ORIGINAL ARTICLE

Veterinary Research Forum. 2023; 14 (11) 625 - 630
doi: 10.30466/vrf.2023.560224.3650
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Effects of dietary resveratrol supplementation on digestive enzymes activities and serum biochemistry of rainbow trout (*Oncorhynchus mykiss*)

Alireza Afzali-Kordmahalleh[1], Saeid Meshkini[2]*

[1] Post-graduate of Veterinary Medicine, Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran;
[2] Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Article Info

Article history:

Received: 24 October 2022
Accepted: 20 June 2023
Available online: 15 November 2023

Keywords:

Digestive enzymes
Rainbow trout
Resveratrol
Serum biochemistry

Abstract

The effects of resveratrol as an anti-oxidant in improving growth and health have been shown in several experiments. This study aimed to evaluate the effects of different dietary resveratrol inclusion levels on digestive enzymes activity and serum biochemistry of rainbow trout (*Oncorhynchus mykiss*). Accordingly, 225 juvenile rainbow trout with an average body weight of 10.00 ± 1.50 g were stocked in nine experimental units. The study was performed as a completely randomized design including three dietary levels of resveratrol as follows: 0.00, 400 and 800 mg kg^{-1} feed. During the experiment, fish were fed based on their respective body weight using standard feeding tables at three feeding times for 8 weeks. Nine fish were randomly selected from each treatment at the end of the 4th and 8th weeks of the experiment. Results revealed that supplementing 800 mg kg^{-1} feed resveratrol significantly increased lipase activity (31.40 ± 0.32 U mg^{-1} protein) compared to the control group (29.92 ± 0.52 U mg^{-1} protein) at the end of week eight. Also, at the same time, it increased serum high-density lipoprotein (123.04 ± 1.57 mg dL^{-1}) compared to the control group (97.055 ± 1.463 mg dL^{-1}). In addition, dietary supplementation of 800 mg kg^{-1} feed resveratrol effectively reduced serum alanine aminotransferase, alkaline phosphatase and aspartate aminotransferase activities along with glucose, cortisol and cholesterol. In conclusion, resveratrol can be used as a suitable food supplement to improve fish health by increasing digestive enzymes activities.

Introduction

Stress is one of the leading causes of growth retardation in fish farms especially in intensive cultures, making the fish use energy in other activities instead of conserving energy for growth and tissue synthesis.[1,2] Furthermore, stress may exhibit general effects in fish, such as changing serum total protein, albumin, glucose, cortisol, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), urea and triglyceride contents.[3,4] Reactive oxygen species (ROS) that cause these stresses are one of the body's most important free radicals mediating cellular mechanisms such as cellular transmission and transcriptional control.[5] Nevertheless, their excessive increase may lead to oxidative changes in cellular macromolecules, such as lipids, proteins and nucleic acids causing growth retardation or death in fish.[6] Moreover, stress decreases the ability of fish immune system and provides suitable conditions for pathogens to cause disease.[7] Studies have reported that increasing digestive enzymes activities may change the proportion of microbiota to beneficial ones by washing out the harmful microbiota in addition to efficient digestion and absorption of food.[8,9] Accordingly, fish breeders prefer using natural compounds to improve the function of fish immune system and confront stresses.[10,11]

Resveratrol is a lipid-soluble phytoalexin being present in fruits and vegetables effectively improving the immune system function. This natural anti-oxidant significantly reduces plasma triglycerides, free fatty acids, cholesterol and triglycerides entering the liver.[1,12] Resveratrol, being mostly as a glucuronide sulfate in plasma, effectively decreases the free radicals in body through enhancing the mitochondrial anti-oxidant enzymes level and changing the kinase activity as well as cellular signaling path improving the fish health.[13-17]

This study was conducted with the aim of investigating the effects of dietary supplementation of resveratrol on

***Correspondence:**

Saeid Meshkini. DVM, PhD
Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
E-mail: s.meshkiniy@urmia.ac.ir

fish health and resistance to environmental stresses by evaluating digestive enzymes, liver enzymes and some of the above-mentioned biochemical parameters in rainbow trout.

