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High levels of vegetable oils in plant protein-rich diets fed to gilthead sea bream (*Sparus aurata L.*): growth performance, muscle fatty acid profiles and histological alterations of target tissues

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Abstract:

The feasibility of fish oil (FO) replacement by vegetable oils (VO) was investigated in gilthead sea bream (*Sparus aurata* L.) in a growth trial conducted for the duration of 8 months. Four isolipidic and isoproteic diets rich in plant proteins were supplemented with L-lysine (0·55 %) and soya lecithin (1 %). Added oil was either FO (control) or a blend of VO, replacing 33 % (33VO diet), 66 % (66VO diet) and 100 % (VO diet) of FO. No detrimental effects on growth performance were found with the partial FO replacement, but feed intake and growth rates were reduced by about 10 % in fish fed the VO diet. The replacement strategy did not damage the intestinal epithelium, and massive accumulation of lipid droplets was not found within enterocytes. All fish showed fatty livers, but signs of lipoid liver disease were only found in fish fed the VO diet. Muscle fatty acid profiles of total lipids reflected the diet composition with a selective incorporation of unsaturated fatty acids in polar lipids. The robustness of the phospholipid fatty acid profile when essential fatty acid requirements were theoretically covered by the diet was evidenced by multivariate principal components analysis in fish fed control, 33VO and 66VO diets.

Keywords: Essential fatty acids; Phospholipids; Soya lecithin; Lipoid liver disease

Introduction

Marine fish farming is mostly based on diets containing high levels of n-3 highly unsaturated fatty acids (n-3 HUFA), particularly eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). However, the continuous expansion of aquaculture and the decreasing global availability of marine oil and fish meal force the industry to explore alternative and sustainable lipid sources[1,2]. In salmonids, the use of vegetable oils to replace the majority of dietary fish oil (FO) is now feasible in practical aquafeeds without loss of growth performance[3-5]. Nevertheless, essential fatty acid (EFA) requirements differ between species. Thus, linoleic acid (LA, 18:2n-6) and α-linolenic acid (LNA, 18:3n-3) can satisfy the EFA requirements of freshwater fish, whereas marine fish require longer chain n-3 and n-6 polyunsaturated fatty acids (PUFA) for optimal growth and health[6]. Supporting this, fatty acid (FA) desaturation and elongation of LA and LNA are well established in freshwater and anadromous fish species[7], but marine fish including European sea bass[8] and gilthead sea bream[9,10] do not show rates for bioconversion of C_{18} PUFA into C_{20} and C_{22} HUFA that would allow n-3 HUFA requirements to be met.

Signs of EFA deficiencies in fish include skin lesions and several neurological alterations linked to reduced growth and survival rates during larval and juvenile on-growing phases[11]. Lipoid liver disease and intense accumulation of intestinal lipid droplets are also documented as metabolic disorders arising from defective supplies of phospholipids[12-14] and n-3 HUFA[15]. Additionally, FA modulate immune responses and eicosanoid production from arachidonic acid (ARA, 20:4n-6) are recognized as inflammatory agents, whereas DHA, and especially EPA-derived eicosanoids exert anti-inflammatory effects in a wide variety of experimental models[16,17]. However, factors other than dietary ones may influence lipid metabolism, and relative rates of fat deposition and mobilisation vary greatly as a result of environmental factors including parr-smolt transformation in salmonids[18,19]. Likewise, gonadal maturation and spawning have a significant impact in the muscle FA profile of gilthead sea bream females[20]. Deposition rates and FA profiles also vary seasonally in wild gilthead sea bream[21], but the feeding regime is a major influence and most of these changes can be overridden by full rations given under intensive aquaculture. Indeed, monitoring studies in various Greek fish farms failed to show a seasonal impact in the muscle fat deposition and profiling of gilthead sea bream[22].

Gilthead sea bream is a major cultured finfish in the Mediterranean area, and extensive research to sustain further growth has proved that vegetable oils can replace up to 60% of the added FO, in fish meal-based diets, without adverse effects on growth, feed efficiency and survival rates[8,23,24]. Additional studies have addressed the extensive replacement of fish meal by plant proteins[25,26], and recently growth-compensatory mechanisms of the somatotropic axis have been

evidenced in short-term trials when juvenile fish were fed during the summer growth spurt with plant protein-based diets and graded levels of vegetable oils[27]. Indeed, with the total replacement of dietary FO some growth reduction occurred, and it was accompanied by decreased production of hepatic insulin-like growth factor-I (IGF-I) not compensated by the local expression (skeletal muscle) of IGFs and/or growth hormone receptors. In humans and other animal models, there is also increasing evidence linking endocrine and metabolic dysfunctions resulting in obesity and insulin resistance with steatosic livers and altered FA profiles of phospholipids and stored triglycerides[28]. In this sense, three major goals were addressed herein in a gilthead sea bream trial conducted over a growth trial of 8 months duration a) the relationship between dietary and muscle FA profiles b) the robustness of the phospholipid FA profile when EFA requirements are theoretically covered in the diet, and c) histological alterations of liver and intestine as sensitive target tissues of lipid-metabolism deregulation.

Materials and methods

Diets

Four isoproteic, isolipidic and isoenergetic plant protein-based diets were made with a low inclusion level (20%) of fish meal and fish soluble protein concentrates (Tables 1 and 2). All diets were supplemented with L-lysine (0·55%) and contained soya lecithin (1%). Added oil was either Scandinavian FO (control diet, CTRL diet) or a blend of vegetable oils, replacing 33% (33VO diet), 66% (66VO diet) and 100% (VO diet) of the FO. The blend of vegetable oils (2·5 rapeseed oil: 8·8 linseed oil: 3 palm oil) provided a similar balance of saturates, monoenes and PUFA to that found in FO, but without HUFA[29,30]. All diets were manufactured using a twin-screw extruder (Clextral, BC 45) at the INRA experimental research station of Donzacq (Landes, France), dried under hot air, sealed and kept in air-tight bags until use.

Growth trial and tissue sampling

Juvenile gilthead sea bream (*Sparus aurata* L.) of Atlantic origin (Ferme Marine de Douhet, Ile d'Oléron, France) were acclimated to laboratory conditions at the Institute of Aquaculture Torre de la Sal (IATS) for 20 days before the start of the growth study. Fish of 16 g initial mean body weight were distributed into 12 fibreglass tanks (500 litres) in groups of 60 fish per tank. Water flow was 20 l/min, and oxygen content of outlet water remained higher than 85% saturation. The growth study was undertaken over 8 months (May 23rd - January 18th), and day-length and water temperature (11-27ºC) varied over the course of the trial following natural changes at IATS latitude (40º 5'N; 0º 10'E).

Each diet was randomly allocated to triplicate groups of fish, and feed was offered by hand to apparent visual satiety twice a day (9.00, 14.00 hours) from May to September, and once a day (12.00 hours) from October to January. No mortality was registered, and feed intake was recorded daily. At regular intervals, fish were counted and group-weighed under moderate anaesthesia (3-aminobenzoic acid ethyl ester, MS 222; 100 μg/ml). At critical step windows over the growth trial (midsummer, August 5th; early autumn, September 27th; and early winter, January 18th), randomly selected fish (4 fish per tank; 12 fish per treatment) were killed by a blow on the head prior to tissue sampling. Portions of dorsal muscle (white muscle) were extracted and rapidly excised, frozen in liquid nitrogen, and stored at –80 ºC until FA analyses of lipid extracts. Liver and intestine samples for fat content determinations and histological samples were taken only in September (20 hours after the last feeding) when fish still show an active feeding behaviour. All

procedures were carried out according to national and institutional regulations (Consejo Superior de Investigaciones Científicas, Institute of Aquaculture Torre de la Sal Review Board) and the current European Union legislation on handling experimental animals.

Histology and tissue lipid content determinations

Tissue fragments of liver and hind gut were fixed in 10% buffered formalin, embedded in Technovit-7100 resin (Kulzer, Heraeus, Germany), and stained with toluidine blue (TB) or hematoxylin-eosin after thin sectioning (1-3 µm). Liver and muscle lipids were extracted according to Folch et al.[31], and determined gravimetrically after the evaporation of the organic solvent under a stream of nitrogen and overnight desiccation.

FA analyses

Muscle total lipids (TL) for FA analyses were extracted by the method of Folch et al.[31], using chloroform:methanol (2:1) containing 0·01% butylated hydroxytoluene (BHT) as antioxidant. Phospholipids (PL) from muscle lipid extracts were isolated by thin layer chromatography (TLC) (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. PL bands at the bottom of plates were scraped and extracted with chloroform:methanol (2:1) containing 0·01% BHT.

After the addition of nonadecanoic FA (Sigma, Poole, Dorset, UK) as internal standard, muscle PL and TL extracts were subjected to acid-catalysed transmethylation for 16.00 hours at 50 ºC using 1 ml toluene and 2 ml of 1% (v/v) sulphuric acid in methanol[32]. FA methyl esters (FAME) were extracted with hexane:diethyl ether (1:1), and those derived from TL were purified by TLC 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 FO standards, and the relative amount of each FA was expressed as a percentage of the total amount of FA in the analysed sample.

