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Research Article

Potential of Macrophytes as Alternative Fish Feed Ingredients for Sustainable Aquaculture

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Abstract

Freshwater macrophytes are gaining importance as nutritious ingredients for fish feed formulation. In this study, the nutritional and anti-nutritional contents of the valuable macrophytes were evaluated. Freshwater macrophytes including *Lemna minor*, *Spirodela polyrhiza*, *Wolffia globosa*, *Pistia stratiotes* and *Ipomoea aquatica* were cultured with organic manures including cattle dung, poultry droppings and mustard oil-cake in 1:1:1 combination @ 1.052 kg/m³. The temperature and light intensity ranged 20-28°C and 4346-5352 lux, respectively during the culture period. The proximate composition study showed that significantly ($P < 0.05$) higher crude protein content present in *L. minor* ($35.94 \pm 0.05\%$) and *S. polyrhiza* (35.21 ± 0.31) among other cultured macrophytes. Significantly ($P < 0.05$) higher crude lipid ($8.13 \pm 0.06\%$) with higher calorific value (3.79 ± 0.01 Cal/g) found in *L. minor* only compared to others. Significantly ($P < 0.05$) higher ash content (21.69 ± 0.23) was present in *P. stratiotes*. The anti-nutritional analysis study showed that phytic acid content significantly ($P < 0.05$) higher in *I. aquatica* ($2.10 \pm 0.02\%$) followed by *P. stratiotes* ($1.85 \pm 0.01\%$). *P. stratiotes* had significantly ($P < 0.05$) higher tannin ($0.96 \pm 0.02\%$) and saponin ($1.59 \pm 0.04\%$) content among other cultured macrophytes. Saponin content was significantly ($P < 0.05$) lower in *W. globosa* compared to others. Significantly ($P < 0.05$) lower tannin content was found in duckweeds *L. minor* and *W. globosa*. These results indicate that some freshwater macrophytes have favorable nutritional properties and may be used to formulate sustainable fish feeds that reduced the costly fishmeal in aquaculture.

Keywords: Macrophytes, Duckweeds, Proximate composition, Anti-nutritional factors.

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Introduction

Aquaculture is one of the major food producing sector globally that providing a significant portion of dietary protein, especially in emerging nations. The increasing demand for fish and income source for millions of people worldwide leads to the rapid growth of

aquaculture sector (FAO, 2024). The key challenges facing the aquaculture industry is use of traditional fishmeal and other animal-based protein sources in feed formulation. These conventional sources are coming with very high cost and also unsustainable in long term due to overfishing and other environmental impact

associated with their production (Tacon & Metian, 2008). These challenges open the necessity to search other alternative protein sources that can ensure the rapid growth of aquaculture industry without disturbing the marine ecosystem further.

