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Comparative Impacts of Plant-Derived Functional Diets (Azolla, Duckweed, and Napier Grass) on Growth, Hematology, and Serum Biochemistry of Grass Carp (*Ctenopharyngodon Idella*)

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ABSTRACT

This study assesses the effects of partial dietary replacement with three plant-based ingredients, Azolla (*Azolla pinnata*), Duckweed (*Lemna minor*), and Napier grass (*Pennisetum purpureum*), on growth performance, hematological indices, and serum biochemical parameters in grass carp (*Ctenopharyngodon idella*). A 60-day feeding trial was conducted using 240 juvenile fish (initial weight 14.38 ± 0.12 g) randomly distributed into four dietary groups: a control (T0) and three experimental diets containing 10% Azolla (T1), Duckweed (T2), or Napier grass (T3). Each treatment was replicated three times. Growth parameters, feed utilization, hematological profiles, and serum biomarkers were assessed post-trial. Results indicated that the T2 (Duckweed) group exhibited significantly superior final body weight (58.1 ± 1.4 g), weight gain (43.8 ± 1.5 g), specific growth rate (2.757 ± 0.044 % day^{-1}), feed conversion ratio (2.171 ± 0.035), and protein efficiency ratio (1.531 ± 0.032) compared to the control and other treatments ($P < 0.05$). Hematological analysis revealed significantly elevated red and white blood cell counts, hemoglobin, and hematocrit values in the T2 group. Serum biochemical profiles indicated higher total protein, albumin, and globulin levels, along with reduced glucose, cholesterol, triglycerides, and hepatic enzyme activities (AST, ALT, and ALP) in Duckweed-fed fish. Azolla supplementation yielded intermediate results, while Napier grass led to the poorest performance across all parameters. The study concludes that Duckweed at 10% inclusion is a highly effective, sustainable feed ingredient for enhancing growth and health in grass carp aquaculture, whereas Napier grass is less suitable as a primary dietary component.

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INTRODUCTION

Aquaculture sectors have developed worldwide to support optimal fish growth and health, contributing significantly to nutritional supply and global food security **(Obiero *et al*., 2019; Reshi *et al*., 2025)**. The fastest aquaculture practices use sustainable, cost-effective, nutritionally balanced feed to advance the quality of fish **(Hossain *et al*., 2024)**. *C. idella* in the freshwater aquaculture of Asia and other parts of the world holds a prominent position due to its consumer acceptability, rapid growth rate, and herbivorous feeding habit **(Hassoun *et al*., 2022; Mohammed *et al*., 2024)**. Its capability to employ plant-based feedstuffs makes it a gifted species for assessing alternative feed resources; predominantly, feed costs account for more than 60% of total aquaculture production expenditures **(Olaleru *et al*., 2024; Iheanacho *et al*., 2025)**. Fish diets' ingredients for the fish growth frequently rely on soybean meal and fishmeal as primary protein sources, but these are expensive, inadequate in supply, and compete with human and livestock food necessities **(Uddin *et al*., 2023)**. Nutritionally rich, low-cost, and eco-friendly feed substitutes such as Azolla (*Azolla pinnata*), Duckweed (*Lemna minor*), and Napier grass (*Pennisetum purpureum*) partially replace conservative feedstuffs in aquaculture diets **(Wang *et al*., 2017; Hossain *et al*., 2024)**. Azolla is documented as a valuable unconventional feed resource, and it is a free-floating aquatic fern with essential amino acids, minerals, and bioactive compounds and high crude protein content (20–30%) **(Naseem *et al*., 2021)**. Partial replacement of soybean meal and fishmeal with Azolla meal in fish diets progresses immune response, feed utilization, growth performance, and is considered a sustainable ingredient for aquaculture feeds that also enhances nutritive value due to its ability to fix atmospheric nitrogen in symbiosis with cyanobacteria **(Korsa *et al*., 2024; Anany *et al*., 2025)**. Duckweed has also been acknowledged as another free-floating macrophyte that contains balanced amino acids, vitamins, and crude protein (25–35%), a promising protein source in aquafeeds with a quick biomass production rate that supports sustainable feed availability **(Ahmad *et al*., 2022; Du *et al*., 2025)**. Duckweed also has positive effects on the dietary inclusion on feed conversion ratio, survival rate, and growth performance in carp species in tilapia without adverse effects on hematological parameters and with better antioxidant and immunomodulatory properties **(Deepti *et al*., 2025)**. Napier grass (*Pennisetum purpureum*) is anticipated as a feed improvement in aquaculture systems and widely cultivated as fodder for livestock **(Islam *et al*., 2024)**. It is rich in vitamins, secondary metabolites, crude fiber, carbohydrates, and contains protein content (8–12%) that is comparatively lower than that of Azolla and Duckweed, which may increase digestive physiology and overall fish health **(Tran *et al*., 2020)**. Based on their natural herbivorous feeding behavior, grass carp were given diets containing Napier grass to improve gut function, reduce production costs, and support sustainable aquaculture practices **(Kurniasih *et al*., 2024)**. Dietary treatments in aquaculture provide valuable insights into growth performance, hemato-biochemical indices and metabolic responses of fish **(Eissa**

