Journal of Agriculture and Rural Development in the Tropics and Subtropics
Vol. 127 (2026) 11–18

https://doi.org/10.17170/kobra-2026011411799 ISSN: 2363-6033 (online); 1612-9830 (print) – website: www.jarts.info

Growth performance, amino acid composition and biochemical parameters of *Anabas testudineus* (Bloch, 1792) fed with *Ipomoea aquatica* supplemented diets

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Abstract

This study investigated the potential for supplementing the diet of the climbing perch (*Anabas testudineus*) with *Ipomoea aquatica* by evaluating its growth performance, amino acid composition and biochemical parameters. A 60-day feeding trial was conducted using five isonitrogenous diets that incorporated varying levels of *Ipomoea aquatica*: IP0 (0 %), IP5 (5 %), IP10 (10 %), IP15 (15 %) and IP20 (20 %). Growth parameters, amino acid profiles and biochemical markers were analysed to determine the optimal dietary inclusion level. The results revealed that 15 % inclusion of *I. aquatica* resulted in the highest final weight (FW), body weight gain (BWG) and specific growth rate (SGR), as well as an improved feed conversion ratio (FCR). This group also exhibited enhanced fish muscle amino acid composition, particularly of essential amino acids such as methionine, phenylalanine, and tryptophan. A similar increase in total immunoglobulin (TIg), lysozyme (LYZ) and alkaline phosphatase (ALP) activities was observed in this group, suggesting improved immunity and health. No significant changes were observed in aspartate aminotransferase (AST), alanine aminotransferase (ALT), superoxide dismutase (SOD) activities or thiobarbituric acid reactive substances (TBARS) across the different levels of inclusion. These findings suggest that *I. aquatica* can serve as a sustainable alternative protein source in *A. testudineus* feed, with a 15 % inclusion level providing optimal growth, nutritional benefits, and immune enhancement. This study highlights the potential of *I. aquatica* as a viable alternative to conventional fishmeal and promotes sustainable aquaculture practices.

Keywords: alternative protein source, essential amino acids, sustainable aquaculture

1 Introduction

Aquaculture has the potential to meet rising global fish demand (Hua *et al*., 2019). However, feed remains a major challenge, accounting for 50–70 % of production costs (Iskander *et al*., 2019; Andriani *et al*., 2019). The climbing perch, *Anabas testudineus* (Bloch, 1792), is a valuable aquaculture species due to its resilience and nutritional quality, making it suitable for sustainable production (Talwar & Jhingran, 1992). Yet its farming is constrained by dependence on costly animal-based protein sources (Devi *et al*., 2022). To address this, studies have examined cost-effective, sustainable plant-based protein alternatives (Dorothy *et al*., 2018; Sonta *et al*., 2019). Among these, freshwater aquatic weeds, particularly macrophytes, have gained attention as substitutes for animal proteins because of their rich nutrient profiles, rapid growth, and broad availability (Naseem *et al*., 2021; Muchahary & Khangembam, 2024). The semi-aquatic *Ipomoea aquatica* has emerged as a promising feed ingredient due to its abundance of essential nutrients, vitamins, and bioactive compounds (Austin, 2007; Adedokun *et al*., 2019). Inclusion of *I. aquatica* in fish diets has been shown to improve growth in species such as *Clarias gariepinus* (Odulate *et al*., 2013) and *Oreochromis niloticus* (Ramzy *et al*., 2019; Yousif *et al*., 2019). However, its potential as a dietary supplement remains untested for air-breathing species like *A. testudineus*.

A key determinant of fish quality and nutritional value is its amino acid composition, which is shaped by dietary in-

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Published online: 20 January 2026 – Received: 23 April 2025 – Accepted: 23 December 2025
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puts. Analysing the amino acid profile of fish fed specific diets provides insights into feed adequacy and physiological effects (Saikia *et al*., 2023). Given the importance of protein quality in aquaculture, evaluating the amino acid composition of *A. testudineus* fed *I. aquatica*-supplemented diets is essential for assessing nutritional efficacy. Beyond growth and nutrition, immune and biochemical parameters are also critical indicators of fish health (Roy *et al*., 2022). Although plant-based diets offer potential benefits, they may introduce anti-nutritional factors and anti-proteases that can affect health. Research on plant-based feeds has produced mixed outcomes: some studies report improved growth and immunity, such as in red sea bream, *Pagrus major* (Dossou *et al*., 2018), while others note reduced immunity in carnivorous species (Maita *et al*., 2006; Daniel, 2018) or no adverse effects, as seen in large yellow croaker, *Larimichthys crocea* (Wang *et al*., 2017). This variability highlights the need for species-specific evaluation.

