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Dietary Effects of Fig (*Ficus carica*) Extract on Growth, Survival and Hemato-immunological Indices in Great Sturgeon (*Huso huso*)

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Abstract

The present research aimed to evaluate the effects of fig (*Ficus carica*) extract on growth and hemato-immunological parameters of great sturgeon (*Huso huso*). Fig extract was added to the basal diet (protein 45.3% and lipid 14.7%) at four levels of 0, 1, 2, and 4 g kg^{-1} and fed in triplicate groups. A total of 84 juveniles weighing 1078.21±64.07 g were introduced into 12 ponds. Results indicated that dietary inclusion of fig extract at 2 g kg^{-1} significantly increased the total length, weight gain, average daily gain, specific growth rate, and condition factor of fish compared to other treatments ($P<0.05$). Food conversion ratio was remarkably ($P<0.05$) improved in fish that received fig extract at 2 g kg^{-1}. Significant effects were recorded in number of red and white blood cells, values of hematocrit, hemoglobin, lysozyme activity, and immunoglobulin M (IgM) in fish fed diet supplemented with fig extract at 2 g kg^{-1} ($P<0.05$). Significant changes were also found in liver enzymes (AST, ALT, and ALP) among treatments. In conclusion, administration of fig extract at 2 g kg^{-1} can improve hematological parameters and stimulates immune responses as well as enhancement of growth performance in great sturgeon.

Introduction

The great sturgeon is the most valuable and largest species of sturgeon which has delicious flesh and very tasty and precious caviar. However, the harmful changes in the Caspian Sea ecosystem in recent decades due to the regulation of river water (construction of dams), destruction of spawning grounds, overfishing, and types of pollutants have caused the reduction of the population of this species (Ivanov et al., 1999; Speer et al., 2000). Therefore, during the past decades, the production of cultivated stocks has been seriously emphasized (Chebanov & Savelyeva, 1999; Burtsev et al., 2002).

Intensive aquaculture has created very stressful conditions for aquatic animals, which can weaken the immune function and spread a variety of diseases (Talpur & Ikhwanuddin, 2014). Genetics, season, temperature fluctuations, pollution, unnecessary handling, density, dietary composition, and food additives such as immunostimulants and herbal promoters or phytobiotics can affect immunity and health (Magnadottir, 2006). Due to the many problems caused by antibiotics, it is necessary to use natural and healthy alternatives to protect the health of aquatic animals and ensure their survival and proper growth during the rearing period (Diab et al., 2002). Recently, the application of plant derivatives such as essential oils, extracts, and powders in fish has received special attention because of their availability, reasonable price, no harmful effects, degradability in the environment, and strengthening the immune function (Galina et al., 2009). In addition, herbal compounds show influential properties of flavoring and appetizing (Zhu, 2020). On the other hand, it has been well documented that phytoadditives improve various defense mechanisms

like serum lysozyme activity, phagocytosis, and activation of immune parameters (e.g. antibody enhancement, serum bactericidal, blood clotting against bacteria, viruses, and fungi) in diverse species of fishes (Chakraborty & Hancz, 2011).

Fig (*Ficus carica*) is one of the most widely consumed fruits in the world, which is eaten both fresh and dried (Polat & Caliskan, 2008). This plant is well known for its immunomodulatory, antiinflammatory, antimicrobial, and antioxidant potential. The antibacterial influence of fig has been proved against harmful bacteria (Tkachenko et al., 2016, 2017). Moreover, fig is a good source of K, Mg, Ca, P, vitamins A, B, C, as well as full of fiber (Singh et al., 2011; Badgujar et al., 2014). This crop is well acclimatized to temperate and tropical climates; hence, it is widely distributed in Mediterranean zones. Turkey is the most important and largest fig supplier country in the world (Polat & Caliskan, 2008; Uzundumlu et al., 2018). According to the latest FAO statistics, global production of fig was 1,264,943 tonnes in 2020. The major five fig- producing countries were Turkey (25%), Egypt (16%), Morocco (11.4%), Algeria (9.1%), and Iran (8.5%), respectively (FAO, 2022).

