

Effects of dietary supplementation with *Saccharomyces cerevisiae* and *Chlorella vulgaris* on body composition, hematological, immune, and biochemical responses of sterlet, *Acipenser ruthenus*

Elnaz Naseri Khalkhali¹, Abbasali Zamini^{1*}, Habib Vahabzadeh Roudsari¹, Hadi Ershad Langeroudi¹, Reza Taati²

1. Department of Fisheries, La. C., Islamic Azad University, Lahijan, Iran

2. Department of Fisheries, Tal. C., Islamic Azad University, Talesh, Iran

* Corresponding author's Email: abbas.ali.zamini@iaui.ac.ir

ABSTRACT

Sturgeons are commercially important fish that provide valuable proteins and fatty acids. *Chlorella vulgaris*, a microalga, and *Saccharomyces cerevisiae*, a main source of nutritional yeast, are commonly used as feed, growth enhancers, and immunostimulants in aquaculture. This study investigated the effects of dietary supplements containing *Chlorella vulgaris* and *Saccharomyces cerevisiae* on blood, immune, and biochemical parameters of sterlet, *Acipenser ruthenus*. A total of 315 juveniles, with a mean initial weight of 24.5 ± 0.2 g, were randomly assigned to seven treatments containing different levels of *Chlorella* and yeast (S_1 , $S_{1.5}$, Ch_3 , Ch_5 , $Ch_5+S_{0.5}$, Ch_3+S_1 , and control). The fish were kept in twenty-one 500-L circular fiberglass tanks (15 fish in each tank) under controlled conditions. At the end of the trial, blood, immune, and biochemical parameters, and carcass composition of the fish were measured, and the obtained data were statistically analyzed. Significant effects were observed in RBC count, Hb, and Hct concentrations, MCV, MCH, MCHC, eosinophil and neutrophil percentages in fish fed diets supplemented with different levels of *Chlorella* and yeast ($p < 0.05$). Additionally, the highest increase in total immunoglobulin, IgM, albumin, and calcium content were recorded in the Ch_3+S_1 and Ch_5 treatments, respectively. The activity of AST and ALT significantly ($p < 0.05$) increased in *Chlorella* and yeast-supplemented groups. In contrast, the value of ALP declined in the supplemented groups ($p < 0.05$). Fish that received Ch_5 and Ch_3+S_1 indicated higher amounts of carcass protein than those fed the control diet. In the S_1 group, the lowest ($p < 0.05$) content of carcass fat was reported. Based on the study findings, it can be concluded that dietary supplements containing *Chlorella* and brewer's yeast can positively affect the body composition, blood, immune, and biochemical parameters in sterlet.

Keywords: Sterlet, *Acipenser ruthenus*, *Saccharomyces cerevisiae*, *Chlorella vulgaris*, Carcass, Blood, Immunity.

Article type: Research Article.

INTRODUCTION

Sturgeons account for the most expensive and economically valuable group of fish due to their high-quality meat and valuable caviar (Song *et al.* 2024). One of the 27 sturgeon species that face global extinction threats is sterlet, *Acipenser ruthenus* (Müller *et al.* 2018; Gessner *et al.* 2022). This species has several advantages over other sturgeons, making it an excellent candidate for freshwater aquaculture. It also matures sexually more quickly and needs less room

in captivity because of its small size (Abdollahpour *et al.* 2018; Mohammadzadeh *et al.* 2021). This fish is currently found in Europe, large parts of Asia, and North America (Paschos *et al.* 2008). Since sturgeon aquaculture is part of the development programs for aquatic production (Vaugeois *et al.* 2022). It is necessary to investigate their physiological status in different aquaculture environments to optimize the use of resources. Growth and disease resistance are two important factors affecting cultured organisms (Lin *et al.* 2007). The use of immunostimulants in aquaculture, especially under intensive production conditions, is a suitable solution to prevent infectious diseases (Elumalai *et al.* 2020). Blood markers are effective diagnostic tools that can be used to monitor the health status of fish and other aquatic organisms in response to nutritional changes, water quality changes, diseases, and treatments (Fazio 2019). The fish immune system consists of two major subsystems: non-specific and specific. The non-specific immune system, which includes physical barriers, humoral immunity, and cellular immunity, is regarded as the primary immune response mechanism in fish. Granulocytes, monocytes, and macrophages are components of cellular immunity; granulocytes are further classified as neutrophils, basophils, and eosinophils (Mokhtar *et al.* 2023; Zapata 2024). These essential cellular components specialize in identifying and eliminating pathogens. Innate immune responses, such as superoxide production, alternative complement activity, normal hemagglutinin levels, respiratory burst activity, and lysozyme activity, are important immune parameters for determining disease resistance. All leukocyte subpopulations are involved in innate and adaptive cell-mediated immune responses, which constitute cellular immunity. Cell-mediated phagocytosis and cytotoxicity are essential processes (Mokhtar *et al.* 2023). The most important liver enzymes are aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). AST and ALT are found in various organs of fish, such as the liver, heart, skeletal muscles, kidney, pancreas, spleen, RBCs, and gills (Center 2007).

ALP is also present in the epithelium of the bile ducts, liver cells, intestinal mucosa, and kidneys. Liver enzymes are critical biomarkers for determining the proper function of body tissues and organs, especially the liver (Guo *et al.* 2023). Microalgae are commonly used in aquaculture as feed, growth enhancers, and immunostimulants (Ahmad *et al.* 2020). *Chlorella vulgaris* is categorized as a green microalga because of the presence of chloroplasts. It is spherical in shape, and its size ranges between 1 and 10 microns (Safi *et al.* 2014). In addition to chlorophyll, this microalga is rich in intracellular proteins, carbohydrates, lipids, vitamin C, beta-carotene, and B vitamins including B₁, B₂, B₆, and B₁₂ (Coronado-Reyes *et al.* 2022). Other bioactive components of *C. vulgaris* include minerals (e.g., potassium, iron, sodium, calcium, and magnesium) and pigments (e.g., chlorophylls and beta-carotene; Ahmad *et al.* 2020; Alagawany *et al.* 2021). Notably, *C. vulgaris* is among the microalgae commercially used in aquaculture (Sankar & Ramasubramanian 2012). Most microalgae contain plenty of antibacterial compounds, such as linolenic acids, which can kill methicillin-resistant *Staphylococcus aureus* (MRSA). Some bioactive compounds, including glycosides, terpenes, unsaturated aldehydes, and derivatives of chlorophyll, mediate the antibacterial properties of many microalgae (Natrah *et al.* 2014). Certain substances secreted by microalgae prevent bacterial growth and viral attachment. *C. vulgaris* possesses antibacterial activity and wound healing properties (Dineshkumar *et al.* 2017; Aldayel *et al.* 2022; Morowvat *et al.* 2023). They have antioxidant activities (Goiris *et al.* 2012; Safafar *et al.* 2015; Chinnasamy *et al.* 2024), increased survival rate, and phagocytic function (Andrews *et al.* 2009). *Saccharomyces cerevisiae*, brewer's yeast, is widely used in the production of proteins and vitamin B₁₂, food processing, alcohol production, food fermentation, and pharmaceuticals and chemical industries (Wang *et al.* 2023). Yeast is a type of probiotic (Sen & Mansell 2020), and probiotics improve the development of fish intestines by increasing the length, width, and surface area of the villi.

They also significantly increase the phosphorus levels (Ziółkowska *et al.* 2020). The most common type of yeast is *S. cerevisiae*, also known as brewer's yeast or baker's yeast, which is used in the fermentation of alcohol, bread, beer, and other carbohydrate sources (Wang *et al.* 2023). Brewer's yeast is a good source of protein, as it contains 40–45% protein, 1% fat, 1–2% fibre, and 6–9% ash. It is rich in B vitamins, including biotin, choline, niacin, folic acid, pantoic acid, pyridoxine, riboflavin, and thiamine. It is also a rich source of potassium, iron, and phosphorus. Brewer's yeast contains a high level of digestible energy (Alboghobesh *et al.* 2015). The phosphorus content of brewer's yeast is available to common carp at 92% and rainbow trout at 91%. Brewer's yeast is a rich source of glutathione, a necessary component of all living cells. The addition of brewer's yeast up to 50% to the diet of river trout caused no negative effect on fish growth. It is also a good source of amino acids but poor in methionine (Hertrampf & Piedad-Pascual

