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# Administration of grape (*Vitis vinifera*) seed extract to rainbow trout (*Oncorhynchus mykiss*) modulates growth performance, some biochemical parameters, and antioxidant-relevant gene expression

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**Abstract** Grape seed, as a main source of polyphenols, has many nutritional and medicinal properties in humans. In the current study, the effects of dietary ethanolic grape seed extract (GSE) on the growth performance, antioxidant activity, and some biochemical parameters in rainbow trout were investigated. Ninety fish (initial weight 78.47 g) were randomly distributed among nine cement tanks (1.8 m × 0.22 m × 0.35 m) with 10 fish per tank. Three experimental diets containing either 0, 10, or 50 g $kg^{-1}$ GSE were prepared and each diet was randomly assigned to three tanks of fish for 60 days. Results showed that feeding GSE enhanced some growth parameters including the specific growth rate and condition factor in comparison with the control group. Among different serum metabolites, the glucose levels in treatment groups significantly decreased compared to the control group. The total product of lipid peroxidation indicated as malondialdehyde significantly decreased in both the GSE-added treatment groups. The gene expression related to the antioxidant enzymes, *catalase*, *glutathione peroxidase 1*, and *glutathione S-transferase A*, were upregulated in the intestine of fish that received a low dose of GSE. The results of the current study suggest that GSE, especially at 10 g $kg^{-1}$, diet had the potential to improve (1) specific growth rate and condition factor, (2) biochemical parameters including glucose and lipid peroxidation product, and (3) upregulated the expression of antioxidant genes including *catalase*, *glutathione peroxidase 1*, and *glutathione S-transferase A* in rainbow trout.

**Keywords** Rainbow trout · Grape seed extract · Growth performance · Antioxidant genes

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## Introduction

The fast development of the aquaculture industry and increase of fish demands resulted in fish culture intensification, highlighting different stressors, such as mismanagement, different pathogens, and chemical pollutants (Nootash et al. 2013). In different fish species, both

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immune and antioxidant systems have protective effects against the mentioned stresses. Antioxidant defenses can detoxify reactive oxygen species (ROS) and reactive nitrogen metabolites (RNMs) which can modify and damage nearly all cell and tissue components in different conditions (Kruidenier et al. 2003). The antioxidant defense system comprises both enzymatic and non-enzymatic antioxidants (Kruidenier et al. 2003).

The administration of environmentally friendly plant products to fish through the diet appears to improve fish antioxidant and immune systems to cope with stresses (Amar et al. 2004; Martinez-Álvarez et al. 2005; Verlhac Trichet 2010). Plant products with active biomolecules have been reported to stimulate the growth performance, and immune and antioxidant systems in fish and shellfish aquaculture (Harikrishnan et al. 2011). Grape seed extract (GSE) is a by-product of the juice industry which contains a range of active compounds such as phenols, catechins, epicatechins, procyanidins, and proanthocyanidins. Positive health benefits of GSE as a dietary supplement, namely, anti-cancer, anti-inflammatory, anti-aging, anti-diabetic, anti-bacterial, and anti-viral effects, have been proven in animal models and humans (Nowshehri et al. 2015); however, only a few studies are available on the effect of dietary GSE supplement in aquatic animals. For example, GSE could decrease the inflammatory responses and mortality rate in zebrafish (*Danio rerio*) challenged with *Staphylococcus aureus* (Kao et al. 2010). Dietary grape seed proanthocyanidins showed beneficial effects on tilapia (*Oreochromis nilotica*) growth, health status, and body composition (Zhai et al. 2014). Positive effects of GSE on the kidney melanomacrophages response in European sea bass (*Dicentrarchus labrax* L.) were also determined (Arciuli et al. 2017). Grape seed oil as a supplement has also the potential to increase antioxidant enzyme activities, the growth performance, and disease resistance in rainbow trout (Arslan et al. 2018). Besides the fish species, the administration of 5% GSE enhanced the productivity, survival rate, and feed intake in greenlip abalone (*Haliotis laevigata* Donovan) (Lange et al. 2014; Duong et al. 2016; Shiel et al. 2017).

