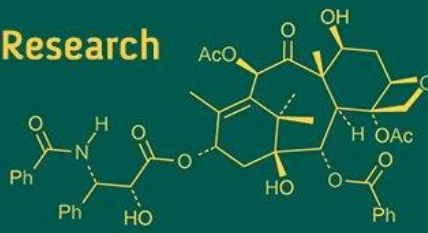


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Effect of azolla-based feed on growth and survivability of Indian Butter catfish (*Ompok bimaculatus*)

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Abstract

Aquaculture is a critical component of global food production, and developing sustainable feed alternatives is essential to support its growth. This study investigated the effects of Azolla supplementation on the growth performance and survival of Indian butterflyfish (*O. bimaculatus*) fingerlings. Four dietary treatments were evaluated over a 60-day trial: a commercial control diet (T1), a commercial diet with 20% Azolla replacement (T2), 30% Azolla replacement (T3), and a 100% Azolla-based diet (T4). Growth parameters were assessed, including daily weight gain, specific growth rate, feed conversion ratio, and survival rate. The results demonstrated that T2 achieved the highest growth performance, with a daily weight gain of 0.29 g, a specific growth rate of 1.75%, and an optimal feed conversion ratio of 1.61. In contrast, T4 exhibited the lowest growth metrics and a reduced survival rate of 83.33%. These findings indicate that partial replacement of commercial feed with 20% Azolla enhances growth efficiency and survivability in *O. bimaculatus*, whereas a complete Azolla diet is suboptimal. The study underscores Azolla's potential as a sustainable, cost-effective supplementary feed in aquaculture.

Keywords: Azolla, *Ompok bimaculatus*, growth, survivability

Introduction

Despite significant advances in global food production, approximately 370 million people, representing over 5% of the world's population, are projected to experience hunger by 2060, particularly in developing nations (Parry *et al.*, 2014) [23]. The accessibility and affordability of protein sources for aquafeed production represent critical factors determining the sustainability and success of aquaculture operations (FAO, 2018). In recent decades, plant-based protein sources have gained recognition as sustainable alternatives to fishmeal in aquafeeds, owing to their environmental benefits, cost efficiency, and reliable availability (Gatlin *et al.*, 2007; Ahmadifar *et al.*, 2019; Elumalai *et al.*, 2020) [2, 14, 18]. Among these alternatives, Azolla, a floating aquatic fern commonly found in stagnant or slow-moving freshwater ecosystems across Africa, Asia, and parts of Australia, has emerged as particularly promising due to its rapid biomass production, high nutritional value, and adaptability to diverse environmental conditions.

Azolla pinnata establishes a symbiotic association with the nitrogen-fixing cyanobacterium *Anabaena azollae*, which inhabits the dorsal leaf cavities of the fern. This unique biological relationship enables Azolla to thrive in nitrogen-deficient environments, making it an excellent candidate for sustainable feed production. With a crude protein content ranging between 13% and 30%, complemented by a rich profile of essential amino acids, particularly lysine, Azolla demonstrates superior nutritional quality compared to many other aquatic macrophytes and conventional green feed crops (Brouwer *et al.*, 2018) [8]. The practical application of Azolla in aquaculture has been validated through its successful incorporation into diets for species such as Thai silver barb (*Barbonymus gonionotus*) and Nile tilapia (*Oreochromis niloticus*), where it has demonstrated positive effects on growth performance and feed utilization efficiency (Das *et al.*, 2018; Sivakumar and Solaimalai, 2003) [11, 26].

Furthermore, unlike seasonal crops, such as soybeans, Azolla offers the distinct advantage of continuous cultivation and harvest throughout the year, providing a consistent and economically viable protein source for aquaculture systems (Anglade *et al.*, 2015)^[3].

