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Research Article

In Vitro Digestibility Study: Evaluating Plant Proteins Digestibility in *Anabas testudineus* and *Channa punctata*

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ABSTRACT

Protein is the most important component of any fish feed for its role in growth, especially during the larval stages, and its high cost. Fish meal continues to be a major source of protein in fish feed production. But its supply cannot keep pace with ever-expanding aquaculture production, leading to its high cost. Plants are being considered as potential replacements in the search for new alternatives to fish meals. But their application depends significantly on their digestibility in target species. The present study aims to determine the protein content of four locally available plants *Moringa oleifera*, *Ipomoea aquatica*, *Lemna minor* and *Salvinia natans*, and test their digestibility in vitro by using the pH drop method in two important food fish *Anabas testudineus* and *Channa punctata*, of Assam, India. The crude protein in all plants ranged from 19-29%, and the highest crude protein was observed in *Lemna minor* (29.9 ± 2.34%). The in vitro digestibility was estimated by calculating the RPD% (relative protein digestibility) using casein as a standard reference. Digestibility of the plant proteins exhibited species-specific variations. The RPD% ranged from 50.39% to 75.39% in *A. testudineus*, and 41.38% to 54.02% in *C. punctata* compared to that of casein (100%). The highest RPD% was observed in *I. aquatica* (75.39%) for *A. testudineus*, and the lowest (50.39%) in *L. minor* whereas, in *C. punctata*, the highest RPD% was observed in *L. minor* (54.02%) and the lowest in *I. aquatica* (41.38%). The digestibility of all plant proteins was comparatively higher in *A. testudineus* than in *C. punctata*. Our results indicate that *I. aquatica* and *L. minor* may be a suitable replacement for animal protein in the diet of *A. testudineus* and *C. punctata*, respectively, because of their good protein content and high digestibility. Moringa may be considered for utilization in the fish feed as it recorded good protein and digestibility. This information may be useful in developing a cost-effective, plant-based protein diet for the two fish species for their mass production.

Keywords: Anabas testudineus, Channa punctata, In vitro digestibility, Ipomoea aquatica, Plant proteins

Introduction

Aquaculture is a fast-growing sector projected to grow by about one-third by the year 2030 [1]. Productive aquaculture depends upon the availability of high nutritional quality, low-cost feeds, contributing nearly 50% of total production cost [2]. Among many factors, a nutrient-rich diet is essential for the high growth rate of the fish required for its successful culture. Protein is the most important component of any fish feed for its role in growth, especially during the larval stages, and also due to its high cost. The aquaculture industry is facing an increasing demand for a protein-rich diet, and the major challenge is to find economically and nutritionally suitable protein sources. Fish meal remains a major source of protein in fish feed, especially for carnivorous species [3]. Currently, it provides the largest supply of dietary protein in the fish feed industry which is considered unsustainable aquaculture as much of the fish meal is obtained from wild catch fishery [4]. The high

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dependence of the aquafeed industry on fish meal and diminishing supply from catch fishery have also led to an increase in the price of the fish feed [5]. Therefore, aquaculture nutritionists are exploring alternative sources of proteins to replace fish meal. Many animal-derived alternative proteins like feather meal [6], poultry meal [7], bone, and meat meal [8, 9] have been investigated, but their mass utilization is hampered by their high cost [10]. Thus, the aquaculture industry has started to focus on plant protein by searching for novel nutrient-rich ingredients [11]. Studies found that plant protein ingredients like oil cake of rapeseed, canola, pea, corn gluten and wheat gluten have high protein content which can be utilized to partially replace animal protein sources like shrimp meal, fishmeal, etc. [4]. Advantages of plant protein sources include greater availability, sustainability, lower price and effectively reducing feed production cost [12]. Aquatic weeds are readily available, have good protein content, and therefore are good potential replacements for costly animal protein in fish feed.

