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Scientific Article

Evaluation of dietary oyster mushroom on intestinal histology, growth performance, and immuno-serological parameters of juvenile rainbow trout

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ABSTRACT

In this study, the effects of nutritional supplementation with oyster mushroom extract on the nonspecific immune response, gut histology, and growth performance of rainbow trout were investigated. Sixty fish (weighing 20 g) were placed in tank groups, and groups were established. The control fish were fed with commercial pellets, and the other two experimental group fish were fed with experimental diets containing oyster mushroom extracts for six weeks. During the trial, biometrical, immunological, and biochemical parameters were assessed in one, two, three, four, five, and six weeks. Also, gut samples were taken at the same sampling weeks. The intracellular respiratory burst, bactericidal activity, total protein, and globulin were significantly enhanced in fish fed 2% experimental diet in three, four, five, and six weeks. Compared to the control group, the fish group which received 2% supplemented diet showed elevated cholesterol levels in serum at the first week followed by decrease in the third and sixth weeks. Some biochemical parameters (glucose, albumin, and triglycerides) were not affected. Moreover, the feeding with dietary extract did not show any negative impact on the histology of the gut and growth performance of fish. These results indicated oyster mushroom extract may effectively enhance non-specific immune parameters in juvenile rainbow trout.

Keywords: Fish; *Pleurotus ostreatus*; Mushroom extract; Non-specific immune system.

Avaliação do cogumelo ostra alimentar na histologia intestinal, no desempenho de crescimento e em parâmetros imuno-serológicos da truta-arco-íris juvenil

RESUMO

Neste estudo, investigaram-se os efeitos da suplementação nutricional com extrato de cogumelo-ostra na resposta imune inespecífica, na histologia intestinal e no desempenho de crescimento da truta-arco-íris. Foram colocados 60 peixes (peso 20 g) em cada tanque. Os grupos foram estabelecidos. Os peixes controle foram alimentados com *pellets* comerciais, e os peixes dos outros dois grupos experimentais foram alimentados com dietas experimentais contendo extratos de cogumelo-ostra por seis semanas. Durante o ensaio, parâmetros biométricos, imunológicos e bioquímicos foram avaliados em uma, duas, três, quatro, cinco e seis semanas. Além disso, amostras de intestino foram coletadas nas mesmas semanas de amostragem. A explosão respiratória intracelular, a atividade bactericida, a proteína total e a globulina foram significativamente aumentadas em peixes alimentados com dieta experimental a 2% em três, quatro, cinco e seis semanas. Comparado ao grupo controle, o grupo de peixes que recebeu dieta suplementada a 2% apresentou níveis elevados de colesterol sérico na primeira semana e depois diminuição na terceira e na sexta semana. Alguns parâmetros bioquímicos (glicose, albumina e triglicerídeos) não foram afetados. Além disso, a alimentação com extrato dietético não apresentou nenhum impacto negativo na histologia do intestino nem no desempenho de crescimento dos peixes. Esses resultados indicaram que o extrato de cogumelo-ostra pode efetivamente melhorar parâmetros imunológicos não específicos em trutas-arco-íris juvenis.

Palavras-chave: Peixe; *Pleurotus ostreatus*; Extrato de cogumelo; Sistema imunológico inespecífico.

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INTRODUCTION

In Turkish aquaculture industry, rainbow trout is the most commonly farmed fish species. However, microbial diseases cause economic losses to the companies. Generally, chemotherapeutics and disinfectants are used to treat these diseases on farms. This might lead to bioaccumulation, drug resistance, and pollution in the environment. Furthermore, as there are no effective treatments for viral diseases, the use of immunostimulants in fish has become an effective disease control measure in aquaculture (Assefa and Abunna, 2018; Wang et al., 2017).

Instead of fighting diseases with drugs, the use of fish immunostimulants against pathogens has received much more attention (Mastan, 2015; Vijayaram et al., 2023). Generally, immunostimulants enhance the non-specific immune response in fish, but this does not lead to an increased survival rate against pathogens. Also, applying immunostimulants in feed for fish at too high concentration or too long time can induce immunosuppressive effects (Esther & Ekundayo, 2022). The compounds in the immunostimulant substances that promote the release of immune affect cells in the body.

