Aquatic plants like A. pinnata the floating fresh water fern offer a relatively cheap alternative feedstuff in most developing countries. Azolla can be used as feed substitute for cattle, fish, pig and poultry. A pinnata is a good source of protein (Basak et al., 2002) as it contains about 24-30 per cent CP (On dry weight basis) and since it is capable of assimilating atmospheric nitrogen due to the presence of symbiotic algae in its leaves.

The present study was therefore planned to assess the effect of feeding of A. pinnata on rumen characteristics and to find adequate replacement value of A. pinnata in complete ration for ruminant animals.

**Materials and Methods**

The processed samples were analyzed for proximate principle (AOAC, 1995). Four iso-nitrogenous and iso-caloric paddy straw based complete rations were formulated by part replacement of nitrogen equivalent of affafal and ground nut cake with, 0, 10, 20, and 30 per cent with A. pinnata represented as T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> respectively.

The Rumen Simulation Technique (RUSITEC) was equipped with eight fermenters each of 100 ml capacity. Inoculum was obtained from three cattle maintained on grazing alone. To begin the experiment, the fermenters were filled with 500 ml of strained rumen liquor and 200 ml of artificial saliva prepared as per McDougall (1948).

The four experimental diets were placed in nylon bag and incubated in eight fermenters to form duplicate measurement. The pore size of nylon bag was 100 µ as suggested by Carro et al. (1995). The digesta bag was replaced by feedbag after 24 hours. Artificial saliva was infused continuously into the fermenters at a dilution rate of 0.55 ml/minute. From the second day onwards the bags that had spent 48 hours was replaced. Six days of adaptation was followed by a collection period of 48 hours. The procedure was replaced once again to get two more replicate samples for next 48 hours.

Microbial protein in the effluents of the RUSITEC was determined as per the method described by Makkar et al. (1982) and protein estimation was done by the method described by Lowry et al. (1951). The total volume of effluent was measured at the end of specific incubation time. Data
were grouped and analyzed by simple mean and standard error for proximate analysis and by Completely Randomized Design (CRD) for in vitro dry matter degradability studies as per the method of Snedecor and Cochran (1967).

**Results and Discussion**

The chemical composition of *A. pinnata* indicates that it is a fairly rich source of CP which ranged between 24.50 – 30.99 per cent in the present study. The average values of CF, EE, NFE and TA contain in *A. pinnata* were 12.06 ± 0.39, 2.74 ± 0.25, 44.03 ± 1.11 and 12.57 ± 0.60 per cent on DMB. The TA content was higher than other conventional fodders.

Table 2 presents the pH, NH₃-N microbial protein and TVFA production data after incubation of experimental diets for 48 hours in RUSITEC. NH₃-N was significantly decreased (P<0.05) with increased level of *A. pinnata* in the ration. Ruminal microbial protein and TVFA production did not differ significantly among the treatment groups but it was numerically higher in T₄.

Mean pH of the experimental ration ranged from 6.5 to 6.78, indicated efficient functioning of microbial ecosystem for enzymatic activity. The non-significant reduction in pH (Tamminga, 1979) with increased levels of *A. pinnata* favors, efficient fermentation of complete diet. Ammonia nitrogen production in the present finding was significantly reduced (P<0.05) with increased level of *A. pinnata*. Khan et al., (2002) reported that *A. pinnata* was having the highest rumen undegradable protein (UDP), this could be the reason for the significant reduction of NH₃-N with increased levels in experimental diets. Rumen microbial protein synthesis was higher (P<0.05) with increased level of the *A. pinnata* (T₄). 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 (Khan et al., *loc.cit*). These conditions could have been better achieved with increased levels of *A. pinnata* in ration and also could be the probable reason for the decrease in the NH₃-N concentration. The elucidation can be further supported by the trends followed by TVFA concentration in the effluent after 48 hours of incubation which was also increased with increased levels of *A. pinnata* in ration (T₄).

**Summary**

A study was undertaken to evaluate...
Azolla pinnata as protein supplement in ruminant's diet by in vitro analysis using Rumen Simulation Technique (RUSITEC). The pH, ammonia nitrogen (NH₃ - N), microbial protein and total volatile fatty acid (TVFA) were studied. The mean pH was not affected. The ruminal NH₃-N was significantly lower 3.13 ± 0.06 (P<0.05) in T₄ than control diet (T₁). The microbial protein recorded a non significantly (P<0.05) higher value at 30 percent inclusion of A. pinnata than other level of inclusion. Similarly, TVFA concentration was non-significantly higher for T₄ diet compare to other. It can be concluded that A. pinnata could be used as protein supplement by replacing 30 per cent of the nitrogen content in complete diet for ruminants.

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