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Investigating the effects of the anti-yersiniosis vaccine using the bath method on some biochemical parameters of the blood serum of *Huso huso*

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Abstract

Yersiniosis, caused by *Yersinia ruckeri*, is recognized as one of the most significant diseases affecting cold-water and sturgeon fish in aquaculture. Vaccination has emerged as the most cost-effective and sustainable method for controlling infectious diseases in fish. This study investigated the effects of a bath vaccine against yersiniosis on certain biochemical parameters in the serum of beluga sturgeon (*Huso huso*). For this purpose, 400 to 500 juvenile fish with an average weight of approximately 10 gr were divided into 14 tanks and raised for 10 to 14 days to acclimatize to environmental conditions. The treatments included: A single bath vaccination with anti-yersiniosis vaccine (with Brand name: Antiyersin) for 10 gr fish, two-stage bath vaccination against yersiniosis for 10 gr fish. two-stage hyperosmotic bath vaccination against yersiniosis (antiyersin) for 10 gr fish. Single injection vaccination against yersiniosis for 10 gr fish and a control group of unvaccinated fish. The impact of the vaccine on the biochemical factors in the blood of beluga sturgeon was also evaluated. The results indicated that in the vaccinated treatments, biochemical blood indices such as glucose, uric acid, urea, cholesterol, total protein, triglycerides, calcium, creatinine, and albumin were measured. The findings from this study demonstrated that the vaccine had a better effect on the health of beluga sturgeon weighing between 10 to 15 gr, with significant differences observed in glucose, uric nitrogen, uric acid, triglycerides, total cholesterol, and calcium levels between the vaccinated groups and the control group. However, there was no significant effect on total protein, creatinine levels, and the gene expression of dismutase among the vaccinated groups compared to the control group.

Keywords: Beluga sturgeon, Yersiniosis, Biochemical factors, Vaccine, Gene expression

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Introduction

Sturgeon fish are considered some of the most valuable economic fish in the world, historically playing a significant role in the fisheries industry of many countries. It is evident that farming environments, especially intensive farming, are highly stressful for fish, leading to a decrease in their immune system performance, which is associated with various viral, fungal, and bacterial diseases (El-Noby *et al*., 2021).

The use of antibiotics is a traditional and common method for combating bacterial diseases in aquatic animals (Harikrishnan *et al*., 2011). However, the trend towards eliminating antibiotics in aquaculture due to high costs, the development of drug resistance, environmental issues, reduced meat quality, and challenges in administration has led to increased attention on vaccination as an alternative to antibiotics (Harikrishnan *et al*., 2011). Yersiniosis, a common bacterial disease in aquaculture farms worldwide, is caused by the gram-negative bacterium *Yersinia ruckeri*, which typically infects and makes fish ill in a systemic manner (Tobback *et al*., 2007). This disease was first reported in 1999 from rainbow trout farms in Iran (Soltani *et al.*, 1999) and has since been reported from various fish farms across the country, becoming one of the most significant bacterial diseases in the aquaculture industry, causing considerable annual losses (Fadaeifard and Simin, 2014; Soltani *et al*., 2014).

Potential mechanisms related to pathogenicity can be suggested based on disease symptoms. Clinical pathological changes in the disease, as first reported by Rucker (1966) in infected rainbow trout, include reduced movement and darkening of color, along with reddening around the mouth, gill covers, and the bases of the fins. Other commonly reported pathological changes include exophthalmia, redness around the mouth, and localized or generalized darkening of the body (Soltani, 1996).

Vaccination, as a crucial tool against pathogens, holds a special place, and its proper and safe preparation and application, combined with essential factors (including high-quality fry, good nutrition, sanitary management, optimal farming practices), can play a significant role in preventing and controlling diseases, ultimately increasing survival rates and profitability in aquaculture. In many cases, injectable vaccines are more effective, but due to the difficulties and challenges associated with this method, bath vaccines are more commonly used (Bowald and Dalmo, 2019). Ensuring the performance and efficacy of the vaccine used is very important and beneficial (Du *et al*., 2017). Finally, after employing various methods to enhance vaccine efficacy, a proper evaluation of the vaccines used is necessary. There is a direct correlation between vaccine efficacy and serum immune response in fish (Liu *et al*., 2016). Since the first reports in the 1940s regarding fish vaccination to prevent diseases (Snieszko and Friddl, 1949), many vaccines have been developed that significantly reduce the impact of bacterial and some viral diseases in fish (Gudding and Goodrich, 2014). Millions

