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

Laura Benedito-Palos[a], Juan C. Navarro[a], Azucena Bermejo-Nogales[a], Alfonso Saera-Vila[a], Sadasivam Kaushik[b], Jaume Pérez-Sánchez[a]

[a]Department of Biology, Culture and Pathology of Marine Species, Institute of Aquaculture Torre de la Sal (IATS, CSIC), 12595 Ribera de Cabanes, Castellón, Spain

[b]UMR Nutrition, Aquaculture & Genomics, INRA, Unité-Mixte INRA-IFREMER-Univ. Bordeaux I, 64310 Saint-Pée-sur-Nivelle, France

Corresponding author: Jaume Pérez-Sánchez

Institute of Aquaculture Torre de la Sal, 12595 Ribera de Cabanes, Castellón, Spain.

Phone: +34 964319500

E-mail address: jperez@iats.csic.es

Abstract

The aim of the study was to undertake whether changes in the flesh fatty acid (FA) profiles follow a simple test dilution model after changing of dietary oil sources in gilthead sea bream (*Sparus aurata* L.). 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 the 33% (33VO) and 66% (66VO) of fish oil. The trial included 3 finishing months with fish oil 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 have any detrimental effect on growth, and all groups reached 520-531 g body weight. Changes in proximate 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 flesh FA changes, and excellent correlations between observed and predicted values were found at the end of finishing period in fish grown out with either 33VO or 66VO diets.

Key words: fish, growth, flesh**,** fatty acids, plant proteins

1. Introduction

Fish oil supplies are finite (FAO, 2006) and the continuous increase in the 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 towards the reduction of contaminant loadings in the flesh 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 flesh fatty acid (FA) composition if added at high inclusion levels (Sargent and Tacon, 1999; Torstensen et al., 2005). Thus, it may be desirable the use of finishing diets formulated with uncontaminated/decontaminated fish oils to restore the wild flesh FA profile of farmed fish. For instance, southern hemisphere fish oils are cleaner than the 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. In this regard, we have found that the essential FA requirements of fast growing juvenile gilthead sea bream are met in practical diets by less than 25% of fish meal and fish oil inclusion (Benedito-Palos et al., 2007). Besides, n-3 LC-PUFA, in particular eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 20:6n-3), are selectively incorporated into the polar lipid fraction resulting in a high phospholipid robustness in FA composition (Benedito-Palos et al., 2008). The same study points to that the muscle fat depots highly reflect the composition of the diet with a minor influence of the natural seasonal, thermal or photoperiodical changes. Earlier

studies in gilthead sea bream 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 shift levels in dietary lipid sources. The same model was re-evaluated with different success in Atlantic salmon (Jobling, 2004a; Jobling, 2004b), red sea bream (Jobling, 2004a) and Murray cod (Turchini et al., 2006). The rationale for the present study was to investigate whether dietary FAs are incorporated in the flesh 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 diets (2/5 mm pellets) with the same basal composition were coated with fish and vegetable oils to contain 53% crude protein and 21% crude lipid on a dry weight basis (Table 1). Fish oil of southern hemisphere was the only lipid source in the reference/finishing diet (FO). The other two diets contained a blend of vegetable oils (rapeseed oil: linseed oil: palm oil), replacing the 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 diet. All diets were manufactured using a

89 twin-screw extruder (Clextral, BC 45) at the INRA experimental research station of
90 Donzacq (Landes, France), dried under hot air, sealed and kept in air-tight bags until
91 use.
92
93 *2.2. Grow-out trial*
94
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
99 litres) in groups of 150 fish per tank. Water flow was 20 l/min, and oxygen content of
100 outlet water remained higher than 5 ppm. The growth study was undertaken over 11
101 months (July 11th 2006 – June 6th 2007), and day-length and water temperature (10-
102 26ºC) varied 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 hours; 6-7days per week)
105 from July 2006 to September 2006, and once a day (1200 hours, 4-6 days per week)
106 from October 2006 to June 2007. At regular intervals, fish were counted and group-
107 weighed under moderate anaesthesia (3-aminobenzoic acid ethyl ester, MS 222; 100
108 µg/ml) (Sigma-Aldrich, Madrid, Spain). Feed distributed and mortalities (< 5% during
109 the 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 once a day (6 days per week) the FO diet from June 2007 to September 2007 (12 weeks). These duplicate groups became 33VO/FO and 66VO/FO, respectively. The remaining fish were 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 phase (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 fillets (denuded from skin and bone) were excised, vacuum packed and stored at -80 ºC until analyses. All procedures were carried out according to national and 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 were analysed by standard procedures (AOAC, 1990). Moisture content was determined by drying in an oven at 105º C for 24 h. Diets and freeze-dried fillets were then blended and used for determinations of lipid, protein and ash contents. Lipid content (0.5 g samples) was determined by the Soxhlet method with extraction in diethyl ether at 120 ºC (Soxhlet 4001046 Auto extraction apparatus; Selecta, Barcelona, Spain). Crude protein content (N x 6.25) was determined using the automated Kjeldhal method (Kjeldhal Auto

4002430 Analyser, Selecta, Barcelona, Spain). Ash contents were determined after heating at 600 ºC in a muffle furnace for 2 h.

2.5. FA analyses

Total lipids for FA analyses were extracted by the method of Folch et al. (1957), using chloroform:methanol (2:1) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant. After the addition of nonadecanoic FA (19:0) as internal standard, total lipids (TL) were subjected to acid-catalysed transmethylation for 16 hours at 50 ºC using 1 ml toluene and 2 ml of 1% (v/v) sulphuric acid in methanol (Christie, 1982). FA methyl esters (FAME) were extracted with hexane:diethyl ether (1:1), and purified by thin layer chromatography (Silica gel G 60, 20 x 20 cm glass plates, Merck, Darmstadt, Germany) using hexane:diethyl-ether:acetic acid (85:15:1.5) as a solvent system. FAME were then analyzed with a Fisons Instruments GC 8000 Series (Rodano, Italy) gas chromatograph, equipped with a fused silica 30 m x 0.25 mm open tubular column (Tracer, TR-WAX; film thickness: 0.25 μm, Teknokroma, Spain) and a cold on-column injection system. Helium was used as a carrier gas, and temperature programming was from 50 to 180 ºC at 40 ºC/min and then to 220 ºC at 3 ºC/min. Peaks were recorded in a personal computer using the Azur software package (version 4.0.2.0. Datalys, France). Individual FAME were identified by reference to well characterized fish oil standards, and the relative amount of each FA was expressed as a percentage of the total amount of FA in the analysed sample.

