EVALUATION OF TWO FRESHWATER MACROPHYTES SUPPLEMENTED FEED ON THE GROWTH PERFORMANCE, DIGESTIVE ENZYMES AND BIOCHEMICAL PARAMETERS OF TWO AIR BREATHING FISH SPECIES

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The growing global population, and increasing demand for nutritious yet sustainable food has made food security a critical challenge today. Aquaculture is regarded as a key solution to meet the rising demand for fish to meet the requirements of the growing population. For successful aquaculture, the supply of high-quality, nutritious feed for the fish is essential. Protein is the most essential and expensive component of fish feed, which greatly affects fish growth and feed cost (Luo et al., 2004). Traditional fish meals remain the most important source of protein in fish feed. However, the high cost and environmental impact of traditional fish meal have driven the search for alternative protein sources. Finding low-cost, nutritious and sustainable alternatives to traditional fish meals remains a major challenge in the successful aquaculture of many species worldwide. Several studies have explored plant-based proteins as sustainable alternatives to fish fishmeal. Some freshwater aquatic macrophytes have also been successfully tested as a partial replacement for traditional fish meal (Naseem et al., 2021). The aquatic macrophytes like *Lemna minor* and *Ipomoea aquatica* have emerged as promising options due to their rich nutritional profiles, rapid growth, and wide availability (Naseem et al., 2021; Sosa et al., 2024). These plants offer renewable resources that reduce reliance on wild fish stocks. However, most researches have focused on herbivorous and omnivorous fish species, with limited studies on carnivorous species (Naseem et al., 2021). *Heteropneustes fossilis* and *Anabas testudineus* are promising fish species in aquaculture, valued for their nutritional, medicinal benefits, and adaptability, making them ideal for sustainable aquaculture in culturally and economically significant regions (Banerjee et al., 2018; Devi et al., 2022), but their production is limited in India which may be attributed to the high cost of feed production. This study, therefore, evaluates the potential utilisation of the two aquatic macrophytes, *L. minor* and *I. aquatica* in the feed of the two air-breathing fish species *viz*., *H. fossilis* and *A. testudineus* by determining the effects of feeds supplemented with the two plants at different levels, on the growth, digestive enzyme activities, and biochemical parameters of the two fish species.

The nutritional properties of the two freshwater macrophytes, *L. minor* and *I. aquatica*, were evaluated for potential use in fish feed. Both plants exhibited promising dietary nutrient profiles, with *I. aquatica* showing slightly higher protein content than *L. minor*. However, *L. minor* showed a higher carbohydrate content than *I. aquatica*, while lipid content was comparable. Both plants exhibited higher levels of essential amino acids relative to non-essential ones, and demonstrated favourable fatty acid profiles with a high

polyunsaturated fatty acid content, which is beneficial for fish nutrition. Additionally, both the plants showed low levels of anti-nutritional factors, such as oxalates, phytic acid, and saponins, supporting their potential as sustainable protein sources for fish diets.

The effects of *L. minor*-supplemented diets on the growth, nutrient utilisation, and biochemical parameters of *A. testudineus* (initial weight: 0.70 ± 0.01 g) and *H. fossilis* (initial weight: 0.51 ± 0.01 g) were evaluated by feeding the juveniles of these fish species for 60 days. The formulated feeds included *L. minor* at 0% (LM0), 5% (LM5), 10% (LM10), 15% (LM15), and 20% (LM20). At the end of the feeding trial, among all the groups, *A. testudineus* fed with LM15 diet showed significantly ($P < 0.05$) improved final weight (FW), body weight gain (BWG), specific growth rate (SGR), feed efficiency (FE), and protein efficiency ratio (PER), with the lowest feed conversion ratio (FCR), indicating enhanced feed utilisation in the fish. Optimal *L. minor* inclusion was identified as 16.25-17.10% through polynomial regression analysis based on SGR and FCR. LM15-fed fish exhibited higher muscle protein, lipid, and ash content, while moisture was highest in LM0. Digestive enzyme activities (amylase, trypsin, protease, pepsin, lipase) were significantly ($P < 0.05$) elevated in LM15-fed fish, with chymotrypsin highest in LM15 and LM20. Essential amino acids (EAAs) and polyunsaturated fatty acids (PUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), were maximum in the LM15 group, achieving the highest PUFA-to-saturated fatty acid (SFA) ratio. The aspartate aminotransferase (AST), alanine aminotransferase (ALT), superoxide dismutase (SOD) and levels of thiobarbituric acid reactive substances (TBARS) remained unaffected, while total immunoglobulin (TIg), lysozyme (LYZ) and catalase (CAT) improved significantly in *L. minor*-fed fish. These findings suggest that incorporating *L. minor* at 15% enhances growth, nutrient utilisation, and digestive enzyme activity in *A. testudineus* without adverse health effects.