Statistical analysis

Growth parameters (tank average values) and the relative amount of FA were checked for normal distribution and homogeneity of variances, and when necessary arcsin transformation was performed. Data were analyzed by one-way ANOVA followed by Student-Newman-Keuls (SNK) test at a significance level of 5%. Also, the percentages of each FA were chemometrically analysed by including them as variables in a multivariate principal components analysis (MPCA) model. With such a parsimonic approach, the data set of variables (FA) is reduced into a smaller set of factors or components. Parsimony is achieved by explaining the maximum amount of common variance in a correlation matrix using the smallest number of explanatory concepts. Factors are statistical entities that can be visualised as classification axes along which measurement variables can be plotted, giving an idea of their correlation with the corresponding factor (loading). Score plots are a graphical representation of individual (dietary groups) scores in the new subset of measurement variables (factors). They illustrate the relationship among individual cases (dietary groups), and the variables, and help in the analysis of data by showing graphical associations, or through new statistical analyses. In the present work, factor scores were subsequently analyzed by one way ANOVA and SNK multiple comparison tests. All analyses were made using the SPSS package version 13.0 (SPSS Inc, Chicago, USA).

Results

Growth performance

Fish grew from 16 g to 240-270 g over a growth trial of 8 months duration under natural light and temperature conditions (Fig. 1). The final body weight of fish fed the CTRL diet did not differ from that of fish fed 33VO and 66VO diets, with overall specific growth rates ranging between 1·12 and 1·16 (see Table 3). By contrast, the total replacement of FO dictated a slight but significant reduction (10%) of final body weight in fish fed the VO diet. A concurrent and significant decrease of voluntary feed intake (g DM intake) was found in fish fed the VO diet. Feed efficiency (0·97-1·01) remained high and unchanged irrespective of dietary treatment.

Tissue fat deposition and histological alterations

After the summer replenishment of energy stores, lipid content of dorsal white muscle (6-8%) was not affected by the dietary treatment. Hepatic fat content in fish fed CTRL and 33VO diets was high and of the same order of magnitude (15% on wet matter basis; 0·23-0·25 g/100g body weight). A progressive and significant increase (up to 25%; 0·44 g/100 g body weight) was found with the graded replacement of FO in fish fed 66VO and VO diets (Fig. 2C). However, signs of initial and localized lipoid liver disease were only found with the total replacement of FO with vegetable oils (Fig. 2A and B). None of the FO-replaced diets produced apparent signs of histological damage in the intestine. Only one fish fed the VO diet had a moderate accumulation of lipid droplets in the intestinal epithelium that was not considered pathological.

Muscle FA profile

The effects of dietary treatment upon muscle FA profiles of TL are shown in a time course basis (Table 4). Overall, fish fed the CTRL diet contained 28% saturates (mainly 16:0 and 14:0), almost 32% monoenes (over half of which were 18:1n-9), 12% n-6 FA (predominantly 18:2n-6), and 18-20% n-3 HUFA (predominantly EPA and DHA). Increased amounts of 18:1n-9, 18:2n-6 and 18:3n-3, in combination with reduced proportions of n-3 HUFA and saturated FA, were found with the progressive replacement of FO by vegetable oils. The two first components of MPCA accounted for the 78% of variation of this data set, although 67·9% of variation was explained by component 1 itself (Fig. 3A). Thus, no grouping was recognized on the basis of sampling time (second factor

score), whereas four groups were significantly separated (SNK, $P<0{\cdot}05$) and identified as VO, 66VO 33VO and CTRL in the first factor score (Fig. 3B).

The FA profile of muscle PL of fish sampled at the end of the trial (January) is shown in Table 5. All experimental groups retained high amounts of saturated FA predominantly 16:0 (>13%) and 18:0 (>8%), but the relative amount of 18:2n-6 increased up to 23% in fish fed the VO diet. A concurrent reduction in n-3 HUFA was also found, decreasing the EPA plus DHA content from 36-28% (CTRL/33VO/66VO fish) to 16% (VO fish). Thus, when data of PL and TL fractions were analysed by MPCA, the two principal components accounted for 67% of variation (Fig. 4A). Component 1 explained 39·6% of variation and separated FA that predominate in TL (on the left) from those characteristic of more unsaturated PL (on the right). Component 2 accounted for 27·8% of variation, and separated FA representative of FO (above the zero line) from those characteristic of vegetable oils (below the zero line). The factor score plot separated TL and PL in the abscise axis, whereas grouping in the ordinate axis was based on the different effects of dietary intervention upon each lipid class. Accordingly, three major clusters were significantly separated (SNK, $P<0{\cdot}05$) and identified in the first factor score plot as a) TL group, b) PL of fish fed the VO diet, and c) a homogenous group corresponding to PL of fish fed CTRL, 33VO and 66VO diets (Fig. 4B).

Discussion

The demand for feed in intensive aquaculture has increased over recent years and extensive research has been done on alternative raw materials of vegetable origin. However, the main constraint for the use of vegetable oils in marine fish feeds is the lack of n-3 long-chain PUFA, particularly EPA and DHA. Moreover, quantitative requirements depend on species and growth rates, and the biological demand for n-3 HUFA was at least 1·6% of dry matter for flatfish larvae[33] decreasing to 0.8-0.6% in juvenile[34,35] and grower fish[36]. Similar requirements were reported for juvenile European sea bass[37] and gilthead sea bream[38]. In the present study the theoretical requirements of EFA were met by 33VO (1·6% EPA +DHA) and 66VO (0·9% EPA + DHA) diets, but not by the VO diet (0·3% EPA + DHA). Thereby, in this and in a previous short-term trial[27], no detrimental effects on growth performance were found with the replacement of up to 66% of the added FO, whereas a slight but significant reduction in feed intake and weight gain was found with the total FO replacement, indicating that a dietary supply of 0·3% of EPA+DHA was not sufficient for normal growth and development of gilthead seabream. However, fish meal itself contains appreciable amounts of FO, and trials conducted in our experimental facilities show that the total replacement of the added FO is feasible without adverse effects on growth in gilthead sea bream diets with a 30-35% fish meal inclusion (unpublished results). Regost et al.[39] also reported the feasibility of the total replacement of FO by vegetable oils in turbot fed fish meal based-diets. Similar results were reported in sharpsnout sea bream by Piedecausa et al.[40]. However, in the present study, we report for the first time, over the production cycle of a marine fish, the use of well balanced plant protein diets with a low inclusion of marine raw materials (<20%) just to cover EFA needs.

It is noteworthy that growth rates in the trial conducted in the present study were excellent and even improved upon the values reported for fish of the same size class under similar experimental conditions[25,26, 41,42]. This fact can be attributed to the genetic improvement of fish strains but also to better fish management, culture conditions and dietary formulation. Since fish meal is also a source of PL, the plant protein mixture in this study was adequately supplemented with amino acids and PL supplied in the form of soya lecithin. This added component is rich in phosphatidylcholine (PC), a polar lipid molecule that is a natural component of lipoproteins and cellular membranes adding fluidity and rigidity to cells as well as being required for lipoprotein synthesis, lipid mobilisation and digestibility. Our experimental design does not delineate unequivocally the beneficial effects of soya lecithin, but it must be noted that signs of intestine damage and transport dysfunction (massive accumulation of lipid droplets) were not found in any experimental group. By contrast, intense accumulation of lipid droplets was reported earlier in the

hind gut of juvenile gilthead sea bream fed plant protein and FO based-diets without phospholipid supplementation[43]. Similar histological alterations have been reported by other authors using transmission electron microscopy[15] and, interestingly, earlier studies in young larvae demonstrated that dietary lecithin increases the appearance of lipoproteins and enhances the lipid transport through the gut[12,44,45]. Likewise, intense accumulation of lipid droplets was seen in the gastrointestinal tract of salmonids fed with plant oils, but this condition was reversed by phospholipid supplementation[13,14].

Defects in FA storage and oxidation are a central initiating factor for metabolic and endocrine alterations, resulting in enhanced FA flux from adipose tissue towards liver and muscle[46,47]. Ration size by itself is also a major disrupting factor, and long-term feeding close to satiation increases hepatic fat deposition in gilthead sea bream juveniles, leading to lipoid liver disease and enterocyte desquamation in fish fed commercial diets[48]. Dietary inclusion of vegetable oils[49,50] and plant proteins[43] also induces lipoid liver disease, and the role of tumour necrosis factor-α (TNFα) and lipoprotein lipase (LPL) as lipolytic cytokines and rate-limiting enzymes in tissue FA uptake has been reported in gilthead sea bream[51,52]. Precise effects of nutrients on the deregulation of lipid metabolic pathways still remain largely unknown, but several studies indicate that soybean PC may alleviate signs of liver diseases, promoting a healthy lipid metabolism[12,53,54]. This notion is supported herein by the observation that hepatic fat deposition varied between 15% and 25% of wet weight, though signs of initial and focal lipoid liver disease were only found with the total FO replacement. By contrast, clear signs of liver disease have been reported with a liver fat deposition below 15% in fish fed 16% lipid diets[43] (22% lipid diets were used in the present study). This finding suggests that the fat threshold level for liver damage was significantly increased in the present study. However, the extent to which this condition is due to PL supplementation with soya lecithin rather than to other poorly defined dietary factors merits more specific research.

