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Research Article

Arthrospira platensis supplementation: A nutritional boost for enhancing survival and hemato-biochemical indices of Persian Sturgeon (*Acipenser persicus*) larvae

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Abstract

The main obstacles to the artificial reproduction of sturgeon can be mentioned the long-term viability of sturgeon aquaculture and the significant mortality rate of fingerlings. Microalgae supplementation has been shown in numerous studies to have a positive effect on improving overall health and survival rates in a variety of fish species larvae. This study combines the findings of other studies with the results of experimental trials to try and elucidate the mechanisms underlying the beneficial benefits of *Arthrospira platensis* on Persian sturgeon fingerlings. It also seeks to provide helpful information on the optimal dosage and administration methods for *A. platensis* supplementation in sturgeon hatcheries. During a 12-week feeding trial, 600 *A. persicus* larvae (200.50± 0.07 g), were used in this study to evaluate the effects of dietary supplementation of *A. platensis* at different concentrations (2.5%, 5%, and 7.5%, compared to a 0% control) on the growth, survival, and hemo-biochemical indices. The 7.5% *A. platensis*-fed group showed significantly higher values of white blood cells, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. The 7.5% *S. platensis*-fed group also showed a significant increase in hemoglobin levels, red blood cells, hematocrit, and mean corpuscular volume values. Regarding blood biochemical indices, a significant decrease was observed in cholesterol, triglyceride, low-density lipoprotein, lactate dehydrogenase, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase levels. In addition, compared to other groups, *A. platensis* at 7.5% resulted in greater serum glucose and HDL levels. In conclusion, Supplementing with *A. platensis* in a concentration of 7.5% of the diet improves the general health and stress tolerance of Persian sturgeon fingerlings, as well as their survival rates during artificial reproduction.

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Introduction

Sturgeons, considered keystone species, are crucial for maintaining the health and stability of the Caspian Sea ecosystem (Haxton and Friday, 2019). Among them, the Persian Sturgeon (*Acipenser persicus*) is an important species inhabiting the Caspian Sea and the connecting rivers, predominantly found in the southern basin of the Caspian Sea. However, sturgeon populations face habitat degradation and loss due to factors like dam construction and pollution, threatening their survival (Assylbekova *et al.*, 2022). In Iran, conservation efforts for sturgeons primarily involve artificial propagation and releasing larvae into natural waters (Akhoundian *et al.*, 2020). The traditional protocol for artificial propagation involves collecting live broodstock before spawning and releasing the larvae into the sea after hatching, aiming to replenish natural resources. A critical challenge in sturgeon aquaculture, particularly during artificial breeding, is ensuring the proper nutrition and optimal feeding practices for larvae survival and healthy growth. Sturgeon larvae have specific nutritional requirements, including high-quality protein, lipids, vitamins, and minerals, essential for their development. However, transitioning sturgeon larvae from yolk sac absorption to external feeding, a critical growth stage, poses challenges, including their reluctance to feed on dry food, leading to high losses in breeding farms. While fish meal has been a traditional protein source in aquaculture, its drawbacks, such as cost fluctuations, reliance on wild-caught fish, and environmental concerns, necessitate the search for alternatives (Jannathulla *et al.*, 2019). *Arthrospira* (*Spirulina*) *platensis*, a type of blue-green algae, emerges as a promising alternative protein source for feeding fish larvae. Its protein content is comparable to or higher than fish meal, providing a balanced profile of essential amino acids, vitamins (including B vitamins), and minerals (such as iron and zinc), crucial for fish growth and health (Akhoundian and Mirhasannia, 2017; Al-Deriny *et al.*, 2020; Zhang *et al.*, 2020). Research indicates that dietary supplementation with Spirulina can significantly improve growth performance and immune responses in various aquatic species (Shao *et al*., 2019; Muahiddah and Affandi, 2023), potentially improving disease resistance (Abdel-Latif *et al.*, 2022). While specific studies on Persian Sturgeon larvae are limited, existing research provides insights into the potential benefits of Spirulina supplementation for sturgeon species. The effects of different dietary formulations, including live foods like Artemia, have been examined in Persian Sturgeon. These studies show that the nutritional composition of live feeds significantly impacts growth and survival rates, indicating that similar benefits could be expected from Spirulina supplementation due to its rich nutrient profile (Simeanu *et al*., 2015) . While direct studies on Persian Sturgeon are still needed, the existing literature suggests that incorporating Spirulina into larval diets could lead to improved growth performance, enhanced immune responses, and overall better health outcomes for sturgeon species in aquaculture practices (Ahsan *et al*., 2008; Ali *et al*., 2023; Saravanan *et al*., 2023). This study aims to

