NUTRITIVE EVALUATION OF *Azolla pinnata* USING *IN VITRO* GAS PRODUCTION TECHNIQUE

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Abstract

A study was conducted to evaluate the rumen fermentation characteristics of Azolla pinnata by in vitro gas production technique.The chemical composition along with the fibre fractions were determined. The in vitro gas production (IVGP) was recorded and the true organic matter and true dry matter digestibility was determined (TOMD, TDMD). Furthermore, microbial biomass production (MBP), partitioning factor (PF) and metabolisable energy (ME) was predicted from the gas production data. The corresponding values to the above said parameters are 9.87±0.27 ml/200mg DM, 58.49 ± 0.72 per cent, 60.37 ± 1.11 per cent, 90.32 ± 2.50 mg/200mg DM, 11.22 ± 0.46 and 5.15 ± 0.05 MJ/kg DM, respectively. The estimated value for total volatile fatty acid concentration and concentration of VFAs like acetate, propionate, butyrate and valerate were 50.9 ± 0.24, *35.17 ± 0.14, 12.1 ± 0.10, 3.42 ± 0.02 and 0.2 ± 0.01 respectively. It is concluded that Azolla pinnata can be considered in the ruminant rations because of its high nutritive value and fermentative abilities.*

Keywords: *Azolla pinnata, In vitro gas production, metabolisable energy, volatile fatty acids.*

A major constraint in the livestock production is the scarcity of fodder and inconsistent quantity and quality of livestock feed for year-round feed supply. This condition is particularly evident during dry season, wherein the natural pastures drop in quality, especially in energy and protein content and also in quantity. As a consequence, it results in low productivity, poor growth and reproduction of animals (Krishnamoorthy and Moran, 2011). 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. Hence, knowledge regarding the nutrient composition of different unconventional feeds helps in preparation of balanced rations for ruminants (Devendra and Leng 2011).

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The nutritive value of ruminant feed is determined by the concentration of its chemical compositions, as well as rate and extent of digestion in the rumen (Kumar *et al*., 2015). Different methods are available to determine the nutritive value of ruminant feed and *in vivo* trial is said to be the best method of evaluation. However, *in vivo* trails are time consuming, laborious, expensive, require large quantities of feed, and a large number of feeds cannot be evaluated (Carro *et al*., 1994). *In vitro* gas production (IVGP) is an alternative technique used to determine the nutritive value of feedstuffs, since rate and extent of degradation, and rumen fermentation can be determined by measuring the volume of gas production (Dhanoa *et al*., 2000). *In vitro* trials, are less time-consuming, cheaper and more efficient when large number of samples are to be handled.

Therefore, the objective of this study was to evaluate the *Azolla pinnata* in terms of chemical compositions, TDMD, TOMD, MBP, PF, ME, total and individual volatile fatty acid production by IVGPT for its effective incorporation in the ruminant feeds.

Materials and Methods

Sample collection and preparation

Azolla pinnata was collected from the local farmers of Thrissur, Kerala. Five kg of sample was dried in a hot air oven at 55 to 60 °C for 48 h, and ground to pass a 1mm screen and stored in air-tight containers.

Chemical analysis

Chemical composition was analysed as per the standard procedures (AOAC, 2012) Fibre fractions was estimated according to the procedure described by Van Soest (1991). Further analysed for their gross energy content using bomb calorimeter (Parr Instrument Company, U.S.A)

In vitro gas production technique

In vitro trials were conducted in quintuplicate to estimate various parameters such as total gas production, *in vitro* dry matter/organic matter (DM/OM) digestibility, metabolizable energy (ME), and partitioning factor (PF). The samples were subjected to *in vitro* trials according to the procedure described by Menke and Steingass (1988). Azolla samples were incubated with the buffered rumen fluid in calibrated glass syringes. The donor animals were fed on green roughage 40kg/ day and concentrate 2kg/day. Rumen liquor was collected before morning feeding in a pre-warmed thermos-flask (39°C) and brought to the laboratory. The rumen liquor was bubbled with CO_2 for about 2 minutes.

About 200 mg of 1 mm milled samples were weighed into 100 ml calibrated glass syringes in quintuplicate. Petroleum gel was applied to the piston to ease movement and to prevent escape of gas. The syringes were pre-warmed (39°C) for 1 h, before addition of 30±1.0 ml of rumen-buffer mixture (1:2) into each syringe. Three blank syringes containing 30 ml of buffered rumen fluid were incubated to estimate gas production due to endogenous substrates for the blank corrections. All the syringes were incubated in an incubator maintained at 39±0.10°C. The syringes were gently shaken every hour during the first 8 h of incubation and readings were recorded at the end of 24 hr incubation. *In vitro* DM and OM digestibility were estimated using methods suggested by Van Soest *et al.* 1991.

Metabolisable energy content of feed

This was calculated by the method of Menke and Stienglass *et al*. (1989)

ME (MJ/kg DM) = 1.06 + 0.1570GP + 0.0084CP + 0.022EE - 0.0081TA

CP = Crude protein

EE = Ether extract

TA = Total ash

GP = Corrected gas production for 24 hrs

On completion of the incubation, the buffered rumen liquor was filtered through four layers of muslin cloth and approximately 0.8 ml of the sample was preserved with 200 µL of 25 per cent metaphosphoric acid. The samples preserved in this way were immediately analysed

or stored at -20 °C temperature for the future analysis. Sample were centrifuged at 20000 rpm at 4°C, for 20 mins and the supernatant was analysed for volatile fatty acids. The analyses were conducted on a 7890A GC System gas chromatograph, Agilent Technologies as per standard procedure described by Filípek and Dvorak (2009).

