Evaluation of Some Aquatic Plants from Bangladesh through Mineral Composition, *In Vitro* Gas Production and *In Situ* Degradation Measurements

M. J. Khan*, H. Steingass[1] and W. Drochner[1]

Department of Animal Nutrition, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

ABSTRACT : A study was conducted to evaluate the nutritive potential value of different aquatic plants: duckweed *(Lemna trisulaca)*, duckweed *(Lemna perpusila)*, azolla *(Azolla pinnata)* and water-hyacinth *(Eichhornia crassipes)* from Bangladesh. A wide variability in protein, mineral composition, gas production, microbial protein synthesis, rumen degradable nitrogen and *in situ* dry matter and crude protein degradability were recorded among species. Crude protein content ranged from 139 to 330 g/kg dry matter (DM). All species were relatively high in Ca, P, Na, content and very rich in K, Fe, Mg, Mn, Cu and Zn concentration. The rate of gas production was highest in azolla and lowest in water-hyacinth. A similar trend was observed with *in situ* DM degradability. Crude protein degradability was highest in duckweed. Microbial protein formation at 24 h incubation ranged from 38.6-47.2 mg and *in vitro* rumen degradable nitrogen between 31.5 and 48.4%. Based on the present findings it is concluded that aquatic species have potential as supplementary diet to livestock. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 4 : 537-542)***

Key Words : Aquatic Plants, Mineral Composition, Gas Production, Rumen Degradable Nitrogen, *In Situ* Degradability

INTRODUCTION

One of the major limiting factors to animal production in Bangladesh is nutrition. The acute shortage of feeds and fodders has stimulated a lot of research aimed at utilizing unconventional feeds in animal ration. A number of reasons, including human population pressure on the land, scarcity of high cost concentrate feeds and the economic need to match livestock production system with available resources, justify the increased use of non-conventional feed resources for animal feeding. Feed costs, the single largest expense in animal production, may be reduced by using aquatic plants as animal feed. Numerous aquatic plants are extensively grown throughout the year in Bangladesh. Among them water-hyacinth, duckweeds, azolla and algae commonly grown in flooded rice fields, ponds, canals, road side ditches and other water reservers. But usage is sometimes limited due to poor understanding of their nutritional and economic value, as well as their proper use in animal ration. In fact, little is known about the nutritive value of this type of aquatic biomass for livestock.

Multiplication rate and nutrients removal capacity of aquatic plants from water is an unique feature. Biomass production of water-hyacinth have been estimated to 100-200 MT/ha/yr. Under ideal conditions, each plant can produce 248 offspring in 90 days (Matai and Bagchi, 1980). Holm et al. (1977) reported that a floating mat of medium sized plants may contain 2,000,000 plants/ha weighing 270-400 MT wet (15-20 MT DM). An acre of water-hyacinth could remove 2.4 tons of ammonium sulfate in one hour. It could also remove toxic heavy metal pollution from water, but there will be accumulation in plant biomass. Duck weed can double their biomass within 16-48 h under optimum nutrient availability, sunlight and water temperature. Growth of this plant is faster than any other higher plant. Annual dry matter yield/ha from duckweed can be up to 28 tons (Chowdhury et al., 2000). Duck weeds grows in water decaying organic material, providing it with a steady supply of nutrients. By efficient removal of mineralized nutrients, duckweed treats wastewater and enriches itself with highly digestible sources of nutrients. Some species of aquatic plants not only thrive on manure rich water, but can be fed back to livestock, thus completing the recycling process. In addition, some species are a potential source of food for humans because they contain 40 percent crude protein (dry weight) and are equivalent to soybeans in their amino acid content, with high levels of all essential amino acids except methionine.

Studies showed that aquatic plants are rich in protein and amino acid contents and consumed by animals and avian species in many countries (Rusoff et al., 1980; Dolberg et al., 1981; Khan et al., 1981 and Singh et al., 1983).