The Indian butterfish (*O. bimaculatus*), an economically important freshwater species, presents considerable potential for aquaculture development due to its high market demand and excellent nutritional profile. However, the cultivation of this species faces significant challenges related to feed formulation and growth optimization. Fish growth represents a complex biological process influenced by multiple dietary and environmental factors, typically quantified through various performance indices including daily weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio, feed acceptability, and survival rate (Lupatsch *et al.*, 2003; Simensen *et al.*, 2000)^[20, 25]. The implementation of Azolla-based feed formulations for *O. bimaculatus* holds promise for enhancing growth performance, given its favorable nutritional composition and cost-effectiveness. Maintaining optimal water quality parameters represents a fundamental requirement for successful aquaculture production, as these conditions directly influence fish health, feed utilization efficiency, and overall growth performance. Critical water quality parameters requiring careful management include temperature, pH, dissolved oxygen concentration, alkalinity, and nutrient levels (Bhatnagar and Devi, 2013)^[6]. Hypoxia, characterized by dissolved oxygen concentrations below 1 to 2 mg/L, can induce severe physiological stress in fish populations, leading to growth impairment and potentially significant mortality events (Silviana *et al.*, 2022)^[24]. A comprehensive understanding of the complex interactions between feed composition, water quality parameters, and growth performance remains essential for developing sustainable culture practices for *O. bimaculatus*.

2. Materials and methods

2.1 Experimental fish and setup

The present study was carried out at the College of Fisheries Sciences, Chaudhary Charan Singh Haryana Agricultural University (CCS HAU), Hisar. The study was conducted to investigate the effects of Azolla-based feed on the growth performance and survivability of Indian butterfish (*O. bimaculatus*) during the advanced fingerling stage. The experiment was carried out in 12 disinfected glass aquarium tanks, each with a capacity of 150 liters, over 60 days from October 5, 2022, to December 4, 2022. The experimental design included four treatments (T1: control; T2: 20% Azolla; T3: 30% Azolla; T4: 100% Azolla), each with three replications. Each replication was stocked with 10 acclimatized fingerlings, arranged in a randomized block design (RBD). Fish survival was monitored daily, and growth data were recorded weekly.

2.3 Preparation of experimental diet

Fresh Azolla was obtained from the Azolla culture unit of the College of Fisheries Science, CCS HAU, Hisar. The

Azolla was thoroughly washed, air-dried under sunlight for 48 hours, ground into fine powder, and stored at 4°C to prevent carotenoid oxidation. The experimental diets were formulated by incorporating Azolla powder at proportions of 0%, 20%, 30%, and 100% into a commercial feed using the Pearson square method to balance protein levels. The feed mixture was combined with a small amount of vegetable oil and water to form a dough, which was extruded through a hand-operated pelletizer (1 mm pore size). The pellets were dried in a hot air oven at 55°C and stored in airtight glass containers in a refrigerator at 4°C.

Table 1: Ingredient composition of the experimental diet

Treatment	Ingredient	Weight (g)	Total weight (g)
T ₁	Commercial feed	500	500
T ₂	Commercial feed + 20% Azolla feed	400 + 100	500
T ₃	Commercial feed + 30% Azolla feed	350 + 150	500
T ₄	100% Azolla feed	500	500

2.5 Feeding Protocol

The experimental fish were fed thrice daily at a rate of 5% of their body weight. Initial measurements of body length (mm) and weight (g) were recorded before the experiment, and feed quantities were adjusted weekly based on the mean weight of the fish. Growth parameters were measured at weekly intervals, and final measurements were recorded after 60 days of rearing.

2.6 Physico-Chemical Analysis of Water

Water quality parameters were monitored fortnightly between 9:00 a.m. and 10:00 a.m. throughout the experimental period using standard methods (APHA, 1998). Temperature was measured using a mercury thermometer, while pH levels were determined with a digital pH meter (Aquasol, AM-P-PH). Salinity was assessed using a microprocessor salinity meter (LT-51), and dissolved oxygen (DO) was estimated through the modified Winkler method using an Aquasol DO kit (AEDO8). Ammonia concentration was quantified using a visual colorimetric method with an AQUASOL testing kit, and alkalinity was determined through titration with sulfuric acid, employing phenolphthalein and methyl orange indicators. These parameters were carefully recorded to ensure the optimal rearing environment for the experimental fish and to evaluate any potential variations across treatments. Alkalinity: Measured through titration with sulfuric acid and indicators (phenolphthalein and methyl orange).

