Acid and re-esterified rapeseed oils as alternative vegetable oils for rainbow trout diets: effects on lipid digestibility and growth

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Abstract

The present study aimed at evaluating the effects of dietary acid and re-esterified rapeseed oils as alternatives to native vegetable oils (VO) on growth performance and feed utilization in rainbow trout. Acid oils are a free fatty acid (FFA)-rich by-product from the refining of VO and re-esterified oils are the final product of a chemical esterification process between acid oils and glycerol. Because re-esterified oils have a high content of mono- and diacylglycerols (MAG and DAG), known for being good emulsifiers, a higher nutritive value than that of the native and the acid oils might be expected. A 72-day feeding trial where triplicate groups of rainbow trout were fed eight

experimental diets formulated to contain a 15% of a native, a re-esterified and an acid rapeseed oil, in addition to a 5% of fish oil (FO), was carried out. Diets with the native or the re-esterified oils blended with the acid oil were also studied. A commercial fish oil was used for the control diet. Fish fed rapeseed acid and re-esterified oils diets (RA and RE, respectively) showed high fat and total fatty acid apparent digestibility coefficients (ADC) (RA: 90.5±0.3%, RE: 92.5±1.0% for total fat and RA: 95.7±0.1%, RE: 95.8±0.2% for total fatty acids). However, the lowest total fatty acid ADC was that obtained in animals fed RA, which was significantly lower ($P<0.05$) than that of fish fed the rapeseed native oil diet (RN: 96.7±0.1%). No significant differences in final weight were obtained between fish fed RA (375.9±2.9g) and RE (381.5±11.1g) and those fed RN (393.7±6.1g), even though both values were significantly lower ($P<0.05$) than that of fish fed the control diet (411.1±3.3g). Nonetheless, fish fed diets including blends of the rapeseed acid and the re-esterified oils (RE/RA and RA/RE) had higher final weights (392.8±4.4 and 394.6±1.6, respectively) than those of RA and RE, although differences were not statistically significant. Furthermore, RA and RE diets did not produce relevant changes in plasma parameters or in the morphology of liver and intestine of fish. Therefore, the inclusion of rapeseed acid and re-esterified oils along with a 5% of FO in aqua feeds does not seem to have negative effects on fat and fatty acid digestibility, growth, plasma parameter or morphology of liver and intestine in rainbow trout. However, before recommending their use, further studies regarding their effects on the final composition and quality of fillets should be carried out.

Keywords: rainbow trout, acid oil, re-esterified oil, growth, digestibility, by-product.

Abbreviations

ADC: Apparent digestibility coefficient(s)

ADG: Average daily growth

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

CF: Condition factor

DAG: Diacylglycerol(s)

FCR: Feed conversion ratio

FFA: Free fatty acid(s)

FO: Fish oil

GGT: Gamma-glutamyl transferase

HDL: High density lipoproteins

HSI: Hepatosomatic index

LDL: Low density lipoproteins

MAG: Monoacylglycerol(s)

MUFA: Monounsaturated fatty acid(s)

PUFA: Polyunsaturated fatty acid(s)

SFA: Saturated fatty acid(s)

SGR: Specific growth rate

TAG: Triacylglycerol(s)

VLDL: Very low density lipoproteins

VO: Vegetable oil(s)

VSI: Viscerosomatic index

WG: Weight gain

1. Introduction

There are many studies reporting the suitability of vegetable oils (VO) as an alternative to fish oil (FO) in fish feeds (Fonseca-Madrigal et al., 2005; Sun et al., 2011; Tocher et al., 2003a; Turchini et al., 2009), as they are sustainable and economically advantageous sources. VO are mainly used in both the food and the feed industries, although their use by the biofuel industry has been rising notably since the early 2000s (Gunstone, 2011). In Europe, this is especially remarkable for rapeseed, which is the predominant feedstock for biodiesel production (Haas, 2005). Thus, the competition among industries has caused an increase of grains and oilseed prices (Behr and Pérez Gomes, 2010), which in turn has led to the need of finding suitable and economically interesting alternatives to the commonly VO used in fish nutrition. In this regard, the interest of the feed industry for the by- and co-products generated during the crude VO processing has also been growing. Indeed, a significant amount of by-products is generated from crude oil refining processes and can be valuable feedstocks for animal feeds (Dumont and Narine, 2007). Of these products, acid oils from the chemical refining of VO, a free fatty acid (FFA)-rich by-product, were found to be quite promising for feeding uses (Nuchi et al., 2009). In rainbow trout, an apparent digestibility coefficient (ADC) of total fatty acids above 95% was obtained for a diet including rapeseed acid oil, which did not differ from that of the native oil diet, the latter referring to the unrefined and unprocessed oil produced from vegetables (Trullàs et al., 2015).

Vegetable acid oils can be chemically re-esterified with glycerol to produce the so-called re-esterified VO. These oils can have a high final content of partial acyglycerols (monoacylglyerols, MAG and diacylglyerols, DAG), amphiphilic molecules that could exert a beneficial effect on digestibility (Fregolente et al., 2009; Martin et al., 2014). Good results in fat absorption and growth performance in broiler chicks and chickens have been obtained when including re-esterified VO in diets (Vilarrasa et al., 2014,

101 2015). Although the digestibility of rapeseed re-esterified oil has been investigated in
102 rainbow trout (Trullàs et al., 2015), growth performance has not yet been assessed. Fatty
103 acid digestibility coefficients of rainbow trout fed re-esterified oils from an unsaturated
104 vegetable source such as rapeseed did not present differences compared to those of fish
105 fed the native oil (Trullàs et al., 2015). Even so, from the economical point of view, acid
106 oils seem to be a more interesting alternative than re-esterified oils since the latter are
107 approximately 100 €/t more expensive due to the added cost of the chemical
108 esterification (Parini, personal communication). The economic viability of re-esterified
109 oils in relation to native oils is variable, since it depends on the price differential
110 between native and acid oils, which is in turn subjected to fluctuation.
111 While digestibility of acid and re-esterified oils is acceptable in rainbow trout (Ng et al.,
112 2010; Trullàs et al., 2015), growth performance and productive parameters have not
113 been investigated (Aliyu-Paiko and Hashim, 2012).
114 Thus, one of the objectives of the present study was to assess the growth performance
115 and the feed utilization of rainbow trout fed acid and re-esterified rapeseed oils in
116 comparison with those of fish fed the native oil. We also aimed at evaluating the partial
117 substitution of the native and the re-esterified oils by graded levels of the more
118 economical acid oil in order to optimize their use.
119 Because diet composition could induce changes in specific plasma haematological and
120 biochemical parameters (Peres et al., 1999), the evaluation of the plasma biochemical
121 parameters and also the morphology of liver and intestine could provide additional
122 information on the effects of the inclusion of these alternative oils.
123

