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Effects of a Commercial Multi-Strain Probiotic on Growth Performance, Muscle Antioxidant Parameters, and Fillet Shelf Life in Rainbow Trout, *Oncorhynchus mykiss*

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Abstract

Background: Probiotics are widely used in aquaculture and can improve the growth, immunity, and antioxidant system of fish; however, they can be used to increase fillet shelf life.

Objectives: The present study investigated the effects of dietary supplementation with a commercial probiotic (Bio-Aqua) on growth performance, muscle composition and shelf life during refrigerated condition.

Methods: Four diets were prepared containing 0 (CTR), 0.3 (0.3B), 1 (1B), and 2 (2B) g/kg of the probiotic. Triplicate groups of fish were fed the diets over 12 weeks, then the dorsal muscle chemical composition and antioxidant enzymes were determined. Fillet quality was determined after 0, 4, 8, 12, and 16 d refrigerated condition.

Results: At the end of the rearing period, there was no significant difference in the growth performance of the fish among the treatments. 0.3B exhibited significantly higher muscle protein content and superoxide dismutase activity; whereas, muscle catalase and glutathione peroxidase activities significantly increased in 0.3B and 1B treatments. Fillet thiobarbituric reactive substances (TBARS), total volatile base nitrogen, total bacterial count, and psychrotrophic bacterial count exhibited elevation over time, during refrigerated condition. Dietary probiotic significantly affected the fillet concentration of TBARS, as 0.3B and 1B showed lower TBARS than CTR and 2B treatments. Total bacterial count in probiotic treatments (particularly 0.3B and 1B) were significantly lower that CRT, after 8 and 16 days in refrigerator. There were no interaction effects of dietary probiotic and refrigerated condition time on the fillet total volatile base nitrogen and psychrotrophic bacterial count.

Conclusion: Based on the results, dietary 0.3 or 1 g/kg of Bio-Aqua can improve antioxidant capacity and decrease lipid peroxidation in the fillet of rainbow trout.

Keywords: aquaculture, fillet quality, peroxidation, refrigerated condition, spoilage

Introduction

Rainbow trout, *Oncorhynchus mykiss*, is an important aquaculture candidate with more than 800000 tons world annual production (Yousefi *et al.,* 2022a). Aquaculture of rainbow trout is an important sector of food supply in Iran (Tulaby Dezfuly *et al*., 2019) with more than 170000 tons annual production (Yousefi *et al.,* 2022c). Although aquaculture can supply high quality food for human, fish/shellfish meat is very susceptible to perishing due to their relatively high protein content, nitrogenous compounds, and unsaturated fats in muscles (Arulkumar *et al.,* 2017). Refrigeration is one of the methods that can be used to increase shelf life of fish meat in fish supply centers or for transferring fish from aquaculture centers to markets. Fish storage in

refrigerator reduces the rate of enzymatic, chemical, and microbial activities. But due to the inability of the refrigerator to reduce fish meat temperature to the necessary level, undesirable changes such as oxidation and hydrolysis of fats occur slowly that deteriorate the quality of the products (Pérez-Alonso *et al.,* 2003). Therefore, the use of methods to improve the quality and increase the shelf life of meat is essential to prevent economic losses and maintain consumer health. In this regard, many studies have been conducted on adding preservatives to fish fillets after harvesting (Hussain *et al.,* 2021); however, improving the antioxidant conditions of fish during rearing may help to improve the quality of fish fillets in the refrigerator after harvesting.

Probiotics are widely used in aquaculture and can improve the growth, immunity, and antioxidant system of fish (Balcázar *et al.,* 2008; Alishahi *et al.*, 2018). For this reason, the use of probiotics in aquaculture leads to increase in economic efficiency. The most comprehensive definition of probiotics was provided by Merrifield *et al.*, (2010); "In general, any microbial cell that enters a living organism's body through feed or water in aquaculture and creates beneficial effects for the fish, and consumer by improving microbial balance is called a probiotic." Probiotics can create an unfavorable environment for pathogens and control them by various mechanisms such as the production and secretion of inhibitory compounds, competition with pathogenic agents, opportunistic consumption of essential nutrients and attachment sites in the digestive system, as well as stimulating the host's immune system (Balcázar *et al.,* 2008; Wang *et al.,* 2010). Studies have shown that probiotics can improve growth performance, feed utilization, and digestibility of dietary components in fish (Rohani *et al.,* 2021).

