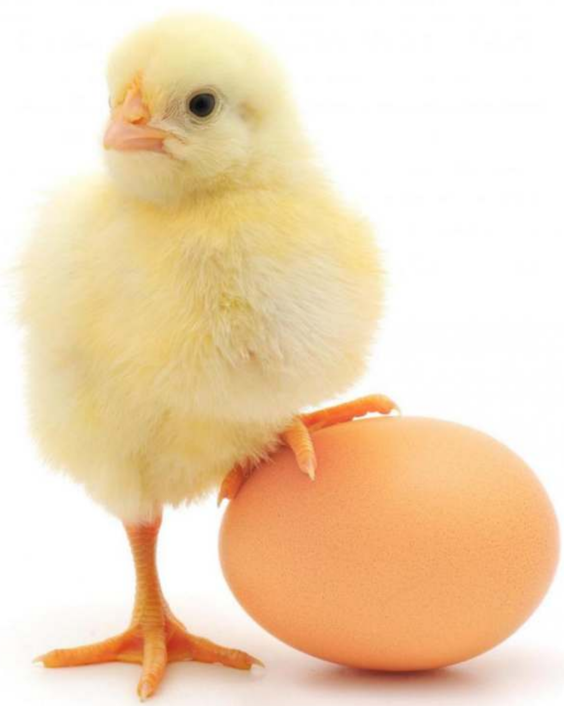
ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 5 (2): 137-141, 2006
ISSN 1682-8356

Chemical Composition and the Feeding Value of Azolla (*Azolla pinnata*) Meal for Egg-Type Chicks

O.A. Alalade and E.A. Iyayi
Department of Animal Science, Faculty of Agriculture and Forestry, University of Ibadan, Ibadan, Nigeria

Abstract: Biochemical and nutritional studies were carried out to evaluate the potential of Azolla (*Azolla pinnata* R. Brown) meal, AZM, as a feed resource in the diet of egg-type chicks. In a completely randomized design, 30 two weeks old Nera brown chicks were assigned to each of the four dietary treatments containing 0, 5, 10 and 15% azolla meal. Chemical analysis indicated that azolla meal contained (%DM) 21.4 crude protein, 12.7 crude fibre, 2.7 ether extract, 16.2 ash and 47.0 carbohydrate. A gross energy value of 2039 kcal kg^{-1} was obtained. The concentrations of calcium, phosphorus, potassium and magnesium were 1.16%, 1.29%, 1.25% and 0.25% respectively, while those of sodium, manganese, iron, copper and zinc were 23.79ppm, 174.42ppm, 755.73ppm, 16.74ppm and 87.59ppm respectively. The chemical score index showed the potential of azolla meal as a good source of protein. Leucine, lysine, arginine and valine were the predominant essential amino acids while tryptophan and the sulphur-containing amino acids were deficient. All AZM diets depressed feed intake. Average weekly weight gains (AWWG) were 95.43g, 95.22g, 98.62g and 93.44g for 0, 5, 10 and 15% AZM respectively. Average weekly feed intake decreased ($p<0.05$) from 286.95g/bird to 224.38g/bird as the level of AZM increased to 15%. A non-statistically better AWWG of 98.62g and feed conversion ratio (FCR) of 2.54 was obtained on diet containing 10% AZM. Based on these results, AZM, as an unconventional feed resource has a potential as a feedstuff for chicks. Above all, for improved performance, diets of chicks can be formulated with inclusion of AZM up to 10%.

Key words: Azolla meal, chemical composition, amino acid content, chicks, weight gain, feed intake

Introduction

Poultry industry as one of the most profitable business of agriculture provides nutritious meats and eggs for human consumption within the shortest possible time. However, availability of quality feed at a reasonable cost is a key to successful poultry operation (Basak *et al.*, 2002). FAO program focuses on increasing the feed base production systems to locally available feed resources in developing countries (Sansoucy, 1993). In addition, for livestock production systems to be sustainable it should be based on the resources available in the country and likewise there should be a balance between crop and livestock, so that these activities are complementary and if possible synergistic (Preston and Murgueitio, 1987).

