



DIETARY EFFECT OF GRAPE SEED PROANTHOCYANIDIN EXTRACT ON GROWTH PERFORMANCE, SERUM BIOCHEMICAL PARAMETERS, SKIN MUCOSAL IMMUNE RESPONSE, AND ANTIOXIDANT CAPACITY IN GOLDFISH (*CARASSIUS AURATUS*)

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Abstract

A trial was conducted to evaluate the effect of dietary grape seed proanthocyanidin extract (GSPE) on goldfish, *Carassius auratus*. In this regard, GSPE was added to a basal diet at four levels including 0, 200, 400, and 600 mg/kg to produce four experimental diets including control, GSPE200, GSPE400, and GSPE600. Three hundred and sixty goldfish (3.75 ± 0.1 g) were stocked in twelve 100 L rectangular tanks (30 fish per tank) and fed with the experimental diets three times a day for nine weeks. During the experimental trial, water temperature was 26.7–28.5°C. The weight gain and specific growth rate in the fish fed with GSPE supplemented diets were higher than the control, meanwhile feed conversion ratio value in these groups decreased compared to the control. Fish fed GSPE-supplemented diets had lower fillet lipid (10–19%), but higher protein levels (7–15%) compared to the control. The levels of serum triglyceride, alanine aminotransferase and aspartate aminotransferase in the fish fed GSPE-supplemented diets were decreased compared to the control group. The highest and lowest levels of serum glucose, and ALP were in the fish fed with control and GSPE600 diets, respectively. The skin mucosal lysozyme activity (24–38%) and protein level (70–96%) were higher in fish fed GSPE-supplemented diets than the control. The highest, and lowest liver antioxidant enzymes including superoxide dismutase, catalase, and glutathione peroxidase were observed in GSPE 600 and control groups, respectively. The findings of the present study indicated that supplementing 400 mg/kg GSPE in diet can improve growth and health condition in goldfish.

Key words: goldfish, grape seed proanthocyanidin extract, physiological parameters, immunity

During last decade, there has been an increasing interest in the use of phytochemicals as therapeutic supplements in aquaculture industry. Phytochemicals are environment-friendly products, and containing bioactive ingredients that contribute to many physiological functions in aquatic animals (Zhu, 2020). Furthermore, the beneficial effects of herbs have been the subject of many studies in fish and crustaceans (Soosean et al., 2010; Wu et al., 2015; Pourmozaffar et al., 2019). Grape seeds are produced in grape juice industry, and winery (Grosu et al., 2019). The high levels (~60–70%) of total extractable phenolic compounds in grape were detected in seeds such as lipids, fatty acids, carbohydrates, amino acids, complex fibers, oligomeric proanthocyanidins, polymerized oligomers, polyphenolic flavonoid, and proanthocyanidins (Hajati et al., 2018; Grosu et al., 2019; Rajput et al., 2017, 2019). Proanthocyanidins are natural compounds

involved in many physiological mechanisms such as antioxidant capacity that are mainly related to their impressive hydrogen donation and effective delocalization of an unpaired electron (Pekkarinen, et al., 1999). An appreciable amount of proanthocyanidins existed in many leguminous, and graminaceous plants such as grape seed proanthocyanidin extract (GSPE) that has many health promoting effects (Pekkarinen et al., 1999; Zhai et al., 2014). Recent studies have demonstrated that administration of GSPE had a great potential in improving the defense system, growth, and protecting broilers, mice, and fish against free radicals, DNA damage, and radical-induced lipid peroxidation (Zhai et al., 2014; Long et al., 2016; Rajput et al., 2017). Moreover, GSPE improved the body weight, and alleviated the immune-mediated injury, and oxidative damage of the spleen induced by aflatoxin B₁ in mice (Long et al., 2016) and fish (Zhai et

