Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi 27 (3):623-628, 2022
(*Mustafa Kemal University Journal of Agricultural Sciences* 27 (3):623-628, 2022)
e-ISSN: 2667-7733
http://dergipark.org.tr/mkutbd

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Effects of dietary grape seed extract supplementation on some biochemical parameters of Rainbow trout

Yemlere ilave edilen üzüm çekirdeği ekstraktının Gökkuşağı alabalığının bazı biyokimyasal parametrelerine etkisi

Mikail ÖZCAN[1], Ünal İSPİR[2], Ayşegül ŞAHİN[3]

[1]*Kahramanmaras Sütçü Imam University, Agriculture Faculty, Department of Fisheries Kahramanmaras, 46040 Turkey.*
[2]*Malatya Turgut Özal University, Doğanşehir Vahap Küçük Vocational School, Department of Aquaculture, 44210 Malatya, Turkey.*
[3]*Elazig Directoriate Fisheries Research Station, Elazig, Turkey.*

MAKALE BİLGİSİ / ARTICLE INFO

Makale tarihçesi / Article history:
DOI: 10.37908/mkutbd.1057918
Geliş tarihi /Received:08.04.2022
Kabul tarihi/Accepted:07.09.2022

Keywords:
Grape seed extract, Rainbow trout, *Oncorhynchus mykiss*, total protein, total lipid, total carbohydrate.

Corresponding author: Mikail ÖZCAN
✉: mikailozcan@ksu.edu.tr

Ö Z E T / A B S T R A C T

Aims: The goal of this research was to investigate the impact of grape seed extract on some biochemical parameters of rainbow trout (*Oncorhynchus mykiss*) with an average weight of 42.5±1.44g.
Methods and Results: Fishes were fed with diets containing 0%, 0.5%, 1% and 1.5% of grape seed extract (GSE) during 42 days. At the end of the experiment, kidney, spleen, muscle and liver tissues were taken from fish and total protein, total lipid and total carbohydrate levels were determined.
Conclusions: The total protein levels of 0%, 0.5%, 1% and 1.5% group were increased importantly by dietary GSE supplementation ($p<0.05$). On the other hand, the total carbohydrate and total lipid levels were not significantly affected by the dietary level of GSE in all tissues.
Significance and Impact of the Study: In conclusion, we demonstrated an improvement of total protein, total lipid and total carbohydrate parameters of *Oncorhynchus mykiss* by dietary GSE supplementation.

Atıf / Citation: Özcan M, İspir Ü, Şahin A (2022) Effects of dietary grape seed extract supplementation on some biochemical parameters of rainbow trout. *Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi,* 27(3) : 623-628. DOI: 10.37908/mkutbd.1057918

INTRODUCTION

The development of fish farming, associated with intensive production, has expanded the demand for superior quality feedstuff in arrange to move forward fish health without any side impacts on consumers (Espe et al., 1999; Tacon and Metian, 2015).
Fish meal has traditionally been used as the most nutritious feed in fish feed regulation because of its protein content and amino acid profile. Due to its deficiency worldwide, coupled with expanded demand and competition for its utilization in poultry and livestock feeds, costs of the fish meal have ended up unreasonably expensive (Tacon, 1993).
Subsequently, sustainable aquaculture connects with an idealized balance between health conditions and the development of fish. The utilization of chemotherapeutics and antibiotics to eliminate fish infections might create bioaccumulation, pathogens, and environmental pollution. Besides, vaccines are costly for fish farming practices and are against particular specific pathogens (Raa, 1992).
Grapes (*Vitis vinifera*), which are usually processed for grape juice or wine, produce seed and skin by-products known as pomace. This pomace is a rich source of flavonoid compounds which are antioxidants. Grape seeds are rich in oligomeric procyanidins such as the (-)-epicatechin, (+)-catechins, monomeric phenolic

compounds and dimeric(-)-epicatechin-3-O-gallate, tetrameric procyanidins and proanthocyanidins and trimeric (Perdicaro et al., 2017). Grapes possess proanthocyanidins in an amount between 0.3 and 0.9 g kg-1 of in (Gu et al., 2004). On average, 60-70% of the extractable phenolic compounds are available in the grape seeds (Wilska-Jeszka, 1996).

