



TAURINE SUPPLEMENTED PLANT PROTEIN BASED DIETS WITH ALTERNATIVE LIPID SOURCES FOR JUVENILE GILTHEAD SEA BREAM, *Sparus aurata*

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Abstract- Two lipid sources were examined as fish oil replacements in fishmeal free, plant protein based diets for juvenile gilthead sea bream, *Sparus aurata*. A thraustochyrid meal plus soybean oil (MSC) and a canola oil (CO+EFA) supplemented with docosahexaenoic (DHA) and arachidonic acids (ARA). A twelve week growth trial was undertaken to examine the performance of the diets and to assess whole body and fillet fatty acid profiles resulting from the use of alternative lipid sources was comparable to fish oil. A finishing period was also utilized in an attempt to recover a fish oil fatty acid profile in the fillets of fish fed the alternative lipid source diets. The MSC diet significantly outperformed the fish oil control diet in terms of weight gain, feed conversion ratio, condition factor and protein efficiency ratio while maintaining similar fillet yield while the CO+EFA diet performed similarly to the control diet. Although fatty acid profiles in the whole bodies and fillets at the conclusion of the growth trial were representative of that of the diet fed, transition toward the fish oil profile was observed at the conclusion of the finishing period. Overall, both alternative lipid sources performed equivalently or better than the fish oil control, a significant finding for this species. Low dietary lipid (~7-9%) was just as effective as high dietary lipid input, observed in other studies and did not result in lowered fillet lipid levels or growth performance. As a note taurine had to be supplemented to the plant protein diets .

Keywords- Aquaculture, Aquatic feeds, Gilthead, Taurine, Fish oil

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Introduction

Fisheries and to a growing extent aquaculture, are responsible for a major source of the human population's supply of n-3 and n-6 polyunsaturated fatty acids (PUFA) [1-3]. Species used for direct human consumption are often high in these essential and human health beneficial fatty acids, as are the species captured for reduction to fishmeal and fish oil, components utilized for the bulk of protein and lipid sources in many aquaculture diets. However, both capture and reduction fisheries have reached production plateaus, with continued decline predicted for most fisheries worldwide. Aquaculture already consumes the bulk of the world's production of both fishmeal (60.8%) and fish oil (73.8%) [1]. In order for aquaculture to satisfy growing global population and protein requirements, alternative protein and lipid sources must be identified and evaluated to significantly reduce the quantities of fishmeal and fish oil used in diets for aquaculture. In fact, with the increase in aquaculture over the past few decades, the availability of fishmeal has steadily declined and costs have steadily increased [4], with predictions that demand will soon be greater than supply.

Gilthead sea bream, *Sparus aurata*, is one of the most heavily cul-

tured species in Europe with ~140,000 metric tons produced annually [1] and the ability to replace high quantities of fishmeal with plant proteins in the diets of juvenile gilthead sea bream has been well established [5-10], although complete fishmeal replacement often results in poorer growth and feed conversion when compared to traditional high fishmeal or commercial feeds for many species [11-14]. Limited synthetic capacity of the essential arachidonic (ARA) and docosahexaenoic (DHA) acids and their absence or low abundance in terrestrial oil sources has presented a formidable challenge in the development of complete fishmeal and fish oil free diets for many marine species [1,15]. Eicosapentaenoic acid (EPA) has been considered an essential fatty acid for marine species, however recent evidence suggests that this may not be the case for all species [16]. Studies attempting to completely or substantially replace fish oil with terrestrial oils in diets for gilthead sea bream have generally observed lower growth, poorer feed conversion, increased stress responses and poorer fillet quality when compared to full or only partially substituted fish oil diets. Much of this decline may be attributed to lack of essential fatty acids in the substituted oils.

The objectives of this study were to examine the effects on growth,

feed conversion, survival and proximate and fatty acid compositions of juvenile gilthead sea bream fed diets varying in lipid source. The base of the diets in the study consisted of a fishmeal free, plant protein based diet which has been used effectively for rearing juvenile cobia, *Rachycentron canadum*, in recirculating aquaculture systems [17]. With fish oil serving as the sole lipid source in the control diet, two alternative lipid sources were selected for the experimental diets; a microbial single celled thraustochytrid derived meal high in DHA [18-20] plus soybean oil and a canola oil supplemented with ARA and DHA at levels to mimic potential genetically engineered canola oil levels [21,22]. In addition to the twelve week growth trial on the complete fish product free experimental diets, a twelve week finishing period was implemented at the conclusion of the growth trial to determine the potential for fillet fatty acid profiles to return to a profile more similar to fish oil (i.e. farmed fish reared on traditional feeds or wild fish).

Materials and Methods

Diet Formulation and Growth Trial

The formulations for the three fishmeal free diets using a blend of plant protein sources are presented in [Table-1]. The plant protein blend supplementation for all three diets is based on a proven formulation for rainbow trout [23] with slight modifications for cobia [17]. Lysine, methionine, threonine, magnesium and potassium chloride were all supplemented to mimic concentrations commonly found in fillet tissues [23]. All ingredients were ground using an air-swept pulverizer (Model 18H, Jacobsen, Minneapolis, MN) to a particle size of <200µm. All ingredients were mixed before extrusion except for the menhaden oil. Pellets were prepared with a twin-screw cooking extruder (DNDL-44, Buhler AG, Uzwil, Switzerland) with an 18 second exposure to 127°C in the extruder barrel. Pressure at the die head was approximately 26 bar and a die head temperature of 71°C was used. The pellets were dried for approximately 15 minutes to a final exit air temperature of 102°C using a pulse bed drier (Buhler AG, Uzwil, Switzerland) followed by a 30 minute cooling period to product temperature less than 25°C. Final moisture levels were less than 10% for each diet. Oils were top-coated after cooling using a vacuum pressure of 25 bar (A7J Mixing, Ontario, CA). Diets were stored in plastic lined paper bags at room temperature and were fed within 6 months of manufacture.