al., 2014). Furthermore, it has been confirmed that GSPE reduced the production of free radicals, and lipid peroxidation by improving the antioxidant system in broilers (Rajput et al., 2019). Most studies have been carried out to investigate physiological role of GSPE in pig and broiler (Wang et al., 2008; Eldamrawy, 2014; Hajati et al., 2018; Grosu et al., 2019; Rajput et al., 2019; Taranu et al., 2019). In recent years, there has been an increasing interest in the potential use of GSPE in the aquatic animals. For example, diet supplemented with grape seed proanthocyanidin enhanced supplementation improved growth performance and antioxidant capacity in common carp (*Cyprinus carpio*) (Mohammadi et al., 2021) and rainbow trout (*Oncorhynchus mykiss*) (Arslan et al., 2018; Mousavi et al., 2020). Anti-inflammatory effect of GSPE on liver of *Ctenopharyngodon idella* was reported by Lu et al. (2020). In Australian abalone (*Haliotis laevis*), GSPE had positive effects on feed intake, antioxidant enzymes activities, and survival after challenge with high water temperature under laboratory condition (Lange et al., 2014). In another study, Zhai et al. (2014) reported that GSPE could significantly improve the growth of Nile tilapia (*Oreochromis niloticus*).

Goldfish (*Carassius auratus*) as an important aquarium fish is a native to East Asia but it has been exported to different countries because of its economic value; hence, it has become one of the commonest aquarium fish species. Early researchers reported that goldfish is a suitable model organism in endocrinological, nutritional, and physiological studies (Mimeault et al., 2005; Blanco et al., 2018; Jahanbakhshi et al., 2021). Moreover, the healthy and fast-growing goldfish can supply market demand in the shortest possible time (Priestley et al., 2006; Gumus et al., 2016). Previous studies demonstrated that different medicinal plants

such as chaste tree (*Vitex agnus-castus*), garlic (*Allium sativum*), *Magnolia officinalis*, *Sophora alopecuroides* can improve physiological parameters of goldfish when added to their diets as a feed additive (Yi et al., 2012; Rashmehi et al., 2020, 2022; Ahmadniae Motlagh et al., 2021). However, there is little information about effect of dietary GSPE supplementation on physiological responses of ornamental fish. In the present study, it was aimed to evaluate the physiological responses of goldfish (*C. auratus*) after receiving different levels of GSPE.

Material and methods

Trial diets

Grape seed proanthocyanidin extract (95% purity, powder form, Hangzhou Joymore Technology Co. Ltd., China) was added to a basal diet free from GSPE (37.69% protein and 6.48% lipid) at four levels including 0, 200, 400, and 600 mg/kg to produce four experimental diets including control, GSPE200, GSPE400, and GSPE600, respectively (Table 1). The selected GSPE dosages were according to the previous study by Zhai et al. (2014). The experimental diets were prepared according to Mohammadi et al. (2021). Briefly, the dry ingredients were mixed with different levels of GSPE for 10 min, then oil was added to the mixture and mixed for 10 min again. Next, distilled water was poured on the mixture and blended for 15 min. Finally, the mixture was passed through a meat grinder with a 2.5-mm diameter module. The prepared diets were dried at room temperature for 24 h till the moisture content was reduced below 100 g kg⁻¹. The dried pellets were packed in plastic bags, and stored at 4°C until used.

Table 1. Formulation, proximate composition and gross energy of basal diets

Ingredients (%)		Proximate composition (% dry weight)	
Casein	32	Crude protein	37.69
Gelatin	8	Crude fat	6.48
Dextrin	28	Crude ash	14.36
Cellulose	19	Gross energy (MJ/Kg)	16.03
Fish oil	6	Moisture	8.13
Carboxy methyl cellulose	2		
Mineral premix ^a	4		
Vitamin premix ^b	1		

^aMineral premix (%): KAl(SO₄)₂, 0.159; CaCO₃, 18.101; Ca(H₂PO₄)₂, 44.601; MgSO₄, 5.216; CoCl₂, 0.07; KCl, 16.553; ferric citrate (H₂O), 1.338; MnSO₄·H₂O, 0.07; KI, 0.014; ZnSO₄, 0.192; NaH₂PO₄, 13.605; and CuSO₄·H₂O, 0.075.