In fish, changes in blood parameters occur at different times, and factors such as manipulation of sampling, type of diet, genetic differences, sexual maturity, season, and age can alter blood condition (Knowles et al., 2006). The effects of dietary fig extract on growth, blood parameters, and immune responses have been investigated in swordfish tail (*Xiphophorus helleri,* Hajibeglou & Sudagar, 2016), (*Ctenopharyngodon idella*, Yang et al., 2015), olive flounder (*Paralichthys olivaceus*, Lee et al., 2015), crucian carp (*Carassius carassius*, Wang et al., 2016), Nile tilapia (*Oreochromis niloticus*, Said et al., 2017), common carp (*Cyprinus carpio*, Yilmaz et al., 2017) and rainbow trout (*Oncorhynchus mykiss*, Yilmaz et al., 2019). Studies on sturgeons are very rare. Due to importance of sturgeon culture, this study aims to investigate the effect of different levels of fig fruit extract on growth, survival, blood parameters, and immunity of great sturgeon.

Materials and Methods

Fish and Experimental Conditions

The great sturgeon juveniles were originally obtained from International Sturgeon Research Institute (Rasht, Iran) and adapted to the basal diet (containing protein 45.3%, lipid 14.7%, and without fig extract) and the new culturing facilities for two weeks. Fish were manually fed three times daily. A total of 84 fish with an average body weight of 1078.21±64.07 g were randomly stocked in 12 circular concrete ponds (diameter 3 m, water depth 0.8 m, water flow rate 0.5 L S^{-1}) equipped with a central aeration system at a stocking density of 7 fish per pond. Photoperiod was regulated to 11 h light and 13 h dark. Water quality indices were monitored daily. Average temperature (15.5±0.3°C), dissolved oxygen (6.55±0.89 mg L^{-1}) and pH (7.95±0.28) were recorded.

Supplementation of Basal Diet with Fig Extract

Four diets were considered in a completely randomized design (4 treatments with 3 replicates), including control (without fig extract) and levels of 1, 2, and 4 g kg^{-1} of concentrated fig fruit extract (*F. carica*) (Soha Jissa plantation industries and medicinal plants processing Co., Mazandaran, Iran) in the diet (Yilmaz et al., 2017; Yilmaz & Er, 2019). The basal diet for great sturgeon (GFS1) was prepared from Sadrdaneh Co. (Ardabil, Iran), which contains 45.3% crude protein, 14.7% crude lipid, 2.6% crude fiber, 9.4% ash, and 8.5% moisture. To prepare the dietary treatments, one kilogram of the ration was poured in a flat layer on a tray covered with aluminum foil. Then, the determined levels of fig extract were separately dissolved in 100 mL of distilled water and sprayed on the feed. When spraying, the pellets were gently inverted so that all surfaces of the pellet are impregnated with the extract. To protect the feed and prevent to release of the fig extract into the water, the diets were coated with 5% cow gelatin powder. First, 5 g of cow gelatin powder was poured into 100 mL of distilled water at 50°C and thoroughly stirred until the solution was clear and smooth, and then sprayed evenly on the pellets (Ramsden et al., 2009). To standardize the test conditions, all the above processes (except adding fig extract) were carried out for the control diet. The diets were dried at room temperature for 3 hours. Finally, the diets were packaged, numbered, and stored in the freezer at -14°C until consumption. Fish were manually fed to satiation three times daily at 0800, 1400, and 2000 h for 60 days. All ponds were daily cleaned to remove feces and uneaten food.