Adding probiotics to the fish diet can increase the antioxidant power of fish, as the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase in various fish tissues increases and the amount of thiobarbituric reactive substances (TBARS) decreases (Tovar-Ramírez *et al.,* 2010; Duan *et al.,* 2017; Gobi *et al.,* 2018). However, few studies have focused on the antioxidant effects of probiotics on muscle antioxidant indices and fillet quality during refrigerated condition. For example, the use of the yeast probiotic, *Rhodosporidium paludigenum,* in diet of white leg shrimp, *Penaeus vannamei*, led to an increase in the activity of muscle antioxidant enzymes and a decrease in TBARS levels (Yang *et al.,* 2010). Adding the probiotic *Bacillus vireti* to diet of freshwater prawn, *Macrobrachium rosenbergii*, improved the antioxidant status of muscle after disease occurrence in animals (Vidhya Hindu *et al.,* 2018). Dietary supplementation with

Bacillus subtilis improved intestinal antioxidant indices and increased physical quality indices of the muscle in crucian carp, *Carassius auratus*, (Cao et al., 2019). Adding a probiotic mixture of *Bacillus subtilis*, *Enterococcus faecium*, *Pediococcus acidilactici*, and *Lactobacillus reuteri* to rainbow trout, diets increased antioxidant enzyme activity and decreased TBARS levels in the fish fillet after 5 days of refrigerated condition (Giannenas *et al.,* 2015). Therefore, adding probiotics to fish diets can not only improve growth and disease resistance in fish but also increase fillet quality and shelf life in refrigerated condition, which is important for consumer health. However, due to limited studies in this area and the variety of probiotics on the market and fish species (with different muscle compositions), further research is needed.

In the present study, a commercial probiotic consortium (Bio-Aqua, Biodep Co., Tehran, Iran) composed of 9 bacterial and yeast strains (*Pediococcus acidilactici*, *Enterococcus faecium*, *Bacillus subtilis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei,* *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, and *Saccharomyces cerevisiae*) was used for dietary supplementation and administration to rainbow trout. This product has been specifically formulated for fish and has a cell density of 3×10^9 cfu/g. The recommended dose of this product is 0.2-0.3 g/kg, although studies have shown that adding 1-2 g/kg of this product to the diet can increase the growth rate of male and female breeders (Akbari Nargesi *et al.,* 2018; Akbari Nargesi *et al.,* 2019). But there is no data regarding its benefits on rainbow trout fillet quality in refrigerator. Therefore, the aim of this study is to investigate the effect of adding Bio-Aqua probiotic to rainbow trout diets on antioxidant indices and biochemical quality of fish fillets during refrigerated condition.

Materials and Methods

Fish diets

In this study, a specific rainbow trout feed (Faradaneh Co., Tehran, Iran) was used. Bio-Aqua was a kind gift from Zist Darman Mahan Co. (Tehran, Iran). To prepare the diets, the probiotic supplement was first mixed with water and then sprayed onto the surface of the pellets. The concentrations used in this study were 0 (control), 0.3, 1, and 2 g/kg of feed. The amount of 0.3 g/kg was selected based on the manufacturer's recommendation, and the amounts of 1 and 2 g/kg were selected based on previous studies on rainbow trout (Akbari Nargesi *et al.,* 2018; Akbari Nargesi *et al.,* 2019). After spraying the probiotic onto the feed surface, a gelatin solution was

sprayed onto it to prevent the probiotic from being washed away. The feed pellets were used for fish feeding after drying.

