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RESEARCH ARTICLE

Effects of *Azolla pinnata* as a Partial Soybean Meal Substitute on Growth, Carcass Traits, Haemato-biochemical Parameters, and Tissue Health of Common Carp (*Cyprinus carpio*)

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ABSTRACT

This study evaluated the potential of *Azolla pinnata* as a partial replacement for soybean meal in carp diets, focusing on growth performance, carcass yield, blood indices, fatty acid composition, and tissue integrity. A total of 600 common carp (Cyprinus carpio) were randomly assigned to three treatments: control, 6% Azolla, and 12% Azolla, each replicated five times. Fish were hand-fed twice daily for 48 days, and growth performance, meat quality, serum biochemistry, and histological parameters were assessed. Results showed that Azolla inclusion had no significant effect on final body weight, weight gain, feed intake, or feed conversion ratio ($P>0.05$). Fatty acid profiling revealed a notable increase in omega-3 fatty acids, particularly EPA and DHA, in Azolla-fed fish. Histological examination indicated normal liver, kidney, pancreas, and muscle structures in the control and 6% groups, whereas mild alterations were detected in the 12% Azolla group. In summary, substituting soybean meal with up to 6% Azolla improved carcass traits, enhanced nutrient deposition, and enriched omega-3 fatty acids without negative health impacts, demonstrating its potential as a sustainable feed component in carp aquaculture.

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INTRODUCTION

Fish is one of the most important sources of animal protein globally, and the growing demand for fish products has increased the need for cost-effective and sustainable aquaculture practices (Maulu et al., 2021; Lamraoui et al. 2023; Verdegem et al., 2023). Feed remains the major expense in aquaculture, representing 50–70% of production costs, with protein sources such as fish meal and soybean meal being the most costly (Hua et al., 2019). Although fish meal is rich in essential amino acids and fatty acids, its reliance on wild fish has raised sustainability and economic concerns (Majluf et al., 2024).

This has prompted efforts to identify alternative protein sources for aquafeed. Among these, *Azolla pinnata*, a fast-growing aquatic fern, is a promising option due to its high protein content (22–34%), essential amino acids, vitamins, and minerals (Sharma et al., 2020; Gupta et al., 2018). It is environmentally friendly, economical, and improves water quality by absorbing heavy metals (Taghilou et al., 2023). Previous studies reported that incorporating Azolla in fish diets enhanced growth performance, feed efficiency, blood parameters, and protein deposition in common carp and other species (Ahmed et al., 2023; Refaey et al., 2023).

Common carp (*Cyprinus carpio)*, a widely cultured species, is valued for its adaptability, nutritional quality, and economic importance (Horvath et al., 2023; Stanivuk et al., 2024). Its meat is rich in protein, essential amino acids, omega-3 fatty acids, and vitamins, making it a desirable food source (Wang et al., 2023; Zhang et al., 2024). Therefore, replacing soybean meal with *Azolla pinnata* in carp diets could provide a sustainable and cost-effective alternative while maintaining growth, performance, meat quality, blood components, and tissue health.

MATERIALS AND METHODS

Experimental Fish: The study was conducted at the Fish Experimental Unit, Department of Aquatic Animal Diseases, Faculty of Veterinary Medicine. A total of 600 common carp (*Cyprinus carpio*) were used over an 8-week trial period including one week of adaptation. Fish were procured from local fish ponds in Kayseri, Turkey, and transported in nylon bags (50 × 100 cm) containing 20 L of oxygenated water and securely wrapped. On arrival, fish were transferred to experimental tanks and acclimatized to the laboratory conditions for one week. Water temperature was maintained between 25–28 °C using a laboratory cooling system.

Experimental design: The rearing system consisted of 15 plastic tanks, each with a 200 L capacity and equipped with a filtration system, pump, cooler, and oxygenation device. The experimental design comprised three dietary treatments (control, 6% *Azolla pinnata*, and 12% *Azolla pinnata*), each applied in five replicates. A total of 600 common carp were used, with 40 fish randomly stocked per replicate tank. Random allocation of fish to replicates minimized variation among treatments. Daily maintenance included siphoning to remove uneaten feed and fecal matter, thereby ensuring proper water quality throughout the trial.

Feed Ingredient and Chemical Composition of Azolla: The proximate composition of *Azolla pinnata* used in this study included dry matter (5.81%), crude protein (27.22%), crude fat (3.40%), crude fiber (12.98%), ash (13.53%), neutral detergent fiber (NDF, 36.28%), and acid detergent fiber (ADF, 27.58%). The nutrient composition of the formulated experimental diets, covering dry matter, crude protein, crude fat, carbohydrates, and ash, is presented in Table 1.

