Utilization of grape seed oil as a dietary lipid source in rainbow trout (*Oncorhynchus mykiss*) diets

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Abstract

A 60-day feeding trial was conducted to determine the effects of different levels of grape seed oil (GO) on growth performance, digestive enzymes activity, fillet proximate and fatty acid composition of rainbow trout (*Oncorhynchus mykiss*) juveniles (40.17 ± 0.04 g). Five experimental diets were formulated where fish oil (FO) was replaced with 0 (D1), 25 (D2), 50 (D3), 75 (D4) and 100 (D5) % GO. Growth performance was significantly improved with increasing GO levels up to 50% after which fish growth decreased ($P < 0.05$). Fillet fatty acid composition was affected by the inclusion level of GO in diets; in particular, n-6 PUFA levels increased with increasing GO in diets, while n-3 HUFA levels, especially EPA and DHA, significantly decreased ($P < 0.05$). Fish fed on diets containing higher levels of GO revealed a decrease in α-amylase activity, whereas trypsin, total alkaline protease, and lipase activities increased significantly with increasing GO levels up to 50% and then decreased ($P < 0.05$). Based on the findings of the present study, it could be concluded that GO could be included in diets for rainbow trout up to 50% where it had the best performance over the other diets tested in the present experiment.

Keywords: Fatty acid; fish oil; grape seed oil; digestive enzymes; rainbow trout

Abbreviations: FO, Fish oil; GO, Grape seed oil; SGR, Specific growth rate; FCR, Feed conversion ratio; VSI, Viscero somatic index; HSI, Hepato somatic index; IW, Initial weight; FW, Final weight; WG, Weight gain; LER, Lipid efficiency ratio; PER, Protein efficiency ratio; FER, Feed efficiency ratio; CF, Condition factor; PUFA, Polyunsaturated fatty acid; HUFA, Highly unsaturated fatty acid; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid; TAP, Total alkaline protease; LC, Long chain; ARA, Arachidonic acid; VO, Vegetable oil; LA, Linoleic acid; LNA, Linolenic acid; PC, Pyloric caeca; I, intestine; FA, Fatty acid; BHT, Butylated hydroxyl toluene; FAME, Fatty acid methyl esters; FID, Flame ionization

detector; BAPNA, N-α-Benzoyl-DL-arginine-4-nitroanilide hydrochloride; ANOVA, Analysis of variance; SPSS, Statistical package for social sciences; MUFA, Mono unsaturated fatty acid; SFA, Saturated fatty acid; PA, Palmitic acid; SA, Stearic acid; OA, Oleic acid, MCFA; mid chain fatty acid

Introduction

Social acceptability of the aquaculture industry growth requires its sustainable development. For achieving this goal, a reliable supply of effective feeds is a prerequisite for fish farming (Piedecausa et al. 2007; Nogales-Merida et al. 2017). Although fish oil (FO) is considered as the main source of high quality lipids in aqua-feeds due to its high content in essential fatty acids and high digestibility, FO production cannot sustain the growing aquaculture industry, which is forecasted to grow dramatically over the next decades (Subasinghe 2017). During the last years, an extensive research has been conducted by the academia and the industry in order to screen and evaluate the potential use of alternative oil sources that guarantee the partial or complete replacement of FO in fish diets. In this context, the production of vegetal oil (VO) sources has dramatically increased in recent years, reaching volumes 100 times than that of FO. Therefore, the substitution of FO with alternative oils could be a viable option for the sustainable development of aquaculture industries (Olsen 2011; Sheperd and Jackson 2013). Vegetal oils are generally considered as valuable ingredients due to their relatively low cost and stable production (Kenari et al. 2011). From a nutrition point of view, VOs are rich in C18 polyunsaturated fatty acids (PUFA) such as linoleic acid (LA; 18:2n-6) and α-linolenic acid (LNA; 18:3n-3), but the main drawback of using VOs as the main lipid source is their fatty acid (FA) profile, which has a different n3:n6 ratio in comparison to FO that is considered as the gold standard in feed formulation. In addition, VOs lack long chain HUFA, particularly eicosapentanoic acid (EPA; 20:5n-3) and

docosahexanoic acid (DHA; 22:6n-3). Biosynthesis of LC-HUFA has been deeply investigated in fish, and it has been shown that many freshwater species are able to convert dietary LA and LNA to HUFA, such as EPA, DHA and arachidonic acid (ARA; 20:4n-6) (Tocher 2003). Although several studies on freshwater fish have reported that FO can be successfully replaced by different VOs without affecting growth performance in fish (Kutluyer et al. 2017; Nayak et al. 2017; Ayisi et al. 2018; Yıldız et al. 2018, among others), the inclusion of VOs in aqua-feeds affects the nutritional quality of the fish by modifying the fatty acid composition of the flesh with an increase in LA and LNA and a decrease in n-3 HUFA (Turchini et al. 2009; Tocher 2010). The above-mentioned changes in the fillet fatty acid profile may be restored by means of the use of finishing diets at the end of the on-growing phase prior to fish harvest (Glencross et al. 2003; Trushenski and Boesenberg 2009)

The grape (*Vitis vinifera*) is one of the world's largest fruit crops (Maier et al. 2009). About 80% of the total crop is used in the wine-making industry, yielding by-products, which include grape skins and seeds (Valiente et al. 1995). Grape seed is valuable for oil extraction that it typically contain 8–15% (w/w) of oil with high levels of unsaturated fatty acids, namely oleic acid (18:1n-9) and LA (Crews et al. 2006). According to Roberts et al. (2008), grape seed oil (GO) does not contain cholesterol and it has a higher ratio of unsaturated to saturated fatty acids than animal fats. In addition, GO spreads and mixes well with different feed ingredients (Arvanitoyannis et al. 2006), which makes GO a potential ingredient for aqua-feeds. Commercial diets for rainbow trout (*Oncorhynchus mykiss*) mostly contain FO as the main lipid source. Therefore, successful replacement of FO with GO would be an alternative for alleviating the absolute dependence on this ingredient and reducing its associated costs. The aim of present study was to investigate the effects of dietary increasing levels of GO replacing FO on rainbow trout growth performance, activity of digestive enzymes, fillet proximate composition and fatty acid profile.

Materials and methods

Experimental diets

Five experimental diets were formulated to be isonitrogenous (ca. 50% crude protein), isoenergetic (ca. 17 MJ kg^{-1}) and isolipidic (ca. 18% crude lipid). Lipids used in diets were FO (anchovy oil) and GO. The control diet contained only FO as the primary lipid source (D1), whereas in the others, FO was partially or totally replaced by GO at 25% (D2), 50% (D3), 75% (D4) and 100% (D5), respectively. The dietary ingredients and proximate compositions are given in Table 1, whereas their fatty acid profile is presented in Table 2. The experimental diets were prepared (2.5 mm pellet diameter) following standardized procedures as previously reported (Brown et al. 2010).

Animals and husbandry

Rainbow trout juveniles with a mean initial body weight (BW) of 40.17 ± 0.04 g (mean ± standard deviation) were obtained from the Fish Culture Center (Borujerd, Lorestan, Iran) and transported to the Sharifabad facility (Malayer, Hamedan, Iran) in a 2,000 L-container. Fish were randomly distributed into fifteen 1,500 L cylindroconical tanks (30 fish per tank) connected to an open-flow water system (3 replicates per each diet). Experimental conditions were as follows: 12:12 h light:dark cycle photoperiod, water temperature at 15.0 ± 1.5°C and mean oxygen concentration of 9.6 ± 0.1 mg L^{-1} (WTW, Multi 3410, Weilheim, Germany). Water quality parameters (pH, ammonia and nitrites) were measured two times per week using Aquamerck test kits (Merck, Darmstadt, Germany); mean water pH values were 8.2 ± 0.1, whereas levels of ammonia and nitrites were below 0.1 mg L^{-1}.