Calculations

The growth indices of juveniles were calculated by the following formulas (Luo et al., 2010; Iqbal et al., 2022):

Weight Gain (WG) % = (final body weight-initial body weight) × 100 / initial body weight

Specific Growth Rate (SGR) = (Ln final weight- Ln initial weight) × 100 / rearing days

Average Daily Gain (ADG) = (final body weight-initial body weight) × 100 / (initial body weight × rearing days)

Feed Conversion Ratio (FCR) = feed consumption / body weight gain

$$\text{Condition Factor (CF)} = (\text{body weight} / \text{body length}^3) \times 100$$

$$\text{Survival Rate (SR)} = (\text{final number of fish} / \text{initial number of fish}) \times 100$$

Hemato-biochemical Measurements

At the end of the experiment, fish were fasted for 24 h before sampling. Blood samples were taken through the caudal vein using a 3.0 mL syringe (3 fish from each treatment). The amounts of 0.5 and 1.5 mL of blood were poured into heparinized and non-heparinized tubes for blood variables and biochemical and immune factors, respectively.

Hematocrit values were investigated by microhematocrit method and hemoglobin concentrations were determined according to tcyanmethhemoglobin method (Klontz, 1994). Red and white blood cells were counted using a neubaur hemocytometer. After preparing the blood smears, leukocytes differential count was obtained based on the percentage of lymphocytes, eosinophils, neutrophils, and monocytes. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated based on the following mathematical equations (Klontz, 1994):

$$\text{MCV} = (\text{Hematocrit} \times 10) / \text{RBC}$$

$$\text{MCH} = (\text{Hemoglobin} \times 10) / \text{RBC}$$

$$\text{MCHC} = (\text{Hemoglobin} \times 100) / \text{Hematocrit}$$

Liver enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured based on kinetic colorimetric method, and alkaline phosphatase (ALP) was detected by kinetic enzymatic assay using commercial clinical kits (Pars Azmoon kit, Tehran, Iran) (Shahsavani et al., 2010).

Immunological Assay

Immunoglobulin M (IgM) values were assayed in accordance with the method suggested by Yamamoto and Yonemasu (1999). Briefly, IgM was formed with polyclonal antibodies in the tampon solution and the color of the compound was cloudy. There was a direct relationship between turbidity and IgM and the intensity of turbidity was measured by spectrophotometer at 340 nm. The turbidimetric assay was applied for lysozyme according to Ellis (1990). First, 1.75 mL of *Micrococcus lysodeicticus* suspension with a concentration of 0.2 mg mL^{-1} was added to 0.02 M sodium citrate buffer and then mixed with 250 µL of serum. Subsequently, the light absorption of the samples was read at intervals of 30 s at 450 nm for 5 min by ELISA.

Data Analysis

A completely randomized design was considered for this project. At the beginning, the normality of numbers was verified through Kolmogorov-Smirnov test and homogeneity test was done by Levene's test. One-way analysis of variance (ANOVA) was applied for homogeneous data and Duncan's multiple range test was used to compare the means between treated groups at 95% confidence level. Kruskal-Wallis non-parametric test was performed for heterogeneous data. The significance of the groups was obtained by Mann-Whitney test at 95% confidence level. All row data were analyzed using the SPSS version 26 (SPSS Inc., Chicago, IL, USA).

Results

Growth performance is summarized in Table 1. In general, fig supplementation improved growth values of fish than those of the control fish. Results revealed that total length, weight gain, average daily gain, specific growth rate, and condition factor were significantly higher in fish fed diet supplemented with fig extract at 2 g kg^{-1} ($P<0.05$). In this treatment, the lowest ($P<0.05$) amount of food conversion ratio was recorded. In all feeding groups, there was no mortality.