Fish culture

In this study, rainbow trout with a weight of ~50 g were purchased from a local farm (Aliabad, Golestan province, Iran). The fish were allowed to acclimatize to the new conditions for 10 days, during which they were fed the control diet and kept in a 2000-L tank with aeration and water flow rate of 5 L/min. Then, a total of 240 fish (61.02 ± 0.13 g) were distributed among twelve 300-liter tanks. Each three tanks was considered as one dietary treatment, and fish were fed daily with the mentioned diets at a rate of 3% biomass (Yousefi *et al*., 2023a). The rearing tanks were equipped with aeration and constant water flow. Every two weeks, the biomass of each tank were measured to adjust the daily feed amount accordingly. After 12 weeks of culture, the final weight of the fish was recorded, and growth and feed efficiency were calculated based on following formula (Mirghaed *et al.,* 2023):

$$\text{Weight gain (\%)} = 100\times \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}}$$

$$\text{Specific growth rate (SGR; \%/d)} = 100\times \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{days}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Consumed feed (g)}}{\text{Gained biomass (g)}}$$

The proximate composition and antioxidant indices of fish muscle

At the end of the culture period, three muscle samples were taken from each treatment. For this purpose, a piece of dorsal muscle was cut and immediately frozen in liquid nitrogen (Ahmadifar *et al*., 2023). These samples were used to investigate the biochemical composition and antioxidant parameters, including the activity of SOD, CAT, and GPx, as well as the amount of TBARS. The biochemical composition of the muscle, including moisture, fat, protein, and ash, were determined according to the AOAC (2005) method. Moisture was measured using an oven at 105°C for 24 h. Fat was measured using ether extraction and a Soxhlet apparatus. Protein was

determined using the Kjeldahl method by measuring the nitrogen content of the samples. Ash were determined using an electric furnace at 550°C for 8 h.

To determine the antioxidant parameters, the samples were first homogenized in phosphate buffer (pH 7.4) and centrifuged at 4°C (15 min and 13,000 g). Then, the upper part of the sample was separated and used to measure the mentioned parameters. The activity of SOD was measured based on the rate of the cytochrome C reduction using a commercial kit from ZellBio Co. (Germany) (Hoseini *et al.,* 2020). CAT activity was measured based on the rate of hydrogen peroxide decomposition. In this method, hydrogen peroxide was used as a substrate, and ammonium molybdate was used as a chromogen and reaction stopper (Goth 1991). GPx activity was measured based on the rate of glutathione oxidation using a commercial kit from ZellBio Co. (Germany) (Hoseini *et al.,* 2019). Measurement of TBARS was performed based on the reaction with thiobarbituric acid at a temperature of 95°C using the method of Moore et al. (1998). In this method, the samples were first deproteinized with trichloroacetic acid, and then the samples were incubated with a thiobarbituric acid solution in the presence of BHT for 2 h at a temperature of 95°C to produce a red color. The absorbance of the samples was recorded at a wavelength of 534 nm and the amount of TBARS was calculated based on the following formula:

TBARS = Sample absorbance - Blank absorbance / 1560000

Investigation of fillet quality indices during refrigerated storage

Three fillets from each treatment were used to investigate the fillet quality indices. For this purpose, the fillets were placed in a refrigerator, and the total bacterial count, psychrotrophic bacterial count, total volatile base nitrogen, and TBARS will be determined on days 0, 4, 8, 12, and 16.

To determine the total bacterial count and psychrotrophic bacteria count, the samples were first homogenized in a 0.85% sodium chloride solution. Then, different dilutions of these solutions were prepared and cultured on nutrient agar medium. The total bacterial count was determined

after 48 h of incubation at a temperature of 37°C, and the psychrotrophic bacteria count was determined after 7 days of incubation at a temperature of 10°C (Ojagh *et al.*, 2010).