Before the onset of the trial, fish were acclimatized to experimental conditions using a commercial diet (crude protein 44%, crude lipid 16%, ash 7 %, crude fiber 2% and moisture 5%, FFT2, Faradaneh, Iran) for 2 weeks. During this period, fish were hand-fed twice a day

up to apparent satiation. After that, fish fed one of the tested diets at a feeding ration of 2% of their body weight (apparent satiation), which divided into two equal portions and given to fish for 60 days.

Sampling procedure

At the beginning of the experiment and every 2 weeks, all fish from each tank were captured with a dipnet, anaesthetized using clove oil (30 mg L^{-1}) (Velisek et al. 2005), their BW measured to the nearest 0.1 g and then returned to their respective tanks and feed ratio was adjusted accordingly. Daily feed intake was recorded by collection of uneaten feed from the effluent water and calculated by the difference between the amount of feed distributed and the quantity of collected uneaten feed pellets as described in Helland et al. (1996). At the end of the experimental period, fish were fasted for 24 h, anaesthetized and individually measured for BW and standard length (SL) to the nearest 0.1 g and 1 mm, respectively. Then, a sample of 10 fish per tank (30 fish per dietary group) was randomly selected, sacrificed with an overdose of clove oil and stored at -80°C for fillet proximate analysis and fatty acid composition. The remaining 20 fish per tank were sacrificed as described above in order to evaluate their hepatosomatic (HSI) and viscerosomatic (VSI) indexes, as well as evaluate the activity of selected pancreatic digestive enzymes.

The following standard formulae were used to calculate different growth and feed utilization parameters:

- Weight Gain (WG, g) = final weight (g) – initial weight (g);

- Specific Growth Rate (SGR, % body weight day^{-1}) = 100 [(Ln final weight (g) - Ln initial weight (g) / time (days)];

- Feed Conversion Ratio (FCR) = feed intake / fish weight gain;

- Condition Factor (CF) = 100 × final weight (g) / [total length (cm)]^3;

Feed intake (FI, g fish^{-1} 60 days^{-1}) = total dry feed given / no. of fish;

Survival rate (SR, %) = 100 × final number / initial number;

- Hepatosomatic Index (HSI, %) = 100 × (liver weight) / [final weight (g)];

- Viscerosomatic Index (VSI, %) = 100 × (viscera weight) / [final weight (g)];

- Lipid Efficiency Ratio (LER) = weight gain / total amount of lipid ingested;

- Protein Efficiency Ratio (PER) = weight gain / total amount of protein ingested;

Proximate and fatty acid analyses

The proximate composition of the experimental diets and fish fillets were performed according to standard procedures Association of Official Analytical Chemists (AOAC) (2005). Briefly, moisture content was obtained by weight loss after drying samples in an oven (Memmert Universal Oven, UN30) at 105 °C until they reached a constant weight. Protein was determined by measuring nitrogen, using the Kjeldahl (Foss, model: KjeltecTM 2300, Foss Tecator, Hoganas, Sweden) technique (N × 6.25). Total lipids were extracted by n-hexane using the Soxhlet method (Foss, Soxtec^{TM} 2050, Foss Tecator) and ash content was determined for each dried sample after incineration in a muffle furnace (Nabertherm model: K, Nabertherm GmbH, Bremen, Germany) at 550 °C for 5h. Feed energy contents were calculated on gross energy values of 23.6 MJ kg $^{-1}$ protein, 39.5 MJ kg $^{-1}$ fat and 17.2 MJ kg $^{-1}$ carbohydrates (National Research Council (NRC) 1993).

Fatty acid analysis was performed in triplicate for each experimental diet and fillet samples. Total lipids from feed samples and fillets were extracted by homogenization in

chloroform/methanol (2:1, v/v) containing 0.01% BHT as antioxidant, according to the method of Folch et al. (1957). Fatty acid methyl esters (FAME) in samples were analyzed using a Philips PU 4400 gas chromatograph (Phillips Scientific, Cambridge, United Kingdom) equipped with a fused silica capillary column BPX-70 (30 m × 0.25 mm, film thickness of 0.22 μm) and a flame ionization detector. The carrier gas and split rate were helium and 1/100, respectively. The temperature program included a gradient from 140 up to 250 °C with an increase rate of 1.5°C min^{-1}. FAME levels were determined by comparison of their retention times with commercial standards (Sigma, St. Louis, MO, USA).

Activity of pancreatic digestive enzymes

For determination of digestive enzyme activities, fish (n = 10) were dissected on chilled trays and their digestive tract were excised and the adherent adipose and connective tissues were removed. Then, the pyloric caeca (PC) and intestine (I) were separated and immediately frozen in liquid nitrogen and stored at -80°C until analysis. Frozen PC and I were partially thawed in the refrigerator at 4 °C for 2 h. Samples were homogenized by separate (dilution 1:20, w/v) in cold buffer (50 mM Tris-HCl buffer, pH 8.0 containing 10 mM $CaCl_2$) on ice at 11,000 rpm for 2 min. Thereafter, the homogenate was centrifuged at 14,000 g for 45 min at 4 °C. The resultant supernatants were collected and aliquots were stored at -80 °C until digestive enzyme analysis following the recommendations provided by Solovyev and Gisbert (2016). For each enzyme activity, assay dilution tests were previously done to ensure optimum ratio between enzyme and substrate (Zamani et al. 2014). All enzyme activities were measured at 37 °C by spectrophotometer (UV/VS Ultro Spec 2000, Pharmacia Biotech, Canada). The specific assay conditions for each enzyme were as follows:

Trypsin (EC 3.4.21.4) activity was determined using BAPNA as substrate according to the method of Erlanger et al. (1961). One unit of activity was defined as the enzyme releasing

1μmol p-nitroaniline per minute at λ = 410 nm. Alpha-amylase (EC 3.2.1.1) activity was estimated using the Bernfeld's (1951) procedure using starch as substrate. One unit of activity was defined as 1μmole of maltose released per minute and absorbance was measured at λ = 540 nm. Bile salt-activated lipase (EC 3.1.1.3) activity was measured by assessing the hydrolysis of ρ-nitrophenyl myristate as substrate according to method of Iijima et al. (1998). One unit of enzyme activity was defined as 1μmol of ρ-nitrophenol released per minute at λ = 405 nm. Total alkaline protease activity (TAP) was determined based on the assay of Kunitz (1947) modified by Walter (1984) using casein as substrate. One unit of activity was defined as the amount of enzyme needed to produce 1 μmol tyrosine per minute at λ = 280 nm. Data were expressed as specific activity (U mg protein^{-1}), and the concentration of soluble protein in extracts was determined by the method of Lowry et al. (1951) using bovine serum albumin (0–1 mg ml $^{-1}$) as a standard. All samples were analysed in triplicate (methodological replicates).

Statistical analyses

Data were presented as means ± standard deviation (mean ± SD), and a probability value of $P< 0.05$ was considered as significant. Following confirmation of normality and homogeneity of variance, ANOVA was performed followed by the Duncan's multiple range test when statistically significant differences were detected among experimental groups. Statistical analyses were performed using the SPSS (Version 21.0, SPSS Inc., Chicago, IL, USA). Broken-line regression method (Robbins et al. 2006) was used to determine the breakpoint that represents the optimum dietary GO requirement of rainbow trout based on weight gain values using GraphPad Prism 5 software.

