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Impact of Azolla on some biochemical traits of carp (*C. carpio*)

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Abstract

Carp fingerlings *C. carpio* weighing (41.50-40.50) grams were obtained from the pond fish hatchery. The fish were then randomly distributed in glass tanks with dimensions of (40×40×60) in the laboratory. The fish were divided into four groups, each group had three replicates. The first group was a control group C1, and the second, third and fourth groups C2, C3, and C4 were supplemented with 3, 6 and 9 g/Azolla powder/kg feed. The fish were fed at three times: 6, 12 and 18 hours/day at a rate of 3% of body weight, the fish were raised for two months. The results shows: significant ($p \leq 0.05$) increase for C2, and C4 in WBC and total protein, significant ($p \leq 0.05$) increase for C3 in AST enzyme, while the C2 group significant ($p \leq 0.05$) heights in albumin, creatinine, and VLDL, as well the C4 group significant ($p \leq 0.05$) increase in globulin, the C1 group was significant ($p \leq 0.05$) heights in urea and cholesterol compared other group.

Keywords: Azolla, biochemical traits, carp (*C. carpio*), sustainable fish farming

Introduction

The success of intensive fish farming depends on many factors, especially the provision of good feed and healthy water that reduces pollution and other essentials for the rapid growth of carp, fish also need many nutritional elements in sufficient quantities to achieve the best growth, in general, these needs vary according to the method of breeding, age and season, in order to provide sustainable fish farming, nutrients must be provided that do not compete with human food, while at the same time providing high-quality feed (FAO, 2007: El-Din *et al.*, 2022) ^[6, 5]. The primary concern of any livestock sector, particularly in developing nations, is the achievement of sustainable output, this has been challenging in the majority of these countries due to their reliance on conventional feed ingredients, which are either imported or expensive where available locally. Furthermore, the common protein ingredients utilized in fish feeding (fish meal, groundnut cake, and soybean) have grown prohibitively expensive due to their high demand, thus, for fish farms to effectively contribute to poverty reduction and improve food security, it is necessary to raise their productivity by enhancing feeding practices through the usage of unconventional local feed resources (Alalade *et al.*, 2006) ^[3]. The need to find locally accessible protein-rich feed supplies that are not directly consumed by humans, like species of the hydrophytes Azolla, is prompted by the high protein requirements of broilers and the restricted capacity to meet these needs from non-protein nitrogen sources (Ara *et al.*, 2015) ^[4]. Growing naturally on stagnant water in drains, canals, ponds, ditches, rivers, and water bodies such as marshes and wetlands, azolla is a free-floating aquatic fern with small leaves. Its natural habitats include warm temperate, tropical, and subtropical regions of Africa, Asia, North and South America, and portions of Australia (Sharma, 2014) ^[13], the genus Azolla comprises seven species of aquatic ferns: *Azolla pinnata*, *Azolla filiculoides*, *Azolla mexicana*, *Azolla nilotica*, *Azolla caroliniana*, *Azolla japonica*, and *Azolla microphylla*. Given its ease of cultivation, low water requirements for propagation, ability to grow in untapped niches, high productivity, and good nutritional value, the water fern *Azolla pinnata* is arguably the most promising of these species (Singh and Subudhi, 1978; Prabha and Kumar, 2010) ^[15, 11]. Therefore, the current study aims to study the effect of adding different levels of Azolla to carp feed on some physiological traits.

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Materials

Carp fingerlings *C. carpio* weighing (41.50-40.50) grams were obtained from the pond fish hatchery. The fish were then randomly distributed in glass tanks with dimensions of (40×40×60) in the laboratory. The fish were divided into four groups, each group had three replicates. The first group was a control group C1, and the second, third and fourth groups C2, C3, and C4 were supplemented with 3, 6 and 9 g/Azolla powder/kg feed. The fish were fed at three times: 6, 12 and 18 hours/day at a rate of 3% of body weight. The fish were raised for two months. The fish were fed a diet consisting of 31% fish meal, 18% soybeans, 38% corn, 10% barley, 1% probiotic and 2% vitamins. Obtained Azolla powder from local markets.