Although aquatic plants are rich in protein contents, the quantity of nitrogen (N) reaching the small intestine and the properties of dietary to microbial nitrogen supply in ruminant animals depends largely on sources of protein supply. For optimizing the efficiency of microbial growth and rate of fermentation availability of nitrogen containing substances in the diet of ruminants must be synchronized with that of energy yielding nutrients. On the other hand, the rate of digestion is related to diet and the extent to

* Corresponding Author: M. J. Khan. Tel: +88-91-55312, Fax: +88-91-55810, E-mail: mjkhan@royalten.net

[1] Institute of Animal Nutrition, University of Hohenheim, 70593-Stuttgart, Germany.

Received July 24, 2001; Accepted November 7, 2001

which dietary nutrients are degraded in the rumen depends both upon their degradation rate and on the time they are retained in the rumen. Degradability of protein in the rumen is an important value to be used for the prediction of protein passing undegraded to the small intestine and for the calculation of protein utilization and protein requirements of ruminants. The *in-vitro* gas production and NH_3-N measurement have been used to assess the *in-vitro* degradability of nitrogen (IVDN) in protein rich diets (Raab et al., 1983) and in poor quality roughage (Getachew et al., 1998).

To suggest possible method of using aquatic plants in animal feeding, nutritional potentialities of the plants should be studied. The work reported here was therefore, undertaken to study mineral composition, *in vitro* gas production and *in situ* nutrient degradability of some aquatic plants.

MATERIALS AND METHODS

Samples

Aquatic plants viz. *Lemna trisulaca*, *Lemna perpusila* (duckweed), *Azolla pinnata* (azolla) and *Eichhornia crassipes* (water-hyacinth) were collected in the month of June, 2000 from the natural habitats located in and around the Bangladesh Agricultural University, Mymensingh. All the samples were collected randomly in a lush green condition. Immediately after collection, samples were washed in tap water to make the plants free from soil if any. After removal of extraneous material, approximately 5 kg of each plant sample was placed under fan for air drying.

Analytical methods

Dry matter and crude protein were determined according to the method of the Association of Official Analytical Chemists (1990).

In vitro rumen degradability of nitrogen (RDN)

In vitro rumen degradability of nitrogen was calculated from the linear regression of NH_3-N concentration (y, mg) versus gas production (x, ml) observed on incubation of plant materials for different hours with and without exogenous energy source (starch) as described by Raab et al. (1983). Incubation of samples for gas production were conducted according to Menke and Steingass (1988). For this purpose air dried samples were ground to pass through 1 mm screen. Feed samples (150 mg), starch (150 mg) were weighed into glass syringes in their order and kept into an oven at a temperature of 40°C before the buffered rumen fluid was added. A mixture containing 475 ml distilled water, 240 ml NH_4 buffer solution, 240 ml macro-mineral solution, 0, 12 ml micro-mineral solution and 1, 22 ml resazurin solution per litre was prepared in an Erlenmeyer flask according to Menke and Steingass (1988). After warming to 38°C, a reducing solution consisting of 47.5 ml distilled water, 20 ml 1 N NaOH and 0.33 mg $Na_2S{\cdot}9H_2O$ was added. Thereafter, the flask with buffer solution was placed in a small water bath (38°C) and gently bubbled with CO_2 until the blue colour turned to pink and then clear. Rumen fluid was taken before morning feeding from two rumen cannulated dairy cows receiving standard diet containing hay and concentrates (70:30). Rumen fluid was collected into a pre-warmed thermos bottle homogenized in a laboratory blender, strained using nylon cloth of 100 µm. All handling was carried out under continuous flushing with CO_2. The incubation volume was 30 ml. Incubation was carried out in triplicate.