^bVitamin premix: thiamin hydrochloride, 10 mg/kg; riboflavin, 20 mg/kg; calcium pantothenate, 40 mg/kg; nicotinic acid, 50 mg kg⁻¹; pyridoxine hydrochloride, 10 mg kg⁻¹; folic acid, 5 mg kg⁻¹; inositol, 400 mg/kg; choline chloride, 2000 mg/kg; menadione, 10 mg/kg; cholecalciferol, 1500 U/kg; biotin, 1 mg/kg; cobalamin, 20 µg/kg; vitamin A, 3000 U/kg; vitamin E, 50 U/kg; and vitamin C, 200 mg/kg.

Experimental setup

Juveniles goldfish, *Carassius auratus* ($n = 360$) were purchased from a private farm, Golestan province, Iran, and transferred to the laboratory (Gorgan University of Agricultural, Science and Natural Resources). Fish were stocked in ten 400 L tanks for ten days prior to the experiment. After the acclimatization period, fish with initial weight of 3.75 ± 0.10 g were transferred into twelve rectangular tanks (100 L) with 30 fish stocked in each. Water in the husbandry system was completely exchanged daily. The fish were fed three times a day to apparent satiation for nine weeks at 08:00, 13:00, and 18:00 h. Feces and uneaten feed were syphoned every day to clean the tanks. The amount of uneaten feed was daily recorded to determine the feed intake. Each tank was continuously aerated by an air stone connected to a central air compressor. During the experimental trial, water temperature was $26.7\text{--}28.5^\circ\text{C}$, dissolved oxygen was $6.3\text{--}7.1$ mg/L, alkalinity was $66.3\text{--}85.4$ mg/L, and pH was $7.3\text{--}7.7$. The photoperiod was 12 h light and 12 h darkness.

Sampling

At the end of experimental period, all fish were starved for 24 h and their weights were measured at the accuracies of 0.1 g. For determination of the fillet composition, fish (six individuals from each replicate) were randomly chosen and then, they were sacrificed with overdose of 2-phenoxyethanol (0.7 ml/L) (Hedayati, 2018), and the fillets were dissected from two lateral sides of the body. For evaluating serum biochemical parameters, fish were anesthetized with the same anesthetic (0.7 ml/L) and blood samples (0.5 ml per fish) were collected from the caudal vein (eight fish per replicate). Blood samples were pooled to provide adequate volume for analysis, as was done with three of the eight sample series in this study. The blood samples were left to clot in room temperature for 1 h, then centrifuged to separate serum (5000 g, 5 min, room temperature). After that, the same fish was eviscerated and its liver was eviscerated on ice, and transferred to a -80°C freezer for evaluating antioxidant enzymes. For skin mucosal extraction, fish (six fish per replicate) were transferred into polyethylene bags containing 10 mL of sodium chloride (50 mmol). After two min, mucus samples were centrifuged (1500 g, 10 min, room temperature). The supernatant was kept in 2.5 mL tubes at -80°C for further analysis (Ross et al., 2000). Other samples ($n = 3$ per group) including liver, fillet, and skin mucus were obtained by pooling tissues from six and eight fish from each replicate, respectively.

Proximate biochemical analyses

The chemical composition of basal diet and fish fillet was determined in triplicate according to AOAC methods (Horwitz et al., 1970). Moisture was measured by drying in an oven (Heraeus, D-63450 Hanau, Germany) at 105°C for 24 h. Gross energy of basal diet was determined using a calorimeter (IKA C6000, Germany). Crude

protein content was estimated by a Kjeldahl (Kjeltec Auto Analyzer; FOSS, Denmark) based on nitrogen levels ($N \times 6.25$) which consisted of three steps (digestion, distillation, and titration). Crude lipid was determined by ether extract using a Soxhlet apparatus (Foss, SoxtecTM 2050; Germany). Crude ash was measured after placing in a muffle furnace (Isuzu, Japan), at 550°C for 6 h.

