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Evaluation of the nutritional quality of four unexplored aquatic weeds from northeast India for the formulation of cost-effective fish feeds

Pallabi Kalita [a], Pratap K. Mukhopadhyay [b], Ashis K. Mukherjee [a,*]

[a] Department of Molecular Biology and Biotechnology Tezpur University, Tezpur 784 028, Assam, India
[b] Wastewater Aquaculture Division, Central Institute of Freshwater Aquaculture, Rahara 743186, West Bengal, India

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Abstract

A study was conducted to evaluate the nutritional potential of four commonly available, unexplored aquatic weeds namely, *Salvinia cuculata, Trapa natans, Lemna minor* and *Ipomoea reptans* from northeast India for ascertaining their suitability for utilization as supplementary fish feed with the aim to reduce the cost of commercial feeds. Results of proximate analysis showed that the crude protein content of the aquatic weeds ranged between 11.0% and 32.2% (w/w), whereas crude fibre and ash contents varied between 4.2% and 20% (w/w), and 13.3% and 31.2% (w/w), respectively. Protein to energy ratio (P/E) of these weeds ranged between 30.7 mg/kcal and 95.3 mg/kcal and the highest value was displayed by *I. reptans*. All these aquatic weeds contained high amounts of vitamins E and C and mineral elements required for the normal growth and development of fish.

Analysis of antinutritional factors showed that the concentration (g%) of trypsin inhibitor (TIA) ranged from 1.1% to 1.5%, calcium oxalate concentration ranged from 0.6% to 3.5%, tannin concentration ranged from 0.25% to 0.93% and phytate concentration ranged from 0.004% to 0.005% in these plant samples, and the amounts of these antinutritional components were within the tolerable limits of fish, particularly for carp. The present study demonstrates that commercial exploitation of these aquatic weeds, particularly *I. reptans* and *L. minor*, for the formulation of cost-effective and balanced artificial fish feeds appears to be highly promising.

Keywords: Antinutrient; Aquatic weeds; Gross energy; *Ipomoea reptans*; *Lemna minor*; Plant nutrients; *Salvinia cuculata*; *Trapa natans*

1. Introduction

The menace of aquatic weeds is reaching alarming problems in many parts of the world, but it is particularly severe in tropical countries, where abundant sunlight and favourable water temperature, increasing numbers of dams, barrage and irrigation channels foster aquatic plant growth. This problem is further aggravated because these unutilized weeds choke the water bodies, thus reducing the carrying capacity for aquaculture purposes. Such water bodies are often left unproductive with impeded light penetration and depletion of dissolved oxygen. Regrettably, there is hardly any simple or cost-effective way to control the infestation of these aquatic macrophytes in an environment-friendly manner. However, a perusal of the available literature shows that some of the aquatic weeds are highly nutritive and, therefore, one alternative solution to check the massive population of these weeds might be their utilization through incorporation as components of feedstuff for fish and prawn, in particular. In fact, significant effort has been directed toward evaluating the nutritive value of different non-conventional feed resources, including terrestrial and aquatic macrophytes, to formulate nutritionally balanced and cost-effective diets for fish and poultry (Edwards, Kamal, & Wee, 1985; Patra & Ray, 1988; Ray & Das, 1995; Wee & Wang, 1987).

The northeast (NE) part of India, which is considered as one of the hotspot regions of the world, has a rich heritage

* Corresponding author. Tel.: +91 3712 267172 (O); fax: +91 3712 267005/267006.
E-mail address: akm@tezu.ernet.in (A.K. Mukherjee).

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of biodiversity. This area contains an abundance of aquatic weeds that grow throughout the year. A few of them are consumed by local people but many remain unutilized and go to waste. Some of the aquatic weeds of the latter category, namely, *Salvinia cuculata, Trapa natans, Lemna minor* and *Ipomoea reptans,* are widely distributed in this part of the country without any agronomic care. Unfortunately, the nutritive value of these weeds, endemic to northeast India, has never been assessed. Fish-culture is one of the important occupations of this region, and therefore the use of some of these weeds as nutrient sources for fish feed formulation will not only at least partly replace the rather expensive, conventional commercial aqua feeds and assist the pisciculture of this region, but might restrict the alarming growth of these weeds that are affecting the ecosystem.

