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Effect of shilajit enriched diet on blood serum enzymes 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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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*) 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 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.

1. Introduction

1.1 Aquaculture status in the world

Production of aquatic animals in 2020 was more than 60 percent higher than the average in the 1990s, considerably outpacing world population growth, largely due to increasing aquaculture production (FAO, 2022). 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).

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). Antioxidants are widely used in the food industry for various reasons, mainly including prevention of oxidation, neutralization of free radicals, preserving the food, enhancing its flavor, smell, dour and colour (Gremski *et al*., 2019).

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 serum enzymes

Liver injury can be diagnosed by certain biochemical markers like serum alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and bilirubin (Anwar, 2021).

1.5 Common carp (*Cyprinus carpio*)

Common carp (*Cyprinus carpio*) is among the most important reared fish species in many parts of the world, representing 71.9% of freshwater production (Abdel-Tawwab and Monier, 2018) and contributes 8% (ca.4.6million tons) of the world's total finfish aquaculture production (FAO, 2018).

Carp is a hardy fish that survives close to anoxic conditions (<1 mg/L), tolerates wide thermal limits (1-35 °C) and a broad pH range (5-9) with mild halotolerance abilities too (0.5-5 practical salinity units-psu). Carp lives at high densities within small ponds, cages or tanks and the dietary regime of carp as an omnivorous fish is diversified comprising water plants, benthic warms, insects, crustaceans, and artificial diets (Ahmed *et al*., 2020).

1.6 Objectives

The aim of the current review articles was to evaluate the effects of dietary supplementation of Shilajit on blood serum enzymes in common carp.

Table 1 World fisheries and aquaculture production, utilization and trade

	1990s	2000s	2010s	2018	2019	2020
	Average per year					
	Million tonnes (live weight equivalent)					
Production						
Capture:						
Inland	7.1	9.3	11.3	12.0	12.1	11.5
Marine	81.9	81.6	79.8	84.5	80.1	78.8
Total capture	**88.9**	**90.9**	**91.0**	**96.5**	**92.2**	**90.3**
Aquaculture:						
Inland	12.6	25.6	44.7	**51.6**	**53.3**	**54.4**
Marine	9.2	17.9	26.8	**30.9**	**31.9**	**33.1**
Total aquaculture	**21.8**	**43.4**	**71.5**	**82.5**	**85.2**	**87.5**
Total world fisheries and aquaculture	**110.7**	**134.3**	**162.6**	**178.9**	**177.4**	**177.8**
Utilization[2]						
Human consumption	81.6	109.3	143.2	156.8	158.1	157.4
Non-food uses	29.1	25.0	19.3	22.2	19.3	20.4
Population (billions)[3]	5.7	6.5	7.3	7.6	7.7	7.8
Per capita apparent consumption (kg)	14.3	16.8	19.5	20.5	20.5	20.2
Trade						
Exports – in quantity	39.6	51.6	61.4	66.8	66.6	59.8
Share of exports in total production	*35.8%*	*38.5%*	*37.7%*	*37.3%*	*37.5%*	*33.7%*
Exports – in value (USD 1 billion)	46.6	76.4	141.8	165.3	161.8	150.5

Source (FAO,2022)

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 2 and 3 shows the ingredient and proximate composition of the experimental diets.

Table 2. 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

*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 3: 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.

2.4 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.

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

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 4. Effect of shilajit concentrations on blood serum enzymes of the common carp after 8 weeks days feeding trial

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±1.21[a]
AST	35.03±2.94 [a]	33.13±0.26 [a]	34.24±0.11[a]	34.52±0.24[a]

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

4. Discussion

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.

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.

6. References

Abdel-Tawwab, M. and Monier, M.N., 2018. Stimulatory effect of dietary taurine on growth performance, digestive enzymes activity, antioxidant capacity, and tolerance of common carp, Cyprinus carpio L., fry to salinity stress. Fish physiology and biochemistry, 44(2), pp.639-649.

Agarwal, S.P., Khanna, R., Karmarkar, R., Anwer, M.K. and Khar, R.K., 2007. Shilajit: a review. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 21(5), pp.401-405.

Ahmed, A.R., Al-Zewar, J.M., Fawzi, N.A.M. and Abulhasan, A.A., 2020. Culture of common carp (Cyprinus carpio L.) in Basrah Governorate, southern Iraq; Current status and suggestions for development. Ecology, Environment and Conservation, 26(2), pp.824-831.