The greens (green plants) have long been recognized as the cheapest and most abundant potential source of proteins because of their ability to synthesize amino acids from a wide range of virtually unlimited and readily available primary materials (Fasuyi and Aletor, 2005). Any assessment of the potential of leaf meals in non-ruminant nutrition necessitates a comparative review of nutrient content (D'Mello and Devendra, 1995). Though the pigmenting efficiency of leaf meals for broiler chickens remain virtually unexplored, the attributes of leaf meals for egg yolk pigmentation have long been recognized (Osei *et al.*, 1990; Udedibie and Igwe, 1989). Aquatic plant species, because of their growth habit, appear not to accumulate secondary plant compounds and therefore offer a greater potential than tree leaves as a source of protein for monogastric animals (Bacerra *et al.*, 1995). Of these species, the water fern Azolla, which grows in association with the blue-green alga *Anabaena azollae*, a nitrogen fixing organism, is perhaps the most promising from the point of view of ease of cultivation, productivity and nutritive value (Lumpkin and Plucknette, 1982; VanHove and Lopez, 1982). The use of Azolla as a feed resource for fish, swine and poultry had been tested with favourable results (Castillo *et al.*, 1981; Alcantara and Querubin, 1985). Besides, the inclusion of aquatic plants at low levels in poultry diets have shown better performance especially when they supply part of the total protein or when included as a source of pigment for egg yolk and broiler skin (Maurice *et al.*, 1984). With these, coupled with the dearth of information on the performance of egg-type chicken fed azolla meal in diets, this study was conducted to determine the chemical composition of azolla meal and to assess its feeding value for egg-type chicks.

Materials and Methods

Sampling: Whole plants of *Azolla pinnata* (R. Brown) were harvested in sufficient quantities from the Oyo State Ministry of Agriculture fishpond in Ibadan, Nigeria. It was sun dried for three days until they become crispy while

Table 1: Gross composition of chicks diets (%)

Ingredients	Replacement levels of azolla meal 0	0.05	0.1	0.15
Soybean meal	23.3	21.84	20.38	18.92
Corn bran	13.35	9.81	6.27	2.73
Azolla meal	0	5	10	15
Maize	50.6	50.6	50.6	50.6
Bone meal	2	2	2	2
Oyster shell	1	1	1	1
Salt	0.3	0.3	0.3	0.3
Palm oil	1.2	1.2	1.2	1.2
*Premix	0.25	0.25	0.25	0.25
Fish meal (72%)	8	8	8	8
Methionine	0.1	0.1	0.1	0.1
Lysine	0.1	0.1	0.1	0.1
Total (kg)	100	100	100	100
Calculated composition:				
Crude protein (%)	22.6	22.6	22.6	22.6
Crude fibre (%)	4.2	4.44	4.55	4.67
Ca (%)	1.63	1.68	1.74	1.79
P (%)	0.74	0.79	0.84	0.89
ME (kcal/kg)	3031.2	2993.1	2955	2916.9

*Embavit premix, RhonePoulenc Lagos contains the following vitamins A, D_3, E, B_{12}, riboflavin, pantothenic acid, nicotinic acid, folic acid, choline chloride; and P, Ca, I, Cu, Mn, Zn, Fe and Se; Terramycin, anti-oxidant and anti-caking agent.

retaining their greenish coloration. The dried leaves were then milled using a hammer mill to produce leaf meal, which was then stored in sacs until used for feeding.

Proximate composition: A sample of the leaf meal was subjected to proximate analysis according to standard methods of AOAC, (1990). Nitrogen-free extract (NFE) was obtained by difference. Nitrogen content was estimated by the micro Kjeldahl method. Gross energy value was determined using a Gallenkamp adiabatic oxygen bomb calorimeter.

Fibre analysis: The method of Goering and Van Soest, (1970) was used to determine the neutral detergent fibre (NDF) and the acid detergent fibre (ADF). Insoluble hemicellulose was calculated as loss in weight of ADF residue after treatment with sulphuric acid. The loss in weight of the above residue upon ashing was used to calculate the lignin content.