In situ dry matter (DM) and crude protein (CP) degradability

Principally, the *in situ* procedure as described by Ørskov et al. (1980) was applied to determine the degradation characteristics of different aquatic plants. Air dried samples were ground to pass a 2 mm sieve. Two rumen fistulated Holstein cows (600 kg) were used for the determination. Cows were fed standard diet containing medium quality hay and concentrates (70:30). Diets were formulated to contain required energy and protein and fed twice daily at 8:00 and 17:00 h. The nylon bags were made out of the nylon-gauze PA 40/30 and the inner size was 6 cm×12 cm. The maximum number of bags incubated was 50 per cow at each time. Before incubation the bags were washed in a washing machine using 50 g of soap powder during prewashing and 100 g of soap powder during final washing at 70°C. After washing, the bags were dried in an oven at a temperature of 65°C for 48 h. Dried bags were weighed one by one on Mettler AE 166 balance. Five grams of sample was poured into each bag. The bags were tightly closed with plastic strips and were placed with the plastic blocks. The bags in duplicate with the block were immersed into the ventral rumen of each cannulated cow. After removing the stopper of the canule, a nylon rope was connected with a hook on the stopper. The bags were incubated in the rumen for 2, 4, 12, 24 and 48 h in each cow. After different hours of incubation the bags with residue were taken out from the rumen, dipped immediately into ice to stop microbial activity and kept in a deep freeze. After completing all collections, the bags were rinsed with cold tap water to remove the particles from outside the bags. Thereafter, the bags were washed in a domestic washing machine with cold water without any detergent for 30 minutes. After washing, the bags were placed into an oven at 65°C for 48 h. To determine the content of water soluble material, bags representing 0 h also underwent the same washing procedure as the incubated bags (Mertens, 1993). The feed

samples and the nylon bag residues were analysed for dry matter. Dried residues from each bag were composited for crude protein analysis.

***In situ* degradability calculation**

From the % of disappearance data, the degradation values (P) were calculated by the exponential equation of McDonald (1981) as $P = a+b\ (1-e^{-ct})$

where

P = percentage disappearance at time t.

a = rapidly soluble fraction.

b = slowly degradable fraction.

c = rate constant of disappearance for b fraction and

t = time

Effective degradability of crude protein (EDCP) was calculated from the rumen outflow rate (K) and the constants a, b and c from the above model. Value of k was calculated at 0.02/h, 0.04/h, 0.06/h or 0.12/h and the EDCP was determined with the following equation.

$EDCP = a+bc/(c+k)$

where k is the estimated rate of outflow from the rumen.

Statistical analysis

Data from the *in situ* bag study were analysed using linear regression to establish rates of degradation (k) of the fraction 'b'. Randomized block design was followed for the study. Data were analysed statistically following Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Mineral concentration

Aquatic plants grow in different types of fresh water and brackish waters (Vries and Wal, 1988). Nutritive value of the plants often varies largely due to variation in nutrient concentration of the media in which it grows (Gijzen and Khondker, 1997; Sultana, 1998).

Macro and micro mineral content of aquative plants are presented in table 1. It is evident in the table that all the species contain remarkably high amounts of mineral matter. Lemna species are exceptionally high in Mn, Fe, Cu and Zn content. All the species are appreciable high in Ca and P content. Topps (1992) reported low values of phosphorus for most of the tree legume forages but P content in the present study are higher than his findings. Calcium is closely related to phosphorus metabolism in the formation of bones and a Ca:P ratio of 2:1 is recommended. The Ca:P ratio in our study is almost 2:1 which proved aquatic species as well balanced source of Ca and P. Information about minerals in aquatic plants are scarce. However, the values obtained in this study are consistent with the wide range of data reported (Khan et al., 2001). Factors such as soil, climate, species and stage of maturity contribute to variations in the concentration of minerals in forages (Spears, 1994). This remark corresponds well with our findings.

Gas production and organic matter digestibility

Table 2 presents the gas production and *in-vitro* organic matter digestibility data. The gas production pattern of duckweed and water-hyacinth were similar unlike the azolla, which showed comparatively higher gas production values. The gas production pattern in this study are comparable with those reported for five acacia species (Abdulrazak et al., 2001). Organic matter digestibility at 24 h of incubation ranged between 529 and 556 g/kg DM among the species studied and azolla was highest in this regard. The higher gas production as well as organic matter digestibility value of azolla could be attributed to a relatively low cell-wall content.

