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PROTEIN FRACTIONATION AND *IN VITRO* DIGESTIBILITY OF AZOLLA IN RUMINANTS

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ABSTRACT: *A study was undertaken to evaluate the nutritive value and digestibility of Azolla in ruminants by in vitro techniques. The crude protein, crude fibre and ether extract contents were at a level of 21.37%, 12.5% and 2.3%, respectively. The neutral and acid detergent fibre levels were about 35.4 and 23.9%, respectively. The average in vitro dry matter digestibility, in vitro organic matter digestibility and metabolozable energy contents were 79.5%, 63.8 mg/200mg and 7.36 MJ/kg DM (1759 kcal/kg), respectively. The various protein fractions A, B_1, B_2, B_3 and C estimated by Cornell net crude protein solubility system were 18.22, 42.56, 15.15, 7.47 and 16.61% of total protein, respectively. The Azolla contained significantly higher B1 fraction followed by A, B2 and C and lowest fraction of C. Thus in view of above, present study indicated Azolla to be a good source protein supplement with 21.37% crude protein with highest B protein fractions, moderate source of energy (1759 kcal ME/kg), high dry matter and organic matter digestibilities and rich in trace minerals thus could be used as an alternate protein supplement or as supplementary protein supplement to ruminants.*

ORIGINAL ARTICLE

Key words: *Azolla, Digestibilities, In Vitro, Protein Fractions, Proximates*

INTRODUCTION

In India, the cost of conventionally used protein supplements in livestock diets like ground nut cake and soya bean have more than doubled over the past few years due to their demand, export policy coupled with low production and more cultivation of other cash crops. The high cost of feed is largely due to the exorbitant price and scarcity of conventional feed ingredients. Thus, depending on groundnut cake and soybean meal as the sole source of protein in livestock diet is gradually becoming economically impracticable in India. Since the cost of feeding is a significant factor dictating the economic viability of livestock industry, it must be reduced by adopting new measures in the ration formulation. Hence to make livestock production as a lucrative enterprise, there is a great need to use alternate feedstuffs replacing the traditional sources. Azolla is a free-floating water fern that floats in water containing 28% crude protein and has a potential to be used as a protein supplement in ruminants (Ahirwar and Leela, 2012). Azolla fixes atmospheric nitrogen in association with nitrogen fixing blue green alga *Anabaena azollae*, making it an excellent source of protein for livestock. The present study was undertaken to evaluate Azolla (*Azolla pinnata*) as protein supplement in terms of chemical composition and nutritive value by *in vitro* techniques. Moreover, the data regarding nutritive value, protein fractionations and digestibility of Azolla appears to be scanty. Hence an attempt was made by applying different *in vitro* techniques to explore Azolla as an alternate protein supplement for livestock.

MATERIALS AND METHODS

The samples of Azolla (*Azolla pinnata*), harvested on 10-15 days of cultivation were procured from different localities in and around the Hyderabad, Andhra Pradesh. The fresh samples of Azolla were collected in two sets, one set for dry matter (DM) estimation and other for sun drying. The dried samples were ground separately to get 1 mm size. Later the ground samples were mixed to get homogeneous sample before subjecting to analysis.

The mixed samples were analyzed in triplicate for proximate principles (AOAC, 1997) and fibre fractions analysis (Van Soest et al., 1991). The calcium (Ca) and phosphorus (P) contents were estimated as per Talapatra method (Talapatra et al., 1940), while the trace minerals (Cu, Fe, Zn and Mn) were estimated using atomic absorption spectrometry (Arenza et al., 1977).

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The samples were screened for *in vitro* DM digestibility (IVDMD) (Goering and Van Soest 1970) and *in vitro* gas production techniques (Menke et al., 1979) using buffalo rumen liquor. The *in vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME) was estimated as per the formulas suggested by Krishnamoorthy et al. (2005) and Menke and Steingass (1988), respectively.