***et al*., 2025)**. Hematological parameters such as hemoglobin concentration, red blood cell count, white blood cell count and hematocrit percentage serve as indicators of oxygen-carrying capacity, general health and immune status **(Nisa *et al*., 2024)**. Similarly, biochemical markers such as serum proteins, glucose, cholesterol, triglycerides, and hepatic enzymes (AST, ALT, ALP) are widely used to assess nutritional status, stress response, and liver function in fish **(Al Sulivany *et al*., 2025)**. Disparities in these indices can imitate the physiological adaptability of fish to different dietary intrusions and are therefore essential in determining the effectiveness of alternative feed ingredients **(Herrera *et al*., 2019)**. Although Azolla, Duckweed, and Napier grass show promise as feed supplements, little is known about their comparative effects on grass carp growth and hemato-biochemical responses. Most earlier studies examined single ingredients rather than evaluating them side by side, and limited attention has been given to their influence on serum biochemical markers. Therefore, this study assesses the impact of these plant meals on growth, feed utilization, and health parameters of grass carp. It is assumed that their inclusion would improve physiological indices, lower feed costs, increase growth performance, and support sustainable aquaculture practices.

MATERIALS AND METHODS

Experimental site

This experiment was designed at the University of Education, Township Lahore, Punjab, Pakistan. The main purpose of this study was to examine the dietary supplementation, viz, Azolla (*Azolla pinnata*), Duckweed (*Lemna minor*), and Napier grass (*Pennisetum purpureum*), on growth parameters, haematology and serum biochemical parameters in *Ctenopharyngodon idella* (Grass carp).

Experimental design and fish rearing conditions

A total of 240 healthy juvenile *C. idella* (average weight 14.38 ± 0.12 g) were purchased from the Government fish hatchery, Lahore and acclimated for 14 days before the start of the feeding trial. During acclimation, fish were maintained in 2000-L fiberglass tanks supplied with continuous aeration and were fed a standard commercial carp diet (32% crude protein) at a rate of 3% body weight twice daily **(Abdulrahman & Al Sulivany, 2025)**. Only clinically healthy individuals displaying uniform size, active swimming behavior, bright red gills, and intact fins were selected for the experiment. Four experimental diets were formulated: a control group (T0, without plant supplementation), an Azolla-based diet (T1), a Duckweed-based diet (T2), and a Napier grass-based diet (T3). Each treatment was tested in triplicate using 12 circular fiberglass tanks (300 L capacity each). Fish were randomly stocked at a density of 20 fish per tank (60 fish per treatment). Tanks were continuously aerated using diaphragm air pumps (Hailea® V-60, China) connected to 8 mm PVC airline tubing and cylindrical diffusers (Boyur AS-02, China) to ensure uniform oxygenation and gentle water circulation. Water quality was maintained within optimal limits during the whole 60-day study trial. The water temperature was maintained between 27–29°C using thermostatically controlled heaters, and above the 6.0mg/ L DO level was maintained. pH was stabilized between 7.3–7.6 using food-grade sodium bicarbonate as needed, while total ammonia nitrogen levels were kept below 0.1mg/ L through partial water replacement (25% daily). Water quality parameters were monitored twice daily using a multiparameter water quality meter (Hanna Instruments HI98194, USA) **(Owais *et al*., 2024b; Asad *et al*., 2025)**.

