Effects of fish oil substitution by hazelnut oil on growth performance, whole body fatty acid composition and enzymes of intermediary metabolism of juvenile meagre (*Argyrosomus regius* Asso, 1801)

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Abstract: The need for new alternative oil sources in the ever-increasing aquaculture of carnivorous species is imperative. We substituted fish oil (FO) with hazelnut oil (HO) in meagre diet at 20, 40, 60, 80and 100%. Triplicate groups of 40 fish/tank juvenile meagre (ca 15 g) were used. For the reduction of FO level in meagre diet, a 40% substitution by HO is attainable without affecting growth performance. However, higher substitutions appear to have a negative effect on growth performance and feed efficiency, although daily feed intake seems to remain unaffected by the reduction in FO. No effects on whole-body protein and lipid concentration were observed for fish fed the experimental diets. Whole-body fatty acid composition reflected the dietary fatty acid composition in all dietary groups. Diets with higher than 40% levels of FO substitution significantly reduce highly unsaturated ω-3 fatty acids in the fish. Although the metabolic relationship between carbohydrate and fatty acid exploitation is extremely versatile, and thus it is difficult to reach a safe conclusion, it seems that FO substitution by HO activates fish carbohydrate, rather than fatty acids, exploitation machinery. These results support the use of HO in meagre diets up to 40% FO substitution with a future thought for more efficient use of 30% FO substitution by HO.

Keywords: diet formulation; feed oils; proximate chemical composition; growth metrics; metabolism enzymes

1. Introduction

The culture of new species and the use of new ingredients in feeds are critical factors for the further development of the aquaculture industry. Optimizing the use of new ingredients in aquafeeds presupposes knowledge of their chemical composition and the effects on growth of the organisms that feed on them (Glencross et al., 2007). Meagre is the most promising new species for the Mediterranean aquaculture due to its high growth rate (2.5 kg in 24 months), good conversion efficiency (0.9 - 1.2), high nutritional value and low lipid content of the fillet (Poli et al. 2003; Grigorakis et al. 2011; Fountoulaki et al., 2017). The large commercial sizes it achieves are beneficial for processing, in a multitude of marketable products (Pérez et al., 2019), giving it a competitive edge in the marketplace compared to gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*). Also, it is a lean species, even in intensive culture conditions, with a high protein content andω−3 (Long Chain) LC- polyunsaturated fatty acids (PUFA) (Monroig, Tocher, Hontoria, & Navarro, 2013;Piccolo et al., 2016). Meagre production has increased dramatically over

recent years, from only a few tons in 2000 to 11770 tons in 2015, as aquaculture contributed to the production of 5471 of those (FAO, 2018; FAO, 2019). The European Aquaculture Technology and Innovation Platform reports that meagre culture will increase in production substantially until 2030 (EATiP, 2012).

Rising demand for fish farming, combined with declining natural resources and climate change, is putting increasing pressure on industry to shift to new sustainable alternatives to farming practices (Alhazzaa, Nichols, & Carter, 2019; Handisyde, Telfer, & Ross, 2017). At the same time, the demand for feed ingredients is expected to increase in order to cover the needs of fish farming and alternative sources to fish oil (FO) should be investigated. For fish farmers, one of the key factors driving the replacement of FO by alternative oil sources is the ever-increasing production cost due to the stagnant supply rate of FO (Arslan et al., 2012). Reducing dependence on FO and minimizing feed costs could still be based on a partial FO substitution from vegetable oil (VO) (Izquierdo et al., 2003). The need to find alternative sources of oil is not only aimed at reducing the final product but is a critical factor for the survival of aquaculture industry (Ng et al., 2007). Hazelnut oil (HO) is the main vegetable oil (VO) produced in Turkey, which supplies 75% of total world hazelnut production (Erdogan, 2018). Relative to other sources of VOs, hazelnut is one of the richest in monounsaturated fatty acids (MUFA) and low in saturated ones (Granata et al., 2017). MUFA and PUFA constitute 79.5% and 12.5% of the total fatty acids (FAs) respectively in HO and it could be considered for partly substitution of FO in meagre feeds. HO contains 74.2–83.1% oleic acid (OA, C 18:1, ω-9) and linoleic acid (LA, 18:2 ω-6) (Yildiz-Turp & Serdaroğlu, 2008). Replacing FO with VO in specific proportions were studied in some marine and fresh water species such as European sea bass (Montero et al., 2005), gilthead sea bream (Izquierdo et al., 2003) and rainbow trout (*Oncorhynchus mykiss*) (Montero et al., 2005). Successful results in terms of growth performance and feed conversion ratio were obtained with the use of VO as partial or total substitution of FO in seabream and seabass diets (Izquierdo et al., 2003; Izquierdo et al., 2005). Further, some researchers reported that there was no effect on growth and feed conversion ratio of salmonids fed VO (Caballero et al., 2002; Rosenlund, Obach, Sandberg, Standal, & Tveit, 2001; J. G. Bell et al., 2001a). It has been suggested that some VO oils can overcome the problem of ω-3 LC-PUFAs deficiency and be compensatory oil sources for fish (X. Peng et al., 2016).

For successful substitution of FO by HO, the essential FA requirements of fish should be covered. Especially, the needs of marine fish in LC-PUFAs must be met because most of them do not have the capacity to convert 18C PUFAs to 20 and 22C highly unsaturated fatty acids (HUFAs) (Mourente, Good, & Bell, 2005a; Tocher, 2003). In fact, it seems that the marine fish dependence on dietary HUFA is due to the inadequacy of one or more basic enzymes activity, Δ5 and Δ6 fatty acid desaturases, and FA elongases, which are essential for HUFA biosynthesis (Tocher, 2003). Depending on the species, the developmental stage, the environment and the trophic level, the demand in PUFAs varies, with increasing demand in LC - PUFA, eicosapentaenoic (EPA, 20:5 ω-3), docosahexaenoic (DHA, 22:6 ω-3) and arachidonic (ARA, 20:4 ω-6) acids to display marine species (Tocher, 2010). Any change in the diet of fish can directly alter the biochemical mechanisms involved in metabolism (Greene & Selivonchick, 1987). The change in the composition of the diet in terms of FAs, especially the ω-3 and ω-6 FAs is of great importance for fish metabolism (Tan et al., 2009). Therefore, the study of metabolic enzymes involved in these mechanisms could provide answers to the way in which any modification of the dietary protocol has a positive or negative effect on fish physiology (Atasever et al., 2014). Previous research in Atlantic salmon (*Salmo salar*) and *Catla catla* have shown that the replacement of FO by VO oils such as rapeseed oil, soybean oil, linseed oil and palm oil at a rate of 80 to 100% is feasible without significantly affecting the growth of fish, but it causes various changes in metabolism and body lipid composition (J. G. Bell et al., 2001b; Rosenlund, 2001; Singh Parihar, 2015). In the past, the use of HO in the diets of mammals (rabbits, lambs) and poultry (hens, broilers) has been used with very positive effects both in growth and productivity of these animals and for its beneficial effects in the reduction of oxidative stress (Hatipoğlu et al., 2004; Cetingul et al., 2009; ÇetİngÜl & Vét, 2009). To our knowledge, although VO supplementation in fish feed is largely investigated, the effects of the partial substitution of FO by HO on the growth performance, feed utilization and fatty acid

composition, and moreover on the intermediate metabolic capacity of meagre has never been studied. To this end, we aimed to investigate the maximum level of substitution of FO by HO oil on the above-mentioned parameters.

2. Materials and Methods

2.1. Fish maintenance

The experiment was conducted at the Institute of Marine Biology, Biotechnology and Aquaculture of the Hellenic Centre for Marine Research (HCMR) in Crete, Greece. HCMR facilities are licensed by the region of Crete (General Directorate of Agricultural and Veterinary under the updated license No 3989/01.03.2017) for operations of breeding and experimental use of fish. Experimental protocols of surgery and sacrifice conditions were approved under the license numbers EL91-BIO-03 and EL91-BIO-04, following the Greek Presidential Degree No 56/2013, Official Journal of the Greek Government No. 106/30 April 2013 on the protection of animals used for scientific purposes.

Meagre were provided by the HCMR hatchery in Crete, Greece. At the beginning of the study, 720 fish (c.a. 15 g) were lightly anaesthetized (with 150ppm 2-Phenoxyethanol), individually weighed with a precision scale and randomly divided into eighteen 500 L tanks. For the nutritional trials, six diets were prepared with 0, 20, 40, 60, 80 and 100% (D0, D20, D40, D60, D80 and D100) substitution of fish oil with hazelnut oil (**Table 1**). Fish were hand-fed to apparent satiation three times a day (09:00-12:00-15:00), six days a week for 90 days. The tanks were supplied with borehole water with a constant temperature of 19 ^{o}C and 35,000 mg L^{-1} salinity. Optimum water flow (200% per hour) was obtained during the study period and oxygen saturation was kept over 80%. Any unconsumed feed would be removed and dried daily, in order to determine feed intake. In the middle and the end of the experimental period, all fish were lightly anaesthetized (with 150ppm 2-Phenoxyethanol) and individually weighed.

Ten fish at the beginning of the experiment and three fish per tank at the end were sacrificed by anaesthesia overdose (with 500ppm of 2-Phenoxyethanol) and placed in the freezer (-20 ^{o}C) for whole body composition analysis. Tissue samples (intestine, liver, heart and white muscle) from three fish per tank (12 fish per diet) were collected for the determination of the hepatosomatic index and the metabolic enzyme activity. All samples were frozen in liquid nitrogen immediately after removal and stored at -80 ^{o}C until they were analysed.

2.2. Diet preparation

The nutrient composition of the feed ingredients was analyzed and the experimental feeds were designed to contain 54.8% crude protein and 18.6% crude fat and to cover the nutritional needs of the meagre (Chatzifotis et al., 2012). All ingredients were obtained by local suppliers (Perseus Specialty Food Products S.A. Stanotopi 20001, Zevgolatio Greece). Experimental feeds were prepared using a press-pellet machine (3 mm dia).

2.3. Analytical procedures

The following growth performance and somatic indexes were calculated:

Specific growth rate (SGR, % body weight x day^{-1})) = 100 x ($\ln^{(\text{final body weight})}$ - $\ln^{(\text{initial body weight})}$)/days of trial

Feed conversion ratio (FCR) = total dry feed consumed / weight gain

Daily feed intake (DFI, % body weight day^{-1})=[total dry feed intake (g) x 100] / [(initial body weight+final body weight x 0.5] x number of days

Hepatosomatic index (HSI) = 100 x (liver weight / body weight)

The proximate composition of feed ingredients, diets and whole bodies were determined. Crude protein was determined using a nitrogen analyzer (FP-528, Leco corporation, St. Joseph, Michigan, USA), according to Dumas method (nitrogen x 6.25), while total lipids were determined according to (Folch et al., 1957). Briefly, the fat was extracted using methanol/chloroform/BHT solution (2:1 methanol/chloroform v/v + 0.01% w/v BHT) (Sigma Aldrich, Darmstadt, Germany) in proportions

1:15 w/v. Dry matter content was determined in a drying oven at 105 °C for 3.5 hours until constant weight, and the ash content was determined after incineration at 550 °C for 4 hours (Horwitz et al., 1990). Fatty acid methyl esters (FAMEs) were prepared according to (AOAC, 1989) following a saponification and BF3 methylation using around 50 mg of lipids and analyzed using a GC-2010 Shimadzu gas chromatography system (Shimatzu Corporation, Kioto, Japan) equipped with a flame-ionization detector (GC-FID) and a SP2330 capillary column (30 m length and 0.25 mm inner diameter). Helium was used as the carrier gas. The injector and detector temperature were both 250 °C. The thermal gradient was 150°C for 1 min, 150 °C to 180 ° Cat 10 °C min^{-1}, 180 °C to 211 °C at 1.5 °C min^{-1}, 211 to 250 at 35 °C min^{-1} and kept at 250 °C for 3 minutes. A known standard mixture (Supelco 37 Component FAME Mix) was used for the identification of the FAMEs. The concentration of FAMEs was done expressed as a percentage of the total FAMEs.

2.4.Metabolic enzymes' activities

Activities of L-Lactate Dehydrogenase (L-LDH - E.C. 1.1.1.27), Citrate synthase (CS – E.C. 2.3.3.1), Pyruvate Kinase (PK – E.C. 2.7.1.40) and Malate Dehydrogenase (MDH – E.C. 1.1.1.37) were determined in intestine, liver, heart and white muscle of meagre according to techniques described by (Driedzic & Fonseca de Almeida-Val, 1996). All assays were performed at 18 °C and were based on well-established protocols for fish tissues (Moon & Mommsen, 1987; Singer & Ballantyne, 1989; Sidell, Driedzic, Stowe, & Johnston, 1987). The L-LDH, MDH and PK activities were measured following the oxidation of NADH at 340 nm (mM extinction coefficient = 6.22) and CS activities were determined based on the reaction of free acetyl-coenzyme A with DTNB [5.5 dithio-bis (2-nitrobenzoic acid)] at 412 nm (mM extinction coefficient = 13.6). Enzyme activities are expressed as micromoles of substrate oxidized/reduced per min and per gr of wet tissue.