2.7 Growth Parameters

Growth parameters like Average daily weight gain (ADG), Feed conversion ratio (FCR), Specific growth rate (SGR), the percentage increase in length and weight, and survival rate, total weight gains were recorded fortnightly and evaluated using the provided formulas –

$$a) \text{ Average daily weight gain} = \frac{\text{Mean final weight} - \text{Mean initial weight}}{\text{culture day}} \times 100$$

$$b) \text{ Specific growth rate (SGR \%)} = \frac{\log \text{ final weight} - \log \text{ initial weight}}{\text{Number of days}} \times 100$$

$$c) \text{ Feed conversion ratio} = \frac{\text{Amount of feed fed}}{\text{weight gain}}$$

$$d) \text{ Survivability} = \frac{\text{Total number of fish harvested} - \text{total number of fish stocked}}{100}$$

$$e) \text{ Percentage increment in length} = \frac{\text{Mean final fish length} - \text{Mean initial fish length (cm)}}{\text{Mean initial fish length}} \times 100$$

$$f) \text{ Percentage increment in weight} = \frac{\text{Mean final fish weight} - \text{Mean initial fish weight (g)}}{\text{Mean initial fish weight}} \times 100$$

2.8 Proximate Composition Analysis

The proximate composition of the experimental diets was analyzed to assess their nutritional quality, following the guidelines of AOAC (1995). Moisture content was determined by drying the feed samples in an oven at 105°C until a constant weight was achieved. Crude protein was estimated using the Kjeldahl method, while crude lipid content was analyzed by Soxhlet extraction. Crude fiber was measured through sequential digestion with dilute acid and alkali, and ash content was determined by combusting the samples in a muffle furnace at 600°C. These analyses ensured that the formulated diets met the nutritional requirements of the fish and provided insights into the role of Azolla in enhancing dietary quality.

2.9 Statistical Analysis

All data were analyzed using a randomized block design (RBD) and ANOVA to determine treatment effects, with a significance threshold of $P < 0.05$. Statistical analyses were performed using OpStat software, while graphs and bar charts were generated using Microsoft Excel 2010.

3. Results and discussion

Growth performance and survival rates of *O. bimaculatus* were significantly influenced by dietary treatments and sampling periods ($p < 0.05$). Among experimental groups, the diet incorporating 20% Azolla (T_2) yielded the highest average daily weight gain (ADG: 0.31 g), followed by T_1 (0.26 g), T_3 (0.24 g), and T_4 (0.04 g). A parallel trend was observed in specific growth rate (SGR), with T_2 achieving the highest SGR (1.75%) and T_4 the lowest (0.39%). The feed conversion ratio (FCR), a critical indicator of nutrient utilization efficiency, varied significantly between control and treatment groups. Particularly, T_2 demonstrated superior FCR (1.61 ± 0.03) compared to T_1 (1.87 ± 0.03), T_3 (1.96 ± 0.02), and T_4 (6.61 ± 0.62), underscoring the efficacy of 20% Azolla inclusion. These results align with established optimal FCR ranges (1.5-2.0) for fish growth (Afia *et al.*, 2019) [1] and corroborate findings in *O. bimaculatus* (1.58-

1.85; Chavez *et al.*, 2011) [9] and other teleosts (Cremer *et al.*, 2003; Jayakumar *et al.*, 2014) [10, 19]. Percentage weight gain varied significantly across dietary treatments ($p < 0.05$), with T_2 exhibiting the highest increase (259.24%), followed by T_1 (232.76%), T_3 (231.47%), and T_4 (37.68%); maximal gains occurred by the 60th day. Length increments mirrored this pattern: T_2 (4.48%) outperformed T_1 (4.21%), T_3 (3.81%), and T_4 (0.64%), peaking by day 8. Survival rates remained stable in T_1 (100%), except for transient declines (96.66%) on days 32 and 40. Similarly, T_2 maintained 100% survival until a minor reduction (96.66%) on day 40. In contrast, higher Azolla inclusion (T_3 : 30%; T_4 : 100%) induced progressive survival declines, with T_3 reduced to 86.66% and T_4 to 83.33% by day 60, consistent with trends reported for *L. rohita* (Panigrahi *et al.*, 2014) [22]. Statistical analysis confirmed significant interactions ($p < 0.05$) between dietary treatments and sampling periods, reinforcing the benefits of 20% Azolla supplementation. These findings align with studies reporting enhanced growth in cyprinids with Azolla inclusion (Ayapan, 1992; Panigrahi *et al.*, 2014; Datta, 2011) [12, 22], though optimal levels are species-specific (e.g., 10-25% for grass carp; Majhi, 2006; Tuladhar, 2003; Gangadhar *et al.*, 2017) [17, 21, 27]. Water quality parameters remained within optimal thresholds throughout the trial, supporting physiological performance. Temperatures (22.73-24.06°C) and dissolved oxygen (5.7-6.07 mg/L) aligned with metabolic requirements for growth (Boyd, 1982; Bailey & Alanara, 2006) [5, 7]. Stable pH (7.33-7.93) and alkalinity (115.83-142.39 mg/L), coupled with non-toxic ammonia (< 0.035 mg/L), nitrite, nitrate, and salinity (0.24-0.28 ppt), ensured species-appropriate conditions (Dubey *et al.*, 2022; Bhatnagar *et al.*, 2013) [6, 13]. Regular waste removal and 20% daily water exchange maintained these parameters, emphasizing the necessity of integrated husbandry practices in aquaculture systems (Majhi *et al.*, 2006; Gangadhar *et al.*, 2015) [17, 21]. Collectively, these findings demonstrate the nutritional viability of 20% Azolla in *O. bimaculatus* diets, optimizing growth and survival without compromising water quality.

