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Amino acid composition of azolla as affected by strains and population density

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Abstract

The total nitrogen and amino acid composition of seven Azolla strains were compared at four different growth phases. Total nitrogen content of the individual strains ranged from 2.6% to 5.7% of dry matter and was not significantly influenced by growth phase or population density. The concentration of the sixteen amino acids determined was maximal during the linear growth stage and specific differences occurred among Azolla strains. An *Azolla microphylla* strain was the best source of amino acids and an *A. filiculoides* strain the poorest under the cultural conditions used. The chemical index score demonstrated the potential of some species, such as the *A. microphylla* strain, as contributor of protein for animals. Strains of other species, such as *A. filiculoides*, had several limiting amino acids and appear more suited for use solely as a green manure. All Azolla strains contained a similar proportion of essential (55%) and non-essential (45%) amino acids. Leucine, lysine, arginine and phenylanine + tyrosine were the predominant essential amino acids whereas the sulfur containing amino acids (methionine and cystine) were present in smaller amounts.

Introduction

Rapid growth and ability of the Azolla-Anabaena symbiosis to fix atmospheric nitrogen are attributes associated with the use of this fern-cyanobacteria association as a green manure. Azolla has also reportedly been used as a feed for pigs, ducks, cattle and poultry in many countries (Moore, 1969; Lumpkin and Plucknett, 1982).

The main characteristics influencing the value of Azolla as feed or fodder are their amino acid composition followed by other compounds like lipids and digestible carbohydrates. While considerable effort has been devoted to research on maintaining and improving the quality of Azolla as green manure for rice crops, the potential importance of Azolla's as a source of proteins in practical diets for animals has received little attention. During the Azolla Workshop in Fuzhou 1985 (IRRI, 1987) the need for further research to define the potential of this fern as a source of feed for animals was recognized.

The possibility that individual species and strains of Azolla might differ in the quality and quantity of their protein constituents is rarely mentioned. Studying the changes which take place during the growth of specific populations/strains of *Azolla* species is an initial approach to obtaining a much clearer understanding of the time at which Azolla has to be harvested to maximize its value as a feed for animals.

The objective of the present work was to compare the amino acid compositon of different Azolla species/strains and the influence of population density during the different growth phases.

Materials and method

Seven strains encompassing six species of Azolla

(*A. caroliniana*: ADUL-8-CA; *A. filiculoides*: ADUL-4-FI; *A. mexicana*: ADUL-70-ME; *A. microphylla*: ADUL-65-MI; *A. nilotica*: ADUL-82-NI; *A. pinnata* var. *imbricata*: ADUL-7-PI; *A. pinnata* var. *pinnata*: ADUL-53-PP) selected from the collection of the plant physiology laboratory of the Catholic University of Louvain, Belgium were grown in plastic boxes (20 × 15 × 8 cm) containing a diluted 2/5strength Hoagland solution in which nitrates are replaced by chlorides. Azolla was inoculated at one gram fresh weight per box (60 g m^{-2}). Two experimental boxes for each species/strain were randomly arranged in a phytotron cabinet at 31°C/20°C (day-night temperature) and a 16/8 h photoperiod. The light intensity was approximately 10 000 lux and the relative humidity varied between 70–90% (day-night amplitude).

The Azolla cultures were maintained free of epiphytic algae for 45 days and their biomass was determined at 5 day intervals from the onset of the experiment. Samples for total nitrogen and amino acids were collected in the exponential, linear, slowing down and constant phases as defined by van Hove *et al.* (1987).

At each harvest period sample for dry matter was determined after drying to constant weight at 65°C. Total nitrogen was estimated using a C, H, N analyser. The amino acids were determined from ground samples (50 mg each) after hydrolysis under reflux with 50 ml 6 *N* HCl acid for 24 h. The HCl was removed from the hydrolysates by distillation under reduced pressure at 55°C and the residue was taken up in a citrate buffer (pH 2.2). Methionine and cystine were determined as methionine sulfur and cysteic acid after oxidizing the samples with perchloric acid at 0°C for 24 hours. The solution was then evaporated under vacuum at 40°C after which the residue was hydrolysed and then treated as described above. All amino acids were analysed using an automated ion exchange chromatography, Dionex D-300 Amino Acid/Peptide Analyser calibrated for each individual type of amino acids.

The nutritional quality of Azolla proteins was evaluated by calculating the chemical index score or protein score according to the Block and Mitchell method (FAO, 1973).

Data were subjected to analysis of variance and the Duncan's multiple range test was performed using the Statistical Analysis System (SAS) computer package.

Results

Data in Table 1 show the total N of the individual *Azolla* species/strains at the four different growth phases. No clear-cut tendency was observed concerning the influence of growth stage of Azolla on total nitrogen. In contrast to the growth phase, a wide variation of total N, ranging from 2.6% to 5.7% occurred among the Azolla strains. The highest values were obtained by ADUL-CA-8 and ADUL-ME-70 and the lowest by ADUL-FI-4. Samples of ADUL-NI-82, ADUL-PP-53 and ADUL-MI-65 were intermediate and similar.