Materials and Methods

Fish husbandry. A total of 225 juvenile rainbow trout with an average body weight of 10.00 ± 1.50 g were obtained from a local fish farm in Urmia, West Azerbaijan province, Iran. Fish were transferred to the Aquaculture Center of the Veterinary Medicine Faculty of Urmia University, Urmia, Iran. After a week of acclimation, fish were weighed to determine biomass and randomly divided into three experimental groups with three replications. Each treatment contained 75 fish in 300-L tanks (25 fish per tank) with a volume of 200 L of water per tank. During the experimental period (8 weeks), fish were hand-fed at 2.50% of their initial biomass three times a day. However, they were fasted for 24 hr before any handling or sampling in order to decrease fish metabolism and ammonia and maintain water quality. Water quality characteristics were as follows: pH: 7.30 - 7.50, dissolved oxygen: 8.50 ± 0.50 ppm and temperature: 14.00 ± 1.00 ˚C being daily monitored. All of the breeding water was replaced with filtered water every day. After an hr of feeding, uneaten feed was siphoned from the bottom of the tanks to maintain the water quality. All the experiments were performed based on the standard animal experimentation protocols of the Veterinary Ethic Committee of Urmia University, Urmia, Iran (Approval ID: IR-UU-AEC-3/34).

Diet preparation. Rainbow trout commercial fingerling food trout-1 (FFT1; including protein: 42.00%, crude fiber: 3.00%, fat: 14.00% and ash: 7.00%) was prepared as a basal diet from Faradaneh Company, Shahrekord, Iran. The experimental diets were consisted of a basal diet with inclusion of 400 and 800 mg kg^{-1} feed resveratrol according to the literature.[17,18] Resveratrol (purity > 99.00%) was purchased from Sigma-Aldrich (St. Louis, USA), weighed and added to the basal diet. Afterward, 4.00% gelatin was sprayed on the commercial food. The food was dried at room temperature and refrigerated at 4.00 ˚C until use. The control group was fed a basal diet containing 4.00% gelatin without any resveratrol supplementation. The experimental diets were weekly prepared.

Sampling. At the end of the weeks four and eight of the experiment, fish were anesthetized by dipping method using 100 ppm eugenol (Sigma-Aldrich) in aerated water 24 hr after the last meal.[19,20] Nine fish from each dietary treatment were sampled randomly (three from each experimental unit). Blood samples were taken from the caudal vein using syringes with a 22-gauge needle and reserved in non-heparinized micro-tubes for hematological analyses. Moreover, the intestine was removed, washed in physiological saline (0.90%) and stored in encoded tubes after removing its contents and visceral fat. All of these steps were performed on ice. Finally, these samples were kept at – 70.00 ˚C until digestive enzymes activities determination.

Digestive enzyme activities determination. Amylase activity was determined by the starch-hydrolyzing method, according to Bernfeld.[21] Starch is degraded to maltose by the amylase enzyme and measured using a dinitrosalicylic acid reagent (Sigma-Aldrich) through colorimetric and color intensity changes.[21] The hydrolysis of p-nitrophenyl myristate (Merck, Darmstadt, Germany) was used to determine the lipase enzyme activity using spectrophotometry (Amersham Pharmacia Biotech Inc., Buckinghamshire, UK).[22] Protease enzyme activity was measured using hydrolysis of 1.50% azocasein substrate in 50.00 mM Tris-HCl buffer (Merck) at pH of 7.50.[23,24]

Liver enzymes activities determination in serum. Blood samples were immediately centrifuged at room temperature (15 min at 3,000 rpm) and stored at – 20.00 ˚C. Determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels was performed by colorimetric method; while, alkaline phosphatase (ALP) measurement was done by enzymatic method.[25]

Blood biochemical parameters measurement. Bradford method was used to measure serum soluble protein content. In this method, bovine serum albumin (Biowest, Nuaillé, France) was used as a standard. Also, to measure other biochemical parameters, blood samples were immediately centrifuged at room temperature (15 min at 3,000 rpm) and kept at – 20.00 ˚C until measurement.[26] Then, glucose, cholesterol, HDL, LDL, creatinine, urea, phosphorus, triglyceride and cortisol levels were measured using the spectrophotometer and Pars Azmoon kits (Tehran, Iran).[25]

Statistical analysis. Normality of data was examined using the Kolmogorov-Smirnov test and Levene's test was used to assess the homogeneity of variances. Data were analyzed using one-way analysis of variance and *post-hoc* Tukey HSD test by SPSS Software (version 20.0; IBM Corp., Armonk, USA). All tests were interpreted at a significance level of < 5.00%. The results were presented as mean ± standard deviation. Microsoft Office Excel (version 15.0; Microsoft Corp., Redmond, USA) was used to draw the charts.