BHT and internal standard (19:0) were obtained from Sigma-Aldrich (Madrid, Spain). All solvents in lipid extraction and FA analyses were HPLC grade and were obtained from Merck (Darmstadt, Germany).

160 *2.6. Dilution model*

161

162 Changes in the flesh 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) flesh
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 flesh 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*

177

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 rates, feed intake and feed conversion were not affected by the dietary treatment over the course of the study. Hence, at each sampling point, all data on body weight and feed intake were put together and represented in the fitting plot as the mean of the 9 experimental tanks (Fig. 1). 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 whole 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 than that of the initial period (324 *vs* 307 g). At the end of the wash-out trial, 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 our experimental model, the usage of the added fish oil in the diet (g/fish) in fish always fed FO, 33VO and 66VO diets (growth- plus wash-out periods) was proportional to the percentage of replacement (Fig. 2). At the end of the fish oil wash-out 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 a 15% in comparison to fish always fed the FO diet.

206 *3.2. Lipid content and flesh FA profile*

207

208 Fillet yield and lipid content of skinned fillets were not significantly affected by
209 the dietary treatment (Table 3). However, lipid deposition increased by a 20–30 % over
210 the course of the finishing period regardless of the dietary treatment (Tables 3-5).
211 As shown in Table 3, no consistent changes on the FA profile were found over
212 the course of the finishing period in fish continuously fed with the same diets (FO,
213 33VO and 66VO groups), and only few FAs (16:1 n-7, 17:0, 18:2 n-6, 21:5) showed
214 significant differences (less than 5-30% of variation) in one or two of the three
215 experimental groups. Regarding the effect of dietary treatment, fish fed the FO diet
216 contained 29% saturates (mainly 16:0 and 14:0), almost 32% monoenes (over half of
217 which were 18:1n-9), 1% n-6 LC-PUFA, and 17% n-3 LC-PUFA (predominantly EPA
218 and DHA). Increased amounts of 18:1n-9, 18:2n-6 and 18:3n-3, in combination with
219 reduced proportions of n-3 LC-PUFA and saturated FAs, were found with the
220 progressive replacement of fish oil by vegetable oils.
221 The time course of changes through the finishing period on the flesh FA profile
222 of fish previously fed vegetable oils are shown in Tables 4 and 5. Both in 33VO/FO and
223 66VO/FO groups, the finishing diet caused a progressive increase in the FAs present in
224 higher amounts in fish oil (i.e. 14:0, 16:1n-7, 20:1n-9, 22:1n-11, EPA and DHA), while
225 those characteristic of vegetable oils (i.e. 18:1n-9, 18:2n-6 and 18:3n-3) decreased in
226 proportion to degree of fish oil replacement in the diet.
227 The MPCA analysis of fillet FA profiles before, during and at the end of the
228 finishing period revealed that the two first components accounted for 62% of the total
229 variation, with 52.5% of the variation being explained by component 1 itself (Fig. 3A).
230 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 the three invariable groups (FO, 33VO and 66VO) 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 trial, convincingly demonstrate that dietary fish oils of northern and southern origin can be replaced up to 66% without negative effects on the growth performance of gilthead sea bream. These original data are pioneer at in a stenohaline marine teleost maximizing the simultaneous replacement of fish meal and fish oil by alternative plant

256 ingredients without any histological sign of damage in the liver and intestine epithelium
257 (Benedito-Palos et al., 2008). Other metabolic effects of feeding diets with alternative
258 oils are complex, interconnected and, to date, not fully understood in gilthead sea bream
259 and other fish species. However, it must be noted that growth-compensatory
260 mechanisms are orchestrated at the local tissue level (skeletal muscle) by the
261 somatotropic axis when fast growing juvenile fish of gilthead sea bream are fed with
262 increased levels of vegetable oils (Benedito-Palos et al., 2007). Besides, n-3 LC-PUFA
263 are selectively incorporated into polar lipids, and the stability of muscle phospholipid
264 FA composition is a useful criterion to assess the suitability of the replacement strategy
265 in fish feeds with low levels of marine derived ingredients (Benedito-Palos et al., 2008).
266 In fish and higher vertebrate species, neutral lipids are less conservative than
267 phospholipids (Tocher, 2003; Skalli and Robin, 2004; Schulz et al., 2005). This is
268 because they are the fat storage form and its FA profile highly reflects that of the diet. In
269 the present study, the muscle lipid content was greater than 10% on wet matter basis,
270 and the FA profile of total lipids and thereby that of triglycerides (TAG) remained
271 mostly unchanged (finishing phase) in fish always fed either FO, 33VO or 66VO diets
272 (Table 3). The result of these temporal series agrees with data on a previous seasonal
273 study (Benedito-Palos et al., 2008), and reinforces the idea that accelerated growth
274 overrides major changes in the FA profile in spite of concurrent changes in body
275 composition through weight gain (Grigorakis, 2007). However, it should be born in
276 mind that the tissue-specific FA profile varies in salmonids with the size and age of fish
277 (Bell et al., 2002; Bell et al., 2003a). Probably, this is also true for gilthead sea bream
278 and FA databases considering the effect of different size class, gender, season,
279 maturation state, and nutritional condition are now under construction. Anyway, fish oil
280 replacement by alternative lipid sources has a pronounced effect on the flesh FA profile

281 of most fish. In the present study, we found in fish always fed 33VO and 66VO diets a
282 22-36 % increase of 18:1 n-9 and 18:2 n-6 with a concurrent 20-65 % reduction in EPA
283 and DHA. Similar results have been reported in gilthead sea bream (Izquierdo et al.,
284 2005) and a wide variety of fish species, including Atlantic salmon (Bell et al., 2002;
285 Bransden et al., 2003; Bell et al., 2003b; Torstensen et al., 2004; Bell, 2004; Nanton et
286 al., 2007), rainbow trout (Drew et al., 2007), turbot (Regost et al., 2003), European sea
287 bass (Montero et al., 2005; Mourente and Bell, 2006), Murray cod (Francis et al.,
288 2007a; 2007b), red sea bream (Piedecausa et al., 2007; Huang et al., 2007) and black sea
289 bream (Peng et al., 2008). Since this feature can compromise the beneficial effects of
290 sea food (Din et al., 2004; Psota et al., 2006) as main source of EPA and DHA in the
291 human diet, there is now increased interest in finfish aquaculture for modelling the
292 time-course of FA changes during fish oil re-feeding.

