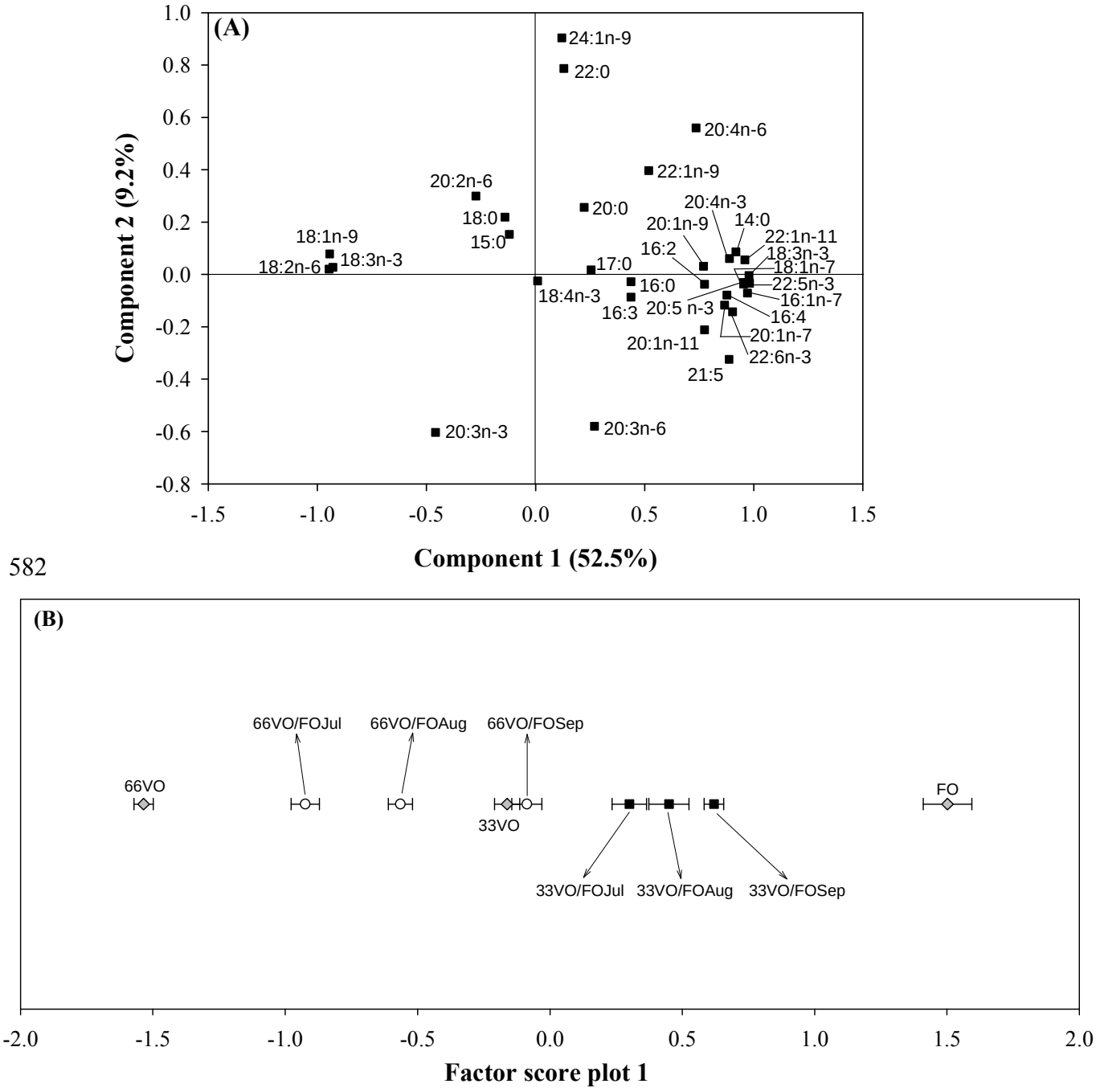

(A)
Temperature (ºC)
Day length (h)
30
25
20
15
10
5
16
15
14
13
12
11
10
09
(B)
DM intake (g / fish): 324.7 307.5
Body Weight (g)
500
400
300
200
100
0
Start
finishing period
Start experimental
diets feeding
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
2007