Up to now, multiple immunostimulants have been evaluated in fish. Immunostimulants such as glucan, chitin, lactoferrin, levamisole, vitamins B, C, and E, as well as the growth hormone, and prolactin applications can cause gaining activity in phagocytic cells (Mehana et al., 2015). The incorporation of edible medicinal mushrooms in fish feed has recently emerged as an immunostimulant in feed (Baba et al., 2015; Baba & Ulukoy, 2022; Dobšiková et al., 2012; Harikrishnan et al., 2012a; Harikrishnan et al., 2012b; Ulukoy et al., 2016). Some edible mushrooms have had medical components, and they have been used in several therapies because of their antibacterial, antiviral, and anti-tumor properties, as well as their enhancement of hematological and immunomodulating effects in the body (Lindequist et al., 2005; Wasser, 2002).

Pleurotus ostreatus is a medicinal mushroom, also known as oyster mushroom. It has several properties, including numerous therapeutic effects (Wang et al., 2000). Especially the immunomodulatory, anti-inflammatory, antioxidant, and analgesic properties of this mushroom have been investigated in several animal and human studies (Bobek & Galbavý, 1999; Bobek & Galbavý, 2001; Katya et al., 2014). This medicinal mushroom has bioactive compounds such as saccharides, proteins, triterpenoids, alcohols, phenols, mineral elements (zinc, copper, iodine, selenium, iron), and vitamins (Elkhateeb et al., 2019). According to the literature, oyster mushrooms are rich in micro- and macroelements such as β-glucan, protein, total fiber, K, P, S, Mg, Ca, Na, Fe, Zn, Cu, low-fat content, vitamins, and chitosan (Akindahunsi & Oyetayo, 2006; Manzi et al., 1999; Reguła & Siwulski, 2007).

Li and Gatlin III (2005) pointed out that a rise in the use of synthetic, inorganic, and organic nutraceuticals as immunostimulants and growth promoters had increased. Many natural plants and their metabolites or products have been shown to have effects on the growth performance and innate immunity of rainbow trout (Tang et al., 2014; Tukmechi & Bandboni, 2014; Wang et al., 2015; Zahran et al., 2014). Baba et al. (2015) recorded increases in immune response in rainbow trout fed with *Lentinula edodes* mushroom extract. Previously, Ulukoy et al. (2016) reported the oyster mushroom extract effects on hemato-immunological parameters in the same species as a part of their study. That study showed that using the oyster mushroom extract as a feed supplement improved the hematological parameters in fish in a significant degree. The feed with the oyster mushroom extract in different concentration levels also modulated the immune response against the *Lactococcus garvieae* pathogen in rainbow trout.

In this study, it was chosen to use juvenile fish, because its immune system needs the enhancement against all kind of threatening issues and pathogens in their environment. The main objective of our study was to investigate the impacts of *P. ostreatus* (oyster mushroom) extract in the feed of juvenile rainbow trout in terms of growth (specific growth rate, weight gain, feed conversion ratio), non-specific immune response (respiratory burst activity, bactericidal activity), biochemical parameters (total protein, glucose, albumin, triglycerides, globulin, and cholesterol), and gut histology.

MATERIALS AND METHODS

Ethics statement

The experiments involving live animals in this study were evaluated by the Ethics Committee of Mugla Sıtkı Kocman University, Turkiye. All methods were carried out by following the use of animals in research in line with the European Union directive 2010/63/EU.

Extraction of dried mushroom

The dried mushroom *P. ostreatus* was purchased and extracted in a solvent according to Yap and Ng (2001). The grounded mushroom was dissolved in water and then put into a 60–65°C-water bath for 24 hours. The obtained solution was filtered by using a 0.45-mM-pore paper. Then, the obtained extract was lyophilized and stored in a fridge.

Preparing feed

In this trial, we used the commercial rainbow trout feed, which has an average of 48% crude protein, 14% crude fat, 12% moisture, 14% crude ash, and 4,000 kcal of digestible energy per kg. The experimental diets were prepared by using 1 and 2% oyster mushroom extract supplements. The feed was blended, and then the extract was added by using water (100 mL of water per 1 kg of feed) to form a dough. The feed was then pelletized again to a suitable pellet size for fish to consume. Pellets were air-dried at room temperature and stored at 4°C in a cold room.

