Iranian Journal of Fisheries Sciences
http:doi.org/ 10.22092/ijfs.2025.133418 2025, 24(3) 527-537

Research Article

Dietary supplementation of savory (*Satureja hortensis* L) extract improves the growth performance, hematological, and immune parameters of stellate sturgeon (*Acipenser stellatus*)

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Keywords

Immunity,
Starry sturgeon,
Acipenser stellatus,
Herbal extract,
Growth performance

Article info

Received: April 2024
Accepted: June 2024
Published: May 2025

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Abstract

The study investigated the effect of dietary supplementation with savory (*Satureja hortensis*) extract (SE) on stellate sturgeon (*Acipenser stellatus*) growth and health status. The fish with an initial body weight of 68.94±2.8 g were allocated randomly into 9 tanks, with a stocking density of 5 fish per tank and were fed with an un-supplemented diet (control, SE0), 1% SE (SE1), and 2% SE (SE2) for 8 weeks in freshwater. After 8 weeks, the feeding was stopped for 24 h and the blood samples were collected to measure immune parameters. The results showed that SE2 exhibited significantly higher values for final weight (164.67 g), specific growth rate (1.55 %/day), and weight gain (95.90 g) as compared to the SE0 (p<0.05). No statistically significant effects were noted among the experimental treatments in terms of the survival rate (p>0.05). The experimental diets significantly affect hematocrit and the highest level was observed in SE2. Total immunoglobulin, lysozyme, and ACH50 levels were significantly higher in the supplemented diets (p<0.05). The present study demonstrates that the administration of savory extract can improve the growth and immunity in stellate sturgeon.

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Introduction

The stellate sturgeon or starry sturgeon belongs to the Acipenseridae family and it shows significant promise in the field of aquaculture. The culture of sturgeon has recently advanced into a flourishing industry due to the high value associated with eggs (caviar) and meat. The practice of intensive culture subjects the fish to various stressors, such as high stock densities and manipulations. It is crucial to develop techniques that can enhance the immune system and prevent infectious diseases (Pourgholam *et al.*, 2016).

For an extended period, antibiotics have been commonly utilized within the aquaculture sector not solely for the prevention and/or management of infectious diseases induced by bacterial pathogens but also as agents to promote growth (Dawood *et al.*, 2018). The public's consciousness regarding the preventive application of antibiotics in aquaculture, which carries the potential for the transfer of pathogenic bacterial strains to humans, has led to their prohibition in aquaculture (Dawood *et al.*, 2015). At present, the primary focus in livestock production lies in nutritional tactics that center around the utilization of phytochemicals to regulate immune and physiological reactions, mitigating a range of stresses, with a particular emphasis on oxidative stress, and enhancing gastrointestinal well-being (Ghafarifarsani *et al.*, 2022). Medicinal plants are abundant in a diverse array of nutrients and have potential applications as chemotherapeutic agents and supplements in animal diets (Ahmad *et al.*, 2011). Savory (*Satureja hortensis* L) is a renowned aromatic and medicinal herb utilized in traditional medicine for the management of a variety of ailments including spasms, muscle aches, nausea, digestive issues, and infectious conditions (Sahin *et al.*, 2003). The extracts and essential oils derived from this botanical species exhibit antioxidant properties, as well as demonstrate antibacterial effects (Sahin *et al.*, 2003) and antifungal capabilities (Mihajilov-Krstev *et al.*, 2009).

The utilization of plant compounds in aquaculture has been documented in numerous research investigations, some of which have demonstrated that unaltered plant materials/extracts enhance the performance and well-being of fish (Hoseinifar *et al.*, 2017; Baba *et al.*, 2018; Paray *et al.*, 2020; Adeniyi *et al.*, 2023). In addition, dietary herbal extract is proven to result in high antioxidant and immune responses in Beluga (Eslami *et al.*, 2022), common carp, *Cyprinus carpio* (Ahmadifar *et al.*, 2022), Siberian sturgeon, *Acpenser baerii* (Ramzani *et al.*, 2021), and sterlet sturgeon, *A. ruthenus* (Lee *et al.*, 2012).

Considering the significance of eco-friendly aquaculture, the avoidance of antibiotics for treating bacterial diseases, and the challenges associated with vaccinating aquatic organisms, the exploration of natural compounds has garnered particular attention. Due to the limited available data on the impacts of savory plant extract on various fish species, this research endeavors to examine the effects of the savory extract on growth efficiency, hematological and immune

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parameters of stellate sturgeon (*A. stellatus*).