The control diet for this study utilizes the same fishmeal free, plant protein base formulated by the USDA's Agricultural Research Service ("ARS Diet") with menhaden fish oil serving as the sole lipid source and its palatability and effectiveness as a feed has previously been examined with positive results for juvenile cobia (120-500g) [17]. The experimental diets for this study examined two possible fish oil replacement sources in the fishmeal free, plant protein based diet. A thraustochytrid meal with additional soybean oil (Aquafauna Biomarine, Hawthorne, CA) ("MSC Diet") constituted the lipid source for one diet and a commercially produced canola oil plus DHA, which was further supplemented with DHA (DHAco, Martek BioSciences, Columbia, MD) and ARA (ARAco, Martek BioSciences, Columbia, MD) constituted the lipid source ("CO+EFA Diet") for the second experimental diet. Proximate compositions of the three diets are presented in [Table-2].

To ensure limited oxidation of the lipids did not occur during the feed manufacturing process or storage and experiment duration, samples were sent to New Jersey Feed Labs (Trenton, NJ) for peroxide analysis.

Table 1- Diet formulations and fatty acid compositions of the diets

Ingredient (g kg ⁻¹)	Diet		
	MSC	CO+EFA	ARS Control
Soy Protein Concentrate	269.3	269.3	269.3
Corn Gluten	199	211	211
Wheat Flour	198.5	226.5	226.5
Soybean Meal	121	121	121
Solvent Extracted Menhaden Oil	0	0	84
Soybean Oil	50	0	0
Algamac 3050	74	0	0
Canola Oil + DHA + ARA	0	84	0
Dicalcium Phosphate	23.7	23.7	23.7
Vitamin Pre-mix ^a	10	10	10
Lysine-HCL	15.5	15.5	15.5
Choline CL	6	6	6
Trace Mineral Pre-mix ^b	1	1	1
Magnesium Oxide	0.5	0.5	0.5
Stay-C	3	3	3
DL-Methionine	5.8	5.8	5.8
Threonine	2.1	2.1	2.1
Potassium Chloride	5.6	5.6	5.6
Taurine	15	15	15
Fatty Acid (g 100g⁻¹)			
12:0	0.15	0.34	0.1
14:0	4.33	1.21	4.88
16:0	17.17	7.73	22.89
17:0	0.16	0.15	0.47
18:0	2.77	3.49	5.31
20:0	0.28	0.82	0.3
22:0	0.24	0.38	0.14
SFA	25.1	14.12	34.08
16:1n-7	0.23	0.46	5.44
18:1n-7	0.87	2.58	2.46
18:1n-9+6	13.86	48.28	19.53
20:1n-15+cis-8	0.06	0.03	0.22
20:1n-9	0.14	0.76	1.21
24:1n-9	0	0.13	0.32
MUFA	15.15	52.24	29.18
16:3n-4	0.05	0.06	0.57
16:4n-1	0.04	0.06	0.79
18:2n-6	33.27	22.86	12.74
18:3n-3	3.94	6.25	1.19
20:2n-6	0.04	0.13	0.18
20:4n-6	0.7	0.32	0.84
20:5n-3	0.42	0.27	8.9
22:5n-6	6.09	0.66	0.31
22:5n-3	0.16	0.16	1.51
22:6n-3	14.93	2.85	8.57
PUFA	59.75	33.64	36.75

^a Contributed per kg diet; vitamin A, 13510 IU; vitamin D, 9.2 IU; vitamin E, 184.4 IU; menadione sodium bisulfite, 6.6 mg; thiamine mononitrate, 12.7 mg; riboflavin, 13.4 mg; pyridoxine hydrochloride, 19.2 mg; pantothenate, DL-calcium, 141.5 mg; cyanocobalamin, 0.04 mg; nicotinic acid, 30.5 mg; biotin, 0.46 mg; folic acid, 3.5 mg.

^b Contributed in mg kg⁻¹ of diet; zinc 37; manganese, 10; iodine, 5; copper, 1.

This study was carried out in accordance with the guidelines of the International Animal Care and Use Committee of the University of Maryland Medical School (IACUC protocol # 0610015). Sea bream eggs were spawned from in-house broodstock and reared at the University of Maryland Center for Environmental Science's (UMCES) Institute for Marine and Environmental Technology (IMET) in Baltimore, MD, USA. Fish were reared on a combination of live feed (rotifers and *Artemia*), Otohime (Reed Mariculture, Campbell, CA) and Zeigler Marine Grower (Zeigler Bros, Gardners, PA) until reaching approximately 11g. Temperature and salinity were maintained at 25°C and 15 ppt throughout larval and juvenile

rearing. One hundred fish each were placed into six identical two cubic meter tanks, connected in pairs that share filtration and life support systems including protein skimming, ozonation, mechanical filtration in the form of bubble-bead filters and biological filtration; and for this reason tanks were randomly assigned diets to avoid having both tanks of one system feed the same diet. Water quality parameters did not differ significantly between systems utilized during the feeding trial and were: salinity, 15.93 ± 2.42 ppt; pH, 7.70 ± 0.51; total ammonia nitrogen, 0.15 ± 0.14 mg l⁻¹; nitrite, 0.14 ± 0.12 mg l⁻¹; nitrate, 236.74 ± 167.72 mg l⁻¹; and alkalinity, 108.93 ± 33.45 mEq l⁻¹.

Table 2- Proximate compositions of the three tested diets

	MSC Mean ± S.D. (NJFL) ³	CO+EFA Mean ± S.D. (NJFL) ³	ARS Control Mean ± S.D. (NJFL) ³
Lipid, g 100g ⁻¹ dm ¹	9.07 ± 0.54	7.32 ± 0.46	7.82 ± 1.05
Ash, g 100g ⁻¹ dm ¹	5.66 ± 0.04 ^a	5.10 ± 0.26 ^b	5.04 ± 0.14 ^b
Protein, g 100g ⁻¹ dm ¹	49.00 ^a	51.38 ^a	44.88 ^b
Carbohydrate, g 100g ⁻¹ dm ²	35.55	35.22	39.75
Fiber, g 100g ⁻¹ dm ¹	(0.72)	(0.98)	(2.51)
Moisture, g 100g ⁻¹ dm	4.02	7.7	5.11
Energy Content, MJ Kg ⁻¹	20.69 ± 0.15	20.29 ± 0.13	19.30 ± 0.77

¹ After lyophilization, ² After lyophilization (100-% lipid, ash, protein, fiber), ³ Values from New Jersey Feed Labs Analysis.

Values in the same row with different superscripts are significantly different (p<0.05); no superscript indicates no significant difference within a category.