However, poor knowledge of digestibility in specific fish species has been a major concern for the utilization of plant proteins in fish feed. Digestibility study is most important for suitable feed formulation [13] because it decides the species' bioavailability of energy and essential nutrients [14]. In vitro digestibility study is an important method as it simulates the digestive process and environment in laboratory conditions. This method has been elaborated in many animals [15, 16], including aquatic animals like fish and prawns [14]. The *in vitro* methods are usually quicker and more inexpensive than *in vivo* methods [13] and species-specific [11]. This method is particularly useful for preliminary screening, where large numbers of test samples are to be evaluated for different species [17]. *In vivo* digestibility tests, on the other hand, are laborious, complex, time-consuming and expensive, and possibly not adequate for application at the industrial level [11]. Among the different in vitro digestibility methods, the pH drop method is simple, effective and relevant in terms of preliminary screening of a large number of test samples for providing digestibility values in a short period of time. *In vitro* digestibility study of experimental feed using fish enzyme extract from a specific species at a certain age could be a practical, quick and reliable method for testing feed quality in growth trials [18].

Most of the in vitro digestibility studies are reported in penaeid shrimp, salmonids, *Sparus aurata*, tuna, *Cyprinus carpio* and *Gadus morhua. Channa punctata* (Bloch, 1793) and *Anabas testudineus* (Bloch, 1792) are two important food fishes in the Northeast region of India, including Assam, and both the species are hugely popular for their taste and cultural significance in the region. These species are known to contain a high amount of digestible protein, an adequate proportion of amino acids, and high omega-3 fatty acids contents [19]. But their culture and production in the region are hindered by the high cost of feed production depending solely on the animal protein which ultimately results in the high market price of the fish. A survey of the literature showed that very few digestibility studies have been reported in the two species, especially on plant protein digestibility. Therefore, the present study aims to determine the crude protein content in four plants found locally in Kokrajhar, Assam, and to test theirs in vitro digestibility in two important fish species of the region *A. testudineus* and *C. punctata* by pH drop method for their potential utilization in the feed of the two species.

Material and Methods

Plant sample collection

Four different plants *Moringa oleifera* (Moringaceae), *Lemna minor* (Lemnaceae), *Ipomoea aquatica* (Convolvulaceae), and *Salvinia natans* (Salviniaceae), were collected from local areas of Kokrajhar, Assam, India. Only the leaf was used in the case of *M. oleifer*, whereas the whole plant except the roots was utilized for the others. The samples were washed with tap water, rinsed thoroughly with distilled water, sundried, and then oven-dried at 50ºC. The dried samples were grounded, sieved (300 µm) to obtain a fine powder, and finally stored in air-tight containers.

Crude protein

Crude protein (%) was determined by multiplying nitrogen (%) by 6.25, where nitrogen was estimated by the advanced Kjeldahl method using automated nitrogen estimating system (Pelican instrument, Chennai, India).

Experimental fish and preparation of crude enzyme extract

Ten fish, each of *A. testudineus* and *C. punc-*

tata, were collected from the local fish markets of Kokrajhar. The total length and weight of *A. testudineus* (11.50 ± 1.20 cm, 9.68 ± 1.0 g) and *C. punctata* (10.01 ± 1.9 cm, 4.13 ± 1.5 g) were recorded. The fish were acclimated at our wet laboratory facility for 7 days at water temperature (28-30ºC), dissolved oxygen ≥ 6.0 mg/L, and pH (7.8-8.5). The fish were fed 40% protein feed at 5% body weight per day during this period.

Fish were anesthetized with MS-222 (tricaine methanesulfonate) and dissected at 4°C. The digestive tract from individual fish of both (0.11 ± 0.03 g and 0.16 ± 0.05 g, respectively) were cleaned, pooled species-wise and homogenized in Tris-HCL buffer, pH-8.0 (1:5 w/v, tissue: buffer). The homogenates were centrifuged (Eppendorf 5425R, Germany) at 10,000 rpm for 15 min at 4°C, supernatants were collected, called crude enzyme extract and stored at -20°C for further study.