124 **2. Materials and methods**

125 **2.1. Experimental diets**

126 Experimental diets (45% protein and 21% lipid) contained the same ingredient
127 composition except for the added lipid source (Table 1). Three different types of
128 rapeseed oil – native (RNO), re-esterified (REO) and acid (RAO) – were included in the
129 diets alone (single oil diets: RN, RE or RA) or blended in graded levels (diet RE/RA:
130 66% RE-33% RA; diet RA/RE: 66% RA-33% RE; diet RN/RA: 66% RN-33% RA and
131 diet RA/RN: 66% RA-33% RN) in a proportion of 15%. A 5% of commercial fish oil
132 (FO) was included in all experimental diets. A diet including only commercial fish oil
133 (20% of the diet) was used as a control (F). Experimental oils were provided by SILO
134 S.p.a. (Firenze, Italy) (RNO and REO) and Cargill (Schiphol, The Netherlands) (RA).
135 The re-esterified oil (REO) was produced by SILO S.p.a. as described in Trullàs et al.
136 (2015). Feeds were produced at the Skretting Feed Technology Plant (Aquaculture
137 Research Center; Stavanger, Norway) as extruded pellets. Yttrium oxide (Y_2O_3) was
138 added to the diets as an inert marker for the apparent digestibility of fatty acids
139 determination. Nutrient composition of experimental diets was determined by standard
140 procedures (AOAC, 2005): moisture (934.01), ash (942.05), crude protein (968.06) and
141 crude lipid (920.39). Unsaponifiable matter was also calculated following AOAC
142 (2005) (933.08) as a quality control. Gross energy of dried feed was determined using
143 an adiabatic bomb calorimeter (IKA-Kalorimeter system C4000, Jankel-Kunkel,
144 Staufen, Germany). Yttrium was analysed in accordance to Austreng et al. (2000). The
145 ingredient formulation and proximate composition of the diets are shown in Table 1.

146 **2.2. Fish husbandry and sampling**

147 All the procedures were conducted in accordance with the Animal Protocol Review
148 Committee of the Universitat Autònoma de Barcelona (UAB) and following the
149 European Union Guidelines for the ethical care and handling of animals under
150 experimental conditions (2010/63/EU). The trial was carried out at the Skretting Italia

SPA (Mozzecane, Italy) facilities. A total of 576 rainbow trout with a mean initial body weight of 101.7±8.80 g were randomly distributed into 24 cylindro-conical tanks of 600 l of capacity (24 fish per tank) in an open freshwater system with a continuous water flow of 24 l min^{-1}. Water temperature (14.3ºC) and dissolved oxygen levels (7.4±0.37 mg/l) were maintained constant throughout all the experimental period. Tanks were subjected to a 24h light photoperiod. Each diet was randomly assigned to three replicate tanks and was fed twice a day by automatic feeders, adjusted to provide the 2.5% of biomass daily. Uneaten feed was collected by filtering effluent water from each tank and collectors were emptied after each meal and feed intake was recorded daily. At day 60, all the fish from each tank were weighed and measured individually before being anaesthetized with clove oil (Phytosynthese, Za de Mozac-Volvic, France; 0.04 ml/l). Faecal samples were collected from the hindgut by manual stripping, after which fish were put into tanks supplied with freshwater to recover from anaesthesia. Samples were pooled by tank and stored at -20°C prior to analysis of yttrium oxide, total fat, fatty acid composition and gross energy. At day 72, five fish from each tank were anaesthetized with clove oil (Phytosynthese, Za de Mozac-Volvic, France), having been previously fasted for 48 hours. Blood samples were then taken from the caudal vein by puncture with a heparinized syringe and collected in 2 ml tubes with heparin (Hospira Inc., CA, U.S.) for further plasma biochemical analyses. Once the blood sampling was finished, five fish from each tank were euthanized in excess anaesthetic and weighed. Liver and viscera were taken and weighed for biometrical measurements. Samples of liver and intestine were also taken and fixed in 10% buffered formalin for histological examination under a light microscopy.

2.3. Total fat and fatty acid composition

Total fat of diets and faeces was determined by Nuclear Magnetic Ressonance (NMR). Fatty acid composition was determined by gas chromatography-flame ionization detector (GC-FID). Fatty acid methyl esters (FAME) were obtained by direct methylation, according to Meier et al. (2006) and analysed using an HP 5890A gas chromatograph. They were identified by comparison of their retention times with those of known standards, and quantified by internal normalization (FAME peak area/total FAME area, in %).

2.4. Lipid class composition

Lipid class composition (TAG, DAG, MAG and FFA) of FO, RNO, REO and RAO, as well as that of all experimental diets, were determined by size-exclusion chromatography on an Agilent 1100 series HPLC chromatograph equipped with a Refractive Index Detector (RID) set at 35Cº. Oils were melted at 55ºC prior to analysis, and a solution of approximately 10 mg of oil/ml of tetrahydrofurane was prepared. The solution was filtered through a Nylon filter (0.45 µm) and injected (20 µl loop) to the chromatograph equipped with two Styragel columns (StyragelHR 1 and Styragel HR 0.5) of 30 cm x 0.78 cm i.d., filled with a spherical styrenedivinylbenzene copolymer of 5µm particle size (Water Associates, Milford, MA, USA), connected in series and placed in an oven set at 35°C. The mobile phase consisted of tetrahydrofuran at 1 ml/min. For diets, fat was previously extracted with diethyl ether following the method 2003.05 from AOAC (2005). Data was expressed as peak area normalitzation (in %), considering the area of the peaks corresponding to TAG, DAG, MAG and FFA.

