

## 1. Introduction

The rapid growth of the global population has intensified the demand for sustainable and nutritious food sources, making food security one of the paramount challenges of the 21st century (FAO, 2022). Alongside population growth, there is a significant rise of the middle class, especially in China and Southeast Asia, where growing affluence is driving a shift toward diets richer in animal-based protein sources (Yotopoulos, 1985; WHO, 2003; Kharas, 2010; Hua et al., 2019). According to FAO (2022), global consumption of aquatic foods has seen a significant increase, rising from an average of 9.9 kg per capita in 1960 to 20.2 kg per capita in 2020. This remarkable surge not only reflects a growing preference for aquatic foods due to their high nutritional value but also underscores the critical role that aquaculture plays in meeting the escalating nutritional needs of the global populace. Aquaculture, the farming of aquatic organisms under controlled conditions, offers a promising avenue to supply this increasing demand for fish and other aquatic products (Hua et al., 2019). It presents opportunities not only for enhancing food security but also for fostering economic development and poverty alleviation, especially in rural and coastal communities where aquaculture activities are often concentrated (Subasinghe et al., 2009).

However, the sustainability and profitability of aquaculture are heavily influenced by the cost and availability of feed, particularly protein sources. One of the primary challenges in aquaculture is the reliance on high-cost and unsustainable traditional fish meals as the main protein source in fish diets (Mukherjee et al., 2010). Protein is not only the most expensive component of fish feeds but also a critical determinant of growth performance and feed cost efficiency (Luo et al., 2004). Feed makes up around 40-70% of the total expenditure in aquaculture (Singh et al., 2006; De Silva & Hasan, 2007; Craig, 2017; Andriani et al., 2019; Naseem et al., 2021). Traditionally, fish meal, derived from wild-caught fish, has been the predominant source of dietary protein due to its balanced amino acid profile and high digestibility (Davis &

Arnold, 2000). However, overfishing and environmental concerns have led to a decline in fish stocks, resulting in high costs, limited availability, and declining quality of fish meal (Naseem et al., 2021). These issues necessitate the exploration of alternative protein sources that are both cost-effective and environmentally sustainable (Kaushik et al., 1995; Fournier et al., 2004). Utilising locally available, sustainable feed resources is essential for the economic viability and environmental sustainability of aquaculture operations (FAO, 2022).

In response to these challenges, researchers have turned their attention to plant-based proteins as potential alternative feed ingredients in aquaculture. Several studies have evaluated the nutritional efficacy and economic feasibility of incorporating plant proteins into fish diets (Awad & Awaad, 2017; Dorothy et al., 2018; Sonta et al., 2019). Freshwater aquatic weeds, particularly macrophytes, have garnered attention as promising replacements for animal proteins in fish diets due to their rich nutritional profiles, rapid growth rates, and wide availability (Naseem et al., 2021). These plants can be cultivated or harvested sustainably, providing a renewable resource that can alleviate the pressure on wild fish stocks used for fish meal production.

Two such aquatic plants, *Lemna minor* L. and *Ipomoea aquatica* Forssk., have been identified for their high protein content, essential amino acids, and other beneficial nutrients, making them suitable candidates for fish feed formulations (Sosa et al., 2024; Adedokun et al., 2019). *L. minor*, commonly known as duckweed, is a small, floating aquatic plant widely distributed across India and other regions with tropical and temperate climates (Falaye et al., 2022). Belonging to the family Lemnaceae, duckweed is characterised by its rapid growth, high protein content and favourable amino acid profile, which includes essential amino acids required for fish growth (Chakrabarti et al., 2018). The feasibility of using *L. minor* as a partial or complete replacement for traditional fish meal has been demonstrated in various fish species, showing positive effects on growth performance and feed utilisation

(Sonta et al., 2019; Irabor et al., 2022; Goswami et al., 2022; Devi et al., 2022). Incorporating *L. minor* into fish feeds could significantly reduce feed costs and contribute to more sustainable aquaculture practices by utilising a readily available natural resource.