Results

The 800 mg kg^{-1} resveratrol showed the highest levels of lipase, amylase and protease activities compared to the other treatments ($p < 0.05$; Fig. 1). At the end of the 8th week, statistical analysis showed a significant increase in lipase enzyme activity between the 800 mg kg^{-1} resveratrol treatment (31.40 ± 0.32 U mg^{-1} protein) and control groups (29.92 ± 0.52 U mg^{-1} protein; $p < 0.05$; Fig. 1). Also, 800 mg kg^{-1} resveratrol (37.53 ± 0.31 U mg^{-1} protein) showed a significant increase in protease enzyme activity

compared to the control group (36.28 ± 0.57 U mg^{-1} protein; $p < 0.05$; Fig. 1). However, no significant difference was observed in the amylase activity of fish between experimental groups ($p > 0.05$; Fig. 1).

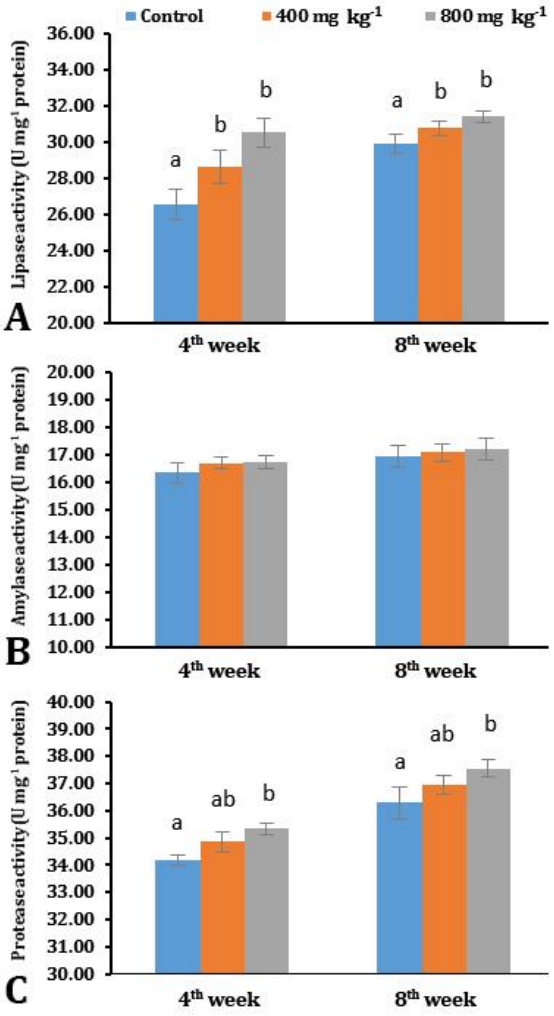

Fig. 1. Digestive enzymes activities at the end of weeks four and eight of the experiment. **A)** Lipase; **B)** Amylase; and **C)** Protease. [ab] Different letters indicate significantly differences at $p < 0.05$.