Digestive enzyme activity

Total protease activity was measured using azocaesin as substrate [20]. The reaction mixture consisted of the substrate azocasein, buffer (Tris-HCL, pH 7.5), and crude extract. Samples were centrifuged (12,500 rpm, 5 mins) after adding trichloroacetic acid (20% w/v) and the absorbance of the supernatant was recorded (366 nm). The specific total protease activity was expressed as Units/mg protein/min and calculated as given in Equation 1 below:

$$\text{Activity Units} = \frac{\text{Absorbance (test}-\text{control)}}{\text{mg protein in reaction mixture}\times\text{time}} \quad \text{…….. Eq. 1}$$

Trypsin activity was measured following Erlanger et al. (1961) [21]. BAPNA (N-α-benzoyl-DL-arginine-p-nitroanilide) was used as the substrate, and the change in absorbance (15 mins, 410 nm) was recorded after adding the crude extract. The specific activity was expressed in U/mg protein/min and calculated using Equation 2 as follows:

$$\text{Activity Units} = \frac{\Delta A\times 1000\times V}{8800\times\text{mg protein in the reaction mixture}} \quad \text{…Eq. 2}$$

where ΔA = change in absorbance per min at 410 nm, and V= volume of the reaction mixture

Protein estimation

The soluble protein in enzyme extract was estimated using Lowry's method [22]. The absorbance was measured at 660 nm and Bovine serum albumin was used as standard (1 mg/mL).

In vitro digestibility

The in vitro digestibility assay was determined using the pH drop method [23]. To 50 ml substrate suspensions (containing plant sample equivalent to 8 mg/mL protein in distilled water and adjusted to pH 8.0 using 0.1 N NaOH), 500 µL of crude enzyme extract (having total protease activity 0.30 U/mg protein, pH 8.0) was added to start the reaction at 25ºC. Then the pH was recorded at every 10-minute interval using a pH meter for a one-hour duration (Figure 1).

The protein digestibility was estimated as the percentage of magnitude of pH drop (Δ pH) ratio of the ingredients to that of casein. Casein was used as a reference protein for comparing the digestibility. A blank sample (distilled H_2O used instead of enzyme) was run for each test ingredient and the values were subtracted from the respective Δ pH of each ingredient. The RPD% (Relative protein digestibility) of different plant proteins was calculated by using the following Equation 3 [24]:

$$\text{RPD (\%)} = \frac{\Delta\text{ pH of ingredients}\times 100}{\Delta\text{ pH of casein}} \quad \text{…… Eq. 3}$$

Statistics

Data values are expressed as mean ± standard deviation (SD). One-way ANOVA (analysis of variance) and Tukey's post hoc test were used to find the significant difference between the means in SPSS 23.0. The level of significance was accepted at $p < 0.05$.

Results and Discussion

The detailed experimental procedure of the experimental work in this study is given as a flowchart in Figure 1. Briefly, crude proteins of test plant protein samples were determined, and subsequently, these samples were tested for in vitro digestibility using the crude enzyme extracted from the two fish. The results are discussed in detail as follows.

Crude protein

Results of the crude protein determination are illustrated in Table 1. The present study observed 19-29% crude protein contents in all four plant samples. Significantly higher ($p < 0.05$) crude protein was observed in *L. minor* (29.9 ± 2.34%) and

