Nutritional Evaluation of Leaf Protein Extracted from Three Aquatic Plants

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Proteins extracted from the leaves of *Alternanthera philoxeroides*, *Lemna minor*, and *Pistia stratiotes* were evaluated for their nutritional quality using rat feeding experiments. It was found that a 3% level of supplementation of leaf protein extracted from *L. minor* and *P. stratiotes* significantly improved the nutritive value of a wheat flour diet, thereby indicating that they could be used as supplements in feeds or foods to improve the quality as well as protein levels of deficient diets.

Keywords: *Aquatic weeds; leaf protein; rat experiments*

INTRODUCTION

Large growths of aquatic plants in lakes and waterways of tropical countries, although a menace, represent a natural resource of green leaves (NAS, 1984). In view of the problems associated with the excessive growth of weeds and the world wide need for additional sources of food, attempts were made in our laboratory to utilize aquatic plants directly as a feed and food source (Banerjee and Matai, 1990; Dewanji et al., 1993). Studies on the feasibility of extracting proteins from aquatic plants have also been reported (Boyd, 1968; Taylor et al., 1971; Rusoff et al., 1980; Dewanji and Matai, 1991). The advantage of the extraction procedure is that in addition to extracting the protein as a concentrate it also helps to reduce the levels of antinutrients present in the original leaf (Rambourg and Monties, 1983; Pirie, 1987; Dewanji, 1993). Thus, leaf protein can be made from aquatic plants which are otherwise toxic and therefore unfit for consumption.

In a previous study, five aquatic weeds, namely, *Alternanthera philoxeroides*, *Azolla pinnata*, *Lemna minor*, *Limnanthemum cristatum*, and *Pistia stratiotes*, were found to be promising for leaf protein extraction based on their low polyphenolic content (below 3%) and *in vitro* digestibility values above 50% (Dewanji and Matai, 1991). The amino acid profile of leaf protein extracted from these weeds was further studied (Dewanji, 1993), and it was found that protein of a uniform composition can be extracted from them with levels of essential amino acids comparable to the FAO reference pattern and chick requirements. The high lysine content of leaf protein can also be useful for supplementing diets of cereal, which is the staple food in many developing countries. The beneficial effects of supplementing cereal diets with leaf protein made from crop plants have been reported (Goel et al., 1977; Hanczakowski and Petzel, 1979; Maciejewicz and Hanczakowski, 1990), but there have been no such studies to evaluate the protein quality of leaf protein extracted from aquatic plants.

This study was, therefore, undertaken to evaluate the acceptability and nutritional effectiveness of leaf protein extracted from three of the five aquatic plants studied previously (Dewanji, 1993), namely, *A. philoxeroides*, *L. minor* and *P. stratiotes*, as a supplement to cereal diets to make possible effective utilization of the plants as a protein source.

MATERIALS AND METHODS

Protein Source. Samples of 10−15 kg of *A. philoxeroides* (alligator weed), *L. minor* (duckweeds), and *P. stratiotes* (water lettuce) were collected, as per their availability, from ponds in and around Calcutta. The samples were transported to the laboratory where they were washed, drained free of water, and hand fed into a specially designed pulper (Davys and Pirie, 1969). The pulper was set with its outflow discharge falling on the belt of a belt press (Davys and Pirie, 1965) where the juice from the pulped material was separated through a perforated roller and the fibrous residue was collected as a byproduct. The protein was precipitated from the juice by steam injection. The protein coagulum obtained was filtered, washed, pressed, freeze-dried, and stored at 4 °C. Vitamin free casein and wheat flour used in the diets were directly purchased from the market, and their nitrogen content was analyzed in the laboratory.

Chemical Analysis. Nitrogen (N) was analyzed by the micro Kjeldahl method and crude protein calculated as N × 6.25. Serum proteins were estimated by the Biuret method (Rajagopalan and Ramakrishnan, 1983) with bovine serum albumin as the reference.