Blood samples were collected from fish and from each replicate from the caudal vein at the age of 60 days. A

portion of it was placed in anticoagulant tubes for the purpose of studying the physical characteristics of blood (the number of red and white blood cells and hemoglobin), and the other portion of blood was isolated from the blood serum for studying the biochemical characteristics of blood (total protein, glucose, liver enzymes AST and ALT, albumin, globulin, creatinine, urea, triglycerides, cholesterol, HDL and VLDL) (Zinkl, 1986) [17]. Statistical analysis was conducted using the SAS program (2012) and using the Duncan multinomial test (1955).

Results and Discussion

In table 1: noted the impact of study in some physical blood traits, there was no significant effect in RBC and Hd, while the C2, and C4 increase significantly ($p \leq 0.05$) compared to C1 and C3.

Table 1: Effect of azolla on some blood traits of common carp (*C. carpio*)

Groups	RBC x 10 ¹²	WBC x 10 ⁹	Hb (g/dl)
C1	1.92±0.01	200.13±4.50 b	103.16±0.65
C2	1.89±0.26	210.63±1.30 a	100.85±1.58
C3	1.82±0.36	198.41±6.10 b	101.09±3.00
C4	1.91±0.51	213.03±2.25 a	104.52±2.00
Significant	N.S	*	N.S

* Shows significant difference at the level ($p \leq 0.05$), N.S: not significant

In table 2: noted significant increase ($p \leq 0.05$) in total protein for the C2, and C4 groups on C1 group, at same time no significant effect in glucose and ALT level, while the

AST enzyme increase ($p \leq 0.05$) in C3 group compared C1 group.

Table 2: Effect of azolla on some biochemical traits of common carp (*C. carpio*)

Groups	Total protein (g/l)	Glucose (mmol/L)	ALT (u/L)	AST (u/L)
C1	41.19±2.10 b	7.52±0.01	17.80±4.20	44.22±2.25 b
C2	46.14±3.01 a	6.97±0.03	18.16±5.30	50.13±1.57 ab
C3	44.30±1.88 ab	7.14±1.00	17.79±3.00	52.59±3.14 a
C4	45.96±0.98 a	7.18±2.10	17.55±4.11	49.70±4.00 ab
Significant	*	N.S	N.S	*

* Shows significant difference at the level ($p \leq 0.05$), N.S: not significant

Table 3: shown the effect of azolla in protein metabolism change, the albumen, and creatinine significant ($p \leq 0.05$) increase in C2 on C1, C3, and C4 groups, while the globulin

was significant heights ($p \leq 0.05$) in C4 compared the C1, and C2 groups, as so the urea significant ($p \leq 0.05$) increase in C1 compared to other groups.

Table 3: Effect of azolla on some biochemical traits of common carp (*C. carpio*)

Groups	Albumen (g/l)	Globulin (g/l)	Creatinine (mg/dl)	Urea (mg/dl)
C1	15.26±1.31 b	25.93±0.55 b	0.60±0.09 b	24.13±4.01 a
C2	22.17±2.18 a	23.97±0.36 b	0.80±0.13 a	18.36±0.47 b
C3	16.31±1.12 b	27.99±0.71 ab	0.43±0.02 c	20.53±1.30 b
C4	17.56±1.26 b	28.40±1.30 a	0.54±0.01 bc	19.38±2.24 b
Significant	*	*	*	*

* Shows significant difference at the level ($p \leq 0.05$)

In table 4: noted no significant effect in triglyceride, and HDL, while significant heights ($p \leq 0.05$) in cholesterol for C1 group on other groups, the VLDL significant heights

($p \leq 0.05$) in C2, and C3 respectively compared to C1, and C4 groups.