Serum biochemical and skin mucosal immune parameters

Glucose, triglyceride, alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase (AST) levels in serum were measured by detection kits (Pars Azmoon, Iran) using an autoanalyzer (Hitachi 917, Japan). The total mucus protein was determined by the Lowry method (Lowry et al., 1951). The protein content was determined at 750 nm in a spectrophotometer (Biochrom, Libra S12). For assessing lysozyme activity of skin mucus a turbid metric assay was used according to Subramanian et al. (2007). Briefly, 40 mM sodium phosphate buffer (pH= 7.4) was added to 100 μL of the mucus sample, then incubated for 15 min in a 96-well plate and then *Micrococcus luteus* (0.75 mg/mL) cells were added to the plate and the absorbance was detected frequently at 30°C for 30 min. Lyophilized *Micrococcus luteus* were suspended in 0.1 M phosphate citrate buffer (pH 5.8). The absorbance was read at 450 nm. A unit of mucus lysozyme activity was defined as the content of the enzyme that caused a reduction in absorbance of 0.001 min/L. The hen egg white lysozyme standard curve was used for calculating units of lysozyme activity.

Antioxidant activities

Liver samples were homogenized in pre-chilled phosphate buffer saline (20 mM, pH 7.4, 4°C). Then, samples were centrifuged (3000 g, 15 min, 4°C) and the supernatant was transferred in microtubes, and stored at -80°C until enzymes assay. The superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities were measured following the methods described by Kosik-Bogacka et al. (2011). To determine SOD, 40 μL of sample was incubated for 1 min with 900 μL 2-amino-2-methyl-1,3-propanediol buffer (pH= 8.8). The absorbance was measured at 525 nm. To determine CAT, 30 μL of sample was mixed with 500 μL 10 mM H_2O_2 for 1 min. 500 μL sodium azide was added to mixture. The absorbance was read at 520 nm. To determine GPx activity, 350 μL of sample was added to 350 μL of Tris-HCl buffer (50 mM). Then, 350 μL 30 Mm tertbutylhydroperoxide was added to mixture. GPx activity was determined by measuring the absorbance at 340 nm.

Statistical analysis

All data were statistically analyzed using one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test using SAS software 9.2 ($P < 0.05$). Normality and homogeneity of variance

of data were performed using the Kolmogorov–Smirnov and Bartlett's tests, respectively.

Results

There was no mortality in any group (Table 2). Growth performance parameters of *C. auratus* fed GSPE-supplemented diets including final weight, weight gain and specific growth rate were higher than the control. In fact, weight gain in fish fed with GSPE-supplemented diets was higher than the control (~24%). The specific growth rate of fish fed GSPE-supplemented diets about ~0.8% (initial body weight/day)[§] was higher than the control. The values of FCR were improved (~23%) in fish fed GSPE-supplemented diets compared to the control.

Fish fed GSPE-supplemented diets had higher protein (~2.0%), but lower fillet lipid (~1.0%) levels compared to the control (Table 3). There were no notable differences in the moisture and ash contents among treatments.

The levels of serum triglyceride, ALT and AST in fish fed GSPE-supplemented diets were significantly decreased compared to the control group (Table 4). In addition, the highest and lowest levels of serum glucose and ALP were in fish fed the control and GSPE600 diets, respectively. The highest and lowest skin mucosal lysozyme activities were in the GSPE600, and the control groups, respectively whereas the other groups showed intermediate values (Figure 1). Skin mucosal total protein content in fish fed GSPE-supplemented diets was higher than the control (Figure 1).