Diet Formulation. The six experimental diets, each containing 10% (w/w) crude protein, used were a wheat flour diet (D_1), three diets of wheat flour supplemented with different LP sources (D_2−D_4), one LP diet (D_5), and a reference diet of casein (D_6). *P. stratiotes* was sampled twice from two different lots since a larger amount was required for two diets (D_4 and D_5). In the supplemented diets (D_2−D_4), LP provided 3% of the protein while wheat flour provided the remaining 7%, a ratio found to give best results in earlier supplementation studies (Garcha et al., 1971; Kawatra et al., 1974). The diets were composed according to AOAC (1984), prepared weekly and stored in airtight containers at 4 °C. Each formulated diet was mixed and sampled for nitrogen analysis prior to feeding. The compositions of the diets are given in Table 1.

Rat Experiments. Weanling albino rats of the Wistar strain were weighed individually and randomly divided into six groups of eight rats each, weighing between 35 and 45 g. The animals were caged individually and provided food and water *ad libitum*. In each group the rats were randomly assigned to experimental diets for a period of 28 days. Food intake was recorded daily while weight gain was recorded weekly. At the end of the 28 day period the protein efficiency ratio (PER) was calculated as

$$\mathrm{PER} = \frac{\text{weight gain (g)}}{\text{protein intake (g)}} \quad (1)$$

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Table 1. Composition of Diets[a] (g kg^{-1} of Diet)

	diet[b]					
	D_1	D_2	D_3	D_4	D_5	D_6
wheat flour	752	526	526	526		
LP (*A. philoxeroides*)		66				
LP (*L. minor*)			78			
LP (*P. stratiotes*)				60	196	
casein						131
salt mixture[a]	40	40	40	40	40	40
vitamin mixture[a]	10	10	10	10	10	10
oil	100	100	100	100	100	100
cellulose	20	20	20	20	20	20
starch	78	238	226	244	634	699

[a] AOAC (1984). [b] D_1, wheat flour (WF); D_2, WF + LP (*A. philoxeroides*); D_3, WF + LP (*L. minor*); D_4, WF + LP (*P. stratiotes*); D_5, LP (*P. stratiotes*); D_6, casein; LP, leaf protein.

Feces excreted during the fourth week of the experimental period were collected daily. The feces of individual rats were pooled and dried at 100 °C for 24 h. The apparent digestibility was calculated from the nitrogen (N) determinations of the food and fecal samples as

$$\text{apparent digestibility} = \frac{\text{N intake (g)} - \text{fecal N (g)}}{\text{N intake (g)}} \times 100 \quad (2)$$

At the end of the experimental period the rats were sacrificed. The animals were killed by a blow to the head and allowed to bleed from the neck. The blood samples were collected and centrifuged at 2500 rpm for 15 min to separate the serum which was then analyzed for total protein and albumin content by the Biuret method. After the animal was sacrificed, the liver was removed, rinsed free of blood with normal saline and distilled water, and trimmed of connective tissue and fat. It was then blotted, weighed, and dried to constant weight at 95 °C. It was finally ground to a fine powder and aliquots taken for nitrogen analysis.

Statistical Analysis. To ascertain group differences for each variable, an analysis of variance (ANOVA) was done (Snedecor and Cochran, 1967) and the least significant difference (LSD) computed for group means which showed statistical significance. PER, being a ratio, was log transformed to have a better approximation to normal distribution, which is the underlying assumption for every variable in ANOVA.

A stepwise discriminant analysis was also performed using the discriminant analysis program 7M in the BMDP statistical package (Dixon et al., 1990) to determine the variables that best discriminate between groups. The seven predictor variables used were PER, apparent digestibility, liver moisture, liver weight, liver nitrogen, serum protein, and serum albumin while the six groups tested were those receiving diets D_1−D_6.

RESULTS AND DISCUSSION

The protein content of the dietary ingredients used was as follows: casein, 76.6%; wheat flour, 13.3%; LP (*A. philoxeroides*), 45.3%; LP (*L. minor*), 38.3%; LP (*P. stratiotes*) in diet D_4, 50.2%; and LP (*P. stratiotes*) in diet D_5, 51.1%.