293 Gilthead sea bream shows in our latitude a pronounced growth seasonality
294 (Mingarro et al., 2002), and the window for fish oil wash-out should take place in the
295 broadly active feeding period of May-October. Thus, after the growth stop of cold
296 season, one month (May) was spent before to start the finishing trial that then continued
297 through the summer growth spurt (June-September). Several variables, including among
298 others the growth and lipid deposition rates, need to be considered to analyse the
299 effectiveness of fish oil wash-out. Therefore, one must be cautious before drawing a
300 definitive conclusion, but the literature is prolific on studies in which a complete
301 restoration of the FA profile is not fully achieved after fish oil re-feeding: 32 days red
302 sea bream (Glencross et al., 2003), 8 weeks turbot (Regost et al., 2003); 8 weeks brown
303 trout (Robin et al., 2003), 12-25 weeks Atlantic salmon (Bell et al., 2003a; Bell et al.,
304 2003b; Torstensen et al., 2004; Bell, 2004); 3 months gilthead sea bream (Izquierdo et
305 al., 2005); 4 months Murray cod (Turchini et al., 2006); 5 months European sea bass

306 (Montero et al., 2005). Most evidences point towards a dilution model, which was
307 proved in turbot, brown trout and Murray cod. Using original data and those derived
308 from red sea bream (Glencross et al., 2003) and Atlantic salmon (Bell et al., 2003a)
309 studies, Jobling (2004a; 2004b) concluded that a dilution process also plays a key role
310 in governing the muscle FA profile of these fish species. Our work in gilthead sea
311 bream points clearly towards the same direction, and gradual changes in the FA profiles
312 of 33VO/FO and 66VO/FO groups were found during the finishing period, making
313 them increasingly similar to the FO group. This is particularly highlighted by the results
314 of the MPCA (Fig. 3) that shows the gradient of fish oil load along the ordinate axis.
315 Therefore, changes in the FA profile arise because the existing stores become
316 diluted as fish grow and deposit increasing amounts of dietary-derived FAs. In other
317 words, nutritional background in fish with no apparent signs of FA deficiencies has a
318 marginal role on FA turnover and flesh FA profiles, although age- and nutritional
319 condition affect the expression pattern of cytokines and key limiting enzymes on tissue
320 FA uptake and mobilization (Saera-Vila et al., 2005; 2007). Thus, using the simple
321 dilution model, a reliable FA prediction was found herein at the end of the fish oil
322 finishing period regardless of the level of fish oil replacement (Fig. 4). The model is in
323 fact a good general descriptor of FAs, and regression curves (predicted *vs* observed
324 values) give slopes nearby to the line of equality when either selected or almost all FAs
325 were considered in the model. In Atlantic salmon, Jobling (2004b) tested three FAs
326 (18:1 isomers, 18:2 n-6 and 18:3 n-3) and confirmed closely the predictions made with
327 the dilution model. Jobling (2004a) again evaluated the dilution model with data from
328 red sea bream studies (Glencross et al., 2003), and a high degree of concordance was
329 found between the predicted and observed values. Turchini et al. (2006) also indicated
330 that deviations for some individual FAs in Murray cod does not invalidate totally the

331 FA dilution model, although FA oxidation and biosynthetic capacities vary from one
332 fish species to another. In spite of these limitations, the dilution model is a useful tool
333 for predicting the FA composition of aquaculture growing fish. This is especially true
334 for gilthead sea bream, and MPCA highlights that fish grow-out with the 33VO diet
335 need more than 12 weeks to revert back the FA composition towards the normal
336 variability of fish fed fish oil-based diets. Besides, low and intermediate levels of fish
337 oil replacement can produce equally acceptable fillets when the latter is accompanied by
338 a fish oil finishing phase. This is because temporal changes on fish oil intake gave a
339 minor effect on the muscle FA profile if the absolute amount becomes equal at the end
340 of the trial. In our experimental model, this was the case of 33VO and 66VO/FO groups
341 (Fig. 2), and the flesh FA profile at the end of the 3-month finishing period was very
342 close in both groups (Fig. 3B).

343 Savings on fish oil resources are therefore limited to a simple dilution, and new
344 approaches are required to improve any mobilisation or turnover of pre-existing FAs.
345 Intake of conjugate linoleic acid complex (CLA) has a lipid-lowering effect in gilthead
346 sea bream juveniles, promotes the diversion of dietary-derived TAG from muscle and
347 adipose tissue to liver, and increase hepatic peroxisomal β oxidation (Diez et al., 2007).
348 At the same time, however, the LC-PUFA biosynthesis is reduced and changes in the
349 muscle FA profile indicate that the inclusion of CLA in aquaculture diets would be of
350 little benefit in gilthead sea bream. Nevertheless, the use of more specific
351 agonists/antagonists of peroxisome proliferators-activated receptors (PPARs) cannot be
352 excluded to improve the retention of n-3 LC-PUFA. Attention also needs to be focused
353 on the transfer from fish oil of PCBs, dioxins and other harmful lipophilic organic
354 chemicals that are now ubiquitous contaminants of marine ecosystems (Sargent et al.,
355 1995; Jacobs et al., 2002; Bell et al., 2005; Domingo, 2007). The effects of feeding

356 strategies on toxic-kinetics will be reported separately to have a more complete
357 framework of nutritional fish tailoring, and to gain public acceptance for the now
358 becoming fish fed with alternative and sustainable diets.
359
360

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.

366 **References**
367

368 Association of Official Analytical Chemists, 1990. Official Methods of Analysis.
369 AOAC, K Heldrich, Arlington, VA, pp. 684.

370 Bell, J.G., 2004. Replacement of dietary fish oil with increasing levels of linseed oil:
371 Modification of flesh fatty acid compositions in Atlantic salmon (*Salmo salar*) using a
372 fish oil finishing diet. Lipids 39, 223-232.

373 Bell, J.G., Henderson, R.J., Tocher, D.R., McGhee, F., Dick, J.R., Porter, A., Smullen,
374 R.P., Sargent, J.R., 2002. Substituting Fish Oil with Crude Palm Oil in the Diet of
375 Atlantic Salmon (*Salmo salar*) Affects Muscle Fatty Acid Composition and Hepatic
376 Fatty Acid Metabolism. J. Nutr. 132, 222-230.