Table 2. Growth performance factors of goldfish fed diets supplemented with GSPE (mean±S.D.) for 9 weeks

	Treatments				
	control	GSPE200	GSPE400	GSPE600	P-value
Initial body weight (g)	3.75±0.10	3.72±0.30	3.77±0.31	3.74±0.20	P = 0.99
Final body weight (g)	6.82±0.23 b	8.32±0.37 a	8.46±0.51 a	8.62±0.52 a	P = 0.02
Weight gain (g) [¶]	3.07±0.13 b	4.60±0.07 a	4.69±0.20 a	4.88±0.32 a	P<0.0001
SGR (% initial body weight/day) [§]	1.02±0.01 c	1.77±0.06 b	1.79±0.02 b	1.87±0.04 a	P<0.0001
FCR [‡]	3.11±0.04 a	2.57±0.05 b	2.53±0.06 cb	2.47±0.04 a	P<0.0001
Survival (%) [*]	100±0.0	100±0.0	100±0.0	100±0.0	

Values in a row with different letters denote significant difference (P<0.05). Values are presented as the mean±SD.

[¶]Weight gain (WG) = final weight – initial weight.

[§]Specific growth rate (SGR) = [(Ln Final weight - Ln Initial weight) ÷ (Experimental days)] × 100.

[‡]Feed conversion ratio (FCR) = [(Feed intake) ÷ (Final weight - Initial weight)].

^{*}Survival (%) = (final number of fish ÷ initial number of fish) × 100.

Table 3. Fillet biochemical composition (% of wet weight) in goldfish fed the experimental diets (n = 3, mean±standard deviation) for 63 days

	Treatments				
	control	GSPE200	GSPE400	GSPE600	P-value
Moisture (% of wet weight)	70.3±0.31	70.5±0.43	70.7±0.32	70.8±0.17	P = 0.3
Crude protein (% of wet weight)	16.3±0.14 c	17.5±0.43 b	18.6±0.48 a	18.8±0.52 a	P = 0.0003
Crude lipid (% of wet weight)	6.42±0.48 a	5.76±0.28 b	5.21±0.28 b	5.17±0.28 b	P = 0.006
Ash (% of wet weight)	5.63±0.26	5.67±0.41	5.70±0.38	5.72±0.29	P = 0.99

Values in a row with different letters denote significant difference (P<0.05).

Table 4. Serum biochemical parameters in goldfish fed diets supplemented the experimental diets (n = 3, mean±standard deviation) for 63 days

	Treatments				
	Control	GSPE200	GSPE400	GSPE600	P-value
Glucose (mg/dL)	76.6±0.42 a	65.3± 0.47 b	62.2±0.19 c	54.5±0.73 d	P<0.0001
Triglyceride (mg/dL)	275.3±2.11 a	248.1±2.11 c	251.4±2.11 c	263.34±2.11 b	P<0.0001
Alanine aminotransferase (U/L)	77.4±1.12 a	65.2±1.32 b	63.7±1.17 cb	62.5±1.34 c	P<0.0001
Aspartate aminotransferase (U/L)	178.5±2.73 a	149.3±2.49 b	147.1±2.41 b	145.8±2.38 b	P<0.0001
Alkaline phosphatase (U/L)	12.8±0.35 a	10.5±0.47 b	10.3±0.62 b	9.10±0.31 c	P<0.0001

Values in a row with different letters denote significant difference (P<0.05).

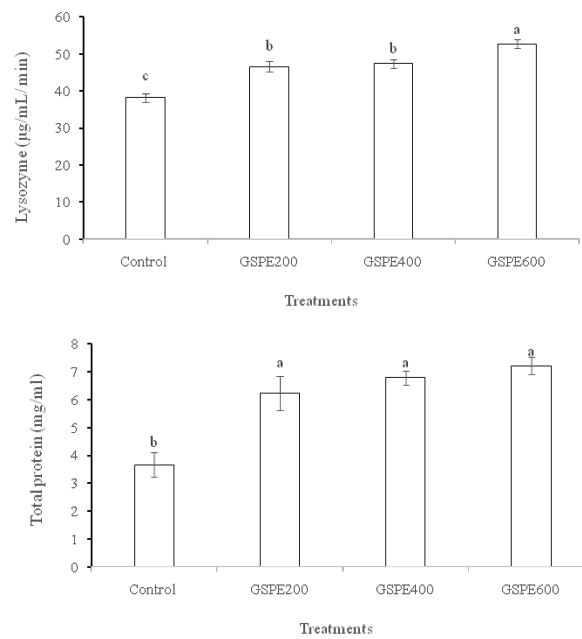


Figure 1. Skin mucosal immune parameters in goldfish fed diets supplemented the experimental diets ($n = 3$, mean $\pm$ standard deviation) for 63 days. Different superscripts on the bars denote differences ($P < 0.05$)