Results

Fatty acid profile of experimental diets

The FA composition of experimental diets is shown in Table 2. The replacement of FO by GO significantly changed the FA profile of diets ($P < 0.05$). Diet 1 contained high levels of HUFA, especially EPA and DHA (8.0 % and 2.4%, respectively), and low levels of total n-6 PUFA (14.9%). In contrast, the D5 diet had higher levels of n-6 PUFA (31.7%), especially LA (29.9%) and lower levels of total n-3 HUFA (3.1%), as well as the lowest DHA/EPA ratio (1.1) among experimental diets. In general, the percentage of total n-6 PUFA gradually increased and the total n-3 HUFA percentage gradually decreased in experimental diets with increasing GO levels in diets ($P < 0.05$). In all experimental diets, the most abundant MUFA, PUFA and HUFA were 18:1n-9, 18:2n-6 and 20:5n-3, respectively.

Growth performance

Growth performance of rainbow trout juveniles was significantly affected by the experimental diets considered (Table 3; $P < 0.05$). In particular, the highest BW and weight gain values were recorded in fish fed D3, while lowest ones were observed in fish fed D5 ($P < 0.05$); the other dietary groups showed intermediate values. However, there were no significant differences in SGR values among groups ($P > 0.05$). When considering weight gain data, the broken-line regression analysis revealed that the optimal dietary level of GO inclusion in diets for rainbow trout juveniles was estimated at 50 % of FO replacement (Figure 2). Values of FCR were significantly lowest in fish fed D3 and highest trouts fed D5 ($P < 0.05$). Condition factor was not affected by experimental diets ($P > 0.05$), while highest HSI and VSI values were observed in fish fed D1. There were no differences in FI values among fish fed different experimental diets ($P > 0.05$), even though FI values in fish fed D5 tended to be higher that then rest of the other groups. No significant differences were found in survival rates among fish fed the experimental diets ($P > 0.05$). The highest LER value was

found in fish fed D3, whereas lowest one observed in fish fed D5 ($P < 0.05$). No differences in PER values were observed among different dietary groups ($P > 0.05$).

Fillet proximate composition and fatty acid profile

As shown in Table 4, the proximate composition of the fillet was not affected by experimental diets with the exception of moisture and crude protein contents ($P < 0.05$). Crude protein content was higher and moisture levels were lower in fish fed D5 in comparison to the other tested experimental diets ($P < 0.05$).

Although experimental diets did not affect the crude lipid content of the fillet, they modified its FA composition at the end of the grow-out phase, a change that reflected the dietary FA composition (Table 5). Levels of SFAs were similar among dietary groups, except for the fish fed D4 that showed a lower content of SFAs ($P < 0.05$). The concentrations of palmitic acid (16:0) and stearic acid (18:0) were the most abundant SFA in fish fillets in all experimental groups, whereas the highest values were found in fish fed D1. MUFAs content was lower in fish fed D5 in comparison to D1 and rest of experimental diets with partial substitution of FO by GO (D2, D3 and D4) ($P < 0.05$). The most abundant MUFA was oleic acid (18:1n-9), whereas its lowest content was found in the fillet of fish fed D5 ($P < 0.05$). The levels of PUFAs significantly increased with increasing GO levels with maximal values in PUFAs found in the fillet of the fish fed D5 ($P < 0.05$). The highest content of LNA was found in fish fed the FO diet, while lowest value was found in fish fed D5 ($P < 0.05$). The highest and the lowest of LA levels were found in fish fed D5 and D1, respectively. The levels of HUFAs decreased with increasing GO levels in diets ($P <0.05$). The amount of total fatty acids from the n-3 series progressively decreased with increasing GO levels in the tested diets ($P < 0.05$). In this sense, fish fed D5 contained the highest concentration of total fatty acids from the n-6 series.

Similarly, highest and lowest EPA and DHA levels were found in fish fed D1 and D5, respectively. The n-3/n-6 ratio decreased with increasing GO levels in the diets ($P < 0.05$); therefore, fish fed D1 had highest values of the n-3/n-6 fatty acids. The level of ARA in fish fillets fed D2 and D4 was lower than in the fillet of fish from the other groups ($P < 0.05$).

Activity of pancreatic digestive enzymes

The specific activities of trypsin, TAP, bile salt-activated lipase and α-amylase showed a similar trend when comparing their values between the samples obtained from the PC and I (Figure 1). Experimental diets had a significant effect on the specific activity of the pancreatic digestive enzymes whatever the region of the digestive tract considered ($P < 0.05$). In particular, trypsin activity in the PC was significantly higher in fish fed D3 in comparison to the other fish groups that showed similar values (Figure 1, a-PC; $P < 0.05$). In addition, highest trypsin specific activities in the intestinal region were found in fish fed D3 and D4 (Figure 1, a-I; $P < 0.05$). Similar to trypsin, highest TAP activity in the PC was found in fish fed D3, whereas no differences were found among the other groups (Figure1, b-PC; $P < 0.05$). The highest and lowest activity values of TAP in the I were found in fish fed D3 and D5, respectively, whereas the others showed intermediate values (Figure 1, b-I; $P < 0.05$). Regarding the specific activity of bile salt-activated lipase in the PC, values progressively increased with increasing GO levels in diets up to 50% (D3), whereas no differences were found between fish fed D4 and D5 (Figure 1, c-PC; $P < 0.05$). The profile of the specific activity of bile salt-activated lipase from the fish intestine was different from that reported from the PC (Figure 1, c-I). In particular, the maximal activity values were found in fish fed D3, whereas the minimal ones were recorded in fish fed D1 and D5 ($P < 0.05$). The activity of α-amylase in the fish PC was lower in fish fed D3, D4 and D5 in comparison to other diets (Figure 1, d-PC; $P < 0.05$). Regarding the activity of α-amylase in the fish intestine, its

highest activity was found in fish fed D1, whereas the lowest values were recorded in fish fed D3, D4 and D5. Fish fed D2 showed intermediate values between the above-mentioned groups (Figure 1, d-I; $P < 0.05$).

Discussion

Last decades of intense research have clearly proven that alternative vegetal protein and oil sources are valid ingredients in aqua-feeds (Gatlin et al. 2007; Sales and Glencross 2011). Within this context where the list of potential ingredients for formulating sustainable feeds for the aquaculture industry does not stop to increase; however, the present study showed that the FO can be successfully replaced with GO in rainbow trout diets without negatively affecting fish somatic growth performance nor the diets utilization.

Fish performance and feed efficiency parameters

Under present experimental conditions, the results obtained with GO in terms of growth performancae were similar to previous studies showing the viability of partially replacing dietary FO with other VOs without negatively affecting somatic growth. These results are similar to those reported using palm oil (Bell et al. 2002), cottonseed oil (Guler and Yildiz 2011), camelina (Hixson et al. 2014; Betancor et al. 2016), flaxseed and sunflower (Wijekoon et al. 2015), canola (Mozanzadeh et al. 2016), linseed (Li et al. 2016; Nayak et al. 2017) and / or a blend of vegetal oils (Piedecausa et al. 2007; Ribeiro et al. 2015; Lopes et al. 2017).

Regarding feed efficiency parameters, the replacement of FO at 75% and 100% by GO negatively affected FCR values. Considering the lack of statistical differences in FI values among rainbow trouts fed different experimental diets, different results in FCR might be attributed to a lower digestibility of D4 and D5 due to their higher levels in GO. When considering the range of FCR values obtained under the present study in rainbow trout (1.26 -

1.54), these results may be slightly higher than those found in other studies conducted in rainbow trout (Caballero et al. 2002; Kutluyer et al. 2017; Yıldız et al. 2018). Our results may be attributed to the larger size of the experimental fish, rearing conditions and/or different diet formulation.

