زانكۆى سەلاحەدین - هەولێر
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Effect of Shilajit enriched diet on blood parameters in common carp

Research Project

Submitted to the department of (Fish Resources and Aquatic Animals) in partial fulfillment of the requirements of the degree of BSc. (Fish Resources and Aquatic Animals)

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April 2024

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Abstract

Natural additives are crucial to maintaining healthy aquaculture practices as they boost fish wellness, reduce related environmental problems, and improve productivity. The current review articles aims to assess the effect of shilajit on common carp (*Cyprinus carpio*) on blood parameters. The integration of natural additives in the common carp diet improves growth performance and general fish health, which can lead to increased production and reduced environmental impact.

1-Introduction

1.1 Aquaculture status in the world

Aquatic foods are increasingly recognized for their key role in food security and nutrition, not just as a source of protein, but also as a unique and extremely diverse provider of essential omega-3 fatty acids and bioavailable micronutrients. Prioritizing and better integrating fisheries and aquaculture products in global, regional and national food system strategies and policies should be a vital part of the necessary transformation of our agrifood systems (FAO,2022).

1.2 Natural antioxidant

Recent studies have shown that adding natural sources like probiotics, prebiotics, and plant extracts to fish diets could improve their growth rate and immune function (Li *et al*., 2022; Rohani *et al*., 2022; Mugwanya *et al*., 2022; Fawole *et al*., 2022). Other studies have reported the positive effects of Shilajit on various aspects of human and animal health (Stohs, 2014).

1.3 Shilajit

Shilajit, also known as salajit, shilajatu, mumie or mummiyo is a pale-brown to blackish-brown exudation, of variable consistency, from layers of rocks in many mountain ranges of the world, especially the Himalayan ranges of the Indian subcontinent (Agarwal *et al.*, 2007). Shilajit plays a vital role as a rejuvenator and potential immuno-stimulant and capable of enhancing the antioxidant properties (Musthafa *et al*., 2016). Moreover, it contains certain organic compounds and vitamins like B1 and B12 (Musthafa *et al*., 2017). Shilajit is able to regulate the activity of body functional components and fluids (Heinrich, 2007; Agarwal *et al*., 2007). It is also used as an immuno stimulant and anabolic food additives (Schepetkin, 2003).

1.4 Blood biochemical parameters

Blood biochemical parameters were considered as indexes in the physiological state of fish and monitoring the physiological status (Anwar *et al*., 2022).

1.5 Common carp (*Cyprinus carpio*)

Common carp (*Cyprinus carpio*) is widely distributed in eutrophic freshwater and is one of the most important cultured species in the cyprinid family, following grass carp, (Ctenopharyngodon idellus), and silver carp (Anwar, 2023). This species also is the fourth most important cultivated species in the aquaculture industry and is mainly produced in many Asian and some European countries (Action, 2020). The species is a highly valuable food source for the ever-growing human population with desirable aquaculture capabilities including a high growth rate, better feed conversion ratio, the higher capability of using carbohydrates and plant protein sources, along with relatively high resistance to variable environmental conditions, and diseases (Tasa, 2020).

1.6 Shilajit composition

The composition of Shilajit samples is typically 13-17% proteins (including a wide range of amino acids), 4-4.5%, lipids 18-20% minerals, 14-20% humidity, 18-20% nitrogen-free substances, 3.3-6.5% steroids, 1.5-2% carbohydrates, and 0.05-0.08% alkaloids (Wilson *et al*., 2011).

1.7 Objectives

Our review articles focuses on the effect of shilajit on the immune system, blood parameters of common carp (*C. carpio*), and a fish with high economic and dietary significance in the aquaculture sector in Kurdistan region. Understanding the possible advantages of shilajit on the development and health of common carp might have profound effects on the aquaculture sector and the larger food supply chain.

2. Materials and Methods

The effect of shilajit on common carp (*Cyprinus carpio*) on blood parameters were investigated (Ahmed *et al.,* 2023).

2.1 Diet preparation

The control diet consisted of fish meal, wheat bran, wheat flour, soybeans, vegetable oil, and starch. To create experimental diets, shilajit powder was added to the control diet at varying concentrations (0, 1, 2, 3) g/kg. Following the mixing of all the dry ingredients, oil and water were combined. Warm water was added to achieve a consistency suitable for extruding into small pellets. The diets were air-dried and stored until use. The proximate composition of the experimental diets was analyzed using the AOAC (2000) method and included crude protein, crude lipid, crude ash, and crude carbohydrate. Table 1 and 2 shows the results ingredient and proximate composition of the experimental diets

Table 1: Ingredient of the experimental diets

Ingredients (%)	Control	T1	T2	T3
*Fish meal	22	22	22	22
Wheat bran	25	24	23	22
Wheat	20	20	20	20
Soybean	30	30	30	30
Vegetable oil	2	2	2	2
Starch	1	1	1	1
Shilajit powder (g/kg)	0	1	2	3

*Fish meal (55% protein) is locally produced by the Agriculture Collage, food industries department, University of Basrah. All other diet ingredients have been purchased from the local market.

Source (Ahmed *et al*., 2023)

Table 2: Proximate composition of experimental diets

Proximate composition	Control	T1	T2	T3
Moisture %	2.84	2.95	2.88	3.06
Protein %	30.45	30.2	31.81	30.54
Lipid %	9.41	9.42	9.52	9.32
Ash %	5.85	6.10	5.88	5.85
Carbohydrate %	50.21	49.5	48.83	48.25

Source (Ahmed *et al*., 2023)

2.2 Experimental fish and husbandry

The nutrition trial was conducted at the fish nutrition laboratory, Agriculture College, University of Basrah, Iraq. After a week of acclimatization, 120 specimens of fish were weighed individually (average initial weight: 108.9±2.20 g) and randomly distributed into 12 indoor plastic tanks with a closed recirculation system (40 L capacity for each tank). The design of the experiment included 10 fish per tank and 3 tanks per treatment. Fish were fed 3% of their body weight twice daily for 60 days. Fish were weighed collectively every week following a 24-hour starvation period.