Fish were anesthetized with Tricaine methanesulfonate (MS-222, 70mg l⁻¹, Finquel, Redmond, WA.) and weighed every six weeks to record growth with feed amounts gradually reduced from 4.4% to 2.2% bw during the 12 week trial. At the conclusion of the twelve weeks, six fish from each tank were euthanized with an overdose of MS-222 (150mg l⁻¹). Three fish from each trial were used for fillet and whole body analyses. Since lipid composition and source plays such an important role in final flavor and acceptance by the consumer, we examined the changes that take place in a twelve week finishing period. For these additional weeks, tanks that had been fed the experimental diets during the growth trial were switched to the ARS control diet with fish oil. Afterwards, three fish from each tank were sampled for fillet lipid analysis to assess the change in fatty acid profile following the diet switch.

Whole body and fillet samples were prepared by lyophilization to constant weight followed by homogenization by blending in a Waring blender (Waring Products, CT, USA). Ash percentage was determined by ashing three replicate samples at 600°C for a minimum of 8 hours. Energy content was determined in triplicate for each sample through bomb calorimetry (Parr instrument company, Moline, IL.) with calibration using benzoic acid over 20 samples. Crude Protein was determined by CHN analysis (Chesapeake Biological Laboratory, Center for Environmental Science, University of Maryland, %N X 6.25).

Lipid Extraction and Analysis of Fatty Acid Methyl Esters (FAMES)

Total lipids were extracted as described in Harvey et al. [24], a modified version of Bligh and Dyer [25]. Samples were subjected to three extractions with 4 ml of 2:1 methylene chloride (MeCl): methanol (MeOH), 1:1 methylene chloride (MeCl): methanol (MeOH) and 1:2 methylene chloride (MeCl): methanol (MeOH) in a sonicating

water bath, the supernatant being removed each time to a clean round-bottom glass test tube. Water was added (0.25 vol) to the lipid extracts. After vortex mixing and phase separation, the organic layer was recovered and dried under N₂ gas. The residue was brought to 1 ml in 1:1 MeCl:MeOH, flushed with N₂ gas and stored at -20°C for less than 1 month.

10% (vol.) of the lipid extract was used to generate fatty acid methyl esters (FAMES) according to procedures described in Ederington, et al. [26]. Five µg of C19:C21 internal standard (2.5 µg of each FA) and the lipid extract were added to glass tubes and dried under N₂ gas. Saponification of lipids was accomplished by adding 2.5 mL MeOH, 1.5 ml KOH-saturated MeOH and 0.5 ml dH₂O to each tube, which was then capped with a teflon-lined screw cap under a stream of N₂ gas and incubated at 70°C for 1 h. After cooling, 0.5 ml of dH₂O was added and neutral lipids (such as sterols) were removed by extracting 3x with hexane:ether (9:1). Fatty acids were then recovered by dropping the pH of the mixture remaining in the tubes to <2 by drop-wise addition of concentrated HCl and repeating the extraction (3x) with hexane:ether (9:1). Neutral lipids were stored frozen or discarded and fatty acids were dried under N₂ gas. Methylation of fatty acids was accomplished using 10% (w/w) BF₃ in methanol (Sigma-Aldrich, Bellefonte, PA USA). To each dried sample, 1 ml of BF₃:methanol were added, the tubes were flushed with N₂ gas, capped with teflon-lined screw caps and incubated at 70°C for 30 minutes. After cooling, 1ml of methanol was added to each tube and the samples were mixed by vortexing. The FAMES were extracted with hexane:diethyl ether (9:1) three times. The upper organic phase containing FAMES was recovered to new glass tubes, dried under N₂ gas, resuspended in 500 µl hexane, transferred to crimp-top GC sample vials containing small-volume sample inserts, capped under N₂ gas and stored at -80°C until analysis. Identification of FAMES was accomplished by comparing gas chromatography retention data with authentic quantitative standards (stds 3B, GLC-68D, GLC-17AA') from NU-CHECK, Inc. (Elysian, MN, USA) and qualitative standards (PUFA No. 1 - Marine Source) from Matreya (Pleasant Gap, PA, USA). Peaks in some samples were also confirmed by GC-MS. The Hewlett-Packard 6890 GC we used was equipped with a 30 m x 0.25 mm I.D. capillary column with 0.25 mm film thickness (DB Wax, J & W Scientific, Folsom CA) and a flame ionization detector at 300°C. The GC was run in 'constant flow rate' mode at 1.5 ml min⁻¹ with H₂ as the carrier gas. The column-temperature profile was as follows: 50°C for 0.5 min, hold at 195°C for 15 min after ramping at 40°C min⁻¹ and hold at 220°C for 7 min after ramping at 2°C min⁻¹. Total runtime was 38.13 min. The mass of FAMES was determined by comparison with the internal C19:C21 standards run with each sample. The relative distribution (% FAMES) was calculated based on the peak area of a given peak divided by the total peak area of identified FAMES in a sample.

Dilution Model

The dilution model of fatty acid turnover in fish is expressed as [Eq-1]:

$$P_t = P_r + (P_r - P_i) \left(\frac{Q_t}{Q_i} \right), \quad (1)$$

where P_t is the percentage of a specific fatty acid in an experimental fillet at time T following the dietary change, P_i and P_r are the percentages of the same fatty acid before the diet change (initially) and in a reference fillet (fish fed the ARS diet throughout) and Q_i and Q_t are the total lipid content initially and at time T, respectively

[27,28]. We compared the fatty acid compositions of fillets from fish that were involved in the twelve week switch to the ARS control diet, Pr, to the fatty acid profiles predicted by the dilution model using the MSC and CO+EFA fillet profiles as the initial, Pi.

Statistics

All statistical tests were run using Aabel v2.4.2 (Gigawiz Ltd., OK, USA) with significance values of $p < 0.05$. Paired t-tests and ANOVA were used to test differences in means between groups and two-way ANOVA was used to test differences in the growth curves from the growth trials. Means were averaged across each treatment if no significant difference was found within each treatment. Statistics were not performed on the carbohydrate and fiber content pool of the tissues since all other components were measured directly, therefore dramatically increasing and pooling the error of this one groups measurement.

