Fatty acid digestibility in gilthead sea bream fed diets containing native, re-esterified or acid vegetable oils

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Accepted for publication in : Aquaculture Nutrition, 2017 (23) 537-547.

https://doi.org/10.1111/anu.12421

Abstract

Re-esterified vegetable oils are obtained from a chemical esterification reaction between vegetable acid oils and glycerol. Due to their properties, it is expected that they have a higher nutritive value than their corresponding acid oils and a better digestibility than their native counterparts. The aim of the present study was to determine the effect of re-esterified oils with a different monoacylglycerol (MAG) and diacylglycerol (DAG) content, produced from palm or rapeseed, on fatty acid digestibility in gilthead sea

bream (*Sparus aurata*). Triplicate groups of fish were fed nine experimental diets containing different oils during 28 days. For each source, four different types of oil were used: native, re-esterified low or high in MAG and DAG and acid. A commercial fish oil was used for the control diet. Diets containing re-esterified oils had better apparent digestibility coefficients (ADC) of total fatty acids than acid oil diets. Re-esterified oils do not negatively affect apparent digestibility coefficients of fatty acids when compared to their corresponding native oils and could be incorporated as a source of energy in diets for gilthead sea bream. An improvement in digestibility compared to the native oil diet was only obtained in palm re-esterified oil high in MAG and DAG.

Keywords: by-product, esterification, diacylgycerol, monoacylglycerol, gilthead sea bream (*Sparus aurata*), digestibility.

Abbreviations

ADC: Apparent digestibility coefficient(s)

DAG: Diacylglycerol(s)

FFA: Free fatty acid(s)

MAG: Monoacylglycerol(s)

MUFA: Monounsaturated fatty acid(s)

PUFA: Polyunsaturated fatty acid(s)

SFA: Saturated fatty acid(s)

TAG: Triacylglycerol(s)

VO: Vegetable oil(s)

1. Introduction

In view of the increasing global demand of fish oil (FO), its decreasing availability and its large use by the aquaculture industry (FAO, 2014) oils from vegetable origin have been widely studied as sustainable and economically valuable FO substitutes in aqua feeds (Bell et al., 2001; Ng et al., 2003; Turchini et al., 2009; Yildiz et al., 2014). As extensively reported, certain vegetable oils (VO) are considered good alternatives to FO in diets for salmonids and freshwater fish (Bell et al., 2001; Caballero et al., 2002; Fountoulaki et al., 2009; Dernekbaşı, 2012). However, in marine fish, many studies have shown the limitation of the inclusion of VO in diets as a sole lipid source due to the low ability of these species to synthesize long-chain polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) from their C18 precursors (Watanabe, 1982). In gilthead sea bream (*Sparus aurata*), oils from different vegetable sources can be included in diets as an efficient source of energy without affecting feed utilization (Izquierdo et al., 2003; 2005; Benedito-Palos et al., 2008; Fountoulaki et al., 2009). Supplies of VO are approximately 100 times higher than those of FO (Bimbo, 1990) and its use as feedstock for energy production by the biofuel industry has greatly increased since the early 2000s. Thus, the livestock and the biofuel industries have undergone a competition for crop feedstocks, with the consequence of the rise of feed grains and oilseed prices. This has also led to a steadily increase of the amount of by-products derived from the production of biofuels (Taheripour et al., 2011). On the other hand, the refining process of VO also generates a considerable amount of fat by-products (Junior et al., 2012), their incorporation in animal diets being a potential way to reutilize them as a cheaper alternative to priced crops. In fact, studies have shown that some of the compounds are valuable and can be recovered for its subsequent use in animal nutrition (Dumont and Narine, 2007; Nuchi et al., 2009). This is the case of acid

oils, a free fatty acid (FFA)-rich by-product. However, as described by Wiseman and Salvador (1991) in broiler chickens, acid oils have a lower nutritive value than that of native oils as a consequence of their high content in FFA. Even so, their nutritive value might be increased when chemically re-esterified with glycerol, a by-product derived from the biodiesel production process (Parini and Cantini, 2009; Vilarrasa et al., 2014; Trullàs et al., 2015). The chemical esterification process does not change the fatty acid composition and the degree of saturation of the processed oil in relation to the original oil. However, the resulting fat has a different positional distribution of fatty acids in the glycerol molecule compared to that in native oils since the chemical esterification process is not regioselective. This means that part of the saturated fatty acids (SFA) present in the oil could be incorporated in the sn-2 position of acylglycerols (Vilarrasa et al., 2014; Trullàs et al., 2015). As it is widely known in mammals, the main products of the hydrolysis by pancreatic lipase during lipid digestion are FFA and 2-monoglycerides (MAG). While 2-MAG are directly absorbed (Schulthess et al., 1994) the rate of absorption of FFA depends on their chain length and degree of saturation (Small, 1991). In fact, free long-chain SFA have a poorer absorption than mono- (MUFA) and PUFA as a consequence of their hydrophobicity, their high melting points and their tendency to form insoluble soaps in the gut (Hunter, 2001).

In VO, SFA are found predominantly in the external positions (sn-1 and sn-3) of the triacylglycerols (TAG) (Berry, 2009) so these SFA are converted to FFA during digestion with the risk of ending up unabsorbed. Then, having more SFA in sn-2 in re-esterified oils could result in a higher digestibility of these oils compared to their native counterparts.