The overall success of aquaculture industry majorly depends on feed formulation. The plant-based protein sources such as legumes, cereals and algae have been explored over the years as an alternative for animal protein (El-Sayed, 2006). Macrophytes, the free-floating aquatic plants are one of the promising groups of plant-based ingredients with high nutrient content and rapid growth that can be served as a protein source with other ingredients in fish diets (Naseem et al., 2021). In many aquatic bodies, the unrestricted spread of these freshwater macrophytes causes an issue. However, from the perspective of human and animal nutrition, proper utilization of these nutrient-rich plants might open the way for new developments. Both human and non-ruminant animals can benefit from the leaf protein that is derived from freshwater macrophytes (Boyd 1970). These macrophytes require not only low cost but can also improve the environmental sustainability of aquaculture by reducing fishmeal use which help to conserve wild fish stocks. The duckweeds are small free floating macrophytes emerged as best ingredient for inclusion in fish feed because of its high yield in relatively short period of time and nutritional profile including high protein content, essential amino acids and optimal level of micronutrients which are beneficial for fish growth (Kumar et al., 2022). *L. minor* is also a rich source of essential fatty acids requires for the proper growth and development of fish (Chakrabarti et al., 2018). The high content of protein and ability of *L. minor* to absorb nutrients directly from water, makes it an excellent option for alternative ingredient to include in fish feed. Besides these nutritional quality, presence of certain anti-nutritional factors (ANFs) such tannin, phytic acid, trypsin inhibitor, lectins, saponins etc., have limited the use of plant derived alternative ingredient to fishmeal and other traditional components (Ghosh and Mandal, 2015; Mandal and Ghosh, 2019). ANFs are secondary metabolites produced by plants for their self-defence but these factors affect the nutrient availability by interfering in absorption on plant consumption. ANFs have the potential to significantly reduce the nutritional value of macrophytes. Unless eliminated or deactivated, tannin and other ANFs have been hypothesized to significantly lower the feed nutritional value of various plant-derived materials (Turner & Liener, 1975; Hossain and Jauncey, 1989). Tannins are the phenolic substances with molecular weight greater than 500 Da. Tannins are produced in the leaves, fruits, and bark of plants (Timotheo and Lauer 2018). Tannins often impact protein digestibility and reduce critical amino acids by forming both reversible and irreversible tannin-protein complexes between the hydroxyl group of tannins and the carbonyl group of proteins (Lampart-Szczapa et al. 2003). Saponins are known to have substantial haemolytic activity because this ANF has been shown to be fatal to cold-blooded animals, including fish (Gemedé & Ratta, 2014).

According to Fleck et al., (2019), saponins have the ability to interact with the cholesterol group in erythrocyte membranes, causing haemolysis. Phytic acid can chelates and affect the micronutrients bioavailability like calcium, iron, copper and zinc. The negatively charged structure of phytic acid typically forms compounds with metal ions of positively charged such calcium, magnesium, iron, and zinc, lowering their bioavailability through decreased absorption rates. Phytic acid is regarded as a highly effective anti-nutrient in foods and reduced ion absorption in both human and animal nutrition, mostly because of its chelating ability (Samtiya et al. 2020; Bora 2014). The objective of the current study is to evaluate the nutritional value and anti-nutritional factors present in freshwater cultured macrophytes that will help to understand the suitability of these macrophytes as feed ingredients to replace costly fishmeal from fish feed.

Materials and Methods

Culture of *Lemna minor*

Freshwater macrophytes viz., water lettuce *P. stratiotes* and water spinach *I. aquatica* and duckweeds including *L. minor*, *W. globosa* and *S. polyrhiza*; were cultured using organic manures following the well-established protocols of Chakrabarti et al., 2018. Macrophytes were cultured in cemented tanks (1.2 m × 0.35 m) maintained at the outdoor facility of Department of Zoology, University of Delhi. Tanks were filled with freshwater up to 30 cm height and 10 cm soil layer was applied additionally in case of *I. aquatica*. Organic manures viz., cattle dung, poultry droppings and mustard oil-cake in combination of 1:1:1 @ 1.052 kg/m³ were mixed thoroughly and allowed to decompose for 5 days in each tank. After 5 days 15 g macrophyte per tank was inoculated. Culture tanks were re-manured in every 10 days to ensure sufficient supply of nutrients. All the macrophytes were cultured in triplicates and harvesting of macrophytes was started when the whole surface of water covered with macrophytes. Harvested macrophytes were washed thrice with tap water and twice with deionized water. After proper washing and cleaning macrophytes were air dried powdered and analyzed for their nutritional and anti-nutritional compositions.

Water quality

During the culture period all the major water quality parameters were monitored at alternate days. Water quality parameters viz., temperature, pH, dissolved oxygen, conductivity and total dissolved solids were monitored using digital multi-parameter (HQ 40d, Hach, USA, Fig. 15 b) while ammonia (NH₃) and nitrate (NO₃⁻) were monitored using Orion Versa Star Pro (Thermo-scientific, USA, Fig. 15 a). Phosphate (PO₄⁻) and nitrite (NO₂⁻) were monitored using spectrophotometer UV-1601, UV-Visible spectrophotometer (Shimadzu, Japan) following the standard methods (APHA, 2017).