The total volatile base nitrogen (TVBN) was determined using the method of Goulas and Kontominas (2005). In this method, magnesium oxide was used to homogenize the samples. Then, the distillation process was performed on the homogenized samples, and the volatile bases were collected in a flask containing boric acid and an indicator. The amount of volatile bases is calculated after titration with sulfuric acid.

Statistical analysis

Normal distribution and homoscedasticity of the data were confirmed by Shapiro-Wilk and Levene tests, respectively. Data of growth performance, feed efficiency, survival, muscle antioxidant enzymes' activities, and proximate composition were subjected to one-way ANOVA and Duncan tests. Fillet quality parameters during refrigerated condition were analyzed by repeated measure ANOVA and Duncan tests. All analyses were performed in SPSS v.22 and expressed as mean ± SE.

Results

Growth performance, feed efficiency, and survival of fish within different treatments are presented in Table 1. There were no significant differences in growth performance and feed efficiency among the treatments. No mortality was observed in different treatments.

Table 1: Growth performance, feed efficiency, and survival of rainbow trout following 12 weeks feeding on diets supplemented with 0, 0.3, 1, and 2 g/kg probiotic (n = 3).

	CTR	0.3B	1B	2B	P-value
Initial weight (g)	61.00 ± 0.10a	61.28 ± 0.36a	61.78 ± 0.07a	61.03 ± 0.15a	0.445

Final weight (g)	258.32 ± 13.17a	280.63 ± 8.96a	267.83 ± 10.83a	267.99 ± 13.88a	0.636
FCR	1.20 ± 0.07a	1.04 ± 0.07a	1.19 ± 0.06a	1.15 ± 0.09a	0.458
SGR (%/d)	1.72 ± 0.06a	1.81 ± 0.03a	1.76 ± 0.05a	1.76 ± 0.06a	0.664
Weight gain (%)	323.54 ± 22.24a	357.85 ± 12.38a	140.71 ± 18.34a	339.06 ± 22.07a	0.671
Survival (%)	100 ± 0.00a	100 ± 0.00a	100 ± 0.00a	100 ± 0.00a	1.000

Proximate composition analysis showed that dietary probiotic had no significant effects on the fish muscle moisture, fat, and ash percentages (Fig. 1). On the other hand, the muscle protein percentage significantly increased in 0.3B, compared to CTR treatment.

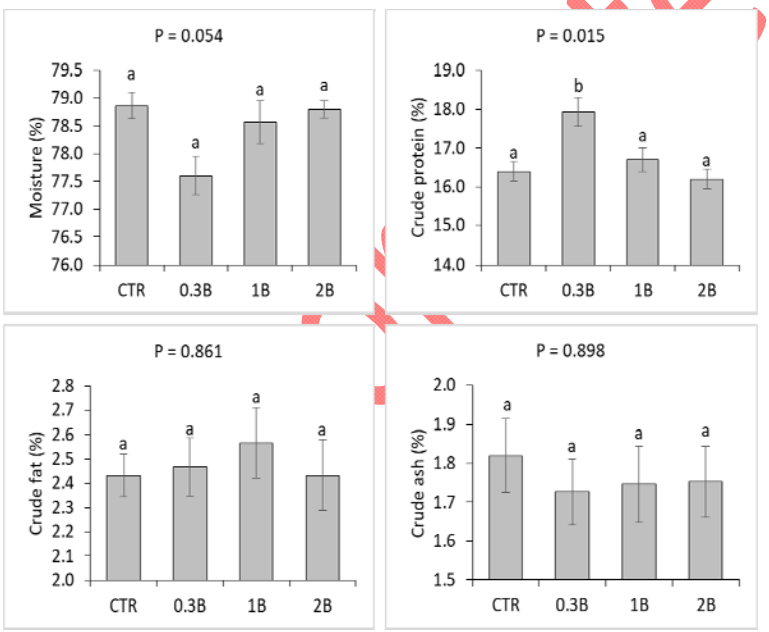

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Figure 1: Proximate composition of rainbow trout muscle following 12 weeks feeding on diets supplemented with 0, 0.3, 1, and 2 g/kg probiotic. Different letters above the bars show significant differences among the treatments (n = 3).