2.5. Statistical Analysis

Statistical analyses were performed with SPSS 11 statistical software (IBM Corporation, Armonk, NY, USA), and the presence of statistical differences among groups were determined with a one-way analysis of variance (one-way ANOVA) at 0.05 significance level and Tukey's test was employed to compare individual means. All data were tested for normality and homogeneity of variance prior to be subjected to one-way ANOVA using Kolmogorov–Smirnov and Levene's tests, respectively. In addition, linear regressions were performed to determine whether data followed a linear response to the dietary hazelnut oil inclusion. Finally, using the Primer 6 program, the hierarchical cluster analysis based on the Bray-Curtis similarity index was applied. In the present work, tissues from the six different feeding groups (liver-0, liver-20, liver-40, etc.) were grouped based on the activity levels of the examined enzymes. The resulting dendrogram provides two axes, the x' axis showing all the samples and the y' axis showing their similarity level.

3. Results

3.1. Fatty acid composition of diets

The fatty acid composition of the diets is presented in **Table 2**. The fatty acid with the highest concentration in all diets was the oleic acid (C 18:1, ω-9) and it was followed by linoleic acid (C 18:2 ω-6). In the D20 the second most abundant fatty acid was palmitic acid (C 16:0). The amount of saturated fatty acids (SFA) was higher in the D20. The D100 had the lowest SFA concentration, but the highest level of MUFA. The D0 was the most plentiful of total poly-unsaturated fatty acids (PUFAs) and ω-3 PUFAs. This diet was also the richest one in both EPA (C 20:5 ω-3) and DHA (C 22:6 ω-3). The decrease in the inclusion of fish oil in the diets, led to a linear decrease of the SFAs, total ω-3 PUFAs, and total long chain PUFAs ($p = 0.019$, 0.004 and 0.008 respectively). On the contrary, an increase in the inclusion of hazelnut oil led to a linear increase of the MUFAs and total ω-6 PUFA ($p = 0.024$ and 0.034 respectively), driven by the high contents of linoleic acid. The highest

concentration of ω-6 was found in D100, which was mainly attributed to the high contents of C 18:2 (ω-6) and C 20:4 (ω-6).

3.2. Growth performance

The growth performance parameters are presented in **Table 3**. At the end of the growth trial (90 days), significant differences were found among the experimental groups where in general significantly lower final weight,weight gain and specific growth rate (SGR), were observed with the increase of the hazelnut oil inclusion ($p = 0.010$, 0.004 and 0.004 respectively). Groups fed with D0, D20 and D40 presented similar final body weight and SGR values, with group fed D20 having the best growth performance ($p > 0.05$). Fish fed the D80 and D100, exhibited the worst growth performance ($p < 0.05$). The lowest FCR corresponds to the D20 group, while the highest one corresponds to the D80 group. Nonetheless, no significant difference was observed for FCR (0.90 – 1.16, $p > 0.05$). Daily feed intake maintained similar values among all the groups (1.13% – 1.25% of body weight per day, $p > 0.05$). Fish fed the D0 had the lowest HSI (1.84±0.10%) and fish fed the D40 had the highest (2.45±0.22%). However, the differences were not statistically significant ($p > 0.05$).

3.3. Whole-body composition

Table 4 presents the whole-body proximate composition of meagre fed the experimental diets. A significant response to the increasing fish oil replacement was observed for the whole-body dry matter, protein and fat content ($p = 0.041$, 0.014 and < 0.001, respectively). However, the inclusion of hazelnut oil did not alter the whole body's protein and fat majorly, thus the differences observed were not statistically significant. The fish of group D0 had the highest dry matter content, significantly higher than the groups D40, D80 and D100 ($p < 0.05$). Finally, fish fed the D0 and D80, had significantly lower ash than the D40, D60 and D100 groups ($p < 0.05$).

3.4. Fatty Acid Composition of Fish

The whole body selected FA composition of meagre is presented in **Table 5**. The total SFA of body composition, in which the most prevailing was the C 16:0, showed a strong negative correlation with the level of fish oil replacement ($r = -0.943$ and $p = 0.017$, for both total SFAs and C 16:0). The amount of total SFAs was higher in D20 group ($p < 0.05$). The D100 group had the lowest SFA concentration but the highest level of MUFA ($p < 0.05$), mirroring the fatty acid composition of the diet. The increase in total concentration of MUFAs, driven by the increase of C18:1 ω-9 content followed the increase of the hazelnut oil inclusion. The highest PUFA content was found in D0 group, while the lowest one in D60 group ($p < 0.05$). The whole-body total ω-3 and n3/n6 ratio followed the pattern of the fatty acid composition in all experimental diets. The contents of DHA (C 22:6ω-3) and EPA (C 20:5ω-3) of fish were significantly reduced when the hazelnut oil levels increased ($p < 0.05$). Linoleic acid (18:2 ω-6) increased in higher fish oil substitution, but the linolenic acid (18:3 ω-3) levels were higher in the hazelnut oil abundant diets ($p < 0.05$).

3.5. Metabolic enzymes' activities

Figure 1 depicts the citrate synthase (CS) activity levels in the intestine, liver, heart and white muscle of meagre. CS is the first of a series of eight enzymes involved in the citric acid cycle, responsible for catalyzing the first reaction of this metabolic cycle (Morrison, 2020). In the digestive system, CS activity was substantially decreased in D60-D100 groups, while in the liver this decrease was observed only for the D40 group. Additionally, while in the heart no change in CS activity was observed, in the muscle D40-D80 groups resulted in CS increased activity. PK is involved in the last step of glycolysis, catalyzing the transfer of a phosphate group from phosphoenolpyruvate (PEP) to ADP, yielding one molecule of pyruvate and one molecule of ATP (Gupta & Bamezai, 2010; Morrison, 2020). Regarding the pyruvate kinase (PK) activity in the intestine and heart (**Figure 2**), a continuous increase, parallel to the increase of fish oil substitution was observed. In the liver, significantly higher PK activity was observed in the fish fed the D60, D80 and D100. On the other hand, in the muscle, significant decrease in PK activity was observed in the D80 and D100 groups. LDH is an enzyme

found in nearly all living cells tissues that utilize glucose for energy, and catalyzes the conversion of lactate to pyruvate and back, as it converts NAD^+ to NADH and back (Morrison, 2020). The HO inclusion did not affect the lactate dehydrogenase (L-LDH) activity of the intestine and liver of meagre (**Figure 3**). However, the fish oil substitution resulted in increased levels of L-LDH activity in the heart of the D80 and D100 groups and in the muscle of the D40 group. MDH is mainly involved in the Krebs cycle (converting malate to oxaloacetate). It has been suggested that this enzyme has also other cellular functions, e.g. participating in a reductive citric acid cycle to protect against oxidative stress and in substrates' transport through the metabolic pathways (Molenaar et al., 2000; Takahashi-Íñiguez et al., 2016). In the heart of meagre, the malate dehydrogenase (MDH) activity remained unchanged (**Figure 4**). However, in the intestine, the D80 and D100 resulted in a sharp decrease in MDH activity. In the liver increased MDH activity was observed under all the hazelnut oil feeding regimes. Finally, the D40, D80 and D100 resulted in an increase of the muscle's MDH activity, with the most intense in the D80 group.

3.6. Hierarchical Cluster Analysis - Similarity in enzymatic activity levels

Figure 5 shows the dendrogram obtained from the Cluster hierarchical analysis based on the activity levels of the investigated enzymes. The analysis showed that the six different states (different diets) for all tissues had a similarity of around 35%. Moreover, dietary patterns of enzymatic activity were divided into 5 subgroups, which responded to a similarity of over 80%. This hierarchical analysis revealed two mainclose-relation clusters, one between heart and muscle, and one between liver and intestine. The results show that the enzymatic activities are more related to tissues' (e.g. muscle and gastrointestinal tissues) function and need in fuels (carbohydrates and / or fatty acids) rather than to each enzyme's specific function. It should be noted though, that it is widely accepted that carbohydrate intake is essential for muscle function, health and growth. The latter is owed to the fact that carbohydrates (glycogen) are essential for muscle building (instead of breaking down muscle proteins for energy). Moreover, it has been shown that in fish dietary carbohydrate intake influences glucose metabolism to a high degree (Hemre et al., 2002).

4. Discussion

Growth performance

Prior to this study, the effect of use HO on farmed meagre had no available information. For a feed to be considered species suitable, it must cover all its nutritional requirements and achieve optimal growth rates, a sufficiently low FCR and at the same time ensuring excellent welfare conditions (Al-Thobaiti, Al-Ghanim, Ahmed, Suliman, & Mahboob, 2018; Yavuzcan Yildiz et al., 2017). Especially for meagre, in addition to meeting the nutritional needs for growth, it is important to study the effects of essential fatty acids (EFA) deficiency, as it could affect the occurrence of hepatic granulomas; one of the most pervasive metabolic disorder in farmed meagre (Marta Carvalho et al., 2019; Tsertou et al., 2020). Different combinations of lipids can be more effective than FO as the supply of high percentages of SFAs and/or MUFA can reduce the use of n-3 LC-PUFA (Turchini et al., 2019). Indeed in fresh water species, such as barred catfish (*Pseudoplatystoma fasciatum*) (Arslan et al., 2008) ,rainbow trout (Rinchard et al., 2007) and yellow drum (*Nibea albiflora*) (Wabike et al., 2020) a better growth has been found in experiments where FO was replaced by VO oils.

PUFAs are essential for fish nutrition, as for all vertebrates, with different demand for each species (M. V. Bell & Tocher, 2009). Their demand also depends on other factors such as the age (M. V. Bell & Tocher, 2009), the dietary lipids content, the EPA/DHA contents (Izquierdo, 1996; Oliva-Teles, 2012) and the levels of the antioxidant nutrients (Mourente et al., 1999). In the present study, a dietary FO substitution up to 40% seems attainable with no significant negative effects on growth performance for meagre. Our results shows that meagre fed with HO diets displayed growth rates comparable to earlier researches for this species fed FO,FM-based diets (Chatzifotis et al., 2012; Chatzifotis et al., 2010; Martínez-Llorens, Espert, Moya, Cerdá, & Tomás-Vidal, 2011). Nevertheless, a future study examining a 30% FO substitution may show clearer results as to the exact upper limit of HO that can be included in meagre diets. Such future data would be very helpful as we observed that in some growth parameters and in body fatty acids composition the effect of 40% substitution is

marginal. Meagre fed D0 featured the best growth performance, while the D20 and D40 groups had similar values. However, further HO inclusion (80-100%) resulted in reduced final body weight (-10.9%) and SGR (-0.2%). In many cases, the decrease in growth performance may be due to the reduced feed consumption, especially in the first few weeks of experimentation, since in such a short period of time the lack of fatty acids may not have such negative effects (Trushenski et al., 2011). In the present study, the DFI index did not change between groups, so a decrease in growth parameters is most likely due to the lack of EFA in diets with high substitution of FO. According to (Montero et al., 2001), the ratio C18:1 ω-9 / (EPA+DHA) is a good indicator of EFA deficiency in fish diets. In our study this ratio is increasing with increasing HO incorporation into diets, reinforcing the view on the reason for the restrained growth in these groups (D60, D80, and D100). Although the increased value of HSI has often been used as another indicator of FA deficiency (Verreth et al., 1994), our results showed that HSI remained unchanged between groups despite the decreasing growth observed in groups fed D60, D80 and D100. It has also been reported that in cases of EFA deficiency, growth retardation is often observed (Trushenski et al., 2011). The same research team achieved 67% substitution of FO from soybean oil without negative effects on Cobia (*Rachycentron canadum*) growth (62.4 g initial body weight), whereas (Benedito-Palos et al., 2009) achieved the same positive results with 67% FO substitution by blended VO oil for farmed gilthead seabream (18 g initial body weight). The differences in growth after a higher FO substitution in Cobia could be due to their higher initial body weight, exhibiting a lower demand of PUFA, compared to our study. However, in line with our results, these studies showed that a complete replacement of FO by alternative VO oil sources causes a reduction in growth with a lower final body weight of fish and reduced feed intake. The aforementioned scientific groups argue that the decline in yield is due to a complete lack of 20:5 ω-3 and 22:6 ω-3 in diets. Indeed, the D100 in our case has significantly lower content rates of 20:5 ω-3 and 22:6ω-3. In Carvalho et al. (2018) the optimum growth of meagre fingerlings was achieved after the inclusion of 2.1% DM ω-3 LC-PUFA in diets containing 16.5% DM lipids, 0.9 EPA/DHA and 0.4% ARA of total FA contents. Another study showed that a 0.4% inclusion of ω-3 LC-PUFA in the diet does not serve the EFA requirements of meagre (El Kertaoui et al., 2017).

In general, supplementation of vegetable ingredients with high content in fish diets has been shown to reduce fish growth in, European sea bass, turbot (*Psetta maxima*), Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (Drew, Ogunkoya, Janz, & Van Kessel, 2007; Mourente, Good, & Bell, 2005b; Regost, Arzel, Robin, Rosenlund, & Kaushik, 2003; Satoh et al., 1998). Conversely, previous study in white sea bream (*Diplodus sargus*) has shown that complete replacement of FO by HO is feasible without interfering with the growth and chemical composition of the body (Taşbozan et al., 2015). However, it has been observed that adding LC-PUFA of plant sources to diets devoid of FM and FO can significantly improve growth in European sea bass (Torrecillas et al., 2017). Another research team with a similar experiment to ours for sea bass fed with increasing peanut oil (PNO) diets (0-100% inclusion), achieved exactly the opposite results; PNO60, PNO80 and PNO100 exhibited better growth performance than FO, PNO20 and PNO40 groups (Dernekbaşı et al., 2020). The process of MUFA β-oxidation is much more efficient than that of ω-6 PUFA due to the better digestibility of the first ones (Yılmaz & Eroldoğan, 2015). Therefore, the difference in these results in relation to ours may be due to the inversely proportional percentages of MUFA and ω-6 PUFA in their diets. Data on the substitution of marine nutrients for fish diets from vegetable sources is, even more, encouraging based on the results of (Ribeiro et al., 2015). This research group found that a concomitant substitution of FO (50%) and FM (60%) with vegetable ingredients without negative effects on meagre growth performance, intestine, intestinal enzyme activity and hematological stress indicators was possible.