Table 1: shows the proportions of the experimental diet composition (%)

Ingredients (%)	Control	Azolla 6%	Azolla 12%
Fish meal	24	24	24
Soybean meal	30	28.2	26.4
Azolla	0	1.8	3.6
Yellow corn	20	20	20
Wheat	10	10	10
Barley	6	6	6
Millet	6.5	6.5	6.5
Plant oil	2	2	2
Vit-Min premix	1	1	1
Salt	0.5	0.5	0.5
Total	100	100	100
Chemical composition of feed			
Chemical Analysis (%)	Control	Azolla 6%	Azolla 12%
Dry matter%	92.62±0.18	92.11±0.11	92.51±0.13
Protein%	29.40±0.31	28.08±0.10	27.98±0.30
Lipid%	4.10±0.18	3.25±0.11	4.20±0.06
Ash%	9.50±0.24	10.50±0.11	10.50±0.11
Carbohydrates %	3.11±0.06	2.71±0.15	2.31±0.08

Premix ingredients Composition: Each gm contains: The premix contained vitamins and minerals as follows: Vitamin A (8000 IU), Vitamin D3 (1500 IU), Vitamin E (1 IU), Vitamin K3 (2 mg), Vitamin B1 (0.50 mg), Vitamin B2 (0.50 mg), Vitamin B6 (0.20 mg), Vitamin B12 (0.008 mg), Calcium-D-Pantothenate (4 mg), Nicotinic acid (6 mg), and Folic acid (0.05 mg). The mineral composition included Manganese sulfate (0.4 mg), Zinc sulfate (0.15 mg), Ferrous sulfate (0.5 mg), Copper sulfate (0.04 mg), and Cobalt chloride (0.01 mg). In addition, the premix supplied essential amino acids (2.8 mg).

Experimental Design and Diets: Three dietary treatments were formulated: Three dietary treatments were prepared: a control diet based on soybean meal, a diet in which soybean meal was partially substituted with 6% *Azolla pinnata*, and a diet with 12% *Azolla pinnata* replacing soybean meal. Each treatment consisted of five replicates, with 40 fish per replicate (Table 1).

Feeding Management: Fish were fed twice daily (08:00 and 20:00) at a rate of 3.5% of body weight. Body weights were measured every two weeks to adjust feed allocation and maintain proper growth and health.

Growth performance: At the start of the trial, all fish were weighed together, and their live weights were measured using an electronic balance (sensitivity: 0.01 g) with a half-filled water container. The fish were then categorized by body weight (BW), assigned into groups, and randomly distributed among the treatments. On the 49th day of the experiment, all fish were weighed again, and body weight gain (BWG) was determined as the difference between the initial and final live weights, using the same electronic balance for consistency:

$$BWG = \text{Final live weight of fish} - \text{Initial live weight of fish}$$

Feed intake was recorded weekly based on the body weight of the fish in each replicate. For each treatment, 5,000 g of the experimental diet was pre-weighed, stored in sealed containers, and refrigerated at +4 °C. Residual feed was weighed at the termination of the experiment, and actual feed consumption was determined for each replicate tank. In cases of mortality, feed allowance was recalculated to account for the reduced number of fish. However, all fish, including mortalities, were considered in the calculation of feed conversion ratio (FCR)

Carcass Characteristics and Meat Quality: At the end of the 48-day experiment, five fish were randomly selected from each tank and their live weights were recorded using an electronic balance. Fish were then dissected, and carcass weight was determined after removal of the viscera and thorough washing. The carcasses were stored at +4 °C for 24 h before further analysis. Carcass pH was assessed with a digital pH meter by inserting the probe into the lateral, abdominal, and caudal muscle regions. Cooking loss (%) was evaluated following the procedure of Honikel (1998): approximately 5 g meat samples were sealed in nylon bags, heated in a water bath at 80 °C for 1 hour, cooled, and then reweighed. Thawing loss (%) was assessed by freezing 5 g meat samples at –20 °C for 24 h, thawing at room temperature, removing excess water, and reweighing (Honikel, 1998).

The chemical composition of meat, including dry matter, crude protein, lipid, and ash, was analyzed following AOAC (2023) procedures. Dry matter was obtained by drying samples at 70 °C for 72 h (UFP 500, Memmert Ltd. Co., Germany). Ash content was measured by incinerating the dried samples at 550 °C for 6 h. Crude protein was determined using a Dumas nitrogen analyzer (NDA 701, Italy), while crude lipid content was assessed with a SER 148 Series Solvent Extractor (VELP Scientifica, Italy) employing petroleum ether as the extraction solvent.

Determination of haemato-biochemistry: Blood samples (2 ml) were collected from the caudal vein of four fish per treatment using 5 ml syringes. For hematological analysis, blood was placed in EDTA tubes and analyzed with a Mindray pathology analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China) to determine RBC, WBC, HCT, HGB, and differential counts. For serum analysis, blood was collected in gel tubes, centrifuged at 2500 rpm for 5 min, and the serum was analyzed using a Reflotron Plus device (Products Health Services Industry Trade Co. Ltd., Türkiye) for total protein, glucose, cholesterol, triglycerides, HDL, urea, creatinine, AST, and ALT.

Determination of Fatty Acids: Fat was extracted following the AOAC (2023) method using a Soxhlet apparatus. The extracted fat was esterified by reacting 1 g of fat with 8 ml methanolic KOH (11.2 g KOH in 100 ml methanol) and 5 ml hexane. Following vigorous shaking and phase separation, the hexane layer containing fatty acid methyl esters (FAMEs) was carefully collected for analysis. Analysis of fatty acids was carried out on a Shimadzu GC-2010 gas chromatograph equipped with an SE-30 capillary column (30 m × 0.25 mm) and a flame ionization detector. The GC was set at an injector temperature of 280 °C, detector temperature of 310 °C, column gradient of 120–290 °C at 10 °C/min, and a carrier gas flow maintained at 100 kPa.