Materials and methods

Preparation of savory ethanolic extract and experimental diets

300 g of the dried plant collected was powdered with a grinder and stirred in 3000 ml of ethanol on a shaker incubator at 40 °C for 48 h. The filtrate was filtered through a paper (0.45 µm) under vacuum pressure, whereas the remaining ethanol in the filtrate was separated using a rotary evaporator running at 45°C. Following this, the resulting filtrate was subjected to pulverization using an electric mill for incorporation into the diet formulation. For the formulation of experimental diets, different levels of ethanolic extract derived from savory (1% and 2%) were integrated into a foundational diet (Table 1) in powder form, and mixed uniformly. After the incorporation of water to create a mixture, the amalgamation underwent another round of pelleting using a meat grinder, after which it was subjected to air drying, grinding, and sieving to produce a fitting crumble. The diets were stored in plastic containers at a temperature of 4°C until they were used.

Fish and culture conditions

The current investigation was conducted at the Qareburoon Sturgeon Culture and Propagation Center in Sari, Iran. Initially, a total of 45 stellate sturgeon juveniles weighing 68.94±2.8 g were placed randomly in 9 tanks of 500 L (filled with 400 L of freshwater) at a stocking density of 5 fish per tank. Subsequently, they underwent a two-week acclimation period, during which they were provided with a basal control diet. Following the acclimation phase, nine circular concrete tanks were randomly distributed among three experimental treatments, each with three replications. The fish were fed diets that included 0% savory extract (SE0), 1% (SE1), and 2% (SE2) ethanolic extract derived from savory. In the present study, a foundational control diet (Table 1) was developed utilizing scientific data to fulfill the nutritional needs of the great sturgeon (Matani Bour *et al.*, 2018; Mirzakhani *et al.*, 2018), comprising 460 g/kg of protein and 196 g/kg of fat. The quantified quantities of dietary components (Caspian Yaqoot Talaei, Eslami, Joybar, Iran) were agitated by mechanical means using an electric stirrer (Pars Khazar, Tehran, Iran) for a duration of 30 minutes. SE was incorporated into the experimental diets at varying concentrations of 0, 1, and 2%. The formulated diets were then processed through an electric meat grinder to form pellets, which were subsequently fragmented to a diameter of 3 mm. During the trial, fish were provided with experimental diets multiple times a day for 8 weeks until they reached a state of apparent satiety at specific time intervals (8:00, 12:00, and 16:00 h). The aeration of each tank was achieved through the utilization of air stones. Daily maintenance of each tank included cleaning, as well as the replacement of 30-40% of the water volume with freshwater before the commencement of the first feeding session in the morning. The freshwater quality parameters such as temperature (21.82±0.6°C), dissolved oxygen (7.8±0.5 mg/L), and pH (7.3±0.2) were meticulously monitored throughout the course of the

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experiment. The temperature was measured by a mercury thermometer (Zomorodazma Company, Iran), dissolved oxygen by Cyberscan Eutech instruments (DO 110, Singapore), and pH (Hanna instrument, 8314, USA).

Table 1: Ingredients and proximate composition (% on dry matter basis) of the basal control diet.

Ingredient	(g/kg diet)
Fish meal (72% protein)	400
Soybean meal (45% protein)	150
Meat and bone meal	120
Wheat gluten	50
Corn flour	119.5
Starch	50
Fish oil	35
Soybean oil	30
Lecithin	20
Di-calcium phosphate	3
Vitamins premix [a] (B1 free)	10
Minerals premix [b]	10
Antifungal	2.5
Chemical composition (%)	
Crude protein	46.18
Crude fat	19.64
Moisture	10.25
Ash	11.5
Crude energy (kcal kg^{-1})	3021

[a] Vitamins mixture was manually provided according to the feed requirements of the fish (NRC, 2011) and ingredients were obtained from Hashtgerd Laboratories (Hashtgerd, Alborz, Iran); each 1000 g vitamin mixture provides: vitamin A, 1,600,000 I.U; vitamin D3, 400,000 I.U; riboflavin, 8 g; niacin, 12 g; pantothenic acid, 40 g; pyridoxine, 4 g; folic acid, 2 g; cyanocobalamin, 8 mg; vitamin C, 60 g; vitamin K3, 2 g; biotin, 240 mg; inositol, 20 g, and vitamin E, 60 g.