Table 2: Average daily weight gain (g) of Indian Butter catfish at different percentages of Azolla diet

Sampling period (days)	Treatment				Mean
	T ₁ (Commercial feed)	T ₂ (T ₁ + 20% Azolla feed)	T ₃ (T ₁ + 30% Azolla feed)	T ₄ (100 % Azolla feed)	
8	0.26	0.37	0.23	0.04	0.23 ^b
16	0.25	0.34	0.23	0.04	0.22 ^{ab}
24	0.25	0.31	0.24	0.04	0.21 ^a
32	0.26	0.29	0.24	0.04	0.21 ^a
40	0.26	0.29	0.25	0.04	0.21 ^a
48	0.25	0.28	0.25	0.04	0.21 ^a
56	0.26	0.29	0.25	0.04	0.21 ^a
60	0.27	0.31	0.27	0.04	0.22 ^{ab}
Mean	0.26	0.31	0.24	0.04	

Values with same superscript do not differ significantly.

CD ($p=0.05$) for Sampling period = 0.01; S E (m) = 0.002

CD ($p=0.05$) for different percent of Azolla diet = N/S; S E (m) = N/S

CD ($p=0.05$) for Sampling period $\times$ different percent of Azolla diet = 0.01; S E (m) = 0.003

Table 3: Specific growth rate (%) of Indian Butter catfish at different percent of Azolla diet