377 Bell, J.G., Tocher, D.R., Henderson, R.J., Dick, J.R., Crampton, V.O., 2003a. Altered
378 fatty acid compositions in atlantic salmon (*Salmo salar*) fed diets containing linseed and
379 rapeseed oils can be partially restored by a subsequent fish oil finishing diet. J. Nutr.
380 133, 2793-2801.

381 Bell, J.G., McGhee, F., Campbell, P.J., Sargent, J.R., 2003b. Rapeseed oil as an
382 alternative to marine fish oil in diets of post-smolt Atlantic salmon (*Salmo salar*):
383 changes in flesh fatty acid composition and effectiveness of subsequent fish oil "wash
384 out". Aquaculture 218, 515-528.

385 Bell, J.G., McGhee, F., Dick, J.R., Tocher, D.R., 2005. Dioxin and dioxin-like
386 polychlorinated biphenyls (PCBs) in Scottish farmed salmon (Salmo salar): effects of
387 replacement of dietary marine fish oil with vegetable oils. Aquaculture 243, 305-314.

388 Benedito-Palos, L., Saera-Vila, A., Calduch-Giner, J.A., Kaushik, S., Pérez-Sánchez, J.,
389 2007. Combined replacement of fish meal and oil in practical diets for fast growing
390 juveniles of gilthead sea bream (*Sparus aurata* L.): Networking of systemic and local
391 components of GH/IGF axis. Aquaculture 267, 199-212.

392 Benedito-Palos, L., Navarro, J.C., Sitjà-Bobadilla, A., Bell, J.G., Kaushik, S., Pérez-
393 Sánchez, J., 2008. High levels of vegetable oils in plan protein-rich diets fed to gilthead
394 sea bream (*Sparus aurata* L.): growth performance, muscle fatty acid profiles and
395 histological alterations of target tissues. Br. J. Nutr., *In press*.

396 Bransden, M.P., Carter, C.G., Nichols, P.D., 2003. Replacement of fish oil with
397 sunflower oil in feeds for Atlantic salmon (*Salmo salar* L.): effect on growth
398 performance, tissue fatty acid composition and disease resistance. Comp. Biochem.
399 Phys. 135B, 611-625.

400 Christie,W.W., 1982. Lipid analysis. Isolation, separation, identification and structural
401 analysis of lipids, 2nd ed. Pergamon Press, Oxford, pp. 207.

402 Diez, A., Menoyo, D., Pérez-Benavente, S., Calduch-Giner, J.A., Vega-Rubín de Celis,
403 S., Obach, A., Favre-Krey, L., Boukouvala, E., Leaver, M.J., Tocher, D.R., Pérez-
404 Sánchez, J., Krey, G., Bautista, J.M., 2007. Conjugated linoleic acid affects lipid

composition, metabolism, and gene expression in gilthead sea bream (*Sparus aurata* L). J. Nutr. 137, 1363-1369.

Din, J.N., Newby, D.E., Flapan, A.D., 2004. Science, medicine, and the future - Omega 3 fatty acids and cardiovascular disease - fishing for a natural treatment. Brit. Med. J. 328, 30-35.

Domingo, J.L., 2007. Omega-3 fatty acids and the benefits of fish consumption: Is all that glitters gold? Environ. Int. 33, 993-998.

Drew, M.D., Ogunkoya, A.E., Janz, D.M., Van Kessel, A.G., 2007. Dietary influence of replacing fish meal and oil with canola protein concentrate and vegetable oils on growth performance, fatty acid composition and organochlorine residues in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 267, 260-268.

Folch, J., Less, N., Sloane-Stanley, G.H., 1957. A simple method for insolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497-509.

Food and Agricultural Organisation (FAO), 2006. State of world aquaculture, FAO Fisheries Technical paper ed. FAO, Rome, pp. 134.

Francis, D.S., Turchini, G.M., Jones, P.L., De Silva, S.S., 2007a. Effects of fish oil substitution with a mix blend vegetable oil on nutrient digestibility in Murray cod, *Maccullochella peelii peelii*. Aquaculture 269, 447-455.

423 Francis, D.S., Turchini, G.M., Jones, P.L., De Silva, S.S., 2007b. Growth performance,
424 feed efficiency and fatty acid composition of juvenile Murray cod, *Maccullochella*
425 *peelii peelii*, fed graded levels of canola and linseed oil. Aquacult. Nutr. 13, 335-350.

426 Glencross, B.D., Hawkins, W.E., Curnow, J.G., 2003. Restoration of the fatty acid
427 composition of red seabream (*Pagrus auratus*) using a fish oil finishing diet after grow-
428 out on plant oil based diets. Aquacult. Nutr. 9, 409-418.

429 Grigorakis, K., 2007. Compositional and organoleptic quality of farmed and wild
430 gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) and factors
431 affecting it: a review. Aquaculture 272, 55-75.

432 Huang, S.S.Y., Oo, A.N., Higgs, D.A., Brauner, C.J., Satoh, S., 2007. Effect of dietary
433 canola oil level on the growth performance and fatty acid composition of juvenile red
434 sea bream, Pagrus major. Aquaculture 271, 420-431.

435 Izquierdo, M.S., Montero, D., Robaina, L., Caballero, M.J., Rosenlund, G., Ginés, R.,
436 2005. Alterations in fillet fatty acid profile and flesh quality in gilthead seabream
437 (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles
438 by fish oil feeding. Aquaculture 250, 431-444.

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

442 Jobling, M., 2004a. 'Finishing' feeds for carnivorous fish and the fatty acid dilution
443 model. Aquac. Res. 35, 706-709.

444 Jobling, M., 2004b. Are modifications in tissue fatty acid profiles following a change in
445 diet the result of dilution?: Test of a simple dilution model. Aquaculture 232, 551-562.

446 Mingarro, M., Vega-Rubín de Celis, S., Astola, A., Pendón, C., Martínez Valdivia, M.,
447 Pérez-Sánchez, J., 2002. Endocrine mediators of seasonal growth in gilthead sea bream
448 (*Sparus aurata*): the growth hormone and somatolactin paradigm. Gen. Comp. Endocr.
449 128, 102-111.

450 Montero, D., Robaina, L., Caballero, M.J., Ginés, R., Izquierdo, M.S., 2005. Growth,
451 feed utilization and flesh quality of European sea bass (*Dicentrarchus labrax*) fed diets
452 containing vegetable oils: A time-course study on the effect of a re-feeding period with
453 a 100% fish oil diet. Aquaculture 248, 121-134.