In marine fish, there are indications that the dominant digestive enzyme could be a carboxyl ester lipase-type (CEL) (Kutovic et al., 2009), also known as bile salt-activated

lipase (Gjellesvik et al., 1989; 1994; Iijima et al., 1998, Nolasco et al., 2011). This enzyme is also present in mammals (Hui and Howles, 2002) and shows strict dependence on bile-salts for hydrolytic activity on insoluble lipid substrates (Gjellesvik et al., 1994). CEL seems to be able to hydrolyze a wide range of lipid classes and is also likely to have a role in hydrolyzing monoglycerides, as reported in mammals (Gjellesvik, 1994; Kurtovic, 2009). Nonetheless, it has been established that the 2-MAG pathway is the predominant for TAG resynthesis in the enterocytes of gilthead sea bream (Caballero et al., 2006; Oxley et al., 2007). Thus, this leads to the possibility that it may possess certain sn-1,3 hydrolytic activity (Bogevik et al., 2008; Bakke et al., 2011).

On the other hand, re-esterified VO can have different proportions of lipid classes – TAG; diacylglycerols, DAG and MAG – which can be used to obtain a final product with specific desired characteristics (Parini and Cantini, 2009). For instance, when a major content of both MAG and DAG is present in the dietary fat, digestibility values might improve because of the emulsifying effect of these partial acylglycerols (Martin et al., 2014).

To the best knowledge of the authors, there is only one study reporting the use of chemically re-esterified VO in fish diets to-date (Trullàs et al., 2015), in which the improvement in fatty acids digestibility of re-esterified oils compared to acid oils was clearly shown in rainbow trout (*Oncorhynchus mykiss*). Re-esterified oils resulted in similar apparent digestibility coefficients (ADC) than those of native VO and were therefore considered potentially suitable for being incorporated as a source of energy in diets for this species. However, there is a wide diversity in the digestive physiology and differences in digestive lipase specificity could exist among fish species (Kurtovic, 2009; Bakke et al., 2011).

The present study aims at giving palm and rapeseed acid oils from the refining industry added value by transforming them to re-esterified oils with different contents of MAG and DAG, and to assess their effect on fatty acid digestibility in gilthead sea bream as a first step to determine if they can be appropriate energy sources for diets for this species.

2. Materials and methods

2.1. Experimental diets

Nine experimental diets were formulated to contain 48% protein and 24% lipid using the same ingredient composition except for the added lipid source. Oils used for the experimental diets originated from two different vegetal sources with different degree of saturation, palm (P) and rapeseed (R). For each source, four different types of oil were used: native oil (N), re-esterified oil low in MAG (EL), re-esterified oil high in MAG (EH) and acid oil (A), all resulting in eight experimental diets (Table 1). A commercial fish oil was used for the control diet (FO). Native, acid and re-esterified oils were provided by SILO S.p.a. (Firenze, Italy). In the process of esterification, the level of the different lipid classes present in the oil (TAG, DAG and MAG) was previously established by fixing the proportion fatty acid:glycerol. The free fatty acidity was determined following the ISO 660:1990 method. Glycerol was calculated according to the following stoichiometric formula: glycerol weight = fatty acid weight · free fatty acid acidity · glycerol molecular weight/fatty acid molecular weight. Once the proportion fatty acids:glycerol was established, both components were put in the reactor at 190-250ºC and 1-3 mm Hg of pressure for 4 – 6 hours. Feeds were produced at the Skretting Feed Technology Plant (Aquaculture Research Center; Stavanger, Norway) as extruded pellets. Yttrium oxide (Y_2O_3) was added to the diets as an inert marker for

apparent digestibility (AD) of fatty acid measure. The ingredient formulation and proximate composition of the diets are shown in Table 1. Nutrient composition of experimental diets was determined by standard procedures (AOAC, 2005): moisture (934.01), ash (942.05), crude protein (968.06) and crude lipid (920.39). Gross energy of dried feed was determined using an adiabatic bomb calorimeter (IKA – Kalorimeter system C4000, Jankel – Kunkel, Staufen, Germany). Samples were analyzed for yttrium by inductively coupled plasma optical emission spectroscopy (ICP OES) (Perkin Elmer spectrometer, model Optima 4300DV) with a previous digestion in a CEM microwave (CEM MARSXpress).

2.2. Fish, experimental conditions and sampling

All the procedures were conducted in accordance with the Animal Protocol Review Commitee of the Autonomous University of Barcelona (UAB) and following the European Union Guidelines for the ethical care and handling of animals under experimental conditions. The trial was carried out at the Institute of Agrifood Research and Technology (IRTA), Sant Carles de la Ràpita, Spain. A total of 702 gilthead sea bream (Piscimar, Spain) with a mean initial body weight of 296±7.2 g were randomly distributed into 27 cylindro-conical tanks of 400l of capacity in a recirculation seawater system IRTAmar®. Water temperature (21.5ºC), salinity (36.1±1.20g·l^{-1}) and dissolved oxygen levels (6.1±1.06 mg·l^{-1}) were maintained constant throughout all the experimental period. The tanks were subjected to a photoperiod of 12h light and 12h dark. Following an adaptation period of a week, fish were fed the experimental diets during 28 days. Each diet was randomly assigned to three replicate tanks and was fed to satiation twice a day by automatic feeders. Feed was supplied in excess of appetite (20%) of measured feed intake. Uneaten feed was collected by filtering effluent water from each tank one hour and a half after each meal so that feed intake could be recorded

daily. At the end of the experimental period, all the animals were euthanized in excess anaesthetic (2-phenoxyethanol) and faeces were collected from the hindgut after laterally opening the peritoneal cavity. Faecal samples were pooled by tank and stored at −20°C prior to analysis of yttrium oxide and fatty acid composition.