There was a significant elevation in SOD activity in 0.3B, compared to the other treatments (Fig. 2). The muscle CAT and GPx activities significantly increased in 0.3B and 1B treatments, compared to CTR and 2B treatments (Fig. 2).

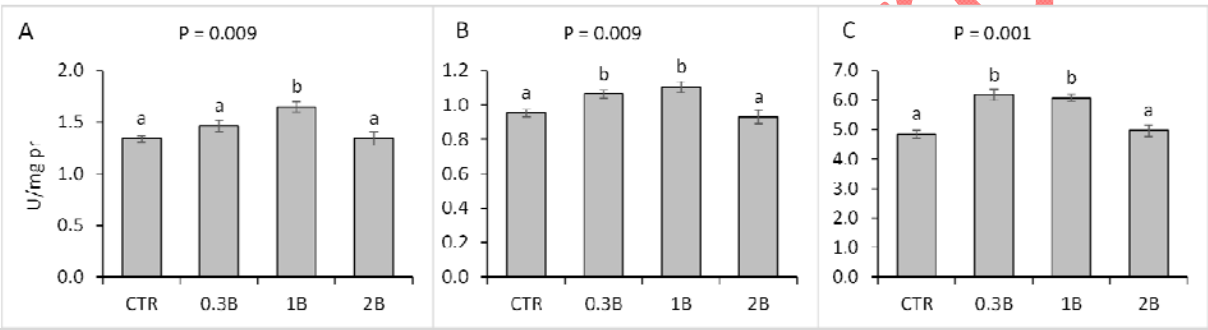

Figure 2: SOD (A), CAT (B), and GPx (C) activities of rainbow trout muscle following 12 weeks feeding on diets supplemented with 0, 0.3, 1, and 2 g/kg probiotic. Different letters above the bars show significant differences among the treatments (n = 3).

Fillet total bacterial count and psychrotrophic bacterial count exhibited elevation over time, during refrigerated condition (Table 2; Fig. 3). An interaction effect of dietary probiotic and sampling time was observed in total bacterial count. The probiotic treatments exhibited significantly lower total bacterial count that CTR, after 8 and 16 days. The lowest total bacterial counts were observed in 1B and 0.3B treatments, after 8 and 16 days, respectively (Fig. 3).

Table 2: Effects of probiotic, refrigerated condition time and their interaction on TBARS, TVBN, total bacterial count, and psychrotrophic bacterial count of rainbow trout muscle

	Repeated measure P-value

Parameters	Time	Probiotic	Time × Probiotic
TVBN	<0.001 (0^{a}, 4^{b}, 8^{c}, 12^{c}, 16^{c})	0.224	0.394
TBARS	<0.001 (0^{a}, 4^{ab}, 8^{c}, 12^{bcd}, 16^{d})	0.012 (CTR/2B > 0.3B/1B)	0.654
Total bacterial count	<0.001	0.001	<0.001
Psychrotrophic bacterial count	<0.001 (0^{a}, 4^{b}, 8^{c}, 12^{d}, 16^{e})	0.817	0.550