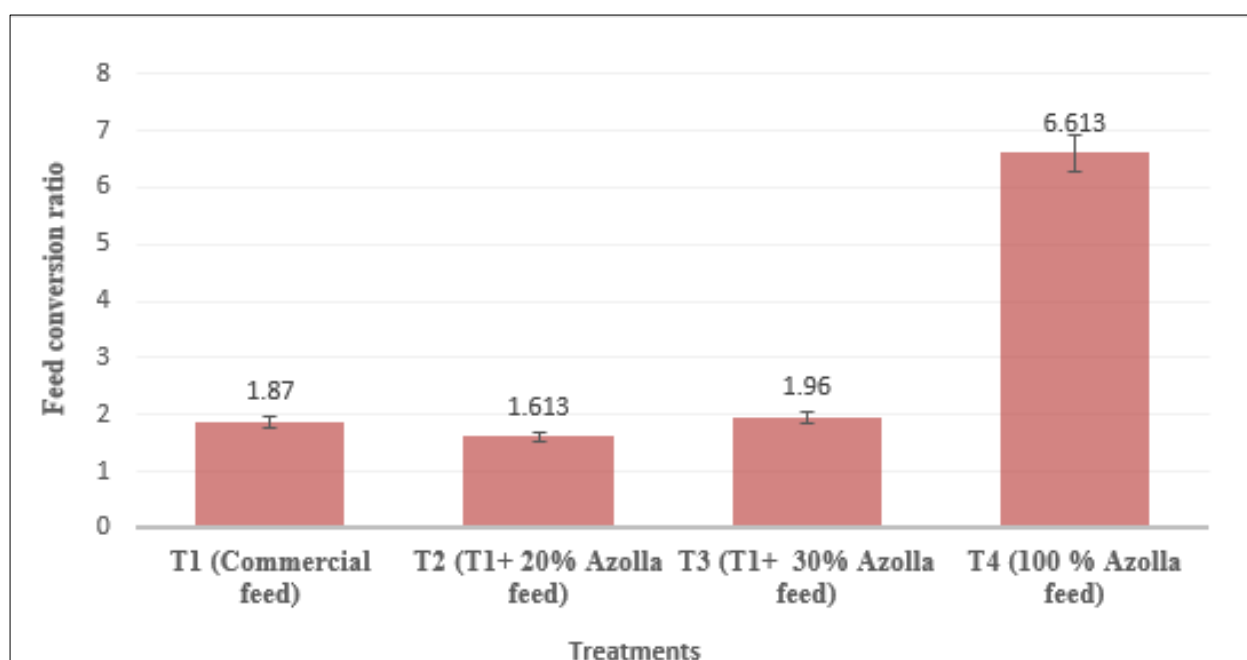
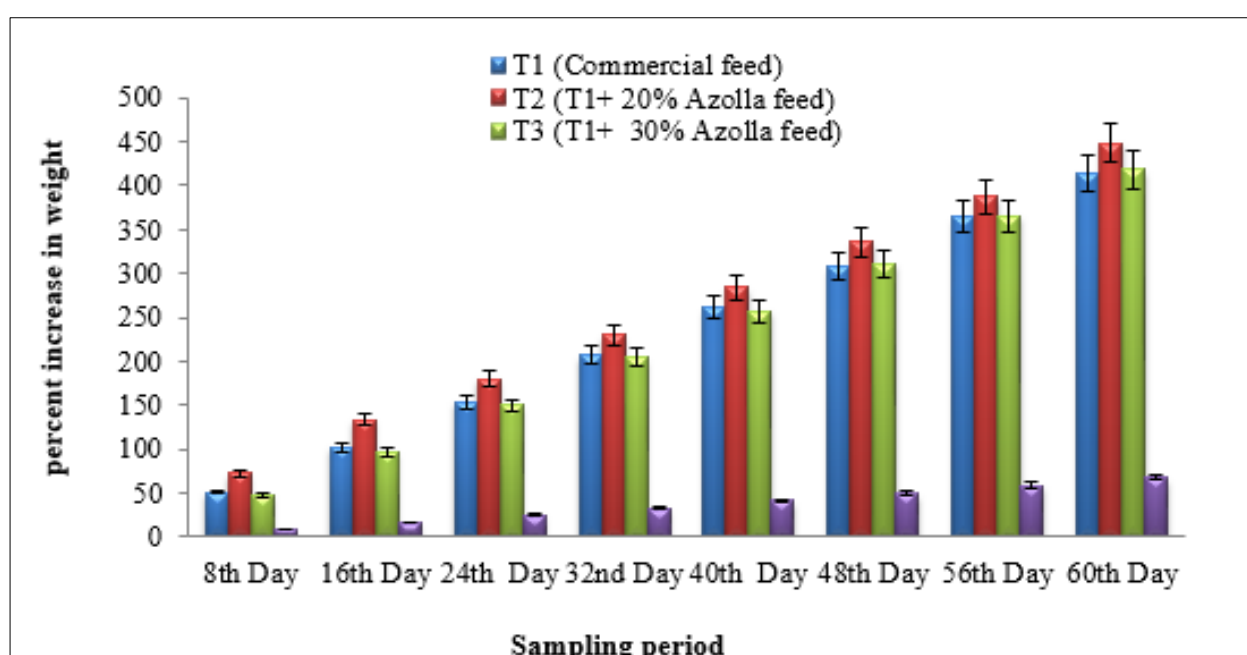
Sampling period (days)	Treatment				Mean
	T ₁ (Commercial feed)	T ₂ (T ₁ + 20% Azolla feed)	T ₃ (T ₁ + 30% Azolla feed)	T ₄ (100 % Azolla feed)	
8	2.26	2.97	2.15	0.45	1.96
16	1.92	2.30	1.84	0.41	1.62
24	1.68	1.87	1.66	0.41	1.40
32	1.52	1.62	1.51	0.39	1.26
40	1.39	1.46	1.38	0.38	1.15
48	1.27	1.33	1.28	0.36	1.06
56	1.19	1.23	1.19	0.36	0.99 ^a
60	1.19	1.23	1.19	0.38	1.00 ^a
Mean	1.55 ^a	1.75	1.53 ^a	0.39	

Values with same superscript do not differ significantly.

