Quality characteristics of fillets of rainbow trout fed acid or re-esterified rapeseed oils as dietary fat sources

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Abstract

Alternatives to the use of native vegetable oils (VO) as fish oil (FO) replacers in aqua feeds were evaluated. Acid oils are a free fatty acid (FFA)-rich by-product mainly from the refining of VO. Re-esterified oils are the final product of a chemical esterification reaction between acid oils and glycerol, and have less FFA and more mono- and diacylglycerols (MAG and DAG), known for being good emulsifiers, than crude VO. Therefore, they could have a higher nutritive value than

that of the native and acid oils. In two earlier studies in rainbow trout (Trullàs et al., 2015; 2016), diets including acid and/or re-esterified VO resulted in total fatty acid apparent digestibility coefficients above 95%. Moreover, no negative effects on growth, plasma biochemical parameters and morphology of tissues were observed when compared to the native oil diet. For all these reasons, the present study aimed at assessing their effects on the final quality of fillets of rainbow trout. Triplicate groups of rainbow trout were fed eight experimental diets containing 15% of different types of experimental rapeseed oils in addition to 5% of FO during 72 days. The experimental rapeseed oils were native (RNO), acid (RAO), re-esterified (REO), or blends (66% RN-33% RAO / 33% RN-66% RAO or 66% REO-33% RAO / 33% REO-66% RAO). Commercial FO was used for the control diet (F). The colorimetric analysis resulted in significant differences only in *b** and *C** in both fresh and thawed fillets, as well as in significant correlations between the colorimetric parameters among diets. For the total fat content, fillets of fish fed the control diet obtained the highest values, which resulted higher than those of fish fed diets containing RNO and the blend 66% REO-33% RAO. No differences in texture, liquid holding capacity and TBARS were found among fillets of fish fed the different diets. Regarding tocopherol concentrations in fillets, fish fed F had a significantly lower concentration of β+γ-tocopherol than the rest, while the concentration of α-tocopherol was significantly higher ($P<0.05$) in fillets of fish fed the control diet than in RA/RE. Even though the aforementioned differences were found, they did not seem to be relevant concerning the final quality of fillet.

Keywords: rainbow trout, rapeseed, acid oil, re-esterification, fillets, quality.

Abbreviations

DAG: Diacylglycerol(s)

FFA: Free fatty acid(s)

FO: Fish oil

HUFA: Highly unsaturated fatty acid(s)

LHC: Liquid holding capacity

MAG: Monoacylglycerol(s)

MDA: Malondialdehyde

MUFA: Monounsaturated fatty acid(s)

PUFA: Polyunsaturated fatty acid(s)

SFA: Saturated fatty acid(s)

TAG: Triacylglycerol(s)

TBARS: Thiobarbituric acid reactive substances

TPA: Texture profile analysis

UFA: Unsaturated fatty acid(s)

VO: Vegetable oil(s)

1. Introduction

The rise in the use of vegetable oils (VO) as a feedstock in the biofuel industry, which started in the early 2000s, generated a subsequent increment in their prices that peaked in 2008 (Gunstone, 2011). This shift in the use of VO towards non-food uses created competition between the feed and the biofuel industries, since both oilseeds and feed grains used as ingredients in diets suffered the increase in their prices, placing the animal feed industry in a difficult situation. This also had a remarkable impact on the aquaculture industry, which had already made a great effort in research on the use of VO to replace fish oil (FO) from fish diets. Consequently, studies focused on finding alternatives to the use of native VO as FO replacers in aqua feeds have been carried out and, among them, those using by- and co-products generated during the crude VO processing are of particular interest (Ng et al., 2006; Bahurmiz et al., 2007; Ng et al., 2010;

2010; Aliyu-Paiko and Hashim, 2012). Most of VO need to be refined to be edible (Vaisali et al., 2015) so an important amount of by-products with low commercial value are generated, being cheaper than their original sources. Vegetable acid oils, a free fatty acid (FFA)-rich by-product from the refining of VO, were pointed out as a promising fat source for feeding uses (Nuchi et al., 2009). Acid oils can be subjected to a chemical esterification process with glycerol to generate the so-called re-esterified vegetable oils, which have fewer FFA and also more mono- and diacylglycerols (MAG and DAG) than the former (Vilarrasa et al., 2014; Trullàs et al., 2015). Partial acyglycerols (MAG and DAG) have emulsifying properties (Redgrave et al., 1988) and so their beneficial effects on digestibility and feed utilization in humans and monogastric animals have been described (Cruz-Hernandez et al., 2012; Garrett and Young, 1975; Martin et al., 2014). Hence, the development of new technical fats obtained from the re-esterification of acid oils with glycerol has been hypothesized as a strategy to valorise these by-products (Trullàs et al., 2015; Vilarrasa et al., 2014, 2015).

It is important to mention that the dietary inclusion of re-esterified oils implies the previous cost of the esterification process and, therefore, acid oils seem to be more interesting from the economic point of view. In fact, the economic viability of re-esterified oils in relation to native oils also depends on the price differential between native and acid oils, which is in turn subjected to fluctuation. Thus, blends of the acid oil with both the native or the re-esterified oils could provide nutritionally interesting fish fillets for human consumption at the maximum feed efficiency and lower cost. Aiming at this, we designed a study (Trullàs et al., 2016) to evaluate the use of rapeseed acid and re-esterified oils (as a single fat source or blended) as the main dietary fat source. High total fatty acid apparent digestibility coefficients, above 95%, were obtained, and no negative effects on growth and health status indicators such as plasma biochemical parameters and morphology of tissues were observed when compared to the native

100 oil diet. However, it is necessary to evaluate whether the inclusion of these two types of oil could
101 have a repercussion on the final product quality.
102 Dietary high percentages of native VO could also affect the sensorial and physico-chemical
103 properties of fish fillets and many studies focused on these aspects have been therefore carried
104 out (Rosenlund et al., 2001; Rosenlund et al., 2011; Izquierdo et al., 2003; Regost et al., 2003;
105 Mørkøre et al., 2007). Even though results of flesh quality parameters when fish are fed VO diets
106 are somewhat contradictory (Rørå et al., 2005; Ng and Bahurmiz, 2009), changes in physico-
107 chemical parameters such as texture, colour, liquid holding capacity (LHC) and lipid
108 peroxidation have been reported in salmonid species (Bjerken et al., 1997; Ng and Bahurmiz,
109 2009; Regost et al., 2004).
110 Given the fact that rapeseed acid and re-esterified oils are by-products with a potential interest as
111 fat sources in aqua feeds (Trullàs et al, 2015), and that satisfactory results were obtained for
112 digestibility and growth (Trullàs et al., 2016), the present work was aimed to assess their effects
113 on the final quality of fillets of rainbow trout.
114