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investigate the potential of *A. platensis* as an alternative food supplement to fish meal for Persian Sturgeon larvae. By studying its effects on the larvae diet, the research intends to improve the overall health status and feeding success of Persian Sturgeon larvae in artificial breeding settings. Further research is necessary to refine feeding protocols and enhance larvae rearing success.

Materials and methods

Ethics statement

All of the experimental procedures involving animals were conducted in accordance with the Guideline for the Care and Use of Laboratory Animals in Iran (Ahmadi-Noorbakhsh *et al*., 2021).

Diet and fish rearing

Six hundred *A. persicus* with an average initial weight of 200.50±0.07 mg were randomly divided into 4 groups, each group with 150 fishes (50 fishes per tank, 3 repetitions). Fish were acclimatized for 2 weeks in a fiberglass tank (2000 L) to adapt and adjust to the laboratory setting. *A. platensis* pure dried powder, was obtained from the Iranian National Algae Culture Collection (INACC) and was mixed with the basal diet to get four experimental diets: Basal diet+0% (Control group), 2.5%, 5%, and 7.5% of *A. platensis*. Dried pellets of the diets were kept at 4˚C until used. Fish were fed (3% of body weight) (Table 1).

The fish were fed on the test diets three times per day until they were satisfied, at intervals of four hours for a total of 90 days. Throughout the experiment period, physicochemical indices of water were measured daily on the site and the details are mentioned in Table 2.

Table1: Dietary formulation and proximate composition of basal diet of *Acipenser persicus*.

Ingredients	Composition (%)
Fish oil	5.6
Soybean oil	5.5
Kilka fish meal	56
Hydrothermic processed wheat	21
Cellulose	2.5
Mineral premix [a]	2
Vitamin premix [b]	3.5
Binder	2
Antifungi [c]	0.4
Antioxidant	0.35
Proximate composition (%)	
Crude protein	41.36
Crude lipid	21.7
Ash	10.1
Moisture	9.2
Fiber	1.9
Energy level of the diet	431.3 kcal/100g.

[a] Mineral mixture (g100 g -1 mineral mix): Fe, 2.5; Mg, 3; K, 3.5; Mn, 1.7; Co, 0.05; Cu, 0.5; Se, 0.25; I, 0.1

[b] Vitamin mixture (g100 g-1 vitamin mix except vitamins A and D3): vitamin A, 180 000 IU; vitamin D3, 60000 IU; vitamin E, 4; vitamin C, 6; K3, 0.3; thiamine, 0.8, riboflavin, 0.6; niacin, 2; pyridoxine, 0.4; pantothenic acid, 3.5; biotin, 0.5; folic acid, 0.3; cyanocobalamin, 0.8; inositol, 2.

c ToxiBan antifungal (Vet-A-Mix, Shenan-doah, IA).

Table 2: Chemical and physical parameters of water entrance (Mean±SD).

Parameter	Mean Value± SD
NH_3(mg L^{-1})	0.21 ± 0.21
NO_{2-} (mg L^{-1})	0.08 ± 0.04
Fe (mg L^{-1})	0.16 ± 0.05
SO_2 (mg L^{-1})	0.01 ± 0.01
$P3O_4$ (mg L^{-1})	0.03 ± 0.01
Total hardness	238.33 ± 15.28
pH	8.10 ± 0.10
Temperature (°C)	19.56 ± 1.03
Dissolved oxygen (mg L^{-1})	11.73 ± 0.35

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Sample collection

All fish were deprived of food for 24 hours before weighing and sampling. 90 fish per treatment (30 fish per tank) were randomly sampled at the end of the feeding trial (12 weeks). Blood samples were taken from the caudal vein and immediately divided into two half parts. Heparinized tubes were used to keep half blood samples for hematological analysis and non-heparinized tubes were used to keep other half blood sample for biochemical studies. The serum samples were separated from the blood using centrifugation at 3000 rpm for 10 min at 4˚C and stored at -80˚C until use (Adel *et al.*, 2015).