Mean values of measured hematological parameters of great sturgeon are presented in Table 2. Significant changes were recorded in number of RBCs, WBSs, values of hematocrit, hemoglobin concentrations, and MCH in fish, which received 2 g kg^{-1} fig extract ($P<0.05$). No statistical differences were obtained in MCV, MCHC, lymphocyte, monocyte, and eosinophil. Nevertheless, fig-containing treatments exhibited a slight increase in number of lymphocyte. Fish that received the control diet had the lowest amount of ALT enzyme which showed a significant difference with those fed fig extract at 2 and 4 g kg^{-1}. Additionally, treatments containing 2 and 4 g kg^{-1} of fig extract revealed the lowest ($P<0.05$) values of AST and ALP enzymes, respectively.

Specific and nonspecific immune reactions were influenced by dietary fig inclusion levels (Table 3). On the basis of the statistical results, lysozyme activity significantly ($P<0.05$) increased in fish fed fig-supplemented diet at 2 g kg^{-1}. Besides, the addition of 2 and 1 g kg^{-1} fig extracts caused statistical raise in the amounts of IgM, respectively ($P<0.05$).

Discussion

Increasing production, reducing costs, and disease resistance have received much attention in sustainable aquaculture. The positive roles of plant components on growth, blood condition, and immunity of fishes have been extensively reported by various scientists (Vallejos-Vidal et al. 2016; Zhu, 2020). In our study, no mortality was recorded. The data presented above

showed that 2 g kg^{-1} of dietary fig increased growth parameters, and also improved FCR ($P<0.05$). In agreement with the present work, growth parameters and survival rate were influenced by the administration of fig in olive flounder (*Paralichthys olivaceus*) (Cho et al., 2011). Similarly, in a study carried out with common carp, dietary inclusion of 1 and 3 g kg^{-1} fig extract caused maximum growth performance. Also, FCR was lower than the control diet (Yilmaz et al., 2017). Contrary to the above findings, some studies have proved that fig extract at different doses was ineffective in olive flounder (Lee et al., 2015) and rainbow trout (*Oncorhynchus mykiss*) (Yilmaz & Er, 2019). The antioxidant, antibacterial, and antiinflammatory properties of plant compounds can enhance the level of nutrient uptake to prevent oxidation reactions (Zeng et al., 2015). In addition, Cho et al. (2014) demonstrated that plant components by reducing microbial fermentation in the gut microbiota cause better and more access to nutrients and organize the density and size of microvilli alignment via changing the microbial population of the intestine. On the other hand, herbal additives present a wide range of bioactive substances and special activities such as stimulating food consumption, producing internal enzymes of the digestive tract, and antimicrobial function based on their purity (Harikrishnan et al., 2011).

Table 1. Growth performance of *Huso huso* fed fig extract in 60-day feeding trial

	Fig extract levels (g kg^{-1})			
Parameters	0 (Control)	1	2	4
Initial weight (g)	95.08±10.30^{a}	96.32 ± 9.82^{a}	95.65±10.11^{a}	95.65±10.11^{a}
Final weight (g)	1567.14±21.43^{a}	1610±20.58^{b}	1670.01±7.14^{c}	1585.71±25.71^{ab}
Final length (cm)	66.35±0.07^{a}	66.06±0.64^{a}	65.35±0.21^{a}	64.85±0.57^{b}
WG (%)	45.11±2.39^{a}	49.28±1.78^{b}	54.41±0.87^{c}	47.85±2.60^{ab}
SGR (% day^{-1})	0.64±0.30^{a}	0.69±0.20^{b}	0.75±0.01^{c}	0.67±0.03^{ab}
FCR	1.61±0.08^{c}	1.48±0.05^{b}	1.34±0.02^{a}	1.52±0.08^{bc}
ADG (g day^{-1})	0.78±0.04^{a}	0.85±0.03^{b}	0.93±0.01^{c}	0.82±0.04^{ab}
CF	0.54±0.01^{a}	0.56±0.01^{ab}	0.59±0.005^{c}	0.58±0.02^{bc}
SR (%)	100±00	100±00	100±00	100±00

*Data are expressed as mean (n=3) ± SD. Means in the same row with different superscripts are significantly different ($P<0.05$).
WG = weight gain, SGR = Specific Growth Rate, FCR = Feed Conversion Ratio, ADG = Average Daily Gain, CF = Condition Factor, SR = Survival Rate.