Organs Histopathology: At the end of the experiment, fish were slaughtered and tissue samples (liver, pancreas, kidney, and muscle) were collected from eight fish per treatment. Samples were fixed in 13% phosphate-buffered formaldehyde and processed for histological examination. Paraffin-embedded tissue sections (4 μm) were cut using a rotary microtome, followed by deparaffinization, rehydration, and staining with hematoxylin and eosin. Histological evaluations of the liver, kidney, and muscle were carried out under an Olympus CX41 light microscope (Olympus Corporation, Tokyo, Japan). To provide a quantitative assessment, lesions were scored using a semi-quantitative scale: 0 = no lesion; 1 = mild; 2 = moderate; 3 = severe. For each tissue type, at least five random fields per slide were evaluated by two blinded observers, and the mean score was calculated for each fish (Bernet et al. 1999).

Statistical Analysis: All statistical procedures were conducted in SPSS v22. Data were checked for normality (Shapiro–Wilk) and variance homogeneity (Levene's test). One-way ANOVA was used to test treatment effects, and Tukey's post hoc test identified pairwise differences at P<0.05.

RESULTS

As shown in Table 2, neither initial nor final body weights of common carp differed significantly among dietary treatments. Likewise, body weight gain and feed intake were unaffected by the inclusion of Azolla at either level. Feed conversion ratio also remained stable, with no notable variation among groups (P>0.05).

Table 2: Effect of Azolla on body weight, weight gain, feed intake, feed conversion ratio

	Control	Azolla 6%	Azolla 12%	P value
BW-initial, g	294.50±4.77	308.25±2.84	298.00±4.26	0.093
BW-last, g	740.25±21.91	769.00±13.42	737.50±27.50	0.547
Feed intake, g	787.46±2.61	783.43±3.73	763.44±10.75	0.069
BWG, g	445.75±19.18	460.75±13.68	439.50±23.97	0.736
FCR	1.78±0.07	1.70±0.04	1.75±0.08	0.759

BW-initial: body weight initial, BW-last: body weight last, BWG: body weight gain, FCR: Feed conversion ratio, g feed/g, P: probability, *, [a, b]: the significance of the means has different letters in the same row were significant P<0.05.

According to Table 3, carcass weight was significantly influenced by dietary treatments (P<0.01). Fish receiving 6% Azolla had the highest carcass weights, followed by those in the 12% Azolla group, both outperforming the control. However, hot carcass weight, cold carcass weight, carcass percentage, and carcass losses showed no significant differences among groups (P>0.05).

Table 3: The effects of Azolla 6% and Azolla 12% addition to the carp fish diet on fish carcass traits

Carcass tests	Control	Azolla 6%	Azolla 12%	P value
Carcass weight, g	109.03±0.35^{c}	113.50±0.61^{a}	110.42±1.00^{b}	0.001
Hot carcass, g	52.17±0.31	53.11±0.67	53.21±0.72	0.412
Cold carcass, g	49.85±0.29	50.47±0.68	50.73±0.74	0.576
Hot carcass, %	47.85±0.27	46.78±0.40	48.19±0.61	0.089
Cold carcass, %	45.72±0.26	44.46±0.43	45.96±0.62	0.068
Loss of carcass, %	2.13±0.09	2.32±0.05	2.24±0.07	0.186

P: probability, *, [a, b]: the significance of the means has different letters in the same row were significant P<0.05.

As presented in Table 4, meat composition and physical traits were affected by Azolla inclusion. Dry matter and crude protein levels were highest in fish fed 6% Azolla, followed by 12% Azolla, both significantly higher than the control (P<0.01). Lipid content increased significantly in the 12% group compared to the other treatments (P<0.05). Ash content and pH, however, were not significantly influenced (P>0.05). In terms of physical properties, cooking loss was lowest in the 12% Azolla group, intermediate in 6%, and highest in the control (P<0.05). Thawing loss followed a similar pattern, with significantly lower values in both Azolla-fed groups compared to the control, and the lowest value observed in the 12% group (P<0.05).

Table 4: The effects of Azolla 6% and Azolla 12% addition to the carp fish diet on fish meat traits

Meat tests	Control	Azolla 6%	Azolla 12%	Total	P value
Dry matter, %	32.94±0.58^{c}	34.91±0.30^{a}	33.08±0.28^{b}	33.64±0.29	0.004
Protein, %	14.27±0.53^{c}	17.88±0.56^{a}	16.15±0.93^{b}	16.10±0.49	0.006
Lipid, %	3.53±0.20^{c}	3.93±0.10^{b}	4.10±0.14^{a}	3.85±0.10	0.043
Ash, %	1.72±0.20	1.89±0.11	1.77±0.19	1.79±0.10	0.772
Physical tests					
PH	6.64±0.10	6.89±0.11	6.97±0.16	6.83±0.08	0.171
Cooking loss,%	4.07±0.31^{a}	3.45±0.19^{b}	3.05±0.20^{c}	3.53±0.16	0.022
Thawing loss,%	3.66±0.15^{a}	3.23±0.14^{b}	3.08±0.11^{c}	3.32±0.09	0.018

[a, b]: the significance of the means has different letters in the same row were significant P<0.05.