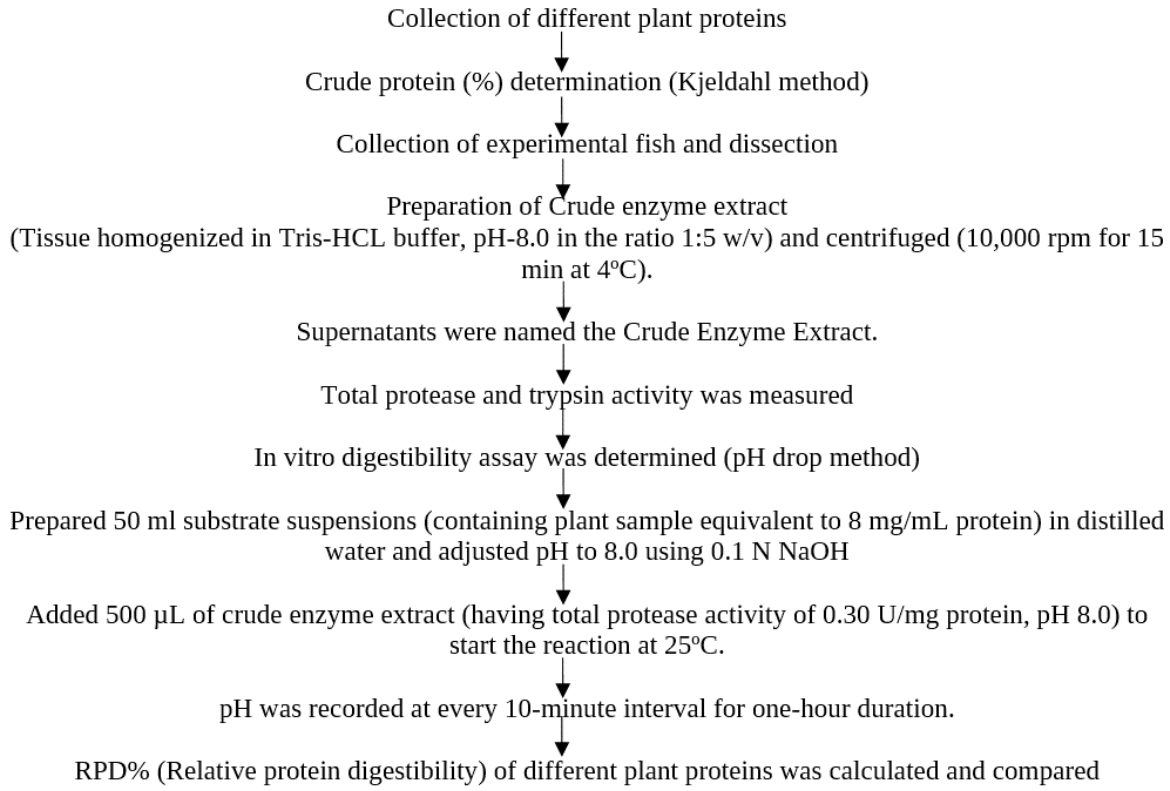

Figure 1. Schematic Flow Chart of the experimental procedure to determine in vitro digestibility of different plant proteins

I. aquatica (27.42 ± 3.25%) compared to the other plants. *M. oleifera* and *S. natans* recorded 19.77 ± 1.53% and 20.81 ± 1.28% crude proteins, respectively. All the investigated plant samples showed moderate (10-20%) to good (20-30%) protein contents, indicating their potential as an alternative protein source. The protein content of different plants observed in the present study agreed with earlier reports.

Significantly higher ($p < 0.05$) crude protein in *L. minor* and *I. aquatica* compared to the other plants may be important because these two aquatic weeds are easily available at a low cost. Because of its high nutritional value, *I. aquatica* has been recommended for use in the aquafeed industry [25]. The potential utilization of *M. oleifera* in aquafeed has been elaborated by Abdel-Latif et al. [26].

M. oleifera has been an important protein source in many studies [26, 27]. Partial replacement of fish meal by this plant in the diet of several fish species has shown good results [28, 29, 30]. In the present study, *M. oleifera* recorded ~20% crude protein, which is similar to earlier reports [31, 32]. *M. oleifera* leaves contain a high proportion of pepsin soluble nitrogen (82-91%), potentially available for digestion [33] in fish, especially those with high pepsin secretion. The protein content in aquatic weed *L. minor* and *S. natans* were in agreement with those reported by Sharma *et al.* [34]. They reported 39.75 ± 0.47 and 20.81 ± 0.10% crude protein in *L. minor* and *S. molesta*, respectively. Dorothy *et al.* [12] have reported in detail about the use of different plant proteins in fish feed, and our findings of crude protein corroborate with their report. Duckweeds (*L. minor*) are known to contain essential amino acids such as threonine, leucine, and lysine [35] which are important for the fish.

Digestive enzyme activity

The digestive enzymes, total protease and trypsin activities, of both fishes, were estimated for in vitro digestibility study. Total protease activities in both the samples were adjusted to obtain a final specific activity of 0.30 U/mg protein. The final total protease activities were 0.36 ± 0.03 U/mg protein for *A. testudineus* and 0.32 ± 0.04 U/mg protein for *C. punctata*. Trypsin activity was 0.26 ± 0.06 U/mg protein f or *C. punctata* and

Table 1. Crude protein (%) content of the four different plant protein sources.