Rainbow trout is cultured in more than 70 countries with distribution in nearly all of the world's continents. More than one third of rainbow trout production belongs to the Asia continent (310,000 MT) with some countries including Iran (140,000 MT) and Turkey (130,000 MT) that are the pioneers in rainbow trout farming. Besides the economic importance, rainbow trout have also been numerously applied as a model of research in different fields including toxicology, immunology, nutrition, physiology as well as developmental and evolutionary biology (Bobe et al. 2016).

Since grape by-products show strong potential as an aquaculture feed ingredient, investigating the effects of GSE on fish health seems to be of great importance. Therefore, this study was undertaken to evaluate the effects of GSE on rainbow trout growth performance, oxidant–antioxidant balance, and some biochemical parameters.

## Materials and methods

### Grape seed extraction

*Vitis vinifera* fruit was collected from Malekan region (East Azerbaijan province, Iran). Grape seeds were separated manually, sun dried, and then powdered in a grinder. The extraction was performed according to the procedure described previously (Mandic et al. 2008) using ethanol 90% and filtration using a filter paper. The ethanol solvent was removed by evaporation under the reduced pressure and maximum temperature of 40 °C.

### Characterization of GSE

The concentration of phenolic compounds of GSE was determined spectrophotometrically according to Folin–Ciocalteau method (Singleton et al. 1999). Gallic acid was used as a calibration standard, and the results were presented as gallic acid equivalents in grams per 1 kg of dry grape seed weight. Total flavan-3-ols amount was determined calorimetrically using (+)-catechin as the standard (Nakamura et al. 2003). Radical scavenging activity of GSE was determined against stable 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) (Brand-Williams et al. 1995). The antioxidant capacity of GSE at concentrations of 50 and 100 ppm was compared to ascorbic acid at the same concentrations. To determine the quantity of phenolic components in GSE, HPLC analysis was performed according to the procedure described in the previous study (Mahdavikia and Saharkhiz 2015).

## Experimental fish and diet

Based on the positive effects of 5% GSE on greenlip abalone (Lange et al. 2014), in the current study dietary inclusion of 50 g $kg^{-1}$ of GSE and one dose lower than this recommended dose (10 g $kg^{-1}$) were used. All the ingredients with three incremental levels (0, 10, and 50 g $kg^{-1}$) of GSE were well mixed and pelletized. The composition of the final experimental diets is shown in Table 1. The modified diets were separately kept in sealed plastic bags at 8–10 °C to be used in the feeding trial.

**Table 1** Formulation and chemical composition of the experimental diets

| Ingredients (g $kg^{-1}$) | GSE in diet | | |
|---|---|---|---|
| | 0 g $kg^{-1}$ | 10 g $kg^{-1}$ | 50 g $kg^{-1}$ |
| Fish meal[a] | 420 | 420 | 420 |
| Soybean oil[b] | 60 | 60 | 60 |
| Wheat flour | 100 | 100 | 100 |
| Soybean meal | 70 | 70 | 70 |
| Wheat gluten | 40 | 40 | 40 |
| Meat meal | 80 | 80 | 80 |
| Cellulose[c] | 50 | 40 | – |
| Grape seed extract | – | 10 | 50 |
| Vitamin mixture[d] | 50 | 50 | 50 |
| Mineral mixture[e] | 50 | 50 | 50 |
| Fish oil | 60 | 60 | 60 |
| Binder | 20 | 20 | 20 |
| Proximate chemical analysis (%) | | | |
| Dry matter | 90.3 | 90.7 | 90.5 |
| Crude protein | 41.9 | 42.1 | 42.3 |
| Crude lipid | 17.1 | 17.1 | 17.3 |
| Ash | 9.8 | 10.2 | 10.1 |
| Crude fiber | 1.3 | 1.2 | 1.3 |
| Gross energy (kcal $g^{-1}$) | 3.8 | 3.8 | 3.9 |

[a] North Sefid Mahi Corporation, Iran

[b] North Sefid Mahi Corporation, Iran

[c] Cellulose (Merck Company, Germany)