Results

Diet Formulation

The protein content of the two fish oil replacement diets are slightly higher [Table-2] than the plant protein fish oil reference diet (ARS Plant Protein Cobia). Crude protein was significantly lower in the ARS diet than either of the experimental diets (ANOVA, $p < 0.05$). There were no significant differences between the diets in overall energy, lipid, or moisture content [Table-2]. The canola oil prior to and post addition of DHA and ARA had peroxide values of 5.2 and 2.0 mep kg^{-1} , respectively. The plant protein diet containing thraustochytrid meal and soybean oil had a peroxide value of 18.0 mep kg^{-1} , the CO+EFA diet had a peroxide value of 2.2 mep kg^{-1} and the ARS diet with fish oil as the lipid source had a peroxide value of 10.0 mep kg^{-1} . All these measures are below the accepted rancid values of 20 Mep kg^{-1} [29].

Growth Trial

At the conclusion of the initial twelve week growth trial, weight gain, percent growth from initial weight, was significantly higher (ANOVA, $p < 0.05$) on the MSC diet (785.69 ± 3.84) than the ARS control diet (696.33 ± 0.05) and the CO+EFA diet (680.34 ± 72.01), however weight gain on the CO+EFA diet (680.34 ± 72.01) was not significantly different than the ARS control ([Table-3], ANOVA, $p > 0.05$). Feed conversion ratio (FCR) and hepatosomatic index (HSI) were both significantly lower on the MSC diet than the CO+EFA or the ARS control diets (ANOVA $p < 0.05$). Protein efficiency ratio (PER) was significantly higher (ANOVA, $p < 0.05$) on the MSC (1.61 ± 0.04) and ARS control (1.64 ± 0.01) diets than the CO+EFA diet (1.42 ± 0.04). There were no significant differences in fillet yield, condition factor, or survival between the three diets ([Table-3], ANOVA $p > 0.05$), with overall averages between the diets of 27.48 ± 1.79 , 1.64 ± 0.14 and 95.8% respectively.

Proximate compositions of the whole body and fillet tissues of three randomly selected individuals from each tank, six per diet, are seen in [Table-4] and [Table-5], respectively. There were no significant differences between the diets in the whole body compositions in terms of lipid, ash, protein, moisture, energy content, or carbohydrates ([Table-4], ANOVA $p > 0.05$). There was a significant difference in fillet protein, g 100g^{-1} , content between the diets with the ARS control diet (78.02 ± 2.51) resulting in significantly higher fillet protein than the MSC (71.54 ± 5.43) or CO+EFA (73.65 ± 2.64). There were no significant differences in fillet lipid, ash, moisture, or energy content between the diets ([Table-5], ANOVA, $p > 0.05$) between the diets.

Table 3- Production characteristics from the twelve week grow out trial

	MSC	CO+EFA	ARS Control
Weight Gain (%) ¹	785.69 ± 3.84 ^a	680.34 ± 72.01 ^b	696.33 ± 0.05 ^b
Fillet Yield (%) ²	27.28 ± 1.30	27.50 ± 2.97	27.66 ± 1.09
FCR ³	1.27 ± 0.01 ^a	1.37 ± 0.04 ^b	1.36 ± 0.01 ^b
PER ⁴	1.61 ± 0.04 ^a	1.42 ± 0.04 ^b	1.64 ± 0.01 ^a
Hepatosomatic Index ⁵	1.41 ± 0.18 ^a	2.14 ± 0.39 ^b	2.11 ± 0.50 ^b
Specific Growth Rate ⁶	2.48 ± 0.00	2.33 ± 0.11	2.36 ± 0.00
Condition Factor ⁷	1.56 ± 0.15	1.67 ± 0.13	1.68 ± 0.13
Survival	97.50%	94.50%	95.50%

¹ Weight gain = (final tank weight - initial tank weight)/ initial tank weight*100.

² Fillet yield = (fillet weight/body weight)*100.

³ FCR=Feed conversion ratio = g fed/g gained.

⁴ PER=Protein efficiency ratio = g gained /g protein fed.

⁵ Hepatosomatic index = (liver weight/ body weight)*100.

⁶ Specific growth rate = ((lnBW_f-lnBW_i)/(days of growth trial))*100.

⁷ Condition Factor = (Weight*100)/(Length³).

Values in the same row with different superscripts are significantly different ($p < 0.05$), no superscript indicates no significant difference within a category.

Table 4- Proximate compositions of whole body tissues from animals fed the three experimental diets

	MSC Mean ± S.D.	CO+EFA Mean ± S.D.	ARS Control Mean ± S.D.
Lipid, g 100g^{-1} dm ¹	26.94 ± 5.55	27.40 ± 4.48	29.90 ± 6.65
Ash, g 100g^{-1} dm ¹	10.14 ± 1.66	10.51 ± 1.29	10.58 ± 1.22
Protein, g 100g^{-1} dm ¹	52.64 ± 5.88	51.42 ± 5.77	52.33 ± 4.52
Carbohydrate/Fiber, g 100g^{-1} dm ²	10.28	10.67	7.19
Moisture, g 100g^{-1} dm	62.88 ± 2.48	64.71 ± 0.81	65.59 ± 1.05
Energy Content, MJ Kg ⁻¹	23.02 ± 1.07	24.11 ± 0.73	24.08 ± 0.84
Body Weight ³	111.33 ± 8.76	94.75 ± 11.06	92.78 ± 13.79

¹ After lyophilization.

² After lyophilization (100-% lipid, ash, protein).

³ For fish used in analyses, g.

Values in the same row with different superscripts are significantly different ($p < 0.05$), no superscript indicates no significant difference within a category.

Table 5- Proximate compositions of fillet tissue from animals fed the three experimental diets

	MSC Mean ± S.D.	CO+EFA Mean ± S.D.	ARS Control Mean ± S.D.
Lipid, g 100g^{-1} dm ¹	15.28 ± 4.54	16.33 ± 4.50	14.46 ± 3.93
Ash, g 100g^{-1} dm ¹	4.23 ± 0.87	4.07 ± 0.77	4.15 ± 1.15
Protein, g 100g^{-1} dm ¹	71.54 ± 5.43 ^a	73.65 ± 2.64 ^a	78.02 ± 2.51 ^b
Carbohydrate/Fiber, g 100g^{-1} dm ²	8.95	5.95	3.37
Moisture, g 100g^{-1} dm	69.69 ± 2.92	71.61 ± 0.62	71.06 ± 2.31
Energy Content, MJ Kg ⁻¹	25.39 ± 0.80	24.64 ± 0.59	24.70 ± 0.84
Body Weight ³	118.72 ± 13.10	104.33 ± 12.52	113.47 ± 11.26

¹ After lyophilization

² After lyophilization (100-% lipid, ash, protein).

