Chapter 6

Summary and Conclusion

6. Summary and Conclusion

The growing global population and the increasing demand for sustainable, nutritious food have positioned food security as a critical global challenge. Aquaculture is widely recognised as a vital solution to meet the rising demand for fish. Successful aquaculture depends on a number of factors, and the supply of high-quality, nutritious feed for the fish is one of them. Among the various components of fish feed, protein is the most expensive yet essential ingredient, significantly influencing fish growth performance and overall feed cost (Luo et al., 2004). However, the high cost and environmental impact of traditional fish meal have driven the search for alternative protein sources. In response to these challenges, researchers are increasingly exploring plant-based proteins as potential alternatives in aquaculture feeds. Numerous studies have assessed the nutritional efficacy and economic feasibility of incorporating plant proteins into fish diets (Awad & Awaad, 2017; Dorothy et al., 2018). Freshwater aquatic macrophytes have emerged as promising substitutes for animal-derived proteins in fish feed due to their rich nutritional profiles, rapid growth rates, and wide availability (Naseem et al., 2021). These plants can be cultivated or harvested easily, offering a renewable resource that helps alleviate pressure on wild fish stocks used as fish meal. Plant-based proteins, particularly those derived from freshwater plants like *Lemna minor* and *Ipomoea aquatica*, present compelling alternatives due to their high nutritional value, rapid growth, and sustainability (Sosa et al., 2024; Adedokun et al., 2019). Many studies have highlighted their potential to enhance fish growth, feed efficiency, and immune health (Sonta et al., 2019; Irabor et al., 2022; Goswami et al., 2022; Odulate et al., 2013; Yousif et al., 2019). However, most studies on *L. minor* and *I. aquatica* have been focused on herbivorous or omnivorous fish like cyprinids and tilapia, with limited studies on its use in carnivorous species, highlighting a need for further exploration (Naseem et al., 2021; Chepkirui et al., 2022; Nandi et al., 2023). *Heteropneustes fossilis* and *Anabas testudineus* are two fish species of significant interest in aquaculture, particularly in Asia. These are 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). Despite its potential, the culture and production of *H. fossilis* and *A. testudineus* remain significantly lower compared to other species like carp. One of the major challenges in aquaculture is the high cost of fish feed, which dominates production expenses (Andriani et al., 2019). Reliance on expensive animal protein sources for feed further limits the economic viability of cultivating *H. fossilis* and *A. testudineus*. Therefore, this study seeks to address these issues by evaluating the effects of *L. minor* and *I. aquatica* supplementation on the growth performance, digestive enzyme activity, and biochemical parameters of these species. The findings aim to promote more sustainable and cost-effective alternatives to animal proteins for feed formulation of the two species.

The thesis consists of six chapters in total. The first chapter provides an introductory overview of the present status and issues of aquaculture nutrition, limitations of traditional fish meals in fish feeds, the need for alternative sources of protein in fish feed for sustainable aquaculture and the potential of plant-based alternative protein sources in fish feed. This chapter also highlights the three objectives of the study. The second chapter offers a comprehensive review of the literature on the role of proteins in aquaculture nutrition. It discusses the use of macrophytes as an alternative protein source in fish diets, highlighting various studies on this topic. This chapter also includes an in-depth analysis of current works evaluating the potential of *L. minor* and *I. aquatica* as potential alternative protein sources in the feed of different fish species. The different published works on the application of different plant-based proteins in the diets of *A. testudineus* and *H. fossilis* are also discussed in this chapter. The third chapter provides a detailed description of the materials and methods used in the study. The nutritional properties of *L. minor* and *I. aquatica* are evaluated in detail in the fourth chapter. The chapter also presents the results of experiments evaluating the effect of feed supplemented with different levels of *L. minor* and *I. aquatica* on the growth, body composition, digestive enzymes and biochemical properties of the two species, respectively. All the results are discussed in the fifth chapter. Finally, the sixth chapter presents the summary and conclusion of the study.

This study assessed the nutritional characteristics of two freshwater macrophytes, *L. minor* and *I. aquatica*, for their potential use in fish feed. Both species exhibited valuable nutritional profiles, with *I. aquatica* showing slightly higher protein content (22.52 ± 0.03%) compared to *L. minor* (20.53 ± 0.01%), making both plants a promising protein source for fish diets. *L. minor* contained a higher carbohydrate content (43.60 ± 0.02%) than *I. aquatica* (43.55 ± 0.01%), while the lipid contents were 7.53 ± 0.01% and 7.34 ± 0.01%, respectively, in *L. minor* and *I. aquatica*, respectively. The amino acid composition of *L. minor* and *I. aquatica* indicated that higher levels of essential amino acids were found in both plants compared to the non-essential amino acids. Both species also demonstrated a favourable fatty acid profile, with a high polyunsaturated fatty acid content, which may be beneficial for fish nutrition. Furthermore, it was observed that both plants contained low antinutritional factors, such as oxalates, phytic acid, tannic acid, alkaloids and saponins, indicating its potential for broader application.