IVOMD (mg) = Gv x 2.2

ME (MJ/kg DM) = 2.2+ 0.136 x Gv + 0.0057 x CPDM/kg

Where, Gv = Net gas volume at 24 hours incubation (ml/200 mg DM)

CPDM = Crude protein on dry matter basis

The protein fractionation was done according CNCP system (Licitra et al., 1996), where in the protein was sub divided in to 5 divisions (Fraction A, B1, B2, B3 and C) according to their degradabilities and passage rate in gastro intestinal tract (Pichard and Van Soest, 1977 and Van Soest, 1994). Fraction A (PA) constitutes non-protein nitrogen (NPN), was separated by using trichloroacetic acid (TCA) according to the method described by Licitra.et al (1996). The fraction B of azolla protein considered as true protein (Pichard and Van Soest, 1977) was further divided into 3 parts namely, fraction B1 (rapidly degraded true protein), B2 (intermediately degraded true protein), B3 (slowly degraded true protein) (Van Soest et al., 1981 and Krishnamoorthy et al., 1983). The fraction B1 (PB_1) was expressed by estimating the true protein soluble in a borate-phosphate buffer at pH 6.7-6.8 (Krishnamoorthy et al., 1982) and the fraction B2 (PB_2) known as neutral detergent soluble protein, was estimated as the difference between buffer insoluble protein (IP) and protein insoluble in neutral detergent (NDICP), and the latter was expressed by estimating the amount of protein recovered in the neutral detergent residue obtained upon standard fibre fraction analysis (Van Soest et al., 1991).

The fraction C (PC) referred as acid detergent insoluble protein (ADIP), measured by estimating nitrogen in ADF residue. The amount of soluble fibre-bound CP (Fraction B_3; PB_3) was calculated as CP in NDF minus ADIP. The data was subjected to one way analysis of variance as per the procedures of Snedecor and Cochran (1980) by using SPSS 17. The differences between the means were tested by significance using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Azolla contained 8.7% dry matter (DM). The proximate constituents and fibre fractions Azollla is given in Table 1. Azolla contained 21.37% crude protein (CP), 35.40% neutral detergent fibre (NDF) and 23.97% acid detergent fibre (ADF) on dry matter basis. The proximate composition of Azolla obtained in the present study was in similar range to values obtained by Ahirwar and Leela (2012). The concentration of calcium, phosphorus, copper, iron, zinc and manganese in Azolla were 0.58%, 0.44%, 17.15 ppm, 710.65 ppm, 77.30 ppm and 207.87 ppm, respectively, indicating to be a rich source of micro nutrients. The CP was comparable, while crude fibre (CF) content was lower in Azolla in comparison to Lucerne (16-25% CP and 20-30% CF, ICAR, 1998). Thus it indicates that Azolla could be good source of protein having low fibre content compared to legume forages.

The *in vitro* dry matter digestibility, *in vitro* organic matter digestibility and metabolizable energy contents were 79.5%, 63.8 mg/200mg and 7.36 MJ/kg DM (1759 kcal/kg), respectively (Table 2). Several *in vivo* experiments indicated improvement in DM digestibility with replacement of 50 % of ground nut nitrogen in diets of buffalos (Indira et al., 2009) and 30 parts of ground nut in concentrate diet of Nellore Sheep (Ravidra reddy et al., 2011).

Table 1 - Nutrient composition of Azolla (*Azolla pinnata)*

Nutrient	% on DMB
Dry matter	8.70±0.49
Crude protein	21.37±0.91
Crude fibre	12.73±0.50
Ether extract	2.36±0.21
Total ash	16.23±0.52
Nitrogen free extract	47.30±1.61
Neutral detergent fibre	35.40±0.64
Acid detergent fibre	23.97±1.01
Cellulose	12.15±1.47
Hemicellulose	11.43±1.47
Lignin	12.57±0.81
Calcium	0.58±0.07
Phosphorus	0.44±0.08
Trace minerals	**ppm**
Copper	17.15± 0.25
Iron	710.65± 23.31
Zinc	77.30± 2.95
Manganese	207.87± 22.46