³ For fish used in analyses, g.

Values in the same row with different superscripts are significantly different ($p < 0.05$), no superscript indicates no significant difference within a category.

Fatty acid Analysis

[Table-6] presents the fatty acid profiles for the fillet and whole body tissues after the twelve week growth trial and the fillet fatty acid

profiles obtained after the additional twelve weeks when tanks fed either the MSC or CO+EFA diets were switched to the ARS diet containing fish oil.

Table 6- Fatty acid compositions (g 100g⁻¹) of fillet and whole body tissues from the twelve week grow out as well as recovery fillets from the additional twelve week period where animals that were on the two fish oil replacement diets were fed the control, fish oil based diet.

Fatty Acid (g 100g ⁻¹)	Fillets			Whole Body			Recovery Fillets	
	MSC	CO+EFA	ARS Control	MSC	CO+EFA	ARS Control	MSC	CO+EFA
12:0	0.10 ± 0.01	0.11 ± 0.01	0.09 ± 0.01	0.11 ± 0.03	0.11 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
14:0	5.48 ± 0.61	2.37 ± 0.20	4.99 ± 0.23	6.50 ± 1.14	2.20 ± 0.19	5.36 ± 0.59	4.18 ± 0.15	2.68 ± 0.11
16:0	24.93 ± 2.01	17.12 ± 0.55	26.08 ± 1.16	29.60 ± 4.21	16.74 ± 1.78	27.82 ± 3.13	22.05 ± 0.73	18.61 ± 0.61
17:0	0.23 ± 0.02	0.11 ± 0.01	0.25 ± 0.01	0.27 ± 0.03	0.13 ± 0.02	0.29 ± 0.03	0.26 ± 0.01	0.21 ± 0.02
18:0	4.08 ± 0.31	3.10 ± 0.13	4.57 ± 0.29	5.15 ± 0.70	3.54 ± 0.43	5.17 ± 0.51	3.87 ± 0.25	3.82 ± 0.11
20:0	0.22 ± 0.04	0.27 ± 0.02	0.24 ± 0.05	0.26 ± 0.04	0.29 ± 0.05	0.27 ± 0.04	0.22 ± 0.03	0.31 ± 0.05
22:0	0.27 ± 0.08	0.20 ± 0.12	0.40 ± 0.07	0.24 ± 0.11	0.25 ± 0.26	0.43 ± 0.05	0.33 ± 0.06	0.28 ± 0.06
SFA	35.32 ± 2.88	23.18 ± 0.60	36.61 ± 1.47	41.99 ± 6.10	23.10 ± 2.43	39.42 ± 4.24	30.98 ± 0.60	26.00 ± 0.79
16:1n-7	6.25 ± 0.55	3.71 ± 0.40	9.13 ± 0.41	6.37 ± 0.69	3.55 ± 0.48	9.08 ± 0.57	6.77 ± 0.61	5.16 ± 0.16
18:1n-7	2.17 ± 0.16	2.43 ± 0.05	3.07 ± 0.11	2.46 ± 0.19	2.58 ± 0.13	3.23 ± 0.16	2.48 ± 0.07	2.64 ± 0.07
18:1n-9+6	22.39 ± 1.48	43.53 ± 0.48	30.20 ± 0.69	23.65 ± 2.04	45.97 ± 2.71	31.13 ± 1.87	23.66 ± 0.85	34.57 ± 1.05
20:1n-15+cis-8	0.11 ± 0.02	0.09 ± 0.03	0.23 ± 0.06	0.16 ± 0.03	0.13 ± 0.07	0.26 ± 0.04	0.15 ± 0.03	0.15 ± 0.03
20:1n-9	0.71 ± 0.08	0.72 ± 0.12	0.81 ± 0.12	0.91 ± 0.08	0.84 ± 0.10	0.91 ± 0.13	0.91 ± 0.06	1.04 ± 0.08
24:1n-9	0.20 ± 0.04	0.20 ± 0.04	0.29 ± 0.07	0.33 ± 0.08	0.26 ± 0.06	0.37 ± 0.08	0.28 ± 0.06	0.33 ± 0.08
MUFA	31.84 ± 2.09	50.67 ± 0.42	43.72 ± 0.76	33.87 ± 3.02	53.34 ± 3.25	44.98 ± 2.46	34.24 ± 1.39	43.89 ± 1.12
16:3n-4	0.39 ± 0.04	0.16 ± 0.04	0.53 ± 0.04	0.31 ± 0.08	0.25 ± 0.10	0.49 ± 0.13	0.34 ± 0.12	0.35 ± 0.03
16:4n-1	0.34 ± 0.04	0.12 ± 0.04	0.38 ± 0.04	0.24 ± 0.05	0.14 ± 0.03	0.42 ± 0.03	0.31 ± 0.01	0.19 ± 0.02
18:2n-6	8.20 ± 0.60	17.12 ± 0.43	6.97 ± 1.99	6.35 ± 3.40	15.59 ± 3.13	6.40 ± 1.98	10.16 ± 0.35	14.45 ± 0.14
18:3n-3	0.73 ± 0.10	3.93 ± 0.13	0.69 ± 0.04	0.76 ± 0.12	3.56 ± 1.01	0.55 ± 0.21	0.89 ± 0.04	2.40 ± 0.08
20:2n-6	0.14 ± 0.03	0.25 ± 0.06	0.30 ± 0.39	0.15 ± 0.04	0.30 ± 0.07	0.15 ± 0.04	0.24 ± 0.03	0.36 ± 0.05
20:4n-6	0.79 ± 0.12	0.22 ± 0.03	0.39 ± 0.06	0.48 ± 0.38	0.23 ± 0.09	0.32 ± 0.13	0.93 ± 0.05	0.58 ± 0.08
20:5n-3	3.42 ± 1.09	0.55 ± 0.16	4.22 ± 0.17	3.21 ± 1.02	0.61 ± 0.35	3.04 ± 1.65	4.82 ± 0.38	3.39 ± 0.29
22:5n-6	3.73 ± 0.59	0.33 ± 0.12	0.33 ± 0.09	1.97 ± 1.66	0.37 ± 0.09	0.29 ± 0.08	2.40 ± 0.22	0.36 ± 0.06
22:5n-3	1.21 ± 0.17	0.25 ± 0.04	1.14 ± 0.14	0.82 ± 0.65	0.27 ± 0.10	1.04 ± 0.37	1.80 ± 0.11	1.39 ± 0.14
22:6n-3	13.46 ± 2.37	3.21 ± 0.41	4.23 ± 0.25	11.92 ± 2.81	2.53 ± 0.98	2.90 ± 1.58	12.33 ± 1.04	6.21 ± 0.57
PUFA	32.84 ± 4.38	26.16 ± 0.74	19.67 ± 2.03	24.14 ± 9.01	23.56 ± 5.55	15.60 ± 6.62	34.78 ± 1.84	30.11 ± 1.13