[b] Aquatic minerals mixture was manufactured by Science Laboratories (Ghazvin, Iran); where each 1000 g contains mineral trace elements: ferrous, 6000 mg; zinc, 10000 mg; selenium, 20 mg; cobalt, 100 mg; copper, 600 mg; magnesium, 5000 mg; iodine, 600 mg, and choline chloride, 6000 mg

Growth performance

Following an 8-week duration, the feeding was stopped for a period of 24 h. Subsequently, all fish in each tank were subjected to sample by anesthetizing with 400-ppm clove powder extract, and individually weighed. The assessment of growth performance was then computed by the following formula:

Weight gain (WG, g) = final weight (g) – (initial weight (g)

Specific growth rate (SGR, %/day) = [(Ln final weight − Ln initial weight) / During the total experimental period (56 days)] × 100

Feed conversion ratio (FCR) = Dry feed consumed (g) / weight gain (g)

Survival rate (%) = 100 × (no. of fish stocked – no. of fish died) / no. of fish stocked

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Sampling

After 8 weeks, the feeding was stopped for 24 h. Subsequently, two fish per experimental repetition were chosen at random. The selected fish were then exposed to an anesthetic agent (with 400-ppm clove powder extract, Ahmadifar *et al.*, 2022) to mitigate the stress induced by manipulation. Initially, blood samples were obtained from the caudal vein using venipuncture, with the samples being aliquoted into tubes with and without heparin. The non-heparinized tubes were subjected to centrifugation at 1600 g for 10 minutes, leading to the separation of serum and supernatant.

Hematology and immunology assays

To quantify the erythrocytes (RBC) and leukocytes (WBC), the blood samples were diluted by adding phosphate-buffered saline (PBS) and analyzed using Hayem and Turk solutions. The cyanmethemoglobin method was utilized to determine the hemoglobin (Hb) concentration. Hematocrit (Hct) percentage was measured by employing micro hematocrit capillary tubes as described by Blaxhall and Daisley (1973). The assessment of serum alternative complement (ACH50) activity, as outlined by Yano *et al*. (1998), involved the use of rabbit red blood cells (RBC) as a target. Serum samples, diluted in a series, were mixed with a suspension of rabbit red blood cells and then incubated at 22°C, pH 7.0, in 10 mM EGTA and 10 mM MgCl2 for 2 h. The hemolytic reaction was terminated by adding gelatin veronal buffer (GVB) containing 10 mM EDTA. Following centrifugation, the supernatant was assessed for optical density (OD) at 414 nm to determine ACH50, indicative of hemolysis extent. Lysozyme activity was determined using *Micrococcus luteus* (Sigma) in a 0.05 M phosphate buffer (pH 6.2) following Ellis's method (2001). Total immunoglobulin (total Ig) levels in serum were determined by polyethylene glycol precipitation of Ig, with adjustments for initial and final total protein levels as per Siwicki and Anderson (1994).

Statistical analysis

This research was conducted in a completely randomized design with three replications (n=3) for all analyses. Data were subjected to one-way ANOVA followed by Tukey’s post-hoc tests to compare the means among treatments. Before the analyses, the normality and homogeneity of variance of the data were assessed using the Kolmogorov-Smirnov and Levene's tests, respectively. The statistical analyses were performed using SPSS software version 23, and a significance level of $p<0.05$ was statistically significant.

Results

Growth performance

Compared to the control diet, dietary levels of SE showed a significant increase ($p<0.05$) in the growth performance of stellate sturgeon. The highest FW, WG, and SGR values were observed by feeding the SE2 diet. Significant differences between the dietary treatments were observed in FCR and the lowest value was recorded in SE2 ($p<0.05$, Table 2).

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Table 2: Efficiency of growth and nutrition parameters of stellate sturgeon (*Acipenser stellatus*) fed with 0 (SE0), 1% (SE1), and 2% (SE2) savory ethanolic extract for 8 weeks.