Dietary supplementation of 800 mg kg^{-1} resveratrol significantly reduced all measured liver enzymes activities compared to the control group in weeks 4 and 8 of the experiment ($p < 0.05$). The results indicated that ALT activity significantly decreased in fish fed with resveratrol ($p < 0.05$). However, no significant difference was observed between 400 and 800 mg kg^{-1} resveratrol treatments ($p > 0.05$; Fig. 2). Unlike 400 mg kg^{-1} treatment, the 800 mg kg^{-1} resveratrol showed a significant reduction in AST activity compared to the control group ($p < 0.05$; Fig. 2). Also, 800 mg kg^{-1} treatment significantly reduced ALP activity compared to the 400 mg kg^{-1} treatment and control groups at the end of the week eight ($p < 0.05$; Fig. 2). The blood biochemical para-meters showed no significant difference between the resveratrol treatment groups and control group regarding total serum protein and albumin contents ($p > 0.05$; Table 1). Furthermore, 800 mg kg^{-1} resveratrol significantly reduced glucose, cortisol, triglyceride and LDL in the blood compared to the control group ($p < 0.05$). However, no significant difference was observed between dietary supplementation of 400 and 800 mg kg^{-1} resveratrol regarding serum glucose and LDL contents ($p > 0.05$). Moreover, dietary 800 mg kg^{-1} resveratrol significantly reduced the serum urea content compared to the 400 mg kg^{-1} resveratrol and control groups at the end of week four ($p < 0.05$). The results also indicated that serum contents of HDL, phosphorus and creatinine were significantly increased in resveratrol treatment groups compared to the control group ($p < 0.05$). Additionally, the 800 mg kg^{-1} treatment showed a significant difference in serum HDL, phosphorus and creatinine compared to the 400 mg kg^{-1} treatment.

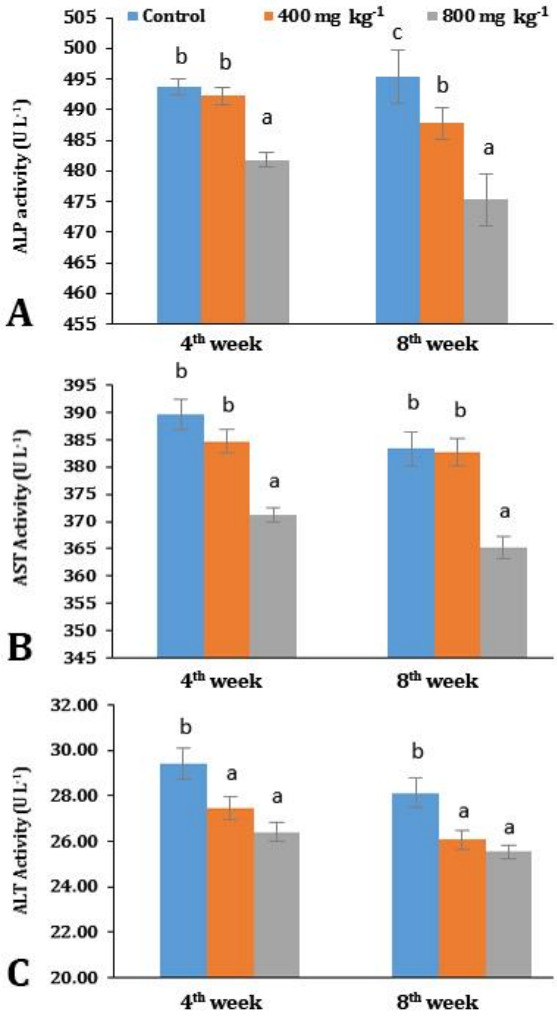

Fig. 2. Liver enzymes activities at the end of weeks 4 and 8 of the experiment. **A)** Alkaline phosphatase ALP; **B)** Aspartate amino-transferase (AST); and **C)** Alanine aminotransferase (ALT). [ab] Different letters indicate significantly differences at $p < 0.05$.

Table 1. Blood biochemical parameters of different experimental groups.