Microbial protein synthesis and rumen degradable nitrogen

Determination of microbial degradability of N is important in formulating a sound supplementation strategy for efficient utilization of supplementary diet components. *In-vitro* rumen degradability of nitrogen (RDN) and microbial protein synthesis (MPS) of different aquatic plants calculated from gas production values at different hours are presented graphically in figure 1 and 2 respectively. Microbial protein formation was highest at 12 h of incubation which ranged from 39.4-57.4 mg. On the other hand, rumen degradable nitrogen was highest at 48 h of incubation and ranged between 43.4 and 63.4%. At 24 h of incubation rumen degradable nitrogen was 31.5-48.4% among different plants with azolla showing the lowest value. The presence of high ADF and ADL content of the plant (Khan et al., 2001) might be the reason for lower RDN

Table 1. Concentration of macro and micro minerals in aquatic plants

Plants	g/kg DM							mg/kg DM	
	Ca	P	Na	K	Mg	Mn	Fe	Cu	Zn
Duckweed *(Lemna trisulaca)*	12.3	10.7	2.9	46.3	3.7	4.8	2.4	8.2	361.7
Duckweed (*Lemna perpusila)*	17.8	8.9	4.0	37.4	5.2	4.6	5.1	18.0	176.6
Azolla *(Azolla pinnata)*	15.3	7.3	15.2	51.0	4.7	0.26	0.86	4.2	50.8
Water-hyacinth *(Eichhornia crassipes*)	18.0	7.3	0.45	48.0	4.7	0.26	0.86	4.2	50.8
Water-hyacinth leaf *(Eichhornia crassipes)*	19.5	7.6	0.64	65.6	5.8	0.43	0.09	6.6	30.6

Table 2. Gas production (ml/200 mg DM) at different hours and organic matter digestibility (OMD) of aquatic plants

Plants	Hours of incubation					
	4	8	12	24	48	+OMD^{24h} (g/kg DM)
Duckweed (*Lemna trisulaca*)	8.0	12.0	13.5	19.0	28.5	539
Duckweed (*Lemna perpusila*)	7.0	10.5	13.0	19.5	29.0	529
Azolla (*Azolla pinnata*)	5.5	14.0	17.5	28.5	43.5	556
Water-hyacinth (*Eichhornia crassipes*)	6.5	11.5	12.5	18.5	28.5	463
Water-hyacinth leaf (*Eichhornia crassipes*)	7.5	10.0	11.0	14.5	25.5	454

+OMD^{24h}: Organic matter digestibility calculated from the equation OMD (g/kg DM)=185.3+9.239 gas production+0.540 crude protein (Menke and Steingass, 1988).