2.3. Fatty acid composition

Fatty acid composition of oils, diets and faeces were determined by gas chromatography – flame ionization detector (GC-FID). For experimental oils, the fatty acid methyl esters (FAME) were previously obtained as described by Vilarrasa et al., (2014) and analyzed using an Agilent 4890D gas chromatograph. For diets and faeces, FAME were obtained by an adaptation of the method of Sukhija and Palmquist (1988) and analyzed using an HP 6890 gas chromatograph (Agilent Technologies). In both cases, fatty acid methyl esters were identified by comparison of their retention times with those of known standards, and quantified by internal normalization (FAME peak area/total FAME peak area, in %). Fatty acid composition of oils and diets is shown in Table 2.

2.4. Lipid class composition of the experimental oils and diets

Lipid class composition (TAG, DAG, MAG and FFA) of oils and diets is shown in Table 3 and was determined following the procedure described by Darnoko et al. (2000), adapted to these samples as reported in Trullàs et al. (2015).

2.5. Sn-2 fatty acid composition of the experimental oils

The composition of fatty acids located at the sn-2 position of the acylglycerols (TAG, DAG and MAG) of the experimental oils is shown in Table 4 and was determined as described in Trullàs et al. (2015).

2.6. Digestibility calculations

ADC of fatty acids was calculated as: ADC (%) = 100 − [100 × (Y in feed/Y in faeces) · (FA in faeces/FA in feed)] (Maynard and Loosli, 1969), where FA = fatty acid ($mg \cdot kg^{-1}$) and Y = yttrium ($mg \cdot kg^{-1}$).

2.7. Statistical analysis

Data were subjected to a one-way analysis of variance (ANOVA) and the significance of the differences between means was tested by Tukey's test. Values are given as means±standard error of a pooled samples each containing faeces samples from 26 fish, analysed in triplicate. Differences were considered significant when $P<0.05$. All statistics were performed by means of the General Lineal Model (Proc GLM) of SAS® software version 9.2 (SAS Institute Inc., Cary, NC, USA).

3. Results

Characterization of experimental oils and diets

As a consequence of the lack of information about chemically re-esterified oils from vegetable origin in the literature, the characterization of the experimental oils was necessary and was already reported in Trullàs et al. (2015). Briefly, differences in the fatty acid composition among the four types of oil (N, EL, EH, A) within each source (P and R) were low (Table 2). Regarding the different lipid classes (Table 3), native oils (FO, PN and RN) were mainly composed of TAG (> 80%) and acid oils consisted of FFA in more than 50%. EL re-esterified oils resulted in MAG and DAG proportions of about 6% and 33-39%, respectively, which increased to 27% MAG and 46-48% DAG in EH oils. 1(3)-MAG constituted the major MAG isomer in most of the oils, and especially in re-esterified and acid oils. Similarly, 1,3-DAG constituted the highest DAG isomer (66-77%). No formation of TAG polymers was observed. In relation to the fatty acid composition of the sn-2 position (Table 4), an increase in the content of SFA

located in sn-2 was clearly observed in both palm and rapeseed EL and EH oils, and especially in EL, compared to the native oil.

Apparent digestibility of fatty acids

The nine experimental diets were well accepted and survival rates were over 97% with all of them. Apparent digestibility coefficients (ADC) of total and individual fatty acids are given in Table 5 for palm and in Table 6 for rapeseed. Significantly lower digestibility values were obtained in palm diets in relation to FO diet. When comparing among palm diets, differences in digestibility were also obtained. ADC of total fatty acids of acid oil diet (PA: 49.6%) was lower than that of native oil diet (PN: 61.8%), but it reached higher values when re-esterified (PEL: 65.7% and PEH: 74.4%). In fact, in PA diet, ADC of both total fatty acids and total SFA were significantly lower than in the rest of diets. As observed, ADC of total SFA seemed to be largely determined by ADC of C16:0, which represented approximately a 40% of the total fatty acids in palm oils and diets (Table 2). Compared to PA, higher digestibility values were also obtained in re-esterified oils diets (PEL and PEH) for total MUFA and PUFA.

When comparing digestibility values of re-esterified oils diets with PN diet, differences were obtained between PEL and PEH. In PEL, ADC of both individual and groups of fatty acids did not present significant differences compared to PN in any case, although they were numerically higher in most cases. Contrarily, ADC of total fatty acids, as well as of both individual and total SFA and MUFA, were significantly higher in PEH than in PN, which was especially remarkable in SFA. If compared to FO, PEH diet obtained similar ADC of total MUFA and PUFA, but not of total SFA.

When PEL and PEH diets were compared between them, only numerically differences in the ADC of the different individual and total fatty acids were observed. However,

digestibility values in PEH were, in general, higher than in PEL. Once more, these differences were particularly notable in both individual and total SFA (PEL: 47.8% and PEH: 62.3% for total SFA).

In rapeseed, digestibility values were higher than those obtained in palm. Similarly, ADC of total fatty acids in REL and REH diets were higher when compared to RA, but similar to values of RN and FO diets. No significant differences were observed in ADC of fatty acids between REL and REH diets.