of fish are vaccinated annually, and in some regions of the world, there has been a shift away from antibiotics towards vaccination. For example, since the introduction of vaccines in Norway, the use of antibiotics in trout farming has significantly decreased (Rodger, 2016), and vaccination has become the most cost-effective and sustainable method for controlling infectious diseases in fish. Information on the molecular and cellular mechanisms of immune response and the expression levels of immune-related genes can help better understand the relationship between protection, host immunization against invading pathogens, and the processes involved in developing resistance to pathogens (Raida and Buchmann, 2008).

Numerous studies have reported the sensitivity of sturgeon fish to yersiniosis. For instance, in a study by Mazandarani and Taheri Mirghaed, the pathogenicity of *Yersinia ruckeri* in Iranian sturgeon was recorded with very high sensitivity (Mazandarani and Taheri Mirghaed, 2016). In a study by Yeganeh and Adel (2018), the pathogenic capability of the *Yersinia ruckeri* strain present in the country was reported for cultured *Huso huso* (Yeganeh and Adel, 2018). Kayis *et al*. (2017) reported this bacterium as pathogenic for sturgeon species such as the Persian sturgeon (*Acipenser gueldenstaedtii*) and Siberian sturgeon (*Acipenser baerii*).

Considering the pathogenicity of *Yersinia ruckeri* in sturgeon fish and the prevalence of this disease in various cold-water and warm-water farms across the country, this disease has the potential to cause significant mortality and damage to the industry. Given the high price of sturgeon fry and the substantial treatment costs, prevention is always more cost-effective and efficient. One of the best methods for disease control is vaccination against common diseases in the region. This study aims to evaluate the efficacy and effectiveness of the anti-Yersinia vaccine in controlling the disease caused by Yersinia in sturgeon fry.

Materials and methods

Sample preparation

For this study, 120 sturgeon fingerlings with an average weight of 15±1 gr were obtained from a breeding farm in Sari. The fish were transported to the Agricultural and Natural Resources University of Gorgan in a fish transport vehicle equipped with aeration. After acclimatization in a 400 liter fiberglass tank for 48 hours, the fish were divided into nine 200 liter fiberglass tanks containing 140 liters of water each and were raised for two weeks to adapt to the experimental conditions. Following treatment, the fish were raised for an additional eight weeks. During this period, the fish were fed twice daily, and 80% of the water in each tank was replaced daily.

Water temperature during the experiment was measured at 21.4±3.5 degrees Celsius, water hardness at 186.2±0.33 mg/Liter, and pH at 7.1±0.1. The light cycle during the research was natural, varying between 10-12 hours of light and 10-12 hours of darkness. At the start of the experiment, feeding was

stopped for 24 hours before transferring the fish to treatments. After conducting biometric operations (initial weight and length measurements), the fingerlings were distributed in a completely randomized design across seven treatments with two replicates in each tank, ensuring no significant differences in length and weight among the experimental tanks. Water temperature was measured at 13±1.3 degrees Celsius, hardness at 58.3±0.33 mg/Liter, and pH at 7.1±0.1. During this period, the water inflow rate for each tank was approximately 3 liters per minute.

Treatment and experimental design

In this study, four experimental groups were considered with two replicates each, including a control group, a group of fish vaccinated once via bath method, a group vaccinated twice via bath method, and a group vaccinated once via injection method. Two-week adaptation period was allowed for the fish to acclimate to the new conditions. The vaccine used in this study was the anti-Yersinia vaccine produced by Boujan Tech Pharmed Co (with Brand name: Antiyersin). This vaccine, which is a suspension of inactivated bacteria, was diluted with 9 liters of rearing water (1 liter of vaccine in 9 liters of water) and used for the bath method for 3 minutes.