Nutritional analysis

The powdered macrophytes were analyzed following the standard methods of Association of Official Analytical Chemists (AOAC, 2000) for moisture, crude protein and total ash in triplicate. Moisture estimated by drying the sample at 105°C for 4hr. The crude protein content was determined using an automated micro-kjeldhal system (Pelican instrument, Chennai, India). The nitrogen content was multiplied with 6.25 to calculate the amount of crude protein. Ash contents of samples were estimated by drying samples in an oven at 100°C for 24hr and then ignition for 8hr at 550°C in a muffle furnace. The crude lipid content was measured gravimetrically after extraction with chloroform/methanol (2:1, v/v) following the method of Folch et al., 1957.

The calorific values were measured using Bomb Calorimeter (Parr 6400, USA). Dry samples 0.5 g was weighed and pellet was prepared with the help of Pelletizer. The pellet was placed in the crucible which then kept inside the bomb head. The bomb head was closed properly. Samples were combusted in the presence of oxygen at pressure 420 psi. Nitrogen gas was used to flush the water automatically inside the calorimeter to clean the bomb head and crucible after sample analysis. Pressure of nitrogen gas was adjusted at 80 psi. Benzoic Acid tablets (Parr™ Calorimeter Standards Benzoic Acid in Pellets, catalogue no. 23-279045) were used to calibrate the instrument. Calorific value results were expressed as Cal/g.

Anti-nutritional analysis

Tannin

The tannin content was assayed following the method of Folin-Denis (Schanderl, 1970). In 1 mL of the plant extract, 7.5 mL of distilled water, 0.5 mL of 10% Folin Denis reagent, 1 ml of 35% of sodium carbonate were added. The mixture was then incubated in dark condition at room temperature for 30 min. Absorbance was recorded at 700 nm. Tannic acid (Sigma-Aldrich, USA) was used as standard to obtain a linear calibration curve for various concentrations (10 - 250 µg/mL), R^2 value of 0.999. The tannin content was expressed as tannic acid equivalent.

Phytic acid

Phytic acid was determined using spectrophotometric method with Wade reagent (Gao et al., 2007). Briefly, 0.5 g powdered sample was mixed with 10 mL of 2.4% (0.64 N) hydrochloric acid in a falcon tube, vortexed

vigorously and the tube was kept in a container on a platform shaker at 220 rpm for 16 h at room temperature. After 16 h, sample was centrifuged and filtered in a new falcon tube containing 1 g sodium chloride solution. Sample was again placed on platform shaker at 350 rpm for 20 min followed by cooling at 4°C for 60 min or at -20°C for 20 min which helped in settle down the salt. Again, centrifuged the sample and supernatant were diluted in distilled water to make a total volume 25 mL. Diluted sample (3 mL) was added in a borosilicate tube and mixed with 1 mL Wade's reagent (0.03% $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ + 0.3% sulfosalicylic acid). Finally, absorbance was measured at 500 nm and result was expressed as equivalent to sodium phytate.

Saponin

Saponin was determined following the method of Hiai et al. (1976). Saponin from the sample was extracted using the Soxhlet apparatus. Briefly, 0.5 mL of 8% vanillin and 5 mL of 72% sulphuric acid were reacted with 0.5 mL aqueous extract of sample in a vessel. Sample was incubated at 60°C for 10 min followed by cooling in ice cold water for 5 - 10 min. A yellow reaction mixture was appeared and the absorbance was measured at 544 nm using a UV-Vis spectrophotometer against a blank containing ethanol in place sample extract. Diosgenin (Sigma-Aldrich, USA) was used as standard to obtain a linear calibration curve for different concentrations of ethanol with R^2 value of 0.999. The saponin concentrations were calculated from standard curve and expressed as diosgenin equivalents (Ncube et al., 2011).