However, before advocating the utilization of these aquatic weeds for supplementation of fish feeds, there is an urgent need to explore their nutritional quality and anti-nutritional composition. The present study was undertaken to investigate the nutritional potential and anti-nutrient components of four commonly available aquatic weeds from northeast India, namely, *Salvinia cuculata, Trapa natans, Lemna minor* and *Ipomoea reptans*, for ascertaining their suitability for use as fish feed.

2. Materials and methods

2.1. Sample preparation

Samples of fresh, tender and green leaves, stem, roots, fruits and flowers of *Ipomoea reptans, Trapa natans, Lemna minor* and *Salvinia cuculata* were collected from ponds at several locations of the north eastern states of India and taxonomically identified. The samples were washed under running water and blotted dry. The moisture content of the leaf samples was determined at 60 °C (AOAC, 1990). The dried matter obtained was ground to a fine powder and stored at −5 °C in air-tight containers prior to further analysis.

2.2. Proximate analysis

Moisture, ash, ether extract (EE), crude fibre (CF) and nitrogen-free extract (NFE) were determined by the methods of the Association of Official Analytical Chemists (AOAC, 1990). The crude protein content was determined by the Kjeldahl procedure, AOAC Method 920; the factor $N \times 6.25$ was used to convert nitrogen into crude protein. The crude lipid was extracted using a Soxhlet apparatus and quantity of lipid was determined gravimetrically. Crude fibre content was determined by the Fibretech system with repeated treatment of dilute H_2SO_4, followed by dilute NaOH and washing by water. The carbohydrate content was estimated as the weight difference using moisture, crude protein, lipid and ash content data.

Determination of α-tocopherol and carotene contents of plant materials first includes extraction of total lipid material from dried plant powder (Folch, Lees, & Sloane-Stanley, 1957), followed by extraction and estimation of α-tocopherol and carotene levels by the procedure of Baker and Frank (1968). For the extraction of ascorbic acid (vitamin C), 5.0 g of plant material were ground using a pestle and mortar in 50 ml of 4% (w/v) oxalic acid solution and filtered through a Whatman filter paper (No. 100). The ascorbic acid content was then determined volumetrically using 2,6-dichlorophenol indophenol dye (Sadasivam & Manickam, 1992). The gross energy was determined by using an adiabatic bomb calorimeter (IKA C-7000), using benzoic acid as a standard.

2.3. Mineral analysis

The mineral elements of the plants, namely, Na, K and Ca, were estimated by flame photometry, whereas Zn, Cu and Mg contents were measured by atomic absorption spectrophotometry (Carl Zeiss) using standard reference chemicals. The total phosphorus content was determined as described by Umoren, Essien, Ukorebi, and Essien (2005).

2.4. Determination of anti nutritional components

The activity of trypsin inhibitors in the samples was determined by using benzoyl-DL-arginine-paranitroanilide (BAPNA) as a substrate.The trypsin inhibitory activity was expressed as the amount of trypsin inhibitor (TI), in grammes, present per 100 g of sample (Kakade, Rachis, McGhee, & Puski, 1974). Total phenols (tannin) from the plants were isolated as described by Makkar (1994) and then estimated by the Folin–Denis reagent (Makkar & Goodchild, 1996). The tannin content of the samples was calculated as tannic acid equivalents from a standard graph. The phytic acid content of the samples was determined spectrophotometrically using a Hitachi U 2000 uv–vis spectrophotometer (Vaintraub & Lapteva, 1988). Phytic acid was used as a standard. For the estimation of calcium oxalate, the procedure of Jones (1988) was followed.

2.5. Statistical analysis

Data are presented as means ± SD. One-way analyses of variance (ANOVA) were carried out to compare the different values.