Many studies have been conducted on the impacts of grape seed extracts (GSE) on terrestrial animals (Dulundu et al., 2007; Yousef et al., 2009; El-Ashmawy et al., 2010; Boghdady, 2013; Hassan and Al-Rawi, 2013). Some limited knowledge is existent about the use of dietary GSE in aquaculture. Kao et al. (2010), reported a noteworthy decrease in inflammatory reactions and mortality in zebrafish infected with *Staphylococcus aureus*. Further studies are required to affirm the useful impacts of GSE in aquaculture (Zhai et al., 2014).

There has been no published report on the biological impacts of GSE on rainbow trout based on the observations from literature survey. Therefore, this study was carried out to study the various biochemical effect of the GSE in rainbow trout (*Oncorhynchus mykiss).*

MATERIALS and METHODS

Fish samples

The research was carried out in the Fish Disease Laboratory of Kahramanmaras University, Faculty of Agriculture, Fisheries Department located in Kahramanmaras province, Turkey. Fish were obtained from a commercial fish farm in Kahramanmaras and It was brought alive to the laboratory, where the research was conducted. Approximately 400 rainbow trout (*O. mykiss*) with an average weight of 42.5±1.44 g were used. Fish were stocked in 600 L fiberglass tanks with the dimensions of 120x10x50 cm. Before starting the experimental study, the fish were acclimated for two weeks to adapt to the study conditions. In the adaptation process, the fish were fed with commercial trout feed (fish feed ABALIOĞLU agricultural production inc. Izmir Turkey)(containing 40% protein, 11% fat, 10.1% crude ash, 1.3% crude fiber, 2.4% calcium, 1.5% phosphorus, 0.3% sodium, 0.6 NM granular fry feed) to satiation twice a day.

Grape seed extract (GSE)

Grape seeds were obtained from a commercial wine production company. extraction, the seeds were sieved through a 4 mm strainer to separate non-seed materials. The seeds were fragmented by utilizing a mill. Later, a solution (150 mL) containing distilled water (9.5 mL), acetic acid (0.5 mL) and acetone (90 mL) were included in each of the partitioned seeds (100 g) (Jayaprakasha et al., 2003). This mixture was incubated in a dark environment and cooled for 24 h. Then, the mix was strained through a vacuum filter system and the filtrate was subjected to purification at 65 °C in an incubator. The grape seed extract (GSE) was stored in vacuum-sealed packs at −20 °C until the use for pellet feed (Kesbiç and Yigit, 2019).

Experimental feeds

GSE were added to the research feed diets with concentrations at 0%, 0.5%, 1% and 1.5%. All feed ingredients were weighed and mixed well by utilizing a mixer. This mixture is pulped with distilled water up to 30% of its weight. The diets, which were turned into pellets by passing through a 3 mm sieve of the meat grinding machine, were dried in an incubator (55 °C) for 12 hours. Then, the product was stored at −20 °C until the start of the feeding experiment.

Experimental fish groups

In this experiment, four fish groups were formed as control 0% GSE (i); 0.5% GSE (ii); 1% GSE (iii) and 1.5% GSE (iv). In each experimental group, 50 fish were stocked in fiberglass tanks. The experiment was conducted with two replications. Therefore, the research was carried out with a total of 400 fish (50 fish/per group; four fish groups, two replications). Fish were fed twice daily with an amount equal to 3% of their body weight for 42 days.

Water quality parameters

Throughout the experiment, average water temperature (WTW, Monoline Oxi 3310), dissolved oxygen (WTW, Monoline Oxi 3310) and pH (HACH, HQ11d) were measured as 12±0.22 °C, 8.5±0.14 ppm and 7.8±0.09 respectively.

Biochemical analysis

At the end of the experimental period of 42 days, the fish were sedated with an anesthetic substance (2-phenoxyethanol). Kidney, spleen, muscle and liver tissues were obtained and kept in storage box at -80 ^{o}C until the analysis of the biochemical parameters. Total protein, total lipid, and total carbohydrates % of spleen, liver, muscle and kidney tissues were measured using the standard methods of the Association of Official Analytical Chemists (AOAC, 1990).