Regarding body condition parameters, HSI and SVI values decreased as levels of GO increased in rainbow trout diets. Thus, fish fed D5 (100% GO) showed the lowest HSI and SVI values, whereas those fed D1 (control diet, 100% FO) had the highest one. When reviewing the literature, there is not a common trend regarding the impact of VOs on HIS values from different species and studies, whereas some studies described an increase in HIS values with increasing dietary levels of VOs, others reported the contrary. For instance, Caballero et al. (2002) reported that no significant differences in HSI and VSI levels were found among rainbow trout fed diets containing VOs (soybean, grapeseed, olive, and palm oils) compared to those of fish fed a FO diet. However, Guler and Yildiz (2011) found that HSI and VSI values in rainbow trout juveniles fed a diet containing 100% FO were significantly lower than those of fish fed diets containing cottonseed oils. By contrast, lower HSI values in Caspian brown trout (*Salmo trutta caspius*) (Kenari et al. 2011) and meagre (*Argyrosomus regius*) (Ribeiro et al. 2015) fed diets containing different VOs. These results may be attributed to the dietary FA profile as different studies have shown that the high dietary levels of 18:2n-6 or 18:1n-9 were apparently leading to the accumulation of these FAs, particularly in the fish liver (Rinchard et al. 2007; Yıldız et al. 2010). Biological availability of dietary lipids is directly related to their chemical and physical properties, including chain length and degree of saturation of triglyceride-bound FAs (Christie 1992). Drastic changes in the FA profile of liver as a consequence of dietary FO replacement with alternative lipid sources may result in changes in lipid metabolism and n−3 LC-PUFA deficiency, which generally leads to changes in the hepatic condition as HSI values indicated (Piedecausa et al.

2007; Mozanzadeh et al. 2016). In the current study, fish fed GO diets had the highest fillet protein content. Vegetable oils like GO, which contain high levels of midchain FAs, such as OA, may promote protein retention, because they can be efficiently oxidized and used for the production of adenosine triphosphate for energy purposes (Sargent et al. 2002; Turchini et al. 2009). In this context, Karalazos et al. (2014) also reported that substitution of dietary FO with rapeseed oil led to increasing whole body protein in Atlantic salmon (*Salmo salar*) because of higher β-oxidation capacity of reactive oxygen in fish tissues.

Fillet proximate and fatty acid composition

Based on present results, a change of the dietary lipid source had no effect on the whole body lipid content in rainbow trout. This was in agreement with previous studies involving FO substitution in salmonids. In this sense, no significant differences on body composition in Atlantic salmon (Rosenlund et al. 2001), brown trout (*Salmo trutta*) (Turchini et al. 2003) and rainbow trout (Martines et al. 2006) fed diets containing plant lipid sources. In general, the lipid content of the fillet is generally largely influenced by the dietary lipid source, as well as its FA profile (Sargent et al. 2002; Piedecausa et al. 2007; Rinchard et al. 2007). In this context, several studies have shown that the high levels of LA (18:2n-6) or OA (18:1n-9) in diets containing VOs lead to the accumulation of these FAs in fish body tissues. Thus, the FA profile of neutral lipids in the muscle closely reflects that of dietary lipids (Turchini et al. 2009; Benedito-Palos et al. 2010). However, many freshwater fish such as rainbow trout are able to convert dietary LA and LNA to HUFA, such as ARA, EPA and DHA, and therefore, FAs of the n-6 series are also required (Caballero et al. 2002; Sargent et al. 2002; Tocher 2010). In the present study, the fillet FA composition clearly reflected the lipid composition of the diet. Replacement of FO with GO, containing mostly OA and LA, resulted in a reduction in the n-3 MUFA, n-3 PUFA and n-3 HUFA contents in the flesh of rainbow trout.

Particularly, LNA, EPA and DHA levels in experimental fish body were strongly influenced by the dietary levels of LNA, EPA and DHA. An increased level of LA was observed in the fillet of rainbow trout fed D5. Several authors like Bell et al. (2002), Geurden et al. (2007), Turchini et al. (2009), Guler and Yildiz (2011), Kenari et al. (2011) and Thanuthong et al. (2011) reported a reduced content of 20:5n-3 and 22:6n-3 in fish muscle that were fed diets containing VOs. In the current study, the high dietary levels of VOs lead to a marked reduction of EPA and DHA, as most VOs are rich in unsaturated 18C FAs like 18:1n-9; 18:2n-6 and 18:3n-3, but they are poor sources of n-3 HUFA (Tocher 2010; Sales and Glencross 2011; Castro et al. 2016). Furthermore, it is reported a reduced percentage of 20:5n-3 in the muscle of trout fed diets containing VOs, suggesting the possible metabolic competition between 18:2n-6 and 18:3n-3 since both fatty acids are substrates for the same enzymes Δ6-desaturases (Caballero et al. 2002; Bell and Dick 2005).

Activity of pancreatic digestive enzymes

The digestive system plays an important role in breaking down nutrients by enzyme secretion and hydrolysis of large molecules into simple molecules, which can then be absorbed and used in metabolic pathways. A drawback associated to plant-based diets is a hindered digestive capacity (Krogdahl et al. 1999; Santigosa et al. 2008), which in extreme cases may be associated to structural alterations of intestinal epithelia, i.e. enteritis (Baeverfjord and Krogdahl 1996; Uran 2008) and affect nutrient absorption. Several studies have evaluated the effect FM replacement by alternative ingredients on the profile of digestive enzymes activity in fish (Santigosa et al. 2008; Rodiles et al. 2012; Gisbert et al. 2016), while few of them have evaluated the impact on digestive enzymes of alternative oils (Castro et al. 2016). In this sense, Castro et al. (2016) showed replacing ca. 70% FO by a blend of VOs (rapeseed, linseed, palm oils; 20:50:30) in diets for European sea bass (*Dicentrarchus labrax*) juveniles

had no effect on the main pancreatic digestive enzymes (i.e., trypsin, α-amylase, lipase and TAP).

In the present study, the α-amylase activity from fish measured in the PC and I decreased with increasing levels of GO in diets, while trypsin, TAP, and bile salt-activated lipase activities increased only in fish fed diets where FO was substituted 25 to 50% by GO (D2 and D3). It is reasonable to assume that differences in dietary FA composition may induce changes in time residence of digestive along the digestive tract and, consequently, distinctive digestive enzyme activity profiles (Castro et al. 2015). In particular, the replacement of FO by GO at high levels (75% and 100% FO replacement) resulted in a decrease in trypsin activity. In senegalese sole (*Solea senegalensis*) larvae fed a diet with soybean oil (Morais et al. 2006) and in yellowtail kingfish (*Seriola lalandi*) juveniles fed a diet with canola oil (Bowyer et al. 2012), trypsin activity was also detected at low levels; however, no effect on trypsin activity was detected with the dietary replacement of FO by VO in diets for European sea bass juveniles (Castro et al. 2016). The presence of antinutritional factors such as tannins in grape seed is well characterized and has been shown to act as enzyme inhibitors (Vallet et al. 1994; Goncalves et al. 2007). However, the increment in proteolytic activity in pyloric caeca was promoted by the dietary fatty acid profile of the GO, which may be attributed to a slower release of proteases into the intestinal lumen due to a decrease in digesta transit rate (Soengas 2014; Castro et al. 2016). These effects may be driven by dietary the regulation of cholecystokinin secretion by free FAs (Guimbaud et al. 1997; Feltrin et al. 2004). In our findings, α-amylase activity decreased with the dietary replacement of FO by GO. In contrast, several studies conducted in gilthead sea bream (Santigosa et al. 2011), yellowtail kingfish (Bowyer et al. 2012) and European sea bass juveniles (Castro et al. 2016) reported that the dietary inclusion of VOs had no marked effects on the α-amylase activity. Such differences between different studies regarding the effects of VOs on carbohydrase activities may be

related to the presence of different antinutritional factors depending on the VO considered (Goncalves et al. 2011), as well as due to the differences in digesta transit time. Bile salt-activated lipase specificity is known to change in function of the unsaturation degree and of the chain length of dietary FA (Tocher 2003). However, present results evidenced a lipase activity modulation in relation to dietary GO. Similarly, in European sea bass larvae, an increment in lipase activity was observed with the ingestion of coconut oil (Morais et al. 2004). On the contrary, no difference on lipase activity was observed in gilthead sea bream juveniles fed diets including FO or a blend of VOs (Santigosa et al. 2011). On the other hand, in yellowtail kingfish, lower lipase activity was observed in fish fed diets with canola oil than with FO (Bowyer et al. 2012). In fish, the digestibility of FAs have been shown to decrease with their increasing chain length and to increase with unsaturation (Olsen et al. 1998). Fish lipases have a preference for PUFA as substrates, followed by MUFA, with SFA being more resistant to lipolysis (Iijima et al. 1998). Therefore, fish oils commonly have a good digestibility, while VOs containing MUFA and particularly SFA show a more reduced digestibility. The present results suggest that lipolytic activity might be stimulated by the MCFA and/or SFA (mainly 16:0 and 18:0) present in GO as shown by Morais et al. (2004) in European sea bass larvae fed with coconut oil.