Experimental design

The total 360 healthy fish, with an average weight of 20 g, were brought from a commercial fish farm. Fish were randomly distributed into three groups following a complete randomized design, with each group consisting of 60 fish. These groups were duplicated utilizing the same design. Before the trial, fish were kept in the tanks for about two weeks for acclimation. Then, they were fed with experimental diets comprising of feed supplemented with oyster mushroom extract at different levels: 0% for the control group, 1 and 2% for other groups. They were fed at 2% of body weight twice daily basis up to six weeks. For blood samples, five fish were taken from each tank. In total, 10 fish were sampled from each group weekly. The water quality was monitored regularly during trial by using a multiprobe (YSI 556MPS). The quality of water showed stable values, and the average measurement was obtained as follows: dissolved oxygen level at 8.00 ± 0.22 $mg \cdot L^{-1}$, water temperature at 15.00 ± 0.32°C, and pH at 7.5 ± 0.17.

Blood sampling

Every week, five fish were taken from each tank and anaesthetized by the application of 2-phenoxy ethanol (0.3 $mL \cdot^{L-1}$) (Velišek & Svobodová, 2004). Blood samples were taken from the fish's caudal vein using a 2-mL sterile syringe. The samples of blood were split into two parts using Eppendorf test tubes to carry out various tests. A portion of blood was run right away for the assays. Another portion of blood in the Eppendorf tube was used to obtain serum, which was separated by centrifugation at 3,500 g for 15 min and stored at -20°C in the freezer.

Respiratory burst activity

Respiratory burst activity was measured according to Anderson et al. (1992) using nitroblue tetrazolium (NBT) glass-adherent assay. To determine neutrophil activity and oxidative radicals production, a drop of blood was placed on coverslips and incubated in a moist chamber for 30 minutes. Briefly, NBT as 0.2% was prepared at the level of 0.85% (w/v) in sterile saline solution. The adherent cells on the slide were incubated with NBT, and then cells on the slides were investigated under the light microscope. The cells were seen as dark blue color and counted as NBT-positive cells 40X magnification. From each blood sample, five coverslips were obtained, and five randomly selected fields were counted on each slide. Under a microscope, it was counted as in 25 fields on the coverslips from one fish, and the results averaged. This was used to calculate the standard error of values per field.

Bactericidal activity

The bactericidal activity of the serum was measured using Kajita et al.'s method (1990). *Aeromonas hydrophila* (ATCC, 7966) was used as a pathogen to investigate the bactericidal efficacy of fish serum. *Aeromonas hydrophila* fresh cultures were harvested by centrifugation, and the pellet was washed and suspended in phosphate buffered saline (PBS). The optical density of the suspension was set to 0.5 at a wavelength of 546 nm. In a microplate, 100 µL of fish serum and 100 µL *A. hydrophila* bacterial suspension were added into each well and slowly mixed before incubating at 25°C for 1 hour. As a control, a blank was prepared by replacing serum with sterile PBS. The serum-bacteria mixture was plated by taking 0.1 mL volume on agar (nutrient) plates at 25°C for 24 hours before the number of viable bacterial colonies was counted.

Biochemical assays

The serum total protein values were measured by Bradford's method (1976). As a standard, the bovine serum albumin (BSA) was used. The levels of albumin, globulin, glucose, triglyceride, and cholesterol were determined with the help of commercial diagnostic test kits (produced by Bioanalytic Diagnostic Industry, Co., Turkiye). All data were obtained by using a spectrophotometer.

Growth parameters

Growth parameters in this study were calculated by using the initial and final weights of fish in each group. The following formulae by Laird and Needham (1988) were used to measure growth performance and feed utilization (Eqs. 1, 2 and 3).

$$\text{Weight gain (\%)} = 100 \text{ (final fish weight – initial fish weight) / initial fish weight} \quad (1)$$

$$\text{Specific growth rate (SGR, \% day}^{-1}\text{)} = 100 \times [\text{Ln (final fish weight) - Ln (initial fish weight)}] / \text{experimental days} \quad (2)$$

$$\text{Feed conversion ratio} = \text{feed intake / weight gain} \quad (3)$$

Diet analysis

The crude protein, crude lipid, moisture, and ash content of experimental diets were determined using standard methods (AOAC, 1990). The Kjeldahl's method was used to obtain crude protein (N × 6.25) by after acid digestion in an auto Kjeldahl system. Crude lipid was also measured by the ether extraction by using a Soxtec system. The moisture content of the diet was pursued by oven drying at 105°C until constant weight was gained. In a muffle furnace at 550°C, the ash content of feed was determined after placing the samples for 24 hours.