Mineral analysis: From the triple digested sample, calcium, magnesium, iron, zinc, manganese and copper were determined using a Perkin Elmer atomic absorption spectrophotometer (Model 5000, Perkin Elmer, USA), while a flame photometer (Elico, India) was used for the determination of potassium and sodium. Total phosphorus was assayed at 630nm following the APHA, (1980) method using a spectrophotometer (Model Spectronic 20D, Milton Roy, USA).

Amino acid analysis: The amino acids were determined from ground samples (50mg) after acid hydrolysis under reflux with 50ml 6N HCl acid for 24h at 110°C. The amino acid analysis was performed using an automated precolumn derivatisation with o-phthaldialdehyde (OPA) using reverse-phase HPLC (Model 23250, ISCO, USA). Tryptophan was determined following the procedure described by Fontaine *et al.* (1998). The contents of the different amino acids recovered were expressed as g per 100g protein. The nutritional quality of AZM was evaluated by calculating the chemical index score or protein score according to the Block and Mitchell method (FAO, 1973).

Experimental diets and birds: From the result of the chemical analysis, four isonitrogenous diets were formulated to contain Azolla meal (AZM) at 0, 5, 10 and 15% respectively. Adjusting the levels of soybean meal and corn bran equalized crude protein content of chick diets. In a completely randomized design, 120 2-weeks old brown Nera chicks were distributed into these four dietary treatment groups having three replicates each. The experimental birds were managed properly in terms of the housing environment, floor space, feeder and waterer space, litter management, lighting management, vaccination and medication. During the experimental period, body weight, feed intake and mortality records were taken. All the recorded and calculated data were subjected to analysis of variance (ANOVA) (Steel and Torrie, 1980) while significant differences were separated using Duncan Multiple Range Test (Duncan, 1955).

Results

Chemical analysis showed that azolla meal (AZM) contained (%DM) 21.4 crude protein, 12.7 crude fibre, 2.7 ether extract, 16.2 ash and 47.0 carbohydrate. A gross energy value of 2039.0 kcal/kg was also obtained (Table 2). The concentrations of calcium, phosphorus, potassium and magnesium were 1.16, 1.29, 1.25 and 0.25 percent respectively while those of sodium, manganese, iron, copper and zinc were 23.79, 174.42, 755.73, 16.74 and 87.59 ppm respectively (Table 2). Leucine, lysine, arginine and valine were the predominant amino acids found in AZM while tryptophan and the sulphur-containing amino acids were deficient (Table 2). Cell wall fraction of AZM contained 47.08% NDF, 36.08% ADF, 10.20% hemicellulose, 12.76% cellulose and 28.24% lignin (Table 2).

The performance of chicks fed graded levels of azolla meal in diets is shown in Table 3. There was no significant difference in the weight gain of chicks. Average weekly weight gains (AWWG) were 95.43, 95.22, 98.62 and 93.44g on 0, 5, 10 and 15% AZM dietary treatments respectively. Diet containing 10% AZM did non-statistically better than the control. Average

Table 2: Chemical composition of azolla meal (AZM)

Nutrient	% DM
Crude protein	21.4
Crude fibre	12.7
Ether extract	2.7
Ash	16.2
NFE	47.0
Cell wall fraction	
Neutral detergent fibre	36.88
Acid detergent fibre	47.08
Hemicellulose	10.20
Cellulose	12.76
Lignin	28.24
Minerals	
Calcium	1.16
Total phosphorus	1.29
Potassium	1.25
Magnessium	0.35
Trace minerals	
ppmDM	
Manganese	174.42
Zinc	87.59
Copper	16.74
Iron	755.73
Sodium	23.79

Table 3: Amino acid composition of azolla meal

Amino acids	% DM	g /100g protein	Chemical score (%)
Lysine	0.98	4.58	130.9
Methionine	0.34	1.59	45.4
Cystine	0.18	0.84	24
Threonine	0.87	4.07	116.3
Tryptophan	0.39	1.82	52
Arginine	1.15	5.37	153.4
Isoleucine	0.93	4.35	124.3
Leucine	1.65	7.71	220.3
Phenylalanine	1.01	4.72	134.9
Tyrosine	0.68	3.18	90.9
Glycine	1.00	4.60	131.4
Serine	0.90	4.21	120.3
Valine	1.18	5.51	157.4

weekly feed intake (AWFI) were similar up to 5% AZM inclusion in diets while 10 and 15% AZM in diets significantly reduced AWFI. Feed/gain ratio (FCR) decreased from 3.13 on control diet to 2.54 in birds fed 10% AZM, and increased to 2.55 on 15% AZM.