CD (p=0.05) for Sampling period =0.03; S E (m) = 0.01

CD (p=0.05) for different percent of Azolla diet = 0.02; S E (m) = 0.01

CD (p=0.05) for Sampling period × different percent of Azolla diet = 0.07; S E (m) = 0.02

**Fig. 1:** Feed conversion ratio of *O. bimaculatus* at different percent of Azolla diet**Fig 2:** Percent increase in weight of *O. bimaculatus* at different percentages of Azolla diet

3.5 Survivability of fingerlings of *Ompok bimaculatus*

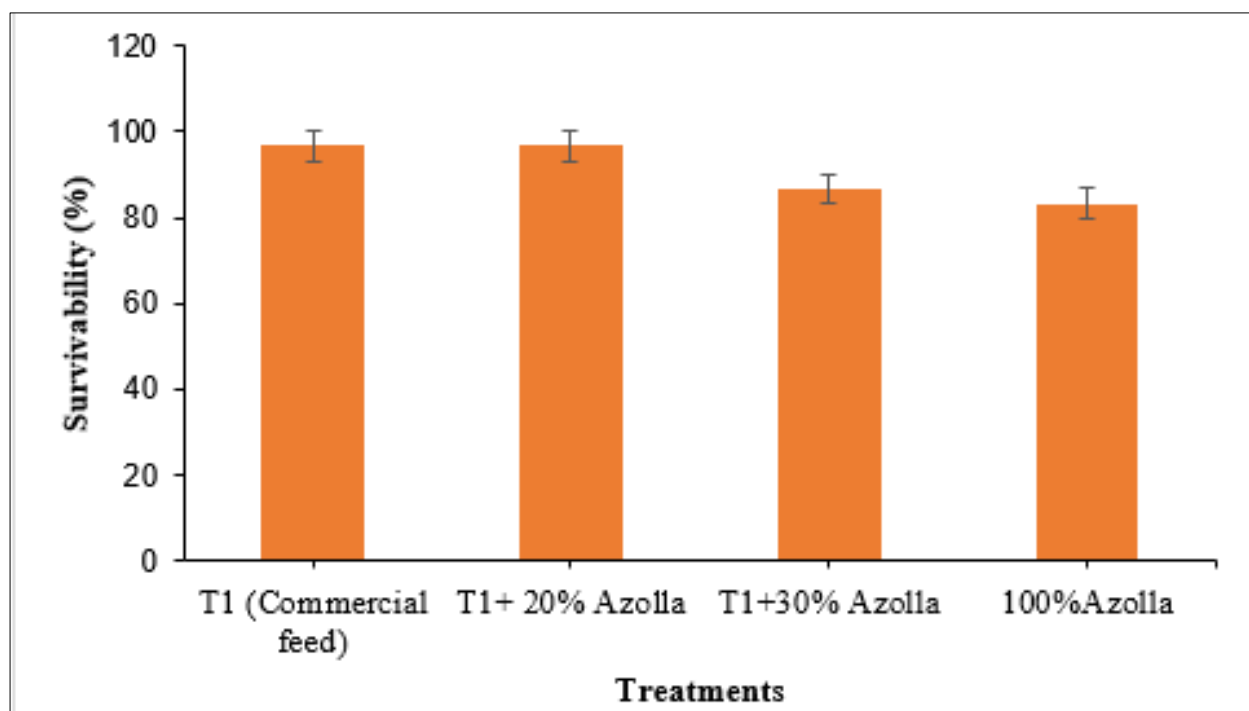


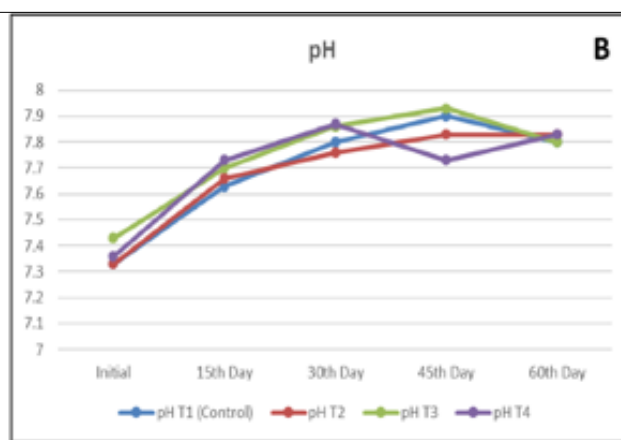
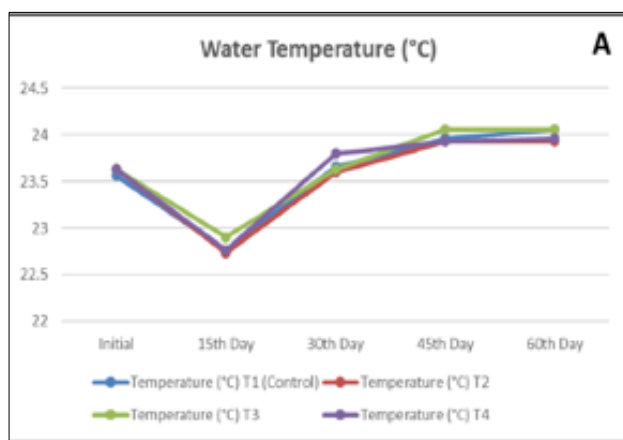
Fig 3: Survivability of fingerlings of *O. bimaculatus* at different percentages of azolla diet

Table 5: Proximate composition (%) of experimental diets.

Experimental feed	Crude Protein (%)	Moisture (%)	Crude Fat (%)	Total Ash (%)