Growth performance and survival rate

Following anesthesia with clove oil (50 mg L^{-1}), sampled fishes weight (with an accuracy of 10^{-4} g, CAS model ME-410) and length (with an accuracy of 10^{-2} Cm) were recorded and the following parameters were measured (Takeuchi *et al.*, 2002):

Survival (%) (final fish number/initial fish number)×100

Specific growth rate (%per day) =100×(Ln final weight (g) - Ln initial weight (g))/time (days)

Condition factor (g cm^{-3})=100×(fish body weight)/(fish body length)^3

Hematological and plasma biochemical indices

The red blood cells (RBC) and white blood cells (WBC) were manually enumerated after being diluted in Natt- Herrick solution with a Neuberger hemocytometer (100), hematocrit (Hct), and hemoglobin (Hb) were determined by the standard microhematocrit method and expressed as percentages were calculated (Adel *et al.*, 2016, Hesser, 1960):

Mean corpuscular volume (MCV (fL)=10×(Hct/RBC)),

Mean corpuscular hemoglobin (MCH (pg $cell^{-1}$)=10×(Hb/RBC))

Mean corpuscular hemoglobin concentration (MCHC (%)=100×(Hb/Hct)) (Chung *et al.*, 2021).

Glucose, Triglyceride, Cholesterol, High-density lipoprotein (HDL), Low-density lipoprotein (LDL), Lactate Dehydrogenase (LDH), Aspartate Aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP) and Phosphate, were analyzed using commercial assay kits (Pars Azmoon, Iran), according to the manufacturer instructions.

Statistical analysis

All the tests were performed in triplicate. The data were subjected to statistical analysis using the SPSS software version 26 (SPSS Inc., Chicago, IL, USA). All data were initially checked for variance, normality, and homogeneity using Levine's test. Then, to compare the mean values across the experimental groups, a one-way ANOVA was used with post-hoc Duncan's

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multiple range tests. P value 0.05 was regarded as statistically significant.

Results

Survival rate

The survival rates of fish fed various experimental diets were monitored over 12 weeks (Fig. 1). Initially, the group fed a diet enriched with 2.5% *A. platensis* exhibited the highest survival rate (70.66%) after 4 weeks. However, by the end of 8 and 12 weeks, the group fed 7.5% *A. platensis* demonstrated the highest survival rates (97.87% and 97.93%, respectively). In contrast, the control group consistently exhibited the lowest survival rates across all three sampling intervals (4, 8, and 12 weeks).

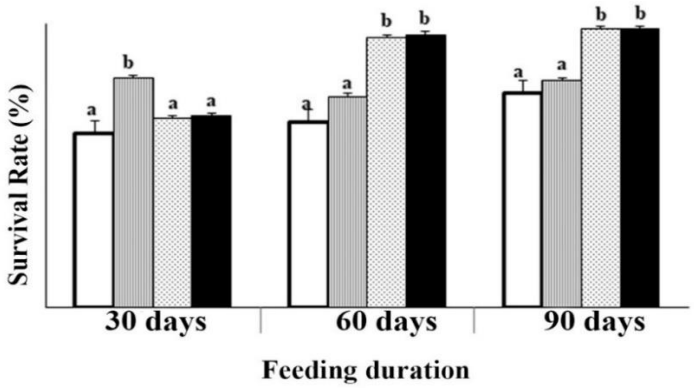


Figure 1: Survival rate of *Acipenser persicus* fingerlings fed different times (30, 60, and 90 days) of feeding fed with different levels (Control (0%), 2.5%, 5%, and 7.5%) of *Arthrospira platensis*. N=150 fish for each group.