Gilthead sea bream, as other poikilotherms, utilizes favourable conditions in summer for rapid growth and replenishment of energy stores, but analyses of FA profiles in this and other fish species including Atlantic salmon[55,56], rainbow trout[57], turbot[39] and European sea bass[58,59] suggest a selective incorporation of n-3 PUFA in polar lipids and perhaps increased oxidation rates of other more easily utilizable FA. Moreover, the seasonal cycling increases in fat storage alter the ratio of polar and neutral lipids, driving the well reported changes in the muscle FA profile seen in wild gilthead sea bream[21]. In addition, there is experimental evidence linking FA profiles of wild brown trout with the trophic level of the species, the location of the catch, and the size and physiological status of the animal[60]. However, feeding regimes under intensive aquaculture production apparently override the impact of the season on the FA profile of farmed gilthead sea

bream[22]. This notion is supported by data from the present study, and the MPCA analysis revealed that the 68% of the total variation in the muscle FA profile of TL is explained by the dietary component. Likewise, alterations in the muscle FA acid profile of cultured Chinook salmon are viewed as a direct consequence of changes in body weight, fat deposition and ration size[61]. This information is of relevance and highlights important nutritional and quality traits, in particular for meeting human requirements for n-3 PUFA and HUFA, which needs to be considered for a proper timing and use of FO finishing diets for the recovery of a marine FA profile in fish fed vegetal oils through most of the production cycle[29,30,39].

The degree of unsaturation of FA mediates the fluidity and structural integrity of cell membranes, which may exacerbate signs of EFA deficiency during fish overwintering[1,62,63]. This is the reason why the analysis of PL FA profiles was focused herein on the cold season. At this time, the factor score plot showed two major clusters corresponding to PL and TL subgroups. In addition, the PL branch of fish fed CTRL, 33VO and 66VO diets appeared as a high homogenous group, which evidenced the robustness of the PL FA profile when EFA requirements were theoretically covered. However, fish fed VO diet were deficient in EFA, and PL-VO appeared as an outlier-group in the MPCA analysis. More detailed analyses revealed the relative enrichment of these fish in 20:2n-6, 20:3n-6 and 20:3n-3. Since vegetable oils are devoid of these FA and they are part of the biosynthetic routes of n-6 and n-3 HUFA, this finding highlights adaptive attempts to alleviate EFA deficiencies. The accumulation of 20:3n-6 indicates increased Δ6 desaturation and elongation of dietary 18:2n-6 that is driven by increased dietary and tissue levels of this FA, derived from vegetable oils, as well as reduced tissue levels of n-3 HUFA[8]. The increased levels of 20:2n-6 and 20:3n-3, which are “dead-end” elongation products of 18:2n-6 and 18:3n-3, respectively, reflect increased levels of dietary C_{18} PUFA although increased levels of 20:3n-9, a marker of EFA deficiency, were not observed. In gilthead sea bream, the expression of Δ-6 desaturase is highly induced in fish fed a HUFA-free diet[10]. There is also now evidence for a regulatory role of conjugated LA acid upon the hepatic and intestine expression of fatty acyl elongase and Δ-6 fatty acyl desaturase[64]. However, a low activity of Δ-5 fatty acyl desaturase activity has been reported either *in vitro*[65] or *in vivo*[9], which may act as a major constraining factor for bioconvertion of C_{18} PUFA into C_{20} and C_{22} HUFA at appreciable rates.

In summary, data on growth performance, tissue histology and FA analysis prompted us to use practical diets with a low inclusion of marine raw materials through most of the production cycle of gilthead sea bream, linking the robustness of the PL FA profile with endocrine, metabolic and somatotropic factors. Precise effects at different developmental stages need to be further evaluated, and interestingly muscle FA profiles and MPCA emerge not only as powerful tools to

understand foraging ecology and food webs, but also to evaluate alternative and sustainable aquafeeds in a global change scenario.

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References

1. Sargent JR & Tacon AGJ (1999) Development of farmed fish: a nutritionally necessary alternative to meat. *Proc Nutr Soc Society* **58**, 377-383.

2. Watanabe T (2002) Strategies for further development of aquatic feeds. *Fish Sci* **68**, 242-252.

3. Bell JG, McGhee F, Campbell PJ & Sargent JR (2003) Rapeseed oil as an alternative to marine fish oil in diets of post-smolt Atlantic salmon (*Salmo salar*): changes in flesh fatty acid composition and effectiveness of subsequent fish oil "wash out". *Aquaculture* **218**, 515-528.

4. Bransden MP, Carter CG & Nichols PD (2003) Replacement of fish oil with sunflower oil in feeds for Atlantic salmon (*Salmo salar* L.): effect on growth performance, tissue fatty acid composition and disease resistance. *Comp Biochem Physiol* **135**B, 611-625.

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

6. Watanabe T (1982) Lipid nutrition in fish. *Comp Biochem Physiol* **73**B, 3-15.

7. Sargent JR, Tocher DR & Bell JG (2002) The lipids. In: *Fish Nutrition*, 3th ed., pp. 181-257 [Halver JE, Hardy RW, editors]. San Diego, CA: Academic Press.

8. Mourente G, Dick JR, Bell JG & Tocher DR (2005) Effect of partial substitution of dietary fish oil by vegetable oils on desaturation and β-oxidation of [1-^{14}C]18:3n-3 (LNA) and [1-14C]20:5n-3 (EPA) in hepatocytes and enterocytes of European sea bass (*Dicentrarchus labrax* L.). *Aquaculture* **248**, 173-186.

9. Mourente G, Tocher DR (1994) In vivo metabolism of [1-^{14}C]linolenic acid (18:3(n - 3)) and [1-^{14}C]eicosapentaenoic acid (20:5(n - 3)) in a marine fish: Time-course of the desaturation / elongation pathway. *Biochim Biophys Acta - Lipids and Lipid Metabolism* **1212**, 109-118.

10. Seiliez I, Panserat S, Corraze G, Kaushik S & Bergot P (2003) Cloning and nutritional regulation of a [Delta]6-desaturase-like enzyme in the marine teleost gilthead seabream (*Sparus aurata*). *Comp Biochem Physiol* **135**B, 449-460.

11. Sargent J, McEvoy L, Estevez A, Bell G, Bell M, Henderson J & Tocher D (1999) Lipid nutrition of marine fish during early development: current status and future directions. *Aquaculture* **179**, 217-229.

12. Fontagné S, Geurden I, Escaffre AM & Bergot P (1998) Histological changes induced by dietary phospholipids in intestine and liver of common carp (*Cyprinus carpio* L.) larvae. *Aquaculture* **161**, 213-223.

13. Olsen RE, Myklebust R, Kaino T & Ringo E (1999) Lipid digestibility and ultrastructural changes in the enterocytes of Arctic char (*Salvelinus alpinus* L.) fed linseed oil and soybean lecithin. *Fish Physiol Biochem* **21**, 35-44.

14. Olsen RE, Dragnes BT, Myklebust R & Ringo E (2003) Effect of soybean oil and soybean lecithin on intestinal lipid composition and lipid droplet accumulation of rainbow trout, *Oncorhynchus mykiss* Walbaum. *Fish Physiol Biochem* **29**, 181-192.

15. Caballero MJ, Izquierdo MS, Kjørsvik E, Montero D, Socorro J, Fernández AJ & Rosenlund G (2003) Morphological aspects of intestinal cells from gilthead seabream (*Sparus aurata*) fed diets containing different lipid sources. *Aquaculture* **225**, 325-340.

16. Abeywardena MY, Head RJ (2001) Longchain *n-3* polyunsaturated fatty acids and blood vessel function. *Cardiovasc Res* **52**, 361-371.

17. Calder PC (2006) Polyunsaturated fatty acids and inflammation. *Prostaglandins Leukot Essent Fatty Acids* **75**, 197-202.

18. Fonseca-Madrigal J, Karalazos V, Campbell PJ, Bell JG & Tocher DR (2005) Influence of dietary palm oil on growth, tissue fatty acid compositions, and fatty acid metabolism in liver and intestine in rainbow trout (*Oncorhynchus mykiss*). *Aquacult Nutr* **11**, 241-250.

19. Fonseca-Madrigal J, Bell JG & Tocher DR (2006) Nutritional and environmental regulation of the synthesis of highly unsaturated fatty acids and of fatty-acid oxidation in Atlantic salmon (*Salmo salar* L.) enterocytes and hepatocytes. *Fish Physiol Biochem* **32**, 317-328.