According to Table 5, most hematological and serum biochemical indices remained unaffected by Azolla supplementation, with the exception of red blood cells (RBC) and total protein. RBC counts were significantly

higher in the 12% Azolla group, followed by the 6% group, while the lowest counts were observed in the control (P<0.05). Similarly, serum total protein was significantly increased in the 12% group compared to both the control and 6% treatments (P<0.05). Other hematological traits (HCT, HGB, WBC) and serum metabolites including glucose, cholesterol, triglycerides, HDL, urea, creatinine, AST, and ALT did not vary significantly across treatments (P>0.05).

Table 5: The impact of the incorporation of Azolla at concentrations of 6% and 12% into the diet of carp on the hematological parameters of the fish.

Blood tests	Control	Azolla 6%	Azolla 12%	Total	P values
HCT, %	36.00±1.96	35.75±3.97	34.00±2.27	35.25±1.52	0.868
HGB, g/l	120.00±8.83	98.00±8.89	109.25±8.72	109.08±5.34	0.262
RBC * 10^6	2.99±0.24^{c}	3.80±0.36^{b}	5.22±0.65^{a}	4.00±0.36	0.019
WBC * 10^3	58.81±4.96	63.48±3.37	61.27±2.67	61.18±2.06	0.695
Blood serum tests					
Glucose (mg/dl)	76.02±6.24	70.16±3.29	66.87±2.71	71.01±2.55	0.366
Cholesterol (mg/dl)	123.19±7.89	122.54±7.81	115.22±3.48	120.31±3.67	0.66
Triglyceride (mg/dl)	74.64±3.53	69.99±6.10	77.36±5.58	73.99±2.86	0.614
Total protein (g/dl)	3.78±0.07^{b}	3.73±0.11^{c}	4.19±0.14^{a}	3.90±0.08	0.029
HDL (mg/dl)	50.13±2.96	53.00±3.81	49.42±1.57	50.85±1.60	0.67
Urea (mg/dl)	16.12±2.63	12.34±0.90	16.31±2.78	14.92±1.31	0.413
Creatin (mg/dl)	0.18±0.02	0.14±0.03	0.15±0.01	0.16±0.01	0.343
AST/GOT (U/L)	42.92±12.02	31.77±13.99	27.00±8.36	33.89±6.43	0.629
ALT/GPT (U/L)	25.00±2.23	21.61±2.95	33.07±5.66	26.56±2.50	0.158

HCT: Hematocrit, HGB: Hemoglobin, RBC: Red Blood Cells, WBC: White Blood Cells, HDL: High-Density Lipoprotein, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, P: probability, *, $^{a, b}$: the significance of the means has different letters in the same row were significant P<0.05.

As indicated in Table 6, the fatty acid profile of carp muscle was markedly affected by dietary Azolla. Saturated fatty acids, particularly palmitic acid (C16:0) and stearic acid (C18:0), were significantly elevated in fish fed 12% Azolla compared with the other groups (P<0.05). Polyunsaturated fatty acids—including linoleic acid (C18:2n-6), alpha-linolenic acid (C18:3n-3), eicosapentaenoic acid (EPA, C20:5n-3), and docosahexaenoic acid (DHA, C22:6n-3)—also increased significantly, with the 12% group consistently recording the highest values, followed by 6% Azolla, while the lowest levels occurred in the control (P<0.05). Additionally, the monounsaturated fatty acid oleic acid (C18:1n-9) was significantly enhanced in Azolla-fed fish, being highest in the 12% group, moderate in 6%, and lowest in the control (P<0.05).

Table 6: The addition of Azolla at concentrations of 6% and 12% into the carp diet on the fatty acid profile of the fish meat

Fatty acids	Control	Azolla 6%	Azolla 12%	Total	P value
Palmitic acid (C16:0)	13.72±0.69^{c}	14.62±0.61^{b}	16.10±0.49^{a}	14.81±0.39	0.034
Stearic acid (C18:0)	2.46±0.29^{c}	3.01±0.42^{b}	4.03±0.27^{a}	3.16±0.23	0.011
Linoleic acid (C18:2n-6)	11.33±0.51^{c}	13.33±0.62^{b}	14.94±1.20^{a}	13.20±0.55	0.02
Alpha-linolenic acid (C18:3n-3)	2.22±0.34^{c}	3.12±0.33^{b}	4.18±0.40^{a}	3.17±0.26	0.004
Eicosapentaenoic acid (C20:5n-3)	19.11±0.77^{c}	22.33±0.72^{b}	23.48±0.80^{a}	21.64±0.57	0.002
Docosahexaenoic acid (C22:6n-3)	16.07±0.85^{c}	17.05±0.81^{b}	18.93±0.63^{a}	17.35±0.49	0.046
Oleic acid (C18:1n-9)	11.06±0.50^{c}	12.56±0.47^{b}	13.73±0.78^{a}	12.45±0.40	0.017

a, b: the significance of the means has different letters in the same row, were significant, P<0.05.

Histopathological examination revealed normal tissue structures in the control and 6% Azolla groups across the liver, pancreas, kidney, and skeletal muscle (Fig 1-4). In contrast, the 12% Azolla group showed clear pathological alterations: non-plastic hyperplasia and venous dilation in the liver, hyperplasia with cellular hyperactivity in the pancreas, renal tubular swelling with desquamation in the kidney, and muscular dystrophy with degeneration, necrosis, and atrophy in skeletal muscle.