2012). Yeast-containing diets can significantly improve growth indices and increase WBCs, total protein, and globulin concentrations (Abu-Elala *et al.* 2018). Yeast supplements increase protein deposition in the fish body; fish fed yeast up to 0.1 g kg⁻¹ of feed show improved biochemical parameters. However, the lowest mortality rate was reported in fish fed 0.5 g kg⁻¹ yeast of feed (Abdel-Tawwab *et al.* 2022 a). Different yeasts, including brewer's yeast (*S. cerevisiae*), produce many different metabolic compounds. They can also positively affect the growth of aquatic organisms by increasing the secretion of digestive enzymes, facilitating the digestion of eaten food, stimulating the immune system, developing the intestines, improving the nutritional performance of larvae, and increasing their survival rate (Ringoe & Birkbeck 1999). Many studies have reported the positive effects of yeast and *Chlorella* on the nutritional, growth, blood, immunity, and biochemical parameters of different fish species, such as Nile tilapia (*Oreochromis niloticus*) (Sutthi *et al.* 2018; Sutthi & Thaimuangphol 2020; Abu-Elala *et al.* 2020; Abdel-Tawwab *et al.* 2022 b), olive flounder (*Paralichthys olivaceus*; Kim *et al.* 2002), gray mullet (*Mugil cephalus*; Akbary & Gorgij 2018), beluga (*Huso huso*; Hoseinifar *et al.* 2011), common carp (*Cyprinus carpio*; Khani *et al.* 2017; Abdel-Tawwab *et al.* 2022 a), and the largemouth bass (*Micropterus salmoides*; Xi *et al.* 2022; Yu *et al.* 2023). This study investigated the effects of dietary *C. vulgaris* and *S. cerevisiae* alone and in combination on hematological indices, immune-related and biochemical parameters, plasma ion concentrations, liver enzyme activities, and whole body composition in juvenile sterlet, *Acipenser ruthenus*.

MATERIALS AND METHODS

In this study, 315 sterlet juveniles, with a mean initial weight of 24.5 ± 0.2 g, were randomly distributed to seven treatments representing nutritional supplements containing different levels of *Chlorella* and yeast (S₁, S_{1.5}, Ch₃, Ch₅, Ch₅+S_{0.5}, Ch₃+S₁, and control) with three replicates each (Table 1). Juveniles were kept in twenty-one 500-liter circular fiberglass tanks (15 fish in each tank) with a central outlet under aeration and controlled conditions. Physicochemical parameters of water, including dissolved oxygen (DO), temperature, and pH were regularly measured and monitored during the study using a digital oxygen meter (WTW, Germany), a thermometer, and a pH meter (AZ8584, China). The mean DO, pH, and temperature during the study were 6.8 mg L⁻¹, 7.2, and 22 °C, respectively.

Table 1. Experimental treatments (diets containing different levels of *Chlorella* (Ch) and brewer's yeast (S).

Treatments	<i>C. vulgaris</i> (%)	<i>S. cerevisiae</i> (%)
Control	0	0
S ₁	0	1
S _{1.5}	0	1.5
Ch ₃	3	0
Ch ₅	5	0
Ch ₅ + S _{0.5}	5	0.5
Ch ₃ + S ₁	3	1

Note: Ch = *Chlorella vulgaris*; S = *Saccharomyces cerevisiae*.

Supplementation levels are expressed as percentages (w/w) of the final diet.

The basic feed was provided by Behsan Arian Nutrition Co. (Beta Group, Alborz, Iran); it was then ground to obtain a powder containing 37% crude protein, 20% fat, 14% ash, 7% fiber, and 10% moisture with a digestible energy level of 3,700 kcal kg⁻¹ (Helrich 1990). Powdered *Chlorella vulgaris* contained 48% protein, 7% fat, 6-8% moisture, 2% fiber, and 2% ash. Specified values of *Chlorella vulgaris* and *Saccharomyces cerevisiae* were added to the basal diet (Table 1). For this purpose, 100 mL distilled water and 5 mL edible oil per kg of feed were added to the diets; the control diet contained only edible oil. Finally, the feed was prepared as pellets (Cerezuela *et al.* 2008). The fish were fed daily at a rate of 3% of their body weight, distributed across five meals, for a period of 8 weeks (Chebanov & Galich 2013).

Measurement of blood and immune parameters

At the end of the trial, blood samples were collected from all treatments at a 45° angle from the caudal vein of the fish using a 2-mL syringe. The blood samples were then transferred to heparinized glass containers. The containers were

shaken until the blood and the anticoagulant were mixed to prevent blood coagulation. The heparinized tubes were centrifuged at 5,000 rpm for 5 minutes to separate the plasma. To measure blood parameters, blood samples were quickly sent to a laboratory in less than three hours in a box filled with dry ice (Filho *et al.* 1992). RBCs and WBCs were manually counted using Neubauer slides. Differential WBCs were counted using Giemsa staining. Hemoglobin (Hb) and hematocrit (Hct) were determined using the cyanmethemoglobin and microhematocrit methods, respectively. MCV, MCH, and MCHC were calculated using mathematical equations (Klontz 1994). Immunological and biochemical parameters, including total immunoglobulin, IgM, total protein, albumin, Ca, Na, and K ions, were also evaluated using an autoanalyzer (Hitachi, Japan) and laboratory kits (Pars Azmoon Co., Iran; Kopp *et al.* 2009).

Measurement of liver enzymes

Liver enzymes (ALP, AST, and ALT) were measured based on the ELISA method using specialized kits provided by Pars Azmoon Co., Iran (Moss & Henderson 1999).

Carcass analysis

At the end of the trial, 3 fish were randomly selected from each replicate to measure the protein, fat, carbohydrate, moisture, and ash contents of carcass. Briefly, the values of protein, fat, and carbohydrate were measured using Kjeldahl method, Soxhlet extraction technique, and Anthrone method, respectively. To measure the ash content of the carcass, samples were put in an electric furnace at 550 °C for 6 hours. Their moisture content was also measured using an oven at 105 °C (Helrich 1990).

Statistical analysis

Since the Shapiro–Wilk test confirmed the normal distribution of the data, One-Way Analysis of variance (ANOVA) was used to compare the mean values of the variables. Tukey's test was also performed to separate homogeneous groups at the 5% significance level. Moreover, Kruskal–Wallis and Mann–Whitney U tests were employed for non-parametric data and pairwise comparison, respectively. All statistical analyses were performed in SPSS-18, and the results were presented as mean $\pm$ SD.

RESULTS

Red blood cell counts and the levels of hematocrit and hemoglobin for fish fed diet containing 3% *Chlorella* were significantly increased compared to those in the control group ($p < 0.05$). Sterlet juveniles fed the control diet represented the highest ($p < 0.05$) counts of WBC, monocyte, and lymphocyte. The values of MCV, MCH, and eosinophil percentage were significantly elevated in the $Ch_5+S_{0.5}$ treatment ($p < 0.05$). Neutrophil percentage and mean corpuscular hemoglobin concentration (MCHC) were affected by *Chlorella* at the levels of 5% and 3%, respectively (Table 2).

Table 3 presents the values of immunological and biochemical parameters in sterlet juveniles. Accordingly, the highest ($p < 0.05$) total protein level was observed in Ch_5 treatment, whereas total immunoglobulin was significantly affected by the combination of 3% *Chlorella*+1% yeast (Ch_3+S_1 ; $p < 0.05$). Furthermore, the levels of albumin and calcium showed an increase ($p < 0.05$) in Ch_3+S_1 treatment. On the other hand, no significant difference ($p > 0.05$) was found in the values of IgM, sodium, and potassium ions across treatments. Liver enzymes activities are summarized in Table 4. The levels of ALT and AST in *Chlorella* and yeast-supplemented groups were significantly higher compared to the control. On the contrary, the values of ALP were significantly elevated in the control group ($p < 0.05$; Table 4). Protein content was significantly higher than in the Ch_5 and Ch_3+S_1 groups in comparison with other groups ($p < 0.05$). The lowest ($p < 0.05$) value of fat was observed in the S_1 group. Ash and moisture contents were significantly elevated in fish fed the control diet. In contrast, $Ch_5+S_{0.5}$ treatment showed the highest level of carbohydrate ($p < 0.05$; Table 5).

Table 2. Hematological indices (Mean $\pm$ SD) in sterlet juveniles fed the diets containing different levels of *Chlorella* and brewer's yeast.