Statistical data analyses

Statistical analysis of the data obtained in the experiment was carried out using the SPSS 10 package statistics program (IBM SPSS Statistics 23.msi). Changes in some biochemical parameters of the control and three experimental groups of fish were tested with one-way analysis of variance (ANOVA) and Duncan's multiple comparison tests. Differences among the groups were considered important if $p< 0.05$.

RESULTS and DISCUSSION

Changes in protein levels in the liver, spleen, kidney and muscle tissues are shown in Figure 1. It was observed that the total protein content of the liver improved with an increase in the grape seed extract (GSE) rate of dietary but the values were significantly higher only at the highest dietary supplemental levels of 1.5% ($p <0.05$). Ratios for the total protein in the liver tissues for the experimental groups (0.5%, 1.0% and 1.5%) and control fish group (0%) were 19.2, 18.79, 21.0 and 18.4, respectively (Figure 1).

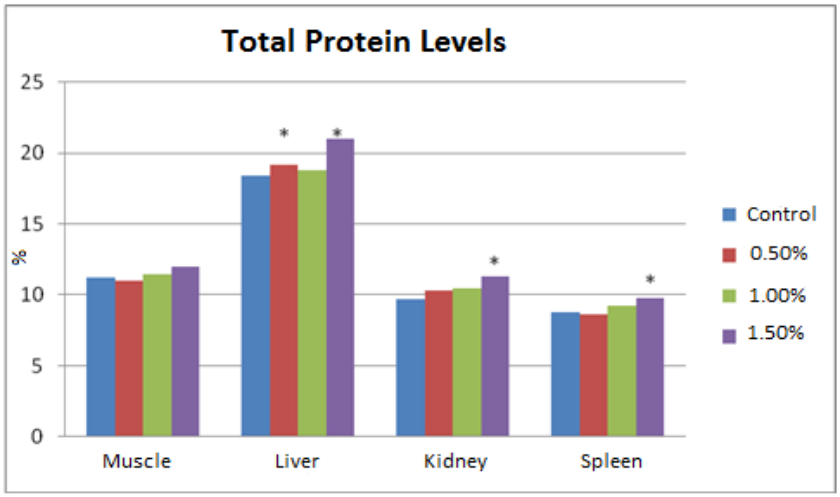

Figure 1. Total protein levels in the liver, spleen, kidney and muscle tissues of the rainbow trout fish fed with different grape seed extracts concentrations (0.0-control, 0.5, 1.0 and 1.5%)

Muscle total lipid quantity was observed as essentially higher in fish group fed with the diet with 1.5% (iv) of GSE than those of the control (i), 0.5% (ii) and 1% (iii) groups (Figure 2). No important differences were observed between the total lipid values for the fish fed with dietary GSE levels of 1% or lower. Liver total lipid amount was most elevated in the fish groups fed with the 1% and 1.5% GSE supplemented diet, however, they were not statistically different from that of the fish group fed with 0.5% GSE diet. But, there were no important contrasts between the values for fish fed basal diets (Figure 2).

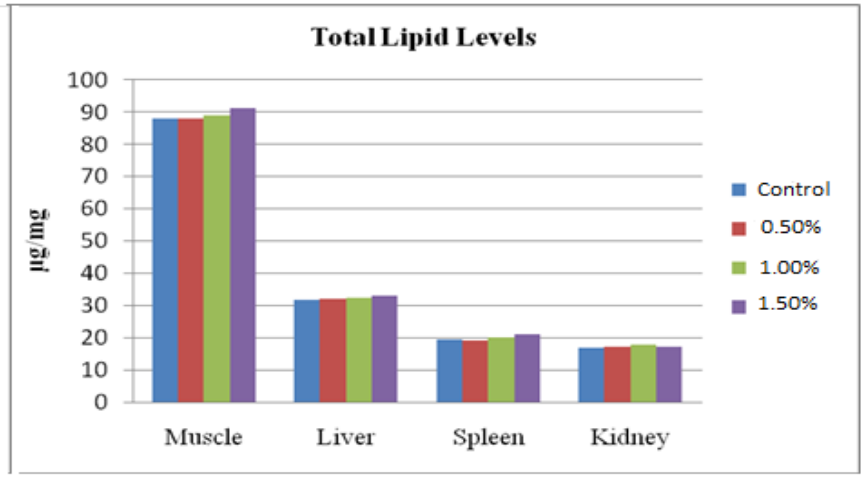

Figure 2. Total lipid levels in the muscle, kidney, spleen and liver tissues of the rainbow trout fish fed with different grape seed extracts concentrations (0.0-control, 0.5, 1.0 and 1.5%)