4. Discussion

In relation to lipid classes, the predominance of 1(3)-MAG and 1(3)-DAG over the rest of MAG and DAG isomers has been related to the higher stability of primary esters (sn-1(3)) than of secondary esters (sn-2) (Cruz Hernandez et al., 2012), which could cause acyl migration of fatty acids from sn-2 to sn-1 or sn-3 positions of MAG and DAG (Destaillats et al., 2010; Martin et al., 2014).

Regarding the higher content of SFA located at the sn-2 position of re-esterified oils compared to the native oil, it was more noticeable in EL than in EH oils. This was probably due to EH oils had more partial acylglycerols (MAG and DAG) and these were mainly 1(3)-MAG and DAG, so less SFA in sn-2 position were present.

The fatty acid composition and degree of saturation of re-esterified oils compared to native oils were unchanged, as many studies on interesterification reactions had previously described (Marangoni and Rousseau, 1998; Scheeder et al., 2003; Berry, 2009; Farfán, 2013).

For more details on the composition of re-esterified oils, readers are addressed to our previous trial in rainbow trout, in which the same oils were used and their characterization was already discussed (Trullàs et al., 2015).

Apparent digestibility of fatty acids

Results indicate that the dietary form of the lipid may be more influential on fatty acid digestibility than the fatty acid composition, as suggested by Ng et al. (2010). Indeed, as observed, esterification of FFA from acid oils with glycerol improves the ADC of fatty acids in both palm and rapeseed.

The higher nutritive value of acid oils when re-esterified to the glycerol molecule could be due to several factors. The lowest fatty acid digestibility of acid oils could be related to a feedback inhibition on the lipase activity caused by FFA, as suggested by Bogevik et al. (2008) for Atlantic salmon. Thus, the richness in FFA of acid oils in our study could reduce the activity of lipase, resulting in a lower hydrolysis of the TAG and the partial acylglycerols present in these oils, which would have a negative effect on its digestibility. On the other hand, in chicks, secretion of bile salts seems to be stimulated by the presence of TAG and 2-MAG, and not FFA, in the intestine (Sklan, 1979). Although no information on this topic is available in fish, it has to be taken into account that bile salts seem to be a requirement for the hydrolytic activity of lipase in many marine species (Gjellesvik et al., 1989; Iijima et al., 1998; Nolasco et al., 2011). Then, in the present study, an impairment in lipase activity would arise in animals fed acid oils, this producing a lower fatty acids emulsification than in the other experimental oils and leading to a reduction in the absorption of fat. Moreover, as already mentioned, divalent ions present in the intestine have a tendency to bind to long-chain free fatty acids and especially free SFA. Then, in acid oils, a high subsequent formation of

insoluble salts would occur in fish, being detrimental in terms of fatty acid digestibility (Ringø, 1991; Olsen et al., 1998).

Comparing re-esterified oils with native oils, the effects on digestibility varied between the oil source (palm and rapeseed) and also between the type of re-esterified oil (PEL and PEH). In palm, contrarily to what was expected, the increase in the content of SFA in sn-2 did not improve digestibility, as no differences in ADC of fatty acids between PEL and PN were observed. Nevertheless, although not significant, higher numerically digestibility values were obtained in PEL than in PN diets, especially for the most quantitatively important fatty acids (SFA and MUFA). Better absorption of SFA when located at the central position of TAG had been reported in chickens, rats, piglets and human infants (Filer et al., 1969; Innis et al., 1995; Lien et al., 1997; Lin et al., 2010). However, in these studies, the minimum percentage of SFA in sn-2 position was 33.9%, so it is possible that the lower content of SFA in sn-2 obtained in re-esterified oils in the present study (maximum 21.4%) was insufficient to have a clear effect on digestibility. Differently, an improvement in digestibility values were obtained in PEH diet compared to PN diet, although they did not always reach those obtained in the control diet (FO). These results seem to point out a positive effect of the partial acylglycerols on digestibility, since PEH had a high content of MAG and DAG. The emulsifying role of partial acylglycerols as amphiphilic molecules in digestion has been widely reported in studies in both human and animal nutrition (Hayes et al., 1994; Da Costa, 2003). Therefore, in the present study, having more MAG and DAG in PEH would have probably helped incorporating a higher amount of hydrophobic FFA in the core of mixed micelles during digestion than in the rest of diets. However, in rainbow trout (Trullàs et al., 2015) no differences among PN, PEL and PEH were found. Differences observed between species could be related to factors such as variations in their digestive

physiology but also to the effect of the different water temperatures at which the two species were reared (14.3 ºC in rainbow trout and 21.5ºC in gilthead sea bream) during the whole experimental period. As reported by Vilarrasa et al. (2014) for palm oils, a re-esterified palm oil high in MAG and DAG had an expanded melting range if compared to its corresponding native oil, which means that this oil has a higher solid fat content than the rest of experimental oils at a given temperature. As observed by the aforementioned authors, a high solid fat content was slightly detrimental in terms of crude fat and fatty acids apparent absorption. Then, water temperature would have had an effect on the melting point of the oils in fish diets. As temperature was higher in sea bream than in rainbow trout studies, the solid fat content of PEH would have been lower in sea bream, producing a beneficial effect on its digestibility.