Plant	Crude Protein (%)
Moringa oleifera	19.77 ± 1.53^{b}
Lemna minor	29.90 ± 2.34^{a}
Ipomoea aquatica	27.42 ± 3.25^{a}
Salvania natans	20.81 ± 1.28^{b}

Remarks: *Values were presented as mean ± SD. Means with different superscripts are significantly different ($p < 0.05$).

tata and 0.75 ± 0.02 U/mg protein for *A. testudineus*, respectively.

Digestion determines the bioavailability of nutrients [36] and the degradation of complex food to absorbable nutrients largely depends on available digestive enzymes [37]. Hence, knowledge about digestive secretions in fish is important for a better understanding of the fish's digestive physiology which is vital in determining the limits and nature of the dietary protein. Proteases are enzymes responsible for breaking down larger proteins into simple peptides and amino acids. Adjusting the specific total protease activity to uniformity (~0.30 U/mg protein) makes it possible to compare the digestibility of the same plant between the two species. Lower total protease and trypsin activity observed in *C. punctata* may be correlated with the difference in their digestive anatomy (shorter gut length of *C. punctata* with a distinct stomach region) and a highly carnivorous dietary habit. Similar observations were made by Banerjee et al. [38], where the gastrointestinal tract of *A. testudineus* showed higher enzymatic activities due to the presence large number of microvilli in the gut lumen and the presence of a large number of digestive enzymes.

The use of species-specific crude enzyme extracts for in vitro study may be important since the catalytic nature of digestive enzymes may differ significantly in different species. It also provides a more similar environment to the digestive environment the food is exposed to inside the species' gut. Hence, a more accurate estimation of the digestibility is achieved which is important in comparing digestibility among different species. More reliable and accurate estimation can be obtained through this process rather than using commercial enzyme cocktails. A significant relationship between in vivo apparent protein digestibility and *in vitro* digestibility of proteins for different feedstuffs using enzyme extracts from digestive organs of the target species has been reported [39, 40] indicating the validity of this method.

In vitro digestibility

In vitro protein digestibility was determined by calculating the pH drop (Tables 2 and 3) and the RPD% of each plant protein relative to that of a pure protein (casein). Casein recorded the highest protein digestibility in both the fish species in our study and its RPD% was taken as 100%. The RPD% of all four plant ingredients ranged from 50.39% to 75.39% in *A. testudineus,* and 41.38% to 54.02%.in *C. punctata* (Table 4). All the plant proteins showed significantly ($p < 0.05$) lower digestibility compared to casein in both the fish. The RPD% was significantly ($p < 0.05$) higher in *I. aquatica* (75.39 ± 3.46%) for *A. testudineus* and the lowest was detected in *L. minor* (50.39 ± 6.03%). The RPD% of *S. natans* and *M. oleifera* were 52.73 ± 6.68% and 62.45 ± 7.19%, respectively. There was no significant ($p > 0.05$) difference in the digestibility of all the tested plants in *C. punctata.* However, the highest RPD% was observed in *L. minor* (54.02 ± 5.54%) and the lowest in *I. aquatica* (41.38 ± 2.44%) for the same fish. The RPD% of *M. oleifera* and *S. natans* in *C. punctata* were found to be 48.28 ± 6.22% and 48.85 ± 5.54%, respectively. In general, it was observed that the digestibility of all tested plants was higher in *A. testudineus* compared to that in *C. punctata*. However, in both the fishes *L. minor* recorded good digestibility (RPD% > 50%).

There is very limited information on the digestibility of plant proteins in *A. testudineus* and *C. punctata* as only a few studies are reported [41, 42]. In vitro digestibility trials are important as they provide a pre-absorption estimation of nutrient bioavailability. These trials are best suited for evaluating the variations in the bioaccessibility of nutrients and the effect of different factors on their potential for intestinal absorption [3, 13]. The drop in pH in the in vitro digestibility test corresponds to the release of protons due to hydrolysis of the peptide bonds by the protease present in the crude extract [13]. All the tested plants were found to have a lower RPD% compared to casein, which may be attributed to the purity and absence of anti-nutritional factors in casein.