The observed mean gain in body weight of experimental rats in various groups, the quantity of food consumed, and PER values are given in Table 2 along with the dietary protein content. The PER of the wheat flour fed group was low when compared to the casein fed group, but with the addition of LP an improvement was seen in all the three supplemented diets (D_2−D_4). Rats fed LP from *L. minor* (D_3) and *P. stratiotes* (D_4) had higher growth rates than those fed LP from *A. philoxeroides* (D_2) which was due to the lower diet intake of rats fed diet (D_2). The only diet tested as a sole plant protein source (D_5) had a PER of 1.7 vs 2.4 of the reference casein diet (D_6). This compared favorably with reported PER values for LP prepared from other crop plants (Oke and Umoh, 1974; Cheeke et al., 1980).

The performance of rats for each diet was further evaluated using variables like apparent digestibility, liver analysis (moisture, weight and nitrogen content), and serum constituents (total protein and albumin). Using ANOVA, the *F* values for all variables were found to be significant, indicating that significant differences did exist in mean values between groups. The group-wise performance of the six groups for all the seven variables is reported in Table 3. From the table, it is interesting to note that no significant difference existed between the growth rate of rats fed LP (*P. stratiotes*) as the sole protein source and those fed the same LP supplemented with wheat flour. This indicates that at very low levels of supplementation LP from *P. stratiotes* could considerably improve the growth of rats on a cereal diet. Apparent digestibility was greater for the casein and LP (*P. stratiotes*) diet when compared to the supplemented wheat flour diets, suggesting a greater efficiency of absorption and utilization of nutrients. As a sole protein source, LP from *P. stratiotes* had 79% digestibility which is comparable to values reported for LP prepared from other crop plants (Jokl et al., 1984). In supplementation studies, wheat + LP (*A. philoxeroides*) had the lowest digestibility, while no significant difference was observed between the other two supplemented groups.

On visual inspection no signs of any abnormalities was observed in the liver samples for all groups. No significant difference was observed in liver weights of animals fed LP (*P. stratiotes*) and wheat + LP (*P. stratiotes*), indicating that the supplemented group performed as well as the group fed a sole protein source. An increase in liver constituents (weight and N content) of animals fed LP supplemented diets (D_3 and D_4) as compared to the wheat flour group (D_1) indicated that organ growth may have resulted from synthesis of new proteins in the supplemented groups. In supplementation studies of lucerne to cereal-based diets it was observed from the liver analysis that animals receiving better protein had greater nitrogen content (Subba Rau and Singh, 1970).

Compared to that of the control, the level of serum constituents was low for all groups. However, there was no significant difference between LP (*P. stratiotes*) and the two supplemented groups (*P. stratiotes* and *L. minor*) for both serum protein and albumin.

Factors Affecting Protein Quality. A significant improvement in protein quality of the wheat flour diet (D_1) was observed when LP prepared from *L. minor* (D_3) and *P. stratiotes* (D_4) was supplemented to it. This was probably the result of a favorable balance of the nutritionally essential amino acids in the supplemented diets. Table 4 gives the essential amino acid content of the diets calculated from previous amino acid analyses (Dewanji, 1993) and from a known amino acid content of wheat (Gopalan et al., 1985). On comparison with the reference pattern for preschool children as given by FAO/WHO (1991), the deficiency of wheat as a sole dietary source is obvious from Table 4 and the optimum improvements brought about by a mixture of wheat and leaf protein is evident. However, a poor response in case of the *A. philoxeroides* supplemented diet (D_2), despite the fact that LP extracted from all the three aquatic plants had a similar amino acid composition (Dewanji, 1993), suggests that other factors could be responsible