Results

During culture period, temperature and light intensity ranged 20 - 28 °C and 4346 - 5352 lux, respectively (Fig.1). Total dissolved solids and conductivity ranged 130.366 - 409.333 mg/L and 291.666 - 757.333 µs/cm, respectively (Fig. 2). Both, total dissolved solids and conductivity were low before applying manure in the culture tanks but there was sudden increase in their values as manure started to decompose. The dissolved oxygen level was 6.124 - 7.196 mg/L before applying manure but it decreased and ranged 0.333 - 2.342 after manure decomposition (Fig. 3). The pH of culture water ranged 5.956 - 7.626 throughout the study. The level of ammonia, nitrite, nitrate and phosphate were increased after applying manure and they ranged 1.473 - 8.373, 0.164 - 0.524, 1.038 - 7.442 mg/L (Fig. 4).

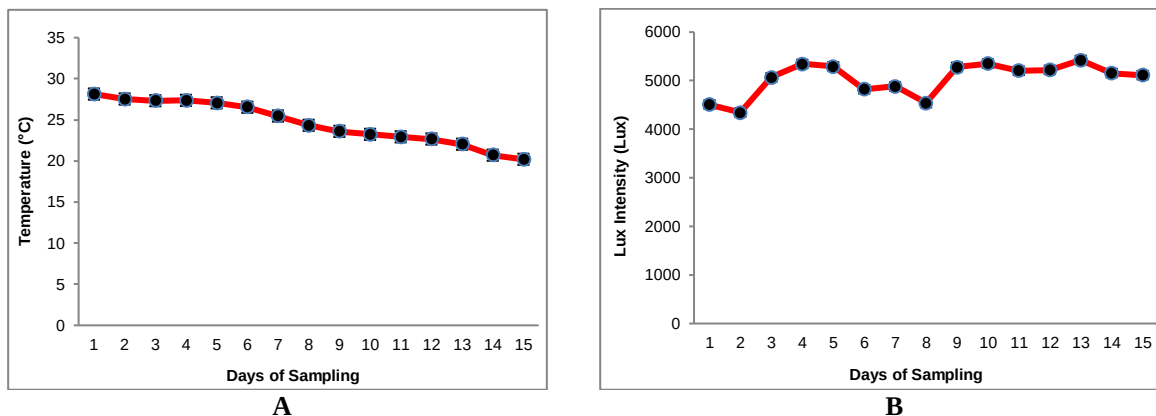


Figure 1: Variations in water temperature (A) and lux intensity (B) during the culture period.

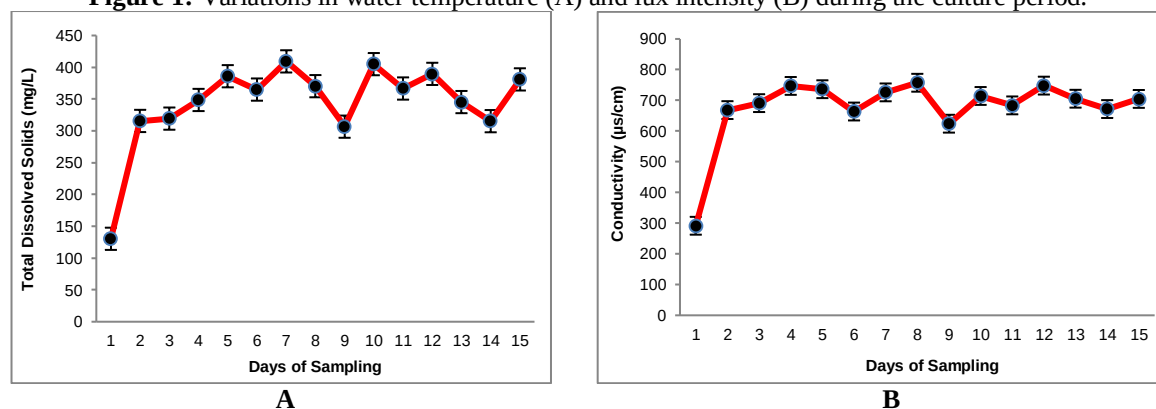


Figure 2: Variations in total dissolved solids (A) and conductivity (B) of water during the study.