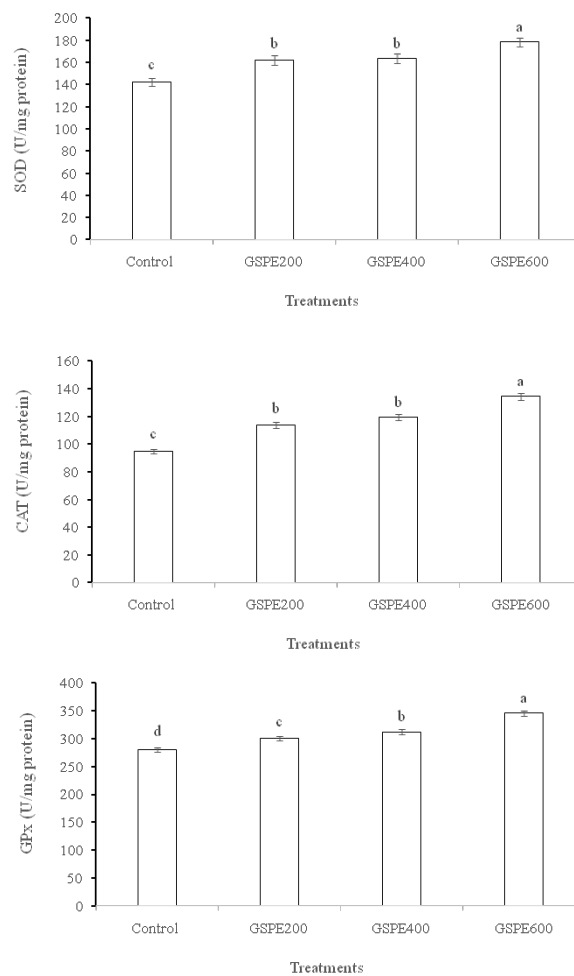


Figure 2. Liver antioxidant enzymes in goldfish fed diets supplemented the experimental diets ($n = 3$, mean $\pm$ standard deviation) for 63 days. Different superscripts on the bars denote differences ($P < 0.05$)

The highest, and lowest liver antioxidant enzymes including superoxide dismutase, catalase, and glutathione peroxidase were in GSPE600, and control groups, respectively (Figure 2).

Discussion

Results of present study showed that the growth performance of gold fish was improved by supplementing diet with GSPE that was associated with increasing feed efficiency, fillet crude protein, enhancing antioxidant enzymes activity in the liver, increment of skin mucosal lysozyme activity, and skin mucosal total protein content. Similarly, Zhai et al., (2014) reported the improvement of growth parameters in *O. niloticus* fed diets containing GSPE (200, 400, and 600 mg/kg) for 49 days. In crucian carp (*Carassius carassius*), diets containing grape seed remarkably increased growth parameters (Huang et al., 2012). Moreover, *C. carpio* fed diet contained GSPE at 200 mg/kg for forty days had better growth rate than fish fed control diet (Mohammadi et al., 2021). Similar results were observed in rainbow trout (*O. mykiss*) and *O. niloticus* fed diets containing GSPE (Arslan et al., 2018; Mousavi et al., 2020). Arslan et al. (2018) reported that the lowest FCR was observed in rainbow trout after feeding with 1000 mg grape seed oil extract per kg feed. Grape seed contains a valuable source of polyphenols (such as catechin, gallic acid, epicatechin, and procyanidin) (Mousavi et al., 2020) which act as powerful antioxidants and enhance the immunity of fish and increase the population of beneficial bacteria in intestine. In addition, the absorption of feed nutrients and digestion in broiler were increased by enhancing crypt depth and villi height in intestine after feeding with GSPE diets (Chand et al., 2021; Viveros et al., 2011; Yang et al., 2017). However, the effects of GSPE supplementation on growth performance of aquatic animals have not been fully clarified (Mousavi et al., 2020; Mohammadi et al., 2021).