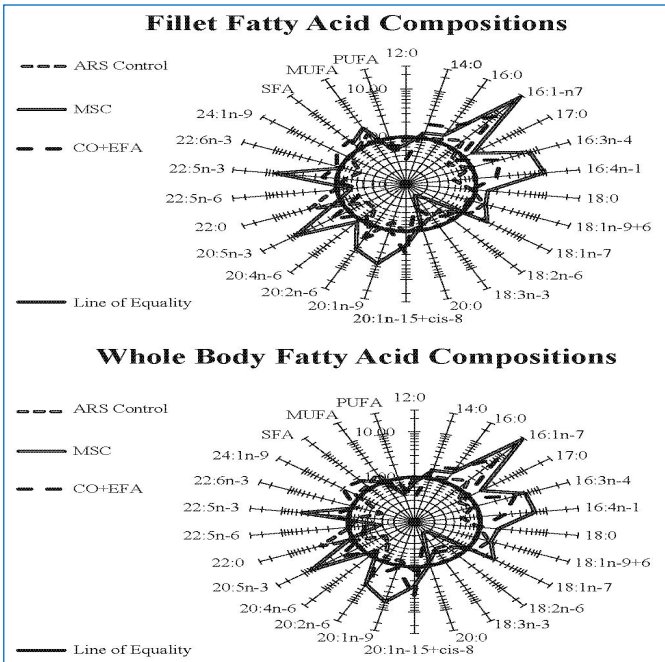


Fig. 1- Fatty acid compositions of fillet and whole body tissues from sea bream experimental fish oil replacement diets expressed as a fraction of dietary total lipid profile. Values were calculated from relative fatty acid methyl ester (FAME) composition (fillet or whole body fatty acid concentration/diet fatty acid concentration). Based on this calculation, a value of 1 represents equality between fillet and dietary fatty acid composition

[Fig-1] presents a radial diagram comparing the fatty acid profiles of the fillet and whole body tissues for fish from each diet at the conclusion of the twelve week growth trial as a ratio of fatty acid concentrations within the diet. Deviations from the line of equality represent enhancement or depletion of each fatty acid when compared to the diet fed, with values greater than 1 indicating an enrichment in the fillet or whole body compared to the diet and values less than 1 indicating a depletion compared to the diet. Euricic acid (22:1n9) is a toxic fatty acid that can be present in canola oil, however it was not detected in the diets, whole body, or fillet tissues of any of the animals fed the CO+EFA diet.

Dilution Model

[Fig-2] presents radial diagrams of the fillet fatty acid (% dw) profiles from this study. The top panel represents the “recovery” of the fatty acid profile of fish fed the MSC diet when switched to the ARS control diet for the twelve week finishing period and the bottom panel represents the “recovery” of the profile of fish fed the CO+EFA diet when switched to the ARS control for the finishing period. [Table-6] contains the fatty acid percentages found in the fillets of fish switched from the MSC and CO+EFA diets to the ARS control diet. [Fig-3] presents the linear regression of the dilution model as applied to either the MSC switch (top panel) or the CO+EFA switch (bottom panel) to the ARS control diet. Linear regression analyses for the comparisons of predicted fatty acid percentages based on the model to actual fatty acid percentages for the individual fatty acids measured resulted in R² values of 0.91 and 0.86 for the MSC and CO+EFA switches to the ARS diet, respectively.

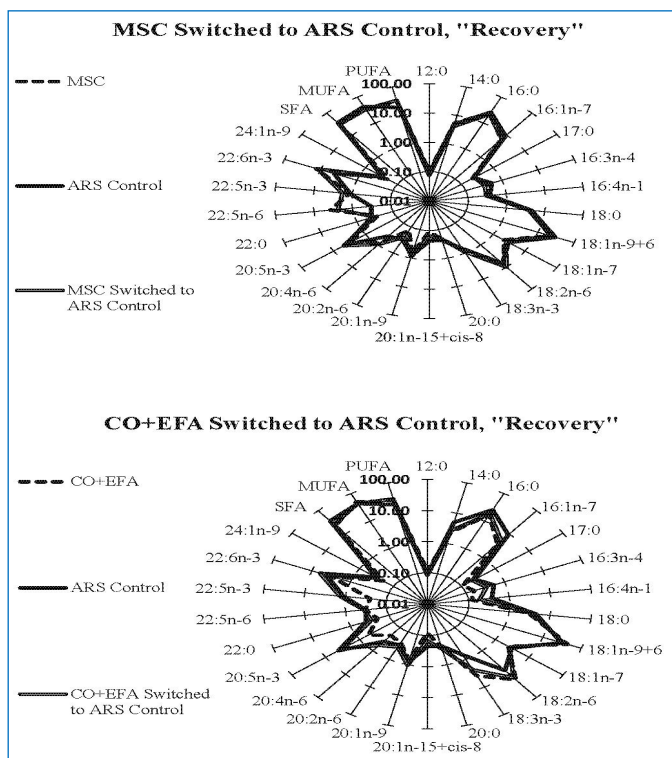


Fig. 2- Fatty acid compositions of fillet tissues from sea bream after twelve weeks on experimental lipid replacement diets, twelve weeks on fish oil based control diet, and twelve weeks on experimental fish oil replacement diet with twelve "recovery" weeks on fish oil based control diet.