Parameters Treatments	RBC ($\times 10^3$ mm^{-3})	WBC ($\times 10^3$ mm^{-3})	Hct (%)	Hb (g dL ⁻¹)	MCV (fL)	MCH (pg)	MCHC (g dL ⁻¹)	Neutrophil (%)	Monocyte (%)	Lymphocyte (%)	Eosinophil (%)
Control	510.0 $\pm$ 0.78 ^b	1792.00 $\pm$ 31.05 ^c	19.47 $\pm$ 0.3	4.37 $\pm$ 0.0	421.17 $\pm$ 1.01 ^f	92.20 $\pm$ 0.2	22.40 $\pm$ 0.4	22.34 $\pm$ 0.8	3.99 $\pm$ 0.2	72.20 $\pm$ 1.2	1.80 $\pm$ 0.1
S ₁	523.1 $\pm$ 1.24 ^d	1368.30 $\pm$ 10.69 ^b	19.97 $\pm$ 0.1	4.47 $\pm$ 0.0	325.13 $\pm$ 0.25 ^c	84.23 $\pm$ 0.3	23.97 $\pm$ 0.1	23.87 $\pm$ 0.9	3.61 $\pm$ 0.0	65.53 $\pm$ 0.9	2.80 $\pm$ 0.1
S _{1.5}	520.0 $\pm$ 0.08 ^c	1368.70 $\pm$ 11.06 ^b	20.40 $\pm$ 0.3	4.40 $\pm$ 0.0	320.50 $\pm$ 0.53 ^b	89.90 $\pm$ 0.4	22.20 $\pm$ 0.3	23.88 $\pm$ 0.9	3.63 $\pm$ 0.0	67.37 $\pm$ 0.8	2.67 $\pm$ 0.0
Ch ₃	586.0 $\pm$ 0.06 ^g	1299.70 $\pm$ 26.73 ^a	23.83 $\pm$ 0.0	5.87 $\pm$ 0.0	384.80 $\pm$ 0.66 ^e	89.23 $\pm$ 0.7	25.43 $\pm$ 0.3	24.32 $\pm$ 0.8	3.75 $\pm$ 0.1	68.03 $\pm$ 0.7	2.93 $\pm$ 0.0
Ch ₅	573.0 $\pm$ 0.55 ^e	1301.30 $\pm$ 27.30 ^a	22.87 $\pm$ 0.0	5.13 $\pm$ 0.0	369.03 $\pm$ 0.97 ^d	85.37 $\pm$ 0.0	24.20 $\pm$ 0.3	24.39 $\pm$ 0.7	3.68 $\pm$ 0.0	68.13 $\pm$ 0.6	2.98 $\pm$ 0.0
Ch ₅ +S _{0.5}	488.0 $\pm$ 0.96 ^a	1258.30 $\pm$ 14.57 ^a	20.80 $\pm$ 0.3	4.43 $\pm$ 0.1	440.08 $\pm$ 0.30 ^g	98.27 $\pm$ 0.1	22.47 $\pm$ 0.2	23.13 $\pm$ 0.7	3.87 $\pm$ 0.1	67.47 $\pm$ 0.9	3.13 $\pm$ 0.0
Ch ₃ +S ₁	578.0 $\pm$ 0.44 ^f	1258.70 $\pm$ 16.92 ^a	22.83 $\pm$ 0.1	5.43 $\pm$ 0.0	288.67 $\pm$ 0.40 ^a	97.37 $\pm$ 0.2	22.30 $\pm$ 1.9	25.19 $\pm$ 0.5	3.80 $\pm$ 0.3	67.50 $\pm$ 0.70	3.10 $\pm$ 0.1

Note: Different lowercase superscript letters within a column indicate significant differences among treatments ($p < 0.05$). RBC, red blood cell; WBC, white blood cell; Hct, hematocrit; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration, S, *Saccharomyces cerevisiae*, Ch, *Chlorella vulgaris*

Table 3. Immunological and biochemical indices (Mean $\pm$ SD) in sterlet juveniles fed the diets containing different levels of *Chlorella* and brewer's yeast

Parameters Treatments	Total Ig (mg dL ⁻¹)	IgM (mg dL ⁻¹)	Total Protein (g dL ⁻¹)	Albumin (g dL ⁻¹)	Calcium (mg dL ⁻¹)	Sodium (mmol L ⁻¹)	Potassium (mg dL ⁻¹)
Control	6.15 $\pm$ 0.52 ^b	0.88 $\pm$ 0.01	3.86 $\pm$ 0.04 ^a	2.20 $\pm$ 0.02 ^a	11.73 $\pm$ 0.14 ^a	136.73 $\pm$ 0.50	3.08 $\pm$ 0.02
S ₁	6.12 $\pm$ 0.40 ^b	0.88 $\pm$ 0.01	3.92 $\pm$ 0.03 ^a	2.32 $\pm$ 0.02 ^b	12.19 $\pm$ 0.01 ^b	136.63 $\pm$ 0.70	3.14 $\pm$ 0.06
S _{1.5}	6.20 $\pm$ 0.23 ^b	0.89 $\pm$ 0.01	4.28 $\pm$ 0.03 ^{cd}	2.36 $\pm$ 0.05 ^{bc}	12.23 $\pm$ 0.05 ^b	139.10 $\pm$ 0.70	3.14 $\pm$ 0.02
Ch ₃	6.30 $\pm$ 0.18 ^b	0.90 $\pm$ 0.00	4.36 $\pm$ 0.02 ^e	2.38 $\pm$ 0.02 ^{bcd}	13.11 $\pm$ 0.00 ^c	138.53 $\pm$ 0.65	3.13 $\pm$ 0.01
Ch ₅	6.50 $\pm$ 0.26 ^{ab}	0.93 $\pm$ 0.05	4.90 $\pm$ 0.09 ^f	2.47 $\pm$ 0.04 ^d	13.17 $\pm$ 0.02 ^{cd}	138.21 $\pm$ 0.73	3.11 $\pm$ 0.00
Ch ₅ +S _{0.5}	6.27 $\pm$ 0.47 ^b	0.90 $\pm$ 0.00	4.13 $\pm$ 0.00 ^b	2.46 $\pm$ 0.10 ^{cd}	13.16 $\pm$ 0.05 ^{cd}	137.80 $\pm$ 1.10	3.14 $\pm$ 0.04
Ch ₃ +S ₁	6.81 $\pm$ 0.59 ^a	0.96 $\pm$ 0.05	4.23 $\pm$ 0.04 ^c	2.64 $\pm$ 0.04 ^e	13.30 $\pm$ 0.02 ^d	137.63 $\pm$ 1.96	3.13 $\pm$ 0.01

Note: Different lowercase superscript letters within a column indicate significant differences among treatments ($p < 0.05$). Total Ig, total immunoglobulin; IgM, immunoglobulin M, S, *Saccharomyces cerevisiae*, Ch, *Chlorella vulgaris*.

Table 4. Liver enzymes activities (Mean $\pm$ SD) in sterlet juveniles fed the diets containing different levels of *Chlorella* and brewer's yeast

Parameters Treatments	ALP (U L ⁻¹)	ALT (U L ⁻¹)	AST (U L ⁻¹)
Control	398.66 $\pm$ 1.45 ^e	13.00 $\pm$ 0.18 ^a	493.91 $\pm$ 4.76 ^a
S ₁	382.24 $\pm$ 6.67 ^d	25.54 $\pm$ 0.19 ^b	520.79 $\pm$ 9.52 ^b
S _{1.5}	369.21 $\pm$ 0.98 ^c	38.64 $\pm$ 1.44 ^c	598.22 $\pm$ 3.19 ^c
Ch ₃	378.14 $\pm$ 5.75 ^{cd}	45.57 $\pm$ 2.76 ^d	690.93 $\pm$ 8.43 ^{de}
Ch ₅	379.49 $\pm$ 0.97 ^{cd}	50.02 $\pm$ 0.57 ^e	700.28 $\pm$ 1.24 ^e
Ch ₅ +S _{0.5}	263.89 $\pm$ 3.69 ^a	77.62 $\pm$ 0.73 ^f	669.20 $\pm$ 12.57 ^d
Ch ₃ +S ₁	280.93 $\pm$ 2.95 ^b	80.72 $\pm$ 0.45 ^f	694.99 $\pm$ 8.46 ^e

Note: Different lowercase superscript letters within a column indicate significant differences among treatments ($p < 0.05$). ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase. S, *Saccharomyces cerevisiae*, Ch, *Chlorella vulgaris*.