20. Almansa E, Martín MV, Cejas JR, Badía P, Jerez S & Lorenzo A (2001) Lipid and fatty acid composition of female gilthead seabream during their reproductive cycle: effects of a diet lacking n-3 HUFA. *J Fish Biol* **59**, 267-286.

21. Özyurt G, Polat A & Özkütük S (2005) Seasonal changes in the fatty acids of gilthead sea bream (*Sparus aurata*) and white sea bream (*Diplodus sargus*) captured in Iskenderun Bay, eastern Mediterranean coast of Turkey. *Eur Food Res Technol* **220**, 120-124.

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

23. Izquierdo MS, Obach A, Arantzamendi L, Montero D, Robaina L & Rosenlund G (2003) Dietary lipid sources for seabream and seabass: growth performance, tissue composition and flesh quality. *Aquacult Nutr* **9**, 397-407.

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

25. Gómez-Requeni P, Mingarro M, Calduch-Giner JA, Médale F, Martin SAM, Houlihan DF, Kaushik S & Pérez-Sánchez J (2004) Protein growth performance, amino acid utilisation and somatotropic axis responsiveness to fish meal replacement by plant protein sources in gilthead sea bream (*Sparus aurata*). *Aquaculture* **232**, 493-510.

26. De Francesco M, Parisi G, Pérez-Sánchez J, Gómez-Requeni P, Médale F, Kaushik SJ, Mecatti M & Poli BM (2007) Effect of high level fish meal replacement by plan proteins in gilthead sea bream (*Sparus aurata*) on growth and body/fillets quality traits. *Aquacult Nutr* **13**, 361-372.

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

28. Kelley DE (2002) Skeletal muscle triglycerides - An aspect of regional adiposity and insulin resistance. *Ann Ny Acad Sci* **967**, 135-145.

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

30. Mourente G, Good JE, Thompson KD & Bell JG (2007) Effects of partial substitution of dietary fish oil with blends of vegetable oils, on blood leukocyte fatty acid compositions, inmune function and histology in European sea bass (*Dicentrarchus labrax* L.). *Br J Nutr* **98**, 770-779.

31. Folch J, Less N & Sloane-Stanley GH (1957) A simple method for insolation and purification of total lipids from animal tissues. *J Biol Chem* **226**, 497-509.

32. Christie WW (1982) *Lipid analysis. Isolation, separation, identification and structural analysis of lipids*, 2nd ed., pp 207 [Christie WW]. Oxford: Pergamon Press.

33. Le Milinaire C, Gatesoupe FJ & Stephan G (1983) Quantitative approach to n-3 long-chain poly-unsaturated fatty-acid requirement of turbot larvae (*Scophthalmus-Maximus*). *C R Acad Sci III, Sci Vie* **296**, 917-920.

34. Gatesoupe FJ, Leger C, Boudon M, Metailler R & Luquet P (1977) Lipid feeding of turbot (*Scophthalmus maximus* L). Influence on growth of supplementation with methyl esters of linolenic acid and fatty acids of ω9 series. *Ann Hydrobiol* **8**, 247-254.

35. Lee SM, Lee JH & Kim KD (2003) Effect of dietary essential fatty acids on growth, body composition and blood chemistry of juvenile starry flounder (*Platichthys stellatus*). *Aquaculture* **225**, 269-281.

36. Leger C, Gatesoupe FJ, Metailler R, Luquet P & Fremont L (1979) Effect of dietary fatty acids differing by chain lengths and ω series on the growth and lipid composition of turbot *Scophthalmus maximus* L. *Comp Biochem Physiol* **64**B, 345-350.

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

38. Kalogeropoulos N, Alexis MN & Henderson RJ (1992) Effects of dietary soybean and cod-liver oil levels on growth and body composition of gilthead bream (*Sparus aurata*). *Aquaculture* **104**, 293-308.

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

40. Piedecausa MA, Mazon MJ, Garcia Garcia B & Hernandez MD (2007) Effects of total replacement of fish oil by vegetable oils in the diets of sharpsnout seabream (*Diplodus puntazzo*). *Aquaculture* **263**, 211-219.

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

42. Gómez-Requeni P, Mingarro M, Kirchner S *et al*. (2003) Effects of dietary amino acid profile on growth performance, key metabolic enzymes and somatotropic axis responsiveness of gilthead sea bream (*Sparus aurata*). *Aquaculture* **220**, 749-767.

43. Sitjà-Bobadilla A, Peña-Llopis S, Gómez-Requeni P, Médale F, Kaushik S & Pérez-Sánchez J (2005) Effect of fish meal replacement by plant protein sources on non-specific defence mechanisms and oxidative stress in gilthead sea bream (*Sparus aurata*). *Aquaculture* **249**, 387-400.

44. Salhi M, Hernández-Cruz CM, Bessonart M, Izquierdo MS & Fernández-Palacios H (1999) Effect of different dietary polar lipid levels and different n-3 HUFA content in polar lipids on gut and liver histological structure of gilthead seabream (*Sparus aurata*) larvae. *Aquaculture* **179**, 253-263.

45. Liu J, Caballero MJ, Izquierdo MS, El-Sayed Ali T, Hernández-Cruz CM, Valencia A & Fernández-Palacios H (2002) Necessity of dietary lecithin and eicosapentaenoic acid for growth, survival, stress resistance and lipoprotein formation in gilthead sea bream *Sparus aurata*. *Fish Sci* **68**, 1165-1172.

46. Browning JD & Horton JD (2004) Molecular mediators of hepatic steatosis and liver injury. *J Clin Invest* **114**, 147-152.

47. Avramoglu RK, Basciano H & Adeli K (2006) Lipid and lipoprotein dysregulation in insulin resistant states. *Clin Chim Acta* **368**, 1-19.

48. Sitjà-Bobadilla A, Mingarro M, Pujalte MJ, Garay E, Alvarez-Pellitero P & Pérez-Sánchez J (2003) Immunological and pathological status of gilthead sea bream (*Sparus aurata* L.) under different long-term feeding regimes. *Aquaculture* **220**, 707-724.

49. Caballero MJ, Izquierdo MS, Kjørsvik E, Fernández AJ & Rosenlund G (2004) Histological alterations in the liver of sea bream, *Sparus aurata* L., caused by short- or long-term feeding with vegetable oils. Recovery of normal morphology after feeding fish oil as the sole lipid source. *J Fish Dis* **27**, 531-541.

50. Wassef EA, Wahby OM & Sakr EM (2007) Effect of dietary vegetable oils on health and liver histology of gilthead seabream (*Sparus aurata*) growers. *Acuac Res* **38**, 852-861.

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

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

53. Canty DJ & Zeisel SH (1994) Lecithin and choline in human health and disease. *Nutr Rev* **52**, 327-339.

54. Ipatova OM, Prozorovskaia NN, Torkhovskaia TI, Baranova VS & Guseva DA (2004) Biological effects of the soybean phospholipids. *Biomed Khim* **50**, 436-450.

55. Bell JG, McEvoy J, Tocher DR, McGhee F, Campbell PJ & Sargent JR (2001) Replacement of fish oil with rapeseed oil in diets of atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. *J Nutr* **131**, 1535-1543.

56. Moya-Falcon C, Hvattum E, Tran TN, Thomassen MS, Skorve J & Ruyter B (2006) Phospholipid molecular species, β-oxidation, desaturation and elongation of fatty acids in Atlantic salmon hepatocytes: Effects of temperature and 3-thia fatty acids. *Comp Biochem Physiol* **145**B, 68-80.

57. Caballero MJ, Obach A, Rosenlund G, Montero D, Gisvold M & Izquierdo MS (2002) Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **214**, 253-271.

58. Richard N, Mourente G, Kaushik S & Corraze G (2006) Replacement of a large portion of fish oil by vegetable oils does not affect lipogenesis, lipid transport and tissue lipid uptake in European seabass (*Dicentrarchus labrax* L.). *Aquaculture* **261**, 1077-1087.

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

60. Kaushik SJ, Corraze G, Radunz-Neto J, Larroquet L & Dumas J (2006) Fatty acid profiles of wild brown trout and Atlantic salmon juveniles in the Nivelle basin. *J Fish Biol* **68**, 1376-1387.

61. Kiessling A, Pickova J, Eales JG, Dosanjh B & Higgs D (2005) Age, ration level, and exercise affect the fatty acid profile of chinook salmon (*Oncorhynchus tshawytscha*) muscle differently. *Aquaculture* **243**, 345-356.

62. Jobling M & Bendiksen EA (2003) Dietary lipids and temperature interact to influence tissue fatty acid compositions of Atlantic salmon, *Salmo salar* L., parr. *Acuac Res* **34**, 1423-1441.

63. Skalli A, Robin JH, Le Bayon N, Le Delliou H & Person-Le Ruyet J (2006) Impact of essential fatty acid deficiency and temperature on tissues' fatty acid composition of European sea bass (*Dicentrarchus labrax*). *Aquaculture* **255**, 223-232.