For the injection vaccination method, each fish received 0.1 cc of the vaccine via intraperitoneal injection. Initially, the fish were anesthetized with 100 mg/Liter eugenol, and then 0.1 cc of the vaccine was injected using an insulin syringe. In the group receiving the two-stage vaccination, the vaccination was repeated 21 days after the first stage. In the first stage of vaccination (day 0) and in the second stage (day 28), the vaccine was diluted in a 1:10 ratio in water for immersion treatments. The fish were immersed in a suspension containing a concentration of 10^9bacteria per milliliter for 1-2 minutes (according to the vaccine protocol). Control groups did not receive vaccination on day 0 or day 28. Four weeks after the second vaccination, blood samples were taken from the fish (12 fish from each treatment). For this purpose, the fish were anesthetized with a concentration of 100 mg/L eugenol, and blood was collected from the caudal peduncle using a 25-gauge needle. In the injection vaccination method, fish received 0.1 cc of the vaccine via intraperitoneal injection. Initially, the fish were anesthetized with 100 mg/L eugenol, and 0.1 cc of the vaccine was injected using an insulin syringe. In the two-stage vaccination group, vaccination was repeated 21 days after the first stage with the same quality. To evaluate the resistance of different groups of vaccinated and non-vaccinated fish against the bacterium *Yersinia ruckeri*, strain PTCC 1888 (Mazandarani) from the Iranian Scientific and Industrial Research Organization was used. The bacterium was enriched in nutrient broth for 48 hours and then inoculated on tryptic soy agar (TSA). After 48 hours, bacteria were collected from the surface of the culture medium to prepare a bacterial suspension. The bacterial load of the suspension was adjusted using

turbidity measurement based on the McFarland standard table and a spectrophotometer at a wavelength of 640 nm, set to an OD of one. Simultaneously, serial dilutions were prepared to determine the viable bacterial load in each cc of the suspension, calculated based on colony-forming units (CFU), which indicated a viable cell count of 4.3×10^8 cells per cc of suspension. Accordingly, 12 to 14 fish from each treatment were divided into glass aquariums measuring 40×30 cm with a water height of 30 cm, with each fish receiving 0.1 cc of the mentioned suspension equivalent to 4.3×10^7 . To assess the injection effect, 12 fish received 0.1 cc of physiological saline intraperitoneally. The fish were monitored daily for 14 days post-exposure, with clinical signs and mortality recorded daily. To confirm the cause of death, bacterial cultures were performed on all dying or freshly dead fish in nutrient agar, isolating and confirming *Yersinia ruckeri* as the causative agent.

Measurement of aerum parameters

Initially, the fish were anesthetized with 100 ppm eugenol, and blood was collected from the caudal peduncle using a 21-gauge syringe. Blood samples were collected in the presence of the anticoagulant heparin at a concentration of 150 units per milliliter of blood. To prepare serum, the blood samples were kept at 4 degrees Celsius for 2 hours to clot and then centrifuged at 1600 × g and 4 degrees Celsius to collect serum samples. Subsequently, biochemical indices such as glucose, uric acid, urea, cholesterol, total protein, triglycerides, calcium, creatinine, and albumin were measured. These parameters were assessed using kits prepared by Pars Azmoon and analyzed with an autoanalyzer following the method by Johnson *et al*. (1999).

Statistical analysis

This experiment was conducted using a completely randomized design. First, the normality of the data was assessed using the Kolmogorov-Smirnov test. Subsequently, one-way analysis of variance (ANOVA) was employed to analyze the data and examine the differences between means using Duncan's test. For the statistical analysis of the results of this study, SPSS software version 22 was utilized with a significance level of 0.05. Additionally, Excel software version 2019 was used for graphing.

Results

Results of statistical analysis of blood biochemical factors

The variables used in this research include both dependent and independent variables. The independent variables in this study were the type of vaccination (single bath, double bath and injection) and the control group without vaccination, while the dependent variables were the levels of blood biochemical factors (glucose, uric acid nitrogen, uric acid, triglycerides, total cholesterol, blood calcium, total protein, and creatinine) in cultured *Huso huso*. Initially, the Kolmogorov-Smirnov test

was conducted to determine the normality of the data, which indicated that the data were normally distributed. To assess the level of significant changes in the blood biochemical factors of catfish and their relationship with the type of vaccination, ANOVA was used (ρ<0.05). The results showed a significant difference between the vaccinated groups and the control group regarding some blood biochemical factors (Table 1).

Table 1: One-way ANOVA results between vaccinated treatments and control group.