115 **2. Materials and methods**

116 **2.1. Experimental oils and diets**

117 Experimental oils consisted of three different types of rapeseed oil – native (RNO), acid (RAO)
118 and re-esterified (REO). The RNO was provided by SILO S.p.a. (Firenze, Italy) and the RAO
119 was provided by Cargill (Schiphol, The Netherlands). The REO was produced by SILO S.p.a by
120 chemical esterification of RAO with glycerol as described and discussed in Trullàs et al. (2015)
121 and it was characterized by a lower FFA content and higher MAG and DAG content than RAO
122 (Table 1), with minor differences in the fatty acid (FA) composition (Trullàs et al , 2016).
123 Experimental diets (45% protein and 21% lipid) had the same ingredient composition except for
124 the added lipid source (Table 2). The three experimental oils RNO, RAO and REO were

included in the diets alone (diets RN, RA or RE) or in blends (diet RN/RA: 66% RNO-33% RAO; diet RA/RN: 66% RAO-33% RNO; diet RE/RA: 66% REO-33% RAO and diet RA/RE: 66% RAO-33% REO) in a proportion of 15%. In all experimental diets 5% of commercial FO was included. A diet including only commercial FO (20%) was used for the control diet (F). Feeds were produced at the Skretting Feed Technology Plant (Aquaculture Research Center; Stavanger, Norway) as extruded pellets. 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) (Table 2). Gross energy of dried feed was determined using an adiabatic bomb calorimeter (IKA-Kalorimeter system C4000, Jankel-Kunkel, Staufen, Germany).

The lipid class composition (TAG, DAG, MAG and FFA) of FO, RNO, RAO and REO oils (Table 1), as well as of experimental diets (Table 2), were determined by size-exclusion chromatography on an Agilent 1100 series HPLC chromatograph equipped with a Refractive Index Detector (RID) set at 35 ºC, as described in Trullàs et al. (2015). Each oil and diet was analysed in triplicate.

2.2. Fish husbandry and sampling

All the procedures were conducted in accordance with the Animal Protocol Review Committee of the Universitat Autònoma de Barcelona (UAB) and following the European Union Guidelines for the ethical care and handling of animals under experimental conditions (2010/63/EU). The trial was carried out at the Skretting Italia Aquaculture Research Centre in Mozzecane, Italy. A total of 576 rainbow trout with a mean initial body weight of 101.7 g ± 8.8 g were randomly distributed into 24 cylinder-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 mg l^{-1} ± 0.37 mg l^{-1}) were maintained constant throughout all the experimental period. Tanks were subjected to a 24 h light photoperiod. Fish were fed the

experimental diets for 72 days. Each diet was randomly assigned to three replicate tanks (8 diets, in triplicate, n=24) and was fed twice a day by automatic feeders, adjusted to provide 2.5% of the biomass daily. Uneaten feed was collected by filtering effluent water from each tank. Collectors were emptied after each meal and feed intake was recorded daily. At day 72 five fish from each tank were sacrificed by an over-dose of anaesthetic and individually gutted and filleted. A colorimetric determination was immediately performed on left fillets (Figure 1). Left fillets were then skinned, bagged and frozen at -20ºC until the corresponding quality analyses were carried out (colour, liquid holding capacity -LHC- and texture). Right fillets were cut in two different specific portions, frontal and central (Figure 1), and also bagged and frozen at -20ºC for carrying out the rest of the analyses (fatty acid composition, pH, total fat, moisture, thiobarbituric acid-reactive substance -TBARS- and tocopherol content).

2.3. Colour evaluation of fillets

Colorimetric determinations were made on fresh fillet (immediately after filleting) and on thawed fillet (after three months of storage at -20ºC) on the Norwegian Quality Cut (NQC) (Figure 1) section (NS9401, 1994). Defrosting of fillets was carried out by letting them thaw overnight in the refrigerator (5ºC). Measurements were performed in the colorimetric space L^*, a^*, b^* (CIE, 1976) using a Minolta Chroma meter (Model CR-410, Minolta Co., Ltd, Osaka, Japan); L^* represents the colour lightness that goes from 0 (black) to 100 (diffuse white), a^* is the position between red and magenta and green and b^* is the position between yellow and blue. Later, values obtained were transformed in the colour appearance parameters L^*, C^*, $H(°)_{ab}$ (Wyszecki and Stiles, 1967); C^* (chroma) expresses the colour intensity and $H(°)_{ab}$ (hue) is the attribute of a visual sensation according to which an area appears to be similar to one of the perceived colours, red, yellow, green and blue, or a combination of two of them (Fairchild, 2005). Three measurements were performed on each of the five fillets per tank, and the mean value of each tank (n = 24) was used for the statistical analysis of the data.

175 **2.4. Texture evaluation of fillets**

176 The texture of the fillet was measured using a TA-TX2 Texture Analyzer (Stable Micro Systems,
177 Surrey, England) texturometer equipped with a 5 kg load cell and the texture data analysis
178 software Exponent 6.1.5.0 (Stable Micro Systems, Surrey, England). Frozen fillet portions were
179 thawed overnight in the refrigerator (5ºC) and were then cut in two standardised pieces (2x2 cm
180 length x width) about 1.5 cm above the lateral line (Figure 1). Each sample was subjected to a
181 texture profile analyses (TPA) followed by a uniaxial compression test. The TPA test was
182 performed using a 100 mm compression plate (type P/100) and the testing conditions were two
183 consecutive cycles at 25% compression (10 mm depth), cross-head movement at a pre-test
184 constant speed of 5 mm/s and a test and post-test constant speed of 1 mm/s. The rest period
185 between cycles was of 15 seconds and the probe always returned to its initial position after the
186 second cycle. Texture variables (hardness, adhesiveness, springiness, cohesiveness and
187 chewiness) were calculated as described by Bourne (1978). The compression test was performed
188 using the same probe and the same pre-test, test and post-test speeds as for the TPA analysis. The
189 work required for the compression of the thickness of the fillet to 90% (5 mm depth) and the
190 force needed to reach the breaking point were measured. Two measurements were performed on
191 each of the five fillets per tank, and the mean value of each tank (n = 24) was used for statistical
192 analyses of the data.