Conclusion

This study showed that FO may be replaced by GO up to 50% in rainbow trout diets with positively affecting growth performance (broken-line analysis), digestive capacity and body composition. The muscle fatty acid profile was modified with increment of GO in diets as n-3 HUFA levels especially EPA and DHA were decreased, while n-6 PUFA levels were increased. Based on the findings of the present study, the enzymatic assessments revealed that the replacement of FO by 50% GO resulted in an increase in bile salt-activated lipase, trypsin,

and TAP activities. The present data suggest that FO can be replaced up to 50% with GO in diets for rainbow trout without major alterations in the digestive function and it would be interesting to analyze the effects of incorporating this oils in rainbow trout diets for other developmental stages like fry.

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Declaration of interest statement:

The authors declare that there are no conflicts of interest in this research paper.

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718 and Its Compatibility with Oxidants and Surfactants. *Journal of aquatic food product*
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720

721 Table 1. Feed ingredients and proximate composition of experimental diets (% dry matter
722 basis).

Ingredients (%)	**Experimental diets**[a]				
	D1	D2	D3	D4	D5
Fish meal (62% crude protein)[b]	46	46	46	46	46
Soybean meal (45% crude protein)[c]	14	14	14	14	14
Meat and bone meal (52.8 % crude protein)[d]	13	13	13	13	13
Wheat flour	8.98	8.98	8.98	8.98	8.98
Fish oil[e]	14	10.5	7	3.5	0
Grapeseed oil[f]	0	3.5	7	10.5	14
Mineral premix[g]	1	1	1	1	1
Vitamin premix[h]	2	2	2	2	2
BHT [i]	0.02	0.02	0.02	0.02	0.02
Toxin Binder [j]	1	1	1	1	1
Total	100	100	100	100	100
Proximate composition (%)					
Dry matter	93.31	93.89	93.72	93.75	93.84
Crude protein	50.45	50.25	50.41	50.45	50.36
Lipid	18.08	18.53	18.11	18.50	18.41
Ash	8.05	8.84	8.51	8.51	8.56
NFE[k]	16.73	16.27	16.69	16.29	16.51
Energy (MJ kg^{-1})[l]	17.01	17.05	17.09	17.17	17.22

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725 [a] Diet abbreviations are as follows: D1, fish oil; D2, D3, D4 and D5 contained grape seed oil of 25, 50, 75 and 100 % instead
726 of fish oil in diets, respectively.
727 [b] Pars kilka (Mazandaran, Iran). The lipid content of fish meal was about 7% and accounted for in the total lipid of diets.
728 [c] Khavardasht Co. (Gorgan, Golestan, Iran).
729 [d] Gohar Daneh Shargh Co. (Mashhad, Iran).
730 [e] Anchovy oil (Havorash: Boshehr, Iran). Fatty acid composition (%): 0.05 (C14:0); 0.7 (C15:0); 20.6 (C16:0); 0.9 (C17); 3.9
731 (C18:0); 21.24 (Σn3); 1.88 (Σn6).
732 [f] Product of Monini company, Italy. Composition: Saturates (12g), Monounsaturates (19g), Polyunsaturates (61g),
733 Carbohydrate, Sugar, Fibre, Protein and Salt (0g). Fatty acid composition (%): Palmitic acid (C16:0), 7.86; Stearic acid
734 (C18:0), 4.45; Oleic acid (C18:1n-9), 22.09; Linoleic acid (C18:2n-6), 63.07.
735 [g] Mineral premix U kg^{-1} of diet: KCl (200), KI (60), $COCL_2$. $6H_2O$ (7), $CuSO_4.5H_2O$ (14), $FeSO_4H_2O$ (400), $ZnSO_4.H_2O$
736 (200), $MnSO_4.H_2O$ (80), $Na_2SeO_3.5H_2O$ (65), $MgSO_47H_2O$ (3000), $Ca(H_2PO_4).H_2O$ (20000), NaCl (136), Zeolit (5840),
737 career up to1kg.
738 [h] Vitamin premix U kg^{-1} of diet: vitamin B1, 12000 mg; vitamin B2, 5000 mg; vitamin B3, 35000 mg; vitamin B5, 30000 mg;
739 vitamin B6, 6000 mg; B7, 60 mg; vitamin B9, 2000 mg; vitamin B12, 50 mg; vitamin A, 80000000 IU; vitamin, D3,
740 200000000 IU; vitamin E, 44000 IU; vitamin K3, 5000 mg; vitamin C, 500000 mg; inositol, 100000 mg; antioxidant
741 (*Ethoxyquin)*, 150000 mg, career up to 1 kg.
742 [i] Antioxidant: Butylated hydroxytoluene (Garmab Shimi, Iran).
743 [j] Antifungal agent: Natural Hydrated Sodium Calcium Aluminium Silicates.
744 [k] Nitrogen-free extract.
745 [l] Calculated on the basis of 23.6, 39.5 and 17.2 MJ kg^{-1} of protein, fat and carbohydrate, respectively (NRC, 1993).
746

747
748 Table 2. Fatty acid composition of experimental diets (% of total fatty acids) [*].