3. Results and discussion

3.1. Proximate composition

The proximate compositions of four aquatic weeds, namely, *S. cuculata, I. reptans, T. natans* and *L. minor,* on a fresh weight basis, are presented in Table 1. Moisture, organic matter and lipid contents were nearly identical in

Table 1
Proximate composition of four aquatic weeds from northeast India on dry matter basis (%)

Component	*S. cuculata*	*I. reptans*	*T. natans*	*L. minor*
1. Moisture	9.8 ± 1.1	10.8 ± 1.5	7.6 ± 0.7	8.8 ± 1.2
2. Organic matter	69 ± 0.1	70.0 ± 0.2	87 ± 1.1	75.0 ± 0.7
3. Crude protein ($N \times 6.25$)	11.0 ± 0.1	32.2 ± 1.0	11.4 ± 0.2	28.0 ± 1.7
4. Crude lipid (ether extract)	7.0 ± 0.1	6.0 ± 0.8	8.0 ± 0.6	5.0 ± 0.1
5. Ash	31.2 ± 1.3	30.0 ± 1.2	13.3 ± 0.7	25.0 ± 1.6
6. Total carbohydrate (NFE + Crude fibre)	50.8 ± 1.1	31.8 ± 1.5	67.3 ± 0.9	42.0 ± 1.9
7. Crude fibre	20.0 ± 1.3	10.0 ± 1.2	4.2 ± 0.6	10.0 ± 0.5

Each value represents mean ± SD of three determinations.

all the plants. Among these four plants, *I. reptans* possessed the highest amount of crude protein (32.2%), followed by *L. minor* (28.0%); *S. cuculata*and *T. natans* had comparatively less crude protein (11–11.4%). The ash content in these plants ranged from 13.3% to 31.2%, the highest amount being displayed by *S. cuculata* and the lowest by *T. natans*. Total carbohydrate (including starch) content of *T. natans* (67.3%) was significantly higher ($p < 0.001$) than the three other plants under study, whereas *S. cuculata* possessed the highest amount of crude fibre (20%, w/w), followed by *I. reptans* and *L. minor* (10%, w/w).

Although carbohydrates are important sources of energy in fish diet, the dietary carbohydrate requirement may vary, depending upon the fish species; e.g. herbivorous fish can metabolize carbohydrates better than can carnivorous species (Cowey & Sargent, 1979; Furuichi & Yone, 1981; Shiemeno, Hosakawa, & Takeda, 1979). It is quite reasonable to assume that because of the high protein and low carbohydrate contents, *I. reptans* and *L. minor* may be used as supplementary feed in commercial fish feed, particularly for the formulation of carnivorous fish diet.

3.2. Energy values, vitamin contents and mineral ion concentrations

As shown in Table 2, all four plants exhibited remarkable similarity in possessing nearly the same gross energy value (338 kcal/100 g to 358 kcal/100 g). P/E value was highest in *I. reptans,* followed by *L. minor* and lowest in *S. cuculata*.

Determination of protein to energy (P/E) ratio in fish diet is very important because the higher this ratio, the better is the diet. Generally, for achieving maximum growth, the P/E ratio in fish diet should range from 80 mg/kcal to 100 mg/kcal (Akand, Hasan, & Habib, 1991; Arockiaraj, Muruganandam, Marimuthu, & Haniffa, 1999; Hasan, Moniruzzaman, & Farooque, 1990). Therefore, on the basis of high gross energy, as well as P/E values, it may be inferred that *I. reptans* and *L. minor* are suitable for incorporation in fish diet to reduce the cost of fish feed.

Vitamin contents of these aquatic weeds are shown in Table 2. Vitamin E (α-tocopherol) content of *T. natans* (61.3 mg/100 g) was significantly higher than those of the other three plants, whereas *I. reptans* was characterized as possessing the highest amounts of ascorbic acid (4.0 mg /100 g) and carotenoids (0.25 mg/100 g) (Table 2). The lowest levels of ascorbic acid and carotenoids were found in *S. cuculata* and *L. minor*.