This study therefore examines the effects of *I. aquatica*-supplemented feed on the growth performance, amino acid composition, and immune and biochemical parameters of *A. testudineus*. By analysing these factors, the study aims to identify the optimal level of *I. aquatica* inclusion that maximises growth, supports immune function, and ensures a balanced nutritional profile to promote sustainable aquaculture practices.

2 Materials and methods

2.1 Feeding unit

The feeding trial was conducted at the wet lab facility of Bodoland University in Kokrajhar, Assam, India, for 60 days. A batch of 450 *A. testudineus* juveniles, averaging 0.75 ± 0.01 g in weight and 4.1 ± 0.03 cm in length, was obtained from Bijni fish farm in Chirang, Assam. These fish were randomly distributed among 15 aquaria (50 litres each), with 30 fish per aquarium in triplicate. Each tank had an inlet and outlet system for water aeration and renewal. Water temperature, dissolved oxygen, and pH were monitored according to APHA (2017) guidelines. The recorded ranges were 26.4–27.6 °C for temperature, 6.53–7.47 mg L^{-1} for dissolved oxygen, and 6.80–7.15 for pH during the feeding trial.

2.2 Experimental diet

Samples of *I. aquatica* were collected from Kokrajhar, Assam, India, and were initially air-dried until the moisture content was below 50 %. These were then oven-dried at 50 °C, crushed, and passed through a 1 mm mesh. Five isonitrogenous diets (40 % crude protein) with different percentages of *I. aquatica* were prepared: IP0 (0 %), IP5 (5 %), IP10 (10 %), IP15 (15 %), and IP20 (20 %), as detailed in Table 1. The feed ingredients (for each diet) were mixed, made into a dough with water, and extruded through a 1 mm mesh to create pellets. These pellets were dried at 50 °C and stored for feeding. Fish were fed twice daily at 5 % of their body weight (9:00 a.m. and 4:00 p.m.) for 60 days. Uneaten feed was collected and oven-dried at 50 °C for one hour to measure feed intake.

The proximate composition of diets was analysed using standard methods outlined by AOAC (2000). Total nitrogen was measured using the micro Kjeldahl method. Samples were air-dried, and their moisture content was measured by heating in a hot oven at 135 °C for two hours. Crude fats were extracted using petroleum ether, followed by a Soxhlet extraction process. Ash content was determined by incinerating samples in a muffle furnace at 550 °C for 16 hours. The crude fibre was measured gravimetrically after chemical digestion and removal of soluble components.

2.3 Sampling and growth parameters

Fish length and weight were recorded weekly throughout the experimental period. After the 60-day trial, the fish underwent fasting for 24 hours, after which they were anesthetised with phenoxyethanol (0.5 mL per litre). Final length and weight measurements were taken. Several growth-related parameters were assessed, including survival rate (SR), body weight gain (BWG), specific growth rate (SGR) and feed conversion ratio (FCR), following the standard protocol (Castell & Tiews, 1980) as follows:

$$\text{SR}\ (\%) = \frac{N_f}{N_i} \times 100 \tag{1}$$

$$\text{BWG}\ (\%) = \frac{W_f - W_i}{W_i} \times 100 \tag{2}$$

$$\text{SGR}\ (\%\ \text{day}^{-1}) = \frac{\ln(W_f) - \ln(W_i)}{t} \times 100 \tag{3}$$

$$\text{FCR} = \frac{\text{Dry feed fed (g)}}{\text{Weight gain (g)}} \tag{4}$$

Where: N_i = initial number of fish; N_f = final number of fish; W_i = initial body weight (g); W_f = final body weight (g); t = duration of the feeding trial (days).

2.4 Amino acid analysis

The procedure for amino acid profiling using LC-MS involves two extraction methods outlined by Nimbalkar *et al*. (2012): one for free amino acids and another for bound

Table 1: *The proximate composition (as a percentage of dry weight) of test diets at varying percentage inclusion of* Ipomoea aquatica.