Whole body composition

Whole-body proximate composition of meagre fed HO diets (D20-D100) did not differ from them who fed FO diet (D0), concerning the protein and fat content. The dry matter content was higher in D0 than in D40, D80 and D100 groups. Also, ash content in D0 and D80 was lower than the D40, D60 and D100 groups. Similar results with only ash content alteration for brown trout (*Salmo trutta*) fed HO diets has been found (Arslan et al., 2012). As reported in Francis, Turchini, Jones, & De Silva

(2007), the apparent digestibility of PUFA is greater than that of MUFA, and this in turn is greater than that of SFA. We found that despite the difference in PUFA proportion in the diets, the whole-body composition, in terms of fat and protein content, remained unchanged in all groups. Our findings agree with previous ones where the proximate body composition of red sea bream (*Pagrus major*) (Huang et al., 2007), black sea bream (*Acanthopagruss chlegeli*) (S. Peng et al., 2008) and white-spotted spinefoot (*Siganus canaliculatus*) (Wang et al., 2018), was not affected when they were fed VO diets. On the other hand, it has been found that diets with VO have led to an increased lipid percentage on body of juvenile golden pompano (*Trachinotus ovatus*) compared to diets rich in ω-3 PUFA or LC-PUFA. They attributed the high lipid content to the ability of ω-3 PUFA to reduce fat deposition, something we did not observe in our results.

One of the main problems in using VO in fish diets is the alteration of their body composition in FAs (Izquierdo et al., 2005). This often affects the consumer's preference, due to the fact that consumption of ω-3 PUFA-rich fish prevents cardiovascular (Herold & Kinsella, 1986) and autoimmune diseases (Connor, 2000; Hwang, 1989). In particular, certain fatty acids such as EPA, DHA and ARA are essential both for optimum growth performance as well as flesh quality index of fish (Drew et al., 2007). The C18:1 (ω-9), which was the dominant FA in the test diets was found in increasing concentrations in the fish body reflecting the increase of HO inclusion in the diet. On the other hand, it is known that certain FAs are selectively maintained or utilized (J. G. Bell et al., 2003). Although there is a tendency for fish to retain C22:6 ω-3 (J. G. Bell et al., 2001b), we observed a decline of C22:6 ω-3 in the flesh of fish fed the HO diets. There was also a decrease in ARA and EPA in the composition of fish fed with increasing levels of HO. Specifically we saw zero value of EPA in fish fed D100, although there was a small content of EPA in this diet. Therefore, the reduction of these FAs in body composition is considered greater than the corresponding reduction in the diets, revealing once again their essential role. The higher ARA levels observed relative to EPA levels in fish fed D100 may be due to increased EPA oxidation as also observed in a previous study (Izquierdo et al., 2005). Regarding groups fed D60-D100, growth retardation was observed but the whole-body FA analysis showed that the percentage of LA and ALA is significantly higher than in the other groups. Therefore, despite the negative consequences of the productivity of these groups for a fish farm, the preference in certain groups of consumers could turn to the consumption of these fish; correlation of occurrence anti-atherogenic and anti-thrombogenic effects due to the high consumption of these two FAs (Ulbricht & Southgate, 1991). According to Grigorakis (2007), a VO based diet leads to a reduction of atherogenic index, conclusion that coincides with our results.

A number of studies on the use of vegetable ingredients in fish diets as substitutes for FM and FO have suggested high amounts of ω-9 MUFA and ω-6 PUFA, increasing C18 FAs such as OA, LA and LNA, while simultaneously reducing ω-3 LC PUFA in the final product (Emre, Kurtoğlu, Emre, Güroy, & Güroy, 2016; Izquierdo et al., 2003; Strobel, Jahreis, & Kuhnt, 2012). In fact, high levels of HO in D60 and D100 groups showed a significant increase in ω-6PUFAswith a simultaneous ω-3 PUFAs decrease, a factor leading to a fairly lowω3/ω6 ratio in body composition of the aforementioned groups. In particular, the ω3/ω6 ratio ranges from 1.11±0.21 for fish fed the control diet (D0) to 0.34±0.12 for those fed D100. The LC-PUFAs play a crucial role in various functional and structural processes of the fish such as membrane structure, metabolism, lipid homeostasis/control and immune responses (Izquierdo, 1996; Tocher, 2015). The concentration of SFA was significantly higher in fish receiving D0 and D20 with a significant and gradual reduction for D60 to D100 groups. It is stated that according to the dietary guidelines for human diets, the PUFA/SFA ratio should be above 0.45 (Wood et al., 2004). Our results based on the fish whole body FA composition showed that the 0.45 ratio in the final product is sufficient for a healthy human diet, and it is covered in the groupsD0, D20 and D40, while the rest of the groups exhibit a lower PUFA/SFA ratio.

A safe approach could be the addition of high levels of VO oils into fish diets with a small incorporation of a rich in ω-3 HUFA source, such as Peruvian anchovy oil (Rosenlund et al., 2001). Another useful practice that is often applied is the re-feeding of fish with FO diets, for a short time period before they reach their commercial size, thus replenishing some of the desired FAs (DHA, EPA, ARA) in their flesh (Yıldız et al., 2018). Consistent with the above, it has been found that fish

have the ability to manage their body composition in FA through feeding management. Specifically, switching the diet of red seabream fed with VO to FO-based diets can rapidly modify the composition of the body FAs in the desired commercial contexts (Glencross et al., 2003). In fact, these findings support the fact that the recovery time of the FAs' body composition relates both to the VO source used in the diets as well as to each FA for which recovery is being studied (Glencross et al., 2003). On the contrary, the results of Regost et al.(2003) showed that the levels of FA in farmed turbot that changed their diet from plant to FO, did not reach those of fish fed on FO during the whole trial period. This may mean that when HO is the only lipid source, a larger amount of EPA source should supplement the diets. In addition to changes made in diet composition of fish in order to incorporate desirable FAs into their flesh, a different feeding strategy has been practiced. In particular, both better growth performance and whole body FA composition have been described in rainbow trout fed with FO and VO in a different cycled feeding (Dernekbasi & Karatas, 2020).

Metabolic enzymes' activities

The metabolic enzymes studied under the different HO inclusion treatments revealed a varied metabolic profile in the examined tissues probably reflecting the role of each organ in metabolism, and preservation of homeostasis. The latter is also exhibited by similarity in the enzymatic activity levels as the result of hierarchical cluster analysis.

In the heart, no changes were generally observed, except for PK and L-LDH, where an increase in activity is observed in D60-D100 groups. It seems, therefore, that for the maintenance of the necessary cardiocirculatory function, a shift in both aerobic and anaerobic glycolysis is observed (Driedzic, Stewart, & Scott, 1982;Giordano, 2005), probably because at these levels of FO replacement, HO cannot be adequately exploited by the cardiac tissue. The intestine appeared to shift mostly to the aerobic component of glycolysis, while the liver seemed to be more dependent to aerobic glycolysis, as indicated by increasing activities of PK (involved in glycolysis) and MDH (involved in gluconeogenesis) respectively, when increased FO substitution is imposed (Driedzic & Fonseca de Almeida-Val, 1996; L. Panepucci, Fernandes, Sanches, & Rantin, 2000; R. A. Panepucci, Fernandes, & Rantin, 2001). Specifically, in the intestine, since statistically significant decrease in the activity of CS (Krebs cycle enzyme) was observed in D60-D100 groups, while no changes were exhibited in L-LDH (glycolytic enzyme) activity levels, it appears that the meagre's metabolic mechanism, in this tissue, targets both on the lipid energy reserves and anaerobicglycolysis for D0-D60 groups, and mainly on anaerobicglycolysis for D80-D100 groups. The activity of metabolic enzymes in the liver of meagre, and the fact that in fish liver is considered to be a major fat and glycogen deposition site (McClelland, Zwingelstein, Weber, & Brichon, 1995; Peres, Gonçalves, & Oliva-Teles, 1999), could probably explain the increased levels of MDH and PK, since FO substitution seems to provoke both gluconeogenesis and glycolysis in this tissue. This seems to be the case for meagre when fed with a high lipid diet of 21% (Antonopoulou et al., 2014). According to Antonopoulou et al. (2014), different dietary lipid contents do not affect the hepatic activity of L-LDH and CS in meagre, whereas this is not the case for MDH. Furthermore, the 17%-lipid diet diminished the hepatic enzymatic activity in MDH and other enzymes such as 3-hydroxyacyl CoA dehydrogenase (HOAD) compared to 13%-lipid diet. On the contrary, meagre fed with the 21%-lipid diet increased the activity of both MDH and HOAD compared to fish fed with the 17%-lipid diet. The elevated MDH and HOAD activity levels confirm the significant role in gluconeogenesis and glycolysis of the liver in meagre (Antonopoulou et al., 2014). However, decreased PK activity was exhibited in the liver of *O. mykiss* fed with increased soybean meal concentration (Martin et al., 2003). These results seem to be in line with the fact that higher levels of FO substitution reduce unsaturated ω-3FAs in the fish body, thus shifting to a more carbohydrate dependent state. The muscle, however, seems to possess a more versatile role since it retains both an aerobic and anaerobic carbohydrate exploitation. However, Chatzifotis et al. (2018) described the fluctuations of the L-LDH, CS and MDH activity levels in the liver as well in the muscle at different time periods of meagre under starvation and different water temperatures.

It has been reported for Atlantic cod (*Gadus morhua*) (Pelletier et al., 1994) that in growth-related tissues, such as muscle, liver and digestive tract, there is an correlation of glycolytic enzymes activity with growth rates. Our results showed an increased PK activity in three (intestine, heart, liver) of the

examined tissues with increasing HO inclusion, whereas the PK activity in muscle for higher HO content was significantly reduced. We also observed a negative correlation of PK activity with ω-3/ω-6 ratio in the intestine, liver and heart, with the lowest activity in D0-D60 groups. In muscle the inverse pattern of PK activity was performed. Our results of liver PK activity are in line with the results of Tan et al. (2009) in juvenile yellow catfish (*Pelteobagrus fulvidraco*). The reduction of PK activity, an enzyme which is directly involved in the glycolytic process, also indicates an inhibition of glucose utilization for D0-D60 groups. According to L-LDH activity, we recorded same values among groups for both intestine and liver, higher activity for D80 and D100 groups in heart but in muscle only D40 group achieved higher L-LDH activity than all the others. We could assume that in groups with increased PK and L-LDH activities, there is a higher dependence on carbohydrate metabolism (Michaelidis et al., 2007).In the present study, the intestine aerobic capacity, estimated by CS activity, was significantly higher for groups with better growth rates (D0-D40), results identical to those for Atlantic cod (Pelletier et al., 1994).

5. Conclusions

In conclusion, our results evince that an adequate HO inclusion of up to 40% in meagre diets to reduce FO levels is attainable, without affecting growth performance. A 30% HO substitution research yields even more discernible positive results for both growth performance and FA body composition of meagre. Dietary substitution above 40% of FO by HO appears to have a negative effect on growth performance, reducing the overall body weight, FCR and SGR. Although the metabolic relationship between carbohydrate and fatty acid exploitation is extremely versatile, and thus it is difficult to reach a safe conclusion, the results herein lead us to assume that FO substitution with HO activates fish carbohydrate, rather than fatty acids, exploitation machinery. This could be attributed to the fact that carbohydrate intake is essential for muscle function, health and growth, since carbohydrates (glycogen) are essential for muscle building (instead of breaking down muscle proteins for energy) and therefore adequate growth. Diets with higher levels of FO substitution (>40%) may inhibit carbohydrate exploitation, but this remains to be elucidated in future studies. The fact that diets with higher levels of FO substitution (>40%) significantly reduce highly unsaturated ω-3 fatty acids in the fish body should be seriously taken into consideration. Therefore, maintaining high levels of PUFAs in the diets of farmed fish is of paramount importance. Resolving this negative impact on the use of vegetable ingredients in fish food is considered essential to maximize the overall benefit to the human consumer.