The total amino acid content (Table 2) is maximum during the 'linear' and the 'slowing down' phases with readily discernible differences among Azolla strains, ADUL-MI-65 showing the highest

Table 1. Azolla total N (% dry matter) as affected by strains and population density during the four phases of growth

Azolla strains	Growth phases			
	Exponential	Linear	Slowing down	Constant
ADUL-MI-65	4.53 ± 0.04	5.04 ± 0.01	5.13 ± 0.03	4.62 ± 0.06
ADUL-CA-8	5.26 ± 0.04	5.59 ± 0.06	4.91 ± 0.04	4.62 ± 0.06
ADUL-PP-53	3.91 ± 0.03	4.54 ± 0.03	4.23 ± 0.04	4.91 ± 0.04
ADUL-ME-70	4.98 ± 0.03	5.51 ± 0.03	4.85 ± 0.03	5.74 ± 0.03
ADUL-NI-82	4.25 ± 0.05	4.41 ± 0.05	5.11 ± 0.03	4.31 ± 0.08
ADUL-PI-7	3.73 ± 0.05	3.55 ± 0.10	4.07 ± 0.07	4.34 ± 0.04
ADUL-FI-4	2.59 ± 0.07	4.34 ± 0.10	3.65 ± 0.06	3.14 ± 0.03
LSD 5% (1)	0.67			
(2)	0.83			

(1) For comparing Azolla strains.
(2) For comparing growth phases.

Table 2. Amino acid (% drÿ matter) of Azolla as affected by strains and by population density during the four phases of growth

Azolla strains	Growth phases			
	Exponential	Linear	Slowing down	Constant
ADUL-4-FI	9.33 ± 0.05	12.00 ± 0.38	10.34 ± 1.06	10.11 ± 0.62
ADUL-53-PP	11.68 ± 0.83	15.56 ± 0.23	16.71 ± 1.23	15.68 ± 0.46
ADUL-65-MI	13.51 ± 1.05	22.43 ± 1.02	22.96 ± 1.09	18.73 ± 0.72
ADUL-7-PI	12.05 ± 1.10	16.27 ± 1.31	16.77 ± 1.52	14.04 ± 1.04
ADUL-70-ME	13.39 ± 0.56	15.59 ± 0.96	17.31 ± 0.85	17.40 ± 0.60
ADUL-8-CA	16.12 ± 1.10	18.69 ± 1.13	19.70 ± 0.42	14.06 ± 0.64
ADUL-82-NI	10.59 ± 0.56	15.85 ± 1.24	16.10 ± 0.59	9.59 ± 0.65
LSD 5% (1)	3.99			
(2)	3.57			

(1) For comparing Azolla strains.
(2) For comparing growth phases.

values and ADUL-FI-4 the lowest. Almost all Azolla strains showed a similar proportion of essential and non-essential amino acids (Table 3). Among the essential amino acids, leucine, lysine, arginine and phenylalanine + tyrosine were predominant. Methionine + cystine and histidine were present in smaller amounts (Table 4). Aspartic acid and glutamic acid represented more than 60% of the non-essential amino acids.

The nutritional quality of Azolla protein at the linear phase calculated using the chemical index score is shown in Table 5. Methionine + cystine were the most limimting for all *Azolla* species used. Amounts of lysine, leucine, arginine and phenylalanine + tyrosine of some species (*e.g.* ADUL-65-MI, ADUL-8-CA) exceeded the levels found in the whole egg. The other amino acids were well represented exceeding the value of 50%. On a percent of dry matter basis Azolla amino acids (22.43% for ADUL-65-MI; 18.69% for ADUL-8-CA) compared favourably with good quality alfalfa (Table 4).

Discussion

One important factor to consider when Azolla is to be used as feed for animals is its protein content and amino acid composition.

This study has shown that the amino acid content varies among different *Azolla* species. *A. microphylla* appeared to be the best source of amino acids, deficient only in methionine and cystine, whilst *A. filiculoides* was the poorest with several deficiencies. Many data have suggested interspecific differences in Azolla composition (De Waha Bail-

Table 3. Proportion (%) of essential and non-essential amino acids during the four phases ot growth of Azolla strains

Azolla strains	Amino acids	Growth phases			
		Exponential	Linear	Slowing down	Constant
ADUL-4-FI	Essential	56	53	57	55
	Non-essential	44	47	43	45
ADUL-53-PP	Essential	56	56	55	55
	Non-essential	44	44	45	45
ADUL-65-MI	Essential	56	55	54	54
	Non-essential	44	45	46	46
ADUL-7-PI	Essential	58	55	55	58
	Non-essential	42	45	45	42
ADUL-70-ME	Essential	55	56	57	56
	Non-essential	45	44	43	44
ADUL-8-CA	Essential	53	55	56	53
	Non-essential	47	45	44	47
ADUL-82-NI	Essential	57	60	54	56
	Non-essential	43	40	46	44

Table 4. Individual essential and non-essential amino acids (% dry matter) of Azolla strains during the linear growth phase