[d] Unit $kg^{-1}$ of mixture: vitamin: retinol acetate (A), 1,600,000 IU; cholecalciferol ($D_3$), 400,000 IU; DL-α-tocopheryl acetate (E), 40 IU; menadione sodium bisulfate ($K_3$), 2000 mg; biotin ($H_2$), 240 mg; thiamin mononitrate ($B_1$), 6000 mg; riboflavin ($B_2$), 8000 mg; calcium *d*-pantothenate ($B_3$), 12,000 mg; niacinamide ($B_5$), 40,000 mg; pyridoxine hydrochloride ($B_6$), 4000 mg; folic acid ($B_9$), 2000 mg; cyanocobalamin ($B_{12}$), 8000 mg; vitamin C, 60000 mg; inositol, 20,000 mg; BHT, 20,000 mg; carrier up to 1 kg

[e] Unit $kg^{-1}$ of mixture: mineral: Fe, 26,000 mg; Zn, 12,500 mg; Se, 2000 mg; Co, 480 mg; Cu, 4200 mg; Mn, 15,800 mg; I, 1000 mg; choline chloride, 12,000 mg; carrier up to 1 kg

Rainbow trout with the mean weight of 78.47 g were purchased from a fish farm in Firuzkuh, Iran. A total of 90 fish were randomly distributed among nine cement tanks (1.8 m × 0.22 m × 0.35 m) supplied with river water and temperature 13.2 ± 2.1 °C, pH 7.5 ± 0.3, dissolved oxygen 8.7 ± 0.4 mg $l^{-1}$, and a flow rate at 0.5 l $s^{-1}$. Water physicochemical parameters inside the tanks were checked with a portable analyzer (Aquacombo, China) daily. After a 10-day adaptation period, three tanks of fish were randomly assigned to one of three treatment groups containing different doses of GSE (0, 10, and 50 g $kg^{-1}$ feed) for a period of 60 days. In this study, fish were administrated at a feeding rate of 20 g $kg^{-1}$ of body weight three times a day.

## Growth performance

The mean of initial and final body weight and length of all 10 fish in each tank were calculated individually at the beginning and the end of the feeding trial. Meanwhile, all fish in each tank were totally weighed every 15 days to adjust the feeding during the trial. Survival (%) and the secondary growth parameters including specific growth rate (SGR) and condition factor (CF) were determined as follows:

$$\mathrm{SGR}\ (\%) = 100\ (\ln \text{final weight} - \ln \text{initial weight}) / \text{number of days in the feeding trial.}$$

$$\mathrm{CF}\ (\%) = 100 \times \text{final weight (g)} / \text{final length (cm)}3.$$

## Serum biochemical parameters

At the termination of the trial on day 60, seven fish in each tank were sacrificed in a clove oil bath (50 ml $l^{-1}$) and fish blood was collected from the caudal vein. After keeping the blood samples at room temperature for 3 h, they were centrifuged at 2000×*g* for 5 min. Collected serum from each fish blood was kept in an Eppendorf tube at − 80 °C which is used for different assays.

Serum metabolites including triglyceride, cholesterol, uric acid, glucose, total protein as well as the activity of alkaline phosphatase (ALP) and aspartate aminotransferase (AST) were determined by commercial kits (Par azmoon, Tehran, Iran) and a biochemical-type automated analyzer (Sheikhzadeh et al. 2012). Total

antioxidant activity in fish serum was calculated by the ferric reducing ability of plasma (FRAP) assay (Benzie and Strain 1996). The total level of lipid peroxidation product was also determined using the thiobarbituric acid test and presented as nanomoles per deciliter of serum (Satho 1978).

### Real-time PCR and gene expression analysis

On day 60 of the trial, three fish in each tank were sacrificed in a clove oil bath (50 ml $l^{-1}$) and the posterior intestine from each fish was immediately removed and stored in liquid nitrogen for real-time PCR analysis. Total RNA was extracted from 50 mg of the intestine tissue using AccuZol® (Bioneer, South Korea). RNA concentration and purity were estimated using a spectrophotometer (Bio-Rad, CA, USA). Extracted RNA (2 μg) was reverse transcribed using GeneAll Reverse Transcription kit (GeneAll Biotechnology, Seoul, Korea). The generated cDNAs were used for real-time PCR analysis using the selected genes (Table 2). The PCR reaction mixture for PCR consisted of 10 μl SYBR® Premix Ex Taq™ II (TaKaRa Biotechnology Co. Ltd., China), 1 μl of synthesized cDNA, and 20 pmol of each forward and reverse primers in a final volume of 20 μl. The amplification was carried out in a Real-Time PCR Detection System (Qiagen, USA) with an initial denaturing step of 95 °C for 3 min, followed by 40 cycles of 95 °C for 20 s and 60 °C for 30 s. The $2^{-\Delta\Delta Ct}$ method was used to calculate the fold change of mRNA expressions for the target genes (Livak and Schmittgen 2001). The *β-actin* gene, as the housekeeping gene, was used to normalize the expression of the selected genes. PCR reactions were performed in triplicates to minimize the experimental error in all samples.