Growth performance

No statistically significant different were observed in condition factor and specific growth rate between the different treatments fed with *A. platensis* and the control group (Table 3).

Table 3: Growth performances of Persian sturgeon (*Acipenser persicus*) larvae fed with different doses (0% (Control), 2.5%, 5%, and 7.5%) of *Arthrospira platensis* for 90 days.

Parameters	Treatments			
	Control	2.5%	5%	7.5%
Initial weight (g)	0.199 ± 0.08^a	0.202 ± 0.064^a	0.199 ± 0.076^a	0.200 ± 0.082^a
Final weight (g)	91.15 ± 29.42^a	79.85 ± 19.57^a	75.2 ± 28.23^a	81.1 ± 29.32^a
Condition factor (g cm^{-3})	0.50 ± 0.05^a	0.51 ± 0.05^a	0.48 ± 0.12^a	0.50 ± 0.11^a
Specific Growth rate (%per day)	6.64 ± 0.40^a	6.61 ± 0.28^a	6.51 ± 0.45^a	6.60 ± 0.41^a

Note: Values are means ± SD of three replications. Derived from 30 fish for each group. Means in the same raw with different superscripts are significantly different by Tukey's Test (p<0.05).

Hematological indices

The results of Table 4 indicate that *A. platensis* could make significant change in some hematological indices of *A. persicus* fingerlings. Significant higher values of WBC, MCH, and MCHC were recorded in 7.5 % ($2.68\pm0.22\times105$ mL^{-1}), (12.78 ± 1.71 pg $cell^{-1}$), and ($17.75\pm2.45\%$) respectively, compared to the control (Table 4). The mean level of Hb was also increased in a

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statistically significant manner on the fish fed diets enriched with 7.5% of *A. platensis* (4.09±0.48 g 100 mL^{-1}).

Plasma biochemical indices

Table 5 depicts the significant variation in the values of glucose, triglyceride, cholesterol, HDL, LDL, and phosphate in fingerlings from the 7.5% group in comparison to the control.

Table 4: Hematological indices of *Acipenser persicus* fingerlings fed with different doses (0% (Control), 2.5%, 5%, and 7.5%) of *Arthrospira platensis* for 90 days.

Parameters	Treatments			
	Control	2.5%	5%	7.5%
RBC (105mL^{-1})	3.22 ± 0.60^{a}	3.10 ± 0.51^{a}	3.23 ± 0.47^{a}	3.32 ± 0.30^{a}
WBC (104 mL^{-1})	1.95 ± 0.54^{c}	2.11 ± 0.52^{b}	2.38 ± 0.44^{ab}	2.68 ± 0.22^{a}
Hb (g 100mL^{-1})	2.49 ± 0.23^{d}	2.86 ± 0.48^{c}	3.27 ± 0.45^{b}	4.09 ± 0.48^{a}
Hct (%)	22.37± 2.06^{a}	22.55 ± 1.66^{a}	22.76 ± 2.16^{a}	23.37 ± 3.60^{a}
MCV (fL)	716.53 ± 137.20^{a}	727.76 ± 129.52^{a}	721.60 ± 134.58^{a}	725.75 ± 95.59^{a}
MCH (pg $cell^{-1}$)	8.04 ± 1.85^{d}	9.39 ± 1.81^{c}	10.61 ± 1.75^{b}	12.78 ± 1.71^{a}
MCHC (%)	11.21 ± 1.35^{d}	13.07 ± 2.39^{c}	14.88 ± 1.88^{b}	17.75 ± 2.45^{a}

Note: Means in the same raw with different superscripts are significantly different by Tukey's Test (p<0.05). RBC: red blood cell; WBC: white blood cell; Hb: hemoglobin; Hct: hematocrit; MCV: mean corpuscular volume (erythrocytes); MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

Table 5: Plasma biochemical indices variables of *Acipenser persicus* fingerlings fed with different doses (control (0%), 2.5%, 5%, and 7.5%) of *Arthrospira platensis* for 90 days.