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Table 1. Ingredients and proximate composition of the experimental diets						
	Levels of fish oil replacement					
Ingredients (%)	**0% (D0)**	**20% (D20)**	**40% (D40)**	**60% (D60)**	**80% (D80)**	**100% (D100)**
Fish meal	40	40	40	40	40	40
Corn gluten	18	18	18	18	18	18
Wheat gluten	12.1	12.1	12.1	12.1	12.1	12.1
Wheat	9	9	9	9	9	9
Wheat bran	7.6	7.6	7.6	7.6	7.6	7.6
Lysine	0.49	0.49	0.49	0.49	0.49	0.49
Premix [a]	0.25	0.25	0.25	0.25	0.25	0.25
Choline	0.15	0.15	0.15	0.15	0.15	0.15
Fish oil (FO)	12.45	9.96	7.47	4.98	2.49	0.00
Hazelnut oil (HO)	0.00	2.49	4.98	7.47	9.96	12.45
Proximate composition (% of dry matter) [b]						
Crude protein	56.7	56.7	56.7	56.7	56.7	56.7
Crude fat	17.9	17.7	17.8	16.9	17.6	18.0
Ash	6.4	6.3	6.3	6.7	6.3	6.9

872 a
873 Premix (kg−1): Choline 90,000 (mg) Vitamin A 0.3 (MIU), Vitamin D3 0.1 (MIU), Vitamin E 20,000
874 (IU), Vitamin K 1030 (mg), Vitamin B1 390 (mg), Vitamin B 960 (mg), Nicotinic acid 2600 (mg),
875 Pantothenic acid 4400 (mg), Vitamin B6 890 (mg), Vitamin B12 15 (mg), Folic acid 290 (mg), Biotin 14
876 (mg),Vitamin C (Stay C 35% MONO) 20,300 (mg), Inositol 15,600 (mg),Total Mn 1200 (mg), Total Ca
877 72,000 (mg), Total Zn 7000 (mg), Total Cu 450 (mg), Total Se 14 (mg), Total I 100 (mg), Betaine (mg)
878 71,250 (mg), BHA (E320) 3000 (mg).

879 b
880 Mean of duplicate analyses.
881

Table 2. Fatty acid composition (% of total fatty acids) of the experimental diets (% of fish oil substitution with hazelnut oil)						
	0% (D0)	**20% (D20)**	**40% (D40)**	**60% (D60)**	**80% (D80)**	**100% (D100)**
C 14:0	3.36	4.47	4.64	2.15	1.48	0.17
C 16:0	8.71	10.72	8.53	8.49	8.74	7.49
C 17:0	0.62	0.72	0.51	0.45	0.30	0.33
C 16:4 (ω-3)	0.36	0.39	0.53	0.30	0.12	0.25
C 16:3 (ω-3)	0.66	0.81	0.59	0.40	0.24	0.05
C 16:3 (ω-1)	0.16	0.12	0.08	0.09	0.02	2.19
C 18:0	1.88	1.56	0.99	1.94	2.01	ND
C 18:1 (ω-9)	23.67	27.61	23.68	39.26	51.79	51.78
C 18:1 (n-7)	0.22	ND	0.52	0.34	0.23	0.18
C 18:2 (ω-6)	10.44	10.45	9.05	12.70	16.15	15.23
C 18:3 (ω-6)	0.92	0.22	0.57	0.40	0.23	0.53
C 18:3 (ω-3)	2.63	2.14	1.41	1.42	1.16	1.31
C 20:1 (ω-9)	5.24	3.08	1.77	2.61	1.91	0.11
C 20:1 (ω-7)	1.78	1.50	1.08	0.92	0.73	0.87
C 20:2	1.60	0.79	1.85	0.73	0.23	ND
C 20:3 (ω-3)	0.86	1.69	ND	0.15	0.02	0.29
C 22:0	0.81	0.06	ND	0.13	0.08	0.52
C 20:4 (ω-6)	0.70	0.68	0.75	0.35	0.26	1.57
C 20:4 (ω-3)	7.08	3.08	4.50	4.10	2.41	0.46
C 20:5 (ω-3)	4.87	2.79	2.37	2.87	2.38	2.08
C 22:5 (ω-3)	2.10	2.85	2.41	2.84	0.43	0.55
C 22:6 (ω-3)	6.82	4.18	4.80	4.91	3.53	3.79
∑ SFA	15.38	17.53	14.66	13.15	12.60	8.51
∑ MUFA	31.06	32.19	27.05	43.13	54.67	52.93
∑ PUFA	39.20	30.18	28.90	31.27	27.17	28.31
∑ ω-3	25.38	17.93	16.60	16.99	10.28	8.79
∑ ω-6	12.06	11.35	10.37	13.45	16.64	17.33
ω3/ω6	2.10	1.58	1.60	1.26	0.62	0.51
ω-3 LC PUFA	21.73	14.59	14.07	14.87	8.76	7.18
(18:1ω-9)/(EPA+DHA	2.02	3.96	3.30	5.04	8.76	8.82

Abbreviations: SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; LC PUFA, long chain poly-unsaturated fatty acids; ND, not detected.
Mean of duplicate analyses.

886

Table 3. Growth performance of meagre (*Argyrosomus regius*) fed diets with different levels of fish oil substitution with hazelnut oil

	0% (D0)	20% (D20)	40% (D40)	60% (D60)	80% (D80)	100% (D100)
Survival (%)	92.22±4.01	95.56±2.94	91.11±5.88	87.78±4.44	92.22±4.84	93.33±1.92
Initial BW (g)	15.36±0.17	15.34±0.61	16.09±0.61	15.51±0.20	15.15±0.17	15.48±0.12
Final BW (g)	56.24±2.63^{a}	57.37±2.69^{a}	55.74±2.69^{a}	48.88±0.83^{ab}	45.03±2.16^{b}	45.99±1.14^{b}
Weight gain (%)	266.0±13.3^{a}	274.0±27.7^{a}	248.3±24.7^{ab}	215.1±1.6^{ab}	197.2±13.1^{b}	197.1±5.1^{b}
SGR (% day^{-1})	1.44±0.04^{a}	1.47±0.09^{a}	1.38±0.09^{ab}	1.28±0.01^{ab}	1.21±0.05^{b}	1.21±0.02^{b}
DFI (% of BW day^{-1})	1.13±0.05	1.14±0.03	1.18±0.03	1.26±0.01	1.25±0.04	1.23±0.04
FCR	0.92±0.05	0.90±0.08	0.99±0.08	1.14±0.02	1.16±0.09	1.14±0.05
HSI (%)	1.84±0.10	2.20±0.13	2.45±0.22	2.01±0.27	1.91±0.16	2.33±0.23

887 Abbreviations: BW, body weight; SGR, specific growth rate; DFI, daily feed intake; FCR, feed
888 conversion ratio; HSI, hepatosomatic index.
889 Mean ± standard error, n = 3 tanks per diet and n = 9 fish per diet for the HSI. Different letters denote
890 statistically significant difference ($p < 0.05$).
891

Table 4. Whole body composition of meagre (*Argyrosomus regius*) fed diets with different levels of fish oil substitution with hazelnut oil						
	0% (D0)	**20% (D20)**	**40% (D40)**	**60% (D60)**	**80% (D80)**	**100% (D100)**
Dry matter	26.57±0.28^{a}	26.02±0.33^{ab}	25.01±0.30^{b}	25.55±0.33^{ab}	25.09±0.32^{b}	25.05±0.3^{b}
Crude Protein	14.31±0.64	14.37±0.51	13.83±0.22	13.71±0.21	13.54±0.57	13.63±0.50
Fat	4.56±0.32	4.58±0.29	4.28±0.12	4.17±0.15	4.03±0.35	3.82±0.17
Ash	2.98±0.16^{b}	3.27±0.10^{ab}	3.58±0.08^{a}	3.63±0.09^{a}	2.87±0.15^{b}	3.20±0.18^{a}

892 Expressed as % of wet matter.

893 Mean ± standard error, n = 9 fish per diet. Different letters denote statistically significant difference
894 ($P< 0.05$).

895

896

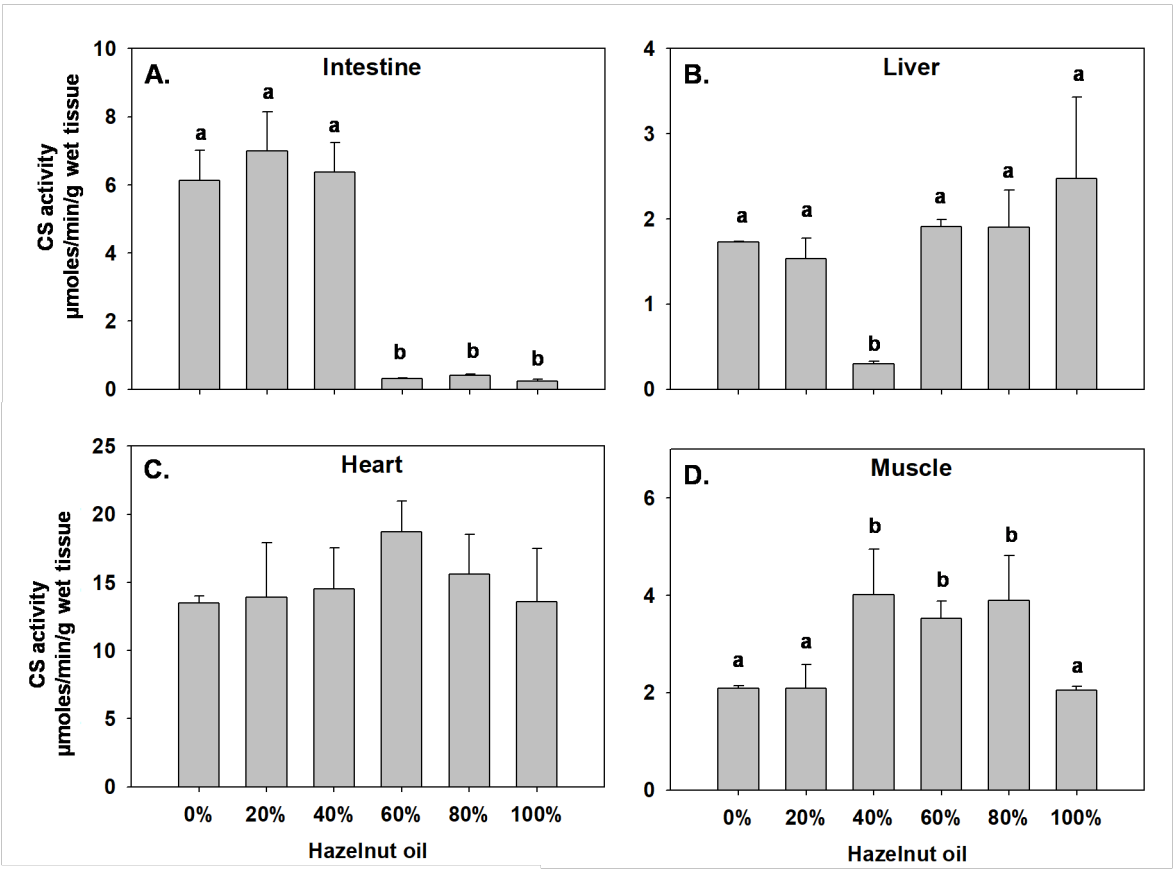
Table 5. Whole body fatty acid composition (% of total fatty acids) of meagre (*Argyrosomus regius*) fed diets with different levels of fish oil substitution with hazelnut oil