It was determined that the GSE ratio in the diet did not importantly affect the carbohydrate values of liver, spleen, kidney and muscle tissues (Figure 3). The total carbohydrate level at low concentration (0.5%) was decreased from that in the fish group of 0% (control group) (i) (p> 0.05). When the addition of GSE supplements to diets was increased to 1.5% and 1.0%, the total carbohydrate level of fish was slightly, however not significantly, higher than those fish with the 0.5% (ii) and %0.0% levels of GSE (control group) (i) (p> 0.05).

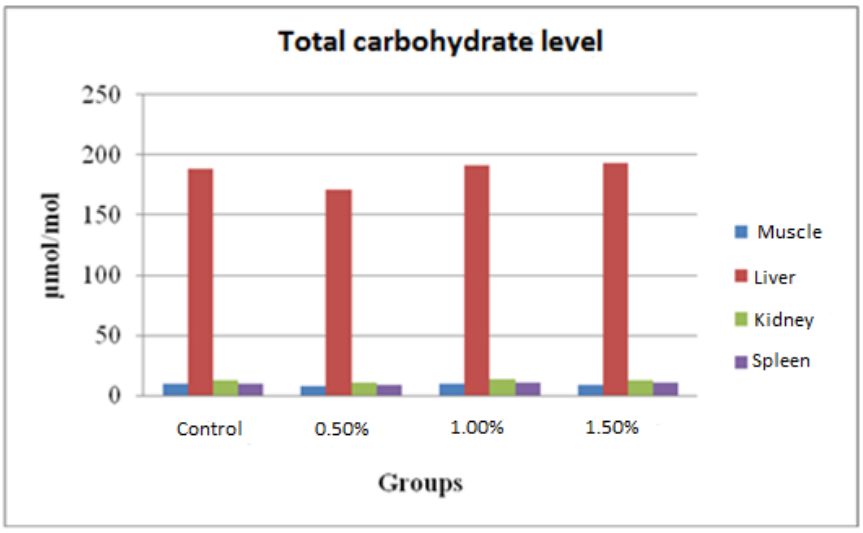

Figure 3. Total carbohydrate levels in the muscle, kidney, spleen and liver tissues of the rainbow trout fish fed with different grape seed extracts concentrations (0.0-control, 0.5, 1.0 and 1.5%)

This research aimed to investigate the impacts of the influential concentrations of grape seed extract (GSE) of 0%, 0.5%, 1% and 1.5% in the feed on total protein, total lipid and total carbohydrate parameters in liver, kidney, spleen and muscle tissues of rainbow trout (*Oncorhynchus mykiss*).

Nutrient ingredients in fish meat are nearly related to their digestibility levels and the nutrient contents of feeds (Jauncey, 1982). Protein amount of liver increased with expanding amounts of dietary in the present study but the values were significantly higher only at the highest dietary supplemental levels (p< 0.05). This increment within the protein substance might propose that the utilization of GSE at 1.5% likely given a positive impact on protein digestibility, conceivably because of the changes in gut microbiota influenced by the use of GSE in the diets. Symbiotic bacteria in the digestive system assist digestion, whereas an increment within the population of commensal bacteria might negatively influence digestion in terms of impact metabolic activities and growth performance (Mehrabi et al. 2012).

Deis (2006) and Min et al. (2006) stated that oxidation of the lipid emerging from the response of lipid with oxygen and its hydrolysis are influenced by the action of lipolytic proteins. In this study, muscle total lipid amount was significantly lower in fish group fed with the diet with 1.5% of GSE than those of the other groups. No important differences were observed among the values of fish-fed dietary GSE levels of 1% or lower. Liver lipid content was highest in the fish group fed with the 1% and 1.5% GSE supplemented diet yet this did not differ from that of fish group fed with the 0.5% GSE diet. Nevertheless, there were no important differences between the values for fish-fed basal diets.

