Progressive Agriculture 25: 31-37, 2014 **ISSN: 1017 - 8139**

Progressive Agriculture
Journal homepage:http://www.banglajol.info/index.php/PA

Effect of tannin from blackberry (*Syzigium cumini)* seed on *in vitro* rumen fermentation

SCK Barma, MR Amin*, M Monirruzzaman, MB Sarker, AKMA Kabir

Department of Animal Science, Faculty of Animal Husbandry, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Abstract

An experiment was conducted to quantify the chemical composition, different forms of tannins and their effects on *in vitro* gas production, organic matter digestibility and metabolisable energy content in *Syzigium cumini* (Blackberry) seed with and without polyethelene glycol (PEG). The DM, OM, ash, CP, NDF and ADF contents of *S. cumini* seeds were 90.1, 87.6, 2.6, 4.6, 32.9 and 15.5%, respectively. The total phenol, total tannin, condensed tannin and hydrolysable tannin content were 5.89, 4.25, 0.42 and 0.05mg/g seed, respectively. *In vitro* gas production, organic matter digestibility and metabolisable energy content of *S. cumini* seed were 54 ml, 42.91% and 6.43 MJ/Kg DM, respectively. Addition of PEG to tannin containing seed was significantly (P<0.05) increased *in vitro* gas production, OMD and ME content. Higher levels of tannins in *S. cumini* seed could limit utilization through impaired digestibility and nutrient utilization but the addition of PEG increased all the fermentation parameters studied. It is predictable from the present findings that *S. cumini* seed could be utilized as natural source of feed additives to alter rumen fermentation parameters especially to protect protein and other nutrients from ruminal degradation, thereby make the nutrients available in the lower tract for higher milk and meat yield.

Key words: Blackberry seed, chemical composition, tannins, gas production, organic matter digestibility, metabolisable energy content

***Corresponding Author:**
aminmr64@yahoo.com

Introduction

In many countries, farmers offer various tree leaves, flowers, pods and twigs to livestock along with conventional grazing as a practical means of rearing animals particularly during scarcity period. With increasing demand for livestock products as a result of rapid growth of population and also for the supply of balanced nutritional food from livestock and feeding the millions and safeguarding their food security will depend on the better utilization of available unconventional feed resources. Many tree seeds contain anti-nutritional factors like polyphenolics and their presence at high level can significantly restrict intake and utilization (Pritchard *et al.* 1992; Kumar, 1992) and also reduce nutritive value of feed (Reed *et al.* 1990).

Tannins are one of the plant secondary metabolites and contain complex phenolic compounds of great structural diversity with a wide variety of biological activities. More specifically, they are naturally occurring polyphenolic compounds of high molecular weight containing sufficient phenolic hydroxyl groups, which have ability to form complexes with numerous types of molecules, including but not limited to, proteins, carbohydrates, other polysaccharides, bacterial cell membranes and enzymes involved in protein and carbohydrate digestion (Deshpande *et al.*, 1986). High concentration of tannins in these tree foliages prevents not only their optimal utilization but also that of the roughages and by-products (Baldwin *et*

al., 1987). The effect of tannins on feed intake, digestibility and animal's response to feeding seems to be unclear but its effect on rumen fermentation is extremely important for utilization of tanniniferous feeds for improving ruminant production systems particularly where livestock live mainly on poor quality roughage.

Syzigium cumini (Blackberry) is a very common multipurpose tree found in many countries. The fruits are eaten by man, stems are used to make furniture and the leaves are sometime fed to livestock. *S. cumini* leaves are rich in tannins and it is expected that the seed may also contains tannin that might have the ability to protect protein from ruminal degradation, thus precipitate protein and increase the availability of essential amino acids for absorption. There is lack of information of the nutritional content, tannin availability and the effect of tannin on fermentation pattern especially on protein spanning ability in the rumen from *S. cumini* seeds. Therefore, a detail baseline investigation as chemical composition, various forms of tannins, *in vitro* gas production, organic matter digestibility and metabolizable energy content and the effect of PEG on tannin activity is essential to know the properties of *S. cumini* seed.

Materials and Methods

Preparation of sample

S. cumini seeds were collected from locality of Mymensingh, Bangladesh, sun dried, ground to pass through 0.5 mm sieve, bottled and covered with aluminum foil and kept in desiccator. Another batch of sun dried seed samples was also ground to pass through 1.0 mm sieve for fiber analysis and *in vitro* digestibility study.