Table 5. Whole body proximate composition of dry matter (Mean $\pm$ SD) and moisture in sterlet juveniles fed the diets containing different levels of *Chlorella* and brewer's yeast

Parameters Treatments	Protein (%)	Fat (%)	Carbohydrate (%)	Ash (%)	Moisture (%)
Control	49.04 $\pm$ 0.12 ^a	19.47 $\pm$ 0.15 ^b	7.23 $\pm$ 0.05 ^a	25.12 $\pm$ 0.02 ^c	85.17 $\pm$ 0.11 ^e
S ₁	49.57 $\pm$ 0.04 ^b	12.63 $\pm$ 0.15 ^a	13.33 $\pm$ 0.15 ^b	24.23 $\pm$ 0.41 ^{bc}	82.43 $\pm$ 0.41 ^d
S _{1.5}	49.30 $\pm$ 0.05 ^{ab}	13.20 $\pm$ 0.35 ^a	14.14 $\pm$ 0.23 ^b	23.95 $\pm$ 0.16 ^b	79.69 $\pm$ 0.56 ^c
Ch ₃	53.22 $\pm$ 0.08 ^c	17.98 $\pm$ 1.16 ^b	14.61 $\pm$ 0.97 ^{bc}	12.93 $\pm$ 0.40 ^a	77.51 $\pm$ 0.49 ^b
Ch ₅	54.18 $\pm$ 0.17 ^d	19.20 $\pm$ 0.65 ^b	16.00 $\pm$ 0.85 ^{cd}	12.70 $\pm$ 0.67 ^a	76.89 $\pm$ 0.05 ^b
Ch ₅ +S _{0.5}	53.30 $\pm$ 0.06 ^c	18.77 $\pm$ 0.06 ^b	16.63 $\pm$ 0.22 ^d	12.36 $\pm$ 0.11 ^a	73.96 $\pm$ 0.13 ^a
Ch ₃ +S ₁	54.14 $\pm$ 0.26 ^d	18.40 $\pm$ 0.61 ^b	15.67 $\pm$ 0.25 ^{cd}	12.41 $\pm$ 0.14 ^a	74.17 $\pm$ 0.02 ^a

Note: Different lowercase superscript letters within a column indicate significant differences among treatments ($p < 0.05$). S, *Saccharomyces cerevisiae*, Ch, *Chlorella vulgaris*.

DISCUSSION

Measurement of blood parameters and analysis of how they change in response to different diseases have always been considered an effective diagnostic tool for many diseases (Fazio 2019). This study showed significant differences across the treatments for haematological indices. RBCs demonstrated a significant difference between all experimental treatments. The increase in blood indices can be attributable to either the protective effect of polyphenols against hydrogen peroxide resulting from RBC oxidation or the antioxidant effects of algae on the reduction of hemolysis caused by fat peroxidation in the membrane of RBCs (Ma *et al.* 2000). Flavonoids and polyphenols, two types of antioxidants abundant in seaweeds, are crucial for preventing oxidation (Onofrejová *et al.* 2010). Compared with other WBCs, lymphocytes live longer, and their numbers typically decrease with pollutant exposure. The activation or suppression of the immune system is attributed to lymphocytes, which are effective biomarkers. As a component of the non-specific cellular immune system, lymphocytes are considered a good indicator of how the body reacts to stressors (Wuertz *et al.* 2021). This study demonstrated that as neutrophil counts increased; they took on more roles in the body, such as phagocytosing small particles, responding to inflammation, decomposing pollutants, and carrying out metabolic activities, such as cell destruction. As a result, it is not surprising that the number of neutrophils increases with exposure to pollutants (Liu *et al.* 2023).

Assessment of indices such as of MCV, MCH, and MCHC can help diagnose various types of anemia (Ferguson et al. 2010). In this study, the mean MCV was lower in S₁, S_{1.5}, Ch₃, Ch₅, and Ch₃+S₁ groups than in the control group. Since the reduced volume of RBCs facilitates their movement in blood vessels and inhibits the formation of clots, a low mean MCV can be considered a positive blood index (Ferguson et al. 2010). The present results demonstrated that the addition of yeast and algae supplements to the diet of the sterlets positively affected the hematopoietic processes and tissues, since they increased the number of RBCs. Some dietary supplements may cause anemia and reduce hemoglobin, hematocrit, and RBCs because of their detrimental effects on hematopoietic tissues (Fails & Magee 2018). Moreover, the use of these supplements increased hematocrit and hemoglobin levels, which is also beneficial, as higher hemoglobin levels translate into higher oxygen-carrying capacity and, ultimately, higher blood oxygen levels (Raji et al. 2019). These results confirm the favorable effect of *Chlorella* and yeast supplements added to the diet of sterlet juveniles. Studies have shown that the addition of algae supplements to the diet of fish species can increase hematocrit levels. Karami et al. (2016) and Zeraatpisheh et al. (2018) also reported that the addition of the aqueous extract of *Sargassum angustifolium* to the diet of the common carp (*Cyprinus carpio*) and the rainbow trout (*Oncorhynchus mykiss*) significantly increased hemoglobin levels compared to the control group. Similar results have been reported about the probiotic effects of *Saccharomyces cerevisiae* on RBCs, WBCs, MCV, and intracellular hemoglobin weight in the beluga (Hoseinifar et al. 2011; Fattahi et al. 2014) and the channel catfish (*Ictalurus punctatus*; Reque et al. 2010) as well as their effects on hematocrit, and neutrophils in the Persian sturgeon (*Acipenser persicus*; Darafsh et al. 2020). Microalgae are valuable nutritional sources used in aquatic nutrition as a suitable replacement for proteins obtained from fish meal and soy, as they are rich in proteins (between 50% and 70%; Alagawany et al. 2021). Furthermore, these algae are potent food supplements because of their high content of biophenols and other bioactive compounds with immunogenic and antioxidant properties (Ahmad et al. 2020). These compounds in the *Chlorella*-containing diets have positive effects on the growth, immune responses, and antioxidant profiles of aquatic organisms (Abdel-Tawwab et al. 2022 b). This study demonstrated that *Chlorella* was more effective than brewer's yeast in increasing the total protein and albumin levels, indicating the positive effects of *Chlorella* and yeast on the immune system of fish. The highest levels of total protein and albumin were observed in Ch₅ treatment. Studies have shown that the addition of *Chlorella* to diets at the levels of 0.8-1.2%, 2%, and 5% increased total protein and albumin in *Sebastess chlegeli* (Bai et al. 2001), *Cyprinus carpio* (Khani et al. 2017; Abdulrahman et al. 2018; Abdel-Tawwab et al. 2022 b), *Oreochromis niloticus* (Abdel-Tawwab et al. 2022 b; Morowvat et al. 2023), and *Carassius auratus* (Xu et al. 2014). Albumin is the most abundant protein of plasma produced in the liver. It regulates body fluid distribution, acid-base physiology, and the binding of essential components in the bloodstream (Sheinenzon et al. 2021). Blood proteins serve a variety of biological functions, such as carriers, enzymes, and protease inhibitors, and are important in humoral immunity (Fails & Magee 2018). All proteins in the blood that trigger particular immune reactions and aid in the production of antibodies are collectively referred to as total protein. A higher serum protein level generally indicates a better state of immunity in fish. Elevated serum protein levels indicate that the liver and other organs involved in plasma protein production are functioning properly (Yavuzcan et al. 2005). As an immunostimulant, *Chlorella vulgaris* significantly increases the IgM and lysozyme levels in the common carp (Khani et al. 2017), Caspian salmon, *Salmo trutta caspius* (Saber et al. 2017), and Nile tilapia, *Oreochromis niloticus* (Abdel-Tawwab et al. 2022 b). The polysaccharides present in the cell wall of *S. cerevisiae* consist of two types of glucans, namely, beta-1-3-glucan (50-60%) and beta-1-6-glucan (10-15%). Studies have demonstrated the positive role of these glucans in enhancing immune function and preventing diseases (Aimanianda et al. 2009). Yeast and *Chlorella* boost protease secretion and protein digestibility, which benefit intestinal microbial flora. Consequently, they act as a barrier against bacterial toxins produced in the intestine and increase total protein levels in fish blood (El-Nobi et al. 2021). *S. cerevisiae* is a natural component of the gut microbiota in rainbow trout (Waché et al. 2006) and beluga (Fattahi et al. 2014). Yeasts contain several bioactive compounds that have strong immunostimulatory effects on aquatic organisms (Kober et al. 2022). This study showed that dietary brewer's yeast increased several immune response-related parameters, including total immunoglobulins and IgM. Numerous cellular components found in yeasts can stimulate an immune response (Martins et al. 2009). IgM is a major immunoglobulin in fish and the primary component of the humoral immune system (Sun et al. 2010). Increased IgM levels improve the immune system of fish. The study results revealed no significant difference between the experimental treatments in terms of IgM levels. It seems that 8 weeks is not sufficient to monitor changes in IgM, and further studies over a longer period