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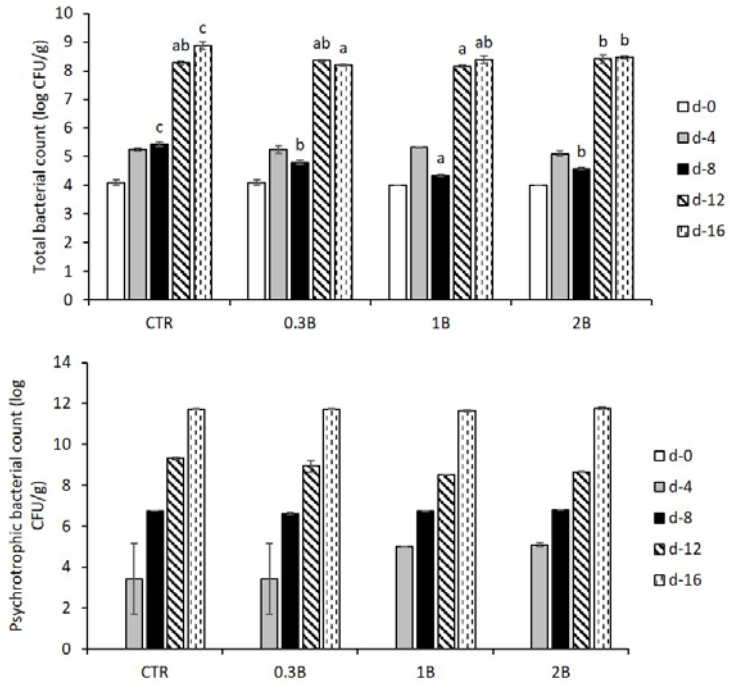

Figure 3: Total bacterial count and psychrotrophic bacterial count of rainbow trout fillet following 12 weeks feeding on diets supplemented with 0, 0.3, 1, and 2 g/kg probiotic and 16 days keeping in refrigerator. Different letters above the bars show significant differences among the treatments within each time (n = 3).

Fillet TBARS and TVBN exhibited elevation over time, during refrigerated condition (Table 2; Fig. 4). Dietary probiotic significantly affected the fillet TBARS concentrations, as 0.3B and 1B showed lower TBARS that CTR and 2B treatments (Fig. 3). There were no interaction effects of dietary probiotic and sampling time on the fillet TVBN and TBARS (Fig. 4).

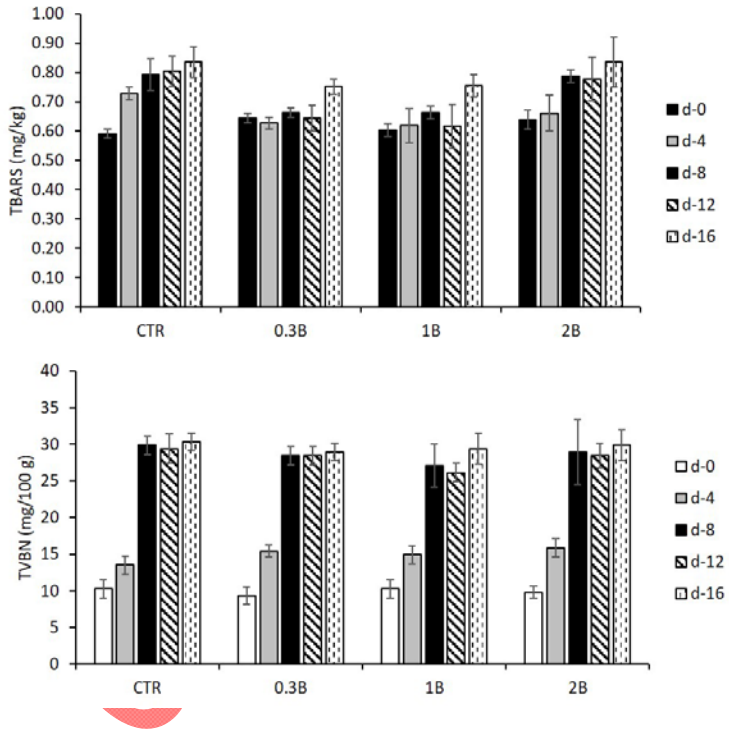

Figure 4: TBARS and TVBN of rainbow trout fillet following 12 weeks feeding on diets supplemented with 0, 0.3, 1, and 2 g/kg probiotic and 16 days keeping in refrigerator (n = 3).