Results and Discussion

The data on the chemical composition and fibre fractions of the *Azolla pinnata*are given in Table 1. Crude protein, crude fat, crude fibre, total ash and nitrogen free extract content of azolla were 21.71, 3.91, 14.94, 1.91 and 57.53 respectively. While NDF, ADF, ADL, cellulose, hemicellulose, non-structural carbohydrate and gross energy content was 52.86, 36.74, 25.48, 11.49, 16.12, 19.61 per cent and 3119.94 (kcal/kg) respectively. The chemical composition azolla in the present study were similar to Kavya *et al*., (2015) who observed the CP, CF, EE, TA and NFE contents of azolla to be 21.66, 15.15, 4.41, 17.84 and 40.79 per cent, respectively. The chemical composition of *Azolla pinnata* obtained in the present study can be compared with some proteinaceous feeds such as berseem, cowpea and mustard cake.

Table 1. Chemical composition of *Azolla pinnata**

Nutrient (%)	*Azolla pinnata*
Crude protein	21.71
Crude fat	3.91
Crude fibre	14.94
Total ash	1.91
Nitrogen free extract	57.53
NDF	52.86
ADF	36.74
ADL	25.48
Cellulose	11.49
Hemicellulose	16.12
Non-structural carbohydrate	19.61
Gross energy (kcal/kg)	3119.94

*on dry matter basis.

Table 2. IVGP, ME content, true DM and OM digestibility, and PF and MBP (mg/200mg) of *Azolla pinnata*.

Parameter	Value
IVGP (ml/200mg)	9.87±0.27
ME (MJ/kg)	5.15±0.05
TDMD (per cent)	58.49±0.72
TOMD(per cent)	60.37±1.11
PF (OM fermented/ml gas)	11.22±0.46
MBP (mg/200mg)	90.32±2.50

Table 3. Individual and total volatile fatty acid concentration (mmol/dL) and acetate to propionate ratio of *Azolla pinnata*.

Parameter	Value
Acetate	35.17±0.14
Propionate	12.1±0.10
Butyrate	3.42±0.02
Valerate	0.2±0.01
Total volatile fatty acid	50.9±0.24
C2:C3	2.92±0.05

IVGP, ME content, DM and OM digestibility, and PF of *Azolla pinnata* is presented in Table. 2. Khan *et al*. (2002) reported 28.5 ml of gas from the incubation of 200 mg of *Azolla pinnata* which is 18 ml more than the present study, and this could be attributed to difference in the strain, chemical composition and the fibre fractions. The values obtained in the present study was more than the reported value by Khan *et al*. (2002). The NDF, ADF and ADL content in the *Azolla pinnata* sample in the present study was 52.86, 36.74 and 25.48 per cent, respectively, which might have attributed to low organic matter (OM) availability for fermentation and subsequent lowered gas production.

The results pertaining to the *in vitro* digestibility of dry matter and organic matter of the azolla in the present study is in agreement with the finding of Khan *et al*. (2002). Incubation of *Azolla pinnata* with buffered rumen fluid from buffalo (Parashuramulu *et al*., 2013) for 24 h resulted in 79.5 and 63.8 per cent digestibility for DM and OM respectively, which was slightly higher than the present study.

Kumar *et al*. 2015 reported that the dry matter digestibility and organic matter digestibility of *Azolla pinnata* was 83.15 and 84.03 per cent respectively. Lower DM digestibility observed in the present study might be due to relatively higher content of NDF and ADF in the *Azolla pinnata* sample.

In the present study the dry matter digestibility and organic matter digestibility of azolla was found to be 58.49±0.72 and 60.37±1.11 respectively. The differences in the digestibility may be due to variation in strains of azolla, method of cultivation and the difference in the composition.

According to Blummel *et al*. (1999), higher PF value of a feed indicates higher efficiency of microbial biomass production and lower methane output by ruminant. The PF for azolla (11.22±0.46) more than the theoretically possible value of 4.41 (Blummel *et al*., 1997). Also azolla had the highest MBP production and these results are supported by the statement given by Blummel *et al*. (1999). Thus, *Azolla pinnata* might also be helpful in mitigating the methane production. The higher PF have been reported for tannins rich feedstuffs which ranged from 3.1 to 16.1 (Getachew *et al*., 2000).

Menke and Steingass, (1988) have demonstrated that there exists a positive correlation between metabolisable energy calculated from *in vitro* gas production along with CP and fat content with metabolisable energy value of conventional feeds measured through *in vivo* experiments. The ME value of *Azolla pinnata* (5.15±0.05 MJ/kg) was found within the range of reported values for a large number of unconventional feedstuffs (Krishnamurthy *et al*., 1995; Kumar *et al*., 2015)

The concentration of acetate, propionate, butyrate and valerate (Table. 3) were 35.17±0.14, 12.1±0.10, 3.42±0.02 and 0.2±0.01 mmol/dL, respectively. Higher acetate concentration in *Azolla pinnata* is due to higher content of soluble and easily fermentable carbohydrates. The non-fibrous carbohydrates (present in many concentrates) promote the production of propionic acid whereas the fibrous carbohydrates (present primarily in forages) stimulate the production of acetic acid in the rumen. In addition, the non-fibrous carbohydrates yield more volatile fatty acids (i.e., more energy) because they are fermented faster and more completely.

Conclusion

The chemical analysis indicated that the *Azolla pinnata* is rich in crude protein content and could be used as a potential natural protein source. The low gas production and high MBP indicates good microbial protein available for the animal body and high production of acetate indicates that the *Azolla pinnata* can be included in the ration of cattle to alleviate the fat depression in early lactating dairy cattle.

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