64. Diez A, Menoyo D, Pérez-Benavente S *et al.* (2007) Conjugated linoleic acid affects lipid composition, metabolism, and gene expression in gilthead sea bream (*Sparus aurata* L). *J Nutr* **137**, 1363-1369.

65. Tocher DR, Ghioni C (1999) Fatty acid metabolism in marine fish: low activity of fatty acyl delta5 desaturation in gilthead sea bream (*Sparus aurata*) cells. *Lipids* **34**, 433-440.

526 **Figure legends**

527

528 Figure 1. Seasonal changes of temperature (solid line) and day length (dashed line) (A). Body
529 weight over the course of trial of fish fed the experimental diets (B). Values are the means and SEM
530 of triplicate tanks. Arrows indicate tissue sampling times.

531

532 Figure 2. Representative histological sections of CTRL (A) and VO (B) livers of fish sampled in
533 September, after 18 weeks of feeding the experimental diets (Staining: toluidine blue; Scale bars =
534 50 µm). Notice the lipoid liver degeneration with breakdown of hepatocyte membranes
535 (arrowheads). Liver fat content (C) of fish fed the four experimental diets (18 weeks). Each bar
536 represents the mean plus the SEM. Different letters stand for statistically significant differences
537 (P<0·05, SNK).

538

539 Figure 3. Component plot (A) and factor score plot (B) of the MPCA for the muscle FA profile of
540 total lipids in fish sampled in August, September and January. Mean values are shown in the factor
541 score plot to simplify the graph representation. Circles stand for different clusters in the factor score
542 1 (P<0·05, SNK).

543

544 Figure 4. Component plot (A) and factor score plot (B) of the MPCA for the muscle fatty acid
545 profile of total lipids and phospholipids (January sampled fish). Mean values are shown in the factor
546 score plot to simplify the graph representation. Circles stand for different clusters in the factor score
547 1 (P<0·05, SNK).

548

Table 1. Ingredients and chemical composition of experimental diets.

Ingredient (%)	CTRL	33VO	66VO	VO
Fish meal (CP 70%)[1]	15·00	15·00	15·00	15·00
CPSP 90 [2]	5·00	5·00	5·00	5·00
Corn gluten meal (CP 63%)	40·00	40·00	40·00	40·00
Soybean meal (CP 46%)	14·30	14·30	14·30	14·30
Extruded wheat (CP 15%)	4·00	4·00	4·00	4·00
Fish oil [3]	15·15	10·15	5·15	0·00
Rapeseed oil	0·00	0·85	1·70	2·58
Linseed oil	0·00	2·90	5·80	8·79
Palm oil	0·00	1·25	2·50	3·79
Soya lecithin	1·00	1·00	1·00	1·00
Binder (sodium alginate)	1·00	1·00	1·00	1·00
Mineral premix [4]	1·00	1·00	1·00	1·00
Vitamin premix [5]	1·00	1·00	1·00	1·00
$CaHPO_4.2H_2O$ (18%P)	2·00	2·00	2·00	2·00
L-Lysine	0·55	0·55	0·55	0·55
Proximate composition				
Dry matter (DM, %)	93·43	94·10	94·79	95·38
Protein (% DM)	48·98	48·74	49·03	48·65
Fat (% DM)	22·19	22·26	22·11	22·31
Ash (% DM)	6·54	6·57	6·62	6·41
EPA + DHA (% DM)	2·31	1·61	0·90	0·30
Gross energy (kJ/g DM)	24·72	24·71	24·65	24·49

[1]Fish meal (Scandinavian LT)
[2]Fish soluble protein concentrate (Sopropêche, France)
[3]Fish oil (Sopropêche, France)
[4]Supplied the following (mg / kg diet, except as noted): calcium carbonate (40% Ca) 2·15 g, magnesium hydroxide (60% Mg) 1·24 g, potassium chloride 0·9 g, ferric citrate 0·2 g, potassium iodine 4 mg, sodium chloride 0·4 g, calcium hydrogen phosphate 50 g, copper sulphate 0·3, zinc sulphate 40, cobalt sulphate 2, manganese sulphate 30, sodium selenite 0·3.
[5]Supplied the following (mg / kg diet): retinyl acetate 2·58, DL-cholecalciferol 0·037, DL-α tocopheryl acetate 30, menadione sodium bisulphite 2·5, thiamin 7·5, riboflavin 15, pyridoxine 7·5, nicotinic acid 87·5, folic acid 2·5, calcium pantothenate 2·5, vitamin B_{12} 0·025, ascorbic acid 250, inositol 500, biotin 1·25 and choline chloride 500.

Table 2. Fatty acid composition of experimental diets (% FAME). Values are the mean of two determinations.

FA %	CTRL	33VO	66VO	VO
14 :0	5·02	3·70	1·89	0·59
15:0	0·35	0·22	0·13	0·12
16:0	16·70	16·90	16·9	16·7
16:1n-7	4·63	2·97	1·96	0·76
16:1n-9	0·22	0·15	tr	tr
16:2	0·49	0·35	0·26	0·14
16:3n-3	0·19	0·13	0·08	tr
16:4	0·40	0·29	0·17	tr
17:0	0·41	0·29	0·23	0·10
18:0	2·55	2·92	3·43	3·73
18:1n-9	12·50	17·50	21·90	25·90
18:1n-7	1·92	1·69	1·49	1·21
18:2n-6	12·10	15·70	19·20	21·30
18:3n-3	1·58	8·94	16·30	23·20
18:4n-3	2·16	1·47	0·82	0·20
20:0	0·30	0·30	0·31	0·29
20:1n-9	7·24	5·12	3·05	1·06
20:1n-7	0·21	0·16	0·09	tr
20:2n-6	0·17	0·12	0·11	tr
20:3n-3	0·08	0·07	tr	tr
20:4n-6	0·31	0·22	0·13	tr
20:4n-3	0·43	0·28	0·15	tr
20:5n-3	6·86	4·68	2·75	0·94
22:0	tr	0·16	0·16	0·17
22:1n-11	10·19	6·74	3·68	0·74
22:1n-9	0·56	0·43	0·29	0·16
22:5n-3	0·64	0·40	0·18	tr
22:6n-3	8·34	5·68	3·38	1·06
Total	96·55	97·58	98·04	98·37
Saturates	25·33	24·33	22·89	21·53
Monoenes	37·47	34·76	32·46	29·83
n-3 HUFA[1]	16·35	11·11	6·46	2·00
n-6 HUFA[2]	0·48	0·34	0·24	tr

tr = trace values < 0·05
[1]Calculated excluding 18 C atoms n-3 series.
[2]Calculated excluding 18 C atoms n-6 series.

Table 3. Data on growth performance of fish fed the four experimental diets during 8 months. Values are the means and standard deviations of triplicate tanks.

	CTRL		33VO		66VO		VO		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P* [1]
Initial body weight (g)	16·10	0·09	16·30	0·01	16·30	0·03	16·10	0·09	0·31
Final body weight (g)	257·80^{ab}	11·84	269·57^{b}	2·41	253·72^{a}	0·16	237·39^{c}	3·07	<0·05
DM intake (g/fish)	238·35^{a}	6·68	256·87^{b}	4·42	241·59^{a}	2·69	226·11^{c}	0·62	<0·001
SGR (%) [2]	1·14^{ab}	0·01	1·16^{a}	0·00	1·13^{b}	0·00	1·11^{c}	0·00	<0·05
FE[3]	1·01	0·02	0·98	0·00	0·98	0·01	0·97	0·01	0·07

[1]*P* values result from one-way ANOVA. Different superscript letters in each row indicate significant differences among dietary treatments ($P<0.05$, SNK).

[2]Specific growth ratio= [100 × (ln final fish wt – ln initial fish wt)] / days

[3]Feed efficiency = wet wt gain / dry feed intake

Table 4. Effects of the feeding regimen on the muscle FA profile of TL (% FAME) in fish sampled in August, September and January. Values are the means and standard deviations of 10 fish. Different superscript letters in each row indicate significant differences over sampling time for each dietary treatment (P<0·05, SNK).