193 **2.5. Determination of total fat, protein, moisture, pH and liquid holding capacity of fillets**

194 Total fat was extracted from fillets (Figure 1) and determined gravimetrically by homogenising
195 them in chloroform/methanol (2:1, v/v) according to the method of Folch et al. (1957). Crude
196 protein from fillets was determined by standard procedure (Method 968.06) and water was
197 extracted by standard procedure for moisture (934.01) (AOAC, 2005). These three
198 determinations were performed on three fillets per tank, and the mean value of each tank (n = 24)
199 was used for statistical analyses of the data.

A pH meter (micropH 2001, Crison, Spain) was used to measure the pH after pooling a portion of the fillets (Figure 1) of five fish per tank and homogenising them in distilled water (ratio 1:10, v/v) (n = 24).

For the LHC evaluation, triplicate muscle samples (Figure 1), were weighed (S) and placed in a tube with a weighted filter paper (Filter-Lab Filtros Anoia, Spain) (V1). The tubes were placed in a centrifuge (Sigma 4K15, Sigma, Germany) at 500 *g* for 10 min at 10 ºC. The wet weight was calculated as $100\% \cdot (V1-V2) \cdot S^{-1}$, water loss as $100\% \cdot (V2-V3) \cdot S^{-1}$ and fat loss as $100\% \cdot (V3-V1) \cdot S^{-1}$, in which V2 corresponds to the weight of the filter paper after centrifugation and V3 to the weight of the filter paper after being dried at 50ºC to constant weight. The LHC was expressed as percentage of water and fat retained, calculated as (% total moisture - % water loss) x % total moisture^{-1} and (% total fat - % fat loss) x % total fat^{-1}, respectively.

2.6. Determination of TBARS and tocopherol concentrations

Fillet TBARS were analysed (Figure 1) as a measure of lipid oxidation by determining equivalents of malondialdehyde (MDA), a secondary product in the oxidation of polyunsaturated fatty acids (PUFA), by spectrophotometry following an adaptation of Sørensen and Jørgensen (1996).

Upon arrival at laboratory, the portions for tocopherol analysis (Figure 1) of the 5 fillets from each experimental group were ground with a knife mill (Grindomix, Restch GmbH, Haan, Germany) at 6000 rpm for 30 s. Then, aliquots of 20g were vacuum packed in high-barrier multilayer bags (Cryovac BB325; permeability to O2, 25 cm3/m2 per day per bar at 23ºC and 0% relative humidity, ASTMD-3985; Cryovac Europe, Sealed Air S. L., Sant Boi de Llobregat, Spain; 20 g meat/bag) and kept at -25ºC until analysis. Alpha-tocopherol of diets and fillets was determined by high-performance liquid chromatography (HPLC) according to Bou et al. (2004).

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 mean±standard error of the mean (SEM) of triplicate groups of five fish fillets in the case of texture and colour, of triplicate groups of three fish fillets in total fat, LHC and tocopherol and of triplicate groups of pooled fillet samples from five fish in pH and TBARS. 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). Data were also subjected to a correlation analysis (Pearson's correlation coefficient) in order to study the relationship between the different parameters. The significance level was also set at 5% ($P<0.05$) (SAS® software version 9.2; SAS Institute Inc., Cary, NC, USA).

3. Results

Colour

Colorimetric values of fresh and thawed fillets from fish fed the experimental diets are shown in Table 3. In respect of the colorimetric space *L**, *a**, *b**, differences were present only in *b** in both fresh and thawed fillets. In fresh fillets, those of fish fed the control diet (F) showed the significantly lowest *b** value along with the two diets with the highest contents of REO (RE and RE/RA). On the other hand, diets with presence of RAO, especially as a single source or when combined with RN, had the highest *b** values. In thawed fillets, the parameters followed a similar trend as in fresh fillets. According to the colorimetric space *L**, *C**, $H(°)_{ab}$, significant differences were only observed in *C** values in fresh and in thawed fillets, that followed the trend observed for *b**. In general, parameters increased from fresh to thawed,

Significant correlations were observed between the different parameters (Table 4). All the parameters in fresh fillets were correlated in a positive way with their corresponding value in thawed fillets. In fresh fillets, *L**, *C** and $H(°)_{ab}$ were positively correlated with *b**. *C** also

displayed a positive correlation $H(°)_{ab}$. In thawed fillets, only with C^* and $H(°)_{ab}$ had a positive correlation with b^*. $L^*$was positively correlated with $H(°)_{ab.}$

At the same time L^*, C^* and $H(°)_{ab}$ from fresh fillets were negatively correlated in a significant way with a^*. In turn, a^* showed a significant negative correlation with b^*. In thawed fillets, L^* and $H(°)_{ab}$ showed a significant negative correlation with a^*.

Texture profile analysis and compression test

No significant differences in any instrumental texture parameter of thawed fillet from rainbow trout fed experimental diets were obtained among diets (Table 5). Only the relationship between springiness and cohesiveness resulted significantly correlated in a positive way ($r = 0.66$, $P<0.05$).

Total fat, protein, moisture, pH and liquid holding capacity of fillets of thawed fillets

Total fat, protein, moisture, pH and LHC values of thawed fillets from rainbow trout fed the experimental diets are shown in Table 5. Only differences in the total fat content of fillets were found, for which fish fed diet F had significantly higher values than those fed diets with presence of RNO (RN, RN/RA and RA/RN), together with those fed diet RE/RA.

The percentage of fat retained of fillets of fish fed the control diet (F) was also the highest, although no statistically significant. Correlations among parameters showed that the percentage of total fat in fillets was positively correlated with the percentage of fat retained ($r = 0.52$, $P<0.05$). In turn, the percentage of fat retained resulted in a significant negative correlation with hardness ($r = -0.43$, $P<0.05$) and compression of fillets ($r = -0.54$, $P<0.05$). Moisture and protein were also significant and positively correlated ($r = 0.52$, $P<0.05$).

Tocopherol and TBARS concentrations of thawed fillets

Concentrations of tocopherol in feeds and fillets (expressed as µg tocopherol/g fillet) are shown in Figure 2. In feeds, the concentration of total tocopherol (α+β+γ) was higher in rapeseed diets than in the control diet (F). oncentrations of α-tocopherol were always higher than those of β+γ-tocopherol and differences in their concentrations were observed among diets (Figure 2.A). RE diet obtained the highest value (143.38±3.84 µg α-tocopherol/g feed), which was significantly higher ($P<0.05$) than that of diet F (100.91±12.63 µg α-tocopherol/g feed) and RN/RA (98.13±2.31 µg α-tocopherol/g feed). Diet F had, in turn, lower ($P<0.05$) concentration of β+γ-tocopherol (29.87±2.23 µg β+γ-tocopherol/g feed) than the rest.