Factor	Sum of Squares	df	Mean Square	F	Sig.
Blood Urea Nitrogen	Between Groups	13.834	3	4.611	7.284
	Within Groups	29.756	47	.633	
	Total	43.590	50		
Calcium	Between Groups	.304	3	.101	3.302
	Within Groups	1.444	47	.031	
	Total	1.749	50		
Total Protein	Between Groups	.088	3	.029	.189
	Within Groups	7.309	47	.156	
	Total	7.397	50		
Uric Acid	Between Groups	4.866	3	1.622	11.616
	Within Groups	6.562	47	.140	
	Total	11.428	50		
Triglycerides	Between Groups	29376.044	3	9792.015	3.971
	Within Groups	115882.583	47	2465.587	
	Total	145258.627	50		
Glucose	Between Groups	482.074	3	160.691	6.120
	Within Groups	1234.083	47	26.257	
	Total	1716.157	50		
Creatinine	Between Groups	.081	3	.027	1.258
	Within Groups	1.004	47	.021	
	Total	1.085	50		
Total Cholesterol	Between Groups	3017.540	3	1005.847	12.266
	Within Groups	3854.146	47	82.003	
	Total	6871.686	50		
Albumin	Between Groups	.646	3	.215	4.474
	Within Groups	2.261	47	.048	
	Total	2.906	50		

There was a significant difference in the levels of glucose, uric acid nitrogen, uric acid, triglycerides, total cholesterol, and blood calcium between the vaccinated

groups and the control group. However, no significant difference was observed in the levels of total protein and creatinine between the vaccinated groups and the control group. Serological evaluations in the different groups of vaccinated fish are illustrated in Figures 1 and 2.

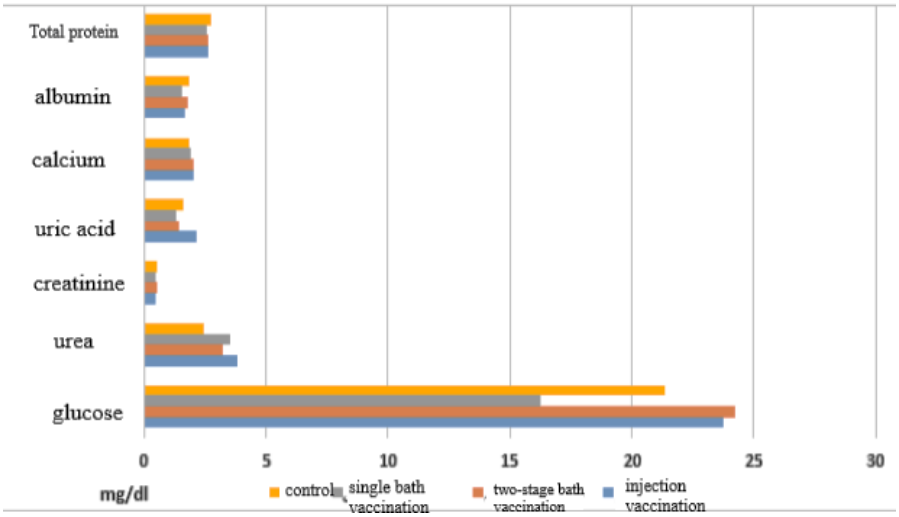


Figure 1: Comparison of blood biochemical factors in vaccinated and control cultured *Huso huso*.

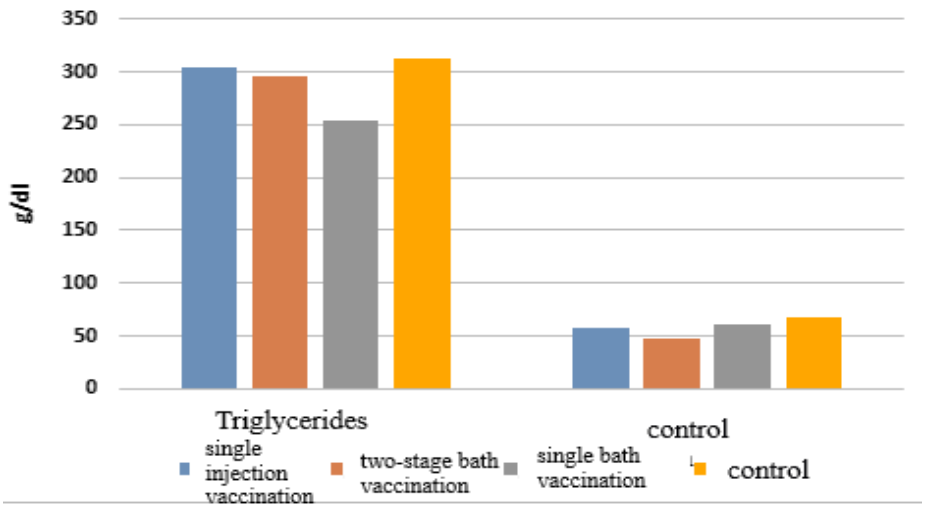


Figure 2: Comparison of blood biochemical factors in vaccinated and control cultured *Huso huso*.