Experimental diets and feeding protocol

The 32% crude protein experimental diets were formulated. The basal diet comprised feed ingredients such as wheat bran, rice bran, fish meal, soybean meal, mustard oil cake, and a vitamin-mineral premix. The control diet (T0) with no plant material, while Azolla meal (T1), Duckweed meal (T2), and Napier grass meal (T3) were incorporated at a fixed inclusion level of 10% (100 g/kg feed). The detailed component of the experimental diets is presented in Table (1). All feed ingredients were dried, ground to a fine powder, and accurately weighed using an electronic balance. Ingredients were systematically assorted by means of a mechanical feed mixer to confirm homogeneity. Warm distilled water (35°C) was added gradually to achieve a workable dough consistency, which was then passed through a pellet mill to produce 2mm diameter pellets. Pellets were dried at 28°C under forced air for 48 hours and stored in airtight polyethylene bags at –20°C until use. During the feeding trial, fish in each tank were fed their respective diets at 3% of their total body weight per day, divided into two equal meals (09:00 and 17:00).

Table 1. Ingredient composition (g/kg) of experimental diets supplemented with Azolla, Duckweed, and Napier (10% inclusion level)

Ingredient	Control Diet (T0)	Azolla Diet (T1)	Duckweed Diet (T2)	Napier Diet (T3)
Fish meal	15	15	15	15
Soybean meal	25	22	22	22
Mustard oil cake	15	15	15	15
Wheat bran	20	18	18	18
Rice bran	20	18	18	18
Azolla meal		10		
Duckweed meal			10	
Napier grass meal				10
Vitamin-mineral premix	2	2	2	2
Binder (gelatin/starch)	3	3	3	3
Total	100	100	100	100

Note. The vitamin–mineral premix was incorporated at a level of 2 g per 100 g of diet. Each 100 g of feed formulated with this premix supplied approximately the following nutrients: Vitamin A – 720 IU, Vitamin D_3 – 160 IU, Vitamin E – 20 IU, Vitamin C – 9 mg, Thiamine (B_1) – 0.15 mg, Riboflavin (B_2) – 1 mg, Pyridoxine (B_6) – 0.8 mg, Cyanocobalamin (B_{12}) – 0.002 mg, Niacin – 5.5 mg, 4 mg (Pantothenic acid), – 0.008 mg (Folic acid), 0.18 mg (Biotin), Copper – 0.6 mg, Iron – 11 mg, Zinc – 3 mg, Manganese – 1.8 mg, Iodine – 0.06 mg, Selenium – 0.02 mg, and Cobalt – 0.012 mg. This premix ensured that all essential micronutrient requirements of grass carp were adequately met throughout the experimental period.

Calculation of Growth Parameters

After the end of the experiment duration, for 24 hours, all the fish in the group were fasted, and then with clove oil (50 mg/L), all the fish were anaesthetized and weighed to determine the final body weight. The subsequent growth catalogues were calculated using standard formulas **(Al Sulivany *et al*., 2024b; Owais *et al*., 2024a)**.

WG, g (Weight Gain) = Final body weight – Initial body weight

SGR, %/day (Specific Growth Rate) = (ln Final weight–ln Initial weight)/Number of days (ln Final weight – ln Initial weight) / Number of days (ln Final weight–ln Initial weight)/Number of days × 100

FI, g/fish (Feed Intake) = Total feed consumed / Number of fish

FCR (Feed Conversion Ratio) = Total feed intake (g) / Total weight gain (g)

PER (Protein Efficiency Ratio) = Weight gain (g) / Protein intake (g)

SR, % (Survival Rate) (= (Final number of fish / Initial number of fish) × 100.