Information about body composition is important to reduce feed waste, increase profitability, improve feed efficiency and the quality of the final product (Espitia-Manrique et al., 2017). For example, muscle lipid content acts as a source of lipid in the ovary, therefore, it is considered as an indicator of the reproductive performance (Ling et al., 2006). Our results showed that fillet protein content increased as GSPE concentrations increased, which was consistent with the results found in *O. mykiss* (Mohammadi et al., 2021), *C. carassius* (Huang et al., 2012) and *O. niloticus* fingerlings (Zhai et al., 2014) after feeding diets containing GSPE. The increase in fillet body protein may be due to improvement of digestive enzyme activities by grape seed polyphenol (Jianmei Yu et al., 2016). Conversely, fillet lipid content exhibited an inverse relationship with dietary GSPE level. Zhai et al. (2014) reported that fish fed GSPE-supplemented diets had lower fillets' crude lipid that coincided with the reduction of serum triglycerides levels in these groups.

GSPE prevented lipid accumulation in whole body by reducing lipid absorption in intestine and inhibiting cellular cholesterol uptake in liver (Bladé et al., 2010; Leifert and Abeywardena, 2008; Ngamukote et al., 2011).

Serum/plasma biochemical parameters provide valuable information in understanding the physiological condition and health status in fish (Wagner and Congleton, 2004; Coz-Rakovac et al., 2005; Zehra and Khan, 2019). In the current research, the level of glucose decreased in fish fed GSPE-supplemented diets suggesting stress mitigation effects of this phytochemical on *C. auratus*. According to Mohammadi et al. (2021) and Mousavi et al. (2020) results, the diet containing GSPE decreased significantly the serum glucose level in rainbow trout (*O. mykiss*). In addition, other studies also reported the positive effects of different phytochemicals on stress resistance of fish such as anthocyanin in *O. niloticus* (Yilmaz, 2019), *Hibiscus sabdariffa* in *O. mykiss* (Yousefi et al., 2021), yucca extract (Dawood et al., 2021) and phytol (Hoseini et al., 2021) in *C. carpio*. Two mechanisms have been proposed to explain the effect of GSPE on serum glucose concentration – modulation of pancreatic b-cell functions and hepatic glucose metabolism (Yang and Chan, 2017). Cholesterol and triglyceride as the most important blood lipid components, vary in the blood of fish depending on feed quality and stress status. According to our results, the level of cholesterol was significantly lower in GSPE groups compared with that of the control group. Similar decreases in cholesterol levels in rainbow trout (*O. mykiss*) fed with grape seed oil supplementation and grass carp (*Ctenopharyngodon idella*) fed intragastrically with GSPE were reported by Arslan et al. (2018) and Lu et al. (2020), respectively. Reduction of serum triglycerides was associated with a decrease in fillets' crude lipid in fish fed GSPE-supplemented diets suggesting hypolipidemic effects of this phytochemical. The results of this research agreed with the findings of Mohammadi et al. (2021). In the current study, serum ALT, AST and ALP significantly decreased in fish fed GSPE-supplemented diets suggesting hepatoprotective effects of GSPE and may be attributed to its antioxidative properties that was manifested in increment of the antioxidant enzymes activity in the liver in these groups. In agreement with the findings of the current research, several studies reported the inclusion of different phytochemicals in aquafeeds remarkably reduced serum/plasma ALT, and AST enzymes following ammonia (Hoseini et al., 2021; Yousefi et al., 2021) or bacterial challenge tests (Saha and Bandyopadhyay, 2020) in fish.