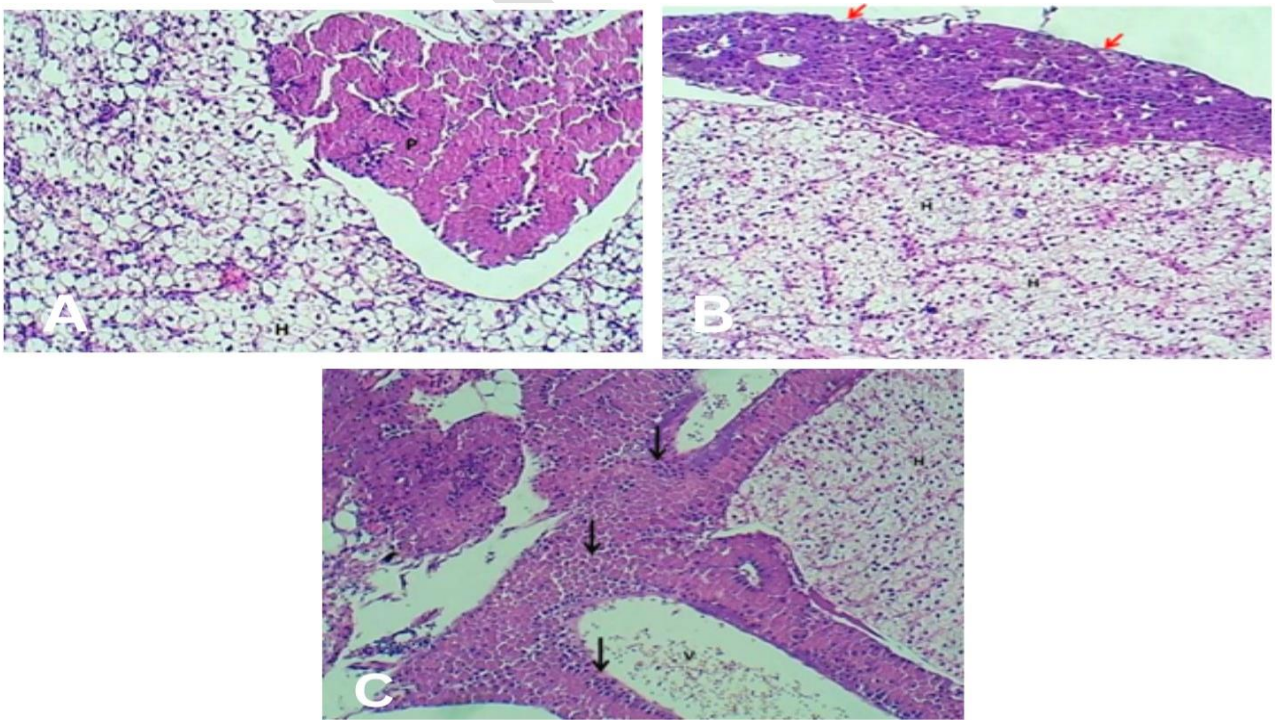

Fig. 1: Liver sections showing normal hepatocyte architecture in the control group (A) and in the 6% Azolla group (B) (H&E, 100X). In contrast, the 12% Azolla group (C) exhibits pronounced non-plastic hyperplasia of acinar cells (arrows) along with marked venous dilation, while normal hepatocytes are still evident.