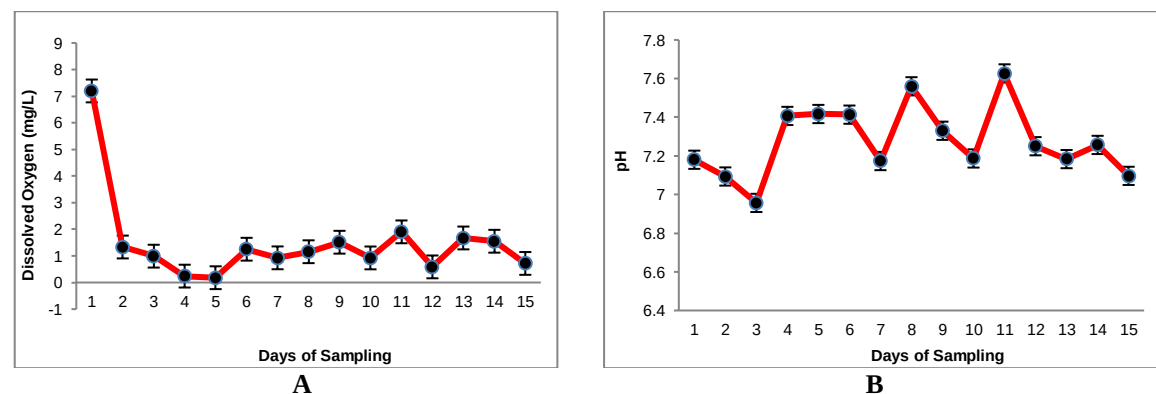
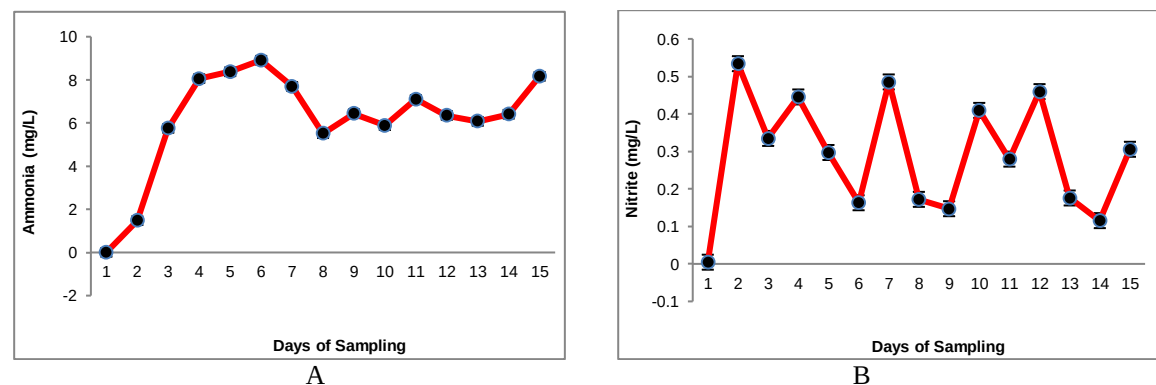


Figure 3: Variations in dissolved oxygen (A) and pH (B) of water during the study.