Chemical analysis

Proximate composition of sun dried seed samples for dry matter (DM), crude protein (CP), and ash were estimated according to the methods of AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were assessed by Faichney and White (1983). Total phenol was determined by Folin-Ciocalteu reagent method and total tannin was determined by subtracting the values of polyvinylpyrrolidone (PVPP) bind tannins from the value of total phenols. The values were expressed as tannic acid equivalent. Condensed tannin was determined by oxidation of condensed tannin in butanol-HCl reagent in presence of iron and gallotannin was determined by hydrolysis of gallotannins to gallic acid with rhodanine. The condensed tannin and gallotannins were expressed as lecucocyanidin equivalent and gallic acid equivalent consequently. The above analyses were conducted according to the laboratory manual of Quantification of Tannins in Tree and Shrub foliage (Makkar, 2003).

In Vitro gas method

Series of experiments were carried out to study the effect of tannins on rumen fermentation using hay and hay-starch as standard. In this study, sun dried seed samples of *S. cumini* was used. The method, in principle, was based on the *in vitro* gas production technique, described by Menke *et al.* (1979) and Menke and Steingass (1988).

Layout of the experiment

Sun dried seed sample of *S. cumini* was assigned into two treatments (with and without PEG). Hay and hay-starch were used as standard and blank was used for correction of gas measurement. PEG was not added with hay standard and blank. Each sample had three replications.

Organic matter digestibility and metaboli-zable energy

The organic matter digestibility (OMD) (%) and metabolizable energy (ME) content (MJ/kg DM) were calculated from the gas volume (Gv) and crude protein value (CP %) using the following equations (Menke and Steingass, 1988).

% OMD = 14.88 + 0.889 x Gv + 0.45 x % CP and ME = 2.20 + 0.136 x Gv + 0.057 x % CP.

Statistical analysis

The computer program Student's "t" test of MSTAT was used for comparison of means of two treatments (with and without PEG). Pearson's correlation coefficient for the tests of interaction between seed samples with and without polyethelene glycol (PEG)

in *in vitro* gas production was calculated by the MSTAT program correlation procedure.

Results

Chemical composition

The chemical composition of the *S. cumini* seed are shown in Figure 1. The DM and ash contents in *S. cumini* seed were 90.13 and 2.57%, respectively in sun dried sample (Figure 1). The CP, NDF and ADF content were 4.60, 32.92 and 15.53%, respectively, in seed samples.

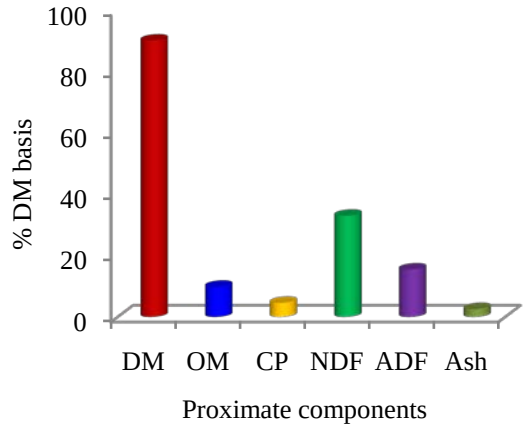

Figure 1**.** Chemical composition of *S. cumini* seed

Composition of tannins in seed

The amount of total phenol and total tannins were calculated by using the tannic acid calibration curve (Figure 2) and the amount of hydrolysable tannin was calculated by using gallic acid calibration curve (Figure 3). Contents of various forms of tannins are shown in Figure 4.