	0% (D0)	20% (D20)	40% (D40)	60% (D60)	80% (D80)	100% (D100)
C 14:0	2.53±0.26^{a}	2.97±0.48^{a}	2.28±0.39^{a}	2.38±0.96^{b}	1.41±0.21^{b}	1.09±0.18^{b}
C 16:0	14.18±0.75^{ad}	15.99±1.08^{a}	13.72±0.62^{ad}	13.13±1.44^{bd}	11.96±1.03^{bc}	10.46±1.04^{c}
C 16:1 (ω-7)	4.71±0.39^{a}	4.58±0.32^{a}	3.34±0.77^{b}	3.30±0.42^{b}	2.45±0.21^{c}	2.10±0.21^{c}
C 17:0	0.62±0.05^{a}	0.56±0.11^{abc}	0.62±0.08^{ac}	0.65±0.10^{ac}	0.54±0.05^{ab}	0.48±0.03^{b}
C 16:4 (ω-3)	0.20 ±0.04^{ac}	0.17±0.01^{ac}	0.16±0.04^{c}	0.25±0.06^{abc}	0.34±0.07^{b}	0.28±0.10^{ab}
C 16:3 (ω-3)	0.42±0.03^{ac}	0.42±0.02^{ac}	0.36±0.06^{ab}	0.37±0.06^{abc}	0.43±0.06^{c}	0.31±0.03^{b}
C 16:3 (n-1)	0.09±0.05^{a}	0.07±0.02^{a}	0.11±0.05^{a}	0.11±0.05^{a}	0.25±0.06^{b}	0.25±0.04^{b}
C 18:0	3.19±0.20	3.27±0.30	3.15±0.20	3.10±0.31	3.36±0.21	3.27±0.18
C 18:1 (ω-9)	33.34±2.25^{a}	34.84±2.07^{a}	40.29±2.03^{b}	41.54±3.56^{bc}	45.33±2.17^{c}	50.89±1.69^{d}
C 18:1 (ω-7)	0.08±0.01^{ab}	0.06±0.01^{a}	0.14±0.05^{bc}	0.14±0.05^{bc}	0.18±0.06^{c}	0.17±0.03^{c}
C 18:2 (ω-6)	12.17±0.36^{ab}	11.85±2.30^{a}	12.96±0.43^{bc}	12.86±1.28^{be}	14.13±0.48^{cde}	15.11±0.45^{d}
C 18:3 (ω-6)	0.17±0.02	0.15±0.02	0.17±0.02	0.10±0.05	0.33±0.24	0.16±0.07
C 18:3 (ω-3)	1.67±0.17^{ab}	1.68±0.25^{abc}	1.45±0.54^{ab}	1.00±0.12^{cd}	0.95±0.09^{cd}	0.73±0.05^{d}
C 20:1 (ω-9)	4.86±0.35^{a}	4.09±0.75^{a}	3.49±0.28^{c}	2.60±0.46^{b}	2.35±0.25^{bd}	1.86±0.24^{d}
C 20:1 (ω-7)	0.52±0.08^{a}	0.50±0.09^{a}	0.35±0.08^{b}	0.37±0.11^{b}	0.28±0.12^{b}	0.26±0.04^{b}
C 20:2	0.64±0.10^{a}	0.56±0.09^{ac}	0.46±0.06^{ab}	0.44±0.11^{bc}	0.45±0.19^{ab}	0.36±0.11^{b}
C 20:3 (ω-3)	0.10±0.01	0.09±0.02	0.07±0.02	0.10±0.10	0.12±0.11	0.07±0.06
C 22:0	0.05±0.02	0.09±0.02	0.10±0.04	0.16±0.15	0.18±0.16	0.10±0.06
C 20:4 (ω-6)	0.49±0.06^{a}	0.33±0.07^{ab}	0.29±0.05^{b}	0.31±0.06^{b}	0.32±0.13^{b}	0.26±0.07^{b}
C 20:4 (ω-3)	4.63±0.42^{a}	3.62±0.90^{ab}	3.09±0.35^{bc}	2.09±0.58^{de}	2.27±0.56^{ce}	1.28±0.23^{d}
C 20:5 (ω-3)	2.05±0.35^{b}	1.08±0.55^{c}	0.46±0.40^{a}	0.23±0.39^{a}	0.20±0.31^{a}	0.00±0.00^{a}
C 22:5 (ω-3)	0.76±0.12^{a}	0.51±0.17^{a}	0.47±0.07^{abc}	0.60±0.30^{ab}	0.43±0.18^{bc}	0.27±0.20^{c}
C 22:6 (ω-3)	4.39±0.75^{a}	2.86±0.95^{ab}	2.94±0.32^{b}	2.72±0.73^{b}	2.93±0.53^{b}	2.32±0.65^{b}
∑ SFA	20.58±1.05^{ab}	22.88±1.44^{a}	19.87±0.73^{b}	19.34±1.92^{bc}	17.45±1.14^{c}	15.28±1.07^{d}
∑ MUFA	43.52±1.73^{a}	43.61±2.03^{a}	47.18±2.31^{ab}	47.93±3.64^{b}	50.58±2.02^{b}	55.28±1.62^{d}
∑ PUFA	26.49±1.78^{d}	22.26±2.09^{a}	21.94±1.01^{ab}	19.97±2.24^{bc}	21.37±1.48^{ac}	20.24±1.10^{ac}
∑ ω-3	14.23±1.65^{d}	10.44±1.87^{a}	8.98±1.08^{ab}	7.29±1.15^{bc}	7.35±1.20^{bc}	5.25±1.02^{c}
∑ ω-6	12.82±0.39^{a}	12.34±0.41^{a}	13.42±0.37^{a}	13.23±1.34^{a}	14.78±0.53^{b}	15.54±0.40^{b}
ω3/ω6	1.11±0.12^{d}	0.84±0.14^{e}	0.67±0.09^{a}	0.55±0.07^{ab}	0.50±0.08^{bc}	0.34±0.07^{c}
(18:1 ω-9)/(EPA+DHA	5.20±0.68	5.18±1.24	6.93±2.27	7.29±2.16	10.13±1.83	7.73±1.11
PUFA/SFA	0.72±0.06^{a}	0.59±0.12^{ab}	0.46±0.12^{ab}	0.40±0.13^{b}	0.43±0.14^{b}	0.36±0.14^{b}

Abbreviations: SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids.

Mean ± standard error, n = 9 fish per diet. Different letters denote statistically significant difference ($P< 0.05$).

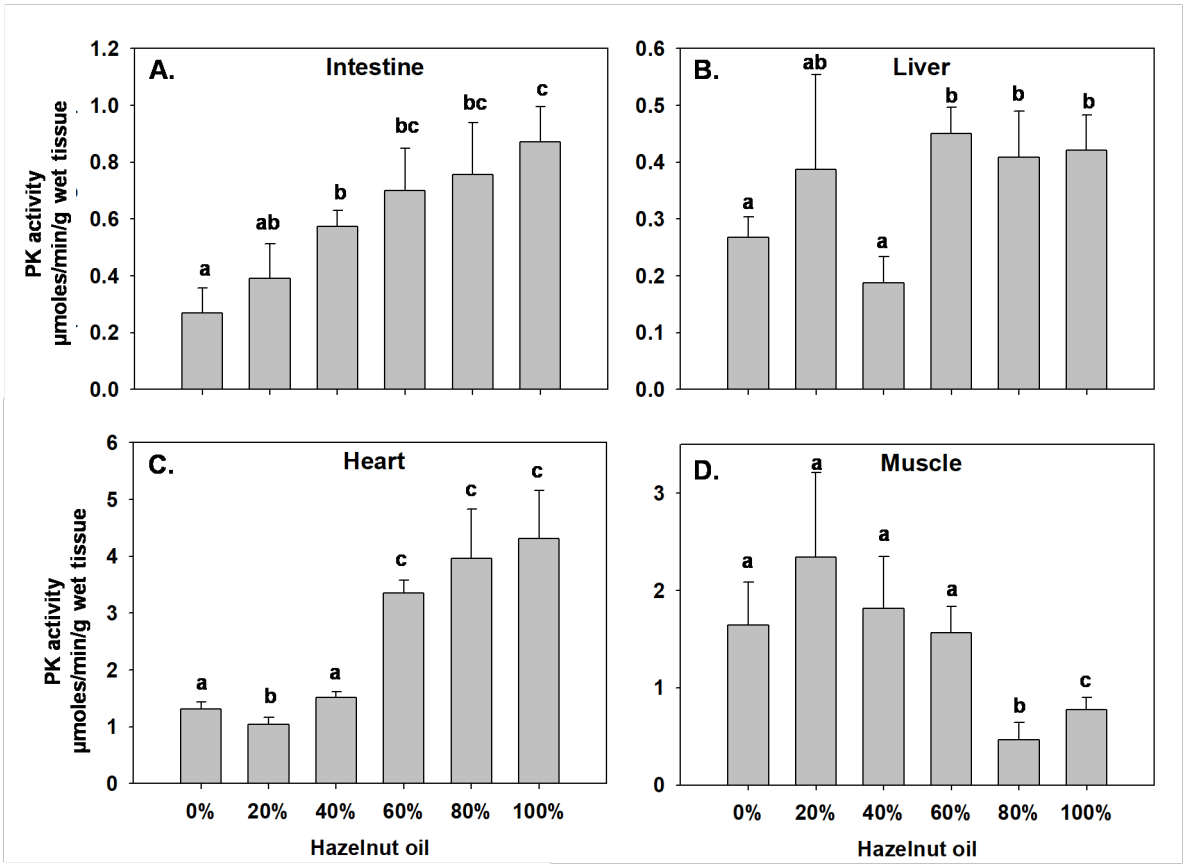
902 **Figure legends**



903

904 **Figure 1.** Effect of fish oil (FO) substitution by hazelnut oil (HO) (0, 20, 40, 60, 80 and 100%) on CS
905 activity in the intestine (A), liver (B), heart (C) and muscle (D) of meagre. Different letters denote
906 significant difference at $P < 0.05$.

907



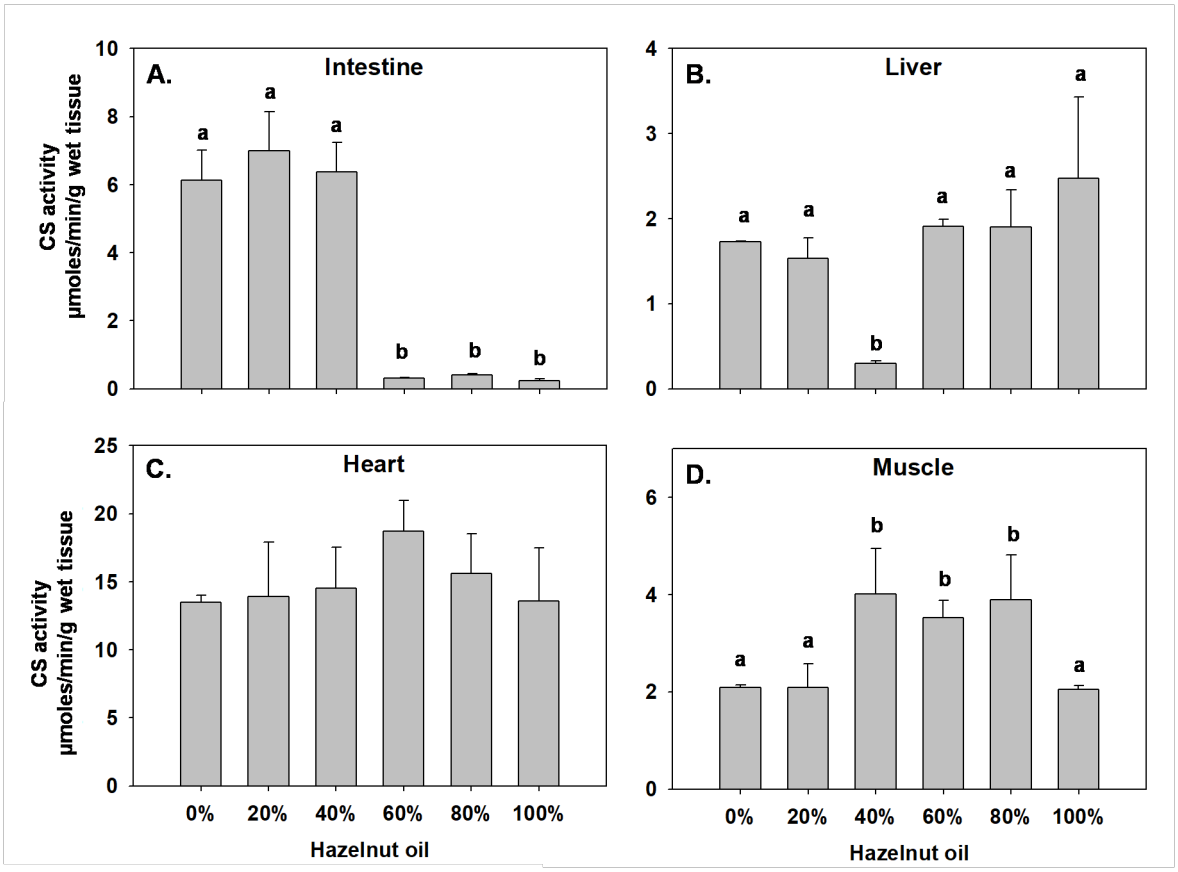
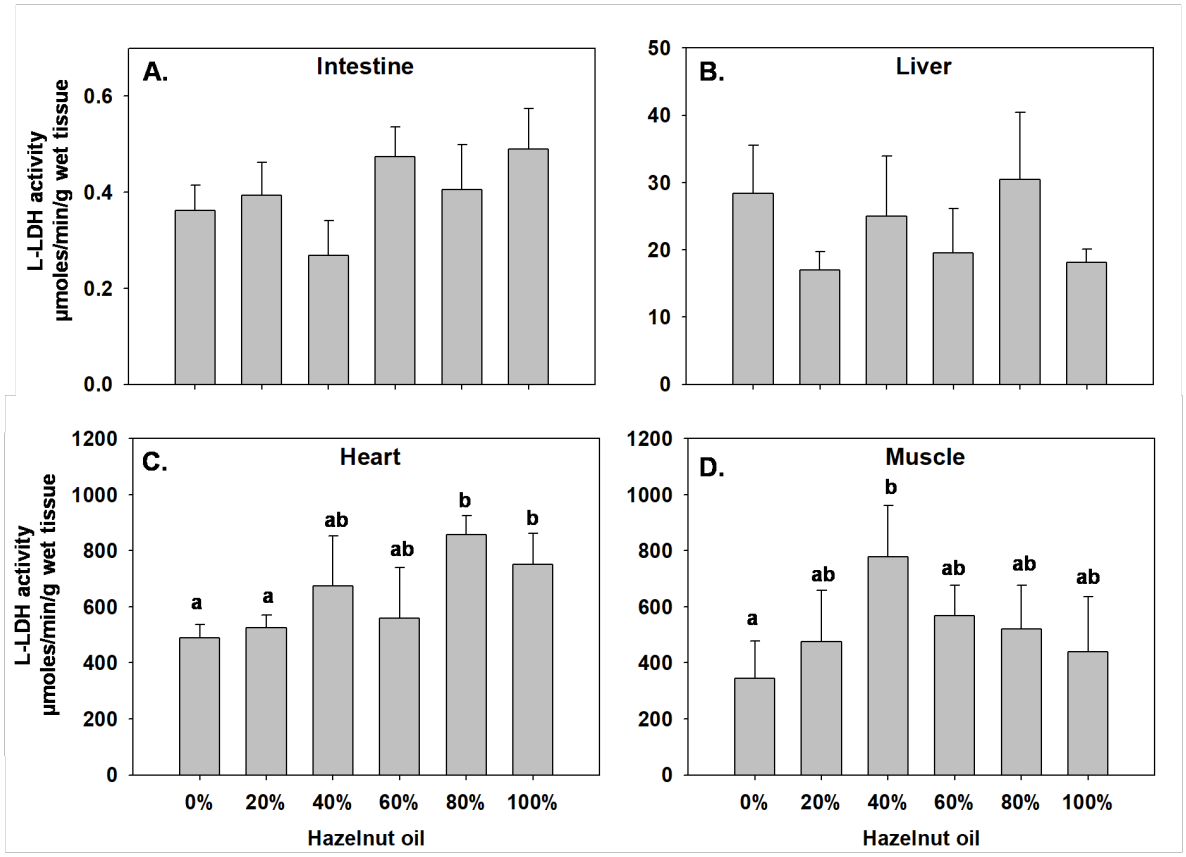
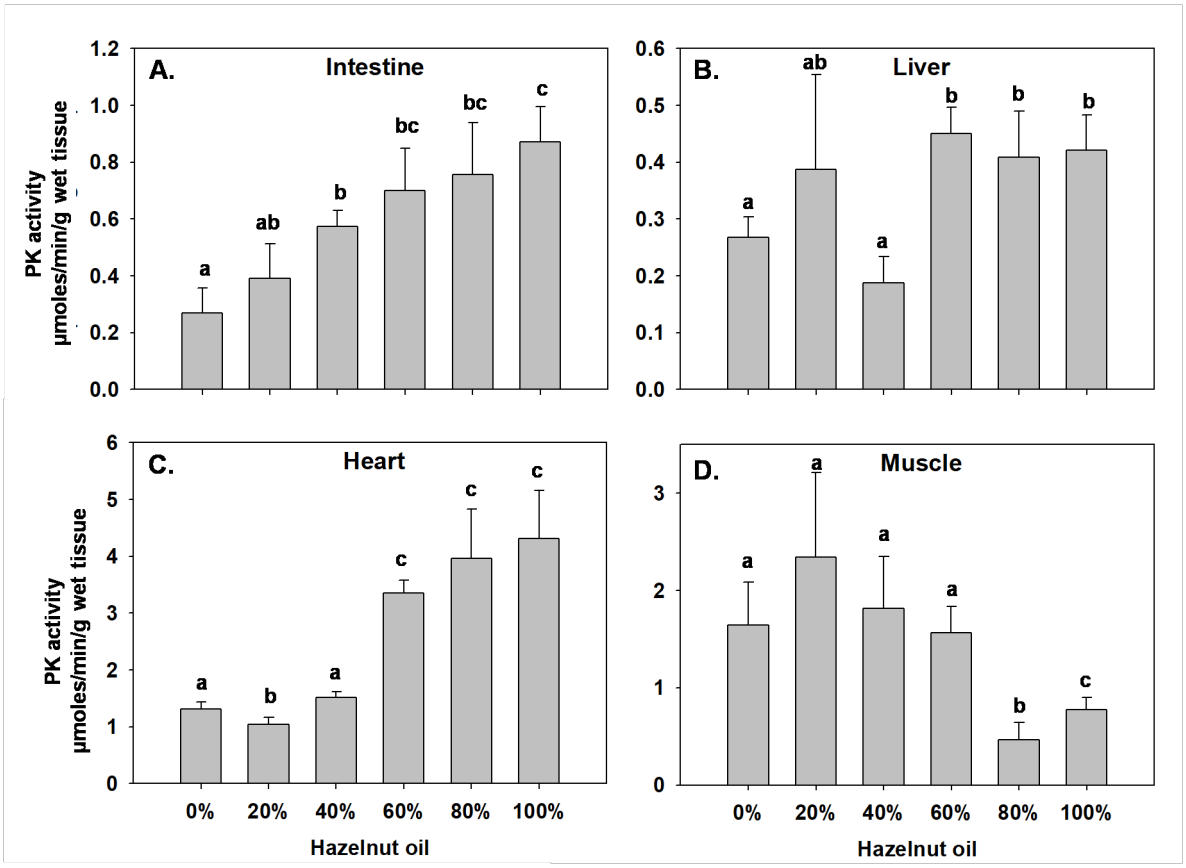