Parameters	Treatments			
	Control	2.5%	5%	7.5%
Glucose (mg dL^{-1})	25.27 ± 2.83^{d}	30.03 ± 3.88^{c}	33.43 ± 3.17^{b}	45.07 ± 3.41^{a}
Triglyceride (mmol L^{-1})	548.07 ± 7.88^{a}	545.20 ± 7.48^{a}	533.93 ± 8.56^{b}	504.17 ± 14.96^{c}
Cholesterol (mmol L^{-1})	149.83 ± 8.59^{a}	131.77 ± 8.32^{b}	106.47 ± 7.87^{c}	98.47 ± 8.46^{d}
HDL (mmol L^{-1})	15.30 ± 2.07^{c}	20.83 ± 1.97^{b}	24.20 ± 3.54^{a}	24.93 ± 4.14^{a}
LDL (mmol L^{-1})	47.60 ± 3.64^{a}	42.27 ± 4.45^{b}	35.33 ± 4.57^{c}	32.33 ± 4.51^{d}
LDH (U L^{-1})	1637.80 ± 307.94^{a}	1562.50 ± 249.55^{a}	1504.30 ± 40.78^{a}	1499.33 ± 358.62^{a}
AST (U L^{-1})	519.17 ± 47.29^{a}	468.03 ± 39.64^{b}	454.20 ± 71.64^{b}	464.27 ± 35.02^{b}
ALT (U L^{-1})	22.80 ± 5.70^{a}	19.60 ± 5.18^{ab}	18.73 ± 4.76^{b}	17.37 ± 3.97^{b}
ALP (U L^{-1})	652.63 ± 47.71^{a}	659.37 ± 77.29^{a}	531.13 ± 81.37^{b}	536.17 ± 94.19^{b}
Phosphate (mg dL^{-1})	14.54 ± 1.62^{a}	14.80 ± 0.80^{a}	14.89 ± 0.95^{a}	16.41 ± 1.86^{b}

Note: Means in the same raw with different superscripts are significantly different by Tukey's Test (p<0.05). HDL: High-density lipoprotein; LDL: Low-density lipoprotein; LDH: Lactate Dehydrogenase; AST: Aspartate Aminotransferase (GOT); ALT: Alanine Amino Transferase (GPT); ALP: Alkaline phosphatase.

Although, the blood serum level of Triglyceride, Cholesterol, and HDL decreased significantly in all fish groups fed *A. platensis* enriched diets, in comparison to the control group; However the lowest amounts were observed increments statistically significant in those fish fed 7.5% *A. platensis* enriched diet (504.17±14.96, 98.47±8.46 and 24.13±4.14 mmol L^{-1}, respectively). The lowest amounts of ALT and ALP (17.37±3.97 and 536.17±94.19 U L^{-1}) were recorded for 7.5% treatment.

Discussion

After 30 days, the fish fed with 2.5% Spirulina platensis demonstrated a higher survival rate compared to those in the 5%

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and 7.5% treatments. This initial higher survival rate could be attributed to the optimal balance between nutritional benefits and the digestive capacity of the fish at this stage. Lower concentrations of Spirulina might have provided an adequate boost to the immune system and overall health without overwhelming the digestive system, which can sometimes occur at higher supplementation levels (Zidan *et al*., 2021). However, as the treatment progressed into the second month, the survival rates for the 5% and 7.5% groups surpassed those of the 2.5% and control groups. This improvement is likely due to the increased weight and overall growth of the fingerlings, which may have enabled them to better utilize the higher Spirulina content in their diet. As fish grow, their digestive systems and metabolic capabilities typically enhance, allowing them to handle and benefit from higher concentrations of dietary supplements like Spirulina (Tan *et al*., 2017). The improved survival and growth performance in the higher Spirulina groups over time could also be linked to the cumulative effects of Spirulina's rich nutritional profile, including its high protein content, essential fatty acids, vitamins, and antioxidants. These nutrients likely contributed to enhanced physiological resilience and a stronger immune response, which became more pronounced with continued supplementation. Similar outcomes have been documented in other fish species, such as *Labeo rohita* (Bhujel *et al*., 2020), *Trichopodus trichopters* (Khanzadeh *et al*., 2016), and Tilapia (*Oreochromis niloticus*) (Takeuchi *et al*., 2002), where varying concentrations of *A. platensis* led to different growth and survival outcomes, largely dependent on the species' growth stage and ability to metabolize and utilize the supplement effectively.