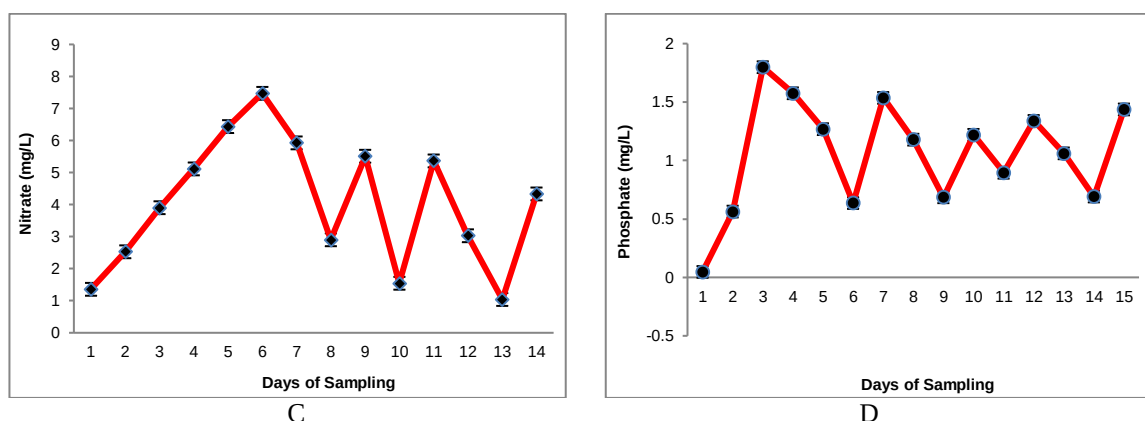


Figure 4: Variations in ammonia (A), nitrite (B), nitrate (C) and phosphate (D) level of water throughout the culture study.

Significant ($P < 0.05$) variations observed in proximate composition of cultured macrophytes viz., duckweeds, water spinach and water lettuce (Table 1). Significantly higher ($P < 0.05$) crude protein found in *L. minor* and *S. polyrhiza* compared to others. The crude lipid and calorific values were observed significantly higher ($P < 0.05$) in duckweed *L. minor* followed by *S. polyrhiza* among others cultured macrophytes. There was significantly ($P < 0.05$) higher ash content found in *P. stratiotes* and *W. globosa* compared to others. Significantly ($P < 0.05$) higher moisture content was found in *P. stratiotes* and *I. aquatica* compared to other cultured macrophytes. There was no significant difference ($P > 0.05$) was observed in the crude lipid and energy value of *I. aquatica* and *W. globosa*. The ash content was not significantly ($P > 0.05$) different between duckweeds *L. minor* and *S. polyrhiza*. There were significant ($P < 0.05$) differences in anti-nutritional factors of the cultured macrophytes.

Significantly ($P < 0.05$) higher saponin content was present in *P. stratiotes* followed by *S. polyrhiza* compared to others. However, significantly ($P < 0.05$) lower saponin content was present in *W. globosa*. Phytic acid was significantly ($P < 0.05$) higher and lower in *I. aquatica* and *L. minor*, respectively compared to others. There was no significant difference ($P > 0.05$) was observed in phytic acid content of two duckweeds *S. polyrhiza* and *W. globosa*. Significantly ($P < 0.05$) higher tannin content was present in *P. stratiotes* followed by *I. aquatica* compared to others. Tannin content was not significantly different in *L. minor* and *W. globosa*. Among duckweeds, *W. globosa* and *L. minor* had significantly ($P < 0.05$) lower amount of tannin and saponin compared to *S. polyrhiza* while higher amount of phytic acid present in *W. globosa* and *S. polyrhiza* compared to *L. minor*.

Table 1: Nutritional composition of cultured macrophytes (% dry matter basis). Data are represented as Mean $\pm$ SE (n = 3).

Macrophytes	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Calorific value (Cal/g)
<i>Lemna minor</i>	8.22 $\pm$ 0.17 ^b	35.94 $\pm$ 0.25 ^a	8.13 $\pm$ 0.06 ^a	19.08 $\pm$ 0.12 ^b	3.79 $\pm$ 0.01 ^a
<i>Spirodela polyrhiza</i>	7.38 $\pm$ 0.13 ^c	35.21 $\pm$ 0.31 ^a	7.19 $\pm$ 0.08 ^b	18.95 $\pm$ 0.15 ^b	3.63 $\pm$ 0.01 ^b
<i>Wolffia globosa</i>	6.36 $\pm$ 0.04 ^d	31.95 $\pm$ 0.22 ^b	6.81 $\pm$ 0.08 ^c	20.95 $\pm$ 0.46 ^a	3.49 $\pm$ 0.02 ^c
<i>Pistia stratiotes</i>	10.77 $\pm$ 0.09 ^a	16.25 $\pm$ 0.06 ^d	5.15 $\pm$ 0.04 ^d	21.69 $\pm$ 0.23 ^a	3.35 $\pm$ 0.04 ^d
<i>Ipomoea aquatica</i>	10.84 $\pm$ 0.29 ^a	28.68 $\pm$ 0.35 ^c	7.12 $\pm$ 0.10 ^c	12.68 $\pm$ 0.27 ^c	3.50 $\pm$ 0.02 ^c
ANOVA	P-value <0.001 F-value 465.210	<0.001 649.845	<0.001 215.548	<0.001 879.621	<0.001 715.231