Parameters	Sampling time (Day)	Control	Resveratrol (400 mg kg^{-1})	Resveratrol (800 mg kg^{-1})
Total protein (g dL^{-1})	28	3.32 ± 0.18^{a}	3.42 ± 0.25^{a}	3.57 ± 0.26^{a}
	56	4.36 ± 0.30^{a}	4.62 ± 0.51^{a}	4.946 ± 0.42^{a}
Albumin (g dL^{-1})	28	1.66 ± 0.38^{a}	1.77 ± 0.35^{a}	1.98 ± 0.25^{a}
	56	1.88 ± 0.24^{a}	1.91 ± 0.27^{a}	2.16 ± 0.20^{a}
Glucose (mg dL^{-1})	28	68.60 ± 0.78^{b}	67.84 ± 0.51^{ab}	67.11 ± 0.42^{a}
	56	67.73 ± 1.09^{b}	66.32 ± 1.43^{ab}	64.74 ± 1.18^{a}
Cortisol (mg dL^{-1})	28	204.59 ± 1.73^{b}	204.38 ± 1.22^{b}	195.13 ± 1.40^{a}
	56	202.52 ± 1.49^{b}	200.40 ± 1.20^{b}	190.57 ± 1.81^{a}
Cholesterol (mg dL^{-1})	28	339.83 ± 4.60^{b}	338.40 ± 4.77^{b}	331.13 ± 3.11^{a}
	56	330.98 ± 9.39^{b}	321.34 ± 7.14^{b}	297.60 ± 9.54^{a}
High-density lipoprotein (mg dL^{-1})	28	94.83 ± 3.95^{a}	101.31 ± 1.12^{b}	104.18 ± 0.85^{c}
	56	97.05 ± 1.46^{a}	109.34 ± 2.54^{b}	123.04 ± 1.57^{c}
Low-density lipoprotein (mg dL^{-1})	28	155.26 ± 2.05^{b}	151.82 ± 1.90^{ab}	148.01 ± 1.64^{a}
	56	152.51 ± 2.35^{b}	150.40 ± 3.00^{ab}	146.38 ± 1.748^{a}
Creatinine (mg dL^{-1})	28	0.27 ± 0.02^{a}	0.36 ± 0.00^{b}	0.43 ± 0.01^{c}
	56	0.28 ± 0.00^{a}	0.30 ± 0.00^{b}	0.33 ± 0.00^{c}
Urea (mg dL^{-1})	28	5.19 ± 0.29^{b}	5.13 ± 0.27^{b}	4.10 ± 0.25^{a}
	56	5.89 ± 0.33^{b}	5.39 ± 0.12^{a}	5.07 ± 0.22^{a}
Phosphorus (mg dL^{-1})	28	15.66 ± 0.27^{a}	16.27 ± 0.12^{b}	16.55 ± 0.05^{c}
	56	17.78 ± 0.21^{a}	18.26 ± 0.00^{b}	18.38 ± 0.00^{c}
Triglyceride (mg dL^{-1})	28	328.01 ± 7.60^{a}	326.60 ± 4.81^{a}	321.63 ± 3.63^{a}
	56	352.79 ± 3.77^{c}	345.45 ± 2.59^{b}	330.83 ± 2.46^{a}

^{abc} Different superscript letters indicate significant differences at $p < 0.05$.

Discussion

Increased digestive enzymes activities lead to efficient digestion and absorption of food and increases in harmful microbiota washing out.[27] Various studies have been performed on herbal supplements or medicinal herbs containing anti-oxidants as gastrointestinal stimulants.[28,29] In the present study, 800 mg kg^{-1} resveratrol treatment significantly increased the activities of lipase and protease compared to the control group confirming the results of Liu *et al.*, showing that combined anti-oxidants (40.00 mg kg^{-1} vitamin C and 80.00 mg kg^{-1} vitamin E) could effectively improve digestive enzymes activities and growth of discus (*Symphysodon haraldi*).[30]

A study on the stem of *Hopea ponga* showed that one of the resveratrol oligomers, alpha-viniferin, had the most significant effect in preventing non-enzymatic reaction of sugars with protein.[31] It increased the activities of digestive enzymes such as glucosidase and amylase. Also, alpha-viniferin and trihydroxyphenanthrene glucoside, resveratrol oligomers, significantly increased glucose uptake into the cells. This raise occurred mainly due to the rearrangement of adenosine monophosphate-activated protein kinase, and eventually, by enhancing glucose transporter 4 activity, glucose transfer to the cell membrane will increase.[32,33]

Previous studies showed that some plant compounds stabilizing cell membranes could protect cells against destructive agents such as free radicals.[34,35] Thus, liver enzymes such as ALT, ALP and AST were significantly lower in fish fed with antioxidant-containing feed than fish fed by a basal diet.[34-36] Arinç *et al.*, investigated the mechanism of five flavonoids including resveratrol, inhibiting cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1) and glutathione S-transferase in the fish liver. They discovered that resveratrol effectively inhibited the ethoxyresorufin-O-deethylase-dependent CYP1A1 enzyme.[37] As a result, this anti-oxidant, being also a potent cancer preventer, can effectively maintain liver health.[38-40] This study found that 800 mg kg^{-1} resveratrol treatment was the most effective one in significant lowering of ALT, ALP and AST activities compared to the control group.