2.5. Calculations

Apparent digestibility coefficient (ADC) of fat, fatty acids and gross energy (GE) was calculated as: ADC (%) = 100 − [100 × (Y in feed/Y in faeces) · (F in faeces/F in feed)] (Maynard and Loosli, 1979), where F = fat ($mg \cdot kg^{-1}$), fatty acid ($mg \cdot kg^{-1}$) or gross

200 energy (kJ g^{-1}) and Y = yttrium ($mg \cdot kg^{-1}$). ADC of GE was used to calculate the
201 digestible energy (DE) of the diets.
202 Growth performance, feed utilization and biometrical parameters were calculated
203 according to standard formulae. Weight gain was calculated from WG (g) = final
204 weight-initial weight, feed intake was determined from [total dry matter intake /
205 (number of fish x number of days fed)], feed conversion ratio from FCR = (dry feed
206 fed) / (wet weight gain), specific growth rate (SGR) from [ln(final weight)-ln(initial
207 weight)] / (number of days) x 100 and average daily growth from AVG = (gain %) /
208 (number of days). Furthermore, condition factor (CF) = 100 x [final weight (g)] / [fork
209 length (cm)]^3, hepatosomatic index (HSI) = (weight of liver) / (total fish weight) x 100
210 and viscerosomatic index (VSI) = (weight of viscera) / (total fish weight) x 100 were
211 also calculated.

212 **2.6. Plasma analyses**

213 Plasma was obtained after immediate centrifugation at 11337 *g* for 2 minutes of the
214 blood samples, pooled per tank and stored at -20 ºC for further analyses. Glucose,
215 protein, triglycerides, cholesterol, free fatty acids, alanine aminotransferase (ALT),
216 aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) were
217 analysed using standard clinical methods with an Olympus AU400 – 3112676 chemistry
218 analyser, (Germany).

219 **2.7. Liver and intestine histology**

220 Samples of liver and intestine fixed in 10% buffered formalin were dehydrated in a
221 graded ethanol series and embedded in paraffin. Sections of 4 mm were stained with
222 haematoxylin and eosin (H&E) (Martoja and Martoja-Pierson, 1970) for morphological
223 observations using a Leica DM5000B microscope (Jenoptik, Germany). Images were
224 taken with ProgRes® CapturePro software (Jenoptik, Germany).

225 **2.8. Statistical analysis**

226 Data were subjected to a one-way analysis of variance (ANOVA) and the significance
227 of the differences between means was tested by Tukey's test. Digestibility values are
228 given as means±standard error of the mean of triplicate values, each being a pooled
229 sample from 24 fish. Values of growth performance, feed utilization and biometrical
230 parameters are given as means±standard error mean of triplicate values, each containing
231 information from 24 fish. Differences were considered significant when $P<0.05$. All
232 statistics were performed by means of the General Lineal Model (Proc GLM) of SAS®
233 software version 9.2 (SAS Institute Inc., Cary, NC, USA).

234

235 **3. Results**

236 *Characterization of experimental oils and diets*

237 Results of fatty acid composition and unsaponifiable matter of experimental diets are
238 shown in Table 2. Lipid class composition of experimental oils and diets are shown in
239 Table 3.

240 Although differences among rapeseed oils were minor with respect to their fatty acid
241 composition, as seen in diets, they were notable in terms of lipid class composition. FO
242 and RNO were constituted by TAG in more than a 90%, while the re-esterified oil
243 (REO) had a considerable amount of partial acylglycerols (35.4% MAG and 34% DAG)
244 and the acid oil (RAO) was rich in FFA (64.3%). No presence of TAG polymers was
245 observed.

246 As in the oils, minor differences in the fatty acid composition were found among diets.
247 Although their lipid class composition mirrored those of the oils in the case of the
248 natives (F and RN; TAG>90 %), differences were observed in RE and RA. Both in the

249 acid oil diet (RA) and in the re-esterified oil diet (RE) higher percentages of TAG but
250 lower of FFA and partial acylglycerols than in their corresponding oils were obtained.
251 In the blended oils diets, an increase in FFA was observed as more RAO was included.
252 Similarly, an increase of MAG and DAG was observed as a higher level of REO was
253 present.
254
255 *Apparent digestibility of fat and fatty acids of the diets*
256 The eight experimental diets were well accepted and total mortality was about 1%.
257 ADC of total fat and total fatty acids of the diets were all above 90% and 96%,
258 respectively (Table 4), being similar or higher than that of F. Minor but significant
259 differences ($P<0.05$) were found among rapeseed diets regarding total fat and total fatty
260 acids digestibility, the latter being slighter than those of total fat.
261 When single rapeseed oil diets were compared, the lowest total fatty acid ADC was that
262 obtained for RA (95.7±0.1), which was significantly lower ($P<0.05$) than that of RN
263 (96.7±0.1). In relation to the different categories of fatty acids, it is worth mentioning
264 that significantly higher ($P<0.05$) ADC values were obtained for SFA (especially
265 palmitic acid, C16:0, and stearic acid, C18:0) in RE.
266 Regarding the replacement of RAO by RNO, no differences due to the level of inclusion
267 of RA were obtained. ADC of total fatty acid of RN/RA and RA/RN resulted in values
268 between those of RA and RN, with no significant differences.
269 Similarly, no differences in total fatty acid ADC were observed as REO was replaced by
270 RAO (RE/RA, RA/RE).
271
272 *Growth performance, feed utilization and biometrical parameters*

Results obtained for the performance parameters (Table 5) followed the trend of those of total fatty acid digestibility. As observed, no significant differences ($P>0.05$) were obtained among the final weights of fish fed RN (393.7±6.1 g), RA (375.9±2.9 g) and RE (381.5±11.1 g). Those of RA and RE were, in turn, significantly lower ($P<0.05$) than that of F (411.1±3.3 g). Similar results were observed for WG and CF, while no statistical differences were obtained for the rest of the performance parameters studied. As obtained in total fatty acid ADC, final weights of fish fed with RN/RA (380.7±20.6 g) and RA/RN (381.2±4.8 g) were in between those of RN and RA. The numerically highest was that of fish fed RN, although this was not statistically higher ($P>0.05$) than those of animals fed RN/RA or RA/RN. RE/RA and RA/RE diets obtained higher final weights (392.8±4.4 g and 394.6±1.6 g, respectively) than RE and RA, although differences were not significant ($P>0.05$). Very similar results were observed in WG in all cases. It is noteworthy that, although final weights and WG of fish fed diets including RE/RA and RA/RE did not result statistically higher than those of animals fed diets RN/RA and RA/RN, they were numerically higher.

In spite of the differences in final weights observed among diets, these were not reflected in SGR or in FCR.