Table 2: Anti-nutritional factors present in cultured macrophytes (% dry matter basis). Data are represented as Mean $\pm$ SE (n = 3).

Sample	Tannin (%)	Saponin (%)	Phytic acid (%)
<i>Lemna minor</i>	0.22 $\pm$ 0.01 ^d	0.66 $\pm$ 0.02 ^d	1.35 $\pm$ 0.02 ^d
<i>Spirodela polyrhiza</i>	0.54 $\pm$ 0.03 ^c	1.32 $\pm$ 0.01 ^b	1.64 $\pm$ 0.02 ^c
<i>Wolffia globosa</i>	0.19 $\pm$ 0.01 ^d	0.11 $\pm$ 0.01 ^e	1.67 $\pm$ 0.04 ^c
<i>Pistia stratiotes</i>	0.96 $\pm$ 0.02 ^a	1.59 $\pm$ 0.04 ^a	1.85 $\pm$ 0.01 ^b
<i>Ipomoea aquatica</i>	0.71 $\pm$ 0.02 ^b	0.93 $\pm$ 0.03 ^c	2.10 $\pm$ 0.02 ^a
ANOVA	P-value <0.001 F-value 348.743	<0.001 431.545	<0.001 1039.571

Discussion

The results of the present research indicates that there were significant variations in the water quality parameters throughout the cultivation period, especially when the manure was applied. For most aquatic species, the ideal temperature and light intensity ranges were between 20 and 28°C and 4346 and 5352 lux, respectively (Rooke et al., 2018). These values are thought to be appropriate for sustaining microbiological and planktonic life and are in line with usual ranges in aquaculture systems (Timmons et al., 2018).

Significant differences ($P < 0.05$) were observed in the proximate composition of cultivated macrophytes; demonstrate the potential of these macrophytes as alternate feed ingredients and nutrition sources in aquaculture. *L. minor* and *S. polyrhiza* were found to have higher crude protein contents, which is in line with earlier research that concluded duckweeds were low-fiber, high-protein plants that might be used as animal feed (Brito et al., 2017; Sharma 2019). This result is consistent with earlier research identifying duckweeds as high-protein sources, with *L. minor* having 36.94% crude protein and 8.13% lipids (Chakrabarti et al., 2018). Duckweeds especially *L. minor* and *S. polyrhiza* are desirable source for incorporation into aquaculture due to their high protein content and quick growth (Savidov et al., 2007; Kumar et al., 2022). Their high efficiency in absorbing nitrogen and steady supply of nutrients required for protein synthesis, may be the causes of this increased protein content (Kadlec & Knight, 1996). It is also noteworthy that *L. minor* has substantially larger lipid content and calorific values than *S. polyrhiza*; supported by earlier study (Kumar et al., 2022). Other research has shown similar patterns, with duckweeds showing high energy content and calorific values, which makes them good candidates for feed formulations (Van Wyk et al., 2020). The nutritional values of *I. aquatica* recorded in this study supports the earlier study that revealed protein $21.45 \pm 0.32\%$ and calorific value 326.98 ± 0.78 kcal/100g in this macrophyte (Satter et al., 2016). However, earlier study showed the crude lipid value of *I. aquatica* 3.82% which reported lower compared to this study. It may be because of the reason that in earlier study macrophytes were collected from natural source. This increased lipid content implies that organic manures could improve the nutritional value of macrophytes. The higher ash level in *P. stratiotes* and *W. globosa* indicates, these species may have a more varied spectrum of important minerals than the other macrophytes. Ash content is frequently used as an indicator of mineral composition. The amount of ash in these macrophytes can reveal important details about the availability of inorganic nutrients, which could help herbivorous species to meet their nutritional needs (Gao et al., 2021). Their higher ash content might also be a sign of their capacity to take up and retain minerals from their surroundings, which can help to enhance water quality by eliminating excess nutrients (Cooney et al., 2020). Both *P. stratiotes* and *I. aquatica* contained much higher moisture content than the rest of the macrophytes. This is consistent with reports that *P. stratiotes* contain a

