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J. Bangladesh Agril. Univ. 5(2): 313–320, 2007 ISSN 1810-3030

Effects of polyethylene glycol on dried *Morus indica* and *Erythrina orientalis* leaves

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Abstract

The experiment was carried out using polyethylene glycol on tannin content, organic matter digestibility and metabolizable energy content in freeze dried and shade dried *Morus indica* (Mulberry) and *Erythrina orientalis* (Mander) leaves. Total phenol, total tannin, condensed tannin and hydrolysable tannin content did not differ significantly (P>0.05) between freeze dried and shade dried *Morus indica* and *Erythrina orientalis* and the mean values were 2.4, 1.3, 0.5, 0.6 vs 1.5, 0.8, 0.2, 0.7 and 2.4, 1.2, 0.5, 0.8 vs 1.8, 0.9, 0.2, 0.7 for *Morus indica* and *Erythrina orientalis,* respectively. In contrast, *in vitro* gas production differed significantly (P<0.05) between freeze and shade dried and the mean values were 37.4, 44.4 vs 41.7, 46.9 and 21.4, 27.2 vs 25.6, 29.1 for *Morus indica* and *Erythrina orientalis,* respectively. Similarly, organic matter digestibility *in vitro* differed significantly (P<0.05) between freeze dried and shade dried and the mean values were 56.6, 63.7 vs 60.5, 64.8 and 45.3, 50.9 vs 49.5, 52.5 for *Morus indica* and *Erythrina orientalis,* respectively. However, metabolizable energy content (MJ/kgDM) was similar (P>0.05) both in freeze dried and shade dried samples of *Morus indica* and *Erythrina orientalis.*

Keywords: *Morus indica, Erythrina orientalis*, Tannin, *In vitro* organic matter digestibility

Introduction

The constraints for low productivity of livestock are the scarcity and poor quality feed that unevenly supplied round the year. Farmers offer various tree leaves, flowers, pods and twigs along with conventional grazing and straw based feeding as practical means of feeding animals particularly during scarcity period. The use of tree foliages as forage is constrained by the presence of some anti-nutritional factors, which are termed as plant defensive components. Phenolics, saponins, alkaloids, free amino acids, steroids, essential oils, glycosides, terpenes and resins are major plant defensive components (Makkar, 2001). Many tree fodders contain anti-nutritional factors like polyphenolics (Lowry *et al.*, 1996) and its presence at high level can significantly restrict intake, utilization and nutritive value of feed (Pritchard *et al.*, 1992; Kumar, 1992 and Reed *et al.*, 1990). Tannins are naturally occurring plant polyphenolic compounds containing sufficient phenolic hydroxyl groups to permit formation of stable cross links with proteins (Deshpande *et al.*, 1986). It has high binding tendency with feed proteins, carbohydrates, and enzymes thus reduce the extent of fermentation of feed in rumen. This binding tendency of tannins with nutrients has negative impact on availability of nutrients for ruminants. The effect of tannins on feed intake, digestibility and animal response to feeding seems complicative but its effect on rumen fermentation is extremely important for utilization of tanniniferous foliages to improve ruminant production systems in Bangladesh where ruminants live on poor quality roughages and leguminous tree foliages. Beneficial as well as detrimental effects of tannins have been reported from herbaceous plants in temperate regions (Makkar, 2001). Number of evidences is available regarding the presence of tannin in some commonly used tree foliages and the methods of reducing toxicity of tannin in ruminants. Therefore, it requires intensive study to investigate the presence of polyphenolics for better utilization of unconventional feed stuffs

and also for identification and introduction of new tree plants that are capable of growing in poor soils, roadsides and riversides to play a vital role as livestock feed as well as to control soil erosion. Considering above facts and findings, the present research was undertaken to observe the effect of using polyethylene glycol on tannin content, organic matter digestibility and metabolizable energy content in freeze dried and shade dried *Morus indica* and *Erythrina orientalis.*

Materials and Methods

Tree leaves of *Morus indica* (Mulberry) and *Erythrina orientalis* (Mander) used to determine the content of various forms of tannin and its effect on *in vitro* digestibility through gas production technique described by Menke *et al.* (1979) and Menke and Steingass (1988).

Freshly flushed leaves approximately three month old including pods from the top of the branch of *Morus indica* and *Erythrina orientalis* were collected from Boira and Darikathal, Mymensingh in January 2003. Samples were carried in dry ice and frozen at -4°C until freeze dried. After completing freeze drying and shade drying, samples were grounded at the size of 0.5 mm and 1.0 mm and were bottled covered with aluminium foil, kept in desiccator for chemical analysis and *in vitro* study. Chemical components were estimated as per AOAC (1980) and Faichney and White (1983).

Briefly, total phenol was estimated by Folion Ciocalteu reagent method and total tannin was estimated by subtracting the values of polyvinylpyrrolidone (PVPP) bind tannins from total phenols. Condensed tannin was estimated by oxidation of condensed tannin in butanol-HCl reagent in presence of iron. Hydrolysable tannin was estimated by hydrolysis to gallic acid with rhodanine. Both total phenols and total tannins were expressed as tannic acid equivalent. The condensed tannin and hydrolysable tannin were expressed as leucocyanidin equivalnet and gallic acid equivalent respectively. The analyses were conducted as per laboratory manual for quantification of tannins in tree foliage (Makkar, 2000).