454 Mourente, G., Bell, J.G., 2006. Partial replacement of dietary fish oil with blends of
455 vegetable oils (rapeseed, linseed and palm oils) in diets for European sea bass
456 (*Dicentrarchus labrax* L.) over a long term growth study: Effects on muscle and liver
457 fatty acid composition and effectiveness of a fish oil finishing diet. Comp. Biochem.
458 Phys. 145B, 389-399.

459 Nanton, D.A., Vegusdal, A., Rørå, A.M.B., Ruyter, B., Baeverfjord, G., Torstensen,
460 B.E., 2007. Muscle lipid storage pattern, composition, and adipocyte distribution in
461 different parts of Atlantic salmon (*Salmo salar*) fed fish oil and vegetable oil.
462 Aquaculture 265, 230-243.

463 Peng, S., Chen, L., Qin, J.G., Hou, J., Yu, N., Long, Z., Ye, J., Sun, X., 2008. Effects of
464 replacement of dietary fish oil by soybean oil on growth performance and liver
465 biochemical composition in juvenile black seabream, *Acanthopagrus schlegeli*.
466 Aquaculture 276, 161.

467 Piedecausa, M.A., Mazon, M.J., Garcia Garcia, B., Hernandez, M.D., 2007. Effects of
468 total replacement of fish oil by vegetable oils in the diets of sharpsnout seabream
469 (*Diplodus puntazzo*). Aquaculture 263, 211-219.

470 Pratoomyot, J., Bendiksen, E.Å., Bell, J.G., Tocher, D.R., 2008. Comparison of effects
471 of vegetable oils blended with southern hemisphere fish oil and decontaminated
472 northern hemisphere fish oil on growth performance, composition and gene expression
473 in Atlantic salmon (*Salmo salar* L.). Aquaculture 280, 170-178.

474 Psota, T.L., Gebauer, S.K., Kris-Etherton, P., 2006. Dietary Omega-3 Fatty Acid Intake
475 and Cardiovascular Risk. Am. J. Cardiol. 98, 3-18.

476 Regost, C., Arzel, J., Robin, J., Rosenlund, G., Kaushik, S.J., 2003. Total replacement
477 of fish oil by soybean or linseed oil with a return to fish oil in turbot (*Psetta maxima*): 1.
478 Growth performance, flesh fatty acid profile, and lipid metabolism. Aquaculture 217,
479 465-482.

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

483 Saera-Vila, A., Calduch-Giner, J.A., Gómez-Requeni, P., Médale, F., Kaushik, S.,
484 Pérez-Sánchez, J., 2005. Molecular characterization of gilthead sea bream (*Sparus*
485 *aurata*) lipoprotein lipase. Transcriptional regulation by season and nutritional
486 condition in skeletal muscle and fat storage tissues. Comp. Biochem. Phys. 142B, 224-
487 232.

488 Saera-Vila, A., Calduch-Giner, J.A., Navarro, I., Pérez-Sánchez, J., 2007. Tumour
489 necrosis factor (TNF)α as a regulator of fat tissue mass in the Mediterranean gilthead
490 sea bream (*Sparus aurata* L.). Comp. Biochem. Phys. 146B, 338-345.

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

493 Sargent, J.R., Bell, J.G., Bell, M.V., Henderson, R.J., Tocher, D.R., 1995. Requirement
494 criteria for essential fatty acids. J. Appl. Ichthyol. 11, 183-198.

495 Schulz, C., Knaus, U., Wirth, M., Rennert, B., 2005. Effects of varying dietary fatty
496 acid profile on growth performance, fatty acid, body and tissue composition of juvenile
497 pike perch (*Sander lucioperca*). Aquacult. Nutr. 11, 403-413.

498 Skalli, A., Robin, J.H., 2004. Requirement of n-3 long chain polyunsaturated fatty acids
499 for European sea bass (*Dicentrarchus labrax*) juveniles: growth and fatty acid
500 composition. Aquaculture 240, 399-415.

501 Tocher, D.R., 2003. Metabolism and Functions of Lipids and Fatty Acids in Teleost
502 Fish. Rev. Fish. Sci. 11, 107-184.

503 Torstensen, B.E., Froyland, L., Ørnsrud, R., Lie, Ø., 2004. Tailoring of a
504 cardioprotective muscle fatty acid composition of Atlantic salmon (*Salmo salar*) fed
505 vegetable oils. Food Chem. 87, 567-580.

506 Torstensen, B.E., Froyland, L., Ørnsrud, R., Lie, Ø., 2005. Tailoring of Atlantic salmon
507 (*Salmo salar* L.) flesh lipid composition and sensory quality by replacing fish oil with a
508 vegetable oil blend. J. Agr. Food Chem. 53, 10166-10178.

509 Turchini, G.M., Francis, D.S., De Silva, S.S., 2006. Modification of tissue fatty acid
510 composition in Murray cod (*Maccullochella peelii peelii*, Mitchell) resulting from a
511 shift from vegetable oil diets to a fish oil diet. Aquac. Res. 37, 570-585.

512 Watanabe, T., 2002. Strategies for further development of aquatic feeds. Fisheries Sci.
513 68, 242-252.
514
515
516

517 L**egends**

518

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

524

525 Figure 2. Effects of diet composition and feeding protocol on fish oil (added fish oil in
526 the diet) intake through grow-out (330 days, black bars) and finishing (88 days, grey
527 bars) periods.

528

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

535

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

540

541 **Table 1.** Ingredients and chemical composition of 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
Protein (% DM)	53.2	52.81	52.62
Fat (% DM)	21.09	21	20.99
Ash (% DM)	6.52	6.69	6.57

542
543 [1]Fish meal (Scandinavian LT)
544 [2]Fish soluble protein concentrate (Sopropêche, France)
545 [3]Fish oil (Sopropêche, France)
546 [4]Supplied the following (mg / kg diet, except as noted): calcium
547 carbonate (40% Ca) 2.15 g, magnesium hydroxide (60% Mg)
548 1.24 g, potassium chloride 0.9 g, ferric citrate 0.2 g, potassium
549 iodine 4 mg, sodium chloride 0.4 g, calcium hydrogen phosphate
550 50 g, copper sulphate 0.3, zinc sulphate 40, cobalt sulphate 2,
551 manganese sulphate 30, sodium selenite 0.3.
552 [5]Supplied the following (mg / kg diet): retinyl acetate 2.58, DL-
553 cholecalciferol 0.037, DL-α tocopheryl acetate 30, menadione
554 sodium bisulphite 2.5, thiamin 7.5, riboflavin 15, pyridoxine 7.5,
555 nicotinic acid 87.5, folic acid 2.5, calcium pantothenate 2.5,
556 vitamin B_{12} 0.025, ascorbic acid 250, inositol 500, biotin 1.25
557 and choline chloride 500.
558

559 **Table 2**. FA composition of experimental diets (% of total FAME).
560

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
21:5	0.4	0.22	0.06
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

561 tr = trace values
562 [1]Calculated excluding 16 C and 18 C.
563 [2]Calculated excluding 18 C.