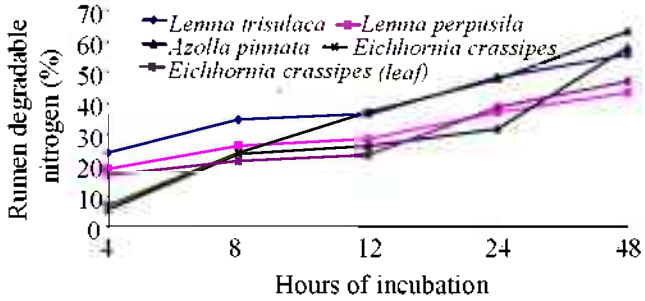

Figure 1. Rumen degradable nitrogen (%) of aquatic plants

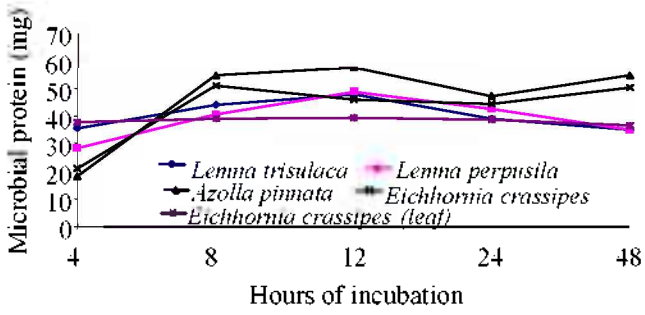

Figure 2. Microbial protein synthesis (mg) of aquatic plants

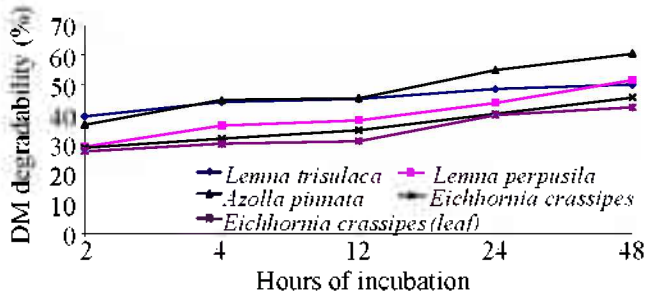

Figure 3. Dry matter degradability of aquatic plants

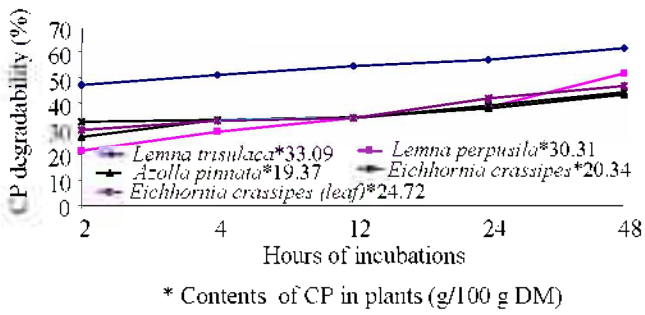

Figure 4. Crude protein degradability of aquatic plants

value. Labri et al. (1998) reported 50.8-95% RDN value for different trees and shrubs. On the other hand. Negiet et al. (1989) have reported 16-40% RDN values for some tree forages.

***In situ* degradation characteristics**

The degradation expressed as loss of dry matter during *in situ* rumen incubation for 2. 4, 12. 24 and 48 h is presented in figure 3. Dry matter disappearance of the plants revealed that aquatic plants are moderate in DM degradability of which azolla showed highest degradability (60%) at 48 of incubation. DM degradability of other species ranged from 39-48% at 24 h and 42-51% at 48 h of incubation. The highest DM degradability of azolla at all incubation time indicates that this species is more susceptible to microbial attack in comparison to other aquatic plants. Lower *in situ* DM degradability of aquatic plants may be due to longer lag phase (Chesson and Ørskov. 1984). Bacteria attachment to the surface of substrates and subsequent formation of colonies does not occur simultaneously hence resulting in lower degradability. Dry matter degradability observed in the present study are lower than the findings of Chaturvedi and Walli (1995).

The crude protein content and CP disappearance of aquatic plants from the nylon bag at different hours is presented in figure 4. The crude protein content of the plants ranged between 135 to 330 g/kg DM and was highest in duckweed and lowest in water-hyacinth. The crude protein content in this study is consistent with what has reported in previous work with aquatic plants (Khan et al.. 2001). Wide differences in CP disappearance was observed among the species at all incubation intervals. At 48 h of

Table 3. Degradation characteristics of CP in nylon bags incubation for 2-48 h in the rumen, P = a+b ($1-e^{-ct}$)

Plants	Degradation characteristics (%)*				
	a	b	c	(a+b)	RSD
Duckweed (*Lemna trisulaca*)	45.29	20.07	0.68	65.36	2.04
Duckweed (*Lemna perpusila*)	22.41	43.06	0.23	65.47	3.68
Azolla (*Azolla pinnata*)	27.80	18.42	0.038	46.22	2.75
Water-hyacinth (*Eichhornia crassipes*)	31.07	19.33	0.025	50.40	1.48
Water-hyacinth leaf (*Eichhornia crassipes*)	28.95	25.97	0.026	54.92	1.87

* a (soluble), b (insoluble but degradable) and c (rate of constant/h) are constants.

Table 4. Effective degradability of crude protein of aquatic plants at different outflow rates, ECPD = a+bc/(c + k)

Plants	Degradability of CP at outflow rate K (%)			
	0.02	0.04	0.06	0.12
Duckweed (*Lemna risulaca*)	58.45	55.60	53.92	51.17
Duckweed (*Lemna perpusila*)	45.47	38.17	34.40	29.37
Azolla (*Azolla pinnata*)	39.82	36.77	34.95	32.25
Water-hyacinth (*Eichhornia crassipes*)	40.30	37.62	35.77	33.80
Water-hyacinth leaf (*Eichhornia crassipes*)	43.40	39.05	36.70	33.55

incubation CP degradability was highest in duckweed (*L. trisulaca*, 61.6%) followed by duckweed (*L. perpusila*, 51.81%) and lowest in azolla (*A. pinneta*, 43%). Wide variation of protein degradability with different time of incubation was noted by Gangadhar et al. (1992). It is reported that protein fractions present in the feed are important factors influencing crude protein degradability (Blethen et al., 1990). CP degradability of aquatic plants are much lower than the common concentrate feeds (Khan et al., 1998) and leguminous and non leguminous forages (Khandaker and Tareque, 1996) although these feeds are high in CP content suggesting that the undegradable fraction of aquatic plant protein are high.