Histology

Gut samples [posterior (distal) region] from each fish (five fish/week) were dissected and fixed in 10% neutral buffered formalin for more than 24 hours. The sampled tissues were embedded in paraffin after using standard protocols preparing tissue for embedding. Tissue sections in a microtome of 5-µm thickness were cut. These sections were mounted onto slides and stained with haematoxyline-eosin. Then, sections were examined under light microscopy (Culling, 1963).

Statistics

Data were subjected to statistical analysis using the Statistical Package for the Social Sciences software version no. 17 (SPSS Inc., Chicago, IL, United States of America). Statistical analysis of the data was performed using analysis of variance and then Tukey's pairwise multiple comparison tests. Data were expressed as arithmetic mean standard error. Statistical significance was set at $p < 0.05$.

RESULTS

Non-specific immune parameters

Data in Table 1 shows that NBT-positive cell numbers were significantly elevated in blood samples starting in the second week up to sixth week in 2% mushroom extract-supplemented fish group compared to the control. Among the groups, the number of NBT-positive cells reached the peak in the 2% group ($p < 0.05$). This result showed that, starting in the second week, adding the oyster mushroom extract as a supplement into feed caused an increase in the phagocytic activity of phagocytes. In fish groups that were fed with all doses of supplemented experimental feed, the serum bactericidal activity was significantly increased against pathogen *A. hydrophila* (ATCC, 7966) compared to control fish serum (Table 1).

Biochemical parameters

The serum biochemical parameters were shown in Table 2. The glucose, albumin, and triglycerides levels in serum were not affected on fish that had dietary oyster mushroom extract in feed ($p > 0.05$). Significant increases in total protein and globulin levels in serum were found especially in treated fish groups compared to the control fish from the third up to the sixth week ($p < 0.05$). No changes were observed in the cholesterol level in fish between treated fish groups and the control group in the first week, whereas it was significantly decreased in fish that received 2% oyster mushroom extract feed in week six (Table 2).

Table 1. Nitroblue tetrazolium (NBT) (+) cell number and bactericidal activity of fish fed different levels of oyster mushroom extracts (one to six weeks)*.

Weeks	Concentration	NBT (+) cell number	Bactericidal activity (CFU/µL)
1	1% Pleurotus	5.00 ± 0.27^{h}	54.60 ± 0.84^{fg}
	2% Pleurotus	6.25 ± 0.30^{h}	46.40 ± 1.22^{h}
	Control	5.90 ± 0.16^{h}	50.80 ± 1.98^{g}
2	1% Pleurotus	9.20 ± 0.66^{fg}	51.60 ± 0.97^{g}
	2% Pleurotus	9.75 ± 0.54^{f}	44.00 ± 1.03^{hi}
	Control	8.00 ± 0.47^{g}	63.40 ± 1.49^{cd}
3	1% Pleurotus	14.50 ± 0.46^{d}	58.00 ± 1.22^{ef}
	2% Pleurotus	20.10 ± 0.64^{b}	40.80 ± 0.90^{i}
	Control	9.90 ± 0.69^{f}	65.20 ± 1.30^{bc}
4	1% Pleurotus	16.70 ± 0.81^{c}	62.60 ± 1.79^{cd}
	2% Pleurotus	21.20 ± 0.31^{ab}	42.40 ± 0.93^{hi}
	Control	12.50 ± 0.54^{e}	58.20 ± 1.44^{ef}
5	1% Pleurotus	16.35 ± 0.85^{c}	56.60 ± 1.33^{ef}
	2% Pleurotus	21.55 ± 0.55^{ab}	40.80 ± 0.74^{i}
	Control	13.60 ± 0.35^{de}	75.40 ± 2.04^{a}
6	1% Pleurotus	16.45 ± 0.58^{c}	60.40 ± 1.42^{de}
	2% Pleurotus	22.05 ± 0.88^{a}	40.80 ± 1.08^{i}
	Control	14.20 ± 0.56^{d}	67.80 ± 2.11^{b}

*Data are represented as mean ± standard error (n = 10); ^{a,b,c,d,e,f,g,h,i}values of means were significantly different ($p < 0.05$) from the control.

Growth performance

Table 3 presents the growth performance of rainbow trout. The fish groups fed with oyster mushroom extracts showed no significant difference compared to the control fish on growth ($p > 0.05$). Among the groups, the average initial weight did not have different values (Table 3). At the end of the trial, the feed conversion ratio, the average final weight, and the specific growth rate were calculated, and the treated fish groups did not show significant increase compared to fish in the control group ($p > 0.05$).