Table 4: Effect of azolla on some biochemical traits of common carp (*C. carpio*)

Groups	Triglyceride (mg/dl)	Cholesterol (mg/dl)	HDL (mmol/L)	VLDL (mmol/L)
C1	2.04±1.13	5.70±1.04 a	1.24±1.05	0.40±0.03 c
C2	2.25±2.00	3.22±0.13 b	1.28±2.00	0.56±0.05 a
C3	2.57±3.44	3.56±0.27 b	1.25±1.96	0.51±0.07 b
C4	2.12±1.36	3.89±1.01 b	1.19±3.05	0.42±0.09 c
Significant	N.S	*	N.S	*

* Shows significant difference at the level ($p \leq 0.05$), N.S: not significant

Aquatic macrophytes are essential nutritional sources for herbivorous-omnivorous fishes and could replace 25% of formulated diets and 50% of commercial feeds without negatively affecting fish growth and body composition. The utilization of alternative and affordable plant protein sources in substitute of expensive and non-sustainable feed ingredients is still in its infancy, according to Cruz-Velasquez *et al.* (2014) ^[18]. Because of their low nutritional potential due to poor digestibility and the presence of anti-nutritional elements, non-conventional plant feedstuffs are likely to be deemed undesirable as ingredients for fish feed. Depending on the source, strain, and growing medium, the aquatic fern *Azolla*, which grows quickly in freshwater environments, has a low protein content (19%-31%). Whether fresh or combined with other feed ingredients, water fern has gained favor as a natural food source for fish species. Our results demonstrate that for *C. carpio* var. *communis* fingerlings, 100% substitution of water fern for fish meal at high degrees of integration is insufficient. One of the useful indicators for monitoring the physiological status and general health of fish is serum biochemical parameters, which are commonly used to evaluate the impact of feed additives on fish health (Parrino *et al.*, 2018; Fazio *et al.*, 2019) ^[9, 7]. These serum properties can significantly contribute in the diagnosis of disease and the efficacy of treatment. In aquaculture, evaluating the serum biochemical parameters is crucial since it enables us to ascertain the normal physiological status of the fish (Patrice *et al.*, 2011) ^[10]. Proteins are one of the primary energy sources for fish and significantly affect their blood glucose levels, according to Shwetha *et al.* (2012) ^[14]. A lower rate of protein synthesis in fish fed greater amounts of dietary *azolla* may be the reason why the *azolla* dietary supplement in our study decreased the total serum protein of *C. carpio* var. *communis* fingerlings. Calcium, phosphorous, and potassium are blood electrolytes that are frequently measured. While potassium ions are mostly found in intercellular fluid and play a major role in the metabolism of carbohydrates in fish nerve fibers, calcium is a component of bones and also regulates neuron and muscle functions in fish. Variations in potassium levels affect the fish's heart and harm its central nervous system neurotoxically (Adedeji, 2010) ^[1]. A comparable significant difference was seen in the calcium and phosphorous activities of fingerling *C. carpio* var. *communis* compared to the group of fish fed a simple diet (C1). On the other hand, cholesterol follows the reverse tendency, rising when dietary *Azolla* intake rises. The enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are found in the liver, kidneys, heart, skeletal muscle, pancreas, spleen, erythrocyte, brain, and gills (Hadi *et al.*, 2009) ^[8]. These blood plasma enzymes are evaluated as markers of liver health in clinical settings. When there is damage, these enzymes are immediately released into the bloodstream, giving information about liver failure. Serum ALT and AST levels sharply increased during our study. Although Xu *et al.* (2018) ^[16] found that blood ALT and cholesterol levels were much lower in *C. carpio* var. *communis* fed *azolla*-replaced meals than in the control group, this suggests that *Azolla* had a negative impact on the liver. According to Xu *et al.* (2018) ^[16], serum glucose levels demonstrated a good level of liver function and normal bodily metabolism. Additionally, it was demonstrated that when dietary *Azolla* intake increased, serum glucose levels rose in all groups. Higher AST and

ALT levels supported our findings that fish were unable to sustain normal glucose metabolism at higher inclusion levels, putting stress on liver metabolism (Ahmed *et al.*, 2023) ^[12].

Conclusion

It is concluded from the results obtained from the study that adding *Azolla* to carp feed may improve its production performance due to the improvement of physiological and immune characteristics, as the treatment caused an improvement in the number of white blood cells, total protein, albumin and globulin, and also significantly reduced creatinine, urea, cholesterol and liver enzymes.