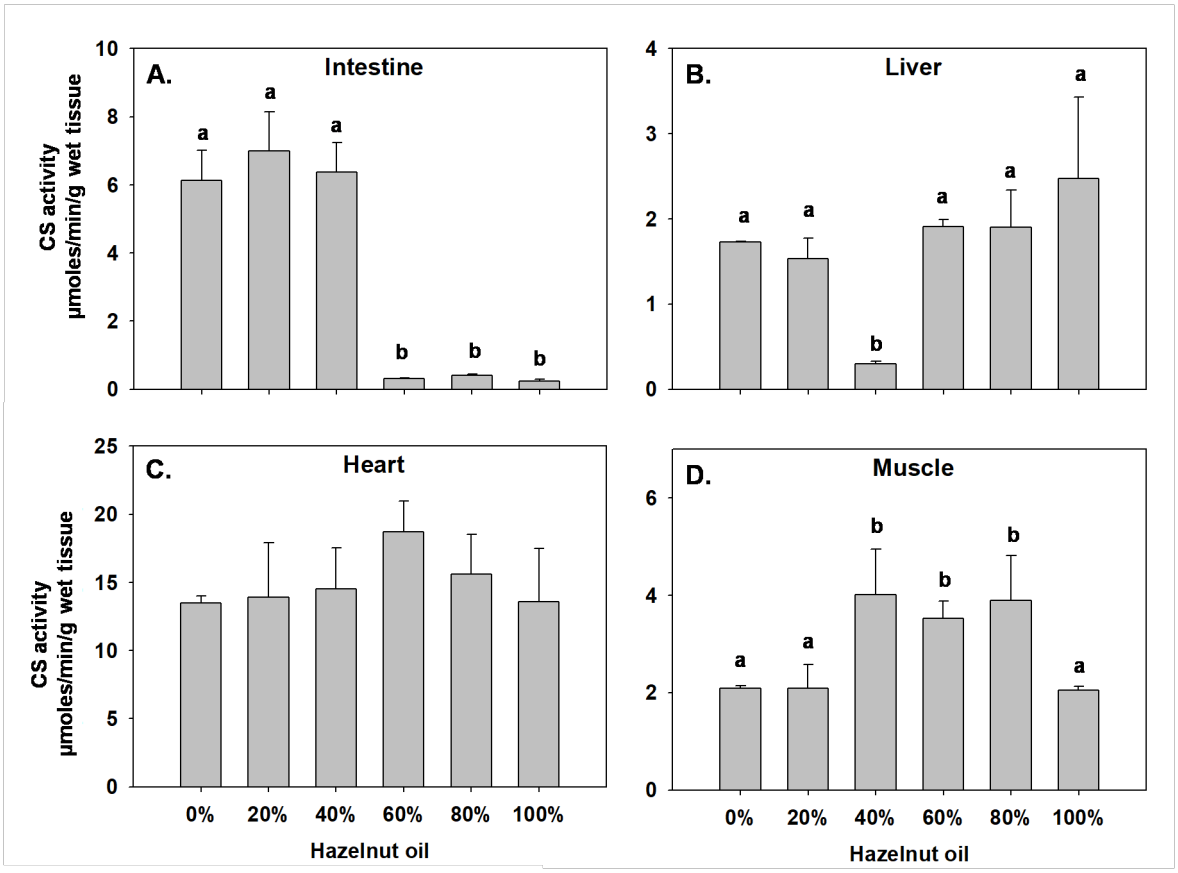
Figure 2. Effect of fish oil (FO) substitution by hazelnut oil (HO) (0, 20, 40, 60, 80 and 100%) on PK activity in the intestine (A), liver (B), heart (C) and muscle (D) of meagre. Different letters denote significant difference at $P < 0.05$.


A.
Intestine
B.
Liver
C.
Heart
D.
Muscle
L-LDH activity
µmoles/min/g wet tissue
0.0
0.2
0.4
0.6
0
10
20
30
40
50
0
200
400
600
800
1000
1200
a
a
ab
ab
b
b
a
ab
b
ab
ab
ab
0%
20%
40%
60%
80%
100%
Hazelnut oil

913

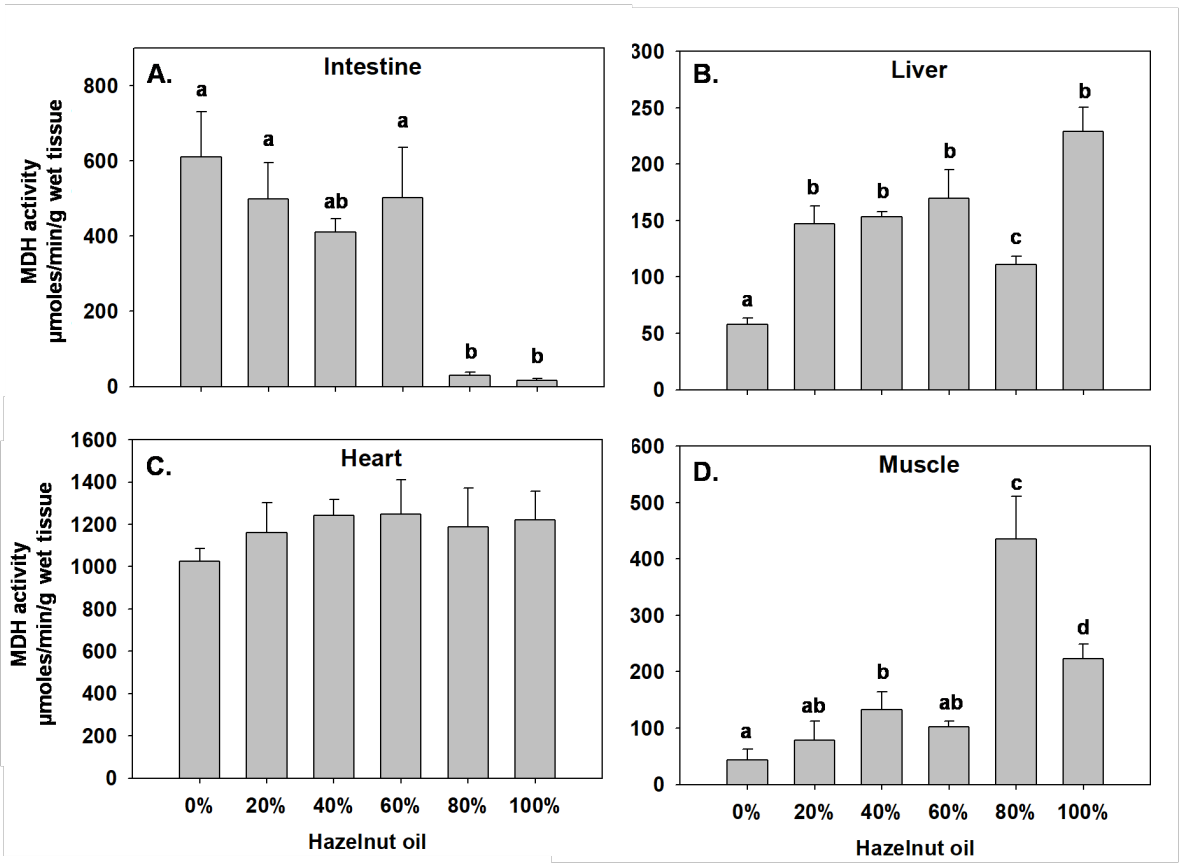

A.
Intestine
B.
Liver
C.
Heart
D.
Muscle
PK activity
µmoles/min/g wet tissue
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
1
2
3
4
5
6
0
1
2
3
a
ab
b
bc
bc
c
a
ab
a
b
b
b
a
b
a
c
c
c
a
a
a
a
b
c
0%
20%
40%
60%
80%
100%
Hazelnut oil