Experiments were conducted on juvenile *A. testudineus* and *H. fossilis* to evaluate the effects of *L. minor*-supplemented diets at graded inclusion levels of 0% (LM0), 5% (LM5), 10% (LM10), 15% (LM15), and 20% (LM20) by feeding the fish for 60 days. In the case of *H. fossilis*, the *L. minor*-supplemented diets significantly enhanced growth performance compared to the control ($P < 0.05$). Fish fed the LM15 diet exhibited significantly higher ($P < 0.05$) FW, BWG, SGR, FE, and PER and the lowest FCR, indicating superior feed utilisation in this group. Polynomial regression analysis based on SGR and FCR determined the optimal inclusion range to be 11.89-12.30%. The LM15 diet also increased the protein, lipid, and ash content of fish carcass. Digestive enzyme analysis revealed significantly higher ($P < 0.05$) amylase and lipase activity in LM10 and LM15, while pepsin activity peaked in LM10, LM15, and LM20 with no significant difference ($P > 0.05$) between these groups. No significant differences ($P > 0.05$) were observed in trypsin, chymotrypsin, or total protease activity among all groups. The LM15 group showed the highest total EAA and NEAA contents. Fatty acid analysis indicated no significant differences in SFA content among LM0, LM5, LM10, and LM20 ($P > 0.05$). MUFA content decreased with increasing *L. minor* levels, significantly higher in LM0 and lowest in LM15.

PUFA content increased with *L. minor* supplementation, peaking in LM15 and LM20, with no significant difference ($P > 0.05$) between these groups. The PUFA/SFA ratio and EPA + DHA content were highest in LM15 for PUFA/SFA and LM15 and LM20 for EPA + DHA. Additionally, ω6/ω3 ratios were significantly lower in LM10, LM15, and LM20, and higher in LM0 and LM5. No significant differences were observed in TIg, CAT, and SOD levels among different groups ($P > 0.05$). LYZ activity was significantly higher in LM0 and LM5 but lower in *L. minor*-fed groups. ALP activity was highest in LM5 and lowest in LM15. TBARS levels showed no significant differences ($P > 0.05$) among LM0, LM10, LM15, and LM20. Similarly, AST levels did not differ significantly ($P > 0.05$) among LM0, LM5, and LM15, while ALT levels showed no significant differences ($P > 0.05$) among LM0, LM15, and LM20. These results indicated that *L. minor*-supplemented diets significantly enhanced growth performance, feed utilisation, enzyme activity, and muscle quality of *H. fossilis* with no adverse impact on its health. These diets improved protein and lipid accumulation, contributing to superior growth and overall quality in *H. fossilis*.

Similarly, in the case of *A. testudineus,* the LM15 diet resulted in significantly ($P < 0.05$) higher FW, BWG, SGR, FE, and PER compared to other groups. It also recorded the lowest FCR, indicating improved feed utilisation. Polynomial regression analysis identified the optimal inclusion range as 16.25-17.10% based on SGR and FCR. The LM15 diet significantly ($P < 0.05$) enhanced protein, lipid, and ash content, while moisture was highest in LM0. Fibre and carbohydrate contents were consistent across all groups. Amylase, trypsin, total protease, pepsin, and lipase were significantly higher in LM15-fed fish, with chymotrypsin activity highest in LM15 and LM20. Essential amino acids and PUFA levels, including EPA and DHA, increased with *L. minor* inclusion, with LM15 achieving the highest PUFA-to-SFA ratio. No significant differences ($P > 0.05$) were observed in AST, ALT, SOD, and TBARS levels across groups, suggesting no adverse health effects. However, TIg, LYZ, and CAT activities were significantly higher in *L. minor*-fed fish. These findings indicate that incorporating *L. minor* up to 15% in the diet of *A. testudineus* enhances growth, nutrient utilisation, and health without adverse effects.