Ingredients (%)	*IP0*	*IP5*	*IP10*	*IP15*	*IP20*
Dry fish powder*	47.27	46.33	45.38	44.44	43.50
Wheat flour*	51.33	47.27	43.22	39.16	35.10
I. aquatica	0	5	10	15	20
Vitamin & mineral premix†	0.4	0.4	0.4	0.4	0.4
Cod Liver Oil‡	1	1	1	1	1
Proximate composition (%)					
Protein	39.94	39.98	39.57	39.85	39.95
Ash	7.34	7.37	7.36	7.38	7.36
Moisture	5.60	5.49	5.43	5.55	5.61
Lipid	4.93	4.96	5.04	5.04	5.13
Fibre	1.86	1.85	1.91	1.95	1.94
Carbohydrate	40.33	40.35	40.69	40.23	40.01
Energy (Kcal/100 g)	365.45	365.96	366.40	365.68	366.01

Note: IP0–IP20 represent feeds supplemented with 0, 5, 10, 15, and 20 % *I. aquatica*. *Local market, Kokrajhar, Assam.
†Vitamins: 1.5 mg Vitamin B6, 1.5 mg Folic Acid, 5 mg Vitamin B1, 5 mg Vitamin B2, 10 mg Calcium D-Pantothenate, 25 mg Tocopheryl Acetate, 50 mg Vitamin B3, 75 mg Ascorbic acid, 150 mcg D-Biotin USP, 400 IU Vitamin D3, 500 mcg Methylcobalamin, 5000 IU Vitamin A. Amino acid: 50 mg L-Glutamic acid. Trace Elements: 5 mg Manganese Sulphate Monohydrate, 25 mcg Sodium Molybdate, 70 mcg Selenium, 250 mcg Chromium Picolinate, 2 mg Copper Sulphate. ‡SEACOD, Cod Liver Oil (Type B) BP Universal Medicare, Mumbai, India.

amino acids through acid hydrolysis. The samples were homogenised with 0.1 % formic acid in 20 % methanol to extract free amino acids, followed by centrifugation and filtration. For bound amino acids, samples were hydrolysed with 6 M hydrochloric acid, processed, and reconstituted before injection. The LC-MS conditions include gradient composition, temperature control, and a PDA detector for monitoring amino acids. The mobile phase for analysis consists of water with 0.1 % formic acid and a mixture of water and methanol (50:50) with 0.1 % formic acid, and the samples were analysed using the Waters Acquity UPLC H (TQD MS/MS, USA) system.

2.5 Immune and biochemical parameters

Mucus and wet muscle tissue samples were collected in triplicate from each dietary group. Alkaline phosphatase (ALP), total immunoglobulin (TIg) and lysozyme (LYZ) activity were measured in the mucus samples, while catalase (CAT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), superoxide dismutase (SOD) activities, and thiobarbituric acid reactive substances (TBARS) were assessed in muscle tissue samples. Mucus collection followed a modified method from Ross *et al.* (2000): fish were starved for 24 hours, anesthetised with phenoxyethanol (0.5 mL L^{-1}), and mucus was collected by placing fish in a polyethylene bag with 10 mL of 50 mM NaCl and shaking gently. The samples were centrifuged at 1500 × g (10 minutes, 4 °C), and the supernatant was stored at −80 °C.

The TIg concentration was measured using the method of Siwicki and Anderson (1993). AST and ALT activity were determined using buffered aspartate-alpha-ketoglutarate and buffered alanine-alpha-ketoglutarate substrates, respectively (Reitman & Frankel, 1957). LYZ activity was determined using the turbidometric assay (Ross *et al*., 2000). ALP activity was measured by the method of Ross *et al.* (2000). The decrease in absorbance at 240 nm measured the CAT activity upon adding the sample to H_2O_2 (Aebi, 1983; Li & Schellhorn, 2007; Vinagre *et al*.,2012). The SOD activity was tested using xanthine oxidase following Roy *et al.* (2020). The TBARS was measured using tetramethoxypropane as the external standard according to the method of Ohkawa *et al.* (1979). The total soluble protein content of mucus and tissue samples was assessed using the method of Bradford (1976), with bovine serum albumin (BSA) at 1 mg ml^{-1} taken as the standard.