Discussion

The use of a feed ingredient in feeding livestock presupposes that the nutritive value in terms of nutrient content and availability are known. The crude protein (CP) content of AZM was 21.4%. This is lower than values reported by Basak *et al.* (2002), Nwanna and Falaye (1997) and Bacerra *et al.* (1995) but higher than 17.59% CP reported by Querubin *et al.* (1986) for a strain of Azolla. VanHove and Lopez (1982) noted that the crude protein content of Azolla might vary from 13.0 to 34.5%. Sanginga and VanHove (1989) attributed variations in the nutrient composition of Azolla meal to differences in the response of Azolla strains to environmental conditions such as temperature, light intensity and soil nutrient which consequently affect their growth morphology and composition. Furthermore, contamination with epiphytic algae could also be important to such a degree as to affect the results of an amino acid composition. Azolla meal crude protein is lower than that of groundnut cake and soybean meal (42 – 48% CP), which are the conventional sources of plant protein in poultry ration. Crude protein content of leaf meals has often been promoted as a major attribute to products of relatively high fibre content. The crude fibre content of AZM is 12.7%. It did not exceed or equal its protein content. Thus the tendency to depress the overall crude protein digestibility when it constitutes a significant portion of the diet is low (Tangendjaja *et al.*, 1990). Lower values obtained for acid detergent fibre (ADF) and neutral detergent fibre (NDF) compared with that report by Querubin *et al.* (1986) indicate a better efficiency of utilization by non-ruminant animals. Thus, in environments where high temperature is accompanied by high humidity, bulky diets might have a useful role, as feed for poultry, provided the utilization of nutrients is not impaired and sufficient dietary energy is metabolized to meet their needs. Azolla meal utilization as a carrier for vitamins and microminerals in premix manufacture can also be exploited. Data on the amino acid analysis indicate that lysine, arginine, isoleucine, leucine, phenylalanine, glycine and valine were predominant. However the sulphur-containing amino acids did not meet the recommended (FAO, 1973) value of 3.5g/100g protein. Amino acid values obtained in this study were lower than those reported by Beckingham *et al.* (1978). Thus any attempt to replace conventional high quality ingredients in diets for non-ruminant animals should give recognition to differences in lysine content and deficiencies of the sulphur-containing amino acids. Sanginga and Van Hove (1989) have indicated that these limiting amino acids be added to make Azolla a complete source of amino acids.

Nutrition is by far the most important environmental influence on animal growth and development before and after birth (Widdowson and Lister, 1991). Warren and Farrel (1990) stressed that young and rapidly growing non-ruminant animals are the most important experimental animals because they are sensitive to small differences in diets and dietary components that are relatively easy to measure as growth and intake responses. Chicks fed diet containing 15% AZM had the lowest growth rate, however, the value was not

Table 4: Performance of pullet chicks fed graded levels of azolla meal in diets

	Replacement levels of azolla meal in diet				
Parameters	0%	5%	10%	15%	SEM
Weight gain (g/week)	95.43	95.22	98.62	93.44	±6.64
Feed intake (g/week)	286.95[a]	270.73[a]	231.28[b]	224.38[b]	±10.61
Feed conversion ratio	3.13[a]	3.05[ab]	2.54[b]	2.55[b]	±0.19
Efficiency of feed utilization	0.35[b]	0.35[b]	0.42[a]	0.41[ab]	±0.02