### Statistical analysis

The statistical analysis of data was performed using SPSS software version 19.0 (IBM Corp., Armonk, USA). Data were presented as mean ± standard error (SEM). One-way analysis of variance (ANOVA) was employed to compare the means, followed by Tukey post hoc test. The $P$ values $< 0.05$ were considered significant.

## Results

### Characterization of GSE

In this study, GSE contained 47.1 ± 2.3 g $kg^{-1}$ total phenolic compounds according to the Folin–Ciocalteu colorimetric method that was expressed as grams gallic acid per kilogram dry seed weight. The total flavan-3-ols content was about 10.47 g $kg^{-1}$ dry seed weight. The concentrations of individual phenolic compounds in GSE are shown in Table 3. Among the identified polyphenols, catechin was the most abundant compound followed by epicatechin and gallic acid. Different procyanidins including procyanidin-B4, procyanidin-B5, procyanidin-C1, procyanidin-B1, procyanidin-B2, and procyanidin-B3 were present at the lower concentrations. The antioxidant activity determined by DPPH method showed that at 50 ppm, ascorbic acid and grape seed exhibited 86.7% and 85% free radical scavenging activity, respectively. At 100 ppm, 96% and 94.7% antioxidant activity was noted for ascorbic acid and grape seed, respectively.

### Growth performance

In this study, there were no significant differences between mean weights in fish fed with different amount of GSE during the 60-day experimental period (Fig. 1). Both initial

**Table 2** Primers used for the expression of the antioxidant genes in the intestine of rainbow trout

| Target gene | Primer sequence | Accession number | Product (bp) |
|---|---|---|---|
| *Catalase* | F: 5′ TGATGTCACACAGGTGCGTA 3′<br>R: 5′ GTGGGCTCAGTGTTGTTGAG 3′ | TC99600 | 195 |
| *Glutathione peroxidase 1* | F: 5′ CGAGCTCCATGAACGGTACG 3′<br>R: 5′ TGCTTCCCGTTCACATCCAC 3′ | TC126469 | 183 |
| *Glutathione S-transferase A* | F: 5′ CAGAGGACAGCTCCCTGCTT 3′<br>R: 5′ CTGAACCGGCTCTCCAGGTA 3′ | NM_001160559.1 | 187 |
| *β-Actin* | F: 5′ ATGGAAGATGAAATCGCCGCAC 3′<br>R: 5′ TGGCCCATCCCAACCATCAC 3′ | AJ438158 | 191 |

**Table 3** Distribution (%) of phenolic compounds in the grape seed extract using HPLC method

| Compound | |
|---|---|
| Catechin | 43 |
| Epicatechin | 30 |
| Gallic acid | 5.2 |
| Procyanidin-B4 | 4.5 |
| Procyanidin-B5 | 3.12 |
| Procyanidin-C1 | 3.1 |
| Procyanidin-B1 | 2.4 |
| Procyanidin-B2 | 2 |
| Procyanidin-B3 | 1.45 |

and final body weights and lengths did not also differ between treatment groups. A significantly higher SGR was noted in both low and high doses of GSE with higher GSR in the lower dose of GSE than the high dose group. CF was higher in the high-dose group compared to the control and low-dose groups. During the 60-day trial, all fish survived in all treatment and control groups (Table 4).

### Serum metabolites

There were no significant differences in the level of metabolites including triglyceride, cholesterol, uric acid, and total protein between all treatment groups; however, the glucose level of fish fed with different doses of GSE was lower than that of the control group (Table 5). Meanwhile, ALP and AST activities in the fish in both low and high doses of GSE groups were almost similar to those fed control diet (Table 6).