Plasma biochemical parameters

Values of the analysed plasma biochemical parameters of fish fed the experimental diets are shown in Table 6. Statistically significant differences in glucose, TAG, LDL-cholesterol, AST and ALT were found among the experimental rapeseed diets. Although fish fed diet RE/RA had significantly higher ($P<0.05$) level of TAG in plasma (565.03±39.52 mg dl^{-1}) than those fed F (384.17±8.09 mg dl^{-1}), RN (431.35±6.25 mg dl^{-1}) and RA (431.60±9.90 mg dl^{-1}), differences did not follow a

298 clear trend related to the type of oil or to their level of inclusion. Similarly, in
299 glucose, animals fed RA (67.27±2.36 mg dl^{-1}) and RA/RN (87.45±3.45 mg dl^{-1}) had
300 a significantly higher ($P<0.05$) glucose plasmatic level than those fed diet RA
301 (67.27±2.36 mg dl^{-1}).
302 Fish fed diets RN and RE had significantly lower ($P<0.05$) LDL-cholesterol levels
303 (114.67±4.30 mg dl^{-1} and 125.48±10.98 mg dl^{-1}, respectively) than those fed F
304 (201.67±16.36 mg dl^{-1}). No differences were found in blended oils diets when
305 compared among themselves or among their corresponding single oil diets. However,
306 RE/RA (125.48±10.98 mg dl^{-1}) and RA/RE (116.22±11.78 mg dl^{-1}) were
307 significantly lower ($P<0.05$) than F. In fact, all diets resulted numerically lower than
308 F.
309 For ALT and AST, animals fed RE (ALT: 3.67±1.20 IU l^{-1}; AST: 5.33±0.67 IU l^{-1})
310 showed significantly lower ($P<0.05$) values than those fed F (ALT:20.50±0.50 IU l^{-1};
311 AST: 20.50±2.50 IU l^{-1}) and RN (ALT: 13.50±2.50 IU l^{-1}; ALT: 11.67±2.40 IU l^{-1}).
312 In relation to RN/RA, RA/RN and their corresponding single oil diets (RN and RA),
313 no differences were observed. Even so, AST and ALT plasmatic levels of fish fed
314 RN/RA (ALT: 10.00±1.73 IU l^{-1}; AST: 9.33±1.45 IU l^{-1}) were significantly lower
315 ($P<0.05$) than those of fish fed F. For diets with blends of RE and RA, the only
316 significant difference ($P<0.05$) was found in ALT between RA (10.33±1.67 IU l^{-1})
317 and RE/RA (2.50±0.50 IU l^{-1}), being RA the highest. Indeed, RA obtained the
318 numerically highest values, although they were not statistically higher, in both
319 parameters. For plasmatic LDL-cholesterol, ALT, and AST, fish fed diet F had the
320 highest values when comparing all treatments.
321
322 *Histology of intestine and liver*

No differences were observed in the morphology of liver or intestine among fish fed the different experimental diets, including F. Normal histology patterns were observed under a light microscope, as presented in Fig 1.

4. Discussion

Minor differences in the fatty acid composition among rapeseed oils were observed. Similarly as it has been described in previous studies (Trullàs et al., 2015; Vilarrasa et al., 2014), the chemical esterification reaction did not have an effect on their fatty acid composition.

Regarding lipid classes, both native and acid oils showed the standard composition described for these types of oils. TAG was the predominant molecule (>95%) in RNO (Flickinger and Matsuo, 2003) and FFA represented a 64.3% in the acid oil (RAO) (Nuchi et al., 2009). On the other hand, a high content of partial acylglycerols (69.4%) was present in REO.

When the lipid class composition of diets was compared to that of their corresponding oils, differences were observed in diets RA and RE. These differences were mostly related to the 5% of FO added to all the experimental rapeseed diets, which was mainly composed of TAG.

Both total fat and total fatty acid ADC of the different experimental diets were high (90.5-96.7%), which is in accordance with authors reporting similar results with diets including rapeseed as a FO replacer in rainbow trout (Caballero et al., 2002; Martins et al., 2006; Turchini et al., 2013). This replacement could even increase lipid digestibility at low water temperatures in salmonid species (Caballero et al., 2002; Karalazos et al., 2007).

As found in a previous study in rainbow trout (Trullàs et al., 2015), a few differences in fatty acid ADC were obtained among rapeseed diets. For the single oil diets, the numerically lowest ADC of RA could be a consequence of its richness in FFA. As it is widely known in mammals, the main products of the hydrolysis by pancreatic lipase during lipid digestion are FFA and 2-MAG. Taking into account that a bile salt-dependent pancreatic lipase with sn-1,3-specific hydrolytic activity has been pointed out as the main lipolytic enzyme in rainbow trout (Bogevik et al., 2007; Gjellesvik et al., 1992; Tocher, 2003b), we would assume that a similar digestion process as in mammals would take place in this species. Then, the main hydrolytic products would be solubilized or emulsified in bile salt micelles, followed by diffusion to the intestinal mucosa (Tocher, 2003b). The large amount of FFA in RA could produce a "saturation effect" at the time of their incorporation into the mixed micelles during digestion, since the amount of FFA would greatly exceed that of MAG and DAG, responsible of expanding the micelle in order to allow the solubilization of other products. However, to our best knowledge, there is a paucity of information regarding this phenomenon in fish. On the other hand, if present, this effect would possibly be more noticeable if the amount of FFA was mainly constituted of SFA, since high levels of SFA have been reported to negatively affect the formation of micelles in the intestinal lumen of Atlantic salmon (Menoyo et al., 2003).

While free MUFA and PUFA are easily absorbed, free long-chain SFA have a poorer absorption as a consequence of their hydrophobicity and high melting points. In native VO, SFA are mainly found in the external positions of TAG (Grundy and Denke, 1990; Karupaiah and Sundram, 2007) and thus are easily converted to FFA during digestion, part of which will form insoluble soaps in the gut to end up excreted in faeces. The fact

that the reduction of ADC in RA was slight could be related to the low amount of SFA present in rapeseed.

Compared to RN and RA, RE had the significantly highest ADC of SFA, which could be a consequence of the emulsifying effect that the partial acylglycerols exert during the digestion process. As amphiphilic intermediate products of TAG digestion, DAG and especially MAG would facilitate the incorporation of hydrophobic FFA in the core of micelles during fat digestion, as described in humans, mammals and poultry (Da Costa, 2003; Krogdahl, 1985; Mattson et al., 1979).