Serum glucose and cortisol levels increase in stress conditions. Anti-oxidants such as resveratrol remove ROS and reduce oxidative stresses.[40] Although in the present study there were no differences between resveratrol treatments and the control group regarding the amounts of total protein and albumin, significant reduction of the amounts of cortisol and glucose was reported. Numerous studies have shown that grape products can increase adiponectin, a hormone being associated with increased blood HDL levels and reduced LDL levels.[41-43] In the current study, HDL levels in all three groups were significantly different, indicating that resveratrol in both doses of 400 and 800 mg kg^{-1} can effectively increase blood HDL. Resveratrol might help prevent damage to blood vessels, reduce LDL and prevent blood clots. There was no study regarding the effects of resveratrol on blood biochemical parameters of fish, such as urea, phosphorus, creatinine and triglyceride. However, several studies have shown that this anti-oxidant is a potent anti-glomerulonephritis food factor in humans, suppressing proteinuria, hypoalbuminemia and hyperlipidemia simultaneously. Moreover, resveratrol significantly lowered cholesterol and triglyceride levels in cholesterol-

fed rats compared to the control group.[12,44] As the results, except ALP, urea and triglyceride, the pattern of changes between experimental groups regarding digestive and liver enzymes as well as blood biochemical parameters was similar with each other in weeks four and eight.

In conclusion, the present study showed that resveratrol increased the activities of digestive enzymes including lipase and protease, which might result in improved digestion efficiency, better nutrients uptake and ultimately better fish performance. Resveratrol also reduced liver enzymes activities via assisting in hepatocytes detoxification, oxidative stress elimination and fish health promotion. In addition, resveratrol improved fish health by increasing HDL, lowering LDL and reducing fish stress through lowering blood glucose. It is recommended to include 800 mg kg^{-1} resveratrol in trout feed to improve fish health and performance.

Acknowledgments

The authors are thankful to the Research Deputy of Urmia University and the Veterinary Medicine Faculty of Urmia University, Urmia, Iran for the financial support.

Conflict of interest

The authors declare no conflict of interest regarding this study.