Compared to rapeseed, ADC of fatty acids in palm diets were lower, which was expected due to the widely known fact that the process of lipid digestion and absorption seem to favor the utilization of unsaturated fatty acids over their more saturated counterparts (Sigurgisladottir et al. 1992; Olsen et al., 1998). In fact, as reported by many authors, high palm oil levels in fish diets significantly reduce fatty acids digestibility, especially in cold water (Torstensen et al., 2000; Ng et al., 2010). In the present study, experimental oils were the only source of fat in the experimental diets, in which the level of SFA of the total fatty acids (45-48%) exceeded the level of SFA (23%) of the total fatty acids up to which digestibility starts to decrease, as described by Hua and Bureau (2009).

In rapeseed oil diets, in spite of the improvement of digestibility of fatty acids when re-esterified, and contrarily to palm, no differences in ADC among RN, REL and REH

diets were obtained. This is in accordance with the previous results obtained in rainbow trout (Trullàs et al. 2015) and could probably be a consequence of the strong effect that the degree of unsaturation had on digestibility, which could have masked both the possible effect of MAG-DAG and the effect of the increased content of SFA in sn-2. Therefore, digestibility of fatty acids of the experimental oils seemed to be more affected by their degree of saturation than by their positional distribution and lipid class composition of the oil. In fact, as observed in palm diets, ADC of individual SFA set the trend of both ADC of total SFA and total fatty acids, clearly showing the importance of the degree of saturation on the overall digestibility.

Independently from the source (palm or rapeseed) and the type of oil (native, re-esterified or acid), digestibility values obtained in the present study for gilthead sea bream were lower than those described in rainbow trout (Trullàs et al., 2015) (mean of 14.3±5.81 for palm and 9.8±5.56 for rapeseed, for total fatty acids; different faeces collection methods were used, -euthanasia followed by collection of faeces directly from the intestine in sea bream and stripping in rainbow trout-). One of the main differences between gilthead sea bream and rainbow trout is their habitat. In marine fish such as gilthead sea bream, the salinity of sea water results in a constant need to drink large amounts of water, rich in calcium and magnesium, to compensate what they lose through osmosis. Insoluble salts can be formed in the presence of these divalent ions and free long-chain fatty acids in the intestine. Thus, this might be affecting digestibility in marine fish in a greater extent than in freshwater fish. In fact, Ringø (1991) found a difference of a 7% in lipid digestibility in Arctic charr when fish were maintained in freshwater compared to sea water.

In conclusion, results from the present study corroborated that the esterification of FFA with glycerol improves the nutritive value of vegetable acid oils. Hence, both palm and rapeseed re-esterified oils could be incorporated as a source of energy in diets for gilthead sea bream without negatively affecting apparent digestibility coefficients of fatty acids when compared to their native oils.

This improvement in digestibility became significantly higher only in rich-in SFA (palm) re-esterified oils high in MAG and DAG, this showing a possible emulsifying effect of the presence of partial acylglycerols. However, this improvement would not reach apparent digestibility coefficients as those found in oils that are mainly unsaturated, such as rapeseed and fish oil. It is important to take into account that all palm experimental diets presented low total fatty acids digestibility values (under 75%) in gilthead sea bream. Therefore, the inclusion of re-esterified palm oils, irrespectively of their type, as a dietary source of energy should be done in combination with oils with a higher degree of unsaturation, as it had been done with native palm oil in previous studies (Benedito-Palos et al., 2008; 2010).

Further studies regarding the inclusion of these oils in diets for gilthead sea bream should be carried out in order to study their effect on growth, metabolism and fillet quality in this species.

Acknowledgements

Authors would like to thank SILO S.p.a. (Firenze, Italy) for providing the experimental fats and Magda Monllaó and Esteban Hernández from IRTA (Sant Carles de la Ràpita, Spain) for the technical assistance. This study was supported by a FPI predoctoral research grant from Ministerio de Ciencia e Innovación del Gobierno de España (BES-2011-046806), a post-doctoral research grant from the Generalitat de Catalunya and the

EU through the Beatriu de Pinós Post-doctoral Program (2011BP_B 00113) and by the financial contribution from Spanish CICYT (project AGL2010-22008-C02).

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Table 1. Ingredient formulation and proximate composition of the experimental diets.

	Diets[a]								
	FO	PN	PEL	PEH	PA	RN	REL	REH	RA
Ingredient composition (g kg^{-1})									
Wheat[b]	162.1	162.1	162.1	162.1	162.1	162.1	162.1	162.1	162.1
Wheat gluten[c]	206.9	206.9	206.9	206.9	206.9	206.9	206.9	206.9	206.9
Soy protein concentrate[d]	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0
North Atlantic fish meal[e]	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0
South American fish oil[f]	210.0	0	0	0	0	0	0	0	
Experimental oils[g]	0	210.0	210.0	210.0	210.0	210.0	210.0	210.0	210.0
Yttrium premix[h]	1	1	1	1	1	1	1	1	1
Mineral and vitamin premix[h]	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Proximate composition (g kg^{-1})									
Dry matter (g kg^{-1})	937.7	947.9	948.2	948.2	941.3	945.6	941.0	940.9	940.5
Crude protein	481.5	489.3	483.8	474.4	482.6	485.2	489.8	471.3	471.0
Crude fat	198.8	219.5	213.8	209.9	214.3	211.9	203.7	204.6	212.6
Ash	89.2	88.4	84.3	76.9	94.1	88.5	83.0	83.2	69.7
Gross energy (kJ g^{-1})	23.1	23.3	23.4	23.3	23.6	23.4	23.2	23.2	23.3

[a]Experimental diets nomenclature: FO: fish oil (control diet); PN: palm native oil; PEL: palm re-esterified oil low in MAG and DAG; PEH: palm re-esterified oil high in MAG and DAG; PA: palm acid oil; RN: rapeseed native oil; REL: rapeseed re-esterified oil low in MAG and DAG; REH: rapeseed re-esterified oil high in MAG and DAG and RA: rapeseed acid oil.
[b]Statkorn, Norway.
[c]Cerestar Scandinavia AS, Denmark.
[d]Selecta, Brasil.
[e]Welcon AS, Norway.
[f]Holtermann ANS, Norway.
[g]Experimental oils.
[h]Skretting standard vitamin and minerals premix, according to requirement data from NRC (2011). Trow Nutrition, The Netherlands.