### Antioxidant activity

In fish that received GSE, total antioxidant activity did not differ from the control group. In contrast, serum lipid peroxidation products of fish fed GSE-supplemented diets were significantly lower than that of fish fed the control diet (Table 6). The gene expression levels of antioxidant enzymes including *catalase*, *glutathione peroxidase 1*, and *glutathione S-transferase A* were upregulated in the intestine of fish that received the low dose of GSE compared to the control group. Conversely, there were no significant changes in the expression of these genes in the intestine of fish that received a high dose of GSE in comparison with the control group (Fig. 2).

## Discussion

The efficient method for extracting different polyphenols from grape seeds is of great importance for many

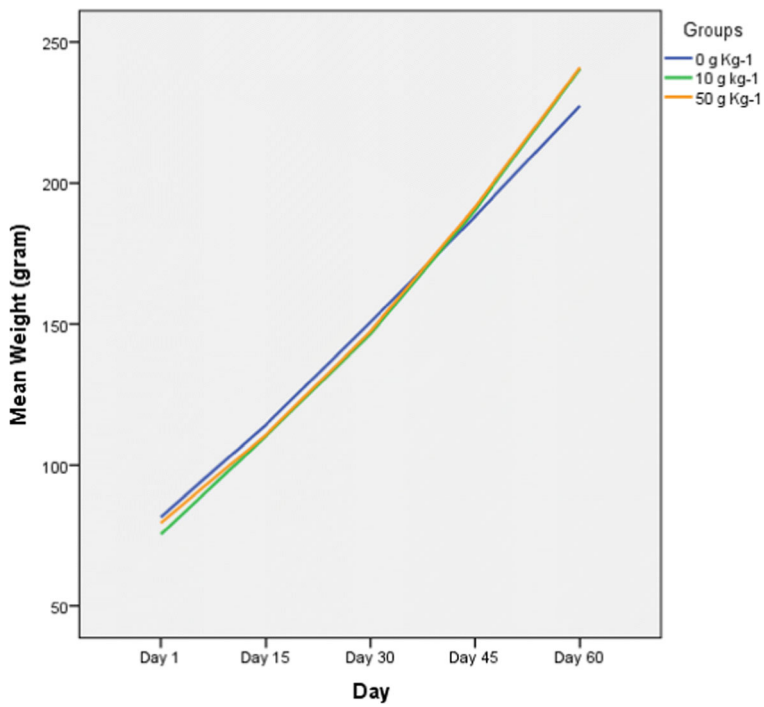


**Fig. 1** Effects of the dietary GSE on mean weight in rainbow trout during the 60-day feeding trial. $n = 3$ in each treatment group

**Table 4** Effects of the dietary GSE on growth performance in rainbow trout at the beginning and the end of the feeding trial

| Grape seed extract in diet | Initial weight (g) | Initial length (cm) | Final weight (g) | Final length (cm) | Specific growth rate (SGR) (%) | Condition factor (CF) | Survival rate (%) |
|---|---|---|---|---|---|---|---|
| 0 g $kg^{-1}$ | 81.69 ± 3.45 | 19.72 ± 0.29 | 227.51 ± 5.49 | 27.70 ± 0.24 | 1.68 ± 0.01[a] | 1.07 ± 0.01[a] | 100 |
| 10 g $kg^{-1}$ | 75.50 ± 3.44 | 19.31 ± 0.28 | 240.50 ± 5.51 | 28.28 ± 0.17 | 1.89 ± 0.01[c] | 1.06 ± 0.01[a] | 100 |
| 50 g $kg^{-1}$ | 79.61 ± 3.90 | 19.80 ± 0.27 | 241.01 ± 14.07 | 26.89 ± 1.43 | 1.79 ± 0.02[b] | 1.26 ± 0.08[b] | 100 |