References

1. Terech-Majewska E, Siwicki AK, Hermańska S, et al. Influence of trans-resveratrol on macrophage and lymphocyte activity in rainbow trout (*Oncorhynchus mykiss*) – *in vitro* study. Cent Eur J Immunol 2018; 43(3): 241-247.
2. Naderi Farsani M, Meshkini S, Manaffar R. Growth performance, immune response, antioxidant capacity and disease resistance against *Yersinia ruckeri* in rainbow trout (*Oncorhynchus mykiss*) as influenced through singular or combined consumption of resveratrol and two-strain probiotics. Aquacu Nutr 2021; 27(6): 2587-2599.
3. Yousefi M, Hoseini SM, Vatnikov YA, et al. Rosemary leaf powder improved growth performance, immune and antioxidant parameters, and crowding stress responses in common carp (*Cyprinus carpio*) fingerlings. Aquaculture 2019; 505: 473-480.
4. Baharloei M, Heidari B, Zamani H, et al. Effects of heat shock protein inducer on Hsp70 gene expression and immune parameters during *Streptococcus iniae* infection in a Persian sturgeon fry. Vet Res Forum 2021; 12(4): 473-479.
5. Amoushahi M, Salehnia M. Reactive oxygen species level, mitochondrial transcription factor A gene expression and succinate dehydrogenase activity in metaphase II oocytes derived from *in vitro* cultured vitrified mouse ovaries. Vet Res Forum 2018; 9(2): 145-152.
6. Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. Biochim Biophys Acta 2016; 1863(12): 2977-2992.
7. Tort L. Stress and immune modulation in fish. Dev Comp Immunol 2011; 35(12): 1366-1375.
8. Dawood MA, Shukry M, Zayed MM, et al. Digestive enzymes, immunity and oxidative status of Nile tilapia (*Oreochromis niloticus*) reared in intensive conditions. Slov Vet Res 2019; 56(Suppl 22): 99-108.
9. Mirghaed AT, Yarahmadi P, Hosseinifar SH, et al. The effects singular or combined administration of fermentable fiber and probiotic on mucosal immune parameters, digestive enzyme activity, gut microbiota and growth performance of Caspian white fish (*Rutilus frisii kutum*) fingerlings. Fish Shellfish Immunol 2018; 77: 194-199.
10. Wunderlich AC, Zica É de OP, Ayres VF dos S, et al. Plant-derived compounds as an alternative treatment against parasites in fish farming: a review. Natural remedies in the fight against parasites. InTech; 2017. doi: 10.5772/67668.
11. Delmas D, Aires V, Limagne E, et al. Transport, stability, and biological activity of resveratrol. Ann N Y Acad Sci 2011; 1215: 48-59.
12. Zhu L, Luo X, Jin Z. Effect of resveratrol on serum and liver lipid profile and antioxidant activity in hyperlipid-emia rats. Asian-Aust J Anim Sci 2008; 21(6): 890-895.
13. Muzzio M, Huang Z, Hu SC, et al. Determination of resveratrol and its sulfate and glucuronide metabolites in plasma by LC–MS/MS and their pharmacokinetics in dogs. J Pharm Biomed Anal 2012; 59: 201-208.
14. Li X, Yao Y, Wang S, et al. Resveratrol relieves chlorothalonil-induced apoptosis and necroptosis through miR-15a/Bcl2-A20 axis in fish kidney cells. Fish Shellfish Immunol 2020; 107(Part B): 427-434.
15. Porcacchia AS, Moreira GA, Andersen ML, et al. The use of resveratrol in the treatment of obstructive sleep apnea and cancer: a commentary on common targets. J Clin Sleep Med 2022; 18(1): 333-334.
16. Tai YS, Ma YS, Chen CL, et al. Resveratrol analog 4-bromo-resveratrol inhibits gastric cancer stemness through the SIRT3-c-Jun N-terminal kinase signaling pathway. Curr Issues Mol Biol 2021; 44(1): 63-72.
17. Torno C, Staats S, Rimbach G, et al. Effects of resveratrol and genistein on nutrient digestibility and intestinal histopathology of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 2018; 491: 114-120.
18. King RE, Kent KD, Bomser JA. Resveratrol reduces oxidation and proliferation of human retinal pigment