Analysis of haematological parameters and blood collection

After measuring the growth parameters, blood samples were collected by using sterile 3mL syringes from the caudal vein. For the haematological analysis, each sample was divided into two parts: one part was sifted into EDTA-coated tubes to prevent coagulation, and the second part was shifted into plain gel tubes for subsequent serum biochemical assessment. The hematological parameters viz Hb, g/dL (hemoglobin concentration), RBCs, $10^6/mm^3$ (red blood cell count), MCV, fL (mean corpuscular volume), hematocrit (%), MCH, pg (mean corpuscular hemoglobin), MCHC, g/dL (mean corpuscular hemoglobin concentration), and white blood cells ($\times 10^3\ \mu L^{-1}$).

These measurements followed the methodology described by **Al Sulivany *et al*. (2024a)**. The following standard formulas were used to calculate red blood cell indices:

MCV (fL) = (10 × PCV) / RBCs

MCH (pg) = (10 × Hb) / RBCs

MCHC (g/dL) = (100 × Hb) / PCV

These procedures were applied consistently to all treatments to ensure accurate assessment of the fish haematological parameters.

Serum biochemical parameters

The second part of blood collected was allowed for clotting at room temperature up to 30 minutes, and then centrifuged at 3500 rpm for 10 minutes to separate the serum. The clear supernatant was extracted and stored at −20°C until biochemical analysis. Glucose, cholesterol, and triglycerides were determined by using commercial diagnostic kits (Merck, Germany) and measured spectrophotometrically (UV–Via spectrophotometer, Shimadzu, Japan) following the manufacturer's instructions **(Xiao *et al*., 2023)**. Albumin levels were estimated via the bromocresol green (BCG) dye-binding method, with readings recorded at 630nm. Globulin concentration was calculated by subtracting albumin from total protein values:

Globulin (g/dL) = Total protein – Albumin

Total protein concentration in serum was quantified using the biuret method, based on the formation of a violet-colored complex between peptide bonds and copper ions in an alkaline medium, with the absorbance at 540nm using a UV-Vis spectrophotometer. Serum enzyme activities such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were assayed using commercially available kinetic enzyme assay kits, which measure the rate of NADH oxidation linked to transamination reactions **(Owais *et al*., 2025)**.

Statistical analysis

ANOVA (One-way analysis of variance) was performed to identify significant differences among treatments by using SPSS software (version 26.0), followed by Tukey's HSD post hoc test **(Șerban *et al*., 2024; Mustafa *et al*., 2025)**.

RESULTS

The significant result of dietary treatment ($P< 0.05$) was recorded on the growth performance and feed utilization of the fish, while the survival rate persisted unaffected across all groups ($P> 0.05$) (Table 2). Final body weight and weight gain were significantly influenced by the experimental diets. Fish fed the T2 diet containing 10% duckweed achieved the highest final weight (58.1 ± 1.4 g) and weight gain (43.8± 1.5 g), which was significantly greater than that of the control group (T0: 47.1 ± 1.3 g and 33.1 ± 1.4 g, respectively) and the T3 Napier grass group (44.1 ± 1.2 g and 29.4 ± 1.8 g, respectively). The T1 Azolla group (54.0 ± 1.2 g and 39.6± 1.4 g) exhibited intermediate values that were not statistically different from the T0 control. This growth pattern was reflected in the specific growth rate (SGR, P = 0.005), with the T2 group again demonstrating the highest SGR (2.757 ± 0.044 % day^{-1}), significantly outperforming the T0 (2.372 ± 0.049 % day^{-1}) and T3 (2.247 ± 0.017 % day^{-1}) groups. Feed intake also varied significantly (P= 0.002). Total feed consumption was the highest in the T2 group (98.1 ± 2.4 g/fish), followed by T1 (97.1 ± 2.6 g/fish); both were significantly greater than the intake of the T0 (92.1 ± 2.3 g/fish) and T3 (91.0 ± 2.7 g/fish) groups. More critically, feed conversion ratio (FCR) was significantly improved (P= 0.001) in the T2 group (2.171 ± 0.035) compared to the control (2.68 ± 0.028). The T3 group displayed the poorest FCR (3.047 ± 0.050). Similarly, the protein efficiency ratio (PER, P = 0.003) was the highest in the T2 group (1.531 ± 0.032), which was significantly superior to the T0 (1.23 ± 0.040) and T3 (1.077 ± 0.043) diets. In contrast, the survival rate was high and not significantly different ($P > 0.05$) among all dietary treatments, ranging from 94.1 to 100.2%. Initial weights were uniform across all groups at the start of the trial ($P = 0.081$).