Table 2. Average values in biochemical parameters of fish fed experimental feed (one, three, and six weeks)*.

W	C	Glucose (mg/L)	Albumin (mg/L)	Globulin (mg/L)	Triglycerides (mg/dL)	Cholesterol (mg/L)	Protein (mg/mL)
1	1%	90.27 ± 1.56^{a}	0.41 ± 0.01^{a}	4.18 ± 0.36^{b}	98.40 ± 1.56^{a}	172.80 ± 3.35^{a}	34.47 ± 0.97^{c}
	2%	93.28 ± 6.12^{a}	0.42 ± 0.01^{a}	4.20 ± 0.14^{b}	96.95 ± 1.76^{a}	175.15 ± 3.82^{a}	37.27 ± 1.75^{c}
	Cont.	93.10 ± 1.05^{a}	0.41 ± 0.01^{a}	4.45 ± 0.22^{b}	99.53 ± 2.26^{a}	177.99 ± 2.15^{a}	35.49 ± 0.84^{c}
3	1%	92.07 ± 0.72^{a}	0.39 ± 0.01^{a}	4.47 ± 0.18^{b}	88.56 ± 2.46^{b}	170.17 ± 2.40^{a}	43.95 ± 0.89^{b}
	2%	90.38 ± 1.99^{ab}	0.39 ± 0.02^{a}	5.20 ± 0.35^{ab}	85.34 ± 2.18^{b}	123.96 ± 4.71^{b}	43.43 ± 1.01^{b}
	Cont.	90.30 ± 1.31^{a}	0.40 ± 0.04^{a}	4.72 ± 0.14^{b}	86.80 ± 1.61^{ab}	172.21 ± 5.50^{a}	37.84 ± 0.84^{c}
6	1%	86.64 ± 1.56^{ab}	0.38 ± 0.03^{a}	4.53 ± 0.27^{b}	85.43 ± 3.90^{b}	174.44 ± 3.46^{a}	45.69 ± 0.64^{ab}
	2%	85.80 ± 2.26^{ab}	0.38 ± 0.01^{a}	5.90 ± 0.17^{a}	84.09 ± 2.16^{b}	114.20 ± 4.95^{b}	48.63 ± 0.78^{a}
	Cont.	87.01 ± 1.29^{ab}	0.42 ± 0.01^{a}	4.88 ± 0.17^{b}	84.42 ± 1.54^{b}	176.21 ± 3.15^{a}	38.18 ± 0.52^{c}

*Data are represented as mean ± standard error (n = 10); ^{a,b,c}values of means were significantly different ($p < 0.05$) from the control.

Table 3. Growth performance of fish fed experimental feed containing different levels of oyster mushroom extract after six weeks*.

Group	Initial weight (g)	Final weight (g)	Weight gain ratio (%)	Specific growth rate (%)	Feed conversion ratio
1% Pleurotus	20.00 ± 0.25^{a}	49.86 ± 3.62^{a}	149.8 ± 1.11^{a}	2.02 ± 0.25^{a}	1.21 ± 0.11^{a}
2% Pleurotus	19.5 ± 0.27^{a}	48.88 ± 3.48^{a}	150.66 ± 1.98^{a}	2.03 ± 0.39^{a}	1.23 ± 0.13^{a}
Control	19.34 ± 0.17^{a}	46.88 ± 4.72^{a}	142.39 ± 2.45^{b}	1.96 ± 0.42^{a}	1.25 ± 0.24^{a}