579 Figure 2

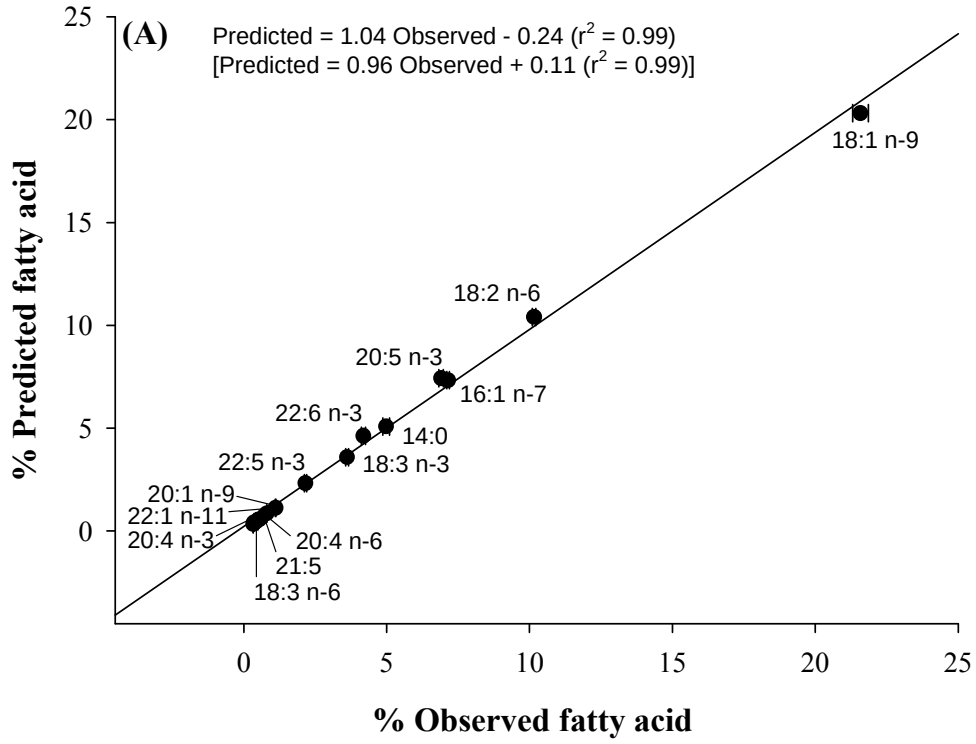


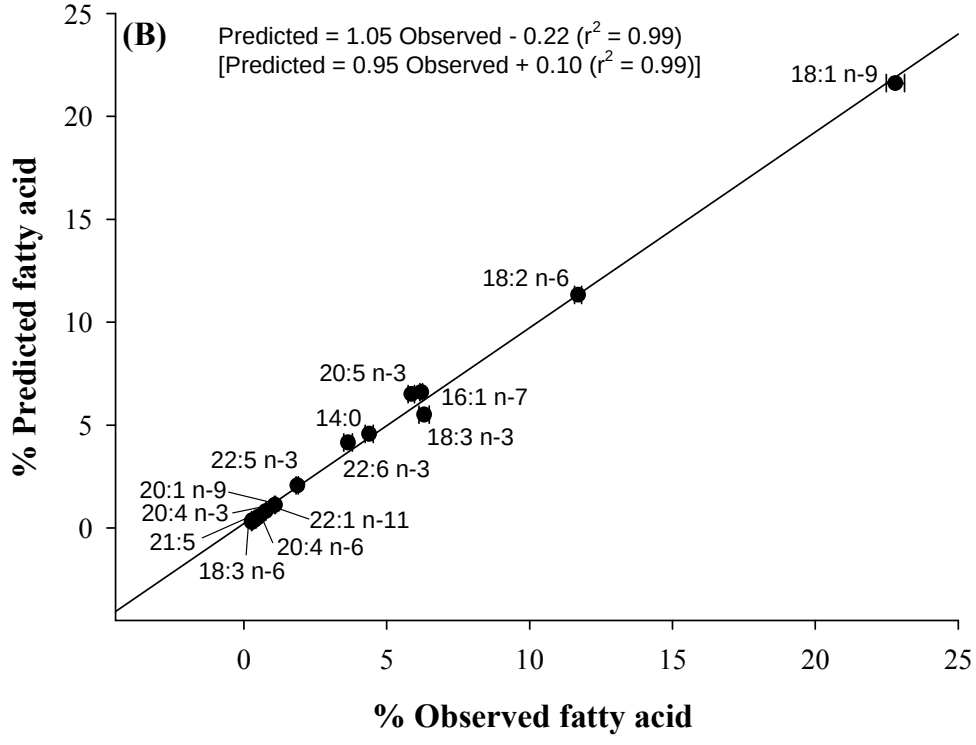
580

581 Figure 3


(A)
Component 2 (9.2%)
Component 1 (52.5%)
24:1n-9
22:0
20:4n-6
22:1n-9
20:2n-6
20:0
20:4n-3
18:0
20:1n-9
14:0
18:1n-9
15:0
16:2
22:1n-11
18:3n-3
18:2n-6
18:3n-3
17:0
18:1n-7
22:5n-3
16:0
18:4n-3
16:1n-7
16:3
20:5 n-3
16:4
20:1n-7
20:1n-11
22:6n-3
21:5
20:3n-3
20:3n-6
(B)
66VO/FOJul
66VO/FOAug
66VO/FOSep
66VO
FO
33VO
33VO/FOJul
33VO/FOAug
33VO/FOSep
Factor score plot 1

583 Figure 4





584