Degradation characteristics and effective degradability of CP are presented in table 3 and 4 respectively. The soluble fraction of CP varied from 224 to 452 g/kg DM. The rate of degradation ranged between 0.023 and 0.068 h^{-1}. The potential degradability varied from 462-654 g/kg DM. The rate of degradation and potential degradability in this study are lower than the values reported for acacia species (Abdulrazak et al., 2000). The soluble fraction (a) is maximum in duckweed (*L. trisulaca*) and minimum in water-hyacinth (*E. crassipes*). Duckweed (*L. trisulaca*) contains more soluble proteins, would be degraded in the rumen very rapidly as evident from the highest "a" value of 45.2%. The EDCP values are higher at rumen outflow rate 0.02 indicating higher protein degradability of the species within few hours of feed consumption.

CONCLUSION

The results have shown that the crude protein content of aquatic plants are sufficiently high to warrant consideration of their use as protein supplement to low quality diets. All species are rich in mineral matter. Results from *in vitro* and *in situ* study have revealed a considerable difference in the pattern of nutrient utilization among different free-floating aquatic plants. This clearly indicates that the availability of nutrient to ruminant animals largely depends on the type of plant. However, more research, especially on animal responses, is needed to affirm the nutritional characteristics reported in this study.

ACKNOWLEDGEMENTS

The senior author is grateful to Deutscher Akademischer Austauschdienst (DAAD), Bonn for awarding scholarship under which this work was carried out. The authors would like to thank laboratory staffs of the Institute of Animal Nutrition, University of Hohenheim, for their help in analysis of samples.

REFERENCES

Abdulrazak, S. A., T. Fujihara, J. K. Ondiek and E. R. Orskov. 2000. Nutritive evaluation of some Acacia tree leaves from Kenya. Anim. Feed Sci. Technol. 85:85-98.

Association of Official Analytical Chemists. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC). Washington, DC.

Blethen, D. B., T. J. E. Wohl, D. K. Jasaitis and J. L. Evons. 1990. Feed protein fractions: Relationship to nitrogen solubility and degradability. J. Dairy Sci. 73:1544-1551.

Chaturvedi, O. H. and T. K. Walli. 1995. Ruminal dry matter and protein degradability of some concentrate ingredients using nylon bag technique. Indian J. Anim. Nutr. 12:133-139.

Chesson, A. and E. R. Orskov. 1984. Microbial degradation in the digestive tract. In straw and other fibrous by-products as feed. (Ed. F. Sundstol and E. Owen). Elsevier, Amsterdom, pp. 305-

339.

Chowdhury, S. A., N. Sultana, K. S. Huque and Q. M. E. Huque. 2000. Manure based duckweed production in shallow sink: Effect of genera on biomass and nutrient yield of duckweed under the same nutritional and management condition. Asian-Aus. J. Anim. Sci. 13(5):686-693.

Dolberg, F., M. Saadullah and M. Huque. 1981. A short review of the feeding value of water plants. Trop. Anim. Prod. 6:322.

Gangadhar, M. A., J. R. Prasad and N. Krishna. 1993. Chemical composition and *in vitro* digestibility of important conventional and unconventional energy supplements. Indian J. Anim. Sci. 63:1004-1005.

Getachew, G., H. P. S. Makker and K. Becker. 1998c. The *in vitro* gas coupled with ammonia nitrogen measurement for evaluation of nitrogen degradability in low quality roughages using incubation medium of different buffering capacity. J. Food Sci. Agric. 77:87-95.

Gijzen, H. J. and M. Khondker. 1997. An overview of the ecology, physiology, cultivation and application of duckweed. Report 08909.