Commercial feed (T1)	32	10	11	8
T1+ 20% Azolla	36.4	9.7	8.4	8
T1+30% Azolla	36.2	10.4	7.6	9.5
100% Azolla	25.3	7.5	5	16

Table 6: Measurements of length and weight of *O. bimaculatus* at different percentages of Azolla diet.

Treatments Parameters	T ₁ (Control)	T ₂ (T1+20%Azolla)	T ₃ (T1+30%Azolla)	T ₄ (100% Azolla)
Initial length (cm)	9.24±0.02	9.34±0.02	9.16±0.03	9.28±0.03
Final length (cm)	12.81 ± 0.03	13.21 ± 0.1	12.54 ± 0.05	9.68 ± 0.09
Final mean body length (cm)	12.74 ± 0.08	12.92 ± 0.05	12.29 ± 0.04	9.46 ± 0.02
Initial weight (g)	4.12±0.01	4.33±0.01	3.91±0.01	4.26±0.01
Final weight (g)	21.02 ± 0.08	23.01 ± 0.13	20.21 ± 0.11	7.01 ± 0.06
Final mean body weight (g)	20.74 ± 0.05	22.64 ± 0.06	19.95 ± 0.09	6.63 ± 0.08
Total weight gain (g)	16.71 ± 0.06	18.51 ± 0.07	16.10 ± 0.09	2.68 ± 0.05



