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The effect of Shilajit on growth performance, blood parameters, and key liver enzymes of the common carp (*Cyprinus carpio*)

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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 study aims to assess the effect of shilajit on common carp (*Cyprinus carpio*) metabolites, such as blood parameters, liver function, and growth performance. The fish were fed a basal diet supplemented with 0, 1, 2, and 3 g/kg shilajit for 60 days. The results showed that shilajit supplementation significantly improved the growth performance of the fish with increasing the shilajit concentration in the diet. Using shilajit in experimentally treated fish enhanced hemoglobin levels, red blood cell count, and hematocrit index. On the other hand, the administration of shilajit did not result in any significant modifications to the liver enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), suggesting no effect on liver function. 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.

INTRODUCTION

The food industry heavily relies on aquaculture worldwide as it is an indispensable sector providing substantial animal protein for human consumption (**Hua *et al*., 2019**). To ensure the success of fish farming, growth performance and immune system enhancement are vital factors to consider (**Assefa & Abunna, 2018**). 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, 2013).**

Shilajit is a sticky substance sourced from the Himalayan regions of India and Nepal known for its exceptional properties with anti-inflammatory, antioxidant, and immune-modulatory effects, helping maintain overall health status **(Stohs, 2013).** Shilajit is a natural ingredient that has been used in traditional Ayurvedic medicine in India for generations, it contains organic and inorganic substances, including minerals, trace elements, fulvic and humic acids **(Al-Salman *et al*., 2020)**

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).** Shilajit samples from different regions have different organic compositions but generally consist of 80-85% humic chemicals (like humins, fulvic, and humic acids) and 15-20% non-humic chemicals **(Carrasco-Gallardo *et al*., 2012; Wilson *et al*., 2011).** About 5% of Shilajit's composition is made up of trace components **(Kwon *et al*., 2004).**

Shilajit has been traditionally used to treat various conditions in humans, including fatigue, diabetes, anemia, digestive issues, and kidney problems **(Wilson *et al*., 2011)**. A previous study on broiler chickens was conducted to evaluate the efficacy of shilajit on body weight, feed consumption, and feed conversion ratio. A significant increase in body weight was observed in different treatment groups of broiler chickens with increasing levels of shilajit supplementation **(Vyas & Kumar, 2014). Musthafa *et al*., (2018)** investigated the effects of a diet enriched with shilajit on growth rate, non-specific immune response, and disease resistance in Mozambique tilapia (*Oreochromis mossambicus*) against *Aeromonas hydrophila*. The results showed that 4.0 g/kg fortified diets significantly increased survival rate, weight gain (WG), protein efficiency ratio (PER), specific growth rate (SGR), and feed efficiency (FE). Another study conducted by **Musthafa *et al*., (2016)** examined the impact of a shilajit-enriched diet on the immunity, antioxidants, and disease resistance of freshwater prawn *Macrobrachium rosenbergii* (de Man) against *Aeromonas hydrophila*. The study found that a 2.0 g/kg supplemented diet significantly increased total hemocyte count and phagocytic activity in the first week.

Our research focuses on the effect of shilajit on the immune system, blood parameters, and growth performance of common carp (*C. carpio*), a fish with high economic and dietary significance in the aquaculture sector in Iraq **(Ahmed, 2022)**. 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. The findings of this study might assist in better understanding shilajit's potential advantages in aquaculture by shedding light on the mechanisms of action underlying such advantages.

MATERIALS AND METHODS

Diet preparation

Table 1. Ingredient and proximate composition 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
Proximate composition				
Moisture	2.84	2.95	2.88	3.06
Protein	30.45	30.2	31.81	30.54
Lipid	9.41	9.44	9.52	9.32
Ash	5.85	6.10	5.88	5.85
Carbohydrate	50.21	49.5	48.83	48.25

*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.

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 shows the results.

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.

Growth performance evaluation

Growth parameters, such as weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER), were evaluated and calculated using standard protocols in each replication following the methodology described in the previous study by **Ahmed *et al*. (2012).**

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).**

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 high-density lipoproteins (HDL).

(LDL) concentration (mg/dl) = Total Cholesterol - (HDL - triglycerides/5) according to **NCHS (2006).**

Blood serum enzymes

The levels of three enzymes, namely aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), were measured. Blood samples were taken from the caudal vein (two fish per tank, six fish per treatment). Next, plasma was separated through centrifugation for 3 minutes at 1500 rpm. The instructions provided with each kit were followed to determine the level of each enzyme.

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.