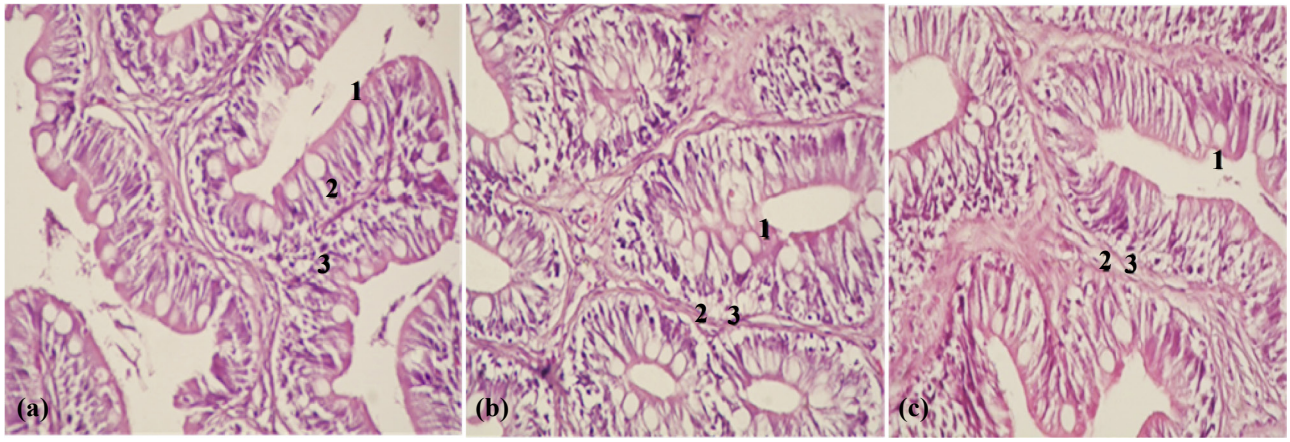
*Data are represented as mean ± standard error; ^{a,b}values of means were significantly different ($p < 0.05$) from the control.

Histology

Sections of the gut tissue of fish were examined under the light microscope. We did not find any abnormal or pathologic structure in the gut tissues in any of the fish samples (Fig. 1).

DISCUSSION

Improving the growth performance and curbing diseases in fish are two of the most important challenges in aquaculture. Several supplements are used in aquaculture to face these



1= Goblet cell; 2= intestinal epithelium; 3= lamina propria.

Figure 1. Light microscopy the gut of fish. Hematoxylin and eosin (40x). (a) 1% Oyster extract; (b) 2% oyster extract; (c) control group.

challenges. Some of them are growth-promoting additives such as nutraceutical health products, yeast, probiotics, antioxidants, enzymes, amino acids, carnitine, lipid derivates, plant extracts, and vitamins (Goda, 2008). To be able to develop alternatives for growth and disease management in aquaculture, natural sources from medicinal plants and mushrooms are receiving attention in contemporary research.

The present study demonstrated the impact of the oyster mushroom extract in feed, on non-specific immune response, the growth performance, and the biochemical parameters in fish. Rainbow trout were fed including 1 and 2% oyster mushroom extract supplemented feed, and some non-specific immune parameters have been stimulated. Intracellular respiratory burst activity of phagocytic cells in blood exhibited a significant increase in all fish that received the experimental diets in the second week.

The present results agree with the previous report on adult Victoria Labeo (*Labeo victorianus*) and juvenile fish fed with a stinging nettle (*Urtica dioica*) diet that significantly enhanced respiratory burst activity (Ngugi et al., 2015). In rainbow trout (*O. mykiss*), the similar findings were reported when fish were fed with mistletoe (*Viscum album*), nettle (*U. dioica*), ginger (*Zingiber officinale*) (Düğenci et al., 2003), and tetra (*Cotinus coggygria*) (Bilen et al., 2011). In this study, *in-vitro* determination of bactericidal activity against *A. hydrophila* presented a significant enhancement in all serum of fish that received supplemented diets starting from the second week up to six weeks compared to the control. Also some findings were documented in rainbow trout fed with black cumin seed oil, and nettle extract (Awad et al., 2013), garlic (Nya & Austin, 2011), and lupin, mango, and stinging nettle (Awad & Austin, 2010). Also, Jagruthi et al. (2014) presented an increase in serum bactericidal activity of common carp that fed with all doses of astaxanthin.

The present study showed that serum glucose levels did not shift at any concentration of oyster mushroom extract fed fish groups. An increase in glucose level is one of the stress indicators in fish (Morgan & Iwama, 1997). Binaii et al. (2014) reported that juvenile beluga (*Huso huso*) supplemented with the nettle showed no significant difference in concentration of glucose in serum. Similarly, no changes in glucose level were found in rainbow trout fed with synbiotic biomin (Mehrabi et al., 2012).