Hoşsu et al. (2001) declared that carbohydrates utilized the foremost effectively by fish is glucose and the as it were sugar within the blood. With the blow down of glucose, the vitality required to preserve crucial exercises is given. In the present study, dietary treatments did not importantly affect the total carbohydrate values of muscle, liver, kidney and spleen tissues. The carbohydrate level at low amount was lower from that in the 0% (control group) (i) (p> 0.05). In the experiment, when the addition of GSE to diets was increased to 1% (iii) and 1.5% (iv), the carbohydrate level of fish was slightly but not importantly (p> 0.05) higher than those fish group fed with the 0.5% (ii) levels of GSE or the 0% (control) diet. Girgin Basusta (2005) underlined that blood glucose is put away as glycogen within the liver of fish and while the living being needs it, it is changed into glucose and given to the blood.

In conclusions, in this study the influence of grape seed extract (GSE) with different concentrations (0.0, 0.5, 1.0

and 1.5%) in the diet was examined on some biochemical properties (total protein, total lipid and total carbohydrate) of rainbow trout (*Oncorhynchus mykiss*).

It was determined that the use of GSE as natural antioxidants have positive effects on biochemical parameters. In conclusion, the GSE may be used as a potent source of piscicidal activity. Because plant-based products are less expensive, easily available and easily soluble in water, they may be preferred for improving the health status of the fish.

ÖZET

Amaç: Bu çalışmada, üzüm çekirdeği ekstraktının (GSE), ortalama ağırlığı 42.5±1.44 g olan gökkuşağı alabalığının (*Oncorhynchus mykiss*) bazı biyokimyasal parametreleri üzerine etkisi araştırılmıştır.

Yöntem ve Bulgular: Balıklar, 42 gün boyunca %0 (i), %0.5 (ii), %1(iii) ve %1.5 (iv) GSE içeren yemlerle beslenmiştir. Deney bitiminde balıklardan dalak, karaciğer, böbrek ve kas dokuları alınarak toplam protein, toplam lipit ve toplam karbonhidrat miktarları belirlenmiştir.

Genel Yorum: Tüm deney gruplarının toplam protein seviyelerinin, diyet GSE takviyesi ile önemli ölçüde arttığı belirlenmiştir ($p<0.05$). Gökkuşağı alabalığının toplam karbonhidrat ve toplam lipid düzeylerinin, tüm dokularda diyetsel GSE düzeyinden önemli ölçüde etkilenmediği tespit edilmiştir.

Çalışmanın Önemi ve Etkisi: Sonuç olarak, diyet GSE takviyesi ile gökkuşağı alabalığının bazı biyokimyasal parametrelerinde bir gelişme olduğu gözlemlenmiştir.

Anahtar Kelimeler: Üzüm çekirdeği ekstraktı, gökkuşağı alabalığı, *Oncorhynchus mykiss*, total protein, total lipid, total karbonhidrat.

CONFLICT OF INTEREST

The authors declare no conflict of interest for this study.

ACKNOWLEDGEMENT

This study was presented as a poster presentation at the FABA, 03- 05/11/2016 in Antalya, Turkey.

ETHICAL APPROVAL

Fish use and experimental protocol were approved by the Animal Ethics Committee of Kahramanmaraş Sütçü Imam University, Faculty of Agriculture (Protocol number: 2016/1).

REFERENCES

AOAC (1990) Official methods of analysis of the AOAC, 15th ed. Methods 932.06, 925.09, 985.29, 923.03. Association of Official Analytical Chemists. Arlington, VA, USA.

Boghdady NA (2013) Antioxidant and antiapoptotic effects of proanthocyanidin and ginkgo biloba extract against doxorubicininduced cardiac injury in rats. Cell Biochem. Funct. 31: 344-351.

Deis RC (2006) The complexity of shelf life stability. 1 st. USA: Virgo pub.

Dulundu E, Ozel Y, Topaloglu U, Toklu H, Ercan F, Gedik N, Sener G (2007) Grape seed extract reduces oxidative stress and fibrosis in experimental biliary obstruction. J. Gastroen. Hepatol. 22: 885-892.

El-Ashmawy IM, Gad SB, Salama OM (2010) Grape seed extract prevents azathioprine toxicity in rats. Phytother. Res. 24: 1710-1715.