Table 2. Dietary Protein Content, Food Intake, Body Weight Gain, and Protein Efficiency Ratio of Rats Fed Various Diets[a]

diet	dietary protein (g)	diet intake (g)	gain in weight (g)	protein efficiency ratio (PER)
D_1, wheat flour (WF)	10.4	267.1(5.0)	26.4(1.3)	0.95(0.03)
D_2, WF + LP[b] (*A. philoxeroides*)	10.0	211.9(5.3)	24.1(1.1)	1.14(0.02)
D_3, WF + LP (*L. minor*)	10.1	304.5(2.4)	44.1(1.0)	1.43(0.02)
D_4, WF + LP (*P. stratiotes*)	10.2	295.9(2.5)	46.9(1.5)	1.55(0.04)
D_5, LP (*P. stratiotes*)	9.9	300.1(2.0)	49.3(1.2)	1.66(0.03)
D_6, casein	10.1	309.0(2.7)	74.8(1.2)	2.40(0.02)

[a] Values are mean (SE) of eight observations. LP = leaf protein.

Table 3. Protein Efficiency Ratio, Apparent Digestibility, and Liver and Serum Constituents of the Six Groups [Mean Values (SE)]

diet	protein efficiency ratio (log values)	apparent digestibility (%)	liver moisture (%)	liver weight (%)	liver nitrogen (%)	serum protein (%)	serum albumin (%)
wheat flour (WF)	−0.02	89.7(0.4)	68.5(0.3)	3.21(0.09)	2.31(0.02)	5.36(0.05)	2.87(0.04)
WF + LP[a] (*A. philoxeroides*)	0.05	71.1(0.5)	68.3(0.6)	3.44(0.08)	2.41(0.02)	5.40(0.06)	2.90(0.05)
WF + LP (*L. minor*)	0.16	77.0(0.3)	68.6(0.6)	4.02(0.10)	2.65(0.02)	5.66(0.08)	3.30(0.05)
WF + LP (*P. stratiotes*)	0.19	77.2(0.6)	69.6(0.4)	4.25(0.23)	2.72(0.03)	5.61(0.05)	3.22(0.08)
LP (*P. stratiotes*)	0.22	79.2(0.5)	68.4(0.7)	4.66(0.13)	2.95(0.04)	5.76(0.08)	3.39(0.07)
casein	0.38	91.6(0.5)	66.4(0.8)	5.27(0.28)	3.28(0.05)	6.03(0.05)	3.62(0.06)
LSD ($P = 0.05$)	0.03	1.4	1.7	0.47	0.09	0.18	0.17

[a] LP = leaf protein.

Table 4. Essential Amino Acid Content[a] of Wheat and Leaf Protein Supplemented Diets

essential amino acid	wheat[b] (D_1)	supplemented diets[c] D_2	D_3	D_4	FAO/WHO reference pattern for preschool child[d] (2−5 years)
isoleucine	35.2	42.7	41.8	42.9	28
leucine	65.6	74.2	74.6	75.4	66
lysine	27.2	42.0	40.8	42.6	58
methionine	14.4[e]	29.5	29.9	29.2	25[e]
phenylalanine[f]	67.6	77.6	78.1	79.8	63
threonine	28.8	35.0	35.6	34.8	34
valine	44.8	51.8	50.7	52.0	35

[a] Milligrams of amino acid per gram of protein. [b] D_2, wheat + *A. philoxeroides* leaf protein diet; D_3, wheat + *L. minor* leaf protein diet; D_4, wheat + *P. stratiotes* leaf protein diet. [c] Gopalan et al. (1985). [d] FAO/WHO (1991). [e] Values for methionine plus cystine. [f] Values for phenylalanine plus tyrosine.

Table 5. Results of a Stepwise Discriminant Analysis of Seven Variables

variable	*F* value to enter	*U* statistic	approximate *F* statistic	degrees of freedom
apparent digestibility	279.12	0.0292	279.12	5, 42.00
protein efficiency ratio	185.56	0.0012	225.00	10, 82.00
liver nitrogen	7.57	0.0006	98.95	15, 110.82

for this. A high ash content has been reported to have a detrimental effect on the nutritive value of a protein (Subba Rau et al., 1972), and LP from *A. philoxeroides* had the highest ash content of 7.3% (Dewanji, 1993).