In fillets, fish fed F obtained a significantly higher ($P<0.05$) concentration of α-tocopherol (7.51±0.80 µg α-tocopherol/g fillet) than those fed RA/RE (4.78±0.35 µg α-tocopherol/g fillet) (Figure 2.B). As in feeds, fish fed F had a lower ($P<0.05$) concentration of β+γ-tocopherol in fillets (0.73±0.23 µg β+γ-tocopherol/g fillet) than the rest.

Lipid oxidation measured as thiobarbituric acid reactive substances (TBARS) concentration (expressed as µg MDA/g fillet) of rainbow trout fed the experimental diets is shown in Figure 3. No significant differences in TBARS values of fish fillets were observed among diets. Fillets from animals fed F tended to have the numerically highest TBARS values, as well as concentration of α-tocopherol.

4. Discussion

Colour

Colour is one of the most important attributes in the perception of flesh quality in salmonids (Bell et al., 1998; Torrissen et al., 2001), being in direct association with the product acceptance or rejection by the consumer (Izquierdo et al., 2005). In rainbow trout, the typical red to pink muscle colour of salmonids is due to astaxanthin, the natural pigment for salmonids flesh and the most efficient carotenoid used in aqua feeds to obtain fillet pigmentation (Torrissen et al. 1989;

299 Storebakken and No, 1992). However, preferences in meat colour vary globally. In Europe and
300 other parts of the world pink meat is preferred, even though white meat is preferred in the USA
301 (FAO, 2005).
302 In the present study, in which no pigment was added in the feeds, no differences in the color of
303 fillets among diets were visible to the naked eye. In spite of this, significant differences in *b**
304 (position between yellow and blue) and *C** (saturation) of fillets were obtained among the
305 different diets, the latter being mainly due to variations in *b**, as it is one of the parameters
306 present in the formula to obtain *C**. As observed, the more RAO was present in diets, the higher
307 *b** tended to be. Indeed, *b** was lower in fillets of fish fed F and RE than in others. These
308 differences could be due to variable proportions of unsaponifiable matter in the experimental
309 oils, which consists of different compounds such as phospholipids, tocopherols, sterols, resins,
310 and pigments, among others (O'Brien, 2008). In fact, acid oils concentrate different compounds
311 from the chemical refining such as FFA, acylglycerols, pigments, and other lipophilic materials
312 (Haas et al., 2003).Therefore, possible different concentrations of pigments among the different
313 rapeseed oils in the experimental diets could explain differences in colour. The fact that fillets of
314 fish fed F had the lowest *b** and *C** values is in accordance with the study by Regost et al.
315 (2004), that reported a decrease in *b** in fillets of salmon fed a fish oil diet than in those fed a
316 rapeseed oil diet.
317 The rise in the values of all parameters in thawed fillets when compared to fresh fillets is in
318 agreement with many authors reporting an evident influence of freezing and thawing processes
319 on the flesh colour (Alizadeh, 2012; Bjerken and Johnsen, 1995; Jensen et al., 1998; No and
320 Storebakken, 1991; Ozbay et al., 2006; Regost et al., 2004). However, results regarding changes
321 in colour in thawed fillets vary greatly among studies. Several factors such as a modification of
322 proteins and the temperature, dynamics, and type of the thawing process (Alizadeh, 2012; Ozbay
323 et al., 2006) have been suggested to affect the colour of fish fillets. Regarding the lightness,

Cristopher et al. (1992) hypothesized that the increase in this parameter in thawed salmonid fish fillets was a result of the dehydration of the fillet surface and of changes in the reflectance properties of ice crystals.

Texture profile analysis and compression test

Texture is an important attribute regarding flesh quality in fish (Ayala et al., 2010) and one of the criteria involved in estimating freshness. Fish fillet texture can be directly affected by diet, although it has been reported that it can be influenced by many other factors: external (feeding regimes, slaughtering procedures, storage conditions, freezing, thawing) and internal (fat and water content, lipid oxidation, pH) (Andersen et al., 1997; Carbonell et al., 2003; Mørkøre et al., 2002). In the present study, the different types of dietary experimental oils did not seem to exert an effect on the texture of thawed fillets, as no significant differences in the TPA or in the compression test were obtained. Indeed, Rosenlund et al. (2011) suggested that the effect of dietary oils on raw fillet texture seems to be very limited, regardless of the species studied. Accordingly, many studies have reported a lack of effects of the partial or total inclusion of different VO on fillet texture in various fish species (Bell et al., 2004; Castro et al., 2015; Morkore et al., 2007; Ng and Bahurmiz, 2009; Richard et al., 2006; Regost et al., 2004; Rørå, 2003, 2005; Torstensen et al., 2004).

Even though a significant correlation between springiness and cohesiveness was obtained in the present study, no information on a positive correlation between these two parameters when determined instrumentally has been found in the literature. The negative correlations obtained between the fat retained in fillets and hardness and compression are in agreement with what other studies reported for different species (Andersen et al., 1997; Ginés et al., 2004; Mørkøre et

al., 2002), all of them suggesting that increasing the fillet fat content leads to a softening of the flesh.

Total fat, protein, moisture, pH and liquid holding capacity

Total fat, protein, moisture, pH and LHC are important quality attributes of salmonid fillets (Hernández et al., 2009; Mørkøre et al., 2002, Rosenlund et al., 2011). The effects of the presence of VO in diets on fillets total fat are not clear. Several authors reported no changes when fish were fed either native VO or FO diets (Bell et al., 2003; Nanton et al., 2007; Ng et al., 2004; Pettersson et al., 2009; Richard et al., 2006; Torstensen et al., 2004, 2005) while others obtained lower values of total fat in fish fed FO than in those fed VO (Turchini et al., 2003). In our study, the higher values obtained in fish fed F compared to those fed diets including RNO was the most remarkable fact. These results coincide with those reported by Yildiz et al. (2015), obtained under very similar experimental conditions to those of the present work. The differences observed among fillets of animals fed the experimental rapeseed diets did not seem to be related to the diets, as their lipid contents did not follow the same trend of variation, and neither to the growth performance of the animals (Trullàs et al., 2016).