Similarly, *I. aquatica*, commonly known as water spinach, is a tropical semi-aquatic plant that thrives abundantly in ponds, rivers, wetlands, and lowlands across Asia (Roy et al., 2022). Often considered a weed due to its invasive growth habit, I. aquatica presents an untapped resource rich in nutritional components beneficial for aquaculture (Austin, 2007). It is abundant in minerals, vitamins, and trace elements (Adedokun et al., 2019; Ramzy et al., 2019). Moreover, it contains both essential and non-essential amino acids, with a substantial portion of its total amino acid content being essential, including lysine-crucial for protein synthesis and growth in fish-as well as phenylalanine and isoleucine in adequate amounts (Saikia et al., 2023). The presence of bioactive phytochemicals in *I. aquatica* may also enhance the immune system of aquatic animals, providing additional health benefits and potentially reducing the need for synthetic additives or antibiotics in aquaculture feeds (Roy et al., 2022). Despite its rich nutritional profile, I. aquatica remains underutilised in fish feed formulations. Several studies have begun to explore its potential, showing promising results. For instance, Odulate et al. (2013) reported improved growth performance in African catfish (Clarias gariepinus) when I. aquatica was included in their diet. Similarly, Yousif et al. (2019) observed positive outcomes in Nile tilapia (Oreochromis niloticus) fed with diets enriched with I. aquatica. These studies suggest that *I. aquatica* could serve as a viable alternative protein source in aquaculture feeds, contributing to sustainable fish farming practices. However, most research on I. aquatica has focused on herbivorous or omnivorous fish species, such as cyprinids (Baruah et al., 2018; Ali & Kaviraj, 2018) and tilapia (Manuel et al., 2020; Chepkirui et al., 2021). Limited research has been conducted on its use in carnivorous fish species (Nandi et al., 2023),

highlighting a gap in the literature and the need for further studies to explore its broader applicability.

Two fish species of particular interest in aquaculture, especially in Asia, are Heteropneustes fossilis (stinging catfish) and Anabas testudineus (climbing perch). These species are highly valued not only for their nutritional and medicinal properties but also for their adaptability to various culture conditions, making them suitable candidates for sustainable aquaculture development in regions where they hold cultural and economic significance. H. fossilis (Bloch, 1794) is a freshwater catfish native to South and Southeast Asia (Talwar & Jhingran, 1991). It is highly valued as a food fish due to its high nutritional content, low fat, and medicinal properties, including traditional medicinal uses for treating various ailments (Fatma & Ahmed, 2020). Known for its hardiness, rapid growth, and capacity to survive in low-oxygen environments, it is wellsuited for intensive culture systems (Chakrabarty & Mirza, 2008). According to the IUCN Red List, the species is classified as least concern (IUCN, 2024). Its ability to utilise both plant- and animal-based feed, along with its market potential, reinforces its importance in aquaculture (Pillay & Kutty, 1990). Research on alternative protein sources for *H. fossilis* is emerging, though limited. Studies have explored various plant-based proteins: Mondal et al. (2011) investigated mulberry leaf meal combined with fish offal meal; Bag et al. (2012) also studied mulberry leaf meal; Siddiqui et al. (2014) and Howlader et al. (2023) examined soybean meal; Ali et al. (2019) evaluated fermented mulberry leaf meal; and Hossain et al. (2023) tested sunflower meal as a fish meal alternative. These studies highlight the potential of plant-based proteins in H. fossilis diets, yet further research is needed to identify and evaluate additional suitable alternative protein sources.

A. testudineus (Bloch, 1792), commonly known as the climbing perch, is another species of significant commercial importance in aquaculture. Belonging to the family Anabantidae, it is widely distributed in Asia, including India, Bangladesh, Myanmar, and other Southeast Asian countries. The climbing