Fatty acids	Experimental diets				
	D1	D2	D3	D4	D5
C14:0	3.51±0.11^{b}	3.43±0.10^{b}	3.35±0.10^{ab}	3.51±0.11^{b}	3.22±0.06^{a}
C15:0	0.39±0.01^{b}	0.31±0.04^{b}	0.28±0.05^{ab}	0.31±0.05^{b}	0.29±0.06^{a}
C16:0	23.76±0.98^{b}	18.23±0.33^{ab}	16.71±094^{ab}	13.25±0.41^{a}	16.71±0.41^{ab}
C17:0	0.41±0.10	0.51±0.02	0.35±0.06	0.51±0.05	0.36±0.02
C18:0	3.86±0.11^{b}	3.52±0.15^{ab}	3.12±0.24^{a}	3.88±0.37^{ab}	3.92±0.13^{ab}
C20:0	1.61±0.16^{b}	1.65±0.06^{b}	1.25±0.05^{ab}	1.28±0.07^{ab}	0.98±0.13^{a}
C22:0	0.19±0.02	0.14±0.02	0.11±0.02	0.10±0.03	0.11±0.01
C24:0	0.29±0.01^{b}	0.11±0.07^{a}	0.12±0.05^{a}	0.13±0.01^{a}	0.20±0.04^{ab}
Σ SFAs	34.02±1.01^{b}	27.90±0.51^{b}	25.47±0.85^{b}	22.97±0.55^{a}	25.79±0.61^{b}
C16:1n-7	4.84± 0.15^{b}	4.35±0.16^{b}	4.30±0.41^{b}	4.39±0.31^{b}	3.43±0.04^{a}
C17: 1n-7	0.29±0.02	0.46±0.04	0.42±0.05	0.49±0.03	0.43±0.01
C18:1n-9	33.71±0.69^{b}	39.06±1.10^{b}	34.23±0.62^{b}	36.40±3.64^{b}	28.62±0.55^{a}
Σ MUFAs	38.84±1.05^{b}	43.87±1.04^{b}	38.95±0.62^{ab}	41.28±1.10^{b}	32.48±0.87^{a}
C18:2 n-6	14.62±1.64^{a}	20.28±1.12^{b}	22.57±0.35^{b}	23.45±1.91^{b}	29.88±1.82^{c}
C18:3 n-6	0.29±0.09^{a}	0.45±0.05^{ab}	0.74±0.11^{b}	0.69±0.07^{b}	0.98±0.21^{c}
C18:3 n-3	1.54±0.11^{b}	1.25±0.07^{b}	1.48±0.19^{b}	1.41±0.12^{b}	0.89±0.61^{a}
Σ PUFAs	16.45±1.07^{a}	21.98±1.39^{b}	24.79±0.49^{b}	25.55±2.11^{b}	31.75±1.93^{c}
C20:3 n-3	1.09±0.21	0.98±0.02	1.01±0.23	0.96±0.04	1.01±0.02
C20:4 n-6	0.80±0.13^{b}	0.43±0.03^{a}	0.64±0.03^{b}	0.54±0.09^{a}	0.70±0.10^{b}
C20:5 n-3	7.98±0.12^{b}	5.49±0.02^{b}	3.11±0.07^{b}	2.17±0.08^{ab}	1.07±0.12^{a}
C22:5 n-6	0.25±0.03^{b}	0.16±0.04^{ab}	0.16±0.03^{a}	0.18±0.05^{ab}	0.15±0.03^{ab}
C22:5 n-3	0.23±0.01	0.14±0.01	0.08±0.01	0.05±0.00	0.02±0.00
C22:6 n-3	2.39±1.26^{b}	1.85±0.24^{a}	1.42±0.14^{a}	1.23±0.52^{a}	1.01±0.03^{a}
Σ HUFAs	12.74±1.12^{b}	9.05±0.33^{a}	6.42±0.11^{a}	5.13±0.21^{a}	3.97±0.02^{a}
Σ n3	13.23±1.31^{b}	9.71±0.23^{ab}	7.10±0.11^{ab}	5.82±0.18^{ab}	4.01±0.15^{a}
Σ n6	15.96±1.30^{a}	21.32±1.29^{ab}	24.11±0.30^{b}	26.09±2.14^{b}	31.71±1.29^{b}
n-3/n-6	0.83±0.06^{c}	0.46±0.02^{b}	0.29±0.01^{b}	0.22±0.02^{ab}	0.13±0.03^{a}
EPA/DHA	3.34±0.02^{ab}	2.97±0.01^{ab}	2.19±0.05^{ab}	1.76±0.05^{b}	1.06±0.02^{a}
PUFAs/SFAs	0.48±0.02^{a}	0.79±0.08^{ab}	0.97±0.01^{ab}	1.11±0.03^{b}	1.23±0.09^{b}
AA/EPA	0.10±0.08^{a}	0.08±0.09^{a}	0.21±0.03^{b}	0.25±0.10^{b}	0.65±0.08^{c}

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750 [*] Data are reported as mean ± SD (n=3). Means with different superscript letter in each row are significantly
751 different ($P < 0.05$). Abbreviations: SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs,
752 polyunsaturated fatty acids; HUFAs, highly unsaturated fatty acids; DHA, docosahexaenoic acid; EPA,
753 eicosapentaenoic acid; AA, arachidonic acid (C20:4 n-6). D1, fish oil; D2, D3, D4 and D5 contained grape seed
754 oil at 25, 50, 75 and 100 % replacing fish oil in diets, respectively.

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Table 3. Growth parameters of rainbow trout (*O. mykiss*) fed experimental diets containing different levels of grape seed oil for 60 days [*].

Parameters	Experimental diets				
	D1	D2	D3	D4	D5
Initial weight (g)	40.10±1.10	40.21±1.51	40.16±1.33	40.20±1.29	40.15±1.47
Final Weight (g)	136.70±7.10^{ab}	137.10±6.90^{ab}	146.20±8.50^{b}	131.00±2.10^{a}	134.90±5.40^{a}
Weight gain (g)	96.69±0.70^{ab}	96.90±0.11^{ab}	106.04±0.80^{b}	90.80±0.20^{a}	94.81±0.50^{a}
SGR (% BW/day) [b]	2.05±0.09	2.04±0.13	2.15±0.09	1.97±0.03	2.02±0.06
Feed Conversion Ratio	1.44±0.10^{ab}	1.44±0.17^{ab}	1.26±0.09^{a}	1.47±0.06^{b}	1.54±0.08^{b}
Feed intake (g fish^{-1} 60 days^{-1})	5.58±0.22	5.66±0.13	5.38±0.42	5.76±0.51	5.83±0.45
K Factor	1.25±0.07	1.25±0.17	1.23±0.06	1.16±0.01	1.23±0.13
Survival rate (%)	83.13±3.32	82.12±1.92	83.45±6.65	81.11±6.93	80.52±5.61
HSI (%)	2.03±0.38^{b}	1.82±0.20^{b}	1.71±0.22^{ab}	1.61±0.90^{a}	1.23±0.13^{a}
VSI (%)	8.14±0.64^{c}	7.26±0.30^{c}	6.60±1.24^{b}	6.18±0.90^{b}	5.78±0.25^{a}
LER	5.25±0.41^{b}	5.23±0.89^{b}	5.85±0.55^{b}	5.24±0.18^{b}	4.91±0.22^{a}
PER	1.92±0.25^{b}	1.93±0.18^{b}	2.10±0.14^{c}	1.79±0.04^{a}	1.88±0.09^{ab}

[*] Data are mean ± SD (n = 3). Means without a common superscript letter in each row are significantly different ($P < 0.05$). Abbreviations: D1, fish oil; D2, D3, D4 and D5 contained grape seed oil at 25, 50, 75 and 100 % replacing fish oil in diets, respectively.

765 Table 4. Fillet proximate composition of rainbow trout (*O.mykiss*) fed experimental diets
766 containing different levels of grape seed oil for 60 days (%) [*].

Parameters	Experimental diets				
	D1	D2	D3	D4	D5
Moisture	74.72 ± 0.94^{b}	73.08 ± 1.05^{b}	72.82 ± 0.79^{b}	72.98 ± 1.28^{b}	70.77 ± 0.85^{a}
Crude protein	16.91 ± 0.29^{a}	17.89 ± 0.50^{ab}	18.30 ± 0.52^{bc}	18.13 ± 0.45^{bc}	19.45 ± 0.55^{c}
Crude lipid	7.19 ± 0.44	7.71 ± 1.13	7.55 ± 0.20	7.55 ± 0.88	8.37 ± 0.85
Ash	1.18 ± 0.009	1.32 ± 0.009	1.33 ± 0.039	1.34 ± 0.100	1.41 ± 0.172

767 [*] Data are reported as mean ± SD (n = 3). Means with different superscript letter in each row are significantly
768 different ($P < 0.05$). Abbreviations: D1, fish oil; D2, D3, D4 and D5 contained grape seed oil at 25, 50, 75 and
769 100 % replacing fish oil in diets, respectively.
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772

773 Table 5. Fatty acid composition (% of total fatty acids) of fillet of rainbow trout (*O.mykiss*)
774 fed experimental diets containing different levels of grape seed oil for 60 days [*].