RESULTS

Growth performance

Table 2 presents growth performance parameters. The study shows significant differences ($P < 0.05$) in growth parameters between the different groups. The T3 group had the best FBW, SGR, and FCR values. The FCR values are also significantly different between the groups, with the control group having the highest FCR.

Table 2. Growth and feed utilization performance of C. carp fed diets containing different levels of shilajit over 8 weeks.

Parameters	Control	T1	T2	T3
IBW (g)	108.55±4.75a	112.15±5.47a	107.43±5.62a	107.51±5.74a
FBW (g)	153.71±6.68a	171.08±9.73b	169.35±8.28b	180.80±10.03c
SGR(g)	0.58±0.02a	0.70±2.02b	0.76±0.02c	0.86±0.02d
FCR	2.72±0.11a	1.64±0.15b	1.60±0.13b	1.55±0.07c

Significant differences ($P < 0.05$) are indicated by different letters, Data are means ± SE. IBW (Initial Body Weight), FBW (Final Body Weight) SGR (Specific Growth Rate), FCR (Feed Conversion Ration)

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.

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

After each mean value, the letters (a, b, c, d) indicate statistically significant group differences. The table shows the mean ± standard error.

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 increase.

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

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

After each mean value, the letters (a, b, c, d) indicate statistically significant group differences. The table shows the mean ± standard error.

Blood serum enzymes

The study results indicate that the graded levels of shilajit in the diets of c. carp did not significantly impact the levels of ALT and AST enzymes. The levels of these enzymes remained relatively stable across all treatment groups, with only minor differences observed between them. On the other hand, the results indicate that ALP enzyme levels were slightly lower in the control group than in the other treatments. However, the differences were not statistically significant.

Table 5. Effect of shilajit concentrations on blood serum enzymes of the common carp after 60 days feeding trial.

	Treatments			
Enzyme (IU/L)	Control	T1	T2	T3
ALP	60.33±1.26 a	62.04±1.68 a	63.20±1.80 a	61.59±1.34 a
ALT	5.32±0.05 a	5.44±1.40 a	5.48±0.42 a	5.21±1.21a
AST	35.03±2.94 a	33.13±0.26 a	34.24±0.11a	34.52±0.24 a

The values in the table are presented as the mean ± standard error.

DISCUSSION

The current study found that shilajit had beneficial effects on *C. carpio* growth performance compared to the control group. This finding is consistent with **Musthafa *et al*., (2018)**, who reported that varied doses of shilajit increased the growth performance of *O. mossambicus*. The high concentration of minerals and trace elements found in shilajit is believed to be a key factor behind this advantage. In this context, **Al-Salman *et al*., (2020)**, reported that shilajit samples from various sources contain minerals and trace elements such as copper, iron, chromium, selenium, and zinc. All these components are required for a variety of biological processes, including fish growth and development (**Watanabe *et al*., 1997**). In addition to a high mineral content (18-20%), shillajit samples contain 13-17% proteins (with a variety of amino acids) and 4-45% lipids **(Wilson *et al*., 2011)**, both of which play a critical role in feed utilization and growth performance. Furthermore, shilajit may include beneficial substances such as fulvic acid, which is known to increase nutrients absorption **(Agarwal *et al.*, 2007)**.

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 anti-inflammatory, 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)**.

Serum metabolic enzymes such as ALT, AST, and ALP are considered as indicators for monitoring fish health **(Samanta *et al*., 2015)**. ALT and AST are involved in protein and amino acid metabolism **(Glusczak *et al*., 2011)**, while Alkaline phosphatase (ALP) is a multifunctional enzyme that serves as a biomarker due to its adaptive response to xenobiotic cytotoxic and genotoxic effects **(Samanta *et al*., 2014)**.

Based on the current results, it appears that feeding graded levels of shilajit to common carp did not significantly affect the levels of ALT and AST. These enzymes are typically used as indicators of liver function **(Joni *et al*., 2020)**. Therefore, the fact that their levels remained relatively stable across all treatment groups suggests that the shilajit did not have a major impact on the liver health of the tested fish.

The results of the current study showed a slight difference in ALP enzyme levels between the control and treatment groups. Specifically, the groups given shilajit had slightly higher ALP levels than the control group, but the difference was not statistically significant. ALP is an enzyme that is involved in bone formation and can also be an indicator of liver function **(Satué *et al*., 2022)**. Therefore, the fact that its levels were not significantly affected suggests that the shilajit did not have a major impact on these areas either.

CONCLUSION

The experiment showed significant differences in growth performance parameters among *C. carpio* fed with varying concentrations of shilajit. The T3 (3g/ kg) group showed the best performance in terms of final body weight, specific growth rate, and feed conversion ratio. 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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