2.6 Statistical analysis

The Shapiro-Wilk test was utilised to assess normality, while Levene's test was employed to examine the homogeneity of variances. Differences among group means were subsequently analysed using one-way ANOVA and Tukey's post hoc tests to determine significant differences, with a significance threshold established at $p < 0.05$. All statistical analyses were conducted using SPSS version 23. Data were presented as mean ± standard deviation.

3 Results

3.1 Growth performance

Growth parameters increased in all groups of fish fed the diet incorporating *I. aquatica*. However, the final weight (4.29 ± 0.01 g), body weight gain (471.71 ± 1.26 %) and specific growth rate (2.91 ± 0.00 % day^{-1}) of fish fed with diet IP15 were noticeably higher ($p < 0.05$) than those of the other groups (Fig. 1a–d). Notably, no mortality was observed throughout the experimental period. Additionally, the IP15 group showed a significantly lower feed conversion ratio (1.36 ± 0.01) than the groups fed other diets ($p < 0.05$).

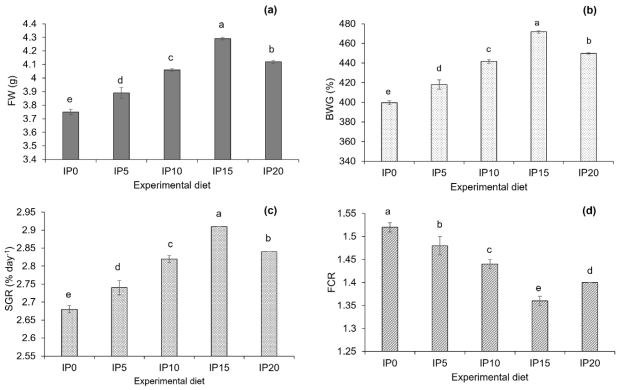

Fig. 1: *The growth performance and nutritional efficiency parameters: (a) final weight (FW, g), (b) body weight gain (BWG %), (c) specific growth rate (SGR % day^{-1}), and (d) feed conversion ratio (FCR) of* Anabas testudineus *fed* Ipomoea aquatica *supplemented diets at different inclusion levels: IP0 (0 %), IP5(5 %), IP10 (10 %), IP15 (15 %), and IP20 (20 %). Values are presented as mean ± SD. Different superscript letters indicate significant differences ($n = 3$, $p < 0.05$).*

3.2 Amino acid composition

The results of amino acid analysis of *I. aquatica* (data available as supplementary material) showed that it is a rich source of amino acids, mainly essential amino acids (EAA), as the total essential amino acid (38.56 ± 0.11 mg g^{-1}) content was higher than the total non-essential amino acid (21.29 ± 0.00 mg g^{-1}). Among the essential amino acids, phenylalanine (8.52 ± 0.08 mg g^{-1}) was the most abundant, followed by lysine (7.80 ± 0.04 mg g^{-1}) and leucine (7.72 ± 0.07 mg g^{-1}). Among the non-essential amino acids (NEAA), proline (4.98 ± 0.01 mg g^{-1}) was found highest, followed by serine (4.16 ± 0.05 mg g^{-1}) and aspartic acid (3.21 ± 0.05 mg g^{-1}).

The amino acid composition (mg g^{-1} dry tissue weight) of the muscle tissues of *A. testudineus* fed diets containing varying levels of *I. aquatica* is presented in Table 2. Essential amino acids (EAA) such as arginine, histidine, lysine, leucine, methionine, phenylalanine, threonine, tryptophan, and valine were significantly affected by the dietary treatments ($p < 0.05$). The highest levels of most EAAs (methionine, phenylalanine, threonine, and tryptophan) were observed in the IP15 group, except for valine, where no significant difference ($p > 0.05$) was observed among the plant-fed groups. Arginine, histidine, and lysine were the highest in IP10, while leucine was found to be the highest in IP5. Non-essential amino acids (NEAA), including alanine, aspartic acid, glycine, glutamic acid, proline, serine, and tyrosine, also showed significant variation among the dietary groups ($p < 0.05$). The total amino acid content was also found to be highest in the IP15 diet group ($p < 0.05$).