564 Table 3. Fillet weight, wet lipid content and FA profile (% of total FAME) in fish always fed
565 FO, 33VO and 66VO diets. Fish were sampled at the beginning (June 2007) and at the end of
566 the finishing period (September 2007). Mean values and standard deviations are presented (n =
567 8). Mean values within dietary groups with unlike superscript letters are significantly different
568 (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
Lipids (%)	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
21:5	0.41[a]	0.05	0.50[b]	0.06	0.31	0.03	0.30	0.04	0.17	0.02	0.13	0.06
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.

569 Table 4. Effects of finishing diet on fillet weight, wet lipid content and FA profile (% of total
570 FAME) in fish fed 33VO diet and then FO diet (33VO/FO group). Fish were sequentially
571 sampled through the finishing period (June, +0; July, +27; August, +55; September, +88).
572 Mean values and standard deviations are presented (n = 8). Raw values with unlike superscript
573 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
Lipids (%)	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
21:5	0.31	0.03	0.32	0.04	0.36	0.05	0.36	0.04
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.

574 Table 5. Effects of finishing diet on fillet weight, wet lipid content and FA profile (% of total
575 FAME) in fish fed 66VO diet and then FO diet (66VO/FO group). Fish were sequentially
576 sampled through the finishing period (June, +0; July, +27; August, +55; September, +88).
577 Mean values and standard deviations are presented (n = 8). Raw values with unlike superscript
578 letters are significantly different over sampling time (P<0.05).
579

	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
Lipids (%)	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
21:5	0.17^{a}	0.02	0.21^{bc}	0.08	0.26^{bc}	0.03	0.29^{c}	0.07
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.

Figure 1

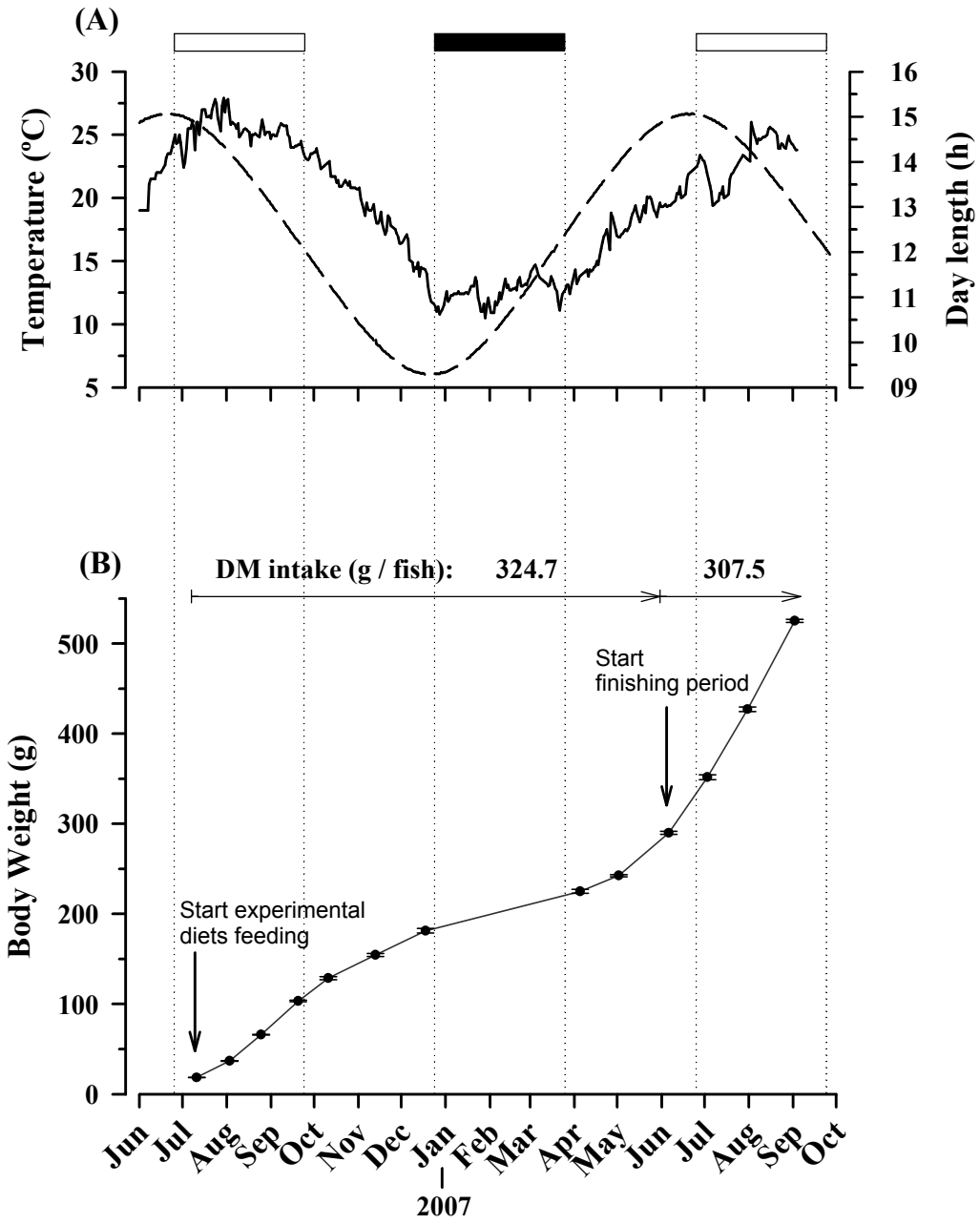

(A)
Temperature (ºC)
Day length (h)
30
25
20
15
10
5
16
15
14
13
12
11
10
09
(B)
DM intake (g / fish): 324.7 307.5
Body Weight (g)
500
400
300
200
100
0
Start
finishing period
Start experimental
diets feeding
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
2007