Table 2. Effect of dietary supplementation with Azolla, Duckweed, and Napier grass on growth parameters, feed efficiency, and survival rate in *C. idella*

Parameter	T0 Control	T1 Azolla (10%)	T2 Duckweed(10%)	T3 Napier (10%)	*P*-value
Initial weight (g)	14.1 ± 0.01[a]	14.4 ± 0.04[a]	14.3 ± 0.06[a]	14.7± 0.01[a]	0.081
Final weight (g)	47.1± 1.3[b]	54.0 ± 1.2[ab]	58.1± 1.4[a]	44.1 ± 1.2[c]	0.002
Weight gain (g)	33.1 ± 1.4[b]	39.6± 1.4[ab]	43.8± 1.5[a]	29.4± 1.8[c]	0.004
SGR (% day^{-1})	2.372 ± 0.04[b]	2.622 ± 0.041[ab]	2.757 ± 0.044[a]	2.247 ± 0.017[c]	0.005
Feed intake	92.1 ± 2.3[b]	97.1 ± 2.6[ab]	98.1 ± 2.4[a]	91.0 ± 2.7[b]	0.002
FCR	2.68 ± 0.028[b]	2.364 ± 0.042[ab]	2.171 ± 0.035[a]	3.047 ± 0.050[c]	0.001
PER	1.23 ± 0.040[b]	1.424 ± 0.048[ab]	1.531 ± 0.032[a]	1.077 ± 0.043[c]	0.003
Survival (%)	96.2 ± 1.3[a]	95.2 ± 1.4[a]	100.2 ± 0.1[a]	94.1± 2.4[a]	0.004

Note. SGR = Specific Growth Rate (% day^{-1}), Feed intake = Total amount of feed consumed per fish during the experimental period (g/fish), FCR = Feed Conversion Ratio, and PER = Protein Efficiency Ratio.

The analysis of hematological parameters revealed significant alterations in response to the different dietary treatments ($P < 0.05$), except MCHC, which remained statistically unchanged across all groups ($P > 0.05$). A significant effect was observed on RBC ($P = 0.004$). The highest RBC was recorded in the T2 Duckweed group, which was significantly greater than that of the T0 and T3 Napier groups. The T1 Azolla group displayed an intermediate value that was not statistically different from the T2 group. Similarly, WBC was significantly influenced by the diets ($P = 0.005$). The T2 group demonstrated the highest value ($32.1 \pm 1.4 \times 10^3$ µL^{-1}), which was significantly elevated compared to the T0 ($26.1 \pm 1.2 \times 10^3$ µL^{-1}) and T3 ($23.1 \pm 1.2 \times 10^3$ µL^{-1}) groups. On the other hand, the Hb concentration varied significantly among treatments ($P = 0.005$). The T2 Duckweed and T1 Azolla groups recorded higher Hb levels, which were significantly superior to the T0 Control group (8.2 ± 0.4 g dL^{-1}). The T3 Napier group yielded the lowest value (7.5 ± 0.2 g dL^{-1}), which was significantly lower than that of the control. This trend was mirrored in the Ht levels ($P = 0.003$). The T2 and T1 groups showed significantly increased hematocrit values compared to the T0 group. The T3 group exhibited a significantly depressed hematocrit value of 25.1 ± 1.1%. Significant differences were found in red blood cell indices. The MCV, $P = 0.004$ was significantly lower in the T2 group (214 ± 0.4 fL) compared to all other groups (T0: 234 ± 0.4 fL; T1: 224 ± 0.3 fL; T3: 227 ± 0.7 fL). Likewise, the MCH, $P = 0.005$, was significantly reduced in the T2 group (59.2 ± 1.7 pg) compared to the T0 (67.4 ± 1.6 pg), T1 (62.4 ± 2.4 pg), and T3 (68.4 ± 2.1 pg) groups. In contrast, the MCHC showed no significant differences between the dietary treatments (Table 3).