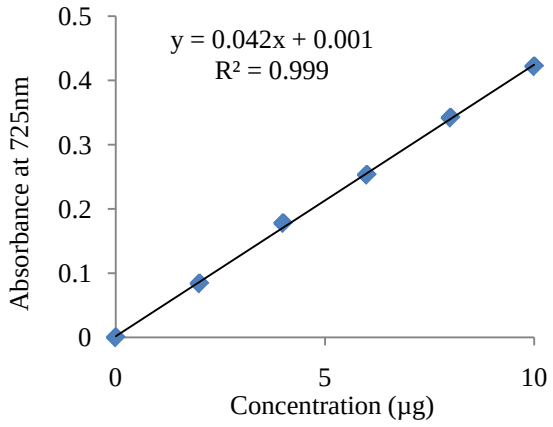

Figure 2. Calibration curve for tannic acid

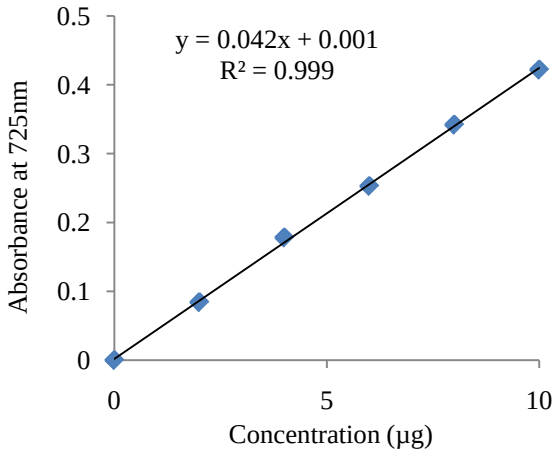

Figure 3. Calibration curve for gallic acid

The total phenols, total tannins, condensed tannins and hydrolysable tannins were 5.89, 4.25, 0.42 and 0.05mg/g seed, respectively in sun dried samples of *S. cumini* seed.

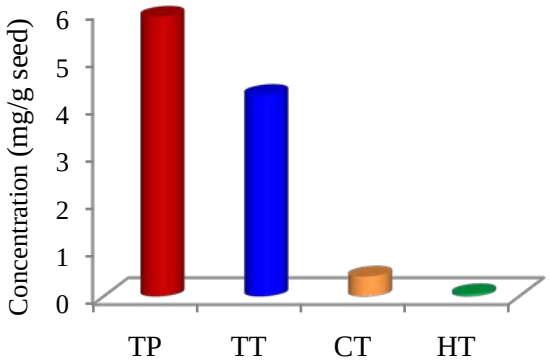

Figure 4. Tannin concentration in *S. cumini* seed (TP=Total phonol; TT=Total tannin; CT=Condensed tannin; HT=Hydrolysable tannin)

In Vitro gas production

The amount of gas produced up to 24 hrs of incubation in rumen buffer with and without Polyethelene glycol (PEG) is presented in Table 2. The effect of PEG on cumulative gas production at different hours of incubation of seed is shown in Figure 5. Addition of PEG increased 28.39% gas production in 24 hrs of incubation. The effect on gas

production was observed significant (P<0.05) in *S. cumini* seed.

Table 2. *In vitro* gas production of *S.cumini* seed with and without PEG

Parameters	Gas Production (ml)				
	3 h	6 h	9 h	12 h	24 h
Seed	6.33	10.17	13.5	23	54
Seed + PEG	11.5	24.84	41.33	50.5	76
Level of significance	**	**	**	**	*
% Increase in gas production	44.96	59.06	67.34	54.46	28.95

** Significant at 1% level; * Significant at 5% level

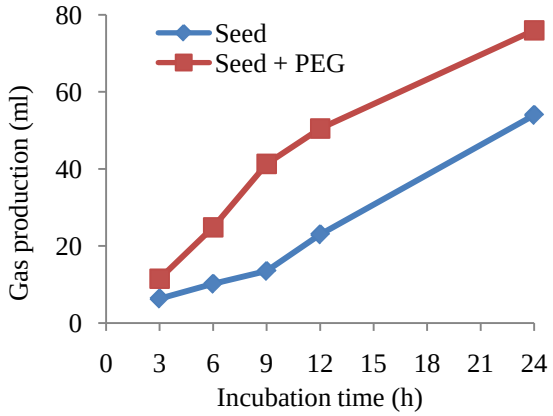

Figure 5. Cumulative gas production of *S. cumini* seed with and without PEG.

In vitro organic matter digestibility and metabolizable energy

The organic matter digestibility (OMD) and metabolizable energy (ME) content are shown in Table 3. Inhibitory effect of tannin by the addition of PEG, resulted increase in OMD and ME content of the seed. Addition of PEG significantly (P<0.05) increased 20.55% OMD and 20.89% ME content.

Table 3. Effect of PEG on OMD and ME content of *S. cumini* seed

Parameters	OMD% ± SD	ME (MJ/Kg DM) ± SD
Seed	42.91 (±0.12)	6.43 (±0.02)
Seed + PEG	54.01 (±0.89)	8.13 (±0.14)
% Increase	20.56	20.89
Significance	*	*

* Significant at 5% level

Discussion

Chemical composition

Chemical composition, alone, as measured by the proximate and elemental analysis system, is an inadequate indicator of nutritive values. These measurements take no account of either the form or availability of nutrients and at best, may provide information on potential nutrient content. This may be due to plant dry matter separated into a completely digestible fraction (neutral detergent solubles, NDS) representing cell contents and partially digestible fraction (acid detergent fiber, ADF), representing plant cell walls (Norton, 1999). It is assumed that drying bind tannins to cell wall and become unavailable for reaction. The sun dried *S. cumini* seeds contained 90.13% DM. The seeds used in this experiment had CP content of about 4.60% but very lower than other seeds (e.g. mango seed kernels 6.7%, sal seed 8.6%, babul seed 16.6%). Feeding of these seed to ruminants is expected to provide about 3% more CP than that of the basal diet. However, the CP content of seeds may not confer any direct nutritional benefit to the animal unless these proteins are protected against ruminal degradation (Norton, 1999).