Table 2. Hemato-biochemical indices of *Huso huso* fed fig extract in 60-day feeding trial

	Fig extract levels (g kg^{-1})			
Parameters	0 (Control)	1	2	4
Hct (%)	22.00±0.58^{a}	23.67±0.88^{ab}	27.00±0.58^{c}	26.33±1.45^{bc}
Hb (g dL^{-1})	4.97±0.15^{a}	5.33±0.22^{a}	6.10± 0.12^{d}	5.90±0.21^{c}
RBC (×10^{6} mm^{-3})	0.55±0.01^{a}	0.59±0.01^{ab}	0.66±0.01^{c}	0.64±0.02^{bc}
WBC (×10^{3} mm^{-3})	4.26±0.08^{b}	4.56±0.23^{a}	5.26±0.32^{b}	4.33±0.08^{c}
MCV (fL)	393.00±1.73^{a}	395.33±3.28^{a}	406.00±2.52^{a}	404.67±7.26^{a}
MCH (pg)	88.67±0.88^{a}	88.67±0.88^{a}	91.67±0.33^{b}	91.00±0.58^{ab}
MCHC (%)	22.57±0.09^{a}	22.50±0.21^{a}	22.60±0.06^{a}	22.43±0.47^{a}
Lymphocyte (%)	80.67±1.86^{a}	81.00±1.15^{a}	83.67±1.45^{a}	82.00±1.15^{a}
Neutrophil (%)	14.67±0.67^{b}	13.00±0.58^{ab}	12.00±0.58^{a}	13.00±0.58^{ab}
Monocyte (%)	4.00±0.58^{a}	5.33±0.33^{a}	3.67±0.67^{a}	4.33±0.88^{a}
Eosinophil (%)	0.67±0.67^{a}	0.67±0.33^{a}	0.67±0.33^{a}	0.67±0.33^{a}
AST (U L^{-1})	403.33±25.46^{b}	364.33±11.26^{b}	272.67±4.84^{a}	383.33±26.96^{b}
ALT (U L^{-1})	15.00±0.57^{a}	18.33±0.33^{ab}	21.33±1.85^{b}	23.00±2.51^{b}
ALP (U L^{-1})	575.00±21.03^{c}	538.00±24.56^{bc}	495.00±6.43^{b}	283.00±18.15^{a}

*Data are expressed as mean (n=3) ± SD. Means in the same row with different superscripts are significantly different ($P<0.05$).
Hct = Hematocrit, Hb = Hemoglobin, RBC = Red Blood Cell, WBC = White Blood Cell, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Hemoglobin, MCHC = Mean Corpuscular Hemoglobin Concentration, AST = Aspartate Aminotransferase, ALT = Alanine Aminotransferase, ALP = Alkaline Phosphatase.

Table 3. Immune factors of *Huso huso* fed fig extract in 60-day feeding trial

	Fig extract levels (g kg^{-1})			
Parameters	0 (Control)	1	2	4
Lysozyme (mL μg^{-1})	24.00±1.53^{a}	28.67±0.88^{ab}	31.67±2.03^{b}	26.00±2.08^{ab}
IgM (dL mg^{-1})	50.00±1.53^{a}	55.67±1.86^{a}	58.33±2.03^{b}	55.00±1.53^{b}

*Data are expressed as mean (n=3) ± SD. Means in the same row with different superscripts are significantly different ($P<0.05$).
IgM = Immunoglobulin M.