Fillet drip formation losses, which include total liquid, water or fat, could result in a drier and tougher cooked product with a decreased nutritive value, bearing the consequence this would have on the processing industry, on the consumer acceptance and on the economy (Elvevoll et al., 1996; Oyelese et al., 2007; Rørå et al., 2003). Losses vary with factors such as size of fish, muscle pH and the amount of fat and handling conditions among others (Johnssen, 2011; Oyelese et al., 2007) and have also been reported to be a direct consequence of frozen storage and thawing due to cell damage and denaturation of proteins (Alizadeh, 2012; Mørkøre et al., 2002). These two factors would in turn result in an increasing fillet hardness (Ng and Bahurmiz, 2009), as observed in our study. As reported, dietary oils can also have an effect on fillets LHC

372 (Regost et al., 2004), although but there are many studies in which no differences in LHC in
373 fillets of fish fed different dietary VO compared to fish fed a FO diet were found (Bell et al.,
374 2004; Ng and Bahurmiz, 2009; Richard et al., 2006; Rørå et al., 2003). According to Rosenlund
375 et al. (2011) data on the effects of dietary oil on the liquid holding capacity of fish fillets are too
376 limited and often contradictory to be able to speculate on how dietary oils affect it. With regards
377 to the fat retained, some authors observed a higher retention in fish fed VO than in those fed FO
378 (Regost et al., 2004; Rørå et al., 2005; Torstensen et al., 2004). In the present study, the retention
379 of fat was higher in fillets of fish fed diet F than in those fed the experimental diets, which could
380 probably be related to the higher total fat content in fillets of fish fed F. Indeed, a significantly
381 positive correlation was obtained between fat retained and total fat ($r = 0.52$, $P<0.05$).
382
383 *Tocopherol and TBARS concentrations of thawed fillets*
384 As widely reported, α-, ß-, γ- and δ-tocopherols together with tocotrienols are fat-soluble vitamin
385 E isomers and the major antioxidants naturally present in VO (Brannan and Erickson, 1996;
386 Kalyana et al., 2003). Vitamin E inhibits lipid peroxidation in biomembranes, lipoproteins and
387 body lipids (Turchini et al., 2009) and this would translate to an extension of the shelf-life of
388 seafood products (Ng et al., 2004).
389 In the present study, the higher concentration of α-tocopherol in the experimental diets in
390 comparison to that of β+γ-tocopherol was in accordance with values reported by Gunstone
391 (1994) and Pettersson et al. (2009) for crude rapeseed oil. The lower β+γ-tocopherol levels of
392 diet F and consequently of fillets of fish fed this diet were due to the high concentration of this
393 isomer in VO (Chu and Kung, 1998), especially in rapeseed oil (Kamal-Eldin, 2005).
394 Differently, although diet F presents one of the lowest concentration of α-tocopherol, its
395 concentration increased in the corresponding fillets, resulting higher than in fillets of fish fed
396 rapeseed diets. As extensively reviewed by Hamre et al. (2011), the metabolism of tocopherols in

fish seems to be similar to that in mammals. The main transport route of tocopherols away from the intestine appears to go through incorporation into chylomicrons. Chylomicrons are transported mainly to the liver, although some transfer of tocopherol to peripheral tissues takes place (Traber et al. 1985; Rigotti 2007). Tocopherols taken up by the liver can be excreted in the bile or returned to the circulation possibly incorporated in very low-density lipoproteins (VLDL). Approximately half of the VLDL is delipidated in the circulation and returned to the liver, while the other half is converted to low-density lipoprotein (LDL). Peripheral tissues can acquire tocopherols from LDL (Cohn et al. 1992). In the liver, there is presence of a hepatic tocopherol transfer (TTP) protein that binds α-tocopherol with a higher affinity than other vitamin E homologues and stereoisomers (Kayden & Traber 1993). As a consequence, α-tocopherol is preferentially secreted from the liver into the blood. Taking this into account, in the present study, given that F has a low content of β+γ-tocopherol, chylomicrons of fish fed this diet probably transferred a higher concentration of α-tocopherol to peripheral tissues than in rapeseed diets. Moreover, given the high affinity of TTP for α-tocopherol, fillets could acquire it through LDL in higher concentrations than others, and so fillets of fish fed F deposited an overall higher concentration of α-tocopherol than those of fish fed rapeseed diets.

However, the total concentration of tocopherols of F treatments was lower than the rest, both for diets and fillets. This, together with the higher presence of long-chain PUFA in F, could be responsible for the slightly higher TBARS value obtained in fillets of fish fed this diet.

Lipid oxidation is a major concern during processing and storage of fish products because it contributes to their quality deterioration (Kamireddy et al. 2011). Lipid oxidation is a balance between substrates (mainly PUFA), antioxidants (such as Vitamin E) and prooxidants (such as mineral elements). Here, we also need to take into account that the TBARS determination measures MDA but also other compounds from fish tissues that might contribute to its value (Botsoglou et al., 1994). In any case, no significant differences in the TBARS value of fillets

between treatments were observed. In this respect, it is important to note that all diets contained a 5% FO along with the experimental oils, and that fillets were stored frozen. Only a tendency of higher TBARS values in fillets of fish fed diet F was observed, which agreed with the higher long-chain PUFA contents in F diets (40.6%) than in rapeseed diets (32.8-35.3%) (Trullàs et al., 2016). Also, although it has been reported that under certain conditions α-tocopherol has a more efficient antioxidant activity than β+γ-tocopherol (Burton and Traber, 1990), F diets and fillets showed lower total tocopherol contents than rapeseed diets. Similar results have indeed been obtained in various studies carried out in different species fed either FO or VO (Menoyo et al., 2004; Regost et al., 2004; Røra et al., 2005; Ng and Bahurmiz, 2009; Qingyuan et al., 2014; Yildiz et al., 2016). Moreover, according to this result, Baron et al., (2013) reported that the use of a rapeseed oil in diets for rainbow trout resulted in a more oxidative stable product after storage on ice when compared to other VO, given that oleic acid is known to be resistant to oxidative rancidity (Satue et al., 1995).

In conclusion, results obtained in the present study indicate that differences in some of the final physico-chemical quality parameters in rainbow trout fed the different experimental diets were found. However, diets including 15% of rapeseed acid or re-esterified oils as a single or blended fat source do not seem to produce relevant changes in the flesh quality of fish. Further studies regarding their effects on the composition of other tissues would be of interest in order to have more information on the metabolism of acid and re-esterified oils in fish.

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Table 1. Lipid class composition of the experimental oils.