Espe M, Sveier H, Hogoy I, Lied E (1999) Nutrient absorption and growth of Atlantic salmon (*Salmo salar* L.) fed fish protein concentrate. Aquaculture 174(1-2): 119-137.

Girgin Basusta A (2005) Balık biyolojisi araştırma yöntemleri (Research techniques in fish biology). Nobel Publication, Ankara, 772, 498s.

Gu L, Kelm MA, Hammerstone JF, Beecher G, Holden J, Haytowitz D, Gebhardt S, Prior RL (2004) Concentrations of proanthocyanidins in common foods and estimations of normal consumption. J. Nutr. 134: 613-617.

Hassan HA, Al-Rawi MM (2013) Grape seeds proanthocyanidin extract as a hepaticreno-protective agent against gibberellic acid induced oxidative stress and cellular alterations. Cytotechnology 65: 567-576.

Hoşsu B, Korkut AY, Fırat A (2001) Balık Besleme ve Yem Teknolojisi (Fish Nutrition and Feed Technology). Ege University Faculty of Fisheries Publications No:50. Basımevi, Bornova, İzmir, 276s.

Jauncey K (1982) The effects of varying dietary protein level on the growth, food conversion, protein utilization and body composition of juvenile tilapias (*Sarotherodon mossambicus*). Aquaculture 27(1): 43-54.

Jayaprakasha GK, Selvi T, Sakariah KK (2003) Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. Food Res. Int. 36(2): 117-122.

Kao TT, Tu HC, Chang WN, Chen BH, Shi YY, Chang TC, Fu TF (2010) Grape seed extract inhibits the growth and pathogenicity of *Staphylococcus aureus* by

interfering with dihydrofolate reductase activity and folate-mediated one-carbon metabolism. Int. J. Food Microbiol. 141: 17-27.

Kesbiç OS, Yigit M (2019) Structural and chemical changes of grape seed extract after thermal processing and its use in rainbow trout (*Oncorhynchus mykiss*) diets as an organic feed supplement. Aquaculture 503: 275-281.

Mehrabi Z, Firouzbakhsh F, Jafarpour A (2012) Effects of dietary supplementation of synbiotic on growth performance, serum biochemical parameters and carcass composition in rainbow trout (*Oncorhynchus mykiss*) fingerlings. J. Anim. Physiol. Anim. Nutr. 96(3): 474-481.

Min S, Rumsey TR, Krochta J (2006) Lysozyme diffusion in smoked salmon coated with whey protein films in corporation lysozyme, China Development Research Foundation, 1: 5-29

Perdicaro DJ, Lanzi CR, Fontana AR, Antoniolli A, Piccoli P, Miatello RM, Diez ER, Prieto MAV (2017) Grape pomace reduced reperfusion arrhythmias in rats with a high-fat-fructose diet. Food Funct. 10: 3501-3509.

Raa J, Rorstad G, Engstad RE, Robertson B (1992) "The use of immunostimulants to increase resistance of aquatic organism to microbial infections" in disease in Asian aquaculture. Proceedings of the First Symposium on Disease in Asian Aquaculture, M. Shariff, R. P. Subasingh, and J. R.Arthur, Eds., vol. 1, pp. 39-50, Asian Fisheries Society, Manila, Philippines.

Tacon AG, Metian M (2015) Feed matters: satisfying the feed demand of aquaculture. Rev. Fish. Sci. Aquac. 23(1): 1-10.

Tacon, AG (1993). Feed ingredients for warm water fish, fish meal and other processed feedstuffs. FAO Fisheries Circular (FAO). no. 856.

Wilska-Jeszka J (1996) Proanthocyanidins: content in fruits and influence on health. Food Chem. 57(1): 57-59.

Yousef MI, Saad A.A, El-Shennawy LK (2009) Protective effect of grape seed proanthocyanidin extract against oxidative stress induced by cisplatin in rats. Food Chem. Toxicol. 47: 1176-1183.

Zhai SW, Lu JJ, Chen XH (2014) Effects of dietary grape seed proanthocyanidins on growth performance, some serum biochemical parameters and body composition of tilapia (*Oreochromis niloticus*) fingerlings. Ital. J. Anim. Sci. 13(3): 3357.