In the present study, feeding fish with fig extract showed an enhancement in the number of white blood cells compared to the control, which was significant at the concentrations of 2 and 4 g kg^{-1}. Published data exhibit that herbal supplements can be considered as immunostimulants and raise the number of white blood cells (Zhu, 2020). The leukocyte cells play distinguished roles in the process of phagocytosis and the body defense responses against infectious agents (Magnadottir, 2006). The increase in leukocyte counts confirms the immunogenicity and antiseptic characteristics of fig (Zhu, 2020) which is in line with the previous research by Taati and Noei Taadoli (2015) that cited increased white blood cells in common carp fed diet containing the herbal additive. The highest number of erythrocytes (RBC) was found in treatment that received 2 g kg^{-1} fig extract. Furthermore, there was a rise in the amounts of Hct and Hb in all fig-containing treatments. This is in agreement with Akrami et al. (2015) and Bazari Moghaddam et al. (2017) reported significant changes in great sturgeon and Siberian sturgeon (*Acipenser baerii*) fed with *Allium cepa* and *Aloe vera* extract, respectively. Blood parameters in Nile tilapia (*Oreochromis niloticus*) fed fig extract and exposed to different concentrations of lead acetate exhibited a slight improvement (Said et al., 2017). In fact, enhanced values of hemoglobin and erythrocytes compared to the control reflect the positive effect of fig on the ability of hemoglobin to transport respiratory gases (Talpur & Ikhwanuddin, 2014). In opposition to the current experiment, Yilmaz and Er (2019) declared that levels of fig extract caused no remarkable changes in the blood factors of rainbow trout.

Liver is the most important organ in the metabolism of drugs and other substances. Generally, liver lesions are associated with the permeability of liver cell membranes and impair the production of enzymes, leading to the release of enzymes into the plasma. In this survey, desirable conditions were achieved in the amounts of AST and ALP in fish treated with fig extract compared to the control. Due to the antioxidant and antiinflammatory effects of the fig, its inclusion in the diet can inhibit lipid oxidation as well as prevent the release of hepatic enzymes into the blood (Al-Snafi, 2017). Lysozyme is a nonspecific immune reaction which is released by white blood cells and secreted into various tissues and blood in response to infections (Sakai, 1999). On the other hand, IgM is a kind of specific immune mechanism that accelerates phagocytosis via opsonization process of pathogens (Vallejos-Vidal et al., 2016). All fig-containing treatments clearly showed elevated levels of lysozyme activity and IgM. According to Taati and Noei Taadoli (2015), in common carp, the highest amounts of lysozyme and IgM were observed in the treatment 1 g herbal mixture. An increase in lysozyme enzyme has been reported in grass carp (*Ctenopharyngodon idella*) after supplementing diets with fig (Yang et al., 2015). Moreover, fig polysaccharides could provoke immune capability and resistance against *A. hydrophila* in crucian carp (Wang et al., 2016). However, unlike this study, the administration of olive pomace at different rates in Siberian sturgeon showed contradictory results in IgM values (Banavreh et al., 2019). In general, contradictions in the results of studies can attribute to species, rearing conditions, water parameters, feeding behaviors, physiological properties of the species, dietary regime, the level of plant additive, and other factors. The reason for the decrease in growth parameters of fig extract at 4 g kg^{-1} level is not clear. It seems that herbal extracts have an optimal level, and higher levels can cause an overdose, disrupt digestive functions, and suppress the immune system. Further investigations are required to elucidate the mechanism in the future.

In conclusion, our findings indicate that supplementation of *Ficus carica* extract at 2 g kg^{-1} level has the potential to promote growth and feed efficiency, as well as improve blood factors and elevate immunity in great sturgeon. Hence, we recommend using fig extract at the mentioned level due to fish health during the experiment, ease of consumption, and low production costs in Iran

Ethical Statement

This work was performed based on the instruction of Ethics Committee of Islamic Azad University, Iran

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Author Contribution

Reza Taati: Conceptualization, Methodology, Analysis, Investigation, Writing, Review, and Editing; Seyedjavad Abolghasemi: References, Writing, Review and Editing.

Conflict of Interest

The authors declare that there is no conflict of interest.

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