Phenolic compounds can also be growth depressant factors because of their inhibitory effect on protein digestion (Pierpoint, 1983). Osuntogun et al. (1987) reported an inverse relationship between PER and tannin content which could probably explain the poor performance of the *A. philoxeroides* diet since its polyphenolic content was found to be 2.9% as compared to 2.1% in LP (*L. minor*) and 1.3% in *P. stratiotes* LP (Dewanji, 1993). The total phenolics:N ratio of the LP preparation was also reported to have an inverse relationship to protein quality (Maliwal, 1983). A ratio above 0.30 was reported to be high (Subba Rau et al., 1972), and LP made from *A. philoxeroides* had a ratio of 0.38. The acceptance of leaf protein in diets as reflected in amounts eaten during feeding cannot be ignored as a factor affecting quality, particularly in view of wide variations in the diet intake and an apparent association between low food intake and nutritional inferiority of the diets as is evident from Table 2. There is a possibility that limited feeding resulted in energy deficiency. In such a situation part of the protein could be deaminated and utilized as an energy source.

Table 6. Proportion of Total Dispersion, Coefficients, and Constants for the Calculation of Two Canonical Variables

	cumulative proportion of total dispersion	
	0.690	0.997
variable	coefficients for canonical variables	
protein efficiency ratio	19.676	21.474
apparent digestibility	0.509	−0.546
liver nitrogen	6.373	2.787
constant	−61.762	33.122

Statistical Analysis. Table 5 gives the results of the stepwise discriminant analysis which shows that three variables, namely, PER, apparent digestibility, and liver nitrogen, were necessary to account for all the observed differences between various groups.

Table 6 gives the coefficients for the computation of the canonical variates (with maximum between group variance relative to their within group variance), and

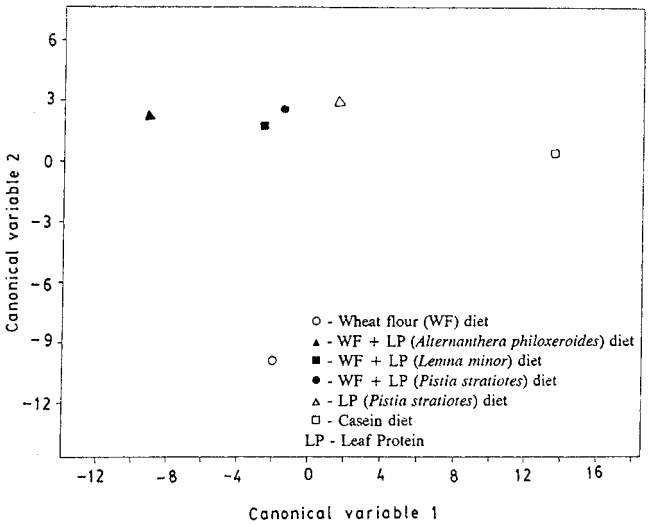

Figure 1. Plot of six group centroids on two canonical variates as given by the stepwise discriminant analysis.

it can be seen that almost all the variability (99.7%) that existed between groups was found to be explained with the inclusion of the second variate. Group differences become more evident from group centroids which have been plotted in a scatter diagram as shown in Figure 1. It can be seen from the figure that the first canonical variate maximally separated the best performing casein group from the rest while the second canonical variate maximally separated the wheat flour group (a cereal source) from the rest. Among the groups clustered together, it is apparent that LP from *L. minor* and *P. stratiotes* when supplemented to wheat flour at a 3% level was almost similar in performance to that of a pure plant protein source, i.e., LP from *P. stratiotes*.

Conclusions. LP extracted from *L. minor* and *P. stratiotes* could be used effectively as a supplement to traditional cereal-based feeds/foods to help alleviate nutritional inadequacies. Exploitation of aquatic weeds which are otherwise of no economic importance would not only be a step toward better resource utilization for additional food production but would also help to solve the weed eradication problem.

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