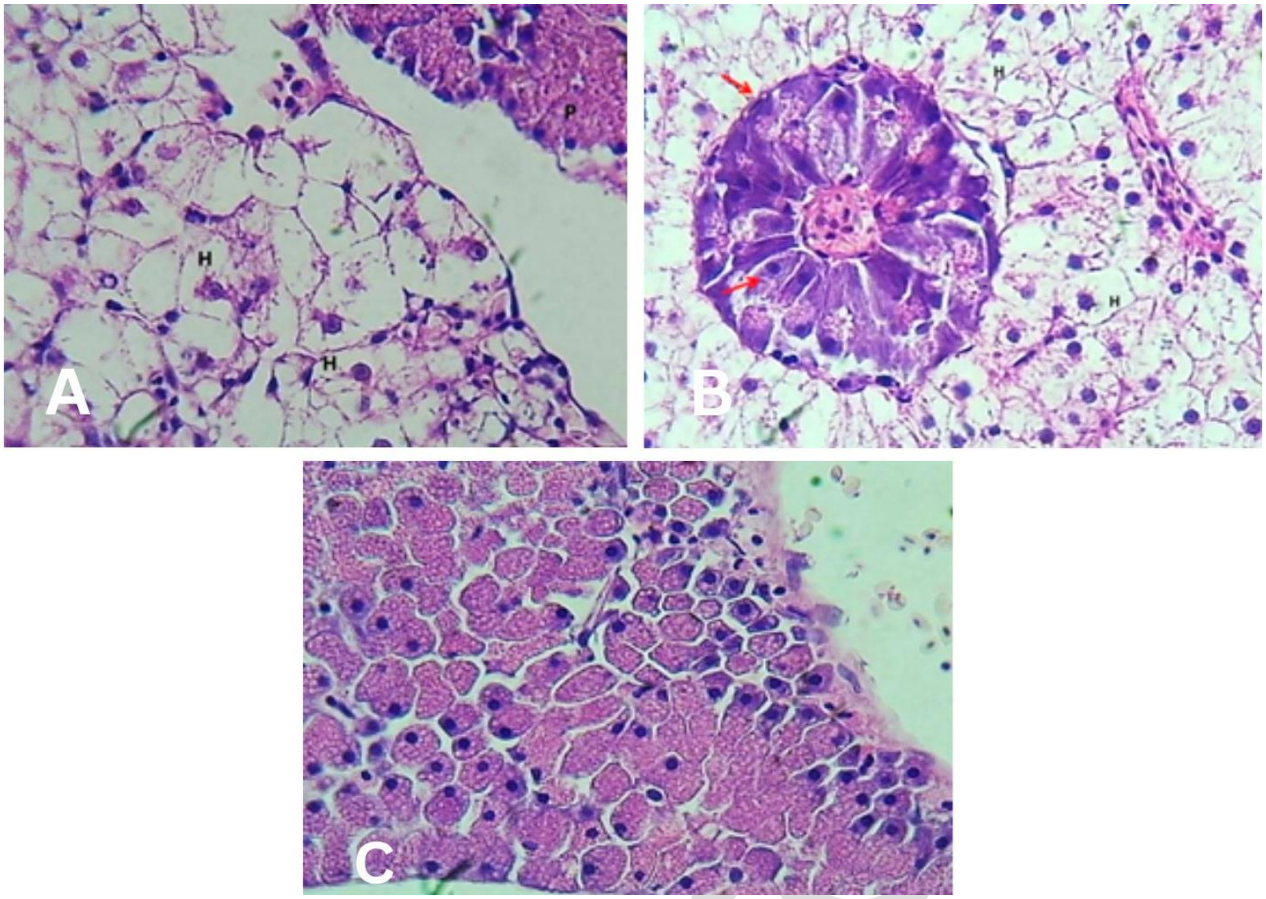

Fig. 2: Pancreatic sections from the control group (A) and 6% Azolla group (B) showing normal cells. In contrast, the 12% Azolla group (C) displays pronounced non-plastic hyperplasia of pancreatic acinar cells with marked cellular hyperactivity (H&E, 400X).

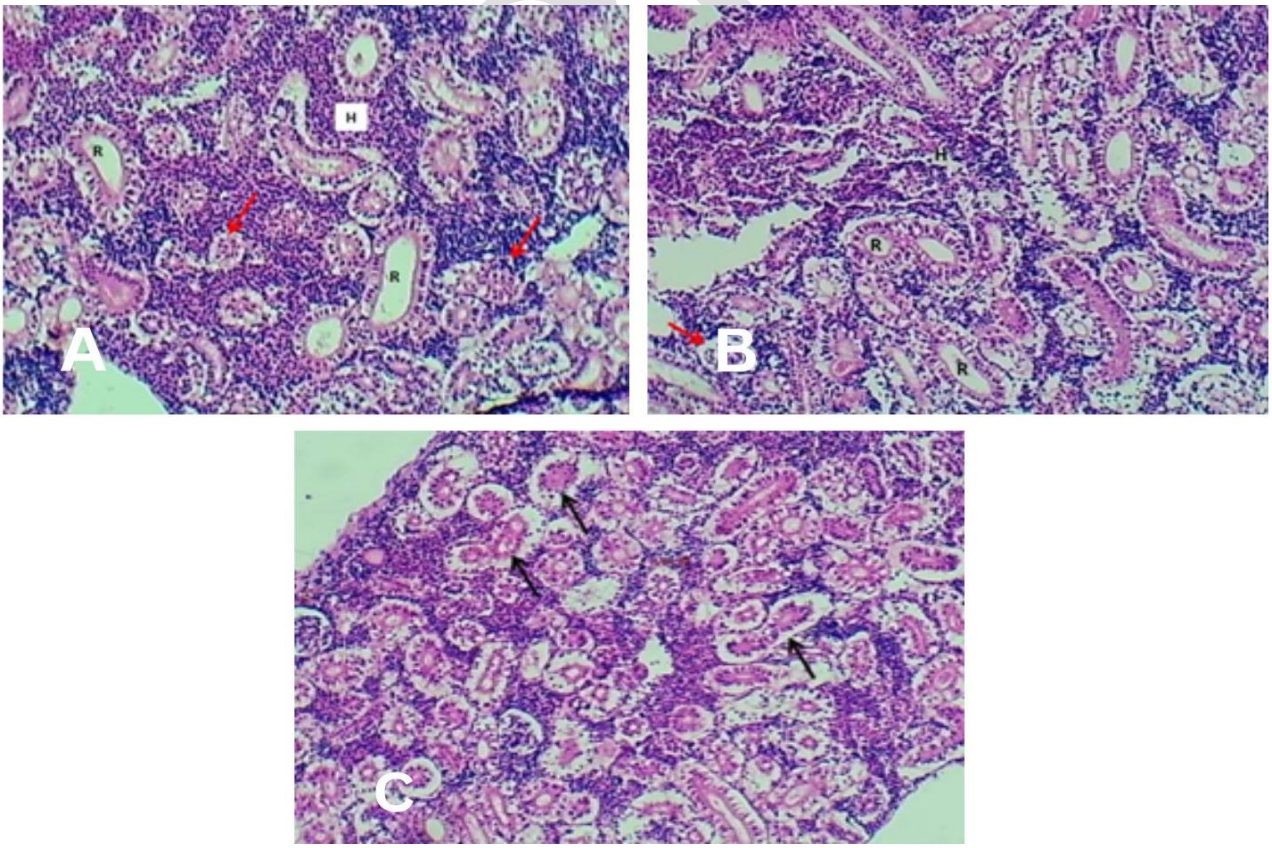

Fig. 3: Kidney sections from the control (A) and 6% Azolla group (B) showing normal renal tubules, glomeruli (red arrows), and hematopoietic tissue (H) (H&E, 400X). In contrast, the 12% Azolla group (C) exhibits marked swelling of renal tubular cells with desquamation of lining cells (arrows), while glomeruli and hematopoietic tissue remain normal.