	CTRL						33VO						66VO						VO					
	Aug		Sep		Jan		Aug		Sep		Jan		Aug		Sep		Jan		Aug		Sep		Jan	
FA %	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14 :0	3·70^{a}	0·22	3·67^{a}	0·63	4·52^{b}	0·36	2·48	0·41	2·54	0·41	2·60	0·42	1·79	0·16	1·62	0·15	1·77	0·31	0·90	0·12	0·79	0·12	1·12	0·45
16:0	20·40^{a}	0·74	20·10^{a}	0·81	18·30^{b}	0·98	20·60^{a}	0·79	19·00^{b}	0·79	19·00^{b}	0·44	17·80^{a}	0·89	19·00^{b}	0·90	17·20^{a}	0·62	15·80	0·58	16·20	0·34	16·10	0·53
16:1n-7	4·75	0·28	4·58	0·74	5·38	0·52	3·60	0·51	3·65	0·51	3·64	0·50	2·93	0·18	2·56	0·21	2·85	0·38	1·77	0·34	1·52	0·21	2·15	0·52
16:2	0·25	0·02	0·25	0·02	0·28	0·02	0·13	0·05	0·18	0·05	0·15	0·02	0·08	0·03	0·13	0·04	0·11	0·03	tr		tr		0·11	0·00
16:3	0·19	0·06	0·22	0·04	0·23	0·02	0·15	0·01	0·16	0·01	0·14	0·04	0·12	0·06	0·08	0·02	0·10	0·04	0·08	0·04	0·09	0·01	0·09	0·01
16:4	0·18	0·02	0·15	0·04	0·15	0·01	0·11	0·03	0·11	0·03	0·10	0·02	0·07	0·01	0·06	0·00	0·08	0·06	tr		0·07	0·02	0·13	0·05
17:0	0·22	0·02	0·26	0·05	0·23	0·01	0·20	0·04	0·18	0·04	0·19	0·02	0·12	0·06	0·18	0·01	0·21	0·06	0·14	0·56	0·14	0·56	0·13	0·01
18:0	3·82^{a}	0·38	3·96^{a}	0·66	3·00^{b}	0·26	4·57	0·74	4·32	0·74	4·10	0·55	4·15	0·56	4·88	0·48	3·92	0·64	4·53	0·36	4·92	1·67	4·40	0·66
18:1n-9	17·40	0·56	16·00	0·86	16·80	0·98	20·40	1·40	20·60	1·41	18·50	2·85	25·00	1·61	23·80	0·91	24·50	2·25	28·20	0·78	27·50	0·08	27·30	3·06
18:1n-7	1·87	0·08	1·84	0·10	1·93	0·07	1·59	0·20	1·75	0·19	1·55	0·05	1·38	0·02	1·30	0·04	1·36	0·04	1·10	0·06	1·09	0·85	1·22	0·13
18:2n-6	10·70^{a}	0·12	10·60^{a}	0·65	11·80^{b}	0·19	12·80^{a}	0·93	13·40^{ab}	0·93	14·90^{b}	1·56	16·30^{a}	0·46	16·60^{a}	0·33	17·40^{b}	0·15	19·40	0·52	20·40	1·54	20·50	1·66
18:3n-3	1·06	0·12	0·98	0·09	1·07	0·05	5·65	0·83	6·42	0·83	5·80	0·64	12·20	1·15	11·00	1·19	12·10	1·50	17·80	0·76	16·80	0·11	15·80	1·75
18:4n-3	1·28	0·08	1·22	0·22	1·38	0·10	0·89	0·20	1·00	0·20	0·83	0·13	0·81	0·12	0·64	0·15	0·77	0·12	0·63	0·08	0·51	0·03	0·55	0·13
20:0	0·18	0·02	0·18	0·02	0·18	0·01	0·17	0·06	0·20	0·06	0·17	0·02	0·16	0·01	0·16	0·01	0·17	0·01	0·15	0·01	0·17	0·10	0·16	0·01
20:1n-9	4·90	0·40	4·79	0·84	5·53	0·22	3·25	0·50	3·15	0·46	3·25	0·01	1·92	0·53	1·86	0·26	1·91	0·29	0·91	0·04	0·92	0·08	0·93	0·52
20:2n-6	0·22	0·00	0·24	0·02	0·25	0·03	0·23	0·06	0·28	0·02	0·26	0·01	0·27	0·01	0·27	0·03	0·27	0·03	0·28	0·02	0·33	0·03	0·33	0·03
20:3n-6	0·17	0·02	0·12	0·06	0·13	0·01	0·16	0·02	0·16	0·06	0·18	0·03	0·19	0·05	0·19	0·04	0·18	0·07	0·18	0·04	0·23	0·10	0·22	0·10
20:3n-3	0·07	0·00	0·09	0·03	0·08	0·00	0·12^{a}	0·05	0·17^{b}	0·05	0·16^{b}	0·01	0·27^{ab}	0·02	0·23^{a}	0·04	0·29^{b}	0·04	0·40	0·06	0·45	0·07	0·48	0·09
20:4n-6	0·49^{a}	0·02	0·54^{a}	0·16	0·38^{b}	0·08	0·49	0·10	0·41	0·02	0·42	0·09	0·26	0·10	0·30	0·05	0·24	0·12	0·18	0·04	0·17	0·09	0·17	0·09
20:4n-3	0·58	0·06	0·59	0·07	0·66	0·04	0·49	0·00	0·52	0·15	0·52	0·06	0·47	0·03	0·39	0·05	0·45	0·05	0·34	0·04	0·34	0·39	0·35	0·05
20:5n-3	6·06	0·42	6·40	0·85	5·02	0·37	4·87	0·93	4·58	0·03	4·34	0·72	2·83	0·55	3·06	0·29	2·56	0·76	1·41	0·24	1·34	0·05	1·55	0·74
22:1n-9	0·62	0·08	0·31	0·08	0·42	0·03	0·22	0·10	0·29	0·93	0·26	0·03	0·20	0·15	0·11	0·02	0·28	0·12	0·10	0·15	0·09	0·03	0·14	0·04
22:1n-11	4·83	0·66	4·73	1·05	5·35	0·51	2·62	0·60	2·91	0·15	2·78	0·46	1·65	0·31	1·46	0·28	1·62	0·40	0·27	0·12	0·30	0·05	0·33	0·09
22:5n-3	1·31	0·10	1·37	0·10	1·51	0·11	1·10	0·07	1·06	0·08	1·25	0·15	0·80	0·17	0·63	0·10	0·69	0·41	0·36	0·1	0·32	0·17	0·45	0·09
22:6n-3	10·80	1·00	12·40	2·79	10·60	2·05	9·74	2·32	8·85	0·63	11·00	2·67	5·75	1·77	6·54	0·82	6·02	2·58	3·11	0·5	3·15	1·15	3·52	1·82
24:1n-9	0·56^{a}	0·08	0·40^{b}	0·04	0·41^{b}	0·04	0·56^{a}	0·03	0·40^{b}	0·07	0·35^{b}	0·08	0·40^{a}	0·04	0·38^{a}	0·04	0·32^{b}	0·03	0·29	0·02	0·29	0·03	0·35	0·06
Saturates	28·32^{a}	1·05	28·17^{a}	0·81	26·23^{b}	0·90	28·02	1·86	26·24	1·22	26·06	0·57	24·02^{a}	1·32	25·84^{b}	1·24	23·27^{a}	0·97	21·52	0·78	22·22	0·77	21·91	0·93
Monoenes	34·93	1·46	32·65	3·61	35·82	1·92	32·24	3·36	32·75	2·93	30·33	4·07	33·48	2·43	31·47	1·09	32·84	3·41	32·64	0·96	31·71	1·97	32·42	3·35
n-3 HUFA[1]	18·82	1·48	20·85	3·59	17·87	2·38	16·32	3·14	15·18	3·26	17·27	3·44	10·12	2·49	10·85	1·23	10·01	3·73	5·62	0·81	5·60	1·61	6·35	2·75
n-6 HUFA[2]	0·88	1·13	0·09	0·11	0·76	0·06	0·88	0·11	0·85	0·22	0·86	0·11	0·72	0·15	0·76	0·13	0·69	0·19	0·64	0·07	0·73	0·18	0·72	0·20

tr = trace value < 0·05. [1]Calculated excluding 18 C atoms n-3 series. [2]Calculated excluding 18 C atoms n-6 series.

Table 5. Effects of the feeding regimen on the muscle FA profile of PL (% FAME) in fish sampled at the end of trial (January). Values are the means and standard deviations of 10 fish. Different superscript letters in each row indicate significant differences among dietary treatments (P<0·05, SNK).