[ab]:Means on the same row with different superscript are significantly ($p<0.05$) different

significantly different from others. The poorer growth rate of birds on 15% AZM diets could be attributed to the lower feed intake and consequently a reduced metabolizable energy intake. It has been reported that birds on high-energy diets consume more metabolizable energy thus resulting in a significant increase in body weight gain (Brue and Latshaw, 1985). Previous reports by Basak *et al.* (2002), Tamany *et al.* (1992) and Beckingham *et al.* (1978) have implicated high levels of ADF and lignin as the main factor limiting the efficient utilization of AZM by monogastric animals. The trend of the growth rate did corroborate previous observations. Basak *et al.* (2002) and Querubin *et al.* (1986) recorded the highest weight gain in birds on diet containing 5% AZM while Cambel (1984) found better result using 10 and 15% AZM. Variations observed in weight gain at different levels of AZM could be attributed to differences in the strain and nutrient composition of Azolla used; and the type and physiological state of the experimental animal used. Feed intake was significantly affected on the 10 and 15% AZM dietary treatments. This observation parallel previous report by Bhuyan *et al.,* (1998), Castillo *et al.* (1981) and Basak *et al.* (2002) that inclusion of AZM in broiler diet did not affect feed consumption. However, Bacerra *et al.* (1995) explained the decrease in dry matter intake as the inability of the birds to eat more of the bulky AZM-based diets. Lower feed conversion ratio (FCR) of the AZM-based diets compared with the control suggests a benefit from the AZM supplementation. Bacerra *et al.* (1995) in studies with growing ducks observed that growth rate was linearly related to protein intake. The correlation was found to be stronger with total protein (Azolla + soybean protein) compared with soybean protein. No mortality was recorded in all the treatments. Thus indicating that AZM had no deleterious effect on pullet chicks.

Conclusion: Based on the results of the study, it could be concluded that AZM as an unconventional feed resource has a potential for use in diets for non-ruminant animals. Above all, for the best performance, diets of pullet chicks can be formulated with the inclusion of AZM up to 10%.

References

Alcantara, P.F. and L.J. Querubin, 1985. Feeding value of *azolla* meal for poultry. Philippine J. Vet. Anim. Sci., 11: 1-8.

A.O.A.C., 1990. Official method of analysis, Association of official Analytical Chemists (15th ed.), Arlington, VA.

APHA, 1980. Standard Methods for the Examination of Water and Waste Water (15th edn). American Public Health Association, Washington, DC, pp: 417-420.

Bacerra, M., T.R. Preston and B. Ogle, 1995. Effect of replacing whole boiled soya beans with azolla in the diets of growing ducks. Livest. Res. Rural Dev., 7: 1-11.

Basak, B., A.H. Pramanik, M.S. Rahmnan, S.U. Taradar, and B.C. Roy, 2002. Azolla (*Azolla pinnata*) as a feed ingredient in broiler ration. Int. J. Poult. Sci., 1: 29-24.

Beckingham, K.W., S.W. Ela, J.G. Moris and C.R. Goldman, 1978. Nutritive value of the Nitrogen-fixing aquatic fern *Azolla filiculoides*. J. Agri. Food Chem., 26: 1230-1234.

Bhuyan, M.A.H., M.R. Hasanat, M.A. Ali and M.A. Rahman, 1998. Effect of feeding Azolla (*Azolla pinnata*) on the performance of broiler. Bang. J. Anim. Sci., 27: 77-82.

Brue, R.N. and J.D. Latshaw, 1985. Energy utilization by the broiler chicken as affected by various fats and fat levels. Poult. Sci., 64: 2119-2130.

Cambel, I.M., 1984. Growth performance of broilers fed with varying levels of azolla meal. Kabacan, North Cotabato (Philippines), pp: 66.

Castillo, L.S., A.L. Gerpacio and F.S.D. Pascual, 1981. Exploratory studies on Azolla and fermented rice hulls in broiler diets. College Laguna (Philippines) pp: 6.

D'Mello, J.P.F. and C. Devendra, 1995. Tropical Legumes in Animal Nutrition. Wallingford, CAB International.

Duncan, D.B., 1955. Multiple Range and F-test. Biometrics 11: 1-42.