Anwar, A.Y., 2021. Dietary antioxidant supplementation ameliorates the physiological toxicity induced by kurdistan crude oil in common carp Cyprinus carpio. Salahaddin University -Iraq

Assefa, A., and Abunna, F. (2018). Maintenance of Fish Health in Aquaculture: Review of Epidemiological Approaches for Prevention and Control of Infectious Disease of Fish. Vet .Med. Int. 26:5432497. doi: 10.1155/2018/5432497.

FAO (2018) The State of World Fisheries and Aquaculture 2018- Meeting the sustainable development goals. Licence: CC BY-NC-SA 3.0 IGO.

Fawole, F. J.; Yisa, R. O.; Jayeoba, O. O.; Adeshina, I.; Ahmed, A. O. and Emikpe, B. O. (2022). Effect of Dietary Polyherbal Mixture on Growth Performance, Haemato-Immunological Indices, Antioxidant Responses, and Intestinal Morphometry of African Catfish, Clarias gariepinus. Aquac. Nutr., 2022:1–11. https://doi.org/10.1155/2022/5502796.

Glusczak, L. V.L.; Loro, A.B.S.; Mor. Prettoaes, A.; Raabe, M.F.; Duarte., M. B., da Fonseca, C.C.de; Menezes and D.M. and Valladão (2011). Acute exposure to glyphosate herbicide affects oxidative parameters in piava (Leporinus obtusidens). Arch.Environ.Contam., Toxicol., 61:624-30.

Hua, K.; Cobcroft, J.M.; Cole A.; Condon, K.; Jerry, D.R.; Mangott A.; Praeger C. and Strugnell, J.M. (2019). The Future of Aquatic Protein: Implications for Protein Sources in Aquaculture Diets. One Earth, 3: 316-329. https://doi.org/10.1016/j.oneear.2019.10.018

Joni, S.S.; Cheshmavar M, Shoureshi P; Zamani Z; Taoosi N; Akbari, M. and Afzali M. (2020). Effects of fingolimod treatments on alanine transaminase and aspartate transaminase levels in patients with multiple sclerosis. Int J Physiol Pathophysiol Pharmacol., 3:88-94.

Li, W.; Huang, X.; Lu, X.; Liu, C.; Huang, Y. and Su, Y. (2022). Effects of dietary Lactobacillus reuteri on growth performance, nutrient retention, gut health and microbiota of the Nile tilapia (Oreochromis niloticus). Aquaculture Rep. 26:

Mugwanya M; Dawood M.A.O.; Kimera F. and Sewilam H. (2022). Updating the Role of Probiotics, Prebiotics, and Synbiotics for Tilapia Aquaculture as Leading Candidates for Food Sustainability: A Review. Probiotics Antimicrob.

Musthafa, M.S., Athaullah, A., Anbumani, S., Ali, A.J., War, M., Paray, B.A., Al-Sadoon, M.K., Muthiah, S.S., Kembeeram, P. and Harikrishnan, R., 2017. Ameliorative efficacy of bioencapsulated Chironomous larvae with Shilajit on Zebrafish (Danio rerio) exposed to Ionizing radiation. Applied Radiation and Isotopes, 128, pp.108-113 Proteins.1:130-157. doi: 10.1007/s12602-021-09852-x.

Rohani M.F; Islam S.M; Hossain M.K; Ferdous Z; Siddik, M.A; Nuruzzaman, M.;Padeniya, U.; Brown, C. and Shahjahan M. (2022). Probiotics, prebiotics and synbiotics improved the functionality of aquafeed: Upgrading growth, reproduction, immunity and disease resistance in fish. Fish Shellfish Immunol.,

Samanta. P.; Pal, S; Mukherjee, A.K. and Ghosh, A.R. (2014). Evaluation of metabolic enzymes in response to Excel Mera 71, a glyphosate-based herbicide, and recovery pattern in freshwater teleostean fishes. Biomed. Res. Int., 2014:425159. doi: 10.1155/2014/425159.

Satué, K.; Miguel-Pastor, L.; Chicharro, D. and Gardón, J.C. (2022). Hepatic Enzyme Profile in Horses. Animals, 7:861. doi.org/ 10.3390/ani12070861.