Discussion

Probiotics are used to increase fish growth performance and well-being (Merrifield and Carnevali 2014; Soltani *et al*., 2023), however, the growth-promoting effects of probiotics are context-dependent. The present results are not in line with Akbari Nargesi *et al.,* (2019) and Akbari Nargesi *et al.,* (2018) who reported growth elevation in male and female rainbow trout at 1 and 2 g/kg, respectively. Although several studies have reported enhancement in growth rate of fish fed probiotics (El-Haroun *et al.,* 2006; Giannenas *et al.,* 2015; Ramos *et al.,* 2015; Standen *et al.,* 2016), the present results are similar to Shakourian *et al.,* (2021), Nasiripour *et al.,* (2018), and Merrifield *et al.,* (2010) who found no growth enhancement in Siberian sturgeon, *Acipenser baeri*, and rainbow trout, as a result of dietary supplementation with probiotic consortium. Probiotic effects on fish growth is time-dependent and many studies have shown that a probiotic/probiotic consortium can show growth-promoting effects over a long time, compared to short time. In the present study, 0.3B treatment exhibited non-significant improvement in fish growth and feed efficiency. Therefore, Bio-Aqua at 0.3 g/kg may increase growth and feed efficiency of rainbow trout over a longer time.

Increase in muscle protein content can be related to various factors. It has been demonstrated that probiotics decrease stress in fish (Gomes *et al.,* 2009); so they may suppress stress-mediated muscle proteolysis as proposed by Sadoul and Vijayan (2016). On the other hand, probiotic may act against myostatin, a protein that blocks muscle growth (Michelato *et al.,* 2017), as observed in European sea bass, *Dicentrarchus labrax*, administered by dietary *Lactobacillus delbrueckii delbrueckii* (Carnevali *et al.,* 2006). The present results are in line with those obtained in mrigal carp, *Cirrhinus mrigala* (Ullah *et al.,* 2018), largemouth seabass, *Micropterus salmoides* (Wang *et al.,* 2021), and Asian seabass, *Lates calcarifer* (Lin *et al.,* 2017), after dietary administration of probiotic consortiums.

Reactive oxygen species are common products of cell metabolism and are neutralized by antioxidant enzymes (Yousefi *et al.,* 2022b; Yousefi *et al.,* 2023b). Fish rely on their antioxidant system, which includes enzymes such as SOD, CAT, and GPx (Abbasi *et al.,* 2023; Koohkan *et al*., 2023). However, if the antioxidant system is overwhelmed, biological materials can become oxidized. Unsaturated fatty acids are particularly vulnerable to oxidation and can lead to the

formation of TBARS, which are toxic and can cause further damage to biological materials (Rajabiesterabadi *et al.,* 2020; Hoseini *et al.,* 2022). Probiotics can increase antioxidant capacity of fish (Hoseinifar *et al.,* 2021) and present results suggest Bio-Aqua improves the activity of SOD, CAT, and GPx in the muscle of rainbow trout. Similar results have been obtained by other researchers, when rainbow trout (Giannenas *et al.,* 2015), white leg shrimp (Yang *et al.,* 2010), and freshwater prawn (Vidhya Hindu *et al.,* 2018) were treated with dietary probiotics.

Dietary Bio-Aqua supplementation (0.3B and 1B) decreased TBARS content during the refrigerated condition, which is in line with a previous study on rainbow trout (Giannenas *et al.,* 2015). Such benefits can be related to higher concentration of antioxidant and lower concentration of pro-oxidant compounds in these treatments that protect lipid peroxidation during refrigerated condition, when the cellular antioxidant system not work. Supporting this hypothesis, it has been found that probiotic administration increases reducing capacity of different organs of fish/shellfish (Xie *et al.,* 2019; Chen *et al.,* 2020).