are needed to identify these changes more precisely. Although total protein and albumin levels increased in this study, IgM levels did not change significantly. These studies demonstrate the beneficial effects of dietary probiotic yeast on the immune system and its potential role as a preventive agent in fish feeds. Ions play a role in most biological processes, such as respiration, muscle contraction, absorption, transmission of nerve messages, osmotic regulation, acid and alkali balance, and excretion; hence, they are important for all organs (Zimmer & Perry 2022). The study results showed no significant difference between the experimental treatments in the concentration of blood minerals, except calcium, exhibiting that the brewer's yeast did not affect mineral absorption. There is insufficient information on the effects of dietary yeast on mineral metabolism in fish. The type of fish, duration of supplementation and probiotic yeast species are factors that determine how dietary probiotics affect blood metabolic parameters. Because of their strong immunostimulatory effects, probiotics have attracted great attention in aquaculture for maintaining the health of farmed aquatic organisms (Dimitroglou *et al.* 2011). The fish in the control group exhibited significantly lower levels of calcium ions than those in the other treatments. In fact, the calcium levels increased with the elevation in the *Chlorella* content of the diet. Due to the loss of small ions through gill exchange, the sodium and chloride levels are lower in fish exhibiting secondary stress responses (Barton 2002). However, the study results revealed no significant difference between the control group and the experimental treatments in blood sodium levels. This may suggest that the sterlet juveniles fed diets containing yeast and *Chlorella* were in a stress-free state. The availability and abundance of calcium sources in water affect fish. The gills, skin, and oesophagus of fish come into direct contact with the water, and calcium is actively absorbed from the water (Flik & Verbost 1993; Guerreiro & Fuentes 2007). Although pituitary hormones, such as prolactin, growth hormone, and somatostatin (Wagner & Congleton 2004), and endocrine hormones, such as cortisol (Flik *et al.* 1995; Guerreiro & Fuentes 2007), have been reported to be effective in regulating blood calcium levels, the factors involved in the regulation of fish blood calcium levels are still unknown. Probiotics improve calcium absorption and fortify the intestinal epithelium. They also facilitate the absorption of minerals such as calcium and iron and boost the production of vitamins such as B₁₂, B₆, and K (Mohammadian *et al.* 2021). This may account for the calcium increase. The liver in fish is the primary organ for the metabolism of nutrients and medications; and is also crucial for the proper functioning of the immune system (Center 2007). The concentration of liver enzymes, which increase primarily as a result of tissue injury, is a good measure of liver health. The diets containing different levels of *C. vulgaris* and *S. cerevisiae* significantly increased AST and ALT and decreased ALP activity compared to the control group. Studies have shown that liver enzymes are affected by physiological and environmental factors, including skeletal muscle disorders, heart failure, dietary habits, water temperature, fish age, and water salinity. As a result, increased activity of liver enzymes in the plasma of fish fed diets containing *C. vulgaris* and *S. cerevisiae* could indicate the presence of liver tissue damage. Sherif *et al.* (2012) reported improved ALT and AST levels in Nile tilapia, *O. niloticus* fed diets containing *Spirulina* algae. The feeding of Nile tilapia with diets containing 5% or 10% *C. vulgaris* also reduced liver histopathological alterations (Zahran *et al.* 2019). Daliri *et al.* (2020) showed that the oral administration of red algae, *Laurencia caspica*, reduced the activity of liver enzymes. The findings of the carcass analysis showed that the highest protein, carbohydrate, and moisture contents in the carcass of fish were observed in the Ch₅, Ch₅+S_{0.5}, and control groups, respectively. Other studies have shown that the addition of 10 g kg⁻¹ of *C. vulgaris* to the diet of the grey mullet significantly increased the crude protein and moisture contents of the fish carcass, while decreasing its fat content. The addition of 5 g kg⁻¹ of *Chlorella* to the diet of mullet significantly increased the protein, ash, and moisture content of the carcass compared to the control group (Akbari & Gorgij 2018). These findings are not consistent with the results of the present study. The addition of *Chlorella* powder to the diet of the olive flounder decreased the fat content of the fish carcass (Kim *et al.* 2002). On the other hand, the addition of yeasts significantly increased the protein content of the carcass in the three-spot cichlid (*Cichlasoma trimaculatum*), but there was no significant change in the fat, moisture, and ash contents (Mohammadi *et al.* 2016). In another study on the Nile tilapia, it was reported that the feed containing yeasts significantly elevated the protein content and reduced the carcass fat content (Sutthi & Thaimuangphol 2020). There are reports that the addition of yeasts to diets increased the protein content of the carcass in rainbow trout (Pooramini *et al.* 2009), rohu, *Labeo rohita* (Tewary & Patra 2011), *Astronotus ocellatus* (Kafilzadeh *et al.* 2013), and beluga, *Huso huso* (Taati *et al.* 2011). Most of these studies are consistent with the findings of the current study. Review of previous research showed that the addition of yeast and algae to fish diets increased the protein content and decreased or caused no change in the fat content of the carcass. Moreover, the trend of alterations in the fat content of the carcass is the opposite of that of the moisture

content, which is consistent with the findings of this study. The increased protein content of the carcass following the addition of *Chlorella* to the fish diet shows that protein metabolism is triggered by the use of *Chlorella* algae to join its primary pathway, the path of tissue synthesis, where it is stored as protein in the tissue (Shalaby & El-Nady 2008). Moreover, 50–60% of the dry weight of *C. vulgaris* is composed of proteins (Zheng et al. 2021). This microalga also exhibits probiotic qualities and enhances the secretion of digestive enzymes and food absorption and digestion, in addition to its other beneficial properties. Furthermore, by boosting the secretion of protease, a digestive enzyme, probiotic *S. cerevisiae* enhances the decomposition of protein components in fish meal, which improves intestinal absorption of these protein components and elevates the protein content of the carcass (Puvanasundram et al. 2021). The present results revealed no significant difference in carcass fat content between juveniles fed diets containing only algae and those fed a combination of algae and yeast. In addition, it was lower in juveniles fed diets containing only yeast. There is an inverse relationship between the moisture and fat content of the carcass in fish species (Yigit et al. 2010). This was also observed in this study in sterlet juveniles fed diets containing different levels of yeast and algae. In fact, the fat content of the carcass increased with decreasing moisture content. The highest protein, fat, and carbohydrate contents were observed in the treatments receiving *Chlorella* or a combination of *Chlorella* and yeast, whereas the highest ash content was observed in those fed different levels of yeast and the control group. The ash content of the carcass indicates the concentration of minerals in organic tissues; minerals are found in the bodies of aquatic organisms in a limited but essential form (Kafilzadeh et al. 2013).

CONCLUSION

The results of this eight-week feeding trial showed that dietary treatments were associated with changes in several hematological indices, immune-related and biochemical parameters, liver enzyme activities, and whole-body composition in juvenile sterlet. The highest mean total protein concentration was observed in fish fed the diet containing 5% *Chlorella*, whereas the highest mean total immunoglobulin, albumin and calcium concentrations were recorded in fish receiving the combined diet containing 3% *Chlorella* and 1% *Saccharomyces cerevisiae*. However, IgM levels did not differ significantly among the treatments. AST and ALT activities increased, whereas ALP activity decreased in the supplemented groups. Therefore, the findings support treatment-related changes in selected physiological parameters but do not, by themselves, establish an overall improvement in fish health.

REFERENCES

- Abdel-Tawwab, M, Abdulrahman, NM, Baiz, AI, Nader, PJ & Al-Refaiie, IH 2022a, The using of *Chlorella pyrenoidosa* and *Daphnia magna* as feed supplements for common carp, *Cyprinus carpio*: growth performance, somatic indices, and hemato-biochemical biomarkers. *Journal of Applied Aquaculture*, 34: 64-78.
- Abdel-Tawwab, M, Mousa, MAA, Mamoon, A, Abdelghany, MF, Abdel-Hamid, EAA, Abdel-Razek, N, Ali, F S, Shady, SHH & Gewida, AGA 2022b, Dietary *Chlorella vulgaris* modulates the performance, antioxidant capacity, innate immunity, and disease resistance capability of Nile tilapia fingerlings fed on plant-based diets. *Animal Feed Science and Technology*, 283: 115181.
- Abdollahpour, H, Falahatkar, B, Efatpanah, I, Meknatkhah, B & Van Der Kraak, G 2018, Influence of thyroxine on spawning performance and larval development of Sterlet sturgeon *Acipenser ruthenus*. *Aquaculture*, 497: 134-139.
- Abdulrahman, NM, Abid, SH, Khidir, AA, Omer, BB, Hama Rasheed, DB & Baha Alddin, LH 2018, Effect of adding microalgae *Chlorella* sp. on some biological parameters and proximate analysis of common carp *Cyprinus carpio* L. *Iranian Journal of Veterinary Medicine*, 12: 199-205.
- Abu-Elala, NM, Younis, NA, AbuBakr, HO, Ragaa, NM, Borges, LL & Bonato, MA 2018, Efficacy of dietary yeast cell wall supplementation on the nutrition and immune response of Nile tilapia. *The Egyptian Journal of Aquatic Research*, 44: 333-341.
- Abu-Elala, NM, Younis, NA, AbuBakr, HO, Ragaa, NM, Borges, LL & Bonato, MA 2020, Influence of dietary fermented *Saccharomyces cerevisiae* on growth performance, oxidative stress parameters, and immune response of cultured *Oreochromis niloticus*. *Fish Physiology and Biochemistry*, 46: 533-545.
- Ahmad, MT, Shariff, M, Md. Yusoff, F, Goh, YM & Banerjee, S 2020, Applications of microalga *Chlorella vulgaris* in aquaculture. *Reviews in Aquaculture*, 12: 328-346.