Vitamins are important constituents of fish diet. Vitamin E, nature's most effective, lipid-soluble antioxidant, present in biological membranes, confers stability to the membranes (De Silva & Anderson, 1995). It has been reported that approximate dietary vitamin E requirements for *C. mrigala* and *L.rohita* ranged between 99 mg/kg and

Table 2
Energy values, vitamin contents and mineral ion concentrations in the leaves of four aquatic weeds

Properties	*S. cuculata*	*I. reptans*	*T. natans*	*L. minor*
Energy values				
Gross energy (kcal/100 g)	358 ± 2.1	338 ± 1.2	347 ± 1.4	358 ± 1.6
P/E (mg protein/kcal)	30.7 ± 0.9	95.3 ± 1.0	32.7 ± 1.2	78.4 ± 1.5
Vitamin content				
Vitamin E (mg/100 g)	28.8 ± 0.5	28.5 ± 0.4	61.3 ± 0.7	26.6 ± 0.3
Vitamin C (mg/100 g)	3.0 ± 0.1	4.0 ± 0.2	3.6 ± 0.1	3.8 ± 0.2
Carotenoid (mg/100 g)	0.2 ± 0.04	0.3 ± 0.01	0.2 ± 0.02	0.1 ± 0.01
Mineral ion concentration				
Zn (mg%)	0.7 ± 0.02	1.7 ± 0.01	1.4 ± 0.08	1.0 ± 0.07
Mg (mg%)	17.8 ± 0.1	31.0 ± 0.02	25.1 ± 0.06	20.3 ± 0.03
Cu (mg%)	0.1 ± 0.01	0.1 ± 0.08	0.1 ± 0.005	0.1 ± 0.01
Ca (ppm)	2.0 ± 0.1	2.0 ± 0.004	2.0 ± 0.04	2.0 ± 0.02
Na (mg%)	6.3 ± 1.0	5.0 ± 1.0	5.0 ± 0.2	3.0 ± 0.2
K (mg%)	17.5 ± 0.5	41.4 ± 0.2	27.7 ± 1.0	20.0 ± 0.4
P (g/kg)	1.0 ± 0.1	1.5 ± 0.5	0.9 ± 0.1	1.0 ± 0.4

Values are means of triplicate determinations.

132 mg/kg of dry diet, for the normal growth and development of fish (Paul, Sarkar, & Mohanty, 2004; Sau, Paul, Mohanta, & Mohanty, 2004), whereas dietary vitamin C requirement for Indian major carps is around 10 mg/100 g diet (Mitra & Mukhopadhyay, 2003). However, carotenoid requirement for Indian major carps can be fulfilled by providing 4 mg–6 mg of carotenoids/kg of diet (ADCP, 1983). Therefore, the present study indicates that sufficient amounts of vitamin E and C are present in these plants to meet the requirements of these vitamins for the proper growth and development of Indian major carps. However, the carotenoid concentration in these weeds was much lower than those of alfalfa meal and artificial astaxanthin (Harpaz, Rise, Arad, & Gur, 1998), but it should be noted that this vitamin is mainly required for ornamental fish for pigmentation purposes (Meyers, 1994), and carps may not require a high content of this vitamin in their diet.

The mineral compositions of the four aquatic weeds are presented in Table 2. A significant variation in metal contents was noticed among these plants, which may be attributed to differences in their genus and species level. As shown in Table 2, potassium and magnesium were the most abundant of the elements considered, followed by sodium and copper. Among these plants, *I. reptans* was shown to possess the highest amounts of K (41.4 mg%), Mg (31.0 mg%) and Zn (1.7 mg%), whereas *S. cuculata* and *L. minor* contained the highest amounts of Na (6.3 mg%) and Cu (0.14 mg%). Interestingly, the Ca content was identical in all the four weeds, but phosphorus level was highest in *I. reptans*. Mineral elements play an important role in regulating many vital physiological processes in the body, such as regulation of enzyme activity (cofactor or metalloenzyme), skeletal structures (e.g., calcium and phosphorus), neuromuscular irritability and clotting of blood (calcium). Magnesium has a relationship with the protein concentrations in the blood serum of fish because 25% of the total serum magnesium is bound to albumin and 8% to globulin (Kroll & Elin, 1985). Non-availability of adequate quantities of minerals in the diet affects fish growth and may cause irrecoverable deficiency diseases (De Silva & Anderson, 1995). Although diet is the main source of minerals for fish, some minerals can be absorbed from the environment (Lall & Bishop, 1977). But even then, despite the large amount of calcium in the water, the presence of a minimum amount of dietary calcium (~2.0 ppm) causes an increase in the final weight of the fish, indicating the absolute requirement for calcium in the fish diet (Chavez-Sanchez, Martinez-Palacios, Martinez-Perez, & Ross, 2000). Yueming Dersjant-Li, Wu, Verstegen, Schrama, and Verreth (2001) have shown that dietary Na/K ratios between 1.5 and 2.5 mol/mol produced the best growth for African catfish. The dietary Na/K ratios of the selected four weeds were also within this range, further suggesting their use in fish diet.