Another factor that could have a beneficial effect on the ADC of SFA is that the chemical esterification reaction increases the amount of SFA located at the sn-2 position of acyglycerols, which would imply SFA being directly absorbed as 2-MAG, improving fatty acid digestibility of VO. This rise was of up to 10 points (as % on the total SFA content) in the rapeseed re-esterified oil in comparison to its corresponding native oil in the study by Trullàs et al. (2015). It is possible, though, that the low content of SFA in rapeseed did not exert a clear effect on the total fatty acid ADC. Related to this, Trullàs et al. (2015) concluded that the lipid class composition of the oil seemed to be of less importance as an influential factor on fatty acid digestibility than its degree of saturation. Certainly, the importance of the degree of saturation and the chain length on digestibility as the major factors affecting fatty acid digestibility in fish had been previously pointed out (Francis et al., 2007; Hua and Bureau, 2009). Thus, the slight differences observed in the present work could be due to the predominance of the degree of unsaturation of rapeseed over other factors.

When RNO or REO were substituted by graded levels of RAO (RN - RN/RA - RA/RN - RA or RE - RE/RA - RA/RE - RA), no significant effect of the level of inclusion of RAO (100%, 33% or 66%) in diets on total fatty acid ADC was observed. However, in

diets with substitution of RN by RAO, there was a slight but progressive decrease of ADC as more RAO was present, which suggests that the ADC of a diet could be in direct relation to the richness of FFA of RA. The detrimental effect on digestibility appears when the level of FFA in diets is of around 30%, regardless of the rest of the lipid classes.

In diets with substitution of REO by RAO, total fatty acid ADC of RE/RA and RA/RE were higher than that of RA, indicating a possible effect of the partial acylgycerols and the higher amount of SFA at sn-2 than the rest of diets.

Final weights of fish fed the experimental single rapeseed oil diets were all high, which is in agreement with many studies in salmonids (Bell et al., 2003; Huang et al., 2008; Pettersson et al., 2009; Turchini et al., 2013). Also, values of FCR and SGR were similar to those obtained in studies including different levels of rapeseed oil in salmonid diets (Caballero et al., 2002; Turchini et al., 2013). As reported for Atlantic salmon, rapeseed oil is an effective substitute of FO in terms of growth rates and feed efficiency, since it provides sufficient energy in the form of monoenoic fatty acids to maintain high growth rates Bell et al. (2001).

As has long been reported, rainbow trout require solely linolenic (C18:3n-3) acid as essential fatty acid (Castell et al., 1972) for maximal growth (Watanabe, 1982). Regarding this, it is important to highlight that all our rapeseed diets included 5% of FO in order to ensure a minimum dietary content of n-3 long-chain PUFA. However, fish fed rapeseed diets had lower final weights than those fed diet F, although these differences were significantly lower only in fish fed RA and RE.

Final weights of fish fed RN/RA and RA/RN were not different from each other but numerically lower than those of fish fed RN. As observed, detrimental effects in growth appeared in diet RA, but not when RAO was blended with RNO.

It is important to remind that the experimental period lasted 72 days, and so a longer period of time could have shown noticeable differences among diets regarding final weight and especially SGR and FCR.

The higher final weights obtained in fish fed RE/RA and RA/RE compared to those fed RE and RA seemed to be caused by a synergism between REO and RAO, the causes for this being probably those previously described for digestibility.

Haematological and biochemical parameters reflect the physiological processes undertaken in an animal and give information about its physiological status (Peres et al., 2012). Moreover, fatty acid structure and also its position on the glycerol backbone have an influence on plasma lipids in both humans (Dubois et al., 2007) and fish (Denstadli et al., 2011).

In the present study, no clear relation was obtained between glucose and TAG among diets regarding the type of oil (native, re-esterified and acid) or their level of inclusion (100%, 66% or 33%). Therefore, the dietary fatty acid composition could have had a greater effect on plasma parameters than these two factors, because oleic acid, the main fatty acid in rapeseed, had been shown to be neutral with regard to plasma lipids in studies in humans (Clarke et al., 1997; Grundy, 1986).

For the lipoproteins cholesterol, LDL-cholesterol was the only one to show significant differences among diets, although they were not clearly related to the different types of oils. This is in accordance with studies reporting a decrease in plasma and LDL-cholesterol in salmonid species fed VO-based diets when compared to fish fed F (Jordal

et al. 2007; Richard et al. 2006). In a study with rainbow trout fed a diet with a high proportion of RO, Richard et al. (2006) suggested the high levels of oleic and linoleic acids in the diet, as well as the presence of phytosterols, as possible causes for the decreased plasma total cholesterol and LDL-cholesterol. Similarly, oleic acid and PUFA had been found to reduce levels of plasma LDL-cholesterol in mammals (Fernandez and West, 2005; Grundy and Denke, 1990).

A similar tendency was observed in total cholesterol, for which the lack of significant differences among diets seemed to be a consequence of the high variability of the data. In fact, Kim et al. (2012) reported that a decrease in the total cholesterol of fish fed a diet containing VO has not been well established.

Values of the two hepatic transaminases (ALT and AST) presented similarities. It is difficult to classify values of hepatic enzymes as normal or pathological, since they vary largely among studies and species. Also, reference values for clinical-normal and non-stressed animals are lacking for most fish species (Peres et al., 2012).

Nevertheless, an increase in the levels of plasma and serum transaminases has been associated with liver damage in marine (Lemaire et al., 1991) and freshwater species (Babalola et al., 2009), which was directly related to histopathological findings. In the present study, the normal morphology of livers of fish fed the different diets might indicate that differences in ALT and AST found among diets were possibly not relevant. Díaz-López et al. (2009) observed a significant decrease in several hepatic enzymes in sea bream after 4 months of feeding with rapeseed diets in relation to fish fed a control diet FO as the main fat source. Then, the higher values obtained for F in comparison with the rest of diets would be in accordance with results found in the aforementioned study, although our trial had half the duration of the trial performed by Díaz-López et al. (2009).

In contrast to the results obtained in the present study, lipid vacuoles accumulation in the intestine and/or in the liver have been reported when VO are the main fat source in fish diets (Caballero et al., 2002, 2004; Lie and Lambertsen, 1987; Olsen et al., 1999, 2000, Ruyter et al., 2006).