- Aimanianda, V, Clavaud, C, Simenel, C, Fontaine, T, Delepierre, M & Latgé, J-P 2009, Cell wall β -(1, 6)-glucan of *Saccharomyces cerevisiae*: structural characterization and in situ synthesis. *Journal of Biological Chemistry*, 284: 13401-13412.
- Akbary, P & Gorgij, S 2018, Effect of dietary *Chlorella vulgaris* supplementation on growth performance, feed and body chemical composition of grey mullet, *Mugil cephalus* Linnaeus 1758. *Journal of Animal Environment*, 10: 153-158.
- Alagawany, M, Taha, AE, Noreldin, A, El-Tarabily, KA & Abd El-Hack, ME 2021, Nutritional applications of species of *Spirulina* and *Chlorella* in farmed fish: A review. *Aquaculture*, 542: 736841.
- Alboghobesh, M, Mohammadi, H, Yavari, V & Zakeri, M 2015, Effect of fish meal replacement by soybean meal and baker's yeast on growth performance and feed utilization of juvenile *Mesopotamichthys sharpeyi*. *Journal of Animal Research*, 28: 136-145.
- Aldayel, MF, Al Kuwayti, MA & El Semary, NA 2022, Investigating the production of antimicrobial nanoparticles by *Chlorella vulgaris* and the link to its loss of viability. *Microorganisms*, 10: 145.
- Andrews, SR, Sahu, NP, Pal, AK & Kumar, S 2009, Haematological modulation and growth of *Labeo rohita* fingerlings: effect of dietary mannan oligosaccharide, yeast extract, protein hydrolysate and chlorella. *Aquaculture research*, 41: 61-69.
- Bai, S, Koo, JW, Kim, KW & Kim, SK 2001, Effects of *Chlorella* powder as a feed additive on growth performance in juvenile Korean rockfish, *Sebastes schlegeli* (Hilgendorf). *Aquaculture Research*, 32: 92-98.
- Barton, BA 2002, Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology*, 42: 517-525.
- Center, SA 2007, Interpretation of liver enzymes. *Veterinary Clinics of North America: Small Animal Practice*, 37: 297-333.
- Cerezuela, R, Cuesta, A, Meseguer, J & Ángeles Esteban, M 2008, Effects of inulin on gilthead seabream (*Sparus aurata* L.) innate immune parameters. *Fish and Shellfish Immunology*, 24: 663-668.
- Chebanov, MS & Galich, EV 2013, Sturgeon hatchery manual (FAO Fisheries and Aquaculture Technical Paper No. 558). Food and Agriculture Organization of the United Nations. <https://www.fao.org/4/i2144e/i2144e00.htm>
- Chinnasamy, R, Chinnaperumal, K, Arumugam, P, Natarajan, M, Govindasamy, B, Jogikalmat, K, Cherian, T, Kannupaiyan, J, Sigamani, S & Willie, P 2024, Phyto-fabrication of AgNPs using leaf extract of *Vitex trifolia*: potential to antibacterial, antioxidant, dye degradation, and their evaluation of non-toxicity to *Chlorella vulgaris*. *Biomass Conversion and Biorefinery*. 14: 14903-14920.
- Coronado-Reyes, JA, Salazar-Torres, JA, Juarez-Campos, B & Gonzalez-Hernandez, JC 2022, *Chlorella vulgaris*, a microalgae important to be used in Biotechnology: A review. *Food Science and Technology*, 42: e37320.
- Daliri, MK, Firouzbakhsh, F & Deldar, H 2020, Effect of oral administration of red alga (*Laurencia caspica*) hydroalcoholic extract on growth performance, hematological indices and serum biochemistry in rainbow trout (*Oncorhynchus mykiss*). *Journal of Veterinary Research*, 75: 328-340.
- Darafsh, F, Soltani, M, Abdolhay, HA & Shamsaei Mehrejan, M 2020, Efficacy of dietary supplementation of *Bacillus licheniformis* and *Bacillus subtilis* probiotics and *Saccharomyces cerevisiae* (yeast) on the hematological, immune response, and biochemical features of Persian sturgeon (*Acipenser persicus*) fingerlings. *Iranian Journal of Fisheries Sciences*, 19: 2024-2038.
- Dimitroglou, A, Merrifield, DL, Carnevali, O, Picchiatti, S, Avella, M, Daniels, C, Güroy, D & Davies, SJ 2011, Microbial manipulations to improve fish health and production: A Mediterranean perspective. *Fish and Shellfish Immunology*, 30: 1-16.
- Dineshkumar, R, Narendran, R, Jayasingam, P & Sampathkumar, P 2017, Cultivation and chemical composition of microalgae *Chlorella vulgaris* and its antibacterial activity against human pathogens. *Journal of Aquaculture and Marine Biology*, 5: 65-71.
- El-Nobi, G, Hassanin, M, Khalil, AA, Mohammed, AY, Amer, SA, Montaser, MM & El-Sharnouby, ME 2021, Synbiotic effects of *Saccharomyces cerevisiae*, mannan oligosaccharides, and β -glucan on innate immunity, antioxidant status, and disease resistance of Nile tilapia, *Oreochromis niloticus*. *Antibiotics*, 10: 567.

- Elumalai, P, Kurian, A, Lakshmi, S, Faggio, C, Esteban, MA & Ringø, E 2020, Herbal Immunomodulators in Aquaculture. *Reviews in Fisheries Science and Aquaculture*, 29: 33-57.
- Fails, AD & Magee, C 2018, Anatomy and physiology of farm animals. John Wiley and Sons Publishing. Hoboken, New Jersey, USA. 592P
- Fattahi, AH, Jafarian, H, Khosravi, A & Gholipour Kanani, H 2014, Effects of *Saccharomyces cerevisiae* and *Aspergillus niger* isolated from beluga adult digestive tract on the feeding efficiency and blood serum enzymes of beluga juvenile sturgeon (*Huso huso*). *Journal of Fisheries Science and Technology*, 6:1-13.
- Fazio, F 2019, Fish hematology analysis as an important tool of aquaculture: A review. *Aquaculture*, 500: 237-242.
- Ferguson, RMW, Merrifield, DL, Harper, GM, Rawling, MD, Mustafa, S, Picchiatti, S, Balcázar, JL & Davies, SJ 2010, The effect of *Pediococcus acidilactici* on the gut microbiota and immune status of on-growing red tilapia (*Oreochromis niloticus*). *Journal of Applied Microbiology*, 109: 851-862.
- Filho, DW, Eble, GJ, Kassner, G, Caprario, FX, Dafré, AL & Ohira, M 1992, Comparative hematology in marine fish. *Comparative Biochemistry and Physiology Part A: Physiology*, 102: 311-321.
- Flik, G & Verboost, P 1993, Calcium transport in fish gills and intestine. *Journal of Experimental Biology*, 184: 17-29.
- Flik, G, Verboost, PM & Bonga, SEW 1995, Calcium transport processes in fishes. In: Wood CM & Shuttleworth, TJ (Eds.). *Fish physiology*. Vol. 14. Academic Press. New York, USA. pp. 317–342.
- Gessner, J, Freyhof, J, Kottelat, M & Friedrich, T 2022, *Acipenser ruthenus*. The IUCN red list of threatened species. e.T227A135062526. <https://dx.doi.org/10.2305/IUCN.UK.2022-1.RLTS.T227A135062526.en> Accessed on 12 October 2026.
- Goiris, K, Muylaert, K, Fraeye, I, Foubert, I, De Brabanter, J & De Cooman, L 2012, Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. *Journal of Applied Phycology*, 24: 1477-1486.
- Guerreiro, PM & Fuentes, J 2007, Control of calcium balance in fish. In: B. Baldisserotto, B, da Silva, EMM & Bianchini, OCML (Eds.). *Fish Osmoregulation*. Science Publishers. pp. 381–414.
- Guo, YH, Ma, JK, Xu, Hz, Li, WL, Liu, CJ, Lu, H, Li, H, Zhai, XL, Xue, Y & Luo, L 2023, Effects of different dietary lipid sources (perilla, fish, and soybean oils) on growth, lipid metabolism, antioxidant, and immune status in Chinese giant salamander (*Andrias davidianus*). *Frontiers in Marine Science*, 10: 1139651.
- Helrich, K 1990, Official methods of analysis of the Association of Official Analytical Chemists. Association of Official Analytical Chemists Inc. Arlington, Texas, USA.
- Hertrampf, JW & Piedad-Pascual, F 2012. Handbook on ingredients for aquaculture feeds. Springer Science and Business Media, Netherlands. 573 p.
- Hoseinifar, SH, Mirvaghefi, A & Merrifield, DL 2011, The effects of dietary inactive brewer's yeast *Saccharomyces cerevisiae* var. *ellipsoideus* on the growth, physiological responses and gut microbiota of juvenile beluga (*Huso huso*). *Aquaculture*, 318: 90-94.
- Kafilzadeh, R, Mousavi, SM & Baboli, MJ 2013, Effects of *Saccharomyces cerevisiae* (Saccharomycetes: Saccharomycetaceae) on *Astronotus ocellatus* as growth promoter and immuno stimulant. *Aquaculture, Aquarium, Conservation & Legislation Bioflux*, 6: 587-598.
- Karami, E, Mesbah, M, Molayem raftar, T, Mohammadian, T, Hoseini, SS & Nazari, M 2016, Effects of aqueous extract of *Sargassum angustifolium* (Agardh, 1820) on some of the hematological parameters in Common carp *Cyprinus carpio* (Linnaeus, 1758). *Journal of Aquatic Ecology*, 6: 124-133.
- Khani, M, Soltani, M, Shamsaie Mehrjan, M, Foroudi, F & Ghaeni, M 2017, Short communication: The effects of *Chlorella vulgaris* supplementation on growth performance, blood characteristics, and digestive enzymes in Koi (*Cyprinus carpio*). *Iranian Journal of Fisheries Sciences*, 16: 832-843.
- Kim, KW, Bai, SC, Koo, JW, Wang, X & Kim, SK 2002, Effects of dietary *Chlorella ellipsoidea* supplementation on growth, blood characteristics, and whole-body composition in juvenile japanese flounder *Paralichthys olivaceus*. *Journal of the World Aquaculture Society*, 33: 425-431.
- Klontz, GW 1994, Fish hematology. In: Stolen, JS, Fletcher, TC, Rowley, AF, Kelikoff, TC, Kaatari, SL & Smith SA (Eds.). *Techniques in fish immunology*. SOS Publications. pp. 121-123.