	Oils			
	FO	RNO	RAO	REO
Lipid classes (%)				
ΣTAG	93.8	95.6	20.5	26.6
ΣDAG	2.9	2.5	12.5	34.0
ΣMAG	0.7	0.2	2.7	35.4
ΣFFA	2.6	1.7	64.3	2.0

FO: fish oil, RNO: rapeseed native oil, RAO: rapeseed acid oil, and REO: rapeseed re-esterified oil.
TAG: triacylglycerols, DAG: diacylglycerols, MAG: monoacylglycerols, and FFA: free fatty acids.

Table 2. Ingredient formulation, proximate composition and lipid class composition of the experimental diets.

	Diets[a]							
	F	RN	RA	RE	RN/RA	RA/RN	RE/RA	RA/RE
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
Soya bean meal[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[g]	150	150	150	150	150	150	150	150
Fish oil[h]	201.3	52	52	52	52	52	52	52
Experimental oils[i]	0	150	150	150	150	150	150	150
Yttrium premix[i]	1	1	1	1	1	1	1	1
Mineral and vitamin premix[i]	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	927.9	929.9	931	928.9	926.8	927.3
Crude protein	472.2	466.1	485.1	468.2	468	466.2	471.7	474.3
Crude fat	204.1	215.7	187.7	210.4	219.5	214.3	191.9	201.4
Ash	64.2	63.3	65	70.6	67.6	65.6	65.2	68.1
Gross energy (kJ g^{-1})	22.8	22.5	22.8	22.4	22.3	22.7	22.4	22.4
Lipid classes (%)								
ΣTAG	92.9	93.4	46	54	77.2	62.2	49	46.6
ΣDAG	3.2	3.1	9.4	21.9	5.3	6.9	19	14.8
ΣMAG	0.8	0.7	2.1	22.3	1.0	1.7	14.8	7.9
ΣFFA	3.1	2.8	42.4	1.8	16.5	29.2	17.2	30.7

[a]Experimental diets nomenclature: F: fish oil (control diet), RN: rapeseed native oil, RA: rapeseed acid oil, RE: rapeseed re-esterified oil, RN/RA: 66% rapeseed native oil - 33% rapeseed acid oil, RA/RN: 66% rapeseed acid oil - 33% rapeseed native oil, RE/RA: 66% rapeseed re-esterified oil - 33% rapeseed acid oil, and RA/RE: 66% rapeseed acid oil - 33% rapeseed re-esterified oil.
TAG: triacylglycerols, DAG: diacylglycerols, MAG: monoacylglycerols, and FFA: free fatty acids.
[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]Vitamin and mineral premix, according to requirement data from NRC (2011). Trow Nutrition, The Netherlands.

Table 3. Colour measurements of fresh and thawed fillets of rainbow trout fed the experimental diets.

	Diets							
Colorimetric values	F	RN	RA	RE	RN/RA	RA/RN	RE/RA	RA/RE
Fresh fillets[a]								
*L**	44.07±0.83	43.67±0.23	44.10±0.21	42.17±0.72	43.25±0.67	43.54±0.36	42.50±0.27	42.92±1.02
*C**	5.97±0.27e	7.75±0.34bcd	8.91±0.18abc	5.90±0.17e	8.21±0.12abc	9.20±0.54a	6.34±0.18de	7.70±0.28cd
$H(^\circ)_{ab}$	61.92±5.29	76.45±1.38	76.63±4.08	58.81±5.42	74.05±2.41	76.76±4.58	65.12±3.20	70.72±1.39
*a**	2.72±0.37	1.77±0.14	2.03±0.64	3.08±0.34	2.20±0.42	2.01±0.72	2.62±0.32	2.46±0.16
*b**	5.21±0.50c	7.40±0.49ab	8.45±0.05a	5.17±0.51c	7.60±0.34a	8.78±0.34a	5.65±0.20bc	7.04±0.16ab
Thawed fillets[a]								
*L**	63.40±1.24	63.58±1.47	62.50±1.13	62.04±0.54	62.13±1.18	62.74±0.52	61.23±0.43	60.25±0.87
*C**	16.24±0.35ab	17.37±0.19ab	17.18±0.31ab	15.56±0.37b	17.72±0.58ab	18.01±0.64a	16.32±0.45ab	17.16±0.09ab
$H(^\circ)_{ab}$	65.79±0.10	71.55±1.48	71.83±2.26	63.98±1.90	68.84±3.33	71.83±1.10	67.90±0.34	64.20±1.91
*a**	6.59±0.20	5.48±0.36	5.33±0.62	6.83±0.63	7.09±0.60	6.44±0.07	7.09±0.60	6.44±0.07
*b**	14.82±0.31bcd	16.45±0.33ab	16.27±0.42abc	13.94±0.11d	16.42±0.21ab	17.07±0.55a	14.64±0.37cd	15.86±0.14abc

Experimental diets nomenclature as in Table 2.

Values are mean±SEM of triplicate groups of five fish fillets. Values within the same row with different letters (a, b, c, d, e) are significantly ($P<0.05$) different, according to ANOVA and Tukey's post-hoc test.

[a]*L**, luminosity; *C** (chroma), saturation = $(a^{*2} + b^{*2})^{1/2}$ (Wyszecki and Stiles, 1967); $H(^\circ)_{ab}$, hue value = arctan *b**/*a** (Wyszecki and Stiles, 1967); *a** = position between red/magenta and green and *b** = position between yellow and blue.

Table 4. Correlations (r) among the different colour parameters on fresh and thawed fillets of rainbow trout fed the experimental diets.

	Colorimetric values									
	Fresh fillets[a]					*Thawed fillets* [a]				
Colorimetric values	*L**	*C**	$H(°)_{ab}$	*a**	*b**	*L**	*C**	$H(°)_{ab}$	*a**	*b**
Fresh fillets										
*L**	-									
*C**	0.33	-								
$H(°)_{ab}$	0.40	0.734*	-							
*a**	-0.26	-0.45*	-0.91*	-						
*b**	0.42*	0.96*	0.84*	-0.58*	-					
Thawed fillets										
*L**	0.46*	0.04	0.26	-0.37	0.14	-				
*C**	0.18	0.72*	0.57*	-0.38	0.70*	-0.09	-			
$H(°)_{ab}$	0.36	0.64*	0.82*	-0.79	0.69*	0.54*	0.33	-		
*a**	-0.31	-0.42*	-0.67*	0.71*	-0.48*	-0.61*	0.00	-0.94*	-	
*b**	0.29	0.82*	0.78*	-0.62*	0.83*	0.13	0.93*	0.66 *	-0.37	-

Colour parameters nomenclature as in Table 3.
Correlated values are means±SEM of triplicate groups of five fish fillets.
[a] In fresh and thawed fillets, n=24, each sample corresponding to a pool of five fish fillets.
* Indicates a significant correlation ($p < 0.05$) (Pearson's correlation coefficient).