Figure 2

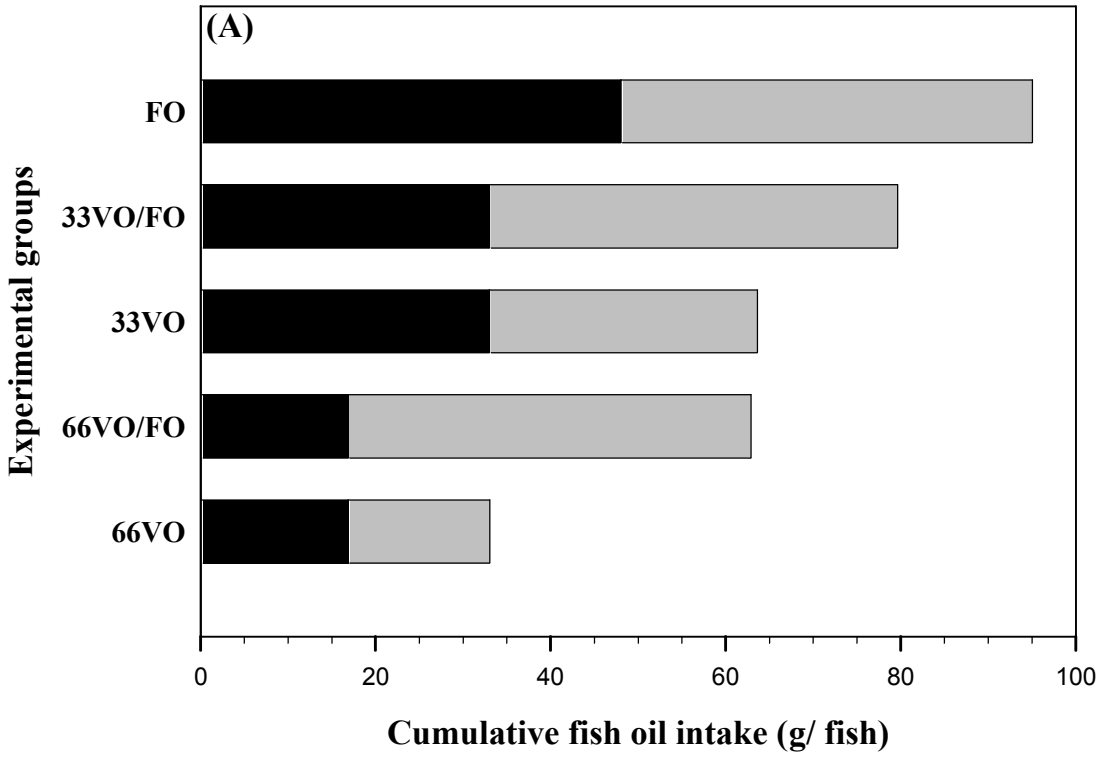

(A)
FO
33VO/FO
33VO
66VO/FO
66VO
Experimental groups
0
20
40
60
80
100
Cumulative fish oil intake (g/ fish)