Data are mean ± SEM. Those within a column superscripted by different letters are significantly different ($P < 0.05$). For SGR $n = 3$ in each treatment group, for remaining parameters $n = 30$ in each treatment group

researchers due to the beneficial effects of phenolic compounds on health improvement and disease prevention in living systems (Nowshehri et al. 2015). The choice of solvent in this study was related to the low toxicity of ethanol which is effective for polyphenol extraction from food material (Mandic et al. 2008; Nowshehri et al. 2015). The Folin–Ciocalteau method is able to detect all available phenols in plants with varying sensitivities. In this study, the total polyphenols of the prepared GSE based on gallic acid was 47.1 ± 2.3 g $kg^{-1}$ dry seed weight which was higher than those obtained in the previous studies (Revilla et al. 1995; Mandic et al. 2008). These various results could be attributed to the differences in the genetic potential of the grape varieties for the biosynthesis of polyphenol besides the variations from season to season (Mandic et al. 2008). The amount of flavan-3-ols in GSE was 10.47 g $kg^{-1}$ dry seed weight which was similar to the previous study by Mandic et al. (2008). HPLC analysis of the extract also showed that catechin (43%) was the most abundant compound followed by epicatechin (30%). These obtained amounts were similar to the findings of the previous studies by Revilla et al. (1995) and Mandic et al. (2008). Meanwhile, the free radical scavenging potentials of GSE at two concentrations were compared with ascorbic acid as a reference antioxidant by the DPPH method. The results showed a similar effect without significant differences between GSE and ascorbic acid. The obtained data revealed that GSE was a free radical inhibitor and an antioxidant with the potential to react with free radicals.

In this study, GSE showed a positive effect on some growth parameters including specific growth rate and condition factor in rainbow trout. Similarly, Zhai et al. (2014) reported that dietary grape seed proanthocyanidins could significantly improve the growth of tilapia. Meanwhile, grape seed oil supplementation could result in positive effects on rainbow trout growth performance (Arslan et al. 2018). In abalone, a type of shellfish, a significant increase in feed intake and meal acceptance was observed after the addition of grape seed to its diet (Lange et al. 2014). The inclusion of GSE in the animal diet provides a diet with high levels of different nutrients such as protein, carbohydrate, and fat, and also a source of bioactive compounds (Shiel et al. 2017). Even though the growth-promoting effect of different compounds in GSE is not fully determined, improved growth performance can happen by different mechanisms. For example, the increase of digestive enzymes following the administration of different additives could result in enhanced growth performance in rainbow trout. Even though GSE can increase the activity of some intestinal enzymes (Xie et al. 2012), the interaction with digestive secretions by some constituents especially tannin in GSE was also shown (Laurent et al. 2007). Improved intestinal microflora following the administration of some additives can also happen. Previously, it was shown that GSE was

**Table 5** Effects of the dietary GSE on serum biochemical parameters at the end of the feeding trial

| Grape seed extract in diet | Triglyceride (mg $dl^{-1}$) | Cholesterol (mg $dl^{-1}$) | Uric acid (mg $dl^{-1}$) | Glucose (mg $dl^{-1}$) | Total protein (g $dl^{-1}$) |
|---|---|---|---|---|---|
| 0 g $kg^{-1}$ | 310.51 ± 32.60 | 308.51 ± 14.65 | 10.81 ± 2.10 | 80.53 ± 4.47[a] | 3.51 ± 0.23 |
| 10 g $kg^{-1}$ | 312.81 ± 33.65 | 294.51 ± 14.09 | 9.80 ± 0.95 | 55.74 ± 6.15[b] | 3.46 ± 0.16 |
| 50 g $kg^{-1}$ | 306.70 ± 18.84 | 291.11 ± 16.07 | 9.83 ± 0.40 | 61.89 ± 3.85[b] | 3.70 ± 0.10 |

Data are mean ± SEM. Those within a column superscripted by different letters are significantly different ($P < 0.05$). $n = 21$ in each treatment group