According to the results, the highest glucose level was recorded in the group of fish vaccinated using the double bath method compared to the other vaccination methods used in this study and the control group, while the lowest glucose level was found in the group of fish vaccinated using the single bath method. Although a significant difference was observed between the groups and the control group. The statistical tests revealed that the serum uric acid nitrogen levels in cultured *Huso huso* showed a significant difference between the vaccinated treatments and the control group, with

the highest-level belonging to the injection treatment and the lowest to the control group. Additionally, significant differences were found in calcium and uric acid levels between the control and treatment groups, with the highest levels associated with the injection vaccination treatment. No significant differences were observed among the treatments and the control group regarding total protein and creatinine levels. Regarding triglycerides and total cholesterol, significant differences were also observed between the treatment and control groups, with the control group exhibiting the highest levels of triglycerides and cholesterol.

discussion and conclusion

regarding diseases represent the most significant barrier to the expansion and stability of aquaculture activities worldwide. Most bacterial pathogens are responsible for severe mortality across a wide range of fish growth stages (Vendrell *et al.*, 2006). Infectious bacterial diseases in aquaculture primarily occur due to gram-negative microorganisms. However, in recent decades, for unknown reasons, diseases caused by gram-positive bacteria have been on the rise and are now considered a challenge in aquaculture. To succeed in the aquaculture industry, one of the prerequisites is to minimize losses due to diseases and reduce the use of antibiotics. Therefore, finding preventive measures to minimize the economic losses from mortality in fish farms and reduce environmental issues caused by excessive antibiotic use seems essential (Hastein *et al*., 2005).

Controlling fish diseases using pharmaceuticals such as antibiotics poses problems, including the development of resistant bacteria in the future and consumer concerns. When diseases occur, the high costs associated with feeding fish and the harsh treatment conditions in ponds often burden farms. For this reason, preventive measures are always considered the most cost-effective and efficient way to control diseases, with vaccination of fish against common diseases in the region being one of the best methods. Vaccination is one of the most economical ways to control diseases in fish. Treating aquatic animals often causes significant harm to the environment and consumer health. Inactivated vaccines are primarily based on killed pathogens or extracts of pathogen components that activate the fish's immune system. In many cases, injectable vaccines are more effective; however, due to the difficulty of use and challenges associated with this method, bath vaccines are more favored (Bowald and Dalmo, 2019).

In this context, ensuring the performance and efficacy of the vaccine used is crucial and beneficial. When using bath vaccines, the duration of bathing and the vaccine dose are very important; failure to adhere to these factors can disrupt the vaccine's efficacy (Du *et al*., 2017). Yersinia is one of the common bacterial diseases in fish, reported with the development of cold-water fish and sturgeon production in farms across different provinces. In this

study, all biochemical blood factors tested and evaluated showed significant differences compared to the control group, with only two factors, creatinine and total protein, not showing significant differences between vaccinated treatments and the control group.

By examining the biochemical indices of fish blood, one can assess environmental conditions and the fish's reactions to these conditions. Hematology, as a fundamental and significant science, is used as an indicator to determine health and disease levels. Measuring biochemical and physiological blood indices can serve as a diagnostic tool in biological monitoring (Xiaoyan *et al*., 2009). Changes in the levels of these parameters can reflect the fish's responses to changes in their living environment (Satheeshkumar *et al*., 2010). Cholesterol is a precursor substance for steroid hormones, which increases in blood under stress conditions and may lead to increased cortisol hormone production. Based on existing scientific reports, elevated plasma cholesterol levels are considered a biological marker of liver and kidney damage (Sharifinasab *et al*., 2016). Cholesterol is a necessary raw material in the synthesis of steroid hormones and plays a significant role in maintaining normal cell membrane conditions. Damage to liver cells causes leakage and release of cholesterol into the blood, increasing its level (Mohiseni *et al*., 2017).