In the intestine, the enterocytic supranuclear lipid droplet accumulation observed in fish fed VO (Olsen et al., 1999, 2000) has been considered a temporary physiological state, due to the presence of a high amount of PUFA and an insufficient lipoprotein synthesis. Certain SFA (mainly C16:0) are required to maintain the cellular synthesis of phosphatidylcholine, necessary for the lipoprotein synthesis. Then, diets containing VO poor in SFA and rich in 18:2n-6 and 18:3n-3, would promote the accumulation of lipid droplets due to the insufficient formation of phospholipids and subsequently of lipoproteins. Nonetheless, in sea bream fed a rapeseed oil diet, poor in SFA and rich in MUFA (mainly C18:1n-9), accumulation of lipid droplets in enterocytes was suggested to be caused by the lower enterocytic reacylation of the oleic acid observed in the polar lipid fraction in comparison with other fatty acids. This fact would be reducing lipoprotein synthesis rates (Caballero et al., 2003). In the present study, no lipid droplet accumulation was observed, but it has to be considered that a different microscopy technique than in Caballero et al. (2003) was used. In addition, the different times of sampling (i.e. 4 h after feeding in Caballero et al., (2003) and after 48 h of fasting in our case) should also be taken into account.

For liver, several studies in gilthead sea bream found a low or non-existent percentage of lipid vacuoles in fish fed rapeseed oil diets compared to those fed diets with only FO (Caballero et al., 2004; Fountoulaki et al., 2009). These studies suggested this low degree of vacuolation could be due to the reduced activity of the fatty acid synthase

enzyme found in these livers in comparison of those of fish fed the F diet, which was consequence of the high 18:1n-9 content in this diet. In relation to this, Caballero et al. (2004) established an order among the characteristic fatty acids in VO and its relationship with the appearance of steatosis in the liver: linoleic acid>linolenic acid>oleic acid.

Considering that lipid vacuolation has been related to the nutritional imbalance due to the high content of n-6 fatty acids present in many VO (Montero and Izquierdo, 2011; Tacon, 1996), the fact that rapeseed contains limited n-6 PUFA could also be a possible explanation for our results.

In conclusion, results from the present study indicate that rainbow trout fed diets including RAO and REO showed acceptable fat and fatty acid digestibility, with no relevant changes in plasma parameters or in the morphology of liver and intestine. However, growth of fish fed these two diets did not reach that obtained in fish fed F, while growth of fish fed diets including a blend of RAO and REO improved when combined with REO at both 33% and 66% levels of inclusion. Therefore, the rapeseed acid oil, which is the most economically advantageous, yields better growth results when blended with the re-esterified oil. It has to be taken into account that the inclusion of these oils should be done with a minimum proportion of 5% of FO in diets. However, before recommending their use, further studies regarding the inclusion of these oils in aqua feeds should be carried out in order to study their effect on the fat content and the fatty acid composition of tissues, as well as on the final product quality parameters in rainbow trout.

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

	Diets[a]							
	FO	RN	REH	RA	REH/RA	RA/REH	RN/RA	RA/RN
Ingredient composition (g kg^{-1})								
Wheat[b]	60	60	60	60	60	60	60	60
Wheat gluten[c]	232.8	232.8	232.8	232.8	232.8	232.8	232.8	232.8
Hi Pro Soya[d]	80	80	80	80	80.0	80.0	80.0	80.0
Soya Protein Concentrate[e]	150	150	150	150	150	150	150	150
Faba beans whole[f]	100	100	100	100	100	100	100	100
Fish Meal North Atlantic[g]	150	150	150	150	150	150	150	150
Fish oil South America[h]	201.3	52	52	52	52	52	52	52
Experimental oils[i]	0	150	150	150	150	150	150	150
Yttrium premix[j]	1	1	1	1	1	1	1	1
Mineral and vitamin premix[j]	24.9	24.9	24.9	24.9	24.9	24.9	24.9	24.9
Proximate composition (g kg^{-1})								
Dry matter	925.7	925.9	929.9	927.9	926.8	927.3	931	928.9
Crude protein	472.2	466.1	468.2	485.1	471.7	474.3	468	466.2
Crude fat	204.1	215.7	210.4	187.7	191.9	201.4	219.5	214.3
Ash	64.2	63.3	70.6	65	65.2	68.1	67.6	65.6
Gross energy (kJ g^{-1})	22.8	22.5	22.4	22.8	22.4	22.4	22.3	22.7
Digestible energy (kJ g^{-1})	20.0	19.5	20.2	19.1	19.8	19.3	18.0	20.2

[a]Experimental diets nomenclature: FO: fish oil (control diet); RN: rapeseed native oil; REH: rapeseed re-esterified oil high in MAG; RA: rapeseed acid oil; REH/RA: 66% rapeseed re-esterified oil high in MAG - 33% rapeseed acid oil; RA/REH: 66% rapeseed acid oil - 33% rapeseed re-esterified oil high in MAG; RN/RA: 66% rapeseed native oil - 33% rapeseed acid oil and RA/RN: 66% rapeseed acid oil - 33% rapeseed native oil.
[b]Statkorn, Norway.
[c]Cerestar Scandinavia AS, Denmark.
[d]IMCOPA, Brasil.
[e]Denofa, Norway.
[f]Ceremis, France.
[g]Welcon AS, Norway.
[h]Holtermann ANS, Norway.
[i]Experimental oils.
[j]Vitamin and mineral premix, according to requirement data from NRC (2011). Trow Nutrition, The Netherlands.

Table 2. Fatty acid composition of the experimental diets.

	Diets							
	FO	RN	REH	RA	REH/RA	RA/REH	RN/RA	RA/RN
Fatty acid (%)								
C14:0	7.5	3	2.4	2.5	2.4	2.5	2.3	2.3
C16:0	17.3	10.3	11	10	10.5	10.3	9	9.3
C16:1n-7	8.1	3.3	2.7	2.8	2.7	2.7	2.6	2.7
C18:0	3	2.3	3.8	2.5	3.4	3.4	2.2	2.3
C18:1n-9	10.1	35.7	26	32.3	28.9	28.9	31	31.8
C18:1n-7	3	3.5	3.3	4	3.4	3.4	3	3.4
C18:2n-6	5.5	16.7	13.8	16.9	14.8	14.8	15.1	15.9
C18:3n-3	1.1	6.2	3.1	4.6	3.7	3.7	5.1	4.9
C18:4n-3	2.2	0.9	0.7	0.7	0.7	0.7	0.7	0.7
C20:1[a]	2.3	2.5	1.8	2	1.8	1.8	2.1	2
C20:4n-6	0.9	0.4	0.3	0.3	0.3	0.3	0.3	0.3
C20:5n-3 (EPA)	13.7	5.2	4.1	4.3	4.1	4.1	4.2	4.1
C22:1[a]	1.9	1.6	1.1	1.3	1.1	1.1	1.4	1.3
C22:5n-3	1.6	0.6	0.5	0.5	0.5	0.5	0.5	0.5
C22:6n-3 (DHA)	10	4.2	3.3	3.5	3.3	3.3	3.5	3.4
C24:1n-9	0.7	0.4	0.5	0.5	0.5	0.5	0.5	0.4
ΣSFA[b]	28.7	16.8	15.9	18.4	17.4	16.9	14.4	14.9
ΣUFA[c]	64.4	83.1	75.1	62.4	67.1	72.3	71.3	72.7
ΣMUFA[d]	26.5	47.7	43.5	35.9	38.9	41.9	41	42
ΣPUFA[e]	37.8	35.3	31.6	26.5	28.2	30.4	30.3	30.7
Σn-6 PUFA[e]	8.2	17.8	17.5	14.6	15.6	16.9	16	16.7
Σn-3 PUFA[e]	29.6	17.5	14.1	12	12.6	13.6	14.3	14
SFA:UFA	0.4	0.2	0.2	0.3	0.3	0.2	0.2	0.2