	Initial weight (g)	Final weight (g)	Weight gain (g)	FCR	SGR (%/day)	Survival rate %
SE0	67.66 ± 0.40^{a}	121.677 ± 10.4^{a}	53.80 ± 10.33^{a}	2.1 ± 0.45^{b}	1.03 ± 0.15^{a}	100
SE1	68.73 ± 2.73^{a}	146.67 ± 12.58^{b}	77.93 ± 10.7^{ab}	1.49 ± 0.19^{a}	1.35 ± 0.10^{ab}	100
SE2	68.76 ± 3.5^{a}	164.67 ± 10.01^{b}	95.90 ± 13.5^{b}	1.21 ± 0.17^{a}	1.55 ± 0.20^{b}	100

FCR: Feed conversion ratio, SGR: Specific growth rate. Different letters designate significant differences as determined by Tukey's post-hoc tests (Mean±SD).

Hematology and immunology assays

No significant differences (p>0.05) between the dietary treatments were observed in hematological factors, except for Hb, and the highest Hb was observed in SE2 (Table 3, p< 0.05).

Table 3: Hematology parameters of stellate sturgeon (*Acipenser stellatus*) fed with 0 (SE0), 1% (SE1), and 2% (SE2) savory ethanolic extract for 8 weeks.

	RBC (10^6/µL)	WBC (10^3 µL)	Hb (g/dl)	Hct (%)
SE0	50.16 ±1.75^{a}	8.33 ± 0.37^{a}	4.83 ± 0.25^{a}	25.3 ± 2.5^{a}
SE1	50.33 ±1.15^{a}	8.4 ± 0.20^{a}	4.8 ± 0.10^{a}	26.0 ± 1.0^{a}
SE2	54.83 ±2.75^{a}	9.3 ± 0.64^{a}	5.3 ± 0.15^{b}	28.6 ± 0.5^{a}

RBC: Red blood cells, WBC: White blood Cells, Hb: Hemoglobin, Hct: hematocrit. Different letters designate significant differences as determined by Tukey's post-hoc tests (Mean±SD).

Notable variances were detected in the levels of lysozyme (Fig 1A), Ig (Fig 1B), and ACH50 (Fig 1C) levels among the various treatments (p<0.05). They were significantly enhanced in the fish fed with SE (SE1 and SE2) compared to the control group (p<0.05).

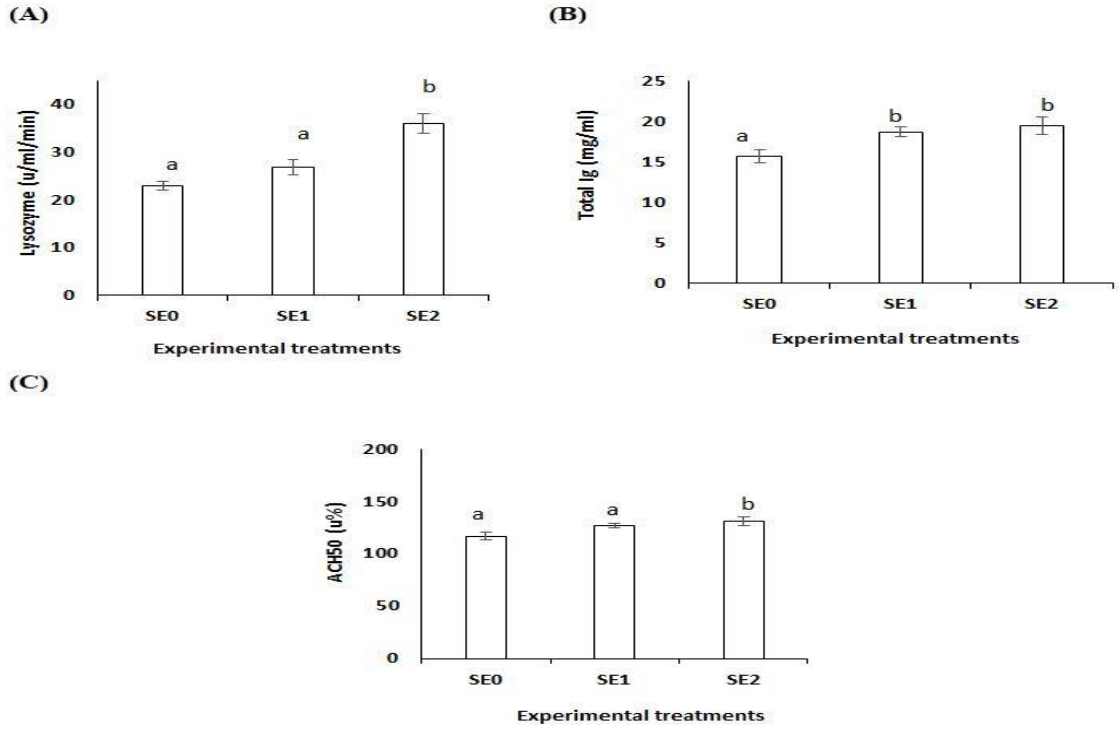

Figure 1: Lysozyme activity (A), total immunoglobulin (B), and ACH50 (C) level of stellate sturgeon (*Acipenser stellatus*) fed with 0 (SE0), 1% (SE1), and 2% (SE2) savory ethanolic extract for 8 weeks.