The serum total protein, globulin, and albumin levels are used as an indicator of innate immunity in fish (Wiegertjes et al., 1996). These proteins are considered important for the humoral immunity of the non-specific immune system and the defense mechanism of fish (Magnadottir, 2006). In this study, the total serum proteins and globulin levels were significantly increased in both groups of fish fed with mushroom extract-supplemented diets compared to the control group. Other studies have documented increases in total protein and globulin levels in rainbow trout following feeding with diets supplemented with ginger, garlic (Nya and Austin, 2009a; Nya & Austin, 2009b), cumin seed oil, and nettle extract (Awad et al., 2013). Similar to the current study, an increase in the total protein was seen in rainbow trout upon feeding with tetra (Bilen et al., 2011) and synbiotics (Mehrabi et al., 2012). Dobsíková et al. (2013) recorded increases in total protein level in common carp fed with oyster mushroom β-1.3/1.6-D-glucan.

Albumin is important for sustaining the osmotic pressure needed for the proper distribution of body fluids, maintaining a healthy immune system and acting as a plasma carrier (Wiegertjes et al., 1996). In this study, neither concentration of the supplemented diet received fish serum showed significant differences in the albumin level. In a similar study, albumin levels were not impacted by dietary bovine lactoferrin in *Acipenser baerii* (Eslamloo et al., 2012).

The current study observed that existing high cholesterol levels in serum in the first week in all groups showed a decrease in fish serum in 2% experimental group compared to the control group after six weeks. Also, triglycerides levels of the fish serum obtained lower levels in all experimental groups at six weeks samples compared to the first week. Akrami et al. (2015) reported a decrease in cholesterol level in juvenile Huso after eight weeks of oral administration of onion (*Allium cepa*). This is in line with the findings of Binaii et al. (2014), who reported a significant decrease in serum cholesterol levels in belugas fed with a basal diet supplemented with nettle. Along similar lines, Al-Salahy (2002) recorded a decrease in cholesterol levels in *Clarias lazera* following oral administration of onion juice. No significant changes were observed in triglycerides. This result is parallel to Yeganeh et al. (2015), who reported that the cholesterol and triglycerides levels were not affected by dietary *Spirulina platensis* in rainbow trout. Similarly, other animal studies have shown that dried mushrooms included in animal diets can significantly decrease serum and liver cholesterol levels (Bobek et al., 1991; Bobek et al., 1995; Hwang et al., 2012; Xu et al., 2008).

In another study, Yang et al. (2013) investigated the effect of *L. edodes* in a mouse model of hypercholesterolaemia. This study found that diets supplemented with *L. edodes* enhanced fat removal in hypercholesterolemic mice.

In this work, the specific growth rate and feed conversion ratio did not significantly increase with any supplemented diet compared to the control group. Dobsíková et al. (2013) studied the effect of a dietary oyster mushroom β-1.3/1.6-D-glucan and found no effect on growth performance in common carp. Similarly, Jagruthi et al. (2014) showed that dietary supplementation with astaxanthin specific growth rate and feed conversion ratio did not significantly increase at any time in common carp. The results of the present study determined no negative effect on fish fed with the dietary-supplemented oyster mushroom extract in rainbow trout feed.

In the present study, the histology of the gut of rainbow trout fed with experimental diets was also studied. Gut samples taken at the end of the feeding period were examined. There was no trace of pathological findings in the appearance of the fish gut among any groups. Similarly, Bonalda et al. (2006) have reported that in the histopathological gut examination of *Solea aegyptiaca* there were no noticeable differences in the appearances of the fish intestines in any of the dietary soybean meal groups. Zahran et al. (2014), who worked on the effect of *Astragalus polysaccharides* on intestinal histology of *Oreochromis niloticus*, found that *A. polysaccharides* had no effect of intestinal histology, but a slight increase in the villi length was recorded. Quanxi et al. (2010) and Merrifield et al. (2011) recorded similar results in tilapia.

CONCLUSION

This study proved that oyster mushroom extract-supplemented diets in juvenile rainbow trout can enhance some non-specific immune responses in fish without causing any negative effect on growth performance. Also, gut histology results support the recorded immunological and biochemical values with no pathology at all. Our results indicate that dietary 2% oyster mushroom extract supplementation can enhance non-specific immune parameters, and improve biochemical parameters in juvenile rainbow trout.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

All datasets were generated or analyzed in the current study.

AUTHORS' CONTRIBUTION

Conceptualization: Baba, E., Uluköy G.; **Writing – original draft:** Baba, E., Uluköy G.; **Formal analysis:** Baba, E., Uluköy G.; **Final approval:** Uluköy G.

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