Table 5. Instrumental texture, total fat, protein, moisture, pH and liquid holding capacity measurements of thawed fillets of rainbow trout fed the experimental diets.

	Diets							
Texture profile analysis	F	RN	RA	RE	RN/RA	RA/RN	RE/RA	RA/RE
Hardness (N)	4.04±0.46	3.81±0.51	4.69±0.34	4.24±0.19	4.45±0.21	3.95±0.28	4.75±0.44	4.64±0.37
Gumminess (N)	2.60±0.23	2.62±0.34	3.06±0.26	2.74±0.14	3.04±0.08	2.63±0.17	3.04±0.34	3.03±0.13
Adhesiveness (N s)	-0.14±0.01	-0.14±0.04	-0.16±0.02	-0.15±0.02	-0.15±0.02	-0.14±0.03	-0.16±0.01	-0.15±0.03
Cohesiveness	0.77±0.01	0.79±0.01	0.77±0.00	0.77±0.01	0.77±0.02	0.79±0.02	0.76±0.01	0.77±0.02
Springiness	0.83±0.00	0.87±0.01	0.86±0.01	0.84±0.01	0.87±0.02	0.86±0.02	0.82±0.02	0.85±0.02
Compression test								
Force (N)	31.51±1.86	35.43±1.37	36.58±1.07	36.74±7.00	34.75±1.64	34.61±1.78	39.82±2.86	41.75±4.73
Total fat (% wet weight)[a]	7.53±0.11^{a}	5.36±0.34^{bcd}	6.02±0.31^{abc}	6.03±0.34^{abc}	4.41±0.28^{d}	5.64±0.25^{bcd}	4.72±0.07^{cd}	6.31±0.38^{ab}
Protein (% wet weight)[a]	20.77±1.96	21.11±0.70	20.74±0.91	21.19±1.58	19.97±0.57	20.92±0.22	21.07±0.61	21.11±0.49
Moisture (% wet weight)[a]	65.93±2.56	66.88±1.22	67.50±1.30	66.57±2.19	69.86±1.52	68.09±0.32	67.30±0.83	67.79±0.57
pH[b]	6.28±0.01	6.22±0.03	6.30±0.04	6.28±0.04	6.33±0.03	6.26±0.01	6.26±0.03	6.32±0.04
Liquid holding capacity (LHC, as % retained)[a]								
Water retained	78.02±0.02	79.24±0.01	76.59±0.00	75.64±0.01	76.13±0.02	77.41±0.01	78.17±0.01	78.00±0.01
Fat retained	38.99±0.09	23.02±0.00	26.28±0.02	30.99±0.08	12.47±0.06	36.51±0.03	19.90±0.08	32.93±0.04

Experimental diets nomenclature as in Table 2.

Values are mean±SEM of triplicate groups of five fish fillets. Lack of letters means no statistical significance obtained ($P<0.05$).

[a]Values are mean±SEM of triplicate groups of three fish fillets. Values within the same row with different letters (a, b, c, d, e) are significantly ($P<0.05$) different, according to ANOVA and Tukey's post-hoc test.

[b]Values are mean±SEM of triplicate pooled fillet samples from five fish. Lack of letters means no statistical significance obtained ($P>0.05$), according to ANOVA and Tukey's post-hoc test.

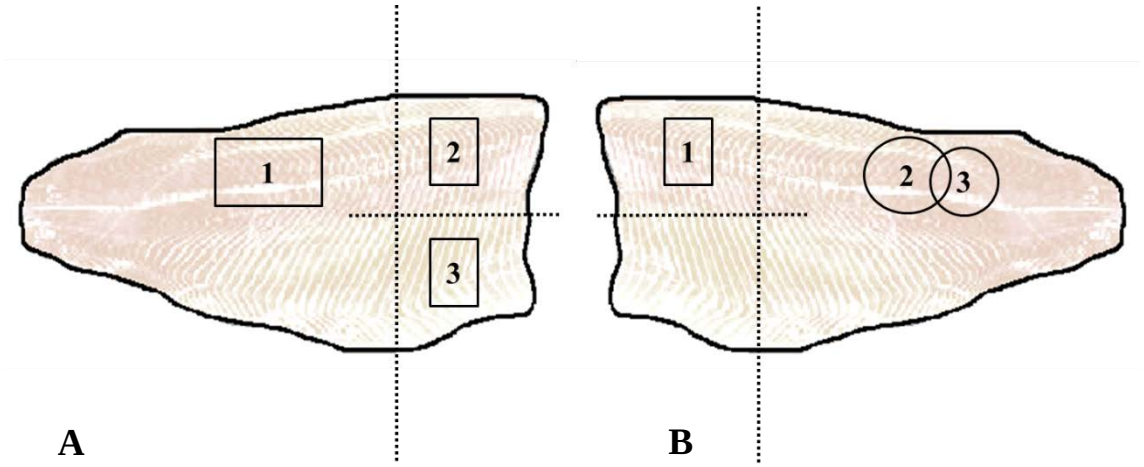

Figure 1. Diagram of the distribution of fillets of rainbow trout regarding the physicochemical analyses performed. Numbers indicate the part of the fillet used for the different determinations: A) Right fillet: A.1. tocopherol and pH; A.2. total fat, protein and moisture; A.3. thiobarbituric acid reactive substances (TBARS); B) Left fillet: B.1. liquid holding capacity (LHC); B.2. colour and B.3. texture.