Reference

1. Adedeji OB. Acute effect of diazinon on blood plasma biochemistry in the African catfish (*Clarias gariepinus*). Journal of Clinical Medicine and Research. 2010;2:1-6.
2. Ahmed I, Khan YM, Lateef A, Jan K, Majeed A, Shah MA. Effect of fish meal replacement by *Azolla* meal on growth performance, hemato-biochemical and serum parameters in the diet of scale carp, *Cyprinus carpio* var. *communis*. Journal of the World Aquaculture Society. 2023;54:1301-1316.
3. Alalade OA, Iyayi EA. Chemical composition and the feeding value of *Azolla pinnata* meal for egg-type chicks. International Journal of Poultry Science. 2006;5(2):137-141.
4. Ara S, Adil S, Banday MT, Khan M. Feeding potential of aquatic fern-*Azolla* in broiler chicken ration. Journal of Poultry Science and Technology. 2015;3:15-19.
5. El-Din EI, Mursy IA, Soliman FM, Soliman HAM, Badrey AEA, Osman AGM. An alternative diet from agriculture wastes as feed for the grass carp (*Ctenopharyngodon idella*) in an integrated agri-aquaculture system in the Eastern Desert and its influences on haematological parameters, blood biochemistry, and immune status. Egyptian Journal of Aquatic Biology & Fisheries. 2022;26(1):257-272.
6. FAO. The State of World Fisheries and Aquaculture 2006. FAO Fisheries and Aquaculture Department, FAO; 2007. p. 162.
7. Fazio F, Saoca C, Ferrantelli V, Cammilleri G, Capillo G, Piccione G. Relationship between arsenic accumulation in tissues and hematological parameters in mullet caught in Faro Lake: A preliminary study. Environmental Science and Pollution Research. 2019;26:8821-8827. doi:10.1007/s11356-019-04343-7.
8. Hadi A, Shokr A, Alwan S. Effects of aluminum on the biochemical parameters of freshwater fish *Tilapia zillii*. Journal of Science and its Applications. 2009;3:33-41.
9. Parrino V, Cappello T, Costa G, Cannava C, Sanfilippo M, Fazio F, *et al.* Comparative study of haematology of two teleost fish (*Mugil cephalus* and *Carassius auratus*) from different environments and feeding habits. The European Zoological Journal. 2018;85:193-199. doi:10.1080/24750263.2018.1460694.
10. Patrice T, Patrice N, Bocioc E, Coada MT. Serum biochemical parameters of farmed carp (*Cyprinus carpio*). Aquaculture, Aquarium, Conservation & Legislation. 2011;4:137-140.
11. Prabha BJ, Kumar K. Dried *Azolla* as a nutritionally rich cost effective and immuno-modulatory feed

- supplement for broilers. Asian Journal of Animal Science. 2010;5(1):20-22.
12. SAS. Statistical Analysis System, User's Guide. Version 9.1. 1th ed. SAS Institute Inc., Cary, NC, USA; 2012.
 13. Sharma K. Biological evaluation of *Azolla* (*Azolla pinnata*) in broiler ration. MSc Thesis, Department of Animal Nutrition, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, India; 2014.
 14. Shwetha A, Hosetti BB, Dube PN. Toxic effects of zinc cyanide on some protein metabolites in freshwater fish, *Cirrhinus mrigala* (Hamilton). International Journal of Environmental Research. 2012;6:769-778.
 15. Singh PK, Subudhi BPR. Utilization of *Azolla* in poultry feed. Indian Farming Journal of Experimental Biology and Agricultural Sciences. 1978;27:37-39.
 16. Xu X, Ji H, Yu H, Zhou J. Influence of replacing fish meal with enzymatic hydrolysates of defatted silkworm pupa (*Bombyx mori* L.) on growth performance, body composition, and non-specific immunity of juvenile mirror carp (*Cyprinus carpio* var. *specularis*). Aquaculture Research. 2018;49:1480-1490. doi:10.1111/are.13603.
 17. Zinkl JG. Veterinary Hematology. Philadelphia: Pahle and Fibiger; 1986. p. 256-260.
 18. Cruz-Velásquez Y, Kijora C, Vergara-Hernández W, Schulz C. On-farm evaluation of Cachama blanca and Nile tilapia fed fermented aquatic plants in a polyculture. Orinoquia. 2014 Dec;18:269-277.