3.3. Anti-nutritional factors

Anti-nutrient contents of the four aquatic weeds are summarized in Table 3. Highest trypsin inhibitory activity was detected in *T. natans* (1.53%), followed by *L. minor,* whereas lowest activity was detected in *S. cuculata* (1.13%). The latter plant had higher amounts of tannins (0.93%) and phytate (0.005%) than had the three other aquatic weeds. Calcium oxalate concentration was highest in *L. minor* (3.5%), followed by *T. natans* (0.9%). Interestingly, among these aquatic weeds, *I. reptans* possessed the least amount of tested anti-nutrients.

Generally, when the composition of a feed is analyzed, attention is directed to those components of the feed which provide nutrition to the cultured species (De Silva & Anderson, 1995). However, in addition to nutrients, a feed may contain anti-nutrients, and the presence of significant amounts of such anti-nutritional factors in the weeds is of great concern since they may have a detrimental influence on the growth of the organisms. For instance, oxalate is a chelating agent, which binds calcium very effectively. Plants with high oxalate content may produce an acute metabolic calcium deficiency syndrome (hypocalcemia) when fed as the main feed to livestock (Checke & Shull, 1985). Trypsin inhibitor (TI) is a widespread anti-nutrient substance in many plant-derived nutritional ingredients that could be used in fish feed. It seems that below the 5 mg/g level of dietary TI, most cultured fish are able to compensate for this anti-nutrient by increasing trypsin production (Francis, Makkar, & Becker, 2001). However, Makkar and Becker (1999) reported that carp are capable of tolerating high levels of TI (24.8 mg/g) in their diet.

Tannic acid is known to cause a growth-depressing effect in tilapia and rohu fish (Jackson, Capper, & Matty, 1982). Phytate chelates with certain metal ions, such as calcium,

Table 3
Concentrations of some anti-nutritional factors in the leaves of four aquatic weeds

Component (g%)	Concentration in plants			
	S. cuculata	*I. reptans*	*T. natans*	*L. minor*
Trypsin inhibitor	1.13 ± 0.1	1.34 ± 0.9	1.53 ± 0.2	1.47 ± 0.5
Calcium oxalate	0.7 ± 0.01	0.6 ± 0.001	0.9 ± 0.2	3.5 ± 0.7
Tannin	0.9 ± 0.1	0.3 ± 0.01	0.5 ± 0.2	0.9 ± 0.3
Phytate	0.005 ± 0.001	0.004 ± 0.001	0.004 ± 0.002	0.004 ± 0.001

Values are means ± SD of triplicate determinations.

magnesium, zinc, copper and iron, to form insoluble complexes that are not readily broken down and may pass through the digestive tract unchanged, thus reducing the bioavailibility of these minerals (Maga, 1982). In addition, phytates also form strong complexes with proteins that can lead to reduced digestibility of the latter component (Richardson, Higgs, Beames, & Mcbride, 1985).

4. Conclusion

The present study has demonstrated that, among the tested aquatic weeds, *I. reptans* and *L. minor* could be important sources of proteins, vitamins and minerals, suitable for incorporation in fish diet. Though anti-nutritional factors were found to be present in these weeds, their levels were within tolerable limits and consumption of these plants would not result in any deleterious effect on the growth of fish, further documenting their possible use for the formulation of balanced fish diet.

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