perch is known for its hardiness and ability to survive in adverse environmental conditions, such as low-oxygen waters and varying salinities, due to its accessory breathing organs (Talwar & Jhingran, 1991). According to the IUCN Red List, it is classified as least concern (IUCN, 2024) due to its wide distribution and adaptability to various habitats (Ahmad et al., 2019). This adaptability makes it an attractive species for aquaculture, particularly in regions with challenging environmental conditions. In Assam, Northeast India, A. testudineus holds cultural significance and is highly preferred by local communities for its superior taste and nutritional value (Devi et al., 2022). Locally known as Kawai in Bodo, it is rich in digestible protein, essential amino acids, and omega-3 fatty acids, which are beneficial for human health (Singh et al., 2016). Despite its potential, the culture and production of A. testudineus remain significantly lower compared to other species like carp. One of the primary obstacles is the high cost of fish feed production, which accounts for the primary cost of total production costs in aquaculture (Mukherjee et al., 2010; Andriani et al., 2019). The reliance on expensive animal protein sources for feed formulation exacerbates this issue, making it less economically viable for farmers to invest in A. testudineus cultivation (Devi et al., 2022).

To address this challenge, studies have explored the use of plant-based proteins as sustainable alternative protein sources in the diets of *A. testudineus*. Panchan et al. (2024) reported the successful replacement of fish meal up to a certain level with soybean meal without adverse effects on growth performance, indicating that soybean meal could be a viable alternative protein source. Similarly, Mishra (2013) found that incorporating *Azolla pinnata*, an aquatic fern rich in protein, positively impacted the growth and feed utilisation of *A. testudineus*. These findings suggest that plant proteins have the potential to reduce feed costs and support sustainable fish farming practices (Naseem et al., 2021). Moreover, Devi et al. (2022) reported that the in vitro digestibility of *I. aquatica* was the highest among the four plant proteins tested in *A. testudineus*. Panchan et al. (2024) studied soybean meal as a fish meal replacement. This

result indicates that *I. aquatica* provides a rich source of nutrients and is highly digestible for this species, making it a promising candidate for inclusion in their diet. The development of cost-effective, nutritious, and digestible feeds is crucial for enhancing the aquacultural production of both *A. testudineus* and *H. fossilis*. By reducing feed costs and improving feed efficiency, farmers can increase profitability and contribute to the sustainability of aquaculture practices.

This study, therefore, aims to assess the impact of a diet supplemented with I. aquatica and L. minor on the growth performance, digestive enzyme and biochemical parameters of A. testudineus and H. fossilis. By providing comprehensive insights into the nutritional and economic benefits of using I. aquatica and L. minor as a feed ingredient, this research seeks to enhance the sustainability and cost-efficiency of A. testudineus and H. fossilis culture. The outcomes could potentially benefit farmers by reducing production costs, improving fish health and growth, and contributing to the broader aquaculture industry by promoting environmentally sustainable practices. As the global population continues to expand, the importance of developing sustainable and nutritious food sources cannot be overstated. Aquaculture stands at the forefront of meeting this increasing demand for high-quality protein sources. However, the success of aquaculture in fulfilling this role hinges on innovative and sustainable feed formulations that are both economically viable and environmentally friendly (FAO, 2022). The utilisation of alternative protein sources like L. minor and I. aquatica offers promising solutions (Naseem et al., 2021). These plants not only provide the necessary nutrients required for optimal fish growth but also contribute to environmental sustainability by reducing reliance on overexploited fish stocks and minimising the ecological footprint of aquaculture operations.

Exploring plant-based alternative protein sources such as *L. minor* and *I. aquatica* represents a significant step toward achieving sustainable aquaculture. This study contributes to the growing body of knowledge aimed at improving feed formulations, enhancing fish growth and health, and reducing

production costs. The findings from this study may not only benefit local communities and economies but also have broader implications for global food security and environmental sustainability.

With the above background information, the following were chosen as objectives of the present study:

- 1. To determine the nutritional characteristics of the two freshwater macrophytes, *Lemna minor* and *Ipomoea aquatica*
- 2. To study the effect of *Lemna minor* supplemented feed on the growth performance, digestive enzymes and biochemical parameters of *Anabas testudineus* and *Heteropneustes fossilis*
- 3. To evaluate the growth, digestive physiology and biochemical parameters of *Anabas testudineus* and *Heteropneustes fossilis* fed with *Ipomoea aquatica* supplemented diet.