moisture content of approximately 83.07% (Wasagu et al., 2013). There were no marked differences in the crude lipid and energy contents between *I. aquatica* and *W. globosa*. This implies comparable lipid contents and energy potentials, as is evident from research showing that *W. globosa* is comprised of 7.62% lipids (Kumar et al., 2022).

Anti-nutritional factors that are crucial for the defense mechanisms of plants can have a detrimental effect on the nutritional value of macrophytes when they are fed to livestock or aquaculture systems. Earlier study conducted on *Salvinia cuculata*, *Trapa natans*, *Lemna minor* and *Ipomoea reptans*, confirmed the presence of tannin and phytic acid in these macrophyte (Kalita et al., 2007). *S. polyrhiza* and *P. stratiotes* have a considerably greater saponin content which is recognized for the foaming and antibacterial qualities (Makkar et al., 2007). Saponins are further known to have the ability to made complex with cholesterol and other sterols, potentially leading to adverse nutrient absorption and growth performance in animals. The higher saponin content in these macrophytes may restrict their direct usage as feed and a prior processing may need to lower these substances to acceptable levels (Bohling et al., 2015). On the other hand, the reduced saponin content in *W. globosa*, suggests that it could be used as a feed ingredient, especially in systems where low saponin levels are desired for better digestibility (Pizzol et al., 2020). Another important anti-nutritional factor is phytic acid, a phosphorus storage form in plants that has ability to chelate minerals, especially calcium, iron, and zinc and reducing their availability for animal absorption (Zhao et al., 2017). Significant ($P < 0.05$) higher phytic acid content in *I. aquatica* revealed higher ability of this macrophyte to store phosphorus. However, this macrophyte need to be processed before using as feed ingredient either it may be more difficult for herbivorous animals to absorb minerals. Lower phytic acid level in *L. minor* raises the possibility that it has improved nutritional availability, especially for micronutrients, which would make it a better choice for animal feed (Miller et al., 2010). *P. stratiotes* compared to *I. aquatica* found to have much more tannins, that have the ability to bind proteins and decrease their digestibility. According to Bharati et al. (2019), tannins are known to possess both anti-nutritional and advantageous qualities, such as antioxidant activity and possible antibacterial actions. However, plants with higher tannin content are less suitable as feed sources if improperly processed, as they can interfere with digestion and decrease the bioavailability of proteins (Salem et al., 2013). Because *P. stratiotes* and *I. aquatica* have higher tannin contents, it is possible that these species will need to undergo further processing, like soaking or fermenting, in order to lower tannin levels and increase their feed value.

Conclusions

The nutritional value of *Lemna minor* and *Spirodela polyrhiza* is quite suitable for their usage in fish feed. The higher crude protein level in these macrophytes suggests that they can be used as alternative protein

source in fish feed to reduce costly fishmeal and other animal protein. The presence of ANFs such as saponins, tannins, and phytic acid in macrophytes is inhibitory to nutrient absorption and overall health in animals. Therefore, processing activities such as cooking and soaking or extrusion may use to reduce ANFs level in these macrophytes, further enhancing them for consumption. Lastly, an understanding of variations in ANF content among different macrophytes is crucial to ensure their fullest utilization in animal feed and human nutrition. Future research needs to be directed towards the development of processing techniques that inhibit the action of these compounds to provide the highest possible nutritional value of the macrophytes.

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