Holm, L. G., D. L. Plunknett, J. V. Pancho and J. P. Herberger. 1977. The worlds worst weeds. Univ. Press of Hawaii, Honolulu.

Khan, M. J., H. Steingass and W. Drochner. 2001. Nutritive evaluation of some aquatic plants from Bangladesh. Anim. Feed Sci. Technol. (Under process).

Khan, M. J., T. Nishida, T. Mijashige, K. Hodate, H. Abe and Y. Kawakita. 1998. Effects of protein supplements sources on digestibility of nutrients, balance of nitrogen and energy in goats and their *in situ* degradability in Cattle. Asian-Aus. J. Anim. Sci. 11(6):673-679.

Khan, M. J., M. A. Razzaque and A. M. M. Tareque. 1981. Effect of feeding water-hyacinth in combination on the growth of bullocks. Bang. J. Agric. 6(1):16-22.

Khandaker, Z. H. and A. M. M. Tareque. 1996. Studies on protein degradabilities of feed stuffs in Bangladesh. Asian-Aus. J. Anim. Sci. 9(6):637-642.

Labri, A., J. W. Smith, I. O. Kurdi, I. O. Adekunle, A. M. Raji and D. O. Lapido. 1998. Chemical composition, rumen degradation characteristics of some multipurpose fodder trees and shrubs during wet and dry season is the humid tropics. Anim. Sci. Feed Technol. 72:81-96.

Matai, S. and D. K. Bagchi. 1980. Water-hyacinth: a plant with profile bioproductivity and photosynthesis, In: Applications of Solar Energy (Ed. A. Gnanam et al.). Proc. Internat Symp on Biol. MacMillan Co., India, Madrass. pp. 144-148.

McDonald, I. 1981. A revised model for the estimation of protein degradability in the rumen. J. Agric. Sci. Camb. 96:251-252.

Menke, K. H. and H. Steingass. 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim. Res. Dev. 28:7-12.

Mertens, D. R. 1993. Rate and extent of digestion. In: Quantative Aspects of Ruminant Digestion and Metabolism (Ed. J. M. Forbes and J. France). CAB International, Wallingford, UK. pp. 13-51.

Negi, S. S., B. Singh and H. P. S. Makkar. 1989. Influence of method of calculation and length of period of rumen fermentation on the effective degradability of dry matter and nitrogen in some tree forages. Anim. Feed Sci. Technol. 26:309-322.

Orskov, E. R., F. D. Hovell and F. Mould. 1980. The use of the nylon bag technique for the evaluation of feedstuffs. Trop. Anim. Prod. 5:195-213.

Raab, L., B. Cafantaris, T. Jilg and K. H. Menke. 1983. Rumen protein degradation and biosynthesis 1. A new method for determination of protein degradation in the rumen fluid *in vitro*. Br. J. Nutr. 50:569-582.

Rusoff, L. L., E. W. Blakeney and D. D. Cully Jr. 1980. Duckweeds (*Lemnaceae spp.*): A potential source of protein and amino acids. J. Agric. Feed Chem. 28:848.

Singh, Y. P., D. G. Naik and G. C. Sharma. 1983. Nutritive value of water fern (*Azolla anabeena*). Indian J. Anim. Res. 17:98.

Snedecor, G. W. and W. G. Cochran. 1967. Statistical Methods, 6th ed. Ames, Iowa: Iowa State University Press.

Spears, J. W. 1994. Minerals in Forages. In: Forage Quality, Evaluation and Utilization (Ed. Jr. G. C. Fahey). National Conferences on Forage quality, Evaluation and Utilization, Lincoln, pp. 281-317.

Sultana, N. 1992. Study on yield, nutrient concentration of duckweed as affected by species, nutrient loading frequency and light intensity. M. S. Thesis, Dept of Animal Nutrition, BAU, Mymensingh.

Topps, J. H. 1992. Potential, composition and usage of legume shrubs and trees as fodder for livestock in the tropics (a review). J. Agric. Sci. (Camb). 118:1-8.

Vries, T. F. de and Van de Wal. 1998. Duckweed in Bangladesh. A synthesis report of studies by the duckweed research project. Ministry of Fisheries and Livestock, Bangladesh.