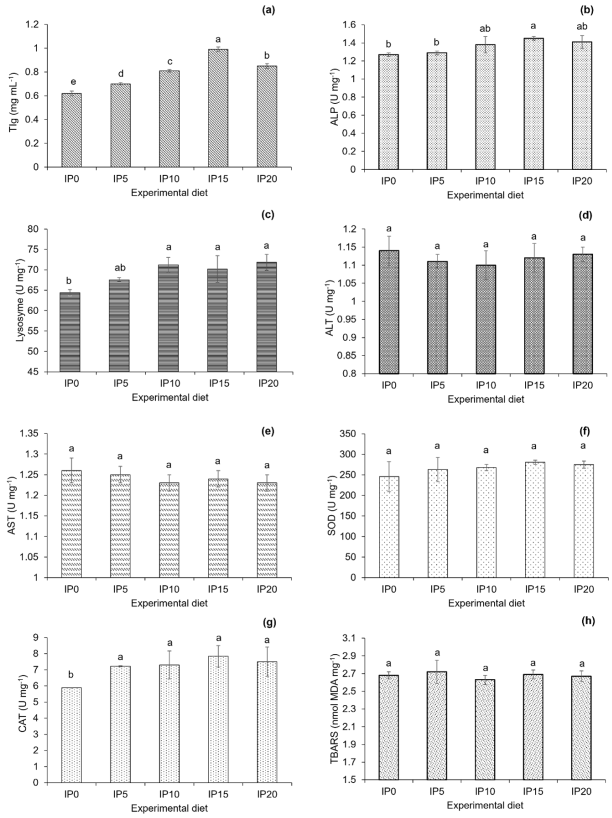

Fig. 2: *Immune and biochemical parameters (a) total immunoglobulin (TIg), (b) Alkaline phosphatase (ALP), (c) lysozyme (LYZ), (d) alanine aminotransferase (ALT), (e) aspartate aminotransferase (AST), (f) superoxide dismutase (SOD), (g) catalase (CAT), and (h) thiobarbituric acid reactive substances (TBARS) of* Anabas testudineus *fed* Ipomoea aquatica *supplemented diets at different inclusion levels: IP0 (0 %), IP5(5 %), IP10 (10 %), IP15 (15 %), and IP20 (20 %). Values are presented as mean ± SD. Different superscript letters indicate significant differences ($n = 3$, $p < 0.05$).*

Table 2: *Composition of important amino acids (mg g^{-1} dry weight) in* Anabas testudineus *fed with* Ipomoea aquatica *supplemented diets at different levels.*

Amino acids (mg g^{-1})	*IP0*	*IP5*	*IP10*	*IP15*	*IP20*
EAA					
Arginine	27.06 ± 0.06^{e}	28.08 ± 0.04^{d}	43.59 ± 0.09^{a}	39.57 ± 0.08^{b}	28.64 ± 0.12^{c}
Lysine	23.06 ± 0.06^{e}	23.84 ± 0.20^{d}	38.43 ± 0.23^{a}	32.81 ± 0.12^{b}	30.85 ± 0.04^{c}
Leucine	58.08 ± 0.07^{d}	77.59 ± 1.55^{a}	71.86 ± 1.17^{b}	67.03 ± 0.80^{c}	60.29 ± 0.96^{d}
Methionine	28.28 ± 0.23^{d}	33.06 ± 0.19^{b}	33.07 ± 0.18^{b}	38.18 ± 0.07^{a}	29.19 ± 0.06^{c}
Phenylalanine	54.09 ± 0.04^{c}	57.95 ± 0.15^{b}	58.27 ± 0.35^{b}	68.25 ± 0.42^{a}	49.64 ± 0.00^{d}
Tryptophan	42.01 ± 0.49^{c}	46.81 ± 0.29^{b}	51.04 ± 0.28^{a}	51.72 ± 0.21^{a}	41.30 ± 0.17^{c}
Valine	16.81 ± 0.31^{c}	18.93 ± 0.21^{ab}	18.99 ± 0.27^{ab}	19.59 ± 0.33^{a}	18.90 ± 0.08^{b}
Total EAA	272.93 ± 0.33^{d}	311.14 ± 1.48^{b}	348.41 ± 1.92^{a}	350.61 ± 0.54^{a}	287.84 ± 1.49^{c}
NEAA					
Alanine	69.78 ± 0.22^{d}	70.83 ± 0.10^{c}	86.84 ± 0.10^{b}	91.68 ± 0.04^{a}	64.50 ± 0.01^{e}
Glycine	1.04 ± 0.01^{c}	1.04 ± 0.07^{c}	1.39 ± 0.05^{b}	1.80 ± 0.16^{a}	0.96 ± 0.01^{c}
Glutamic acid	46.23 ± 0.23^{d}	45.53 ± 0.25^{d}	55.54 ± 1.21^{b}	52.01 ± 0.22^{c}	63.26 ± 0.14^{a}
Proline	60.09 ± 0.04^{c}	68.79 ± 0.82^{b}	57.13 ± 1.06^{d}	77.52 ± 0.52^{a}	58.03 ± 1.11^{cd}
Serine	35.36 ± 0.21^{d}	31.25 ± 0.22^{e}	39.74 ± 0.12^{c}	46.06 ± 1.03^{b}	53.90 ± 0.19^{a}
Total NEAA	257.78 ± 0.50^{e}	266.05 ± 0.04^{d}	295.10 ± 1.65^{b}	319.01 ± 0.83^{a}	289.16 ± 1.32^{c}
Total amino acids	530.71 ± 0.84^{d}	577.19 ± 1.44^{c}	643.51 ± 3.57^{b}	669.62 ± 1.37^{a}	577.00 ± 2.80^{c}