Table 3. Effect of dietary supplementation with Azolla, Duckweed, and Napier grass on the hematological parameters of *C. idella*

Parameter	T0 Control	T1 Azolla	T2 Duckweed	T3 Napier	*P*-value
RBC ($\times 10^6$ µL^{-1})	1.22 ± 0.03^b	1.48 ± 0.02^{ab}	1.59 ± 0.03^a	1.18 ± 0.02^c	0.004
WBC ($\times 10^3$ µL^{-1})	26.1 ± 1.2^b	27.1 ± 1.4^{ab}	32.1 ± 1.4^a	23.1 ± 1.2^b	0.005
Hemoglobin (g dL^{-1})	8.2 ± 0.4^b	9.4 ± 0.3^{ab}	9.7 ± 0.1^a	7.5 ± 0.2^c	0.005
Hematocrit (%)	27.1 ± 0.5^b	34.1 ± 0.7^{ab}	35.1 ± 0.6^a	25.1 ± 1.1^c	0.003
MCV (fL)	234 ± 0.4^b	224 ± 0.3^b	214 ± 0.4^a	227 ± 0.7^b	0.004
MCH (pg)	67.4 ± 1.6^b	62.4 ± 2.4^b	59.2 ± 1.7^a	68.4 ± 2.1^b	0.005
MCHC (g dL^{-1})	29.1 ± 0.4^a	28.2 ± 0.1^a	29.6 ± 0.4^a	30.1 ± 0.3^a	0.002

The dietary regimens exerted a significant influence on the serum biochemical profile of the fish, reflecting alterations in protein metabolism, energy status, and liver function (Table 4). An important effect was observed on total serum protein levels (P = 0.003). The highest concentration was recorded in the T2 group (4.1 ± 0.14 g dL^{-1}), which was significantly greater than that of the T0 (3.24 ± 0.17 g dL^{-1}) and T3 (3.1 ± 0.17 g dL^{-1}) groups. Both albumin and globulin levels were significantly elevated in the T2 (2.1 ± 0.03 g dL^{-1} and 2.1 ± 0.15 g dL^{-1}) and T1 (1.92 ± 0.14 g dL^{-1} and 1.92 ± 0.12 g dL^{-1}) groups compared to the control (1.67 ± 0.03 g dL^{-1} and 1.62 ± 0.03 g dL^{-1}) and T3 (1.52 ± 0.12 g dL^{-1} and 1.54 ± 0.14 g dL^{-1}) groups. Serum glucose levels were significantly modulated by the diets (p = 0.002). The T2 and T1 groups demonstrated significantly lower glucose concentrations compared to the T0 and T3 groups. A similar trend was observed in lipid metabolites. Both cholesterol and triglycerides were significantly lower in the T2 group compared to the control. The T3 group recorded the highest values for cholesterol (157.2 ± 0.2 mg dL^{-1}) and triglycerides (91.2 ± 0.4 mg dL^{-1}). The activities of hepatic enzymes, AST, ALT, and ALP, were significantly affected. The T2 Duckweed group consistently showed the lowest enzyme activities (AST: 36.6 ± 0.2 U L^{-1}; ALT: 23.1 ± 0.3 U L^{-1}; ALP: 122 ± 0.2 U L^{-1}), which were significantly lesser than control group (AST: 44.4 ± 0.3 U L^{-1}; ALT: 32.1 ± 0.4 U L^{-1}; ALP: 152.4 ± 0.5 U L^{-1}). Conversely, the T3 Napier group exhibited significantly elevated activities for all three enzymes (AST: 53.5 ± 0.4 U L^{-1}; ALT: 36.1 ± 0.4 U L^{-1}; ALP: 163.1 ± 0.6 U L^{-1}).