The consumption of microalgae in the diet had no effect on the growth of the fish fry in *A. persicus*, as shown by the lack of significant differences in the growth parameters between the control group and the groups fed *A. platensis* (Table 3). A prior study, which found that *A. platensis* had no influence on *A. transmontanus* growth, also revealed comparable results (Palmegiano *et al.*, 2008). The disparities in fish development performance, the variations in the meals' chemical makeup, and the variations in the nutritional value of the *Spirulina* sp. utilized can all contribute to these disparate outcomes. Additionally, the results are impacted by variations in fish size and age as well as by environmental factors (Kim *et al.*, 2013; Takeuchi *et al*., 2002).

Fish hematological parameters are dependable markers of overall health status, and can be crucial in the identification of hematological disorders, infectious illnesses, and aquatic poisonings (Soliman and Badeaa, 2002). By analyzing blood indices, it is also possible to verify the improvement of aquatic physiological indices resulting from a different diet. WBC analysis is a crucial sign of the health and immune system of fish since WBC is one of the body's first lines of defense against infectious diseases brought on by pathogens and chemical agents (Telli *et al.*, 2014). The current study found that dietary *A. platensis* had significant effects on the WBC value of *A. persicus*. Fish fed 7.5% *A. platensis* showed the largest increments in

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the dose-dependent rise that was seen (Table 4). Similar to our results, varied dietary doses of *A. platensis* significantly increased the WBC count in *A. stellatus* (Salehi-Farsani *et al.*, 2016), *Cyprinus carpio* and *Cyprinus rubrofuscu*s (Marino *et al.*, 2016) and *A. baerii* (Falahatkar, 2018). Consistent with our results, supplementing with *S. platensis*, increased hemoglobin in *A. ocellatus* (Mohammadiazarm *et al.*, 2021), *Oplegnathus fasciatus* (Kim *et al.*, 2013), *Oncorhynchus mykiss* (Yeganeh *et al.*, 2015), and *Plectropomus leopard* (Yu *et al.*, 2018) in comparison to the control group.

The fish fed *A. platensis* diets have higher WBC and Hb levels, which are linked to the presence of a phycocyanin polypeptide. In addition to enhancing the immune system and increasing blood hemoglobin, phycocyanin influences bone marrow stem cells (Tan *et al.*, 2017; Mohammadiazarm *et al.*, 2021). Moreover, phycocyanin has a high iron content, and by raising the level of Hb, it can benefit RBC health and fish health (Talpur *et al.*, 2013). The presence of vitamins in *A. platensis* has acted as an immune-stimulant in fish and it led to the increase of WBCs in cultured fish species to create a quick recovery of its body against some stress factors (Amira *et al.*, 2021).

A rise in plasma glucose is typically linked to an increase in cortisol levels (Malini *et al.*, 2018). To give fish the energy they need to react to stressors, cortisol releases energy reserves, including glucose. As a result, glucose is a quick energy source that stimulates the body's muscles and heart. Consequently, the increased plasma glucose level in fish fed a diet containing 7.5% *A. platensis* in the current study shows that, this group's stress-management system is functioning, which can help them cope with stressful situations more successfully. Our current findings concur with those that Adel *et al.* (2015) and Alofa *et al.* (2020) previously published.

The consumption of *A. platensis* in the fish diet had a significant impact on the plasma levels of cholesterol and triglycerides in Persian sturgeon in the current study, which is consistent with Adel *et al.* (2016) and Yu *et al.* (2018) findings. *A. platensis* supplementation in fish has the potential to reduce cholesterol and triglyceride plasma levels because of its composition, which includes α-linolenic acid, linoleic acid, and β-carotene. These compounds lower cholesterol and triglyceride levels and prevent hypercholesterolemia and atherosclerosis (Xiang *et al.*, 2002). The results also indicated that these effects are dose-dependent, in line with previous research on the subject (Yeganeh *et al.*, 2015; Yu *et al.*, 2018; Hagiaghaei Ghaazi Mahalleh and Imanpour Namin, 2023; Rahman *et al.*, 2023).