In the case of *H. fossilis* also, the FW, BWG, SGR, FE, and PER showed significant ($P < 0.05$) enhancement in fish fed with the LM15 diet. The FCR was also reduced in this group, which indicates superior feed utilisation. Polynomial regression analysis determined the optimal inclusion level of *L. minor* to be 11.89-12.30%. LM15-fed *H. fossilis* exhibited significantly higher ($P < 0.05$) muscle protein, lipid, and ash content. Amylase and lipase were significantly elevated in LM10 and LM15 groups, while pepsin activity was highest in LM10-LM20. Fatty acid analysis of fish muscle

showed increased PUFA content, the highest PUFA-to-SFA ratio, and lower ω6/ω3 ratios in LM15 and LM20. The LM15 group also had the highest total EAA and non-essential amino acid (NEAA) levels, contributing to improved muscle quality. Health parameters, including TIg, CAT, SOD, and TBARS, were unaffected across groups. However, LYZ activity was higher in LM0 and LM5 but reduced in *L. minor*-fed groups, while ALP activity was highest in LM5 and lowest in LM15. Stable AST and ALT levels further confirmed no adverse health effects. Overall, *L. minor* supplementation, particularly at 15%, significantly improved growth, feed utilisation, enzyme activity, and fatty acid profiles in *H. fossilis* without compromising health. Together, these findings demonstrate the potential of *L. minor*-based diets to enhance growth and sustainability in aquaculture for both *A. testudineus* and *H. fossilis*.

The effects of *I. aquatica* supplementation on the growth, nutrient utilisation, and biochemical parameters of *A. testudineus* (initial weight: 0.75 ± 0.01 g) and *H. fossilis* (initial weight: 0.65 ± 0.01 g) were evaluated over 60 days with inclusion levels of 0% (IA0), 5% (IA5), 10% (IA10), 15% (IA15), and 20% (IA20). In *H. fossilis*, the IA10 diet resulted in the highest FW, BWG, SGR, FE, and PER, with the lowest FCR, indicating enhanced feed utilisation. Polynomial regression analysis identified the optimal inclusion range as 11.73-11.97%. Fish-fed IA10 exhibited significantly higher ($P < 0.05$) protein, lipid, and ash content, with lower moisture than the control. Carbohydrate content was higher in IA20, while fibre content showed no significant differences ($P > 0.05$). Amylase, trypsin, chymotrypsin, total protease, and pepsin were significantly higher ($P < 0.05$) in IA10-fed fish, with lipase activity peaking in IA10 and IA15. The IA10 group also exhibited the highest total EAA and NEAA in the fish muscle. Muscle fatty acid analysis revealed significantly higher ($P < 0.05$) PUFA levels, a higher PUFA-to-SFA ratio in IA10 and IA15, and a lower ω6/ω3 ratio than the control. Higher TIg levels were observed in *I. aquatica*-supplemented groups. No significant differences ($P > 0.05$) in LYZ, ALP, AST, ALT, CAT, SOD, and TBARS were found. These findings suggest that *I. aquatica* supplementation, particularly at the IA10 level, significantly improves growth performance, nutrient utilisation, and overall nutrient quality in *H. fossilis*.

In the case of *A. testudineus,* the IA15 diet significantly improved FW, BWG, SGR, FE, PER, and FCR, indicating optimal feed utilisation. Polynomial regression analysis identified the optimal inclusion range as 16.64-17.50%. Fish-fed IA15 also had

higher protein, lipid, and ash content, with lower moisture than IA0. Carbohydrate content was higher in IA5, IA10, and IA20. Digestive enzyme activities were enhanced in IA15, with higher amylase, trypsin, total protease, pepsin, and lipase activity ($P < 0.05$). Chymotrypsin activity was higher in IA15 and IA20. Amino acid content, both EAA and NEAA, was highest in IA15 ($P < 0.05$). Fatty acid analysis showed reduced SFA and increased PUFA levels in fish fed with *I. aquatica*-supplemented diets, with the highest PUFA/SFA ratio and EPA + DHA content in IA15. The fish muscle ω6/ω3 ratio remained stable ($P > 0.05$) among all groups. TIg was higher in IA15, and ALP activity was elevated in IA10, IA15, and IA20. No significant changes ($P > 0.05$) were observed in AST, ALT, CAT, SOD, and TBARS. Overall, *I. aquatica* supplementation, especially at the IA15 level, improved growth, feed efficiency, digestive enzyme activity, and muscle quality in *A. testudineus* without negative effects on its health.

Results of this study indicates that both *L. minor* and *I. aquatica* are promising, sustainable, and cost-effective alternatives to traditional feed ingredients in aquaculture of the two air breathing species. Their optimal inclusion enhances fish growth, feed utilisation, and nutritional quality, without any adverse effect on the fish health. This study underscores the potential for development and wider application of these two plant-based fish feeds to reduce reliance on fishmeal, promote sustainable and enhance production of the two fish species.