Values are average of triplicate

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Table 2 - *In vitro* dry matter digestibility and gas production

Attribute	Content
In vitro dry matter digestibility (%)	79.55±0.26
In vitro organic matter digestibility (mg/200mg)	63.8±3.36
Metabolizable energy (MJ/Kg)	7.36±0.21

Values are average of triplicate

Table 3 - Protein fractions of Azolla analyzed as per CNCPS system

	a	b_1	b_2	b_3	c	P value
% Protein	18.22^{b}±4.30	42.56^{a}±2.54	15.15^{b}±1.04	7.47^{c}±0.48	16.61^{b}±2.32	0.001

Values are average of triplicate

Azolla being evaluated as protein supplement, its protein fractions were evaluated under CNCPS system (Table 3). The fraction A of protein (PA) in Azolla (18.22±4.30 % CP DMB) signifies the instantaneously degradable protein in the ruminant digestive system (amino acids, peptides) i.e. non protein nitrogen (NPN) (Pichard and Van Soest, 1977). Most of the reported concentrations of NPN in grasses and legume forages are having the ranges as fresh material (10-15%), hay (15-25%) and silage (30-65%) (Hughes, 1970; Krishnamoorthy et al., 1982; Xu et al., 1996). This indicated Azolla had rumen degradability similar to grasses and legumes and their hays. The Fraction B_1 (PB_1) referred as true soluble protein (globulins and some albumins) with Rumen degradation of 200 – 300 %/hr, was found to be 42.56±2.54 % CP and was higher ($P<0.001$) than other protein fractions. Elizalde et al. (1999) reported 17.1% CP of PB_1 in alfalfa which was much lower than that observed in Azolla. The PB_1 in oil seed cakes ranged from 13.22% to 49.37% (Kamble et al., 2010). The PB_2 with Rumen degradation of 5 – 15 %/hr was found to be 15.15 ±1.04 % CP in Azolla which was higher ($P<0.001$) than PB_3. The fractions PB_1 and PB_2 (approx. 58% for Azolla) have 100% Intestinal degradability which signifies the potency of Azolla as a protein supplement. The protein fraction B_3 (PB_3) having 80% Intestinal degradability in ruminants was 7.47% for Azolla. This finding is in accordance with Krishnamoorthy et al. (1982) who reported that protein supplements contain a small amount of PB_3 which mainly included prolamine proteins such as zein protein in corn (Van Soest et al., 1981). According to Van Soest (1994), metabolizable protein is defined as the amount of true protein or amino acids absorbed in the small intestine and specifically in ruminants, are represented by the amount of amino acids or protein of microbial or dietary origin absorbed from the intestine. In this study, the metabolizable protein in Azolla was approx 84% of CP ($PA+PB_1+PB_2+PB_3$) which implies the capability of Azolla as a protein supplement.

The fraction C of protein (PC) varied significantly ($P<0.001$) with other fractions and was found to be 16.61 ±2.32 % CP, which contains protein associated with lignin, tannin-protein complexes, and maillard products that are highly resistant to microbial and mammalian enzymes and does not provide amino acids postruminally to the ruminants (Krishnamoorthy et al., 1982). So PC is considered as undegradable protein fraction i.e. PC is resistant ruminant degradation and digestion. Sniffen et al. (1992) reported wide variability in PC content in protein supplements i.e 0 to 20%. The present finding falls in the range that reported by Sniffen et al. (1992) for protein supplements.

CONCLUSION

The study indicated Azolla to be a good source protein supplement with 21.37% crude protein with highest B protein fractions, moderate source of energy (1759 kcal ME/kg), high digestibility of dry matter and organic matte (79.55%) and rich in trace minerals thus could be used as an alternate protein supplement or as supplementary protein supplement to ruminants.

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