(N)EAA: (Non)essential amino acids. Different superscript letters indicate significant differences within a row ($p < 0.05$, n = 3). Diet codes: IP0 (0 %), IP5 (5 %), IP10 (10 %), IP15 (15 %), and IP20 (20 %) *Ipomoea aquatica* inclusion. The full amino acid profile is provided in the supplementary material.

3.3 Immune and biochemical parameters

Fig. 2 (a–h) shows the immune and biochemical parameters of *A. testudineus* fed on a diet containing different concentrations of *I. aquatica*. The group with a 15 % *I. aquatica* inclusion had significantly higher total immunoglobulin levels than the other groups ($p < 0.05$). Similar trends were observed for alkaline phosphatase and lysozyme activities, with significantly higher values in this group ($p < 0.05$) indicating enhanced immune responses. There were no discernible variations in ALT, AST or SOD activities between the different dietary regimens ($p > 0.05$). However, CAT activity increased substantially in the IP15 group compared to the others ($p < 0.05$). TBARS levels were similar across all treatments, indicating no significant difference in lipid peroxidation.

4 Discussion

The results of this study showed that inclusion of *I. aquatica* in the diet of *Anabas testudineus* significantly improved growth performance, with the 15 % inclusion level (IP15) yielding the best results. These findings align with previous reports on other fish species where moderate incorporation of the aquatic macrophytes improves fish growth (Ali *et al*., 2018; Yousif *et al*., 2019). The better growth performance of the fish at this level can be attributed to the balanced nutrient composition (Roy *et al*., 2022) and the species' improved digestibility of *I. aquatica* (Devi *et al*., 2022), both of which enhance metabolic efficiency and nutrient utilisation. However, reduced growth parameters observed at higher inclusion levels of the plant suggest a threshold beyond which its inclusion may not be beneficial for the fish. This may be due to interference in the nutrient absorption and metabolism of the fish, possibly due to the presence of antinutritional factors typical of plant-based ingredients (Francis *et al*., 2001). Although relatively lower levels of these factors are known to be present in *I. aquatica*, their significance may increase at higher inclusion levels.

Effective amino acid utilisation in animal feed is crucial for sustainable protein production (Kaushik *et al*., 2010). The amino acid composition of *I. aquatica* demonstrates its potential as a valuable ingredient in aquaculture diets, providing a balanced profile of essential and non-essential amino acids. The findings in this study are consistent with Adedokun *et al*. (2019) and Saikia *et al*. (2023), who also reported higher levels of total EAA than NEAA in the plant. Additionally, the optimal inclusion of the aquatic macrophyte enhanced the essential and non-essential amino acids in *A. testudineus*. This indicates the effectiveness of this plant in supporting protein synthesis and metabolism at moderate inclusion levels. High-quality protein in *I. aquatica*, including a higher amount of crucial amino acids (Saikia *et*