Table 2. Fatty acid composition of the experimental oils and diets.

	Oils[a]									*Diets*[a]								
	FO	PN	PEL	PEH	PA	RN	REL	REH	RA	FO	PN	PEL	PEH	PA	RN	REL	REH	RA
Fatty acid (%)																		
C14:0	7.9	1.2	1.3	1.4	1.3	0.1	0.1	0.1	0.1	7.0	1.6	1.7	1.8	1.6	0.5	0.7	0.7	0.6
C16:0	16.5	40.3	39.5	40.5	44.5	4.4	7.4	7.4	6.1	19.0	38.6	36.0	35.4	40.2	6.8	8.9	8.7	8.1
C18:0	2.2	4.5	8.5	8.4	6.0	2.0	2.7	2.6	2.4	2.8	4.2	7.6	7.4	5.4	2.2	2.5	2.4	2.5
C16:1n-7	6.2	0.4	0.2	0.2	0.2	0.2	0.3	0.3	0.3	6.5	0.8	0.7	0.8	0.6	0.6	0.7	0.8	0.7
C18:1n-9	12.1	38.6	39.6	39.0	37.1	60.7	53.0	52.3	54.7	17.5	33.5	34.8	34.9	33.3	53.1	45.4	45.2	47.8
C18:1n-7	2.5	0.9	0.8	0.8	0.7	3.2	3.5	3.5	3.4	2.8	1.0	0.9	1.0	0.8	2.8	3.1	3.1	3.1
C20:1n-9	7.6	0.2	0.1	0.1	0.1	1.2	0.9	0.9	1.0	6.0	1.2	1.1	1.2	1.0	1.8	1.7	1.7	1.7
C18:2n-6	1.9	11.4	8.1	7.8	8.5	19.1	25.8	26.0	23.6	8.9	13.6	11.8	11.8	12.0	20.7	26.9	27.0	24.2
C18:3n-3	2.2	0.4	0.2	0.2	0.3	8.1	5.0	5.8	7.4	2.5	0.8	0.7	0.9	0.7	7.3	5.3	5.8	6.7
C20:4n-6	0.8	ND	ND	ND	ND	ND	ND	ND	ND	0.7	ND	ND	ND	ND	ND	ND	ND	ND
C20:5n-3 (EPA)	10.5	0.3	ND	ND	ND	ND	ND	ND	ND	11.3	1.3	1.0	1.1	1.0	0.9	1.1	1.1	0.9
C22:6n-3 (DHA)	10.2	0.7	ND	ND	ND	ND	ND	ND	ND	11.3	2.0	1.4	1.5	1.4	1.4	1.7	1.7	1.4
ΣSFA[b]	26.9	46.6	50.7	51.7	52.8	7.2	10.8	10.7	9.2	30.1	45.3	47.4	46.6	48.7	10.7	13.3	12.9	12.6
ΣUFA[c]	73.1	53.4	49.3	48.3	47.2	92.8	89.2	89.3	90.8	69.9	54.7	52.9	53.7	51.5	89.3	86.7	87.1	87.4
ΣMUFA[d]	30.2	40.2	41.0	40.3	38.4	65.5	58.3	57.4	59.7	34.5	36.9	38.0	38.4	36.4	58.9	51.6	51.4	54.0
ΣPUFA[e]	42.9	13.2	8.3	8.0	8.8	27.3	30.9	31.9	31.1	35.3	17.8	14.9	15.2	15.1	30.4	35.1	35.7	33.4
Σn-6 PUFA[e]	2.7	11.5	8.1	7.8	8.5	19.1	25.9	26.0	23.7	9.9	13.6	11.8	11.8	12.1	20.8	27.0	27.1	24.3
Σn-3 PUFA[e]	40.2	1.7	0.2	0.2	0.3	8.2	5.0	5.8	7.4	25.3	4.2	3.1	3.5	3.1	9.6	8.1	8.6	9.1
SFA:UFA	0.4	0.9	1.0	1.1	1.1	0.1	0.1	0.1	0.1	0.4	0.8	0.9	0.9	0.9	0.1	0.2	0.1	0.1

ND not detected.

[a]Experimental oils and diets nomenclature as in experimental diets (Table 1).

[b]SFA: saturated fatty acids. It includes other SFA of small quantity.

[c]UFA: unsaturated fatty acids. It includes other UFA of small quantity.

[d]MUFA: monounsaturated fatty acids. It includes other MUFA of small quantity.

[e]PUFA: polyunsaturated fatty acids. It includes other PUFA of small quantity; n-6 PUFA: omega 6 polyunsaturated fatty acids; n-3 PUFA: omega 3 polyunsaturated fatty acids.