Figure 3

Figure 3

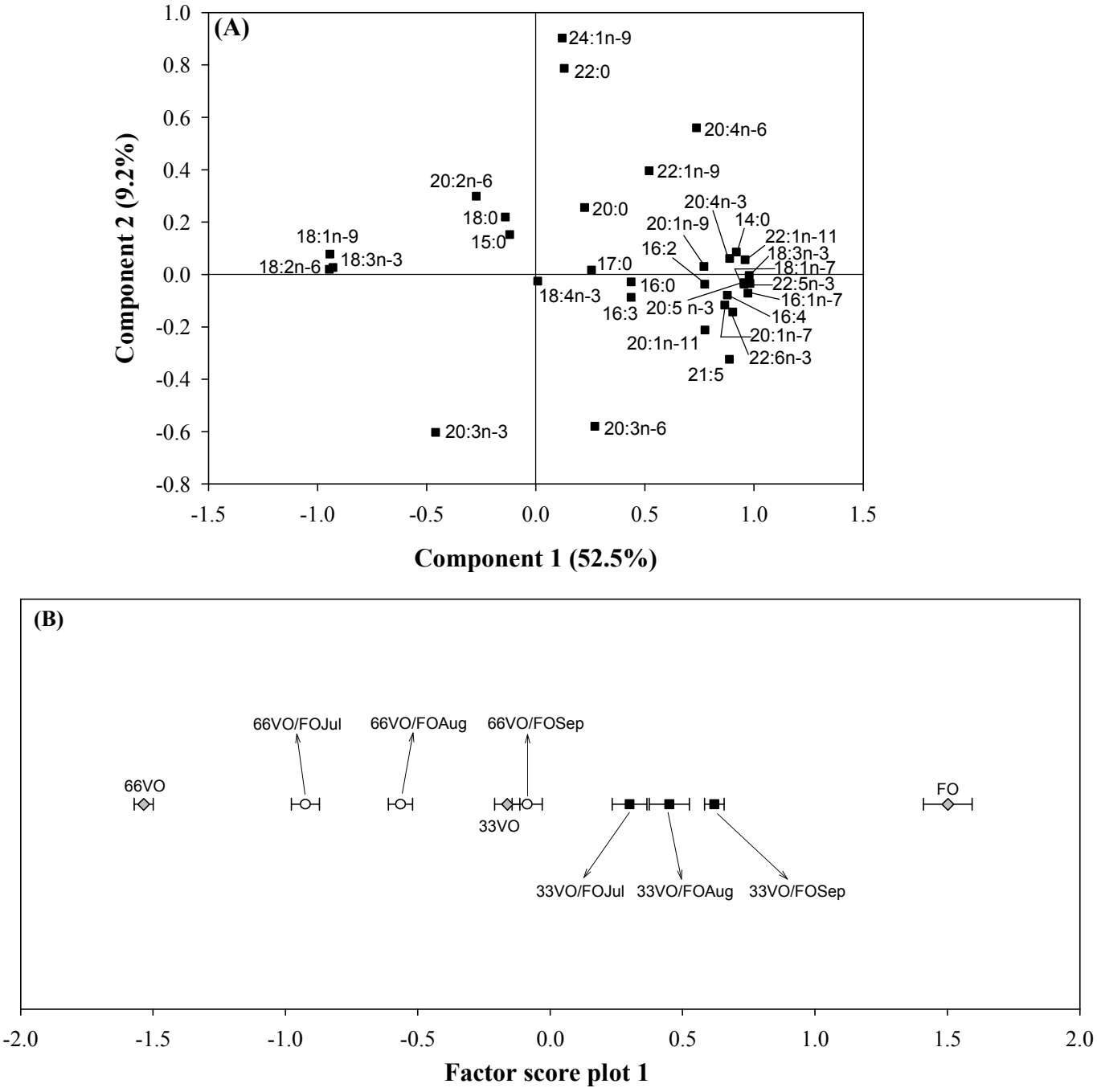
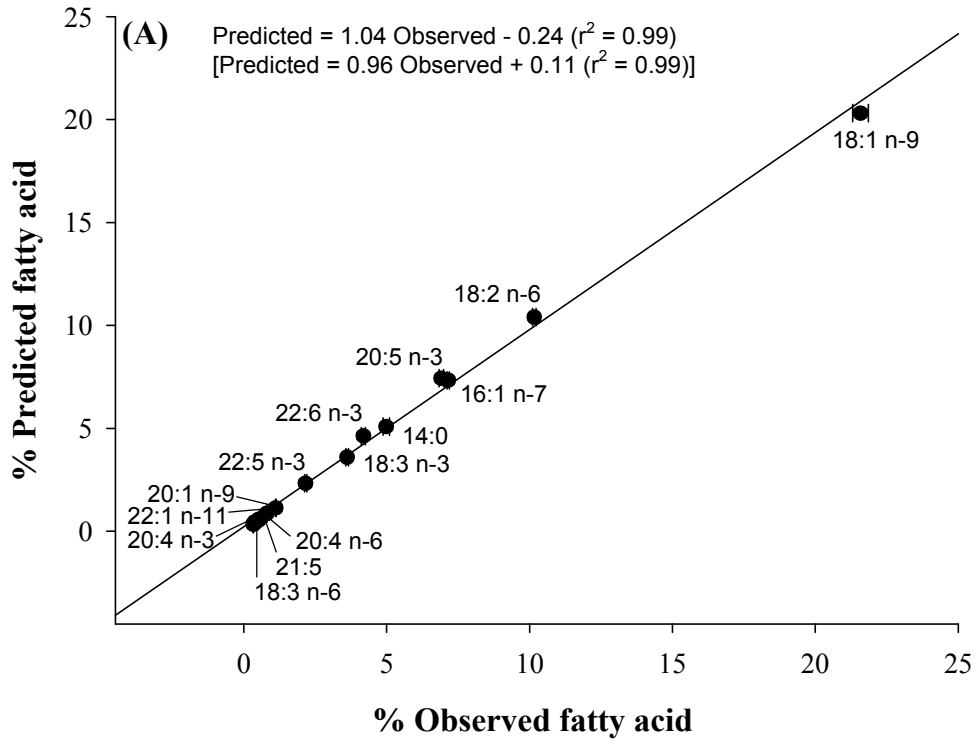
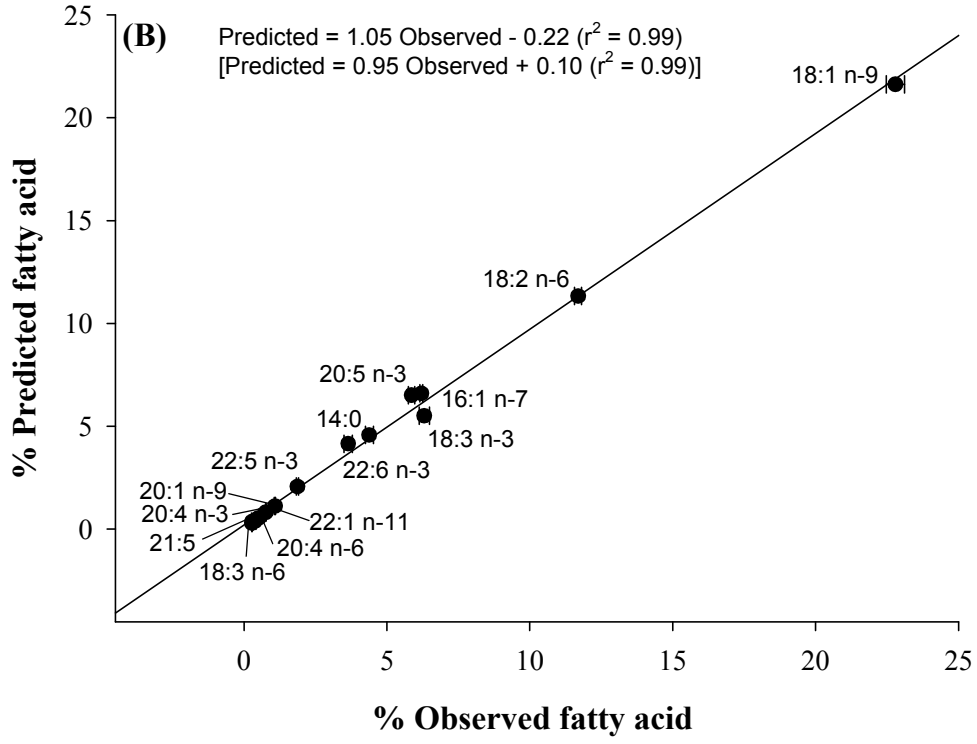


Figure 4


(A)
Predicted = 1.04 Observed - 0.24 (r^2 = 0.99)
[Predicted = 0.96 Observed + 0.11 (r^2 = 0.99)]
% Predicted fatty acid
% Observed fatty acid
18:1 n-9
18:2 n-6
20:5 n-3
16:1 n-7
22:6 n-3
14:0
22:5 n-3
18:3 n-3
20:1 n-9
22:1 n-11
20:4 n-3
20:4 n-6
21:5
18:3 n-6
0
5
10
15
20
25


(B)
Predicted = 1.05 Observed - 0.22 (r^2 = 0.99)
[Predicted = 0.95 Observed + 0.10 (r^2 = 0.99)]
% Predicted fatty acid
% Observed fatty acid
18:1 n-9
18:2 n-6
20:5 n-3
16:1 n-7
14:0
18:3 n-3
22:5 n-3
22:6 n-3
20:1 n-9
20:4 n-3
22:1 n-11
21:5
20:4 n-6
18:3 n-6
0
5
10
15
20
25