Fatty acids	Groups of experimental fish					
	Initial	**D1**	**D2**	**D3**	**D4**	**D5**
C14:0	1.50 ± 0.01^{b}	1.53 ± 0.17^{b}	1.46 ± 0.12^{b}	1.42 ± 0.16^{ab}	1.59 ± 0.21^{b}	1.15 ± 0.09^{a}
C15:0	0.32 ± 0.01^{b}	0.33 ± 0.03^{b}	0.33 ± 0.01^{b}	0.28 ± 0.05^{ab}	0.33 ± 0.03^{b}	0.25 ± 0.02^{a}
C16:0	16.21 ± 1.21^{a}	22.26 ± 1.33^{b}	19.83 ± 0.57^{ab}	18.94 ± 1.04^{ab}	15.36 ± 0.57^{a}	18.81 ± 0.50^{ab}
C17:0	0.50 ± 0.03	0.51 ± 0.16	0.62 ± 0.06	0.46 ± 0.10	0.62 ± 0.09	0.48 ± 0.03
C18:0	4.79 ± 0.52^{ab}	4.96 ± 0.41^{b}	4.50 ± 0.12^{ab}	4.25 ± 0.36^{a}	4.95 ± 0.47^{ab}	4.88 ± 0.22^{ab}
C20:0	1.70 ± 0.11^{b}	1.73 ± 0.26^{b}	1.75 ± 0.10^{b}	1.63 ± 0.02^{ab}	1.62 ± 0.08^{ab}	1.15 ± 0.50^{a}
C22:0	0.22 ± 0.01	0.25 ± 0.01	0.19 ± 0.06	0.16 ± 0.14	0.15 ± 0.09	0.13 ± 0.02
C24:0	0.46 ± 0.06^{b}	0.39 ± 0.01^{b}	0.16 ± 0.04^{a}	0.18 ± 0.09^{a}	0.20 ± 0.01^{a}	0.29 ± 0.07^{ab}
Σ SFAs	26.05 ± 0.98^{a}	31.96 ± 1.37^{b}	28.84 ± 0.68^{b}	27.32 ± 0.75^{b}	24.82 ± 0.65^{a}	27.14 ± 0.97^{b}
C16:1n-7	1.22 ± 0.12^{a}	4.45 ± 0.17^{c}	4.03 ± 0.27^{c}	3.99 ± 0.50^{c}	4.05 ± 0.57^{c}	3.16 ± 0.06^{b}
C17: 1n-7	0.20 ± 0.01^{b}	0.27 ± 0.01^{a}	0.40 ± 0.06^{a}	0.38 ± 0.04^{a}	0.43 ± 0.07^{a}	0.37 ± 0.05^{a}
C18:1n-9	39.21 ± 1.89^{b}	38.81 ± 0.75^{b}	38.26 ± 1.50^{b}	36.40 ± 0.62^{b}	38.87 ± 3.64^{b}	32.62 ± 0.87^{a}
Σ MUFAs	40.63 ± 1.13^{ab}	43.53 ± 0.75^{b}	42.69 ± 1.14^{b}	40.77 ± 0.62^{ab}	43.35 ± 1.10^{b}	36.15 ± 0.87^{a}
C18:2 n-6	22.31 ± 1.22^{b}	19.71 ± 1.74^{a}	25.37 ± 1.60^{c}	27.97 ± 0.50^{c}	28.55 ± 3.01^{c}	33.58 ± 2.15^{d}
C18:3 n-6	0.40 ± 0.09^{a}	0.49 ± 0.14^{a}	0.68 ± 0.09^{ab}	0.92 ± 0.16^{b}	0.86 ± 0.08^{b}	1.28 ± 0.32^{c}
C18:3 n-3	1.86 ± 0.10^{c}	1.74 ± 0.16^{b}	1.58 ± 0.12^{b}	1.62 ± 0.26^{b}	1.60 ± 0.11^{b}	1.09 ± 0.87^{a}
Σ PUFAs	24.57 ± 1.11^{b}	21.94 ± 1.47^{a}	27.63 ± 1.62^{c}	30.51 ± 0.63^{c}	31.01 ± 3.01^{c}	35.95 ± 2.19^{d}
C20:3 n-3	2.10 ± 1.16^{b}	1.39 ± 0.39^{a}	1.19 ± 0.09^{a}	1.36 ± 0.52^{a}	1.18 ± 0.07^{a}	1.29 ± 0.01^{a}
C20:4 n-6	0.79 ± 0.07^{a}	0.89 ± 0.23^{b}	0.67 ± 0.09^{a}	0.84 ± 0.07^{b}	0.78 ± 0.10^{a}	0.89 ± 0.11^{b}
C20:5 n-3	0.38 ± 0.04^{b}	0.55 ± 0.21^{b}	0.40 ± 0.05^{b}	0.39 ± 0.05^{b}	0.37 ± 0.02^{ab}	0.17 ± 0.11^{a}
C22:5 n-6	0.14 ± 0.03^{b}	0.19 ± 0.01^{c}	0.11 ± 0.02^{a}	0.09 ± 0.0^{a}	0.11 ± 0.04^{a}	0.10 ± 0.01^{a}
C22:5 n-3	0.22 ± 0.05^{c}	0.29 ± 0.02^{d}	0.17 ± 0.03^{b}	0.10 ± 0.01^{a}	0.08 ± 0.01^{a}	0.05 ± 0.01^{a}
C22:6 n-3	2.73 ± 0.21^{a}	4.27 ± 1.38^{b}	2.94 ± 0.38^{a}	2.75 ± 0.14^{a}	2.22 ± 0.72^{a}	1.83 ± 0.02^{a}
Σ HUFAs	6.39 ± 1.07^{ab}	7.58 ± 1.20^{b}	5.48 ± 0.58^{a}	5.53 ± 0.19^{a}	4.74 ± 0.32^{a}	4.33 ± 0.01^{a}
Σ n3	7.29 ± 1.21^{b}	8.24 ± 1.52^{b}	6.23 ± 0.35^{ab}	6.22 ± 0.19^{ab}	5.45 ± 0.28^{ab}	4.43 ± 0.25^{a}
Σ n6	23.64 ± 1.28^{a}	21.28 ± 1.30^{a}	26.75 ± 1.78^{ab}	29.82 ± 0.50^{b}	30.30 ± 3.04^{b}	35.85 ± 2.35^{b}
n-3/n-6	0.31 ± 0.06^{c}	0.39 ± 0.09^{d}	0.23 ± 0.01^{b}	0.21 ± 0.01^{b}	0.18 ± 0.01^{ab}	0.12 ± 0.01^{a}
EPA/DHA	0.14 ± 0.02^{ab}	0.13 ± 0.01^{ab}	0.14 ± 0.01^{ab}	0.14 ± 0.01^{ab}	0.17 ± 0.07^{b}	0.09 ± 0.006^{a}
PUFAs/SFAs	0.94 ± 0.09^{ab}	0.69 ± 0.02^{a}	0.96 ± 0.07^{ab}	1.12 ± 0.04^{ab}	1.25 ± 0.06^{b}	1.32 ± 0.14^{b}
AA/EPA	2.08 ± 0.67^{b}	1.62 ± 0.11^{a}	1.68 ± 0.12^{a}	2.15 ± 0.09^{b}	2.11 ± 0.15^{b}	5.24 ± 0.14^{c}