FAO, 1973. Energy and protein requirements. Report No. 52.

Fasuyi, A.O. and V.A. Aletor, 2005. Protein replacement value of cassava (Manihot esculenta, Crantz) leaf protein concentrate (CLPC) in broiler starter: Effect on performance, muscle growth, haematology and serum metabolites. Int. J. Poult. Sci., 4: 339-349.

Fontaine, J., S. Bech-Andersen, U. Butikofer and I. de Froidmont-gortz, 1998. Determination of tryptophan in feed by HPLC-Development of an optimal hydrolysis and extraction procedure by the EU commission DG XII in three international collaborative studies. Agribiol. Res. 51: 97-108.

Goering, H.K. and P.J. Van Soest, 1970. Forage fibre analysis (USDA Handbook no. 279). US Printing Office, Wasington, DC, USA, p: 379.

Lumpkin, T.A. and D.L. Plucknette, 1982. Azolla as a green manure: use and management in crop production. Series No. 15, pp 230, Westview Press, Boulder, Colorado, U.S.A.

Maurice, D.V., J.E. Jones, C.R. Dillon and J.M. Weber, 1984. Chemical composition and nutritive value of Brazilian Elodea (*Egeria densa*) for chicks. Poult. Sci., 63: 317-323.

Nwanna, L.C. and A.E. Falaye, 1997. Substitution of Azolla meal for groundnut cake in diets for Nile tilapia, *Oreochromis niloticus* (L). Appl. Trop. Agri. 2: 139-143.

Osei, S.A, R.S. Opoku and C. Atuahene, 1990. Gliricidia leaf meal as an ingredient in layer diets. Anim. Feed Sci. Tec., 29: 303-308.

Preston, T.R. and E. Murgueitio, 1987. Trees and shrub legumes as protein sources for livestock. In: Forage legumes and other local protein sources as substitutes for imported protein meals, D. Walmsley (ed), CTA: Wageningen and CARDI: Trinidad, pp: 94-104.

Querubin, L.J., P.F. Alcantara and A.O. Princesa, 1986. Chemical composition of three Azolla species (*A. caroliniana, A. microphylla and A. pinnata*) and feeding value of azolla meal (*A. microphylla*) in broiler ration 11. Philippine Agriculturist, 69: 479-490.

Sanginga, N. and C. VanHove, 1989. Amino Acid Composition of Azolla as affected by strains and population density. Plant and Soil, 117: 263-267.

Sansoucy, R., 1993. The FAO program for better utilization of local feed resources in developing countries. In proc. 7th world conference Animal Production. Edmonton, Canada, pp: 77-80.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and procedures of statistics, McGraw-Hill, New York, pp: 137- 269.

Tamany, Y., G. Samanta, N. Chakraborty and L. Mondal, 1992. Nutritive value of Azolla (*Azolla Pinnata*) and its potentiality of feeding in goats. Environment and Ecology, 10: 755-756.

Tangendjaja, B., Y.C. Rahardjo and J.B. Lowry, 1990. Leucaena leaf meal in the diet of growing rabbits: evaluation and effect of a low mimosine treatment. Anim. Feed Sci. Tec., 29: 63-72.

Udedibie, A.B.I. and F.O. Igwe, 1989. Dry matter yield and chemical composition of pigeon pea (*C. cajan*) leaf meal and the nutritive value of pigeon pea leaf meal and grain meal for laying hens. Anim. Feed Sci. Tec., 24: 111-259.

VanHove, C. and Y. Lopez, 1982. Fisiologia de Azolla. In: Workshop on the assessment of Azolla use in tropical Latin America. Chicklayo: Peru.

Warren, B.E. and D.J. Farrel, 1990. The nutritive value of full-fat and defatted Australian rice bran 11. Growth studies with chickens, rats and pigs. Aust. Feed Sci. Tec., 27: 229-246.

Widdowson, E.W. and D. Lister, 1991. Nutritional Control of Growth. In: Pearson, A.M. and Dutson, T.R. (Eds). Growth Regulation in Farm Animals: Advances in eat research pp: 67-94. Elsvier, London.