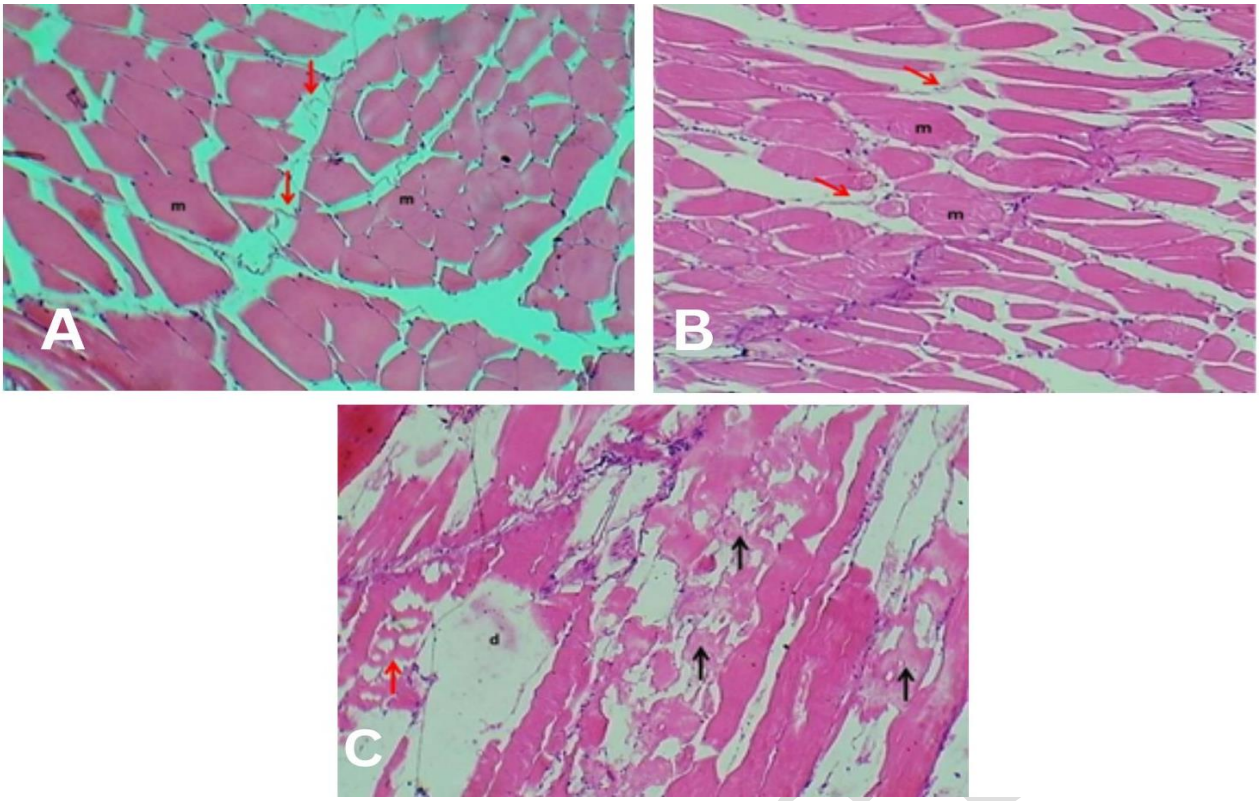

Fig. 4: Skeletal muscle sections from the control (A) and 6% Azolla group (B) showing normal myofibers, endomysium, and loose connective tissue (H&E, 400×). In contrast, the 12% Azolla group (C) displays pronounced muscular dystrophy characterized by degeneration and necrosis of myofibers (black arrows), atrophy (red arrow), and tissue depletion (d) (H&E, 100X).

No detectable lesions were observed in the liver, pancreas, kidney, or skeletal muscle of the control group. Fish receiving the 6 % Azolla diet exhibited only occasional, very mild changes, reflected in mean lesion scores below 0.3 for all tissues. In contrast, the 12 % Azolla group showed pronounced tissue damage, with average scores ranging from 2.3 to 2.6, indicating moderate to severe pathology. Statistical analysis confirmed that lesion scores in the 12 % group were significantly higher ($P<0.05$) than those in both the control and 6 % groups, while the latter two did not differ significantly from each other.

DISCUSSION

The present study evaluated the effects of dietary Azolla supplementation (6% and 12%) on growth, carcass traits, meat quality, hematological indices, fatty acid composition, and tissue histopathology in common carp. Consistent with our results, several studies have reported that partial replacement of fishmeal with Azolla meal does not negatively affect body weight gain, feed intake, or feed conversion ratio when used at moderate levels. Ahmed et al. (2023) and Can (2015) showed that inclusion of Azolla up to 10% did not impair growth performance in carp. Similarly, Al-Dubakel et al. (2021) reported that replacing 15% of fishmeal with Azolla meal in grass carp diets maintained growth rates. However, Naji and Alzurfi (2023) observed growth depression at higher Azolla levels, indicating that low inclusion levels are more favorable. These findings agree with our study, where no significant effects on growth parameters were observed, confirming Azolla's potential as a cost-effective feed ingredient without impairing performance.