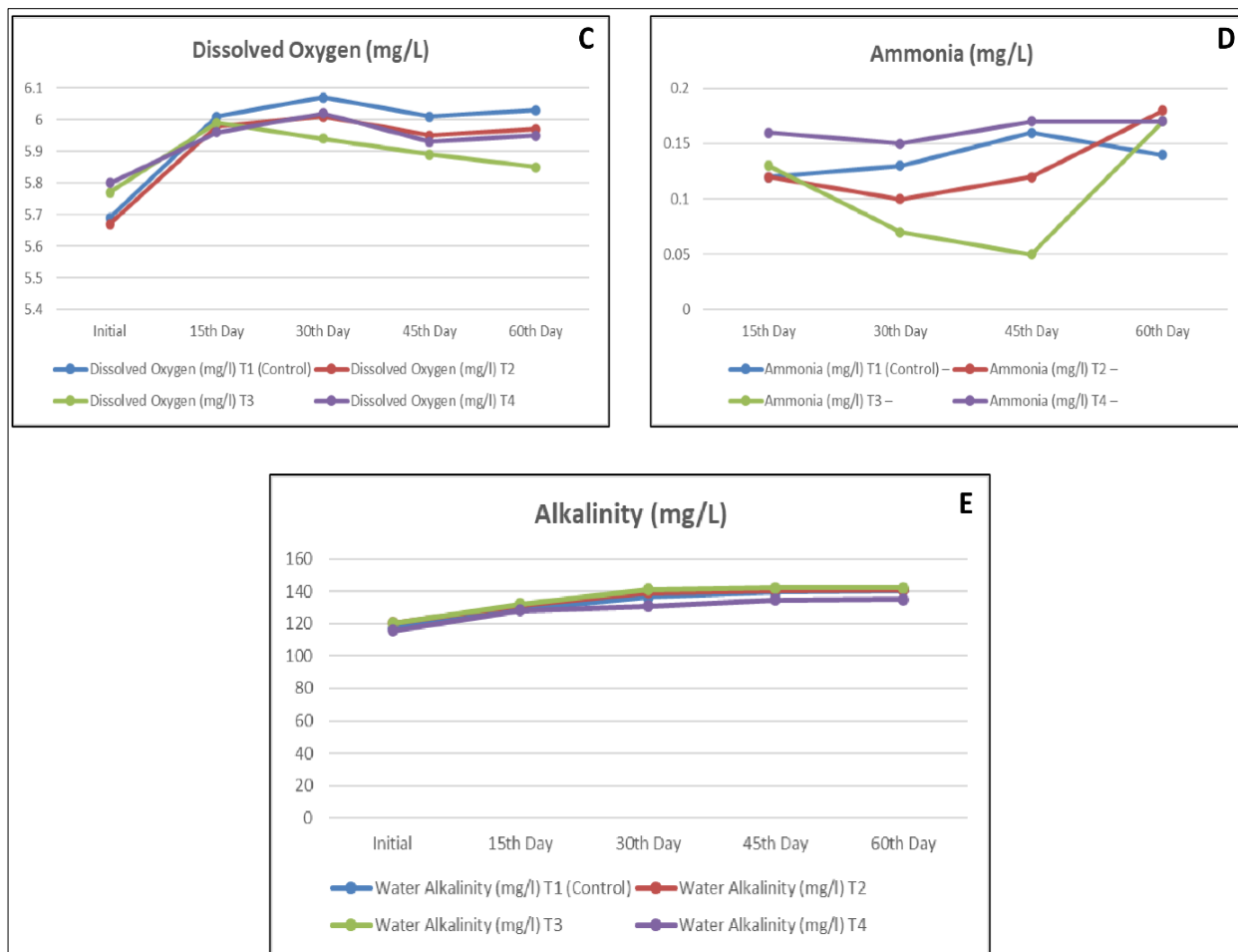


Fig 4: Variations in different water quality parameters: (A) temperature, (B) pH, (C) dissolved oxygen, (D) ammonia, (E) alkalinity in the experimental unit.

Conclusion

This study conclusively demonstrates that a 20% Azolla inclusion (T₂) optimizes growth performance (ADG: 0.31 g; SGR: 1.75%), feed efficiency (FCR: 1.61 ± 0.03), and survival in *O. bimaculatus* fingerlings, significantly outperforming diets with higher Azolla concentrations. The severe decline in growth and survival observed at 100% inclusion (T₄), coupled with the significant treatment $\times$ time interaction ($p < 0.05$), underscores the critical need for moderated supplementation, aligning with optimal ranges (10-25%) established for cyprinids (Majhi, 2006; Panigrahi *et al.*, 2014)^[21, 22]. Stable maintenance of water quality parameters, temperature (22.73-24.06°C), dissolved oxygen (5.7-6.07 mg/L), pH (7.33-7.93), and ammonia (<0.035 mg/L) within species-optimal thresholds (Boyd, 1982; Bhatnagar *et al.*, 2013)^[6, 7] was fundamental to these outcomes, highlighting the essential synergy between nutritional strategy and environmental management. We therefore recommend incorporating 20% Azolla into commercial *O. bimaculatus* diets to maximize cost-efficiency and productivity, while strongly advising against inclusions exceeding 30% due to significant survival compromises. Future research should prioritize defining species-specific tolerance thresholds, evaluating long-term physiological and product quality impacts, and assessing ecological implications of scaled Azolla utilization, alongside standardized water management protocols to ensure replicability.

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