	CTRL		33VO		66VO		VO	
FA %	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14 :0	0·62	0·20	0·57	0·51	0·54	0·24	0·23	0·07
16:0	18·4^{a}	1·22	17·50^{ab}	1·19	16·50^{b}	0·96	13·2^{c}	0·38
16:1n-7	1·06	0·36	0·80	0·12	0·70	0·17	0·76	0·23
16:2	0·30^{a}	0·15	0·25^{ab}	0·12	0·23^{b}	0·00	0·22^{b}	0·00
16:3	0·34	0·00	0·20	0·14	0·36	0·06	0·16	0·13
16:3 n-3	1·76	1·43	0·62	0·48	1·12	1·01	0·81	0·33
16:4	0·30	0·07	0·29	0·10	0·39	0·09	0·42	0·07
17:0	0·38	0·13	0·30	0·17	0·34	0·04	0·26	0·14
18:0	10·10	1·24	8·42	0·75	10·20	1·12	8·44	0·69
18:1n-9	7·59^{a}	0·14	9·33^{b}	0·02	10·20^{b}	0·06	13·40^{c}	0·08
18:1n-7	1·84	0·41	1·66	0·62	1·54	0·92	0·82	0·61
18:2n-6	7·26^{a}	0·74	10·90^{b}	1·42	14·20^{c}	0·92	23·30^{d}	1·79
18:3n-3	0·45^{a}	0·26	2·29^{b}	0·18	4·88^{c}	0·41	10·20^{d}	1·11
18:4n-3	0·31	0·25	0·29	0·29	0·30	0·11	0·29	0·11
20:0	0·27	0·00	0·16	0·03	0·26	0·00	0·30	0·13
20:1n-9	2·42^{a}	0·25	1·67^{b}	0·20	1·09^{c}	0·18	0·57^{d}	0·27
20:2 n-6	0·40	0·14	0·44	0·30	0·65	0·26	0·80	0·60
20:3n-6	0·54	0·44	0·38	0·20	0·41	0·08	0·68	0·06
20:3n-3	0·53^{a}	0·48	0·24^{a}	0·21	0·34^{a}	0·12	0·87^{b}	0·19
20:4n-6	0·94	0·05	1·15	0·07	0·87	0·28	0·65	0·08
20:4n-3	0·43	0·43	0·51	0·20	0·57	0·14	0·53	0·18
20:5n-3	7·08^{a}	0·64	7·52^{a}	0·44	6·32^{b}	0·42	3·72^{c}	0·19
22:1n-11	0·68	0·38	0·40	0·31	0·37	0·28	0·36	0·29
22:5n-3	1·93^{a}	0·07	2·05^{a}	0·15	1·64^{b}	0·22	1·23^{c}	0·20
22:6n-3	29·00^{a}	3·62	27·80^{a}	3·26	21·40^{b}	2·06	12·60^{c}	0·52
24:1n-9	0·76	0·24	0·52	0·25	0·59	0·07	0·41	0·18
Saturates	29·77^{a}	1·75	26·95^{b}	0·55	27·84^{ab}	1·94	22·43^{c}	0·95
Monoenes	14·35^{a}	0·83	14·38^{a}	0·73	14·49^{a}	0·84	16·32^{b}	0·33
n-3 HUFA[1]	38·97^{a}	3·39	38·12^{a}	3·17	30·27^{b}	2·62	18·95^{c}	0·78
n-6 HUFA[2]	1·88	0·79	1·97	0·68	1·93	0·47	2·13	0·96
n-3/n-6 ratio[3]	4·34^{a}	0·33	3·16^{b}	0·09	2·19^{c}	0·06	1·15^{d}	0·02

[1]Calculated excluding 18 C atoms n-3 series.
[2]Calculated excluding 18 C atoms n-6 series.
[3]Calculated taking into account all n-3 and n-6 FA series.

Figure 1

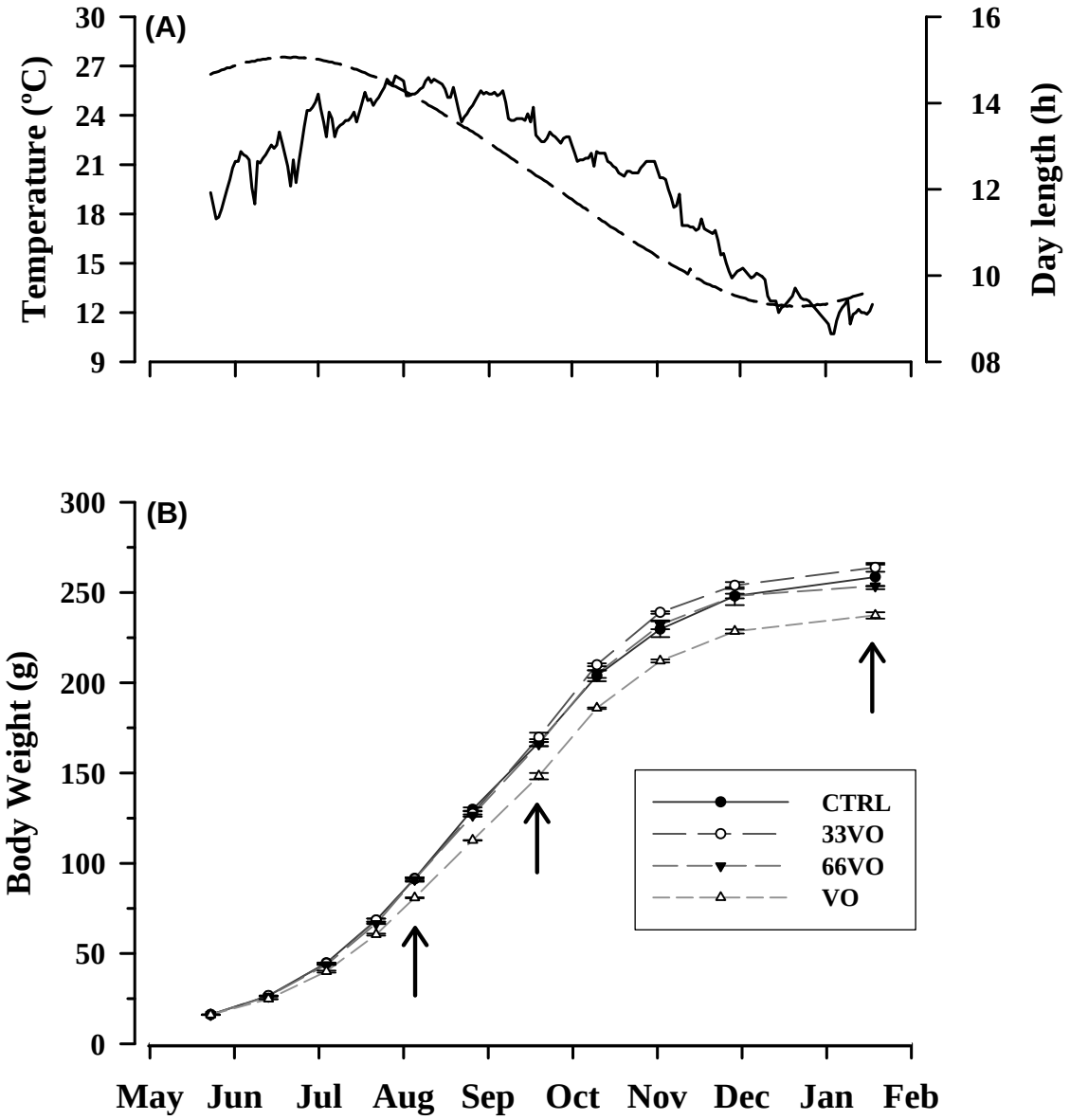
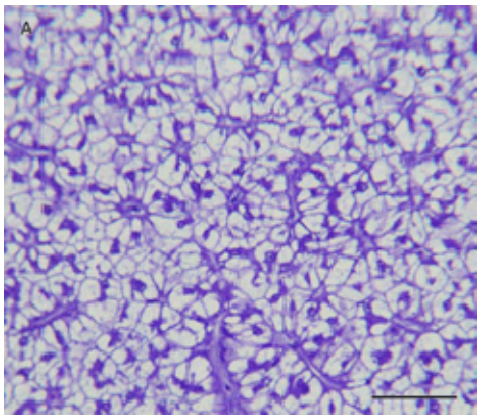
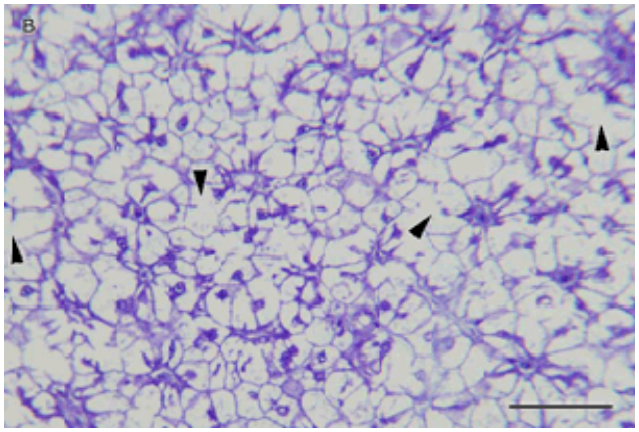

(A)
Temperature (°C)
30
27
24
21
18
15
12
9
Day length (h)
16
14
12
10
08
(B)
Body Weight (g)
300
250
200
150
100
50
0
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
CTRL
33VO
66VO
VO