Table 3. Lipid class composition of the experimental oils and diets.

Oils[a]	FO	PN	PEL	PEH	PA	RN	REL	REH	RA
Lipid classes (%)									
ΣTAG[b, c]	86.0	82.3	59.6	23.6	28.8	95.6	49.9	23.3	32.2
ΣDAG[b, c]	4.1	10.6	33.0	48.1	12.1	2.5	39.2	46.3	12.1
1 (3), 2-DAG[b, d]	40.0	28.6	24.7	22.9	37.9	33.3	24.3	28.5	25.6
1, 3-DAG[b, d]	60.0	71.4	75.3	77.1	62.1	66.7	75.7	71.5	74.4
ΣMAG[b, c]	0.3	0.9	5.7	27.0	3.7	0.2	5.8	27.5	2.3
1(3)-MAG[b, d]	50.0	75.0	87.5	93.1	89.5	50.0	90.9	91.6	90.9
2-MAG[b, d]	50.0	25.0	12.5	6.9	10.5	50.0	9.1	8.4	9.1
ΣFFA[b, c]	9.6	6.2	1.7	1.3	55.4	1.7	5.1	2.9	53.4
Diets[a]									
Lipid classes (%)									
ΣTAG[b, c]	91.9	82.1	61.1	30.7	34.3	94.5	54.2	26.9	40.1
ΣDAG[b, c]	3.2	10.1	30.8	44.3	12.3	2.9	35.9	45.3	11.0
ΣMAG[b, c]	0.4	0.8	4.5	23.2	3.5	0.2	4.7	25.0	1.6
ΣFFA[b, c]	4.5	6.9	3.6	1.8	49.9	2.4	5.2	2.8	47.3

[a]Experimental oils and diets nomenclature as in Table 1.
[b]TAG (triacylglycerols), DAG (diacilglycerols), MAG (monoacylglycerols) and FFA (free fatty acids).
[c]Values determined by size-exclusion chromatography. Values are given as wt% of the total lipid classes (TAG, DAG, MAG and FFA).
[d]Values determined by ^{1}H-NMR. Values of each isomer are given as wt% of the total corresponding fraction (DAG or MAG).

Table 4. Selected fatty acid composition of the sn-2 position of the experimental oils.

	Oils[a]								
sn-2 (%)	FO	PN	PEL	PEH	PA	RN	REL	REH	RA
C16:0	42.5 (7.0)	7.8 (3.1)	20.93 (8.3)	11.9 (4.8)	3.7 (1.6)	2.7 (0.12)	19.5 (1.4)	11.7 (0.9)	6.8 (0.4)
C18:0	17.1 (0.4)	8.3 (0.4)	25.1 (2.1)	17.1 (1.4)	6.1 (0.4)	4.1 (0.08)	30.9 (0.8)	16.6 (0.4)	8.0 (0.2)
C18:1n-9	23.8 (2.9)	49.0 (18.9)	27.9 (11.0)	19.7 (7.7)	21.2 (7.9)	28.8 (17.5)	29.3 (15.6)	16.5 (8.6)	12.2 (6.7)
C18:2n-6	44.3 (0.8)	55.5 (6.3)	32.0 (2.6)	20.9 (1.6)	21.8 (1.9)	52.0 (9.9)	31.4 (7.7)	18.9 (4.9)	13.9 (3.3)
ΣSFA	38.0 (10.0)	8.0 (3.7)	21.4 (10.9)	12.5 (6.4)	4.0 (2.1)	3.2 (0.2)	29.8 (2.5)	13.4 (1.5)	6.9 (0.7)
ΣMUFA	23.4 (7.1)	47.8 (19.2)	28.3 (11.6)	19.8 (8.0)	21.9 (8.3)	27.3 (17.9)	29.9 (17.4)	16.7 (9.5)	11.8 (7.0)
ΣPUFA	28.7 (12.1)	52.3 (6.9)	32.3 (2.6)	21.0 (1.7)	21.5 (1.9)	51.0 (13.9)	29.5 (9.1)	18.9 (6.0)	14.0 (4.3)

Values are given as the % of each fatty acid at the sn-2 relative to its content in the oil. Values in brackets are given as the % of each fatty acid at the sn-2 position relative to the total fatty acid amount.

[a]Experimental oils nomenclature as in Table 1.

Table 5. Apparent digestibility coefficient (ADC %) of selected fatty acids in gilthead sea bream fed the experimental palm diets.