775 [*] Data are reported as mean ± SD (n=3). Means with different superscript letter in each row are significantly
776 different ($P < 0.05$). Abbreviations: SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs,
777 polyunsaturated fatty acids; HUFAs, highlyunsaturated fatty acids; DHA, docosahexaenoic acid; EPA,
778 eicosapentaenoic acid; AA, arachidonic acid (C20:4 n-6). FO, fish oil; GO25, GO50, GO75 and GO100
779 contained grape seed oil (GO) at 25 %, 50 %, 75 % and 100 % replacing FO in diets, respectively.
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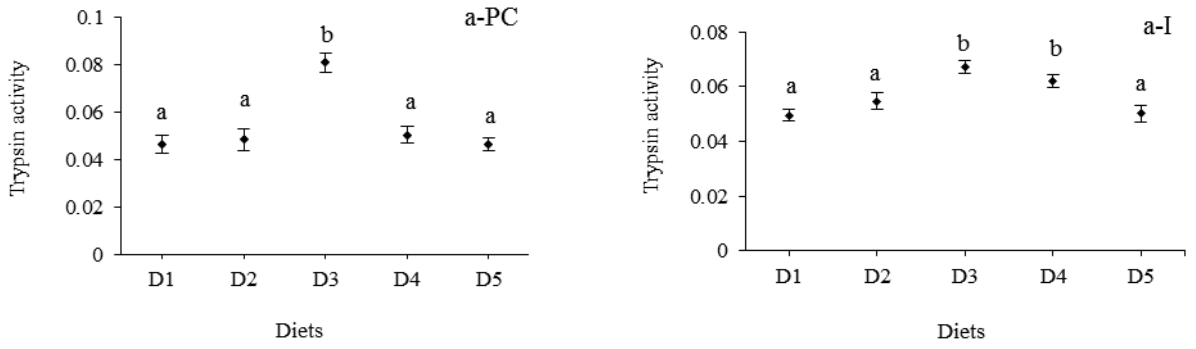

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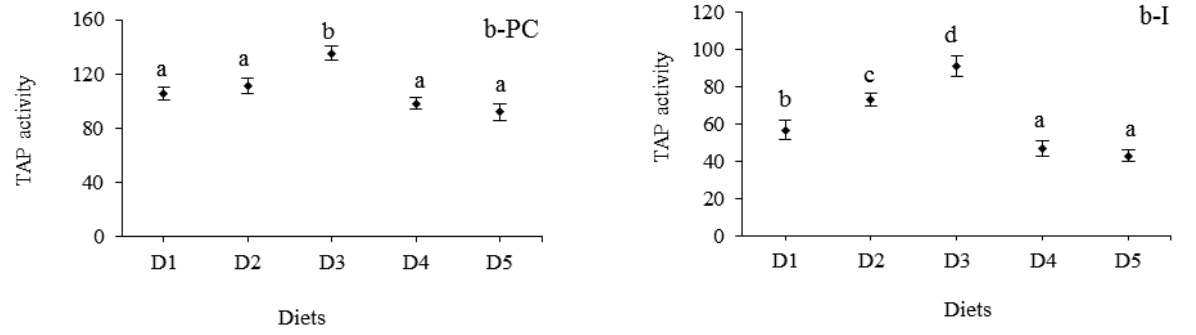

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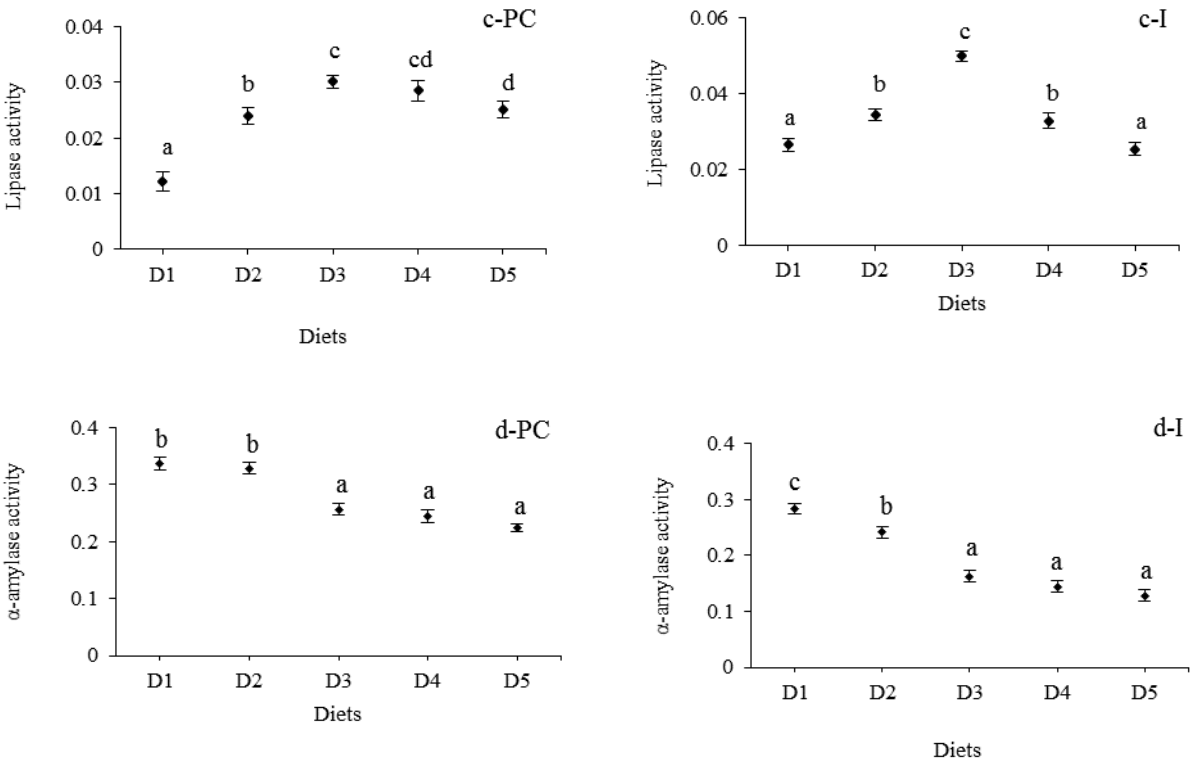

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785 Figure 1. Specific activity (U mg protein^{-1}) of trypsin (a), TAP (total alkaline protease)(b), lipase (c) and α-
786 amylase (d) from the pyloric caeca (PC) and intestine (I) of rainbow trout (*O.mykiss*) fed one of the five
787 experimental diets (D1, fish oil; D2, D3, D4 and D5 contained grape seed oil of 25, 50, 75 and 100% instead of
788 fish oil in diets, respectively). Values are means ± SD (n=3). Values without a common alphabetical letter
789 among diets indicate a significant difference (One-way ANOVA, Duncan test, $P<0.05$).
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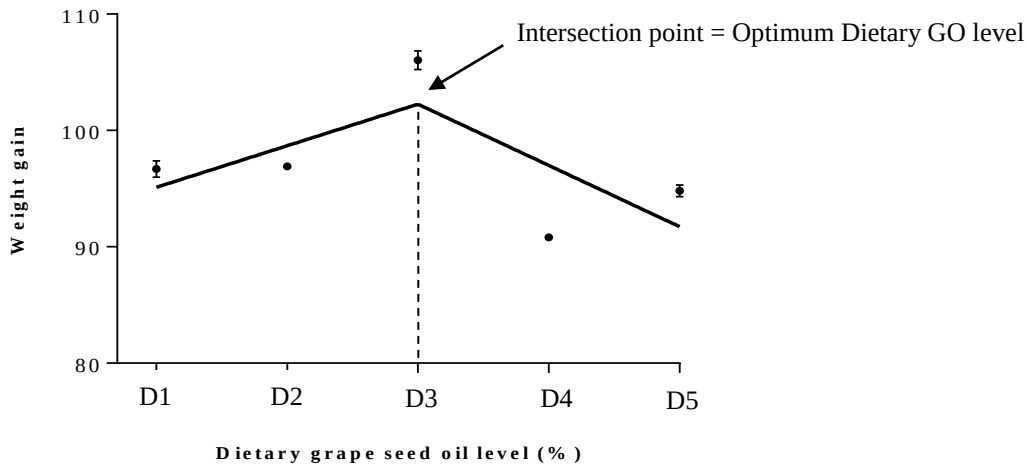

Figure 2. Broken-line analysis of mean weight gain (WG) for rainbow trout (*O.mykiss*) fed the diets containing varying level of grape seed oil for a 60-day feeding trial. D1 (fish oil); D2, D3, D4 and D5 contained grape seed oil of 25, 50, 75 and 100 % instead of fish oil in diets, respectively.