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Discussion

Feeding strategies are widely acknowledged to play a crucial role as the primary determinants with the capacity to impact production outcomes in intensive aquaculture. Thus, the application of functional feed additives is viewed as a practical approach that holds promise for improving both the growth rate and the well-being of farmed fish. Within the confines of the current investigation, statistically significant impacts on growth factors were observed between various treatments and the highest growth was recorded in fish fed with SE compared to the control group. Previous studies have illustrated the beneficial impacts of diverse herbal extracts on the development of goldfish (*Carassius auratus*) (Rashmeei *et al.,* 2022), common carp (*Cyprinus carpio*) (Ahmadifar *et al.,* 2022), and rainbow trout (*Oncorhynchus mykiss*) (Ali *et al.,* 2022). The efficacy of herbal treatments is thought to originate from the existence of biologically active compounds, specifically polyphenols, which are characterized by the presence of hydroxyl groups. These compounds contribute to the extensively documented and health-promoting antioxidant characteristics (Rubio *et al.,* 2013). The enhanced growth performance observed in SE can be attributed to the diverse array of compounds present, such as polysaccharides, flavonoids, vitamins, and alkaloids (Kawashty *et al.,* 1994; Rezaeipoor *et al.,* 2000). Research has indicated that herbal extract may function as a probiotic, thereby enhancing digestive and absorptive processes within the intestines (Xing *et al.,* 2017).

The current study showed the crucial significance of dietary SE in augmenting hematological parameters. The favorable effects of SE on blood morphology may be ascribed to its potent ability to enhance antioxidant capacity through the neutralization of reactive oxygen species and free radicals, in addition to enhancing the activity of antioxidant enzymes, as documented by Ghorbani *et al*. (2019) and Khamse *et al*. (2020).

Lysozyme, total immunoglobulin, and ACH50, as commonly humeral immunity indices, are investigated to evaluate the impacts of dietary feed additives on aquatic animal health (Awad and Awaad, 2017). The primary function of lysozyme, a nonspecific immune parameter, is to counteract the adverse effects caused by pathogenic microorganisms (Oliver and Wells, 2015). Total immunoglobulin serves as an indicator of both innate and acquired immunity in fish, involving complement activation that contributes to the lysis and opsonization of pathogens (Mashoof and Criscitiello, 2016). Furthermore, variations in ACH50 concentration are indicative of the innate immune status of fish, serving as a valuable indicator to assess health status or stress levels across different fish species. Enhancement of all aforementioned immune parameters was observed in the fish-consuming diets containing SE compared to the control group. Similar findings were reported in common carp (Hoseinifar *et al.*,2020), Nile tilapia (Naiel *et al.,* 2020), and rainbow trout (Aqmasjed *et al.,* 2023). Herbal extract represents a rich source of iridoid glucosides, polysaccharides, flavonoids,

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hydroxycinnamic acids, alkaloids, terpenoids, terpenes, and other compounds (Madgulkar *et al.*, 2015). These compounds potentially function as modulators of active sites and receptors within the innate immune system of fish, thereby activating immune-related signaling pathways to enhance both humoral and cellular immune parameters (Ahmadifar *et al.*, 2021). The present findings could be attributed to herbal compounds recognized by their potential immune-stimulating functions which support fish health status and immune system response (Ahmadifar *et al.*, 2021).

In conclusion, the findings of the current research demonstrate that the provision of SE-enriched diets to Stellate Sturgeon increased fish growth and feed efficiency. Based on the results presented in this study, the incorporation of SE (2%) into fish diets is sufficient to promote fish well-being and optimize their performance. Consequently, it can be considered a cost-effective, environmentally friendly, and natural therapeutic intervention for addressing anemia and improving both the performance and welfare of fish.

Acknowledgments

We are grateful to Qareburoon Sturgeon Culture and Propagation Center for their help with this research.

Conflicts of interest

The authors have no conflict of interest.

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