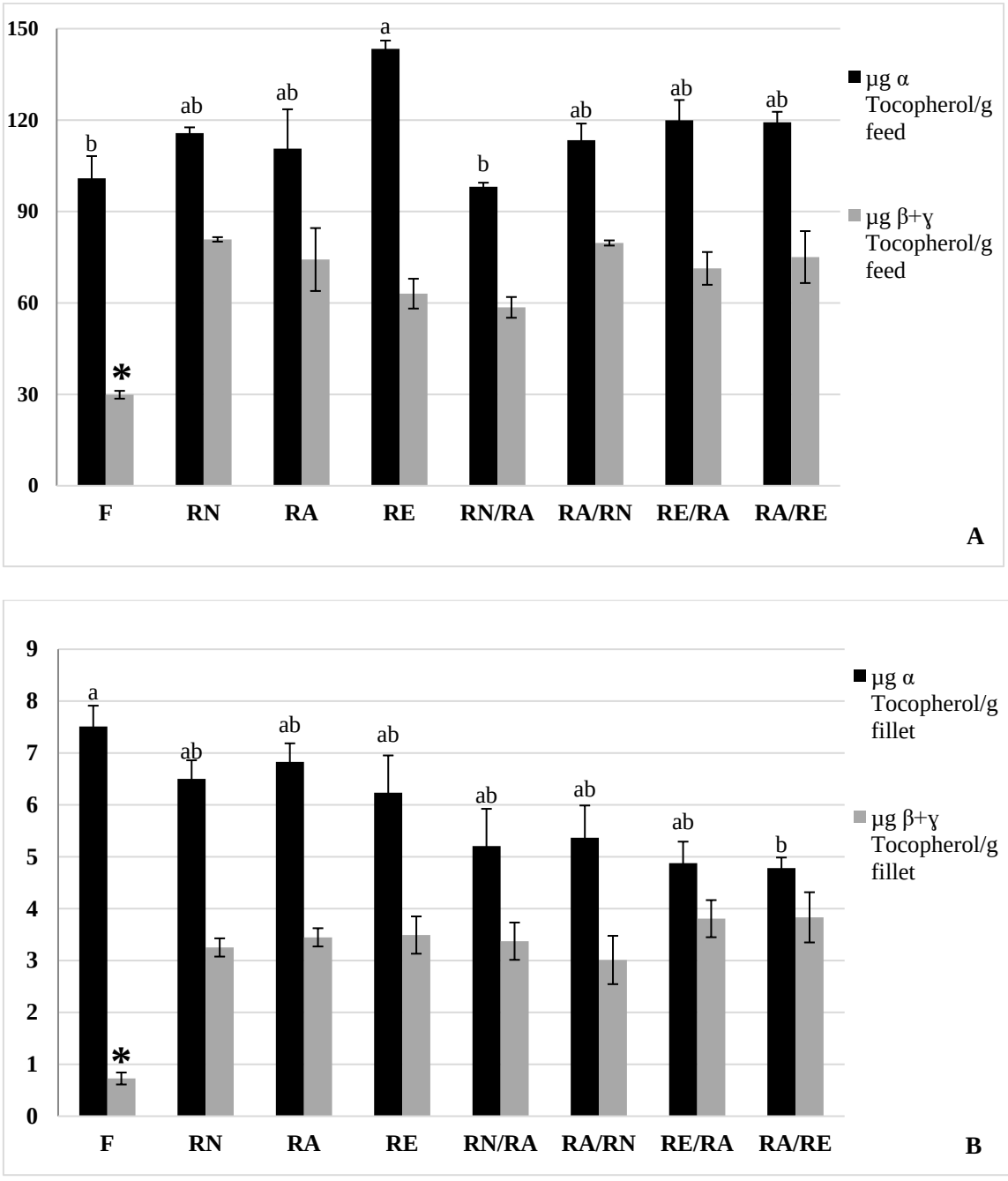

Figure 2. (**A)** α- and β+γ-tocopherol concentrations in experimental diets [F: fish oil (control diet); RN: rapeseed native oil; RA: rapeseed acid oil; RE: rapeseed re-esterified oil; RE/RA: 66% rapeseed re-esterified oil - 33% rapeseed acid oil; RA/RE: 66% rapeseed acid oil - 33% rapeseed re-esterified oil; RN/RA: 66% rapeseed native oil - 33% rapeseed acid oil and RA/RN: 66% rapeseed acid oil - 33% rapeseed native oil] expressed as µg of tocopherol per gram of feed and (**B)** α- and β+γ-tocopherol concentrations in fillets from rainbow trout fed the experimental diets expressed as µg of tocopherol per gram of fillet. Different letters or the sign (*****) indicate significant differences ($P<0.05$), according to ANOVA.

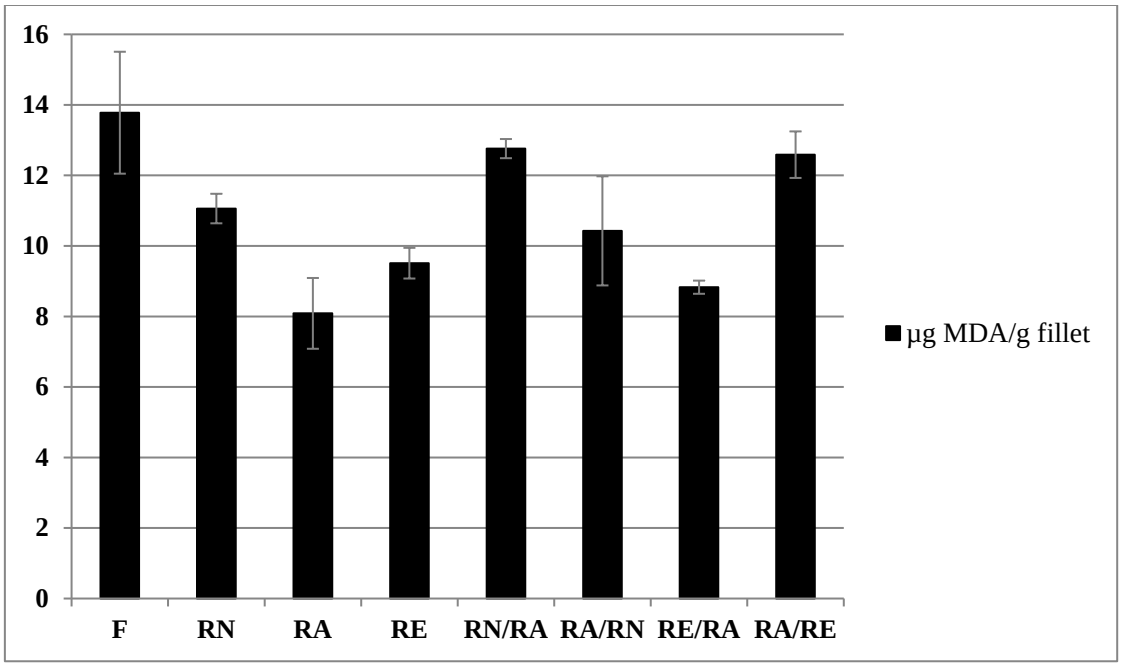

Figure 3. Thiobarbituric acid-reactive substances in fillets from rainbow trout fed the experimental diets (nomenclature as in Figure 2) expressed as micrograms of MDA per gram of fillet. The lack of letters or signs indicates no presence of significant differences (P>0.05), according to ANOVA.