**Table 6** Effects of the dietary GSE on serum metabolic enzyme activity and total antioxidant status at the end of the feeding trial

| Grape seed extract in diet | ALP (U $l^{-1}$) | AST (U $l^{-1}$) | Total antioxidant activity (μmol $l^{-1}$) | Lipid peroxidation product (nmol $dl^{-1}$) |
|---|---|---|---|---|
| 0 g $kg^{-1}$ | 489.82 ± 79.70 | 831.12 ± 48.87 | 30.39 ± 2.43 | 10.97 ± 1.41[a] |
| 10 g $kg^{-1}$ | 449.90 ± 35.29 | 792.52 ± 49.59 | 31.87 ± 1.07 | 5.88 ± 1.08[b] |
| 50 g $kg^{-1}$ | 505.42 ± 74.01 | 817.76 ± 75.26 | 32.41 ± 1.08 | 6.55 ± 0.80[b] |

Data are mean ± SEM. Those within a column superscripted by different letters are significantly different ($P < 0.05$). $n = 21$ in each treatment group

*ALP* alkaline phosphatase, *AST* aspartate aminotransferase

effective in increasing the intestinal populations of microbiota in humans and animals (Yamakoshi et al. 2001; Viveros et al., 2011). Even though the exact mechanism is not clear, it was assumed that GSE can improve the enteric flora by its antioxidative and immunomodulation effects. Meanwhile, enhanced growth performance in fish species can sometimes be related to improving the function and structure of the gut which caused an increased digestive capacity of the intestine. Similarly, dietary polyphenol-rich GSE was able to modify the gut morphology in broiler chicks (Viveros et al., 2011). Noteworthy, evaluating the effects of feeding GSE on rainbow trout intestinal histology is currently under investigation in our laboratory.

Analyzing the serum metabolic enzyme activity of ALP and AST has the capability to show the hepatotoxicity changes. These enzymes activity values were not affected by the administration of GSE, so it can be assumed that this extract could be a safe additive even though more toxicological and histological evaluations in fish species are needed to prove it. In this study, feeding fish with GSE could decrease the serum glucose level. Similarly, the anti-diabetic activity of GSE and its bioactive constituents was previously shown (Nowshehri et al. 2015). Even though the exact mechanism of lower glucose in fish species is unknown, enhancing the glucose uptake in insulin-sensitive cell lines, lowering hyperglycemia in the streptozotocin (STZ)-induced diabetic rats, and inhibiting intestinal pancreatic α-amylase, α-glucosidase activities have previously been shown (Nowshehri et al. 2015).

Oxidative stress is a sign of different health problems. Therefore, the usage of natural antioxidants in order to decrease the oxidative damage is increasing. In this study, GSE could decrease the lipid peroxidation product in rainbow trout serum. In parallel, the improvement in some antioxidant-relevant gene expression was noted in fish intestine fed the low dose of GSE. The antioxidant activities of GSE have been previously studied (Nowshehri et al. 2015). Grape seed is a good source of active antioxidant compounds including phenols, catechins, epicatechins, procyanidins, and proanthocyanidins (Lange et al. 2014). The high antioxidant potential and health benefits of GSE have led several researchers to suggest that it can serve as a nutritional supplement and food additive (Gonzalez-Paramas et al. 2004). A clinical report has shown that the antioxidant power of proanthocyanidins from GSE is 20 and 50 times higher than vitamin C and vitamin E, respectively (Uchida 1980), even though our synthesized GSE showed fairly similar antioxidant potential to vitamin C by DPPH method. Although there are numerous studies on the potent antioxidant effect of dietary GSE in humans and animal models, limited information is available about the effects of grape seed oil supplement in rainbow trout (Arslan et al. 2018). Similarly, resveratrol, a dietary polyphenol, exists in grape and red wine, suppressed the oxidative stress, and increased the expression of antioxidant-relevant enzyme genes in tilapia serum and liver (Jia et al. 2019). Since the mucosal tissues of fish are the first line of defense against numerous aquatic pathogens, this study was carried out to investigate the effects of GSE on the antioxidant-genes expression in fish intestine tissue. It was revealed that fish that received the low dose of GSE had a higher expression of the antioxidant genes in their intestine than the other groups. Higher activity of the antioxidant enzymes could maintain an efficient pro-oxidant and antioxidant balance which results in the better health status of animals. In the previous studies, it was reported that the increased *superoxide dismutase* and *catalase* activities in abalone fed with grape seed resulted in more tolerance to handling stress during sampling (Lange et al. 2014; Shiel et al. 2017). Besides, the antioxidants in GSE caused effective responses in abalone during the heat stress with higher expression of potentially vital oxidative defense genes (Shiel et al. 2017).