Microbial spoilage is a significant cause of food loss worldwide, accounting for a remarkable portion of annual production losses (Mirsadeghi *et al.,* 2022). Fresh and lightly-preserved seafood are particularly susceptible to spoilage due to microbial activity (Li *et al.,* 2013). To address this issue, monitoring microbial loads in fish fillet during refrigerated condition is inevitable. There were progressive increase in total bacterial count, psychrotrophic bacterial count, and TVBN over the refrigerated condition period, which were expected (Li *et al.,* 2013; Nowzari *et al.,* 2013; Ramezani *et al.,* 2015). Interestingly, dietary 0.3 and 1 g/kg probiotic decreased total bacterial count after 8 and 16 days storage in refrigeration, which indicate the role of Bio-Aqua in decreasing microbial count during refrigeration conditions. There is no similar study for comparison, but feeding chicken with diets containing *Saccharomyces serevisiae* decreased bacterial load during keeping in refrigerator (Aksu *et al*., 2005). Application of probiotics in packing fish fillet also showed similar effects (Samiullah *et al*., 2020). On the other hand, probiotics can produce antibacterial compounds with antagonistic effects against harmful microbes (Hamad *et al*., 2022).

In conclusion, dietary supplementation with bio-Aqua has no significant effects on growth performance of rainbow trout, but 0.3 and/or 1 g/kg of this probiotic increase muscle protein

content and activity of antioxidant enzymes. These concentration, also, decrease lipid peroxidation during refrigerated condition storage.

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اثرات یک پروبیوتیک تجاری چند سویه بر عملکرد رشد، پارامترهای آنتی اکسیدانی عضلانی و ماندگاری فیله در ماهی قزل آلای رنگین کمان، *Oncorhynchus mykiss*

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چکیده

زمینه مطالعه: پروبیوتیک ها به طور گسترده در آبزی پروری استفاده می شوند و می توانند رشد، ایمنی و سیستم آنتی اکسیدانی ماهی را بهبود بخشند. با این حال، می توان از آنها برای افزایش عمر مفید فیله استفاده کرد.

هدف: مطالعه حاضر به بررسی اثرات مکمل غذایی با یک پروبیوتیک تجاری (Bio-Aqua) بر عملکرد رشد، ترکیب عضلانی و ماندگاری در یخچال پرداخت.

روش کار: چهار جیره حاوی صفر (CTR)، 0/3 (0.3B)، 1 (1B) و 2 (2B) گرم بر کیلوگرم پروبیوتیک تهیه شد. گروه‌های سه‌گانه ماهی طی یک دوره 12 هفته‌ای با جیره‌های غذایی تغذیه شدند، سپس کیفیت عضلات پشتی پس از 0، 4، 8، 12 و 16 روز در یخچال تعیین شد.

نتایج: در پایان دوره پرورش تفاوت معنی‌داری در عملکرد رشد ماهی در بین تیمارها مشاهده نشد. در تیمار 0.3B پروتئین و فعالیت سوپراکسید دیسموتاز عضله به طور معنی داری افزایش یافت. در حالی که، فعالیت کاتالاز و گلوتاتیون پراکسیداز عضله به

طور معنی داری در تیمارهای 0.3B و 1B افزایش یافت. شاخص تیوباربیتوریک اسید، نیتروژن فرار بازی کل، تعداد کل باکتری‌ها و تعداد باکتری‌های سرمادوست عضله با گذشت زمان یخچال گذاری افزایش معنی داری داشتند. پروبیوتیک به طور معنی داری بر شاخص تیوباربیتوریک اسید تأثیر گذاشت، به طوریکه تیمارهای 0.3B و 1B مقادیر کمتری نسبت به تیمارهای CTR و 2B نشان دادند. هیچ اثر متقابل پروبیوتیک و زمان نگهداری در یخچال بر نیتروژن فرار بازی کل ، تعداد کل باکتری ها و تعداد باکتری های سرمادوست عضله وجود نداشت.

نتیجه‌گیری نهایی: بر اساس نتایج، جیره غذایی حاوی 0/3 یا 1 گرم بر کیلوگرم بیو آکوا می‌تواند ظرفیت آنتی‌اکسیدانی را بهبود بخشد و پراکسیداسیون لیپیدی را در عضله قزل‌آلای رنگین‌کمان کاهش دهد.

واژگان کلیدی: یخچال گذاری، پراکسیداسیون، فساد، آبزی پروری، کیفیت فیله