al., 2023), may contribute to the enhanced amino acid profile of the fish. This finding aligns with previous results suggesting that plant-based diets can adequately meet the amino acid requirements of fish when formulated correctly (Yousif *et al*., 2019; Goswami *et al*., 2022). Higher concentrations of specific amino acids such as arginine, leucine, and phenylalanine highlight the potential of *I. aquatica* to enhance the growth and metabolic functions in the fish. Arginine plays a crucial role in protein synthesis and immune function, while leucine is vital for muscle protein synthesis and repair (Francis *et al*., 2001). In contrast, the slight decline in the amino acid composition of the fish fed higher inclusion of the plant reinforces the importance of maintaining optimal supplementation levels to avoid reduced nutrient bioavailability or potential interference from plant secondary metabolites. The restricted inclusion of plant-based ingredients at optimal levels to avoid adverse effects on fish has also been reported in other species (Muchahary *et al*., 2023).

The immune and biochemical responses observed in this study also highlighted the functional benefits of *I. aquatica* on *A. testudineus*. Increased immunoglobulin observed in the plant-fed group can be associated with enhanced immunity, which may contribute to better health and disease resistance in fish (Ali & Kaviraj, 2018). Also, the enhanced activity of CAT in the *I. aquatica* supplemented diet-fed fish indicates better management of oxidative stress, which is beneficial for the overall health and resilience of the fish (Francis *et al*., 2001), as SOD and CAT are essential antioxidant enzymes that protect cells against free radicals (Fridovich, 1995). Similar response of plant protein-supplemented diets on the fish antioxidant defence system was reported in different fish species such as *L. crocea* (Wang *et al*., 2017), juvenile, red sea bream, *Pagrus major* (Dossou *et al*., 2018), and *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* (Aslam *et al*., 2021). These effects may result from bioactive compounds and micronutrients present in the plant that stimulate immune and antioxidant pathways. These results, therefore, suggest the broader applicability of such plant ingredients in aquafeed for sustainable aquaculture.

Furthermore, the stable levels of AST and ALT across different dietary treatments indicate that *I. aquatica* can be safely supplemented up to 20 % without adverse effects. AST and ALT in fish can indicate liver health and function (Kari *et al*., 2020). The elevated ALP and LYZ activities in the plant-fed group suggest improved innate immune response of *A. testudineus*, as ALP is involved in crucial cellular signalling and immune responses, while LYZ acts as an enzyme to fight off bacterial infections (Chamchuen *et al*., 2014). The unchanged TBARS levels across different groups in this study show controlled lipid peroxidation, implying no oxidative damage (Oakes *et al*., 2003). Similar results were also reported when *C. idella* and *H. molitrix* were fed *L. minor* (Aslam *et al*., 2021). The overall results of this study indicated that *I. aquatica* can replace fishmeal in the feed of *A. testudineus* with an optimal replacement of 15 % without adversely affecting the growth and health status of the fish.

5 Conclusion

This study identifies *I. aquatica* as an effective, sustainable, and economical alternative to fishmeal in the feed of *A. testudineus*. This may, in turn, offer practical benefits for farmers and the aquaculture industry as its inclusion can lower feed costs, improve fish health, and promote environmentally responsible aquaculture practices. Future research may focus on strategies to enhance the level of supplementation through the mitigation of antinutrients commonly present in plants. More long-term feeding trials under field conditions may be promoted to assess its effects on reproductive performance, product quality, and applicability to other species. Utilisation of *I. aquatica* as a viable protein source can reduce dependency on conventional animal-based proteins, thereby contributing to more economical and sustainable aquaculture.

Supplement

The amino acid composition (mg g^{-1} dry weight) of *Ipomoea aquatica* used in the study is provided as supplementary material. The complete amino acid profile of *Anabas testudineus* muscle across different groups fed with graded levels of *I. aquatica* supplemented diets is also available as supplementary material. This supplement is available online on the same landing page at: https://doi.org/10.17170/kobra-2026011411799 .

Acknowledgements

The authors express their gratitude to the Ministry of Tribal Affairs, Government of India, for supporting ST students through the National Fellowship and Scholarships, particularly funding Sanraja Muchahary (Award No.- 201920-NFST-ASS-00711). They also express their appreciation to the Head of the Zoology Department at Bodoland University for providing laboratory facilities.

Conflict of interest

The authors declare that they have no conflict of interest.

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