- Kober, AH, Riaz Rajoka, MS, Mehwish, HM, Villena, J & Kitazawa, H 2022, Immunomodulation potential of probiotics: A novel strategy for improving livestock health, immunity, and productivity. *Microorganisms*, 10: 388.
- Kopp, R., Mareš, J, Palíková, M, Navrátil, S, Kubiček, Z, Ziková, A, Hlávková, J & Bláha, L 2009, Biochemical parameters of blood plasma and content of microcystins in tissues of common carp (*Cyprinus carpio* L.) from a hypertrophic pond with cyanobacterial water bloom. *Aquaculture Research*, 40:1683-1693.
- Lin, S, Mai, K & Tan, B 2007, Effects of exogenous enzyme supplementation in diets on growth and feed utilization in tilapia, *Oreochromis niloticus* × *O. aureus*. *Aquaculture Research*, 38: 1645-1653.
- Liu, W, Wang, D, Zhou, Q, Wang, J & Lian, S 2023, Effect of mineral element imbalance on neutrophil respiratory burst function and inflammatory and antioxidant responses in sheep. *Veterinary Sciences*, 10: 241.
- Ma, L, Liu, Z, Zhou, B, Yang, L & Liu, Z 2000, Inhibition of free radical induced oxidative hemolysis of red blood cells by green tea polyphenols. *Chinese Science Bulletin*, 45: 2052-2056.
- Martins, FS, Silva, AA, Vieira, AT, Barbosa, FHF, Arantes, RME, Teixeira, MM & Nicoli, JR 2009, Comparative study of *Bifidobacterium animalis*, *Escherichia coli*, *Lactobacillus casei* and *Saccharomyces boulardii* probiotic properties. *Archives of Microbiology*, 191: 623-630.
- Mohammadi, F, Mousavi, SM, Zakeri, M & Ahmadmoradi, E 2016, Effect of dietary probiotic, *Saccharomyces cerevisiae* on growth performance, survival rate and body biochemical composition of three spot cichlid (*Cichlasoma trimaculatum*). *Aquaculture, Aquarium, Conservation and Legislation*, 9: 451-457.
- Mohammadian, T, Nejad, AJ, Badiei, E, Momeni, H, Tabandeh, M & Khorasani, SPM 2021, Effect of autochthonous probiotics, isolated from fish intestine on biochemical parameters and hepatic enzymes in rainbow trout (*Oncorhynchus mykiss*). *Journal of Veterinary Research*, 76: 22-30.
- Mohammadzadeh, S, Yeganeh, S, Moradian, F, Milla, S & Falahatkar, B 2021, Spawning induction in Sterlet sturgeon (*Acipenser ruthenus*) with recombinant GnRH: Analysis of hormone profiles and spawning indices. *Aquaculture*, 533: 736108.
- Mokhtar, DM, Zacccone, G, Alesci, A, Kuciel, M, Hussein, MT & Sayed, RK 2023, Main components of fish immunity: An overview of the fish immune system. *Fishes*, 8: 93.
- Morowvat, MH, Kazemi, K, Jaber, MA, Amini, A & Gholami, A 2023, Biosynthesis and antimicrobial evaluation of zinc oxide nanoparticles using *Chlorella vulgaris* biomass against multidrug-resistant pathogens. *Materials*, 16: 842.
- Moss, DW & Henderson, AR 1999. Clinical enzymology. In: Burtis, CA and Ashwood, ER (Eds.). Tietz Textbook of Clinical Chemistry. W.B. Saunders Company, Philadelphia, USA. pp. 617-721.
- Müller, T, Ittész, I, Szóke, Z, Hegyi, Á, Mészáros, E, Lefler, K, Bokor, Z, Urbányi, B & Kucska, B 2018, Attempts on artificial induction of sexual maturation of sterlet (*Acipenser ruthenus*) and identification of late spermatogenesis stage in hermaphroditic fish. *International Aquatic Research*, 10: 293-297.
- Natrah, FM, Bossier, P, Sorgeloos, P, Yusoff, FM & Defoirdt, T 2014, Significance of microalgal–bacterial interactions for aquaculture. *Reviews in Aquaculture*, 6: 48-61.
- Onofrejšová, L, Vašíčková, J, Klejdus, B, Stratil, P, Mišurcová, L, Kráčmar, S, Kopecký, J & Vacek, J 2010, Bioactive phenols in algae: The application of pressurized-liquid and solid-phase extraction techniques. *Journal of Pharmaceutical and Biomedical Analysis*, 51: 464-470.
- Paschos, I, Perdikaris, C, Gouva, E & Nathanailides, C 2008, Sturgeons in Greece: a review. *Journal of Applied Ichthyology*, 24: 131-137.
- Pooramini, M, Kamali, A, Hajimoradloo, A, Alizadeh, M & Ghorbani, R 2009, Effect of using yeast (*Saccharomyces cerevisiae*) as probiotic on growth parameters, survival and carcass quality in rainbow trout *Oncorhynchus mykiss* fry. *International Aquatic Research*, 1: 39-44
- Puvanasundram, P, Chong, CM, Sabri, S, Yusoff, MS & Karim, M 2021, Multi-strain probiotics: Functions, effectiveness and formulations for aquaculture applications. *Aquaculture Reports*, 21: 100905.
- Raji, AA, Junaid, QO, Oke, MA, Taufek, NHM, Muin, H, Bakar, NHA, Alias, Z, Milow, P, Simarani, K & Razak, SA 2019, Dietary *Spirulina platensis* and *Chlorella vulgaris* effects on survival and haemato-immunological responses of *Clarias gariepinus* juveniles to *Aeromonas hydrophila* infection. *Aquaculture, Aquarium, Conservation and Legislation*, 12: 1559-1577.