Triglycerides and cholesterol are primary indicators of the health status of high-quality bony fish. Changes in cholesterol concentration indicate metabolism in the liver. Excessive cholesterol levels indicate disorder in fat and lipoprotein metabolism, particularly dysfunction in liver physiology (Zhu *et al*., 2018). In the present study, the cholesterol and triglyceride levels in the blood of vaccinated catfish were lower than in the control group, with the lowest triglyceride and cholesterol levels belonging to the one-bath and two-bath treatments, respectively. The reduction in cholesterol and triglycerides may be due to decreased stress.

The relationship between triglycerides and total protein may be due to the insolubility of triglycerides in water and their transport in plasma. Therefore, they form a complex with some lipoproteins and cholesterol that can be transported in plasma, and increased production of these lipoproteins affects total protein levels (Maleknia and Shahbazi, 2021). In this regard, various studies have emphasized the increase in plasma cholesterol levels in response to environmental pollutants (Rabitto *et al*., 2005). Environmental stressors, including heavy metals, cause changes in biochemical parameters, including enzymes in animal bodies (Metwally, 2009).

The concentration of blood cholesterol in fish can vary between and within species depending on diet, activity intensity, and sexual growth stage (Zhu *et al*., 2018). Glucose is the primary substance obtained from carbohydrate metabolism (Ahmadifar *et al*., 2011). The serum glucose level is a

suitable indicator of fish's secondary stress responses to inappropriate environmental conditions (Cicik *et al*., 2005; Yousefi *et al*., 2011). Increased glucose levels can occur due to glycogenesis and the conversion of glycogen to glucose, leading to glucose accumulation in the blood. The results of this study showed that the average blood glucose level in vaccinated cultured *Huso huso* using the injection and two-bath methods increased compared to the control group, while the one-bath vaccinated fish had the lowest blood glucose levels. The increase in blood glucose in the two-bath and injectable vaccinated fish may be due to the fish's secondary stress response to vaccination and environmental conditions. Glucose concentration can vary with size, age, reproductive stages, and diet. Generally, it can be stated that factors such as age, species type, and gender significantly influence changes in blood and biochemical parameters, although the role of environmental and dietary factors cannot be overlooked. Blood tissue and determining blood factors, along with hematological and biochemical plasma analysis in fish, can serve as good indicators for diagnosing and determining health or infectious diseases in fish (Khoshbavar-Rostami *et al*., 2006).

Increased serum protein levels are considered a suitable indicator for assessing the fish's immune defense status. Total plasma protein includes albumin and globulin proteins. It is believed that increased levels of albumin, globulin, and serum protein are more related to the stimulation of the host's nonspecific immune system (Wiegertjes *et al*., 1996). The results of the present study indicated that there were no significant differences in total protein and creatinine levels between the treatments and the control group, but the treatments differed significantly in blood albumin levels. This means that the highest albumin levels belonged to the control group, while the lowest belonged to the one-bath treatment. Therefore, it seems that the type of vaccination and conditions led to differences among treatments and stimulated the nonspecific immune system of cultured *Huso huso.*

Regarding total protein, the lowest protein levels were found in the one-bath treatment group, while the highest levels were in the control group. Blood protein is one of the essential components of metabolism in aquatic organisms. The total protein level in blood plasma is used as a clinical indicator to measure health, stress, and body condition in aquatic organisms, and measuring blood protein can predict cellular damage (Riche, 2007).

Kazemi *et al*. (2010) stated that total plasma protein is not a specific indicator but can reflect a metabolic or pathological change. On the other hand, changes in plasma albumin levels are due to decreased sodium permeability (Alkahemal *et al*., 2011). Since albumin is one of the blood proteins and its primary function is to maintain blood osmotic pressure, reduced sodium permeability in plasma increases osmotic pressure, thus disrupting

albumin regulation. The decrease in total protein levels follows the reduction in albumin levels since albumin constitutes 12% of total protein (Yousefi *et al.*, 2011). Plasma proteins, except for immunoglobulins, are synthesized in the liver. Given the destructive effect of metals on liver tissue, the synthesis of liver proteins decreases; moreover, stress from poisoning and starvation leads to a decrease in total serum protein in fish (Gluth and Hanke, 1985). Proteins, fats, and carbohydrates are the main sources of energy in fish (Mazon *et al*., 2002). Therefore, changes and fluctuations in protein and triglyceride levels may relate to their consumption for the energy required for vital body activities (Emad *et al*., 2005).