914

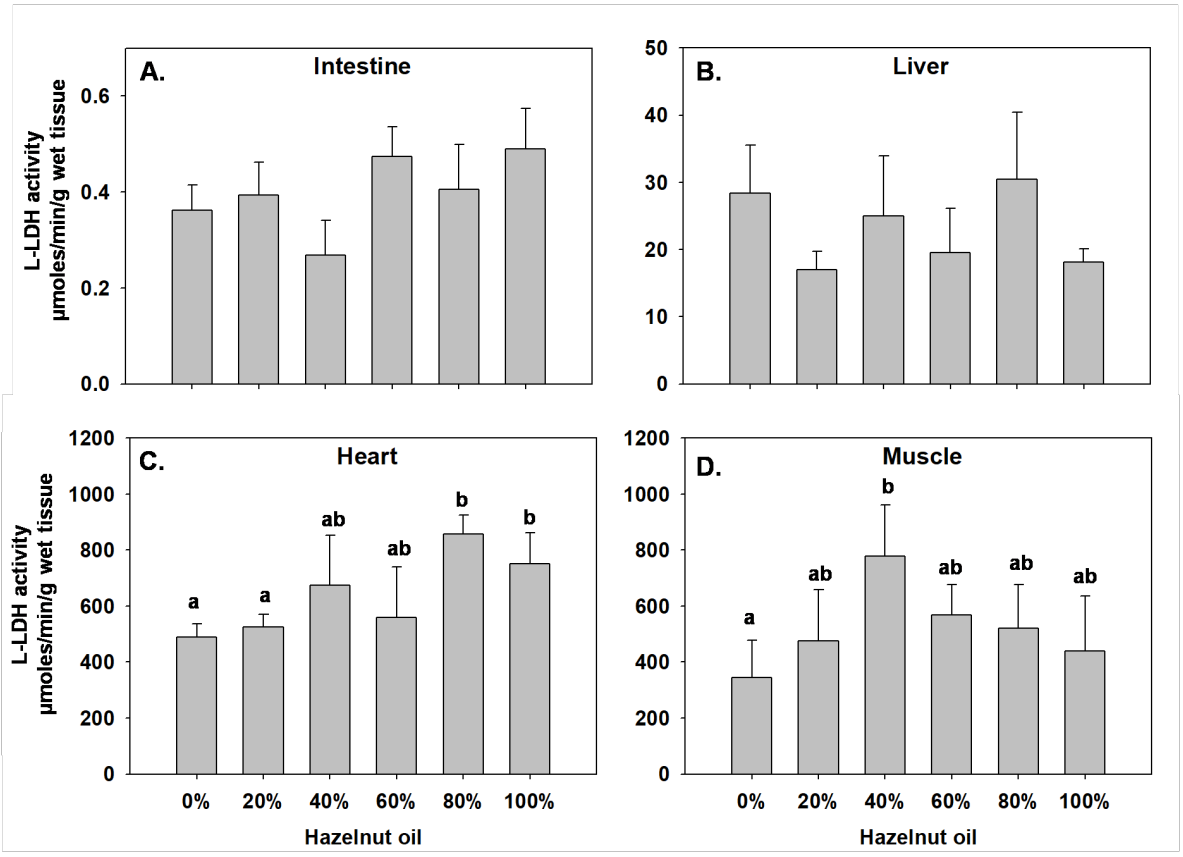


915

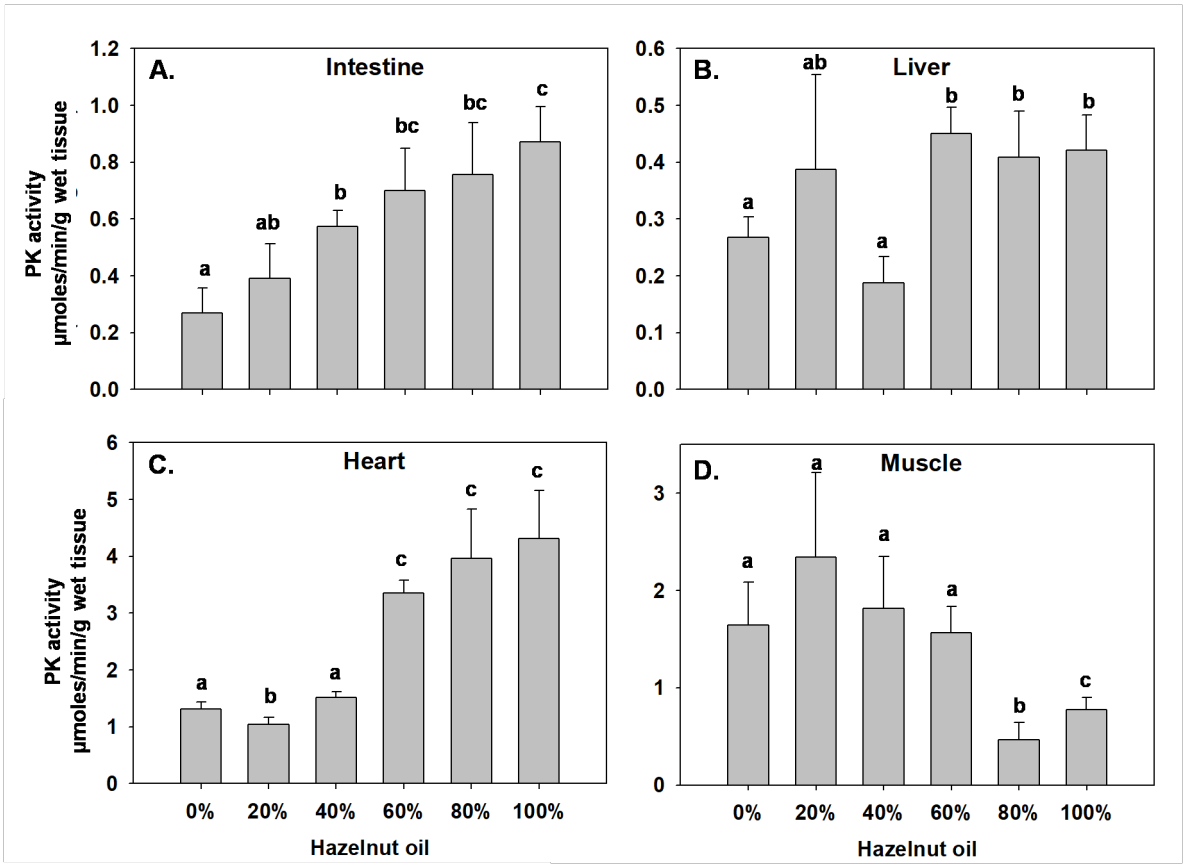
916 **Figure 3.** Effect of fish oil (FO) substitution by hazelnut oil (HO) (0, 20, 40, 60, 80 and 100%) on L-
917 LDH activity in the intestine (A), liver (B), heart (C) and muscle (D) of meagre. Different letters denote
918 significant difference at $P < 0.05$.


A.
Intestine
B.
Liver
C.
Heart
D.
Muscle
MDH activity
µmoles/min/g wet tissue
0%
20%
40%
60%
80%
100%
Hazelnut oil

919


A.
Intestine
B.
Liver
C.
Heart
D.
Muscle
L-LDH activity
µmoles/min/g wet tissue
0%
20%
40%
60%
80%
100%
Hazelnut oil

920



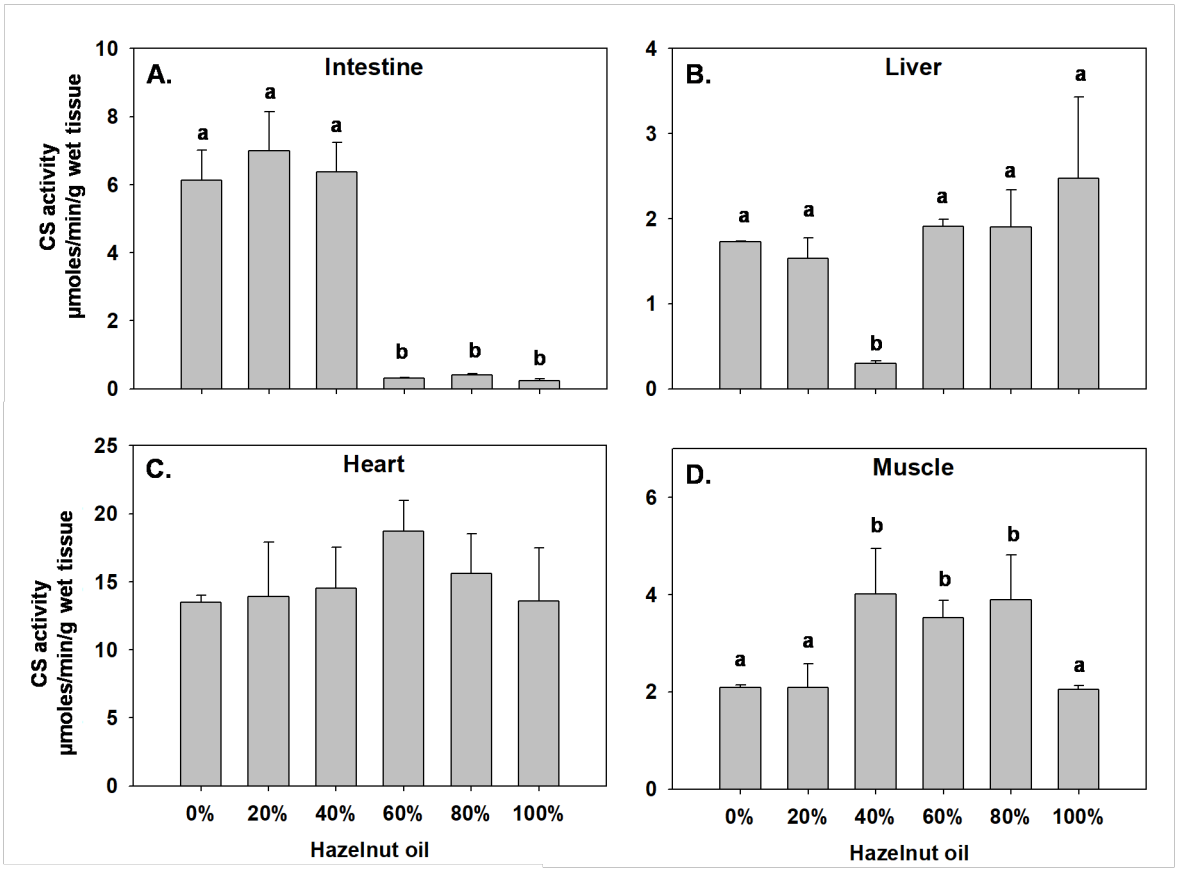
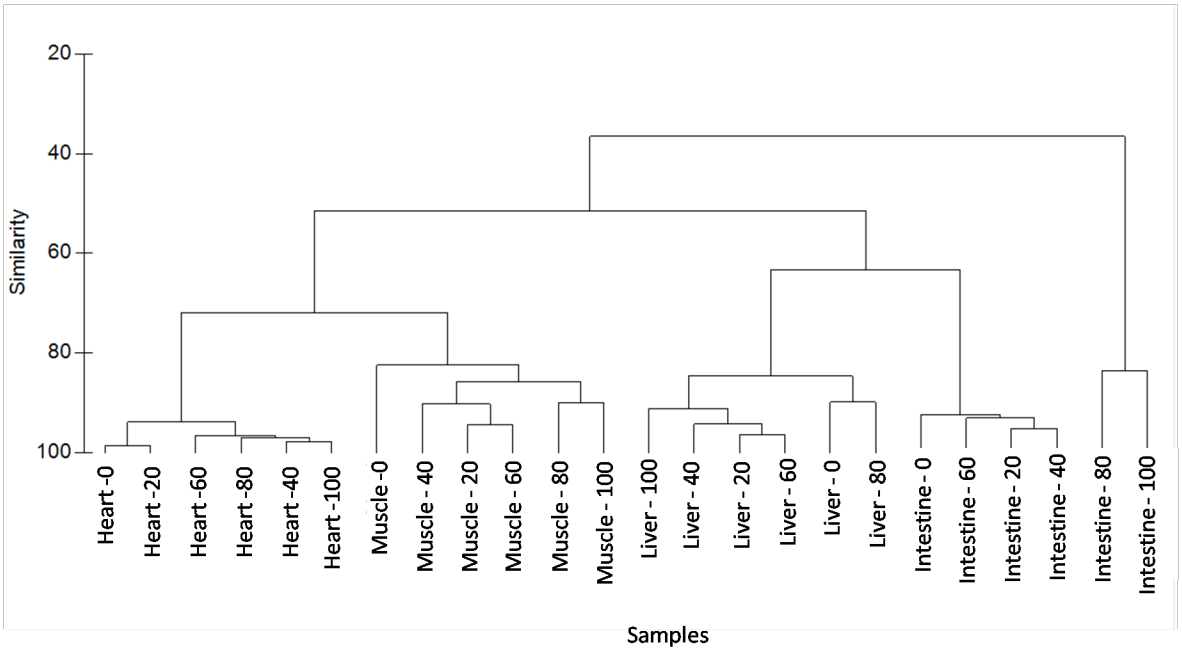
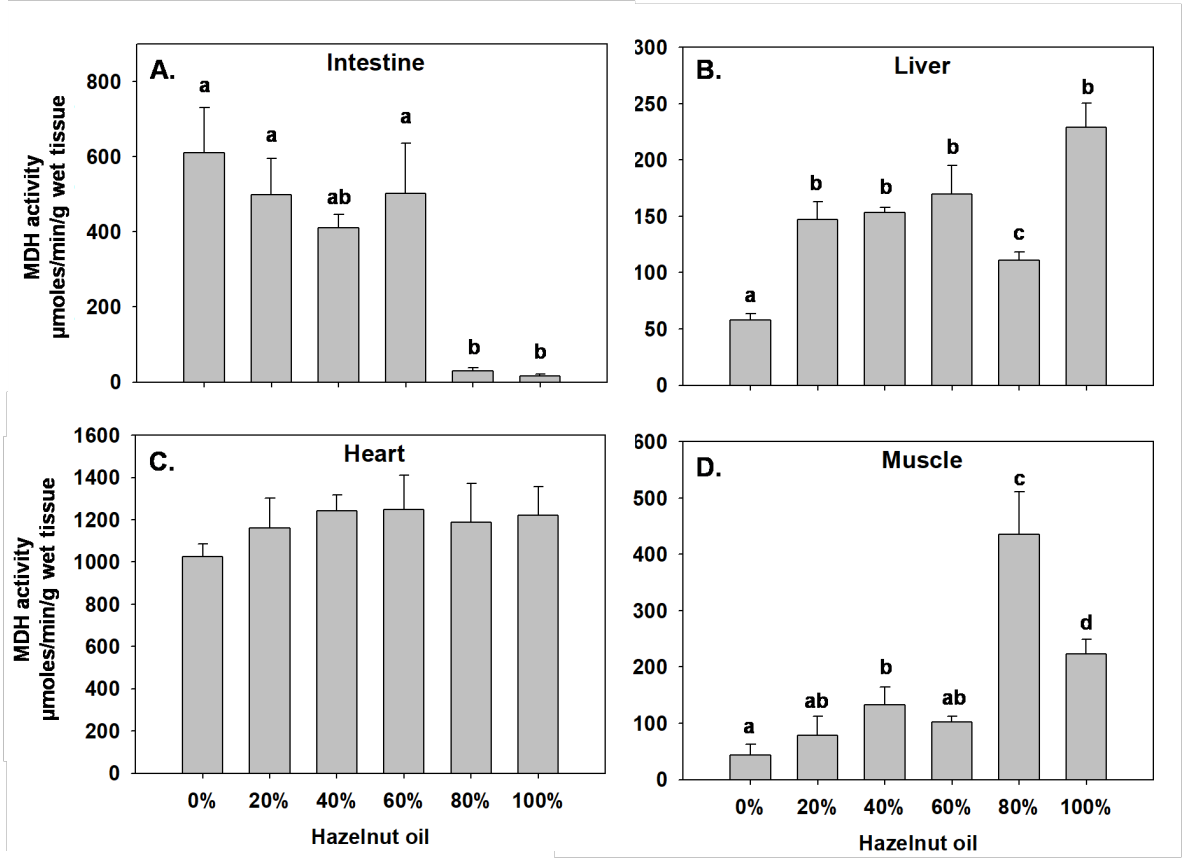