In general, it was observed that the digestibility of all tested plants was higher in *A. testudineus* compared to that in *C. punctata*. However, in both the fishes, *L. minor* recorded good digestibility (RPD% > 50%), indicating its potential as a pro-

Table 2. Change in pH of different substrate suspensions when treated with crude enzyme extract of *A. testudineus*

Time (min)	Change in pH				
	Casein	*M. oleifera*	*L. minor*	*I. aquatica*	*S. natans*
0	8.11 ± 0.08	8.09 ± 0.05	8.11 ± 0.06	8.12 ± 0.08	8.03 ± 0.07
10	7.74 ± 0.03	7.78 ± 0.04	7.87 ± 0.06	7.88 ± 0.06	7.87 ± 0.02
20	7.63 ± 0.05	7.69 ± 0.04	7.75 ± 0.07	7.75 ± 0.07	7.71 ± 0.02
30	7.49 ± 0.06	7.61 ± 0.06	7.61 ± 0.06	7.63 ± 0.07	7.66 ± 0.03
40	7.41 ± 0.03	7.56 ± 0.07	7.51 ± 0.06	7.54 ± 0.07	7.61 ± 0.03
50	7.34 ± 0.04	7.49 ± 0.08	7.41 ± 0.04	7.44 ± 0.09	7.56 ± 0.04
60	7.25 ± 0.07	7.39 ± 0.05	7.33 ± 0.04	7.38 ± 0.09	7.52 ± 0.05

Table 3. Change in pH of different substrate suspensions when treated with crude enzyme extract of *C. punctata*

Time (min)	Change in pH				
	Casein	*M. oleifera*	*L. minor*	*I. aquatica*	*S. natans*
0	8.07 ± 0.11	8.15 ± 0.04	8.07 ± 0.05	8.13 ± 0.06	8.08 ± 0.05
10	7.86 ± 0.10	7.93 ± 0.11	7.71 ± 0.05	7.82 ± 0.03	7.82 ± 0.06
20	7.72 ± 0.09	7.79 ± 0.05	7.60 ± 0.08	7.73 ± 0.06	7.71 ± 0.04
30	7.58 ± 0.14	7.70 ± 0.09	7.50 ± 0.04	7.68 ± 0.05	7.70 ± 0.04
40	7.50 ± 0.18	7.64 ± 0.10	7.44 ± 0.04	7.66 ± 0.07	7.68 ± 0.04
50	7.40 ± 0.14	7.59 ± 0.10	7.39 ± 0.04	7.59 ± 0.03	7.66 ± 0.04
60	7.27 ± 0.09	7.55 ± 0.07	7.35 ± 0.06	7.56 ± 0.30	7.64 ± 0.06

Table 4. RPD% of different plant proteins in *A. testudineus* and *C. punctata*

Protein source	*A. testudineus*		*C. punctata*	
	Δ pH	RPD%	Δ pH	RPD%
Moringa oleifera	0.40 ± 0.05	62.45 ± 7.19^{bc}	0.28 ± 0.04	48.27 ± 6.22^{b}
Lemna minor	0.32 ± 0.05	50.39 ± 6.03^{b}	0.31 ± 0.03	54.02 ± 5.54^{b}
Ipomoea aquatica	0.48 ± 0.02	75.39 ± 3.46^{c}	0.24 ± 0.01	41.38 ± 2.44^{b}
Salvinia natans	0.34 ± 0.04	52.73 ± 6.68^{b}	0.28 ± 0.03	48.85 ± 5.54^{b}
Casein	0.64 ± 0.07	100.00 ± 0.01^{a}	0.62 ± 0.05	100.00 ± 0.05^{a}

Remarks: *Values are represented as mean ± SD. Means with different superscripts within the same column are significant ($p < 0.05$).

tein source. *L. minor* enriched diet was also reported to show excellent growth performance in Grass carp, *Ctenopharyngodon idella* [43] which establishes the nutritional importance of the plant.