Table 4. Serum biochemical parameters of *C. idella* fed experimental diets supplemented with different herbal ingredients

Parameter	T0 Control	T1 Azolla	T2 Duckweed	T3 Napier	p-value
Total protein (g dL^{-1})	3.24± 0.17^{b}	3.82± 0.21^{ab}	4.1± 0.14^{a}	3.1± 0.17^{c}	0.003
Albumin (g dL^{-1})	1.67± 0.03^{b}	1.92± 0.14^{a}	2.1± 0.03^{a}	1.52± 0.12^{b}	0.004
Globulin (g dL^{-1})	1.62± 0.03^{b}	1.92± 0.12^{a}	2.1± 0.15^{a}	1.54 ± 0.14^{b}	0.003
Glucose (mg dL^{-1})	62.2± 0.1^{a}	57.1± 0.4^{b}	53.6± 0.3^{b}	66.4±0. 3^{a}	0.002
AST (U L^{-1})	44.4± 0.3^{b}	39.3±0. 4^{ab}	36.6± 0.2^{a}	53.5±0.4^{c}	0.001
ALT (U L^{-1})	32.1± 0.4^{b}	26.1± 0.5^{ab}	23.1 ±0.3^{a}	36.1±0. 4^{c}	0.004
ALP (U L^{-1})	152.4 ±0. 5^{b}	137.3 ±0.6^{ab}	122±0. 2^{a}	163.1±0.6^{c}	0.003
Cholesterol (mg dL^{-1})	148.9±0.6 ^{b}	142.4±0.7^{ab}	138.1± 0.3^{a}	157.2±0. 2^{c}	0.004
Triglycerides (mg dL^{-1})	87.1 ±0.4^{b}	79.4 ±0. 4^{ab}	76.7 ±0.8 ^{a}	91.2±0. 4^{c}	0.002

Note. AST = Aspartate Aminotransferase (U L^{-1}), ALT = Alanine Aminotransferase (U L^{-1}), and ALP = Alkaline Phosphatase (U L^{-1}).

DISCUSSION

The present study was conducted on *Ctenopharyngodon idella* to examine the effect of dietary supplementation with Azolla, Duckweed, and Napier grass on growth performance, feed utilization, hematological indices, and serum biochemical parameters. among all the dietary supplements, Duckweed (10% inclusion) produced the most promising results in terms of growth performance and other parameters, while Napier grass supplementation produced the least desirable outcomes. These results highlight the potential of Duckweed and Azolla, to a lesser extent, as sustainable and nutritionally beneficial feed components for grass carp aquaculture. The duckweed-fed group, compared with the control and Napier-fed fish, highlights the significant enhancement in final body weight, weight gain, and specific growth rate. This is consistent with **Achoki *et al*. (2024)** and **Vulpe *et al*. (2025)**, who described improved growth performance and feed efficiency in carps fed diets encompassing *Lemna minor*. Similarly, **Goswami *et al*. (2022)** and **Sarkheil *et al*. (2024)** described improved antioxidant responses and feed conversion ratios in tilapia supplemented with Duckweed meal. Duckweed, recognized as a high-nutritional-quality protein source, contains a balanced amino acid profile, 25–35% crude protein, which supports muscle deposition and protein synthesis in fish **(Can Karaca *et al*., 2023)**. The Azolla did not differ significantly from the control in most growth parameters and produced intermediate results, although a trend toward improvement. Previous studies have shown that Azolla meal, without damaging growth, can partially substitute fishmeal or soybean meal in aquafeeds **(Rahmah *et al*., 2022; Sallam *et al*., 2024)**. **Oyedara *et al*. (2022)** explained that Azolla has moderate beneficial effects compared with Duckweed and less pronounced growth-promoting effects relative to Duckweed. However, differences in antinutritional factors and protein digestibility encompass 20–30% crude protein, essential amino acids, and bioactive compounds **(Shea *et al*., 2024).**