Experimental oils and diets nomenclature as in experimental diets (Table 1).
[a]Sum of isomers.
[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							
	FO	RN	REH	RA	REH/RA	RA/REH	RN/RA	RA/RN
Lipid classes (%)								
ΣTAG[a]	93.8	95.6	26.6	20.5	7.2[b]	13.8[b]	69.9[b]	45.1[c]
ΣDAG[a]	2.9	2.5	34.0	12.5	10.1[b]	11.2[b]	5.8[b]	9.1[c]
ΣMAG[a]	0.7	0.2	35.4	2.7	60.4[b]	31.5[b]	1.0[b]	1.8[c]
ΣFFA[a]	2.6	1.7	2.0	64.3	21.2[b]	42.4[b]	22.3[b]	49.8[c]
Diets								
Lipid classes (%)								
ΣTAG[a]	92.9	93.4	54	46	49	46.6	56.1	62.2
ΣDAG[a]	3.2	3.1	21.9	9.4	19	14.8	12.4	6.9
ΣMAG[a]	0.8	0.7	22.3	2.1	14.8	7.9	7.1	1.6
ΣFFA[a]	3.1	2.8	1.8	42.4	17.2	30.7	24.4	29.2

Experimental oils and diets nomenclature as in Table 1.

[a]TAG (triacylglycerols), DAG (diacylglycerols), MAG (monoacylglycerols) and FFA (free fatty acids).

[b]Values calculated as the sum of the corresponding lipid class proportions in each of the two constituent oils.

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

	Oils							
	FO	RN	REH	RA	REH/RA[a]	RA/REH[a]	RN/RA[a]	RA/RN[a]
sn-2 (%)								
C14:0	10.1	26.0		78.3			43.0	52.0
C16:0	13.9	23.4		12.1			19.4	8.3
C16:1n-7	13.4	19.4		0			12.8	0.3
C18:0	8.5	29.3		11.3			23.1	7.8
C18:1n-9	11.7	28.8		7.8			21.6	5.5
C18:2n-6	58.1	51.5		10.5			37.5	7.3
C18:3n-3	22.3	45.2		9.3			32.9	6.5
ΣSFA	12.3	21.5		19.9			20.7	13.4
ΣMUFA	12.4	24.5		10.1			19.5	7.0
ΣPUFA	22.2	39.4		13.4			30.4	9.1

Experimental oils nomenclature as for diets in Table 1.
Values are given as the % of each fatty acid at the sn-2 relative to its content in the oil.
[a]Values calculated as the sum of the corresponding proportions of the % of each fatty acid at the sn-2 relative to its content in the two constituent oils.

Table 5. Apparent digestibility coefficient (ADC %) of selected fatty acids in rainbow trout fed the experimental diets.