The fish skin mucosal immunity has a wide range of humoral (*i.e.*, proteins, peptides and enzymes), and cellular (*i.e.*, leucocytes) parameters with antipathogenic activities (Lazado and Caipang, 2014). The composition of the mucus is affected by various endogenous (*e.g.* developmental stage and sex) and exogenous parameters (*e.g.* food, pH, and stress) (Dash et al., 2018). Lysozyme contributes to the innate immunity of an organism through the anti-inflammatory and bactericidal properties (Ai et

al., 2004; Lin and Shiau, 2005; Zhai et al., 2014). Also, total protein reflects the health status of fish cultured (Sesay et al., 2016). Improvement in skin mucosal total protein and lysozyme activities in fish fed GSPE-supplemented diets indicated the immunostimulatory effects of this phytochemical on *C. auratus*. Elevation in serum lysozyme activity with dietary GSPE inclusion was also reported in *O. niloticus* (Zhai et al., 2014). Similar results were obtained after addition of different levels of chasteberry (*Vitex agnus-castus*) extract and mixed herbal supplementation feed into goldfish diet (Harikrishnan et al., 2010; Rashmeei et al., 2020). Moreover, Baba et al. (2016) reported improvement of lysozyme activity and infection resistance against *Aeromonas hydrophila* disease when *Avena sativa* extract is added to carp diets (*Cyprinus carpio*). Lysozyme breaks β -1,4 glycosidic acids and N-acetyl glucosamine in the peptidoglycan of bacterial cell walls (Harikrishnan et al., 2010).

Generation and removal of reactive oxygen species (ROS) is a dynamic balance in cells under an optimum physiological state. The CAT, GPx, and SOD can remove excessive ROS, thus reducing the lipid peroxidation (Asaikkutti et al., 2016; Liang et al., 2017). GSPE is a powerful antioxidant due to its ability to absorb oxygen radicals, thereby providing significant protection against DNA damage and free radical-induced lipid peroxidation, and it has anti-cancer and anti-inflammatory effects as well (Bagchi et al., 2000; Ouédraogo et al., 2011; Sayin et al., 2014; Xu et al., 2015; Rajput et al., 2017). The improvement in SOD, CAT and GPX activities in fish fed GSPE supplemented diets was demonstrated by improving the antioxidant capacity in goldfish. Likewise, an earlier study has shown that GSPE can be a potent antioxidant to improve the antioxidant system status of *O. niloticus* due to aflatoxin toxicity (Zhai et al., 2014). In another study, dietary supplementation of GSPE (200 mg/kg) enhanced the GPx and SOD activities in the liver of *O. mykiss* (Mohammadi et al., 2021). Grape is known as a powerful antioxidant due to existence of bioactive compounds such as fatty acids, minerals, vitamins, and phenolic compounds (Taranu et al., 2019). Some proteins including Nrf2, NQO1, GCLC, and HO-1 have protective effect against oxidative stress. GSPE could activate these proteins through the Nrf2 signaling pathway in the liver (Rajput et al., 2019).

Conclusions

The findings of the present study demonstrate that administration of GSPE in *C. auratus* diet can improve growth performance that attributed to better FCR. On the other hand, hypolipidemic effects of GSPE were highlighted by significant reduction of the fillets' crude lipid and serum triglycerides in fish fed GSPE-supplemented diets. Inclusion of GSPE in goldfish diet had relevant functions including antioxidant, immune-stimulatory, stress mitigation, and hepatoprotective effects. According to the results of the present study, inclusion of 400 mg/kg diet is recommended for improvement of health condition in *C. auratus*.

Acknowledgments

The authors are thankful to the Taleghani Hospital of Gorgan for providing the necessary facilities for conducting this experiment.

Ethics approval

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

Conflict of Interest

The authors declare no conflict of interest.

Data availability statement

The data that supports the findings of this study are available from the corresponding author, upon reasonable request.

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Received: 28 III 2022

Accepted: 4 VIII 2022