In contrast, Napier grass supplementation compared with the other groups caused significantly higher feed conversion ratio, lower growth performance, and reduced protein efficiency ratio. **Kuangkam *et al*. (2024)** described that Napier grass is widely used as livestock fodder and has inadequate nutrient digestibility and assimilation in grass carp with lower crude protein content (8–12%). **Klahan *et al*. (2024)** recommended the use of Napier grass as a partial roughage source rather than a primary dietary supplement in aquaculture feeds. In the current study, hematological catalogues serve as sensitive biomarkers of physiological adaptability, immune status, and fish health. Duckweed supplementation significantly improved hematocrit levels, WBC, RBC counts, and hemoglobin concentration compared with the control and Napier groups. Additionally, higher WBC counts in the Duckweed and Azolla groups recommend a delicate immune response in stress adaptation and pathogen defense. These enhancements upgraded oxygen transport and immune competence in Duckweed-fed fish. Similar findings were reported by **Li *et al*. (2021)** and **Bardhan *et al*. (2024)**, who highlighted that raised RBC and Hb values are characteristics of improved oxygen-carrying capacity and erythropoiesis under nutritionally balanced diets. Azolla supplementation upgraded hematological catalogues to a lesser extent than Duckweed. These results align with **Abu-Zahra *et al*. (2025)**, who reported improved immune parameters in fish fed Azolla meal. In contrast, Napier grass supplementation caused significantly miserable RBC, Hb, and hematocrit values, potentially reflecting mild physiological stress and poor nutrient utilization. Concentrated hematological catalogues in fish have been linked to nutritional shortages, impaired erythropoietic activity **(Aaqillah-Amr *et al*., 2024)**. Duckweed-fed fish group in this study showed significantly higher globulin concentrations, total protein, and albumin, compared with control and Napier-fed fish, indicating boosted immune function and protein metabolism. Similar outcomes were observed in tilapia; Duckweed supplementation raised improved disease resistance and serum protein fractions **(Tadesse *et al*., 2024)**. Azolla supplementation produced comparable but slightly lower enhancements, documenting the findings of **Minahal *et al*. (2024)**, who distinguished increased improved immune status and serum protein in rohu fed Azolla diets. Serum glucose levels were significantly lower in Duckweed- and Azolla-fed groups. **Liu *et al*. (2024)** described stress-induced hyperglycemia and impaired carbohydrate metabolism in nutritionally challenged fish in the Napier-fed group, with higher glucose levels. cholesterol and triglyceride levels were significantly reduced in Duckweed-fed fish compared with control and Napier groups. Lower serum lipid concentrations are associated with better energy utilization and lipid utilization, as supported by **Jiang *et al*. (2024)**, who observed similar decreases in serum lipids following dietary inclusion of plant-based extracts. Conversely, Napier-fed fish showed raised cholesterol and triglycerides, representing a potential risk of fatty liver growth due to inefficient lipid metabolism. Hepatic enzyme activities (AST, ALT, ALP) were significantly reduced in Duckweed-fed fish compared with control and Napier groups. Similar trends were reported by **Trejo-Escamilla *et al*. (2021)**, who described lower AST and ALT activities with better liver fish integrity in fish receiving balanced plant-based diets. **Guzmán-Carrasco *et al*. (2024)** studied reduced enzyme activities in the Duckweed group, inferring that this ingredient provides hepatic health.

CONCLUSION

This study deduces that Azolla, Duckweed, and Napier grass have different impacts on the growth and physiology of grass carp (*Ctenopharyngodon idella*). Duckweed at 10% inclusion produced the best results for growth, feed use, and blood health, while Azolla provided moderate benefits, and Napier grass was the least effective due to its lower protein and higher fiber content. Overall, Duckweed and Azolla appear to be promising, low-cost feed resources for sustainable carp farming, whereas Napier grass may serve only as a partial roughage ingredient.

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