2.3 Haematological and serological analysis

Following the trial, two fish per tank were gently sedated with tricaine methane sulfonate (MS222) at a concentration of 150 mg/L. Blood was collected from the caudal vein with a 25-gauge needle and a 1 mL syringe. The hematocrit was determined using the microhematocrit method according to Brown (1988) and presented as the percentage of packed cell volume (% PCV). A hemacytometer was used to count leukocytes and erythrocytes after diluting whole blood in Dacie's solution (1:50 dilution). Hemoglobin levels were determined by Drabkin's cyanide-ferricyanide solution (Sigma Aldrich Ltd), following the protocol outlined by Brown (1988).

2.4 Serum lipid profile

Two ml of fish blood was collected from the caudal vein for each treatment and transferred to a 5 ml tube without anticoagulant (EDTA). Centrifugation at 3000 cycles for 15 minutes separated the serum, which was carefully deposited in different tubes. A Mindray laboratory kit and a BS-230 device at a wavelength of 510 nm were used to assess the concentrations of total cholesterol (T.CHO), triglycerides (TG), and highdensity lipoproteins (HDL). (LDL) concentration (mg/dl) = Total Cholesterol - (HDL - triglycerides/5) according to NCHS (2006).

2.5 Statistical Analysis

Statistical analysis was conducted using one-way analysis of variance (ANOVA), followed by Fisher's LSD post hoc test to determine if significant group differences occurred.

3. Results

3.1 Blood parameters

Table 3 presents the blood parameters of carp fed on different shilajit concentrations. No significant difference was found in leukocyte values between the experimental groups. It appears that T3 has the highest values of RBC and Hb. The values gradually increase as shilajit concentration increases, with the T3 group having the highest values for RBC and Hb.

Table 3. Blood parameters of c. carp feed on diets containing graded levels of shilajit.

Parameter	Control	T1	T2	T3
Leukocyte (10^3/mm^3)	2.19±0.11^a	2.21±0.14^a	2.24±0.09^a	2.24±0.11^a
RBC (10^3/mm^3)	0.94±0.04^a	1.09±0.08^b	1.37±0.05^c	1.54±0.07^d
Haemoglobin (mg/dl)	6.09±0.30^a	6.37±0.47^a	8.49±0.59^b	9.50±0.58^c
Haematocrit (10^9/l)	18.27±0.89^a	19.09±1.39^a	25.48±1.81^b	28.52±0.11^c

Source (Ahmed *et al*., 2023)

3.2 Lipid serum profile

Table 4 presents the lipid serum profile of c. carp that were fed diets containing varying levels of shilajit for 8 weeks. According to the results, the control group had the highest total cholesterol (TC) and triglyceride (TG) levels, while the T3 group had the lowest. The T3 group also had the highest high-density lipoprotein (HDL) level, whereas the control group had the lowest levels. On the other hand, low-density lipoprotein (LDL) levels decrease when shilajit dosage increases.

Table 4. Lipid serum profile of c. carp fed on diets containing graded level of shilajit for 8 weeks.

Parameter	Control	T1	T2	T3
TC **mg/dl**	240.11±2.48[a]	190.21±2.42[b]	167.52±2.65[c]	132.49±1.73[d]
TG **mg/dl**	127.54±4.62[a]	89.43±2.37[b]	87.82±2.53[b]	44.81±2.24[c]
HDL **mg\dL**	73.05±3.13[a]	85.43±3.35[b]	90.31±3.21[c]	95.53±3.35[d]
LDL **mg\dL**	71.69±2.24[a]	71.69±2.41[a]	59.44±1.23[b]	54.49±2.54[b]

Source (Ahmed *et al*., 2023)

Data in the same row with different subscript are significantly different (P≤0.05)

4. Discussion

Traditionally, shilajit has been used for the long-term treatment of disorders such as anemia due to its iron and mineral content (Velmurugan *et al*., 2012). According to the current results, shilajit displays a promising ability to enhance blood parameters in fish. Shilajit contains bioactive elements such as humic acid and fulvic acid that possess antiinflammatory, antioxidant, and immunomodulatory characteristics, which may help protect the blood cells from damage caused by free radicals and other harmful agents (Pant *et al.*, 2012; Arif *et al*., 2019).

The findings of the present investigation also indicated that shilajit at graded levels considerably enhanced the lipid profile of *C. carpio*. In this regard, the study of Sharma *et al*., (2003) on healthy human subjects highlights significant benefits of using shilajit for health improvement. The study observed that shilajit significantly decreased serum triglycerides and cholesterol while improving HDL cholesterol, indicating that it has hypolipidemic and cardioprotective effects. Shilajit may also aid in the regulation of lipid metabolism by enhancing the activity of lipid metabolism-related enzymes Sharma *et al*., (2003).

5. Conclusion

Shilajit supplementation, particularly at the T3 concentration, positively influenced blood parameters, and lipid serum profile. The T3 concentration showed the most favorable outcomes under the conditions of the current research. Further research should explore shilajit's impact on gene expression, hormonal regulation, and antioxidant activity. Long-term studies and field trials are necessary to evaluate the sustainability, economic feasibility, and potential effects of shilajit supplementation on fish health and the ecosystem

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