	Diets[a]				
	FO	PN	PEL	PEH	PA
Fatty acid			*ADC (%)*		
C14:0	88.1 ± 0.9^{a}	51.6 ± 3.7^{cd}	57.8 ± 2.3^{bc}	66.0 ± 2.2^{b}	44.3 ± 1.7^{d}
C16:0	82.7 ± 1.0^{a}	42.4 ± 2.9^{c}	47.6 ± 5.0^{bc}	62.6 ± 2.2^{b}	23.8 ± 4.3^{d}
C18:0	75.3 ± 0.9^{a}	37.7 ± 3.2^{c}	43.3 ± 5.8^{bc}	58.4 ± 2.6^{b}	26.6 ± 3.7^{c}
C18:1n-9	88.4 ± 0.7^{a}	77.1 ± 2.4^{cd}	81.6 ± 1.0^{bc}	86.6 ± 0.9^{ab}	71.7 ± 1.0^{d}
C18:2n-6	89.7 ± 1.1^{a}	84.4 ± 1.8^{b}	85.7 ± 1.1^{ab}	86.8 ± 0.8^{ab}	78.3 ± 0.2^{c}
C18:3n-3	92.9 ± 0.7^{a}	72.9 ± 3.4^{b}	75.5 ± 3.0^{b}	76.9 ± 1.1^{b}	67.3 ± 3.0^{b}
C20:4n-6	96.4 ± 0.4	ND	ND	ND	ND
C20:5n-3 (EPA)	97.3 ± 0.3^{a}	78.8 ± 2.7^{b}	77.3 ± 4.9^{b}	74.5± 1.3^{b}	69.6 ± 4.4^{b}
C22:6n-3 (DHA)	96.7 ± 0.4^{a}	82.8 ± 2.2^{b}	80.9 ± 4.1^{b}	78.4 ± 1.4^{b}	74.0 ± 2.1^{b}
ΣSFA	82.9 ± 1.0^{a}	42.0 ± 3.0^{c}	47.8 ± 5.0^{bc}	62.3 ± 2.2^{b}	25.2 ± 4.1^{d}
ΣMUFA	89.3 ± 0.31^{a}	75.7 ± 2.5^{cd}	80.5 ± 1.1^{bc}	85.0 ± 1.1^{ab}	70.6 ± 0.7^{d}
ΣPUFA	85.4 ± 1.6^{a}	78.8 ± 2.5^{ab}	81.0 ± 2.1^{a}	80.7 ± 1.1^{a}	72.2 ± 0.8^{b}
Σn-6 PUFA	90.1 ± 1.0^{a}	83.9 ± 1.8^{b}	85.5 ± 1.3^{ab}	86.1 ± 0.8^{ab}	77.9 ± 0.2^{c}
Σn-3 PUFA	96.6 ± 0.4^{a}	79.6 ± 2.6^{b}	78.6 ± 4.1^{b}	76.8 ± 1.3^{b}	71.1 ± 3.0^{b}
Total FA[b]	89.3 ± 0.1^{a}	61.8 ± 2.6^{c}	65.7 ± 2.8^{bc}	74.4 ± 1.6^{b}	49.6 ± 2.2^{d}

Values represent mean ± SEM of pooled samples from 26 fish analyzed in triplicate. Values in the same row with different letters are significantly different (P<0.05), according to ANOVA.
[a]Experimental diets nomenclature: abbreviations as in Table 1.
[b]Total FA: total fatty acids.

Table 6. Apparent digestibility coefficient (ADC %) of selected fatty acids in gilthead sea bream fed the experimental rapeseed diets.

	Diets[a]				
	FO	RN	REL	REH	RA
Fatty acid			*ADC (%)*		
C14:0	88.1 ± 0.9a	69.0 ± 2.0b	71.0 ± 3.9b	64.2 ± 2.8b	56.1 ± 1.5b
C16:0	82.7 ± 1.0a	76.0 ± 3.5a	83.2 ±2.7a	83.7 ± 0.4a	57.1 ± 1.2b
C18:0	75.3 ± 0.9ab	67.2 ± 1.8b	80.8 ± 3.1a	81.4 ± 0.1a	40.4 ± 2.0c
C18:1n-9	88.0 ± 0.7ab	90.0 ± 0.8ab	92.7 ± 1.2ab	93.2 ± 0.2a	77.6 ± 1.7b
C18:2n-6	88.0 ± 1.1a	91.5 ± 1.1a	94.0 ±0.8a	94.3 ± 0.2a	83.6 ± 1.2a
C18:3n-3	91.9 ± 0.7a	93.7 ± 4.0a	94.7 ± 0.6a	95.5 ± 0.3a	86.0 ± 1.2a
C20:4n-6	95.8 ± 0.4	ND	ND	ND	ND
C20:5n-3 (EPA)	97.3 ± 0.3a	81.8 ± 0.1b	78.3 ± 3.1b	75.4 ± 2.8b	73.8 ± 0.1b
C22:6n-3 (DHA)	96.7 ± 0.4a	84.3 ± 1.2b	82.5 ± 2.2b	79.5 ± 2.1bc	72.1± 2.2c
ΣSFA	82.9 ± 0.9a	81.2 ± 8.6a	82.1 ± 3.3a	82.9 ± 0.8a	51.6 ± 0.8b
ΣMUFA	89.6 ± 0.3ab	89.2 ± 1.3ab	91.9 ± 1.4ab	92.4 ± 0.2a	76.4 ± 1.7b
ΣPUFA	85.4 ± 1.6bc	90.0 ± 1.3ab	92.1 ± 1.1a	92.2 ± 0.5a	84.5 ± 1.1c
Σn-6 PUFA	88.4 ± 1.0a	91.2 ± 1.0a	93.8 ± 0.9a	94.1 ± 0.3a	83.1 ± 1.3a
Σn-3 PUFA	96.6 ± 0.4a	89.9 ± 3.2ab	90.0 ±1.2ab	89.5 ± 0.9ab	85.2 ± 0.3b
Total FA[b]	89.3 ± 0.1a	89.7 ± 1.5a	90.6 ± 1.1a	91.1 ± 0.4a	78.3 ± 1.2b

Values represent mean ± SEM of triplicate pooled samples from 26 fish. Values in the same row with different letters are significantly different (P<0.05).

[a]Experimental diets nomenclature: abbreviations as in Table 1.

[b]Total FA: total fatty acids.