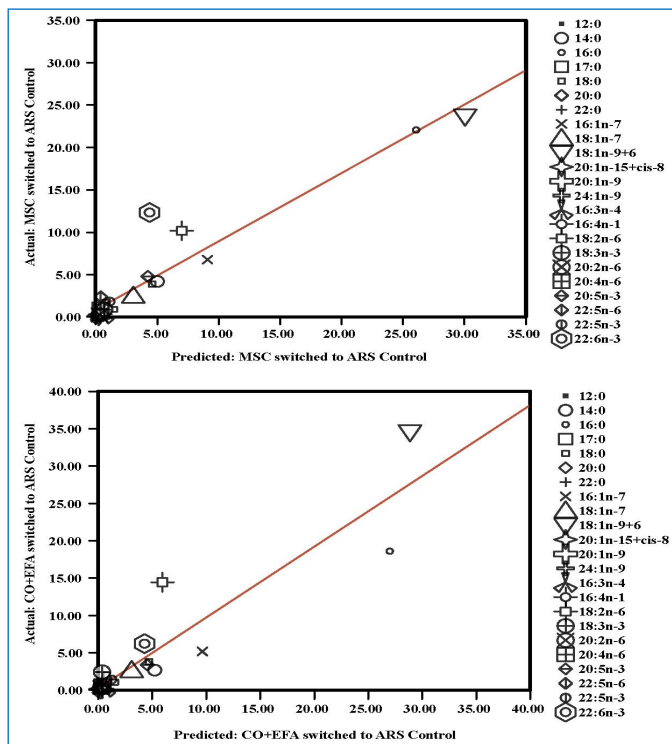


Fig. 3- Relationship between the predicted and actual fillet fatty acid compositions (% dw) following switch from either the Plant Protein: CO+EFA (A) or MSC (B) diets to the fish oil based ARS control diet (12 week duration). The dilution model is described in materials and methods with predicted results based on a standard model [27].

Discussion

Both experimental complete fish oil replacement diets performed as well or better than the fish oil, ARS, control diet. All three diets are also completely fishmeal free, with this study representing one effective fishmeal free and two effective complete fish product replacement diets for juvenile sea bream resulting in excellent growth rates, feed conversion ratios and survival from 11-100g over the initial twelve week growth trial. Performance of all three diets are similar to the results obtained by other researchers utilizing partial fish oil replacement in low fishmeal diets [5, 30-32]. However in this study, complete fish oil replacement in a fishmeal free diet was just as effective as full fish oil. It is important to note that the feeds used in this study were relatively low in overall lipid level (7.32-9.07%), a significant reduction from the 14-16% range utilized for many diets for this species [30, 33-36]. The low dietary lipid level did not result in lower lipid levels in the fillet when compared to other studies, with gilthead sea bream in this study maintaining 14-16% lipid in the fillet tissue, potentially indicating a strong ability for this species to modulate its tissue lipid content compared to dietary input.

In addition to the performance of the fish on the diets, the fatty acid profiles obtained for fillet and whole body tissues display unique characteristics similar to those of the diets, with the ARS fish oil diet being the most similar to the 1:1 line of equality for both fillets and whole body tissues [Fig-1]. This indicates the importance of attempting to match a fish oil profile when utilizing alternative oil sources, even if the supplementation with exogenous EFAs is necessary. However, even given the differences observed due to dietary input, results from the finishing period [Fig-3] give another indication that gilthead sea bream may have a strong ability to regulate the fatty acids being deposited in their tissues. For most fatty acids measured, there is little difference between the concentration in fillets of fish fed the ARS control diet compared to either the MSC or CO+EFA diets and where large differences were detected, after the twelve week finishing period, concentrations had transitioned towards the ARS fish oil type profile. One major exception to this trend was the effect of DHA in the MSC fed fish. The MSC diet is very high in DHA (~14.93% of fatty acids) and after the twelve week finishing period, DHA remained high in the fillets (12.33% vs 13.46%), while the ARS diet only contains ~8.57% DHA. This is potentially an added human health related benefit to the MSC diet, with or without the use of a finishing period. The same trend is seen in docosapentaenoic acid (DPA), an intermediary between EPA and DHA that has been shown to have its own roles in benefiting human health [37-39]. Even with these exceptions, the simple dilution model accurately predicted the overall transitions observed when switching fish fed either the MSC or CO+EFA diets to the ARS control diet [Fig-3]. The minimum g EFA kg⁻¹ (EPA+ARA+DHA in this instance) provided in the diet for marine fish has been suggested to be in the range of 0.5-1, with requirement levels for various species falling in the 5-10 g kg⁻¹ range depending on overall lipid level [3]. As mentioned previously, the addition of DHA and ARA to the canola oil source was selected to mimic potentially available genetically modified crop levels. This resulted in an EFA level of 0.26 g kg⁻¹ diet for the CO+EFA diet. In contrast the MSC diet has an EFA level of 1.37 g kg⁻¹ and the ARS diet has an EFA level of 1.93 g kg⁻¹. Even with the low level of overall EFAs in the CO+EFA diet, growth was equivalent to the ARS control diet, indicating the EFA requirement for this species may be lower than other marine species, potentially due to some synthetic capacity. The fishmeal free, plant protein based diets in this study rely upon and success with these and simi-

lar plant protein blends have largely been attributed to, the supplementation of taurine in the absence of fishmeal. Taurine is a non-protein amino acid that has multiple important physiological roles [40], however it is not found in any terrestrial plant sources and must therefore be supplemented to feeds in the same manner as lysine and other essential components. Taurine has been shown to be the only amino acid used by marine teleosts as a bile conjugate, with conjugated bile salts being critical for efficient lipid digestion [41]. This may be a partial explanation as to why the low lipid diets in this study were still effective, especially considering the use of alternative lipid sources in these fishmeal free diets.