- Reque, VR, de Moraes, JRE, de Andrade Belo, MA & de Moraes, FR 2010, Inflammation induced by inactivated *Aeromonas hydrophila* in Nile tilapia fed diets supplemented with *Saccharomyces cerevisiae*. *Aquaculture*, 300: 37-42.
- Ringoe, E & Birkbeck, T 1999, Intestinal microflora of fish larvae and fry. *Aquaculture Research*, 30: 73-93.
- Saberi, A, Zorriehzahra, MJ, Emadi, H, Kakoolaki, S & Fatemi, SMR 2017, Effects of *Chlorella vulgaris* on blood and immunological parameters of Caspian Sea salmon (*Salmo trutta caspius*) fry exposed to Viral Nervous Necrosis (VNN) virus. *Iranian Journal of Fisheries Sciences*, 16: 494-510.
- Safafar, H, Van Wageningen, J, Møller, P & Jacobsen, C 2015, Carotenoids, phenolic compounds and tocopherols contribute to the antioxidative properties of some microalgae species grown on industrial wastewater. *Marine Drugs*, 13: 7339-7356.
- Safi, C, Zebib, B, Merah, O, Pontalier, PY & Vaca-Garcia, C 2014, Morphology, composition, production, processing and applications of *Chlorella vulgaris*: A review. *Renewable and Sustainable Energy Reviews*, 35: 265-278.
- Sankar, M & Ramasubramanian, V 2012, Biomass production of commercial algae *Chlorella vulgaris* on different culture media. *E-Journal of Life Sciences*, 1: 56-60.
- Sen, S & Mansell, TJ 2020, Yeasts as probiotics: Mechanisms, outcomes, and future potential. *Fungal Genetics and Biology*, 137: 103333.
- Shalaby, MES & El-Nady, MF 2008, Application of *Saccharomyces cerevisiae* as a biocontrol agent against Fusarium infection of sugar beet plants. *Acta Biologica Szegediensis*, 52: 271-275.
- Sheinenzon, A, Shehadeh, M, Michelis, R, Shaoul, E & Ronen, O 2021, Serum albumin levels and inflammation. *International Journal of Biological Macromolecules*, 184: 857-862.
- Sherif, A, EL-Sheekh, M & Abdel-Halim, S 2012, Effect of *Spirulina* algae on the health status and growth performance of Nile Tilapia (*Oreochromis niloticus*) cultured at Kafr El-Sheikh governorate. *Egyptian Journal of Basic and Applied Sciences*, 11: 57-68.
- Song, H, Dong, T, Wang, W, Yan, X, Jiang, B, Xu, S & Hu, H 2024, Whole-genome resequencing of Russian sturgeon (*Acipenser gueldenstaedtii*) reveals selection signatures associated with caviar color. *Aquaculture*, 582: 740545.
- Sun, YZ, Yang, HL, Ma, RL & Lin, WY 2010, Probiotic applications of two dominant gut Bacillus strains with antagonistic activity improved the growth performance and immune responses of grouper *Epinephelus coioides*. *Fish and Shellfish Immunology*, 29: 803-809.
- Sutthi, N & Thaimuangphol, W 2020, Effects of yeast (*Saccharomyces cerevisiae*) on growth performances, body composition and blood chemistry of Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) under different salinity conditions. *Iranian Journal of Fisheries Sciences*, 19: 1428-1446.
- Sutthi, N, Wigraiboon, S & Supakankul, P 2018, Effects of yeast (*Saccharomyces cerevisiae*) supplements on growth performance and blood chemical profiles of Nile tilapia (*Oreochromis niloticus*) at different stocking density. *Burapha Science Journal*: 23: 649-668.
- Taati, R, Soltani, M, Bahmani, M & Zamini, A 2011, Growth performance, carcass composition, and immunophysiological indices in juvenile great sturgeon (*Huso huso*) fed on commercial prebiotic, Immunoster. *Iranian Journal of Fisheries Sciences*, 10: 324-335.
- Tewary, A & Patra, BC 2011, Oral administration of baker's yeast (*Saccharomyces cerevisiae*) acts as a growth promoter and immunomodulator in *Labeo rohita* (Ham.). *Journal of Aquaculture Research and Development*, 2:109.
- Vaugeois, M, Venturelli, PA, Hummel, SL & Forbes, VE 2022, Population modeling to inform management and recovery efforts for lake sturgeon, *Acipenser fulvescens*. *Integrated environmental assessment and management*, 18: 1597-1608.
- Waché, Y, Auffray, F, Gatesoupe, FJ, Zambonino, J, Gayet, V, Labbé, L & Quentel, C 2006, Cross effects of the strain of dietary *Saccharomyces cerevisiae* and rearing conditions on the onset of intestinal microbiota and digestive enzymes in rainbow trout, *Onchorhynchus mykiss* fry. *Aquaculture*, 258: 470-478.
- Wagner, T & Congleton, JL 2004, Blood chemistry correlates of nutritional condition, tissue damage, and stress in migrating juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences*, 61: 1066-1074.

- Wang, S, Zhao, F, Yang, M, Lin, Y & Han, S 2023, Metabolic engineering of *Saccharomyces cerevisiae* for the synthesis of valuable chemicals. *Critical Reviews in Biotechnology*, 44: 163-190
- Wuertz, S, Schroeder, A & Wanka, KM 2021, Probiotics in fish nutrition-long-standing household remedy or native nutraceuticals? *Water*, 13: 1348.
- Xi, L, Lu, Q, Liu, Y, Su, J, Chen, W, Gong, Y, Han, D, Yang, Y, Zhang, Z, Jin, J, Liu, H, Zhu, X & Xie, S 2022, Effects of fish meal replacement with *Chlorella* meal on growth performance, pigmentation, and liver health of largemouth bass (*Micropterus salmoides*). *Animal Nutrition*, 10: 26-40.
- Xu, W, Gao, Z, Qi, Z, Qiu, M, Peng, JQ & Shao, R 2014, Effect of dietary *Chlorella* on the growth performance and physiological parameters of gibel carp, *Carassius auratus gibelio*. *Turkish Journal of Fisheries and Aquatic Sciences*, 14: 53-57.
- Yavuzcan, HY, Bekcan, S, Benli, AK & Akan, M 2005, Some blood parameters in the eel (*Anguilla anguilla*) spontaneously infected with *Aeromonas hydrophila*. *Israel Journal of Veterinary Medicine*, 60: 91-93.
- Yigit, M, Ergün, S, Türker, A, Harmantepe, B & Erteken, A 2010, Evaluation of soybean meal as a protein source and its effect on growth and nitrogen utilization of Black Sea turbot (*Psetta maeotica*) juveniles. *Journal of Marine Science and Technology*, 18: 682-688.
- Yu, H, Ge, X, Huang, D, Xue, C, Ren, M & Liang, H 2023, Dietary supplementation of *Chlorella vulgaris* effectively enhanced the intestinal antioxidant capacity and immune status of *Micropterus salmoides*. *Antioxidants*, 12: 1565.
- Zahran, E, Awadin, W, Risha, E, Khaled, AA & Wang, T 2019, Dietary supplementation of *Chlorella vulgaris* ameliorates chronic sodium arsenite toxicity in Nile tilapia, *Oreochromis niloticus* as revealed by histopathological, biochemical and immune gene expression analysis. *Fisheries Science*, 85: 199-215.
- Zapata, AG 2024, The fish spleen. *Fish and Shellfish Immunology*, 144: 109280.
- Zeraatpisheh, F, Firouzabakhsh, F & Khalili, K J 2018, Effects of the macroalga *Sargassum angustifolium* hot water extract on hematological parameters and immune responses in rainbow trout (*Oncorhynchus mykiss*) infected with *Yersinia ruckeri*. *Journal of Applied Phycology*, 30: 2029-2037.
- Zheng, M, Dai, J, Ji, X, Li, D, He, Y, Wang, M, Huang, J & Chen, B 2021, An integrated semi-continuous culture to treat original swine wastewater and fix carbon dioxide by an indigenous *Chlorella vulgaris* MBFJNU-1 in an outdoor photobioreactor. *Bioresource Technology*, 340: 125703.
- Zimmer, AM & Perry, SF 2022, Physiology and aquaculture: A review of ion and acid-base regulation by the gills of fishes. *Fish and Fisheries*, 23: 874-898.
- Ziółkowska, E, Bogucka, J, Dankowiakowska, A, Rawski, M, Mazurkiewicz, J & Stanek, M 2020, Effects of a trans-galactooligosaccharide on biochemical blood parameters and intestine morphometric parameters of common carp (*Cyprinus carpio* L.). *Animals*, 10: 723.

Bibliographic information of this paper for citing:

Naseri Khalkhali, E, Zamini, A, Vahabzadeh Roudsari, H, Ershad Langeroudi, H, Taati, R 2026, Effects of dietary supplementation with *Saccharomyces cerevisiae* and *Chlorella vulgaris* on body composition, hematological, immune, and biochemical responses of sterlet, *Acipenser ruthenus*, Caspian Journal of Environmental Sciences, 24: 1101-1114.