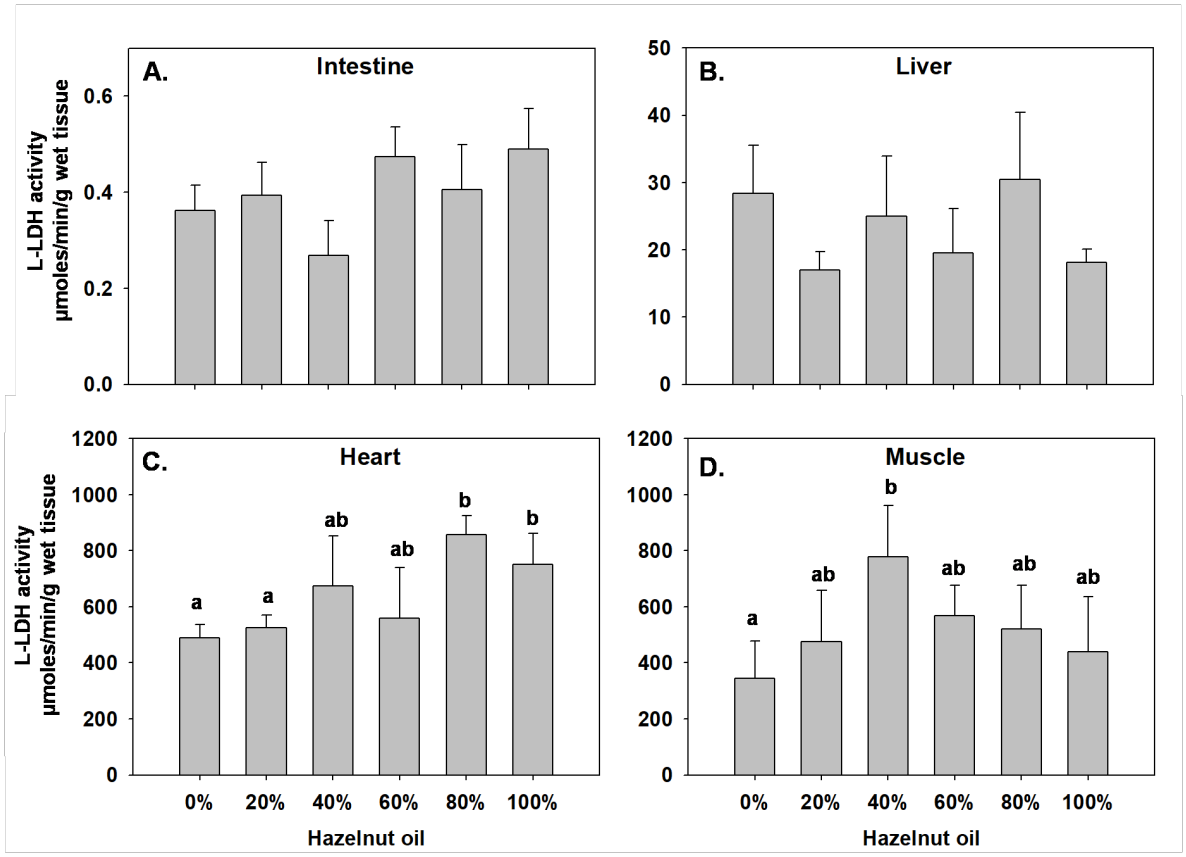
Figure 4. Effect of fish oil (FO) substitution by hazelnut oil (HO) (0, 20, 40, 60, 80 and 100%) on MDH activity in the intestine (A), liver (B), heart (C) and muscle (D) of meagre. Different letters denote significant difference at $P < 0.05$.


Similarity
20
40
60
80
100
Heart -0
Heart -20
Heart -60
Heart -80
Heart -40
Heart -100
Muscle -0
Muscle - 40
Muscle - 20
Muscle - 60
Muscle - 80
Muscle - 100
Liver - 100
Liver - 40
Liver - 20
Liver - 60
Liver - 0
Liver - 80
Intestine - 0
Intestine - 60
Intestine - 20
Intestine - 40
Intestine - 80
Intestine - 100
Samples

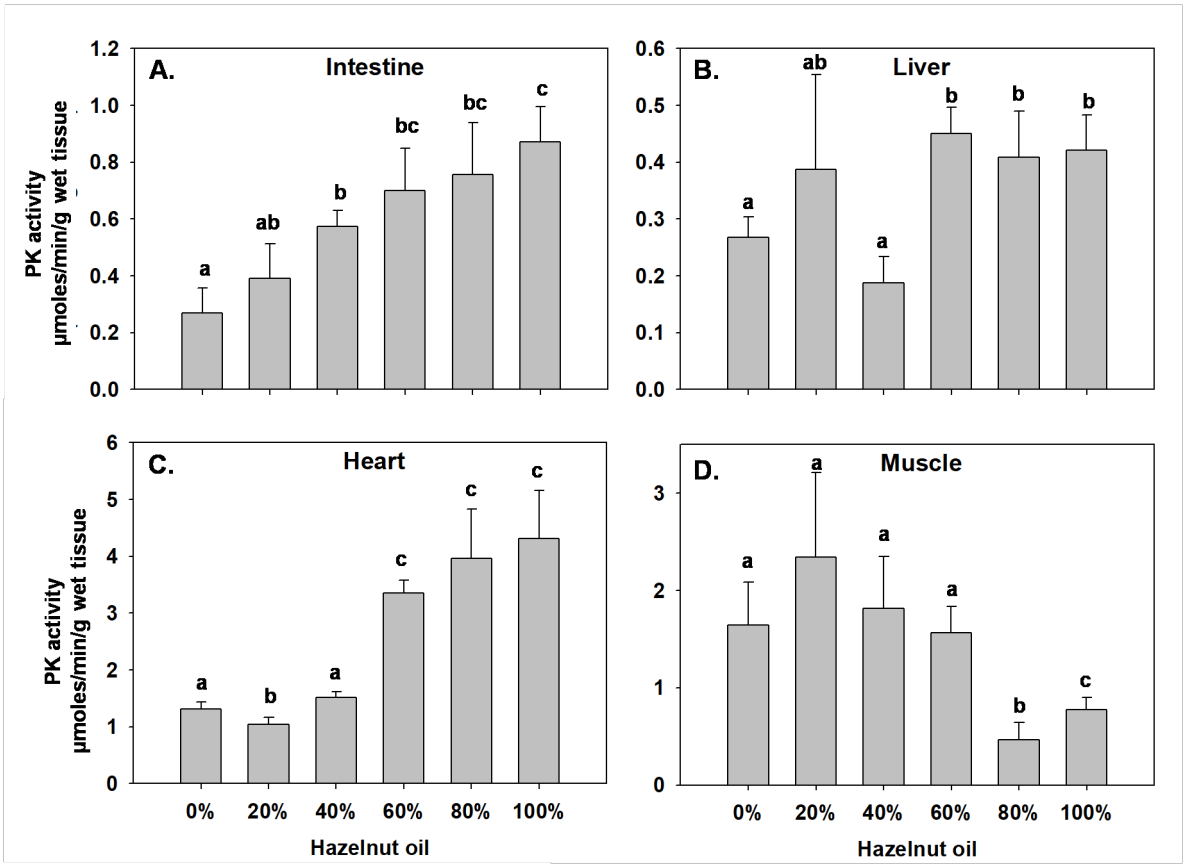
926


A.
Intestine
B.
Liver
C.
Heart
D.
Muscle
MDH activity
µmoles/min/g wet tissue
Hazelnut oil
0%
20%
40%
60%
80%
100%

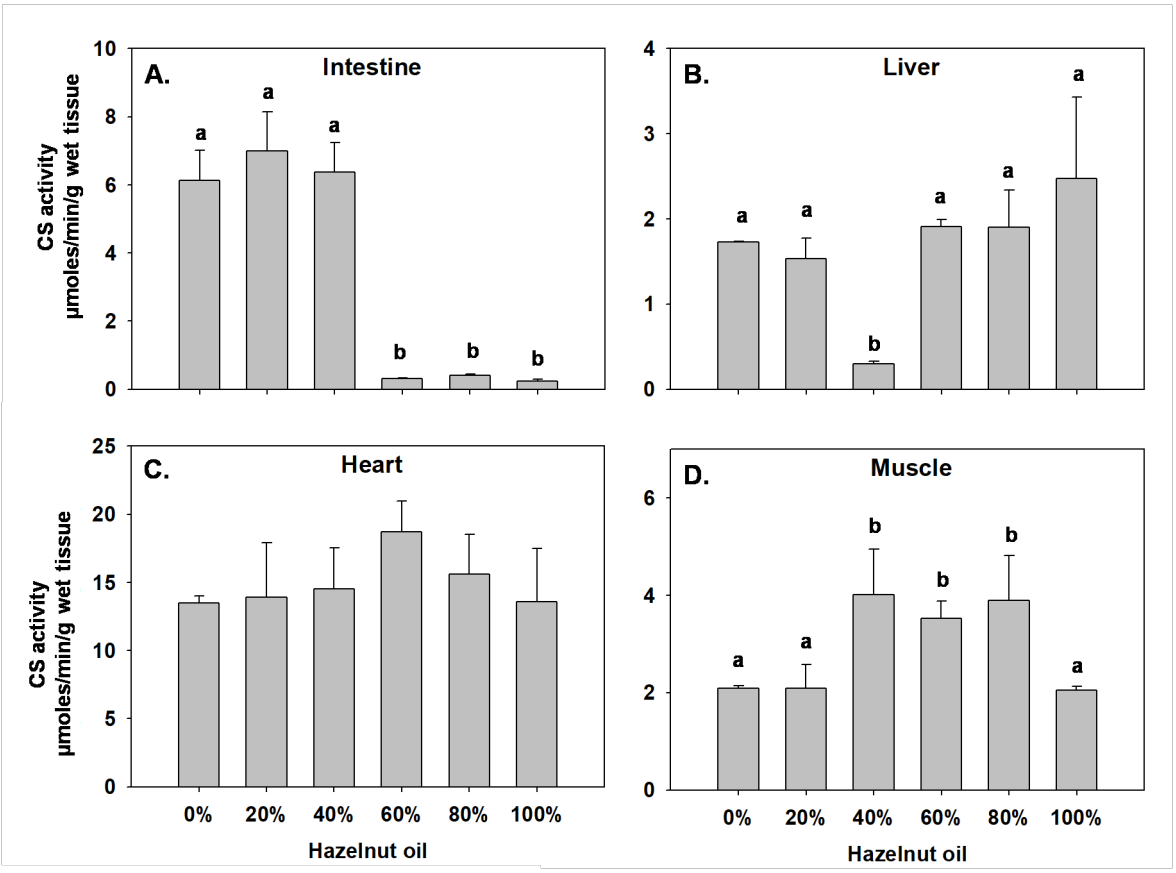
927


A.
Intestine
B.
Liver
C.
Heart
D.
Muscle
L-LDH activity
µmoles/min/g wet tissue
0.0
0.2
0.4
0.6
0
10
20
30
40
50
0
200
400
600
800
1000
1200
a
a
ab
ab
b
b
a
ab
b
ab
ab
ab
0%
20%
40%
60%
80%
100%
Hazelnut oil

928


A.
Intestine
B.
Liver
C.
Heart
D.
Muscle
PK activity
µmoles/min/g wet tissue
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
1
2
3
4
5
6
0
1
2
3
a
ab
b
bc
bc
c
a
ab
a
b
b
b
a
b
a
c
c
c
a
a
a
a
b
c
0%
20%
40%
60%
80%
100%
Hazelnut oil

929



930
931 **Figure 5.** Dendrogram from the cluster analysis, where the x' axis contains the tissue samples for each
932 feeding group (0, 20, 40, 60, 80 and 100% hazelnut-HO concentration), while the axis y' (similarity) is
933 shown in descending order from the bottom up to the level of similarity of the samples with respect
934 to the variables. The activity levels of the enzymes CS, PK, L-LDH and MDH were determined.
935