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References

- [1] The Food and Agriculture Organization (2012) *The State of World Fisheries and Aquaculture*, FAO, United Nations Rome, Italy.
- [2] Gjedrem T., Robinson N. and Rye M. (2012) *Aquacult.*, 350-353, 117-129.
- [3] Glencross B.D. (2009) *Aquacult. Rev.*, 1(2), 71-124.
- [4] Tacon A.G.J. and Metian M. (2008) *Aquacult.*, 285, 146-158.
- [5] Benedito-Palos L., Saera-Vila A., Calduch-Giner J.A., Kaushik S. and Perez-Sanchez J. (2007) *Aquacult.*, 267, 199-212.
- [6] Rigos G., Zonaras V., Nikolopoulou D., Henry M., Nikoloudaki X. and Alexis M. (2011) *Aquacult.*, 311, 31-35.
- [7] Sitjà-Bobadilla A., Peña-Llopis S., Gómez-Requeni P., Medale F., Kaushik S. and Perez-Sanchez J. (2005) *Aquacult.*, 249, 387-400.
- [8] Sánchez-Lozano N.B., Martínez-Llorens S., Tomás-Vidal A. and Cerdá M.J. (2009) *Aquacult.*, 298, 83-89.
- [9] Robaina L., Moyano F.J., Izquierdo M.S., Socorro J., Vergara J.M. and Montero D. (1997) *Aquacult.*, 157, 347-359.
- [10] Lupatsch I., Kissil G.W., Sklan D. and Pfeffer E. (1997) *Aquacult. Nutr.*, 3, 81-89.
- [11] Luo L., Xue M., Wu X., Cai X., Cao H. and Liang Y. (2006) *Aquacult. Nutr.*, 12, 418-424.
- [12] Zhou Q.C., Mai K.S., Tan B.P. and Liu Y.J. (2005) *Aquacult. Nutr.*, 11, 175-182.
- [13] Kader M.A., Bulbul M., Koshio S., Ishikawa M., Yokoyama S., Nguyen B.T. and Komilus C.F. (2012) *Aquacult.*, 350-353, 109-116.
- [14] Xie S., He X. and Yang Y. (1998) *Aquacult. Nutr.*, 4, 187-192.
- [15] Rust M.B., Barrows F.T., Hardy R.W., Lazur A., Naughten K., and Silverstein J. (2011) *The Future of Aquafeeds*, NOAA Tech Memo NMFS-F/SPO-124, 103.
- [16] Trushenski J., Schwarz M., Bergman A., Rombenso A. and Delbos B. (2012) *Aquacult.*, 326-329, 81-89.
- [17] Watson A.M., Kissil G.W., Barrows F.T. and Place A.R. (2012) *Internat. Aquafeed*, 15, 34-38.
- [18] Lippmeier J.C., Crawford K.S., Owen C.B., Rivas A.A., Metz J.G. and Apt K.E. (2009) *Lipids*, 44, 621-630.
- [19] Lewis T., Nichols P. and McMeekin T. (1999) *Mar. biotech.*, 1, 580-587.
- [20] Carter C.G., Bransden M.P., Lewis T.E. and Nichols P.D. (2003) *Mar. biotech*, 5, 480-492.
- [21] Qi B., Fraser T., Mugford S., Dobson G., Sayanova O., Butler J., Napier J.A., Stobart A.K. and Lazarus C.M. (2004) *Nature biotech.*, 22, 739-745.
- [22] Venegas-calcrón M., Sayanova O. and Napier J.A. (2010) *Prog. lipid res.*, 49, 108-119.
- [23] Barrows F.T., Gaylord T.G., Sealey W.M., Smith C.E. and Porter L. (2010) *Aquacult. Nutr.*, 16, 654-661.
- [24] Harvey H.R., Eglinton G., Hara S.C.M.O. and Corner E.D.S. (1987) *Geochimica et Cosmochimica Acta.*, 51, 3031-3040.
- [25] Bligh E.G. and Dyer W.J. (1959) *Can. J. Biochem. Phys.*, 37, 911-917.
- [26] Ederington M.C., Mcmanus G.B. and Harvey H.R. (1995) *Limn. Ocean.*, 40, 860-867.
- [27] Robin J., Regost C., Arzel J. and Kaushik S. (2003) *Aquacult.*, 225, 283-293.
- [28] Trushenski J.T. and Boesenberg J. (2009) *Aquacult.*, 296:277-283.
- [29] Turchini G., Ng W.K. and Tocher D.R. (2011) *Fish oil replacement and alternative lipid sources in aquaculture feeds*. CRC Press, Boca Raton FL.
- [30] Fountoulaki E., Vasilaki A., Hurtado R., Grigorakis K., Karacostas I., Nengas I., Rigos G., Kotzamanis Y., Venou B., Alexis M.N. (2009) *Aquacult.*, 289, 317-326.
- [31] Glencross B., Hawkins W. and Curnow J. (2003) *Aquacult. Nutr.*, 9, 305-315.
- [32] Montero D., Grasso V., Izquierdo M.S., Ganga R., Real F., Tort L., Caballero M.J. and Acosta F. (2008) *Fish. Shellfish Immun.*, 24, 147-155.
- [33] Liu J., Caballero M.J., Izquierdo M., El-Sayed Ali T., Hernandez-Cruz C.M., Valencia A. and Fernandez-Palacios H. (2002) *Fisher. Sci.*, 68, 1165-1172.
- [34] Izquierdo M.S., Obach A., Arantzamendi L., Montero D., Robaina L. and Rosenlund G. (2003) *Aquacult. Nutr.*, 9, 397-407.
- [35] Izquierdo M.S., Montero D., Robaina L., Caballero M.J., Rosenlund G. and Gines R. (2005) *Aquacult.*, 250, 431-444.
- [36] Dias J., Conceição L.E.C., Ribeiro A.R., Borges P., Valente L.M.P. and Dinis M.T. (2009) *Aquacult.*, 293, 255-262.
- [37] Herold P.M. and Kinsella J.E. (1986) *Amer. J. Clin. Nutr.*, 43, 566-598.

- [38]Hino A., Adachi H., Toyomasu K., Yoshida N., Enomoto M., Hiratsuka A., Hirai Y., Satoh A. and Imaizumi T. (2004) *Atherosclerosis*, 176, 145-149.
- [39]Sun Q., Ma J., Campos H., Resrode K.M., Albert C.M., Mozafarian D. and Hu F.B. (2008) *Amer. J. Clin. Nutr.*, 88, 216-223.
- [40]Schuller-Levis G.B. and Park E. (2003) *FEMS Microbio. Lett.*, 226, 195-202.
- [41]Kim S.K., Matsunari H., Nomura K., Tanaka H., Yokoyama M., Murata Y., Ishihara K., Takeuchi T. (2008). *Fisher. Sci.*, 74, 875-881.