The synthesis of plasma proteins occurs in the liver, and evidence suggests that in many diseases and physiological disorders, some plasma proteins are rapidly excreted from the body (Mohiseni *et al*., 2017). If the plasma amino acid levels fall below normal, amino acids are transferred from cells to the blood to restore plasma amino acid concentrations to normal levels. If tissues become protein-deficient, plasma proteins can serve as a source for the rapid replacement of essential tissue proteins. Additionally, proteins play a crucial role in providing energy for fish (Binukumari *et al*., 2016). The chemical composition of fish varies according to age, sex, environmental stress conditions, and season. However, under identical age and environmental conditions, their differences may relate to sex and dietary intake. Therefore, analyzing blood biochemical parameters can be a source for assessing health status, diagnosing anemia, poisoning, diseases, nutritional deficiencies, and fish physiology (Yousefian *et al*., 2010).

Overall, researchers believe that blood and serum factors in different fish species vary and are closely related to environmental conditions, fish size and age, species type, and the quantity and quality of feeding (Abdelhamid *et al*., 2019). Similar results were obtained by Hosseini *et al*. (2023) in their study of the effects of dual vaccination against Streptococcosis and Yersiniosis on some blood and immune parameters of rainbow trout. In that research, administering the vaccine led to improvements in some immune indices, although blood parameters did not change significantly due to vaccination. The results of this study showed that the red blood cell count, hematocrit, and hemoglobin levels in vaccinated fish and the control group did not differ significantly, but the white blood cell count in treatments on day 30 of vaccination in the Yersinia vaccine and dual vaccine groups was higher than in other treatments, with this increase being significant in the Yersinia vaccine group. The increase in white blood cell count may indicate the vaccine's effect on stimulating both the nonspecific and specific immune systems. The study concluded that given the lack of changes in blood indices in the present study and similar studies mentioned, vaccination does not affect red blood cell-related blood indices and likely does not play a role in hematopoiesis in blood-forming

tissues or the lifespan of red blood cells. The administration of the dual Streptococcosis/Yersiniosis vaccine in rainbow trout not only provides adequate protection against the disease but also effectively induces an immune response against these two diseases, comparable to each vaccine alone. A similar study regarding the effects of the dual Streptococcosis vaccine on some immune indices in rainbow trout fry was conducted by Nemoudi *et al*. (2023).

In this research, four experimental groups were considered: one-bath vaccinated fish, two-bath vaccinated fish, hypertonic environment + two-bath vaccine by bath method, injected vaccinated fish, and a control group. According to the results of the above study, total serum protein levels in vaccinated fish were higher than in the control group, and based on statistical analyses, the highest levels were found in the group of fish vaccinated by injection and the two-bath method. Therefore, it can be concluded that vaccination enhances the antibacterial effects of Yersinia in fish.

Vaccines are used as a strategy to prevent bacterial infections. Killed vaccines are relatively suitable and economical for production, and due to their high antigen content, they can play a significant role in immunization and protection. In aquaculture, due to this economic advantage, killed vaccines with formalin are commonly used to protect fish against bacterial diseases (Bercovier *et al*., 1997). Given the importance of *Huso huso*, farming in the country and its economic and social implications, vaccination aimed at immunization with inactivated *Yersinia ruckeri* vaccine (antiyersin) is strongly felt. Immunization with the *Yersinia ruckeri* vaccine has not negatively impacted the growth performance of the tested fish. The comparison of three vaccination methods in this study showed that the injection method has better performance in immunization, which is consistent with the findings of other researchers (Alishahi *et al*., 2020; Morshedi *et al*., 2023).

Considering the pathogenicity of *Yersinia ruckeri* in sturgeon fish and the widespread occurrence of this disease in various cold-water and warm-water farms across the country, this disease has the potential to cause significant losses and damages to the industry. Given the high price of sturgeon fingerling and the substantial treatment costs, prevention is always much more cost-effective and efficient. One of the best ways to control the disease is through vaccination against common diseases in the region. The results obtained in the current study indicated that the vaccine had a better impact on the health of sturgeon at weights between 10 to 15 grams, showing a significant difference in glucose levels, uric acid nitrogen, uric acid, triglycerides, total cholesterol, and blood calcium between the vaccinated groups and the control group. However, there was no significant effect on total protein, creatinine levels, and the expression of superoxide dismutase between the vaccinated groups and the control treatment.

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