Species-specific variations in digestibility of the plant proteins observed in the present study may be due to combinations of many factors such as the difference in characteristics and action of digestive enzymes in the two species, the variation in the amino acid composition, and/or interaction with anti-nutritional factors present in plants. Differences in solubility, buffering capacity of specific proteins, susceptibility of amino acids to cleavage by protease, and susceptibility of peptide bonds to protease may also be important in causing variations in digestibility among different species [33]. The *in vitro* digestibility method measures the susceptibility of amino acids to cleavage by proteases, which depends on their accessibility and flexibility. The quantitative level of highly susceptible amino acid in a given protein could determine the extent of its enzymatic hydrolysis [33]. The degree of digestibility of any ingredients indicates the bioavailability of all essential nutrients to the fish. In the case of *A. testudineus*, the digestibility was significantly ($p<0.05$) higher in *I. aquatica* compared to all the other ingredients except *M. oleifera*. These results indicate that both the plants may be a good source of alternative protein in the artificial diet of *A. testudineus* because of their good protein digestibility. However, comparatively lower digestibility of all the plant proteins was observed in *C. punctata*. This may be correlated with the lower protease activity, and its highly carnivorous habit consisting mostly of animal protein in *C. punctata*. Gut pro-

tease activities are important for protein digestion. The lowest digestibility observed for *L. minor* in *A. testudineus*, and *I. aquatic* in *C. punctata* may be due to species-specific characteristics of the digestive proteases in the two fish. Our results indicate that *I. aquatica* may be best utilized in feed formulation for *A. testudineus*. This is in agreement with Baruah *et al.* [44], where the optimal growth of *L. rohita* was achieved by using 30-40% fermented *I. aquatic* leaf meal in its feed. Findings by Sharma *et al.* [33] confirmed the nutritional value of *S. molesta* for fish feed in the aquafeed industry and recommended *S. molesta* and *L. minor* as a potential replacement for fish meal in the feed of *L. rohita* and *C. carpio*, respectively, for production of cost-effective protein-rich feed. Considering the good protein content and digestibility observed in our study, *M. oleifera* can also be considered for further investigation. Similar utilization of *M. oleifera* (up to 10%) was reported by Hussain *et al.* [27] in the formulation diet for *L. rohita*. The results of the pH drop RPD% observed in the present study were comparable to those reported in other feed ingredients, including animal proteins in *Puntius gonionotus* (79.41 to 91.18% RPD%) by Ali *et al.* [45]. The high RPD% of *I. aquatic* in *A. testudineus* observed in our study is similar to the RPD% of fish meal (78.26%) observed in the same species in earlier reports [41] indicating the value of the plant. Our results indicate that *L. minor* can be considered for the replacement of fish meal in the diet of *C. punctata* as it recorded high RDP% (54.02 ± 5.54%) and is also rich in protein (29.90 ± 2.34). Similar observations were made by Mohapatra and Patra [46], where up to 15% inclusion of *L. minor* in the feed of common carp, *C. carpio* showed no negative growth effect.

In vitro studies are relevant as many studies have reported a significant agreement between in vitro trials and in vivo digestibility in several fish species [3]. Although the pH drop method is very helpful in the preliminary investigation of protein digestibility, some of its criticisms include buffering capacity of some components present in feeds, and its simplicity, considering the complex processes taking place in the different parts of the digestive tract. In addition, the interactions between hydrolysis of protein and other feed components should also be considered [13]. Nevertheless, this method is still prevalent and widely used for digestibility tests. Low digestibility in some plant proteins in the present study may be an indication of the presence of such anti-nutritional or anti proteases in those plants. The presence of indigestible carbohydrates, mainly non-starch polysaccharides, is a major drawback for the utilization of plant proteins in aquafeeds [3], as these may affect the feed utilization and growth performance of the fish. A proper understanding of these characters through further studies may be useful in determining the appropriate treatments to remove them. Additional studies may be required to determine a possible relationship between in vitro digestibility and individual amino acid availability. This may be significant for targeting the improvement and utilization of these plants in the feed of the two species. The high protein content, good digestibility, and easy availability make *I. aquatica* and *L. minor* good alternative protein sources for the feed of *A. testudineus and C. punctata*, respectively.

Conclusion

The present study has established important information regarding the in vitro digestibility of some plant proteins for the first time in the two economically important fish species of northeast India, *A. testudineus* and *C. punctata*. Considering the low cost, easy availability, high protein, and good digestibility, *I. aquatica* and *L. minor* may replace animal protein in the diet of the two fish species, respectively. Further investigations may be aimed toward their efficient utilization for sustainable aquaculture. This study may be useful in the development of a cost-effective, high-protein, and digestible feed for the two species for mass production.

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