epithelial cells via extracellular signal-regulated kinase inhibition. Chem Biol Interact 2005; 151(2): 143-149.
19. Holloway AC, Keene JL, Noakes DG, et al. Effects of clove oil and MS-222 on blood hormone profiles in rainbow trout *Oncorhynchus mykiss*, Walbaum. Aquacu Res 2004; 35(11): 1025-1030.
20. Guénette SA, Uhland FC, Hélie P, et al. Pharmaco-kinetics of eugenol in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 2007; 266(1-4): 262-265.
21. Bernfeld P. Amylases, α and β. Meth Enzymol 1955; 1: 149-158.
22. Iijima N, Tanaka S, Ota Y. Purification and characterization of bile salt-activated lipase from the hepatopancreas of red sea bream, *Pagrus major*. Fish Physiol Biochem 1998; 18: 59-69.
23. García-Carreño FL, Haard NF. Characterization of proteinase classes in langostilla (*Pleuroncodes planipes*) and crayfish (*Pacifastacus astacus*) extracts. J Food Biochem 1993; 17(2): 97-113.
24. Chong AS.C, Hashim R, Chow-Yang L, et al. Partial characterization and activities of proteases from the digestive tract of discus fish (*Symphysodon aequifasciata*). Aquaculture 2002; 203(3-4): 321-333.
25. Borges A., Scotti LV, Siqueira DR, et al. Hematologic and serum biochemical values for jundiá (*Rhamdia quelen*). Fish Physiol Biochem 2004; 30: 21-25.
26. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72: 248-254.
27. Xia S, Zhao W, Li M, et al. Effects of dietary protein levels on the activity of the digestive enzyme of albino and normal *Apostichopus japonicus* (Selenka). Aquac Res 2018; 49(3): 1302-1309.
28. Zeilab Sendijani R, Abedian Kenari A, Smiley AH, et al. The effect of extract from dill *Anethum graveolens* on the growth performance, body composition, immune system, and antioxidant system of rainbow trout. N Am J Aquac 2020; 82(2): 119-131.
29. Bilen S, Altief TAS, Özdemir KY, et al. Effect of lemon balm (*Melissa officinalis*) extract on growth performance, digestive and antioxidant enzyme activities, and immune responses in rainbow trout (*Oncorhynchus mykiss*). Fish Physiol Biochem 2020; 46(1): 471-481.
30. Liu HP, Wen B, Chen ZZ, et al. Effects of dietary vitamin C and vitamin E on the growth, antioxidant defence and digestive enzyme activities of juvenile discus fish (*Symphysodon haraldi*). Aquac Nutr 2019; 25: 176-183.
31. Sasikumar P, Lekshmy K, Sini S, et al. Isolation and characterization of resveratrol oligomers from the stem bark of *Hopea ponga* (Dennst.) Mabb. and their antidiabetic effect by modulation of digestive enzymes, protein glycation and glucose uptake in L6 myocytes. J Ethnopharmacol 2019; 236: 196-204.
32. Rose PM, Saranya J, Eganathan P, et al. *In vitro* evaluation and comparison of antioxidant and anti-bacterial activities of leaf extracts of *Hopea ponga* (Dennst.) Mabberly. Int J Green Pharm 2013; 7: 177-181.
33. Lin MH, Hung CF, Sung HC, et al. The bioactivities of resveratrol and its naturally occurring derivatives on skin. J Food Drug Anal 2021; 29(1): 15-38.
34. Engwa GA. Free radicals and the role of plant phytochemicals as antioxidants against oxidative stress-related diseases. Phytochemicals: source of antioxidants and role in disease prevention. InTech; 2018. doi: 10.5772/intechopen.76719.
35. Ghafarifarsani H, Hoseinifar SH, Adorian TJ, et al. The effects of combined inclusion of *Malvae sylvestris*, *Origanum vulgare*, and *Allium hirtifolium boiss* for common carp (*Cyprinus carpio*) diet: Growth performance, antioxidant defense, and immunological parameters. Fish Shellfish Immunol 2021; 119: 670-677.
36. Adeli A, Shamloofar M, Akrami R. Dietary effect of Lemon Verbena (*Aloysia triphylla*) extract on growth performance, some haematological, biochemical, and non-specific immunity and stocking density challenge of rainbow trout juveniles (*Oncorhynchus mykiss*). J Appl Anim Res 2021; 49(1): 382-390.
37. Arinç E, Yilmaz D, Bozcaarmutlu A. Mechanism of inhibition of CYP1A1 and glutathione S-transferase activities in fish liver by quercetin, resveratrol, naringenin, hesperidin, and rutin. NutrCancer 2015; 67(1): 137-144.
38. Singh CK, George J, Ahmad N. Resveratrol-based combinatorial strategies for cancer management. Ann N Y Acad Sci 2013; 1290(1): 113-121.
39. Wu SL, Sun ZJ, Yu L, et al. Effect of resveratrol and in combination with 5-FU on murine liver cancer. World J Gastroenterol 2004; 10(20): 3048-3052.
40. Alfonso S, Gesto M, Sadoul B. Temperature increase and its effects on fish stress physiology in the context of global warming. J Fish Biol 2021; 98(6): 1496-1508.
41. Sierksma A, Patel H, Ouchi N, et al. Effect of moderate alcohol consumption on adiponectin, tumor necrosis factor-alpha, and insulin sensitivity. Diabetes Care 2004; 27(1): 184-189.
42. Pischon T, Girman CJ, Rifai N, et al. Association between dietary factors and plasma adiponectin concentrations in men. Am J Clin Nutr 2005; 81(4): 780-786.
43. Ghaedi E, Moradi S, Aslani Z, et al. Effects of grape products on blood lipids: a systematic review and dose-response meta-analysis of randomized controlled trials. Food Funct 2019; 10(10): 6399-6416.
44. Nihei T, Miura Y, Yagasaki K. Inhibitory effect of resveratrol on proteinuria, hypoalbuminemia and hyperlipidemia in nephritic rats. Life Sci 2001; 68(25): 2845-2852.