Figure 2





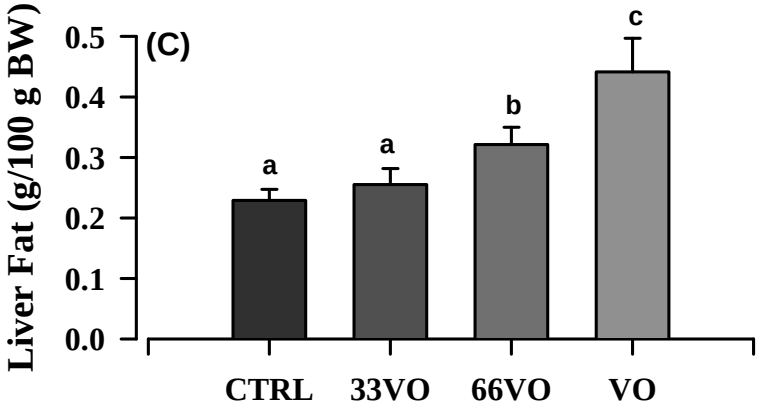
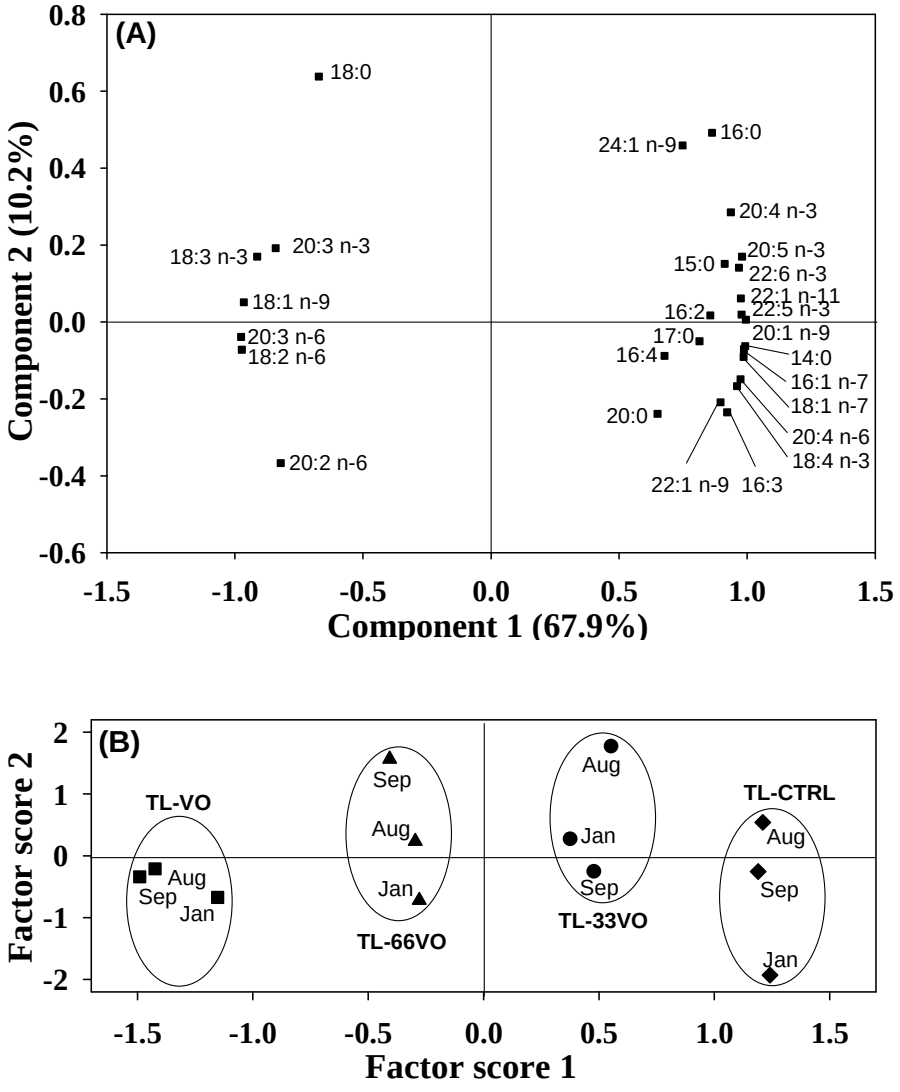
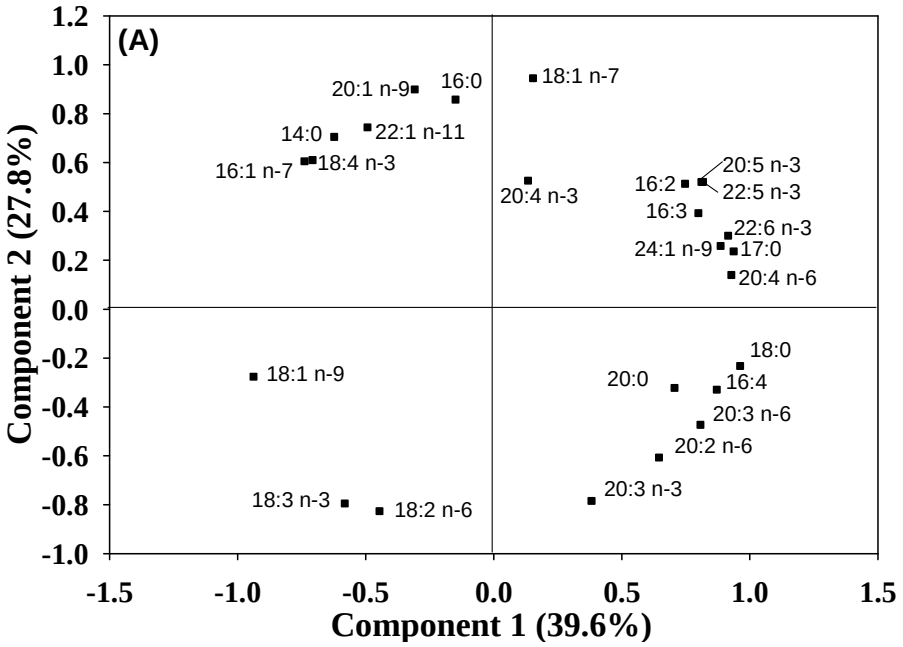


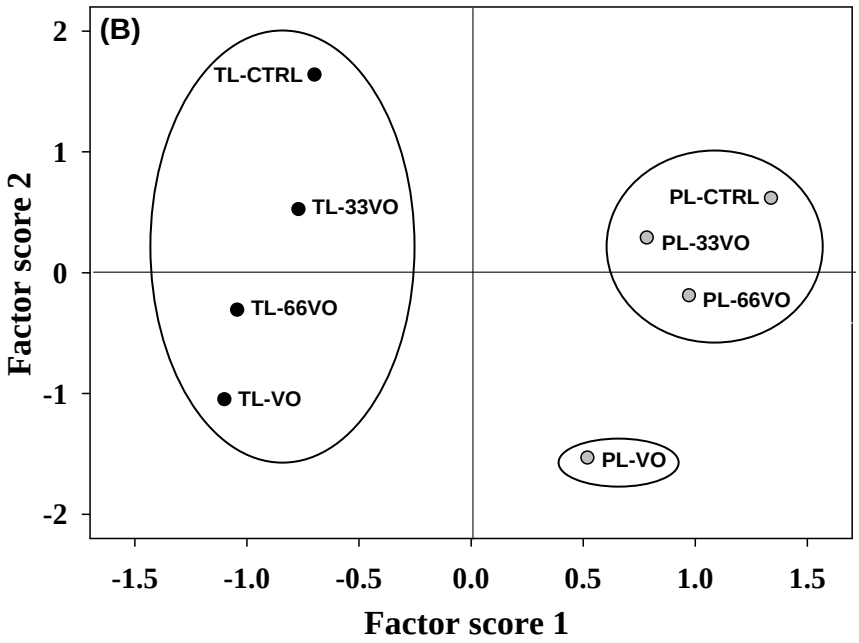
Figure 3


(A)
Component 2 (10.2%)
Component 1 (67.9%)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
18:0
24:1 n-9
16:0
20:4 n-3
18:3 n-3
20:3 n-3
15:0
20:5 n-3
22:6 n-3
18:1 n-9
22:1 n-11
16:2
22:5 n-3
20:3 n-6
17:0
20:1 n-9
18:2 n-6
16:4
14:0
16:1 n-7
18:1 n-7
20:0
20:4 n-6
18:4 n-3
20:2 n-6
22:1 n-9
16:3

(B)
Factor score 2
Factor score 1
2
1
0
-1
-2
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
TL-VO
Aug
Sep
Jan
Sep
Aug
Jan
TL-66VO
Aug
Jan
Sep
TL-33VO
TL-CTRL
Aug
Sep
Jan

Figure 4


(A)
Component 2 (27.8%)
Component 1 (39.6%)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
20:1 n-9
16:0
18:1 n-7
14:0
22:1 n-11
16:1 n-7
18:4 n-3
20:4 n-3
16:2
20:5 n-3
22:5 n-3
16:3
22:6 n-3
24:1 n-9
17:0
20:4 n-6
18:0
18:1 n-9
20:0
16:4
20:3 n-6
20:2 n-6
18:3 n-3
18:2 n-6
20:3 n-3


(B)
Factor score 2
Factor score 1
2
1
0
-1
-2
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
TL-CTRL
TL-33VO
TL-66VO
TL-VO
PL-CTRL
PL-33VO
PL-66VO
PL-VO