Various forms of tannin

Tannin levels in plants are known to vary greatly between species, within species, stages of development or growth, between various tissues of the same plant, from location to location (environmental conditions under which the plant grow) and from year to year due to enhance the synthesis of polyphenolic compound by light intensity. In the present experiment condensed tannin concentration was found 8 times higher than hydrolysable tannin. Higher tannin concentration in leaves are reported in the summer as opposed to the winter (Baldwin *et al.*, 1987) due to higher temperature and light intensity but in case of *S. cumini* seed, the harvesting period is only in summer and once in the year. From the values of condensed tannins and hydrolysable tannin and the effect on rumen fermentation, it appeared that proper supplementation of *S. cumini* seed as additives may

be beneficial to protect protein and other feed nutrients from ruminal degradation. This protected protein may pass through rumen to small intestine and finally may utilized by the animal for increased production of milk and meat and also may reduce methane and CO_2 production and nitrogen excretion to the environment, thereby reducing environmental pollution.

In vitro gas production

Total gas produced in this trial by incubation of hay was 45 ml, which is close to 44.43 ml used for standard hay and hay-starch was 65.5 ml, which is close to 59.8 ml. Therefore, the results obtained for the seed were under optimal rumen environment. Much of the earlier work on gas measurement (McBee, 1953; El-Shazly and Hungate, 1965; Czerkawski and Breckenridege, 1969; Czerkawski and Breckenridege, 1970) centered on investigations of rumen microbial activities using manometric measurements. McBee (1953) developed a manometric method of gas measurement for the evaluation of rumen microbial activity with respect to cellulose and hemicellulose fermentation and concluded that the rate of fermentation of various substrates in the rumen is not constant but subject to wide fluctuations following changes in the diet of the animal.

The increase in gas production by addition of PEG was achieved by inhibiting the action of tannins on digestion in the rumen and resulted in increased microbial fermentation (Makkar and Becker, 1996). In the presence of PEG about 35% of the total gas was produced in the first 12h of incubation and the remaining 65% during the last 12h of incubation. The rate of gas production was less for the first 3h but increased from 6 to 9h of incubation. This was probably due to the fact that the rumen microbes need some times for adaptation before increasing microbial activity and fermentation. Higher the biological activity of tannins on rumen microbes, higher is the gas production (Makkar *et al.*, 1995).

In vitro organic matter digestibility and metabolizable energy

Nutritive value of seeds fed to ruminants is influenced substantially by the protein content of the seed and by the extent to which it is degraded in the rumen. *In vitro* techniques for determination of rumen degradability of organic matter offer considerable advantages in terms of saving time and resources in routine feed analysis. Higher gas volume was achieved in seeds having low CP content. The organic matter digestibility and metabolizable energy content were also affected digestibility of tanniniferous seed by reducing microbial effect on organic matter and its digestibility (Palmer and Jones, 2000).

The present study further validated the finding that the addition of PEG increased organic matter digestibility and metabolizable energy content. Differences in organic matter digestibility in the presence or absence of PEG may be explained partly for fibers which may have either decreased or prevented from ruminal fermentation (Barry and Manley, 1984).

The decrease in the rate of digestion of feeds by tannin could help synchronizing the release of various nutrients, which in turn might be responsible for increase in microbial efficiency. It is an accepted concept in ruminant nutrition to minimize substrate fixation into microbial cells to increase the efficiency of microbial protein synthesis and decrease in the degradability of feed protein in the rumen. As they increase the supply of protein to the lower intestine for production purpose resulting higher milk and meat. Peptide and amino acids accumulation would be expected to be beneficial, because rumen microorganisms would be able to assimilate performed amino acids, saving the energy required for biosynthesis and secondly, because some of these compounds may escape undegraded from the rumen and be utilized by the host. The inhibition of amino acids degradation in the rumen is an obvious objective for manipulation. Therefore, amino acid breakdowns may be nutritionally expensive not only because amino acids are lost, but also because of the high energetic cost of breakdown. *S. cumini* seeds are rich in tannins and the tannin properties have shown to alter rumen fermentation characteristics. Therefore, it is highly expected that *S. cumini* seeds, those are thrown elsewhere could be used as additives to protect protein from ruminal degradation

and increase the availability of protein and amino acids for absorption in the small intestine. Further studies are required to know the nutrients especially protein saving capacity of tannins in the rumen from *S. cumini* seeds in order to have their positive or negative responses to ensure animal productivity.