Under the third objective, the inclusion of *I. aquatica* was evaluated at incorporation levels of 0% (IA0), 5% (IA5), 10% (IA10), 15% (IA15), and 20% (IA20) in the diets of juvenile *A. testudineus* and *H. fossilis*. For *H. fossilis*, the IA10 diet yielded the highest FW, BWG, SGR, FE, and PER and the lowest FCR, indicating superior feed utilisation at this inclusion level. Polynomial regression analysis determined the optimal inclusion range to be 11.73-11.97%. Fish fed the IA10 diet exhibited significantly higher ($P < 0.05$) protein, lipid, and ash content, while moisture content was lower in *I. aquatica*-supplemented groups compared to the control (IA0). Fibre content showed no significant differences ($P > 0.05$) across groups, but carbohydrate content was significantly higher in IA20. Enzymatic activities, including amylase, trypsin, chymotrypsin, total protease, and pepsin, were significantly higher ($P < 0.05$) in IA10-fed fish, while lipase activity peaked in IA10 and IA15. The IA10 group also exhibited the highest total EAA, NEAA, and overall amino acid content. Fatty acid analysis revealed lower SFA and MUFA content in *I. aquatica*-fed groups compared to the control, while PUFA levels were significantly higher ($P < 0.05$), with the highest values observed in IA10. The PUFA-to-SFA ratio was significantly higher ($P < 0.05$) in IA10 and IA15, and the $\omega6/\omega3$ ratio was lower in *I. aquatica*-fed groups compared to the control. Higher TIg levels were observed in *I. aquatica*-supplemented groups compared to IA0. No significant differences ($P > 0.05$) were found in LYZ, ALP, AST, ALT, CAT, SOD, or TBARS across the groups. These results suggest that *I. aquatica* supplementation, particularly at the IA10 level, improves growth performance, nutrient utilisation, and overall quality in *H. fossilis*.

In the case of *A. testudineus*, the IA15 diet resulted in the highest FW, BWG, SGR, FE, and PER, with the lowest FCR, indicating improved feed utilisation. Fish fed the IA15 diet also showed significantly higher ($P < 0.05$) protein, lipid, and ash content. Polynomial regression analysis determined the optimal inclusion range to be 16.64-17.50%. Moisture content was lower in *I. aquatica*-supplemented groups compared to the control, while fibre content remained consistent across dietary treatments ($P > 0.05$). Carbohydrate content was significantly higher in IA5, IA10, and IA20, with no significant differences ($P > 0.05$) among these groups. Digestive enzyme activity was notably enhanced in the IA15 group, with significantly higher (*P*

< 0.05) amylase, trypsin, total protease, pepsin, and lipase activity than other groups. Chymotrypsin activity was significantly higher ($P < 0.05$) in IA15 and IA20. Total EAA and NEAA contents were significantly elevated in the IA15 group, with the highest total amino acid content also recorded in IA15. Fatty acid analysis revealed decreased SFA content with increasing *I. aquatica* inclusion, with the highest levels in IA0 and the lowest in IA15. MUFA content was highest in IA0 and IA20. PUFA content increased with higher *I. aquatica* levels, peaking in IA15. The PUFA-to-SFA ratio and EPA + DHA content were significantly higher ($P < 0.05$) in IA15. The ω6/ω3 ratio remained stable across all treatments ($P > 0.05$). No significant differences ($P > 0.05$) were observed in AST, ALT, CAT, SOD, or TBARS levels across the groups. TIg was significantly higher ($P < 0.05$) in IA15, while LYZ activity was elevated in IA5, IA10, IA15, and IA20, with no significant differences among these groups ($P > 0.05$). ALP activity was significantly higher ($P < 0.05$) in IA10, IA15, and IA20. Overall, *I. aquatica* supplementation, particularly at the IA15 level, significantly improved FW, BWG, FCR, digestive enzyme activity, and muscle quality without adverse effects on fish health.

In conclusion, the findings of this study suggest that both *L. minor* and *I. aquatica* offer promising, sustainable, and cost-effective alternatives to traditional sources of protein like fish meal in aquaculture. Their optimal inclusion enhances fish growth, feed efficiency, and nutritional quality, providing a viable strategy to improve both the sustainability and economic viability of aquaculture. This research underscores the potential for broader adoption of plant-based feeds to meet the increasing demand for environmentally responsible aquaculture practices. Incorporating these plants into aquafeeds reduces reliance on traditional fishmeal and supports environmental and economic objectives for sustainable aquaculture. Further studies on long-term feeding trials, large-scale production feasibility, and the best application of these macrophytes in aquaculture may be encouraged to maximise their effectiveness. By harnessing the nutrition of freshwater macrophytes, the aquaculture industry can make significant progress toward a more sustainable, resilient, and inclusive approach to global food security.