The HDL level of fish treated with *A. platensis* significantly increased (Table 5), but the LDL plasma level significantly dropped in treatment groups when compared to control fish. There have also been reports of a similarly notable impact of *A. platensis* on HDL and LDL plasma levels in *Oncorhynchus mykiss* (Yeganeh *et al.*, 2015), *Huso huso* (Adel *et al.*, 2016), and *Labeo rohita* (Paramanik *et al.*, 2023). To support cellular processes, low-density lipoprotein (LDL) carries cholesterol from

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the liver to peripheral tissues. High-density lipoprotein (HDL) in fish is considered "good cholesterol" because it aids in reverse cholesterol transport, which helps cells get rid of excess cholesterol and improves cardiovascular health. Achieving optimal cholesterol metabolism and lowering the risk of atherosclerosis necessitates keeping LDL and HDL in balance (Chen *et al.*, 2003).

The fish health status was found to be positively affected by the consumption of *A. platensis* in the diet, particularly at doses of 5% and 7.5%, as evidenced by the simultaneous reduction of both ALT and AST values, which are liver enzymes and are referred to as general health indicators. *A. platensis* may have detoxifying properties, according to certain studies, which may facilitate the liver's more effective processing and removal of toxins. Lower ALT and AST levels could be a result of this detoxification process (Carneiro *et al.*, 2022). Previous studies have also indicated that *A. platensis* possesses bioactive compounds that possess anti-inflammatory and antioxidant characteristics. These characteristics might help shield liver cells from harm, diminish inflammation, and eventually result in decreased ALT and AST levels (El-Araby *et al.*, 2022). As with ALT, a drop in ALP levels may indicate better liver function. Elevated ALP can also be linked to other tissues, such as the intestines and bone, and is not as specific to the liver as ALT (Siddik *et al.*, 2022). Analogous findings have been documented regarding the impact of a diet consisting of *spirulina* sp. on the decrease of liver enzyme activity in *A. ocellatus* (Mohammadiazarm *et al.*, 2021) and Nile tilapia (Shalata *et al.*, 2021). While the assessment of liver damage cannot be accurately determined by measuring ALT, AST, or ALP, a decrease in their activity does signify an improvement in the overall state of health and an increase in fish resistance to pathogenic factors and stress (Gopalraaj *et al.*, 2023). Proper nutrient balance, including phosphorus, supports the development and function of immune cells, helping fish resist diseases and infections. Also, plasma phosphorus level has a positive correlation with adenosine triphosphate concentration in plasma and muscle. In addition, since several enzymatic processes in cells are induced by phosphorus, a drop in plasma phosphorus concentration has been reported to cause disturbances in energy-mediated metabolism in fish fed with insufficient phosphorus diets. Although plants have high amounts of phosphorus in the form of phytate, it is unusable for fish because they lack phytase enzyme to digest it, while *A. platensis* as a microalgae, have various enzymes including cellulases, galactosidases, proteases, lipases, phytases, laccases, amylases, antioxidant enzymes and enzymes related to the accumulation of carbohydrates and carbon concentration (Brasil *et al.*, 2017). Similar results have been observed in other fish species fed diets containing *S. platensis*, including Silver perch (Yang *et al.*, 2006), *Sparus aurata* (Pereira *et al.*, 2020), *Labidochromis*, and *Chelon labrosus* (Garcia-Marquez *et al.*, 2022).

Conclusions

In conclusion, *A. platensis* supplementation enhanced the health, survival, and

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immunological response of A. persicus, with the 5% and 7.5% doses demonstrating the greatest advantages. Although the 2.5% dose produced higher survival rates at first, as the fish grew acclimated to higher supplementation, the 5% and 7.5% groups eventually outperformed it in terms of growth and health outcomes. Overall, the results suggest that the 5% dose is the best compromise between immunological stimulation and digestive capability; however, for larger or more mature fish, the 7.5% dose may have longer-term advantages. Further research should investigate mechanisms for potential commercial implementation in sturgeon culture.

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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