Conclusion

The result of this study revealed that crude protein content of *S. cumini* seed is very low compared with other seeds and constitute a considerable amount of active tannins that restrict digestibility and ME content in the rumen but inclusion of PEG improved rumen fermentation as an increased efficiency of feed utilization. Further investigation is needed for *in vitro* and *in vivo* assessment of tanniniferous seeds for their ability to protect protein and other nutrients which may increase by-pass nutrients to the animals

References

AOAC (1990). Official Methods of Analysis. 15th ed., Association of Official Analytical Chemists, Washington, DC.

Baldwin IT, Schultz JC, Ward D (1987). Pattern and sources of leaf tannin variation in yellow birch (Betula allegheniensis) and lignin in Lotus sp. And their possible consequences in ruminant nutrition. J. Sci. Food Agric. 37:248-254.

Barry TN, Manley TR (1984). The role of condensed tannins in the nutritional value of L. pedunculatus for sheep. 2. Quantitative digestion of carbohydrates and proteins. Br. J. Nutr. 51:493-504.

Czerkawski JW, Breckenridge G (1969) The fermentation of sugar-beet pulp and sucrose in an artificial rumen, and the effect of linseed oil fatty acids on the fermentation. Br. J. Nutr. 23:51-66.

Czerkawski JW, Breckenridge G (1970). Small scale apparatus for studying rumen fermentation. Lab Practice 19:717-728.

Deshpande SS, Cheryan M, Salunkhe DK (1986). Tannin analysis of food products. CRC. Critical Rev. Food Sci. Nutr. 57: 401-449.

El-Shazly K, Hungate RE (1965). Fermentation capacity as a measure of net growth of rumen microorganisms. Appl. Microbiol. 13:62-69.

Faichney GJ, White GA (1983). Methods for the analysis of feeds eaten by ruminants. CSIRO, Australia.

Kumar R (1992). Anti-nutritional factors, the potential risks of toxicity and methods to alleviate them. In: Legume trees and other fodder trees as protein sources for livestock (Ed. A. Speedy and P. L. Pugliese) FAO Animal Production and Health Paper 102. Food and Agriculture Organisation of the United Nations, Rome.

Makkar HPS, Becker K (1996). A bioassay for phenols (Tannins). Polyphenols Communications, Bordeaux (France), 96:15-18.

Makkar HPS, Blummel M, Becker K (1995). Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins, and their implication in gas production and true digestibility in *in vitro* techniques. Br. J. Nutr. 73:897-913.

Makkar HPS (2003). Quantification of tannins in tree and shrub foliage. A laboratory manual. Kluwer academic publisherd. Dordrecht/ Boston/London. pp., 43-57.

McBee RH (1953). Manometric method for the evaluation of microbial activity of rumen with application to utilization of cellulose and hemicellulose. Appl. Microbiol. 1:106-110.

Menke KH, Raab L, Salewski A, Steingass H, Fritz D, Schneider W (1979). The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro*. J. Agric. Sci. (Camb) 93:217-222.

Menke KH, Steingass H (1988). Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim. Res. Develop. 28: 7-55.

Norton BW (1999). The significance of tannins in tropical animal production. In: Tannins in livestock and human nutrition (Ed. D. Brooker). ACIAR Proceedings no. 92, Canberra, Australia, pp. 14-23.

Palmer B, Jones RJ (2000). The effect of PEG addition in vitro on dry matter and nitrogen digestibility of Calliandra calothyrsus and Leucaena leucocephala leaf. Anim. Feed Sci. Tech. 85:259-268.

Pritchard DA, Martin PR, O'Rourke PK (1992). The role of condensed tannins in the nutritional value of mulga (Acacia aneura) for sheep. Aust. J. Agric. Res. 43:1739-1746.

Reed JD, Soller H, Woodward A (1990). Fodder tree and straw diets for sheep: intake, growth, digestibility and the effect of phenolics on nitrogen utilization. Anim. Feed Sci. Technol. 30:39-50.