	Azolla strains						
	ADUL-65-MI	ADUL-8-CA	ADUL-53-PP	ADUL-70-ME	ADUL-82-NI	ADUL-7-PI	ADUL-4-FI
Essential amino acids							
Met + Cyst	0.43	0.46	0.23	0.52	0.51	0.21	0.47
Lys	1.62	1.34	0.96	1.27	1.06	1.15	1.04
Val	1.07	0.86	0.97	0.82	0.75	0.88	0.79
Ile	1.07	0.85	0.81	0.84	0.75	0.76	0.57
Thr	1.13	1.03	0.84	0.91	0.85	0.86	0.68
Leu	2.29	1.96	1.71	1.71	1.66	1.79	1.42
Arg	1.90	1.58	1.32	1.56	1.33	1.43	1.04
His	0.47	0.40	0.32	0.37	0.32	0.33	0.28
Phe + Tyr	2.17	1.93	1.45	1.51	1.45	1.57	1.29
Non-essential amino acids							
Asp	2.33	2.04	1.69	1.71	1.82	1.80	1.39
Ser	1.07	0.93	0.85	0.83	0.85	0.91	0.64
Glu	4.04	3.10	0.85	2.28	2.61	2.33	1.91
Gly	1.26	1.08	0.98	0.94	0.98	1.00	0.80
Ala	1.45	1.28	1.18	1.14	1.11	1.25	0.92
Total	22.43	18.69	15.56	15.59	15.85	16.27	12.00

* FAO, 1970

Table 5. Chemical score (%) of different Azolla strains at the linear phase

Essential amino acids	ADUL-65-MI	ADUL-8-CA	ADUL-53-PP	ADUL-82-NI	ADUL-70-ME	ADUL-7-PI	ADUL-4-FI
Met + Cys	33	36	18	40	40	16	36
Lys	105	86	62	82	68	74	67
Val	70	57	64	54	49	58	52
His	87	74	59	69	59	61	52
Ile	76	61	58	60	54	54	41
Thr	99	90	74	80	75	75	60
Leu	117	100	87	87	98	105	76
Arg	140	116	97	115	98	105	76
Phe + Tyr	100	89	67	69	67	72	59

lonville *et al.*, 1984; IRRI, 1983; Peters *et al.*, 1980) and intra-specific differences may also occur. These findings suggest that *Azolla* species/strains should be screened for the protein quality to select those best suited for specific use in agriculture.

Our data indicated that as growth proceeds through the linear phase, the total amino acid contents and most individual amino acid, increased prior to decreasing sharply at the constant phase. Knowing that productivity is maximal during the linear growth phase (Van Hove *et al.*, 1987), harvesting Azolla at the end of this period seems most appropriate, if it is to be used as feed for animals.

The mean protein content of Azolla was 28% which is comparable to that of some rich legumes *e.g.*, alfalfa (FAO, 1970). The potential nutritional quality of Azolla protein was assessed by using the chemical or protein score index. Adequate amounts of essential amino acids were present in most of the Azolla strains at the linear phase but the sulfur containing ones (methionine + cystine) did not reach the recommended values of $3.5\,g.100\,g^{-1}$ protein (FAO, 1973) or $2.3\,g.100\,g^{-1}$ protein for pig (Blum, 1984). This is in agreement with the results of Buckingham *et al.* (1978) and indicates that these limiting S-amino acids should be added to make Azolla a complete source of amino acid. Alternatively, as suggested by Eppendorfer *et al.* (1979) in the case of potato, increasing application of sulfur compounds in the nutrient growth solution might help to overcome the methionine and cystine deficiencies in Azolla.

Determination of the total amino acids expressed as a percentage of the total crude protein (N × 6.25) showed that on average only about 60% was recovered. The unhydrolysed residues were negative for all amino acids determined. This is presumably due to the presence of other N-compounds in Azolla some of which also play role in nutrition.

There have been many attempts to introduce Azolla in various tropical and subtropical countries for its utilization as green manure and feed; these have had variable success (Lumpkin and Plucknett, 1982). One of the questions to be resolved in such introductions is which species or strains are best suited for use as either a green manure or an animal feed in a particular region.

This study has demonstrated that important variation occurs among Azolla strains. *Azolla microphylla* appears to be a much better potential source of essential amino acid for animals, than a strain like *A. filiculoides* ADUL-4-FI. In addition to its low amino acid content, strain ADUL-4-FI has been reported to have a low coefficient of digestibility and a high lignin content (Van Hove *et al.*, 1987). This may explain the poor results obtained with this last species as feed for sheep (de Waha Baillonville, 1983).

However, *Azolla* species/strains will respond differently to environmental conditions such as temperature, regimes, light intensity and soil nutrient constraints. These may affect their growth morphology and perhaps their composition. Contamination with epiphytic algae could also be important to such a degree as to affect the results of an analysis of amino acid composition. Our results showing changes in amino acid during the 4 phases of growth suggest that any factor affecting growth may affect also its amino composition. Further studies are needed to confirm these results under field conditions and to assess the effects of the above factors on the amino acid composition of *Azolla* species.

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