	Diets							
	FO	RN	RA	REH	REH/RA	RA/REH	RN/RA	RA/RN
Fatty acid								
C14:0	95.5±0.2^{c}	97.7±0.1^{a}	96.6±0.2^{b}	97.1±0.2^{ab}	97.6±0.2^{a}	97.1±0.1^{ab}	96.5±0.2^{b}	97.1±0.2^{ab}
C16:0	91.8±0.3^{d}	95.4±0.1^{ab}	93.8±0.3^{c}	95.7±0.2^{a}	96±0.4^{a}	95.6±0.1^{ab}	94.2±0.5^{bc}	94.8±0.4^{abc}
C16:1n-7	98.8±0.2	99±0.2	98.1±0.5	98.8±0.2	98.9±0.2	98.8±0.1	98.8±0.1	99±0.0
C18:0	86.9±0.3^{bc}	83.7±0.2^{d}	88.9±0.3^{b}	93.5±0.4^{a}	94.6±0.2^{a}	93.3±0.2^{a}	84.1±1.0^{cd}	88.1±1.0^{b}
C18:1n-9	97.4±0.1^{b}	98.9±0.2^{a}	98.1±0.3^{ab}	98.3±0.1$^{b\ a}$	98.5±0.2^{a}	98.6±0.1^{a}	98.8±0.1^{a}	98.9±0.1^{a}
C18:1n-7	97.7±0.2^{bc}	98.6±0.1^{a}	96.9±0.2^{c}	97.4±0.1^{bc}	97.4±0.2^{bc}	97.4±0.1^{bc}	97.8±0.2^{ab}	97.6±0.2^{bc}
C18:2n-6	95.4±0.1^{b}	98.1±0.1^{a}	97.4±0.2^{a}	97.6±0.1^{a}	97.6±0.2^{a}	97.7±0.1^{a}	98±0.1^{a}	98±0.2^{a}
C18:3n-3	96.9±0.2^{b}	99.3±0.1^{a}	98.6±0.2^{a}	98.5±0.1^{a}	98.8±0.2^{a}	99±0.1^{a}	99.2±0.1^{a}	99.1±0.1^{a}
C18:4n-3	99.4±0.1^{a}	92.8±3.1^{ab}	97.3±0.0^{ab}	77.9±3.7^{cd}	86.1±0.7^{bc}	73.1±0.5^{d}	86.7±0.2^{abc}	87.6±0.4^{abc}
C20:1	96.4±0.2^{b}	97.5±0.2^{a}	97.2±0.3^{ab}	97.6±0.3^{a}	97.6±0.2^{a}	97.8±0.2^{a}	97.6±0.1^{a}	97.8±0.2^{a}
C20:4n-6	80.6±0.3^{a}	67.2±0.6^{ab}	35.6±0.8^{c}	38.1±3.0^{c}	65.7±1.3^{b}	67.3±0.6^{ab}	67.9±0.4^{ab}	67.9±0.8^{ab}
C20:5n-3 (EPA)	99.6±0.1	99.5±0.1	99.1±0.1	99.4±0.0	99.4±0.1	99.5±0.1	99.5±0.1	99.5±0.1
C22:1	95.1±0.3^{b}	96.1±0.1^{ab}	96.5±0.2^{a}	97.1±0.1^{a}	96.9±0.2^{a}	97±0.2^{a}	96.2±0.2^{ab}	96.9±0.3^{a}
C22:5n-3	98.8±0.3^{a}	84.1±5.1^{ab}	84.3±0.7^{ab}	60.6±1.9^{bc}	60±1.5^{c}	61.9±0.6^{bc}	82.3±0.2^{abc}	62.5±1.0^{bc}
C22:6n-3 (DHA)	98.8±0.2^{a}	98±0.3^{ab}	96.6±0.0^{c}	96.9±0.1^{bc}	96.9±0.1^{bc}	97±0.1^{bc}	97.2±0.5^{bc}	97.3±0.4^{bc}
ΣSFA	92.1±0.3^{b}	92±0.1^{b}	92.8±0.3^{b}	95±0.2^{a}	95.5±0.4^{a}	94.8±0.1^{a}	91.2±0.6^{b}	92.9±0.5^{b}
ΣMUFA	96.9±0.2^{c}	98.3±0.2^{a}	97.4±0.2^{bc}	97.4±0.1^{bc}	97.6±0.2^{abc}	97.8±0.1^{ab}	98±0.2^{ab}	98±0.2^{ab}
ΣPUFA	97.2±0.2	97±0.2	96±0.3	95.8±0.7	96.1±0.2	95.9±0.5	96.7±0.5	96.5±0.0
Σn-6 PUFA	91.9±1.2^{b}	96.8±0.3^{a}	95.1±0.4^{a}	95±0.2^{a}	95.9±0.2^{a}	96.3±0.3^{a}	96.3±0.1^{a}	96.4±0.2^{a}
Σn-3 PUFA	98.6±0.0^{a}	96.9±0.1^{b}	95.7±0.6^{bcd}	94.2±0.1^{d}	94.5±0.5^{cd}	94.4±0.1^{d}	96.1±0.4^{bc}	95.4±0.3^{bcd}
Total FA[a]	95.4±0.1^{b}	96.7±0.1^{a}	95.7±0.1^{b}	95.8±0.2^{ab}	96.3±0.3^{ab}	96.3±0.1^{ab}	96.2±0.3^{ab}	96.4±0.3^{ab}
Total fat	93±0.4^{ab}	93.9±0.4^{a}	90.5±0.3^{b}	92.5±1.0^{ab}	91.7±0.6^{ab}	90.8±0.2^{b}	94.2±0.6^{a}	93.7±0.2^{a}

Experimental diets nomenclature as in Table 1.

Values represent mean±SEM of triplicate pooled samples from 24 fish. Values in the same row with different letters are significantly different (P<0.05).

[a]Total FA: total fatty acids.

Table 6. Growth performance, feed utilization and biometrical parameters of rainbow trout fed the different experimental diets.

	Diets							
	FO	RN	RA	REH	REH/RA	RA/REH	RN/RA	RA/RN
Initial weight (g)	101.6±0.2	101.5±0.2	101.6±0.0	101.7±0.1	101.5±0.1	101.8±0.2	101.8±0.5	101.8±0.2
Final weight (g)	411.1±3.3a	393.7±6.1ab	375.9±2.9b	381.5±11.1b	392.8±4.4ab	394.6±1.6ab	380.7±20.6b	381.2±4.8b
Weight gain (g)[a]	309.4±3.1a	292.1±5.9ab	274.3±2.8b	279.8±11.2ab	291.3±4.5ab	292.8±1.5ab	278.8±21.1ab	279.4±4.9ab
Weight gain (%)[b]	304.5±2.6	287.6±5.3	269.9±2.6	275.3±11.3	287.1±4.5	287.6±1.0	274.0±22.2	274.4±5.1
Feed intake (%)[c]	42.0±2.5	40.4±0.1	42.0±1.9	47.0±1.4	45.6±2.7	42.6±1.7	43.2±1.5	41.6±1.1
FCR[d]	0.87±0.0	0.86±0.0	0.93±0.0	1.08±0.0	0.97±0.1	0.94±0.1	0.94±0.1	0.90±0.0
SGR (%/day)[e]	2.36±0.0	2.29±0.0	2.21±0.0	2.22±0.1	2.28±0.0	2.29±0.0	2.21±0.1	2.23±0.0
ADG (%/day)[f]	5.07±0.0	4.79±0.1	4.50±0.0	4.59±0.2	4.78±0.1	4.79±0.0	4.57±0.4	4.57±0.1
CF[g]	1.83±0.0a	1.79±0.0ab	1.75±0.0b	1.78±0.1ab	1.78±0.0ab	1.77±0.1ab	1.79±0.0ab	1.75±0.0b
HSI (%)[h]	1.09±0.0	1.0±0.0	1.07±0.0	1.08±0.0	1.11±0.0	1.07±0.0	1.10±0.0	1.06±0.0
VSI (%)[i]	12.3±0.6	11.0±0.5	11.7±1.1	12.3±0.4	12.2±0.7	10.9±0.4	12.1±0.9	11.1±0.7

Values represent mean±SEM (n = 3; N = 24). Values in the same row with different letters are significantly different (P<0.05).

[a]Weight gain: (final weight-initial weight).

[b]Weight gain: (final weight-initial weight) / (initial weight) x 100.

[c]Feed intake: [total dry matter intake / (initial weight+final weight)$^{0.5}$ / number of days fed] x 100.

[d]Feed conversion ratio: (dry feed fed) / (wet weight gain).

[e]Specific growth rate: [Ln(final weight)-Ln(initial weight)] / (number of days) x 100.

[f]Average daily growth: (gain %) / (number of days).

[g]Condition factor (K): 100 x [final weight (g)] / [fork length (cm)]^3.

[h]Hepatosomatic index: (weight of liver) / (total fish weight) x 100.

[i]Viscerosomatic index: (weight of viscera) / (total fish weight) x 100.