Carcass weight was significantly increased in Azolla-supplemented groups, particularly at 6% inclusion, while hot and cold carcass percentages remained unaffected. Similar trends have been reported by Yohana et al. (2023), who noted improved muscle yield in fish fed Azolla-based diets. Meat composition was also improved, with higher dry matter and protein content in the 6% Azolla group and increased lipid content in the 12% group. Previous studies attributed this to the rich nutrient profile of Azolla, including high-quality protein, amino acids, and bioactive compounds (Chekol et al., 2024; Gupta et al., 2018). Our results confirm earlier reports that Azolla inclusion enhances meat protein and lipid levels (Bharti et al., 2024; Elrasoul et al., 2020).

Physical meat traits also improved, as cooking and thawing losses were significantly reduced in Azolla-fed groups, particularly at 12%. Refaey et al. (2023) similarly found that Azolla supplementation reduced cooking loss in Nile tilapia meat, thereby improving water-holding capacity and overall flesh quality. The lack of differences in meat pH across treatments is consistent with earlier findings that Azolla supplementation does not alter postmortem biochemical stability in fish meat (Lougovois, 2005; Sari et al., 2020).

Most hematological indices remained unchanged, except for a significant increase in RBC count and serum total protein in the Azolla-fed groups, particularly at 12%. This suggests enhanced oxygen-carrying capacity and protein metabolism, possibly linked to Azolla's rich amino acid composition. Similar improvements in RBC and protein levels were reported in tilapia and carp supplemented with Azolla (Magouz et al., 2020; Naji & Alzurfi, 2023). Other parameters such as glucose,

cholesterol, urea, and liver enzymes remained unaffected, confirming that Azolla did not compromise fish health, in agreement with findings from Nekoubin (2012) and Refaey et al. (2023).

Azolla supplementation significantly improved the fatty acid profile of carp meat, with higher concentrations of both saturated (palmitic and stearic) and unsaturated fatty acids, particularly PUFA such as linoleic acid, ALA, EPA, and DHA. These improvements were more pronounced at 12% inclusion. Previous studies have shown that Azolla is a rich source of long-chain PUFA (Abou et al., 2013; Ibrahim et al., 2022), which can be incorporated into fish tissue, thereby enhancing the nutritional quality of fillets. Importantly, the increased n-3 fatty acid content and improved n-3/n-6 ratio observed in Azolla-fed fish are beneficial for human health, supporting earlier reports by Caruso et al. (2023) and Yohana et al. (2023).

As shown in Table 7, fish receiving the control diet or the ration containing 6 % Azolla displayed normal tissue architecture, whereas those given the 12 % inclusion developed clear pathological changes in several organs. The liver showed venous dilation and areas of hyperplasia, the pancreas presented acinar cell proliferation, the kidney revealed tubular swelling with epithelial loss, and the skeletal muscle exhibited signs of fiber degeneration and atrophy. These lesions are most likely linked to the chemical composition of Azolla when fed at a high proportion. The plant is known to contain anti-nutritional factors such as tannins, oxalates, and phytic acid, and it can accumulate trace metals from water. Both types of compounds may promote oxidative stress and disturb normal cellular metabolism, which in turn can damage membranes and trigger inflammatory responses. Comparable organ changes have been described in other fish species when Azolla was included at excessive levels (Altaee et al., 2024; Refaey et al., 2023). Earlier reports also note the plant's strong capacity to concentrate heavy metals (Sood et al., 2012; Tahseen et al., 2022).

Table 7: Semi-quantitative histopathological lesion scores (mean±SE,n=8 fish per group)

Tissue	Control	Azolla 6 %	Azolla 12 %
Liver	0.0 ± 0.0^{c}	0.2 ± 0.1^{b}	2.4 ± 0.3^{a}
Pancreas	0.0 ± 0.0^{c}	0.1 ± 0.1^{b}	2.5 ± 0.3^{a}
Kidney	0.0 ± 0.0^{c}	0.3 ± 0.1^{b}	2.3 ± 0.2^{a}
Skeletal muscle	0.0 ± 0.0^{c}	0.2 ± 0.1^{b}	2.6 ± 0.3^{a}

Values represent mean lesion scores on a 0–3 scale (0 = no lesion, 1 = mild, 2 = moderate, 3 = severe). Values bearing different superscripts in a row differ sign

Conclusions: Overall, Azolla supplementation at 6% improved carcass weight, meat quality, and fatty acid profile without adverse effects, while 12% inclusion enhanced PUFA deposition but induced histopathological changes. These findings suggest that moderate inclusion levels of Azolla (around 6%) can be safely used to improve fish productivity and fillet nutritional quality, whereas higher inclusion may pose risks to tissue health.

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Data availability statement: The relevant data are provided in the paper. The data of the current experiment can be obtained from corresponding author when needed.

Authors contribution: YWH and RKM designed the study. YWH, RKM, and YSN conducted the experiment and collected the data. YWH and GYS organized and analyzed the data. YWH drafted the manuscript. YWH, RKM, YK, GYS, YSN, and RUK critically revised the manuscript. YK and RUK managed the resources and supervised the research. All authors read and approved the final manuscript.

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