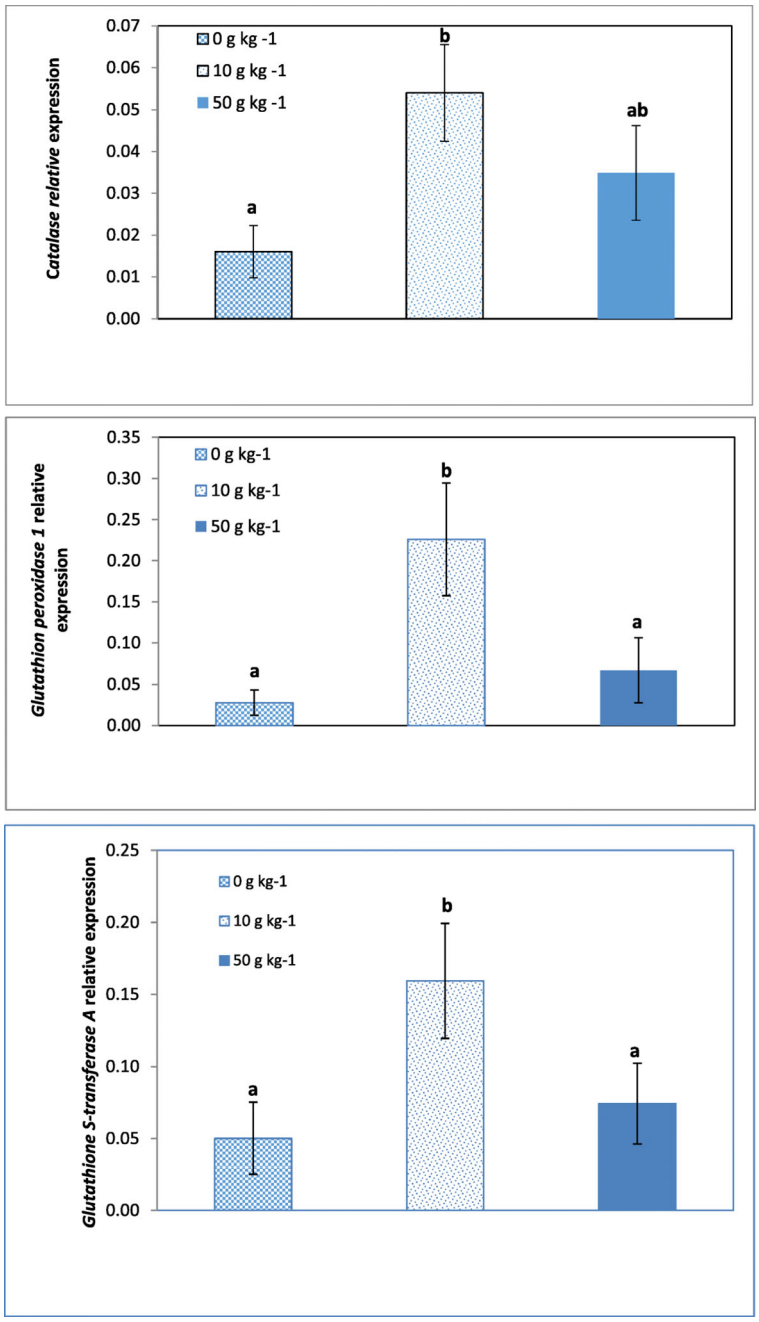


**Fig. 2** Effects of the dietary GSE on *catalase*, *glutathione peroxidase 1*, and *glutathione S-transferase A* gene expressions in rainbow trout intestine at the end of the feeding trial. Results presented are normalized against *β-actin*. Bars superscripted by different letters are significantly different ($P < 0.05$). $n = 9$ in each treatment group

In conclusion, the current study showed that ethanolic GSE, especially at 10 g $kg^{-1}$ diet, could enhance some growth parameters, glucose, and lipid peroxidation product levels as well as antioxidant-relevant gene expression in rainbow trout. Considering the potential beneficial effects of grape seed in rainbow trout, further studies are warranted to evaluate the different aspects of this additive on growth and health status of different fish species.

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