Samples were selected as fresh, heat treated, chemical treated freshly dried and chemical mixed heat treated. Hay was used as standard and blank was used for correction of gas measurement. Each sample had three replications.

A series of experiments were carried out to investigate the effect of tannins on rumen fermentation with and without PEG using hay standard. Shade and freeze dried sample of *Morus indica* and *Erythrina orientalis* leaves were used. The methodology of *in vitro* gas production technique used was as described by Menke *et al.* (1979) and Menke and Steingass (1988).

Mean gas production from blank tests (Gp_0) was calculated by using the formula Gp_9-Gp_0 /3. The net gas production was corrected for differences in sample weight (mg DM), if different from 200mg DM. When the piston of the syringe was moved back to 40 ml at 9 h of incubation (V_9) then the formula was:

1. Gp (ml/200 mg DM) = 44.43 / (Gp_9-Gp_0) + (Gp_{24}-40) - Mean blank gas production x 200 /weight of the sample (mg DM).

The effect of tannins on gas production was calculated from the following formula:

1. Effects of tannins on gas production = (Corrected gas production with PEG/Corrected gas production without PEG) x 100

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

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

The computer program (SAS, 1988) was used for multiple regression procedures to assess *in vitro* parameters. The significant differences between means were compared using Duncan's Multiple Range Test (DMRT) after analysis of variance (ANOVA) for one way classified data. For comparison of means of two groups, Student's "t" test was applied. Pearson's correlation coefficient tests were done to measure interaction between different forms of polyphenolic compounds and *in vitro* gas production was calculated by the STAT program.

Results and Discussion

The chemical composition of *Morus indica* and *Erythrina orientalis* is given in Table 1 . The dry matter contents in *Morus indica* and *Erythrina orientalis* were 92.9% vs 89.9% and 96.2% vs 91.6% for freeze and shade dried samples, respectively. It was observed that the dry matter contents in freeze dried samples were higher than that in shade dried sample. The organic matter contents of freeze and shade dried sample were 86.2% vs 82.9% and 86.2%vs 87.8% in *Morus indica* and *Erythrina orientalis,* respectively. The nitrogen contents of freeze dried sample were 2.9% vs 3.1% and of shade dried samples were 4.1% vs 4.2% in *Morus indica* and *Erythrina orientalis,* respectively. The NDF content in freeze dried samples were 16.5% vs 14.5% and in shade dried samples were 37.1% vs 23.8% in *Morus indica* and *Erythrina orientalis,* respectively. The Acid Detergent Fibre (ADF) content in freeze dried sample were 14.8% vs 15.0% and in shade dried samples were 23.2% vs 21.7% in *Morus indica* and *Erythrina orientalis,* respectively. Lignin contents were 14.5% vs 13.9% and 12.2% vs 20.4% in freeze dried and shade dried samples of *Morus indica* and *Erythrina orientalis,* respectively.

Table 1. Chemical composition of freeze dried and shade dried *Morus indica and Erythrina orientalis*

Parameter (g/100g)	*Morus indica*		Level of significance	*Erythrina orientalis*		Level of significance
	Freeze dried	Shade dried		Freeze dried	Shade dried	
Dry Matter	92.9	89.9	NS	96.2	91.6	NS
Organic Matter	86.2	82.9	NS	86.2	87.8	NS
Nitrogen	2.9	3.1	NS	4.1	4.2	NS
Neutral Detergent Fiber	16.5	14.5	NS	37.1	23.8	NS
Acid Detergent Fiber	14.8	15.0	NS	23.2	21.7	NS
Lignin	14.5	13.9	NS	12.2	20.4	NS

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 of availability of nutrients, and at best, may provide information on potential nutrient content. Both DM and OM contents in *Erythrina orientalis* are higher than in *Morus indica.* The N percentage of *Erythrina orientalis* is also higher than *Morus indica* and the freeze dried contents were lower than shade dried sample although not significant. The NDF and ADF contents in *Erythrina orientalis* are also higher than *Morus indica.* The lignin percentage of *Erythrina orientalis* in shade dried sample was higher than freeze dried sample. This may be due to plant dry matter separated into a completely digestible fraction (neutral detergent soluble, NDS) representing cell contents and a partially digestible fraction (neutral detergent fibre, NDF), representing plant cell walls (Norton, 1994). It is assumed that drying bind tannins to cell wall and become unavailable for reaction. The CP content of the foliages used in this experiment was of above 18%. The result of this study and that reported by Rittner and Reed (1992) and Makkar *et al.*, (1998) indicates that most of the tropical tree species are high in CP content. Unlike grasses, the protein content of tree leaves remains relatively constant throughout the year. However, the high CP content of fodder tree leaves may not affect any direct nutritional benefit to the animal unless these proteins are protected against ruminal degradation (Norton, 1994).

The total phenols in freeze dried and shade dried samples were 2.4% vs 1.5% and 2.4% vs 1.8% in *Morus indica* and *Erythrina orientalis,* respectively. Total tannins were 1.3% vs 0.8% and 1.2% vs 0.9% in freeze dried and shade dried samples of *Morus indica* and *Erythrina orientalis,* respectively. Condensed tannins (CT) were 0.5 vs 0.2% and 0.5% vs 0.2% in freeze dried and shade dried samples of *Morus indica* and *Erythrina orientalis,* respectively. The hydrolysable tannins were 0.6% vs 0.7% in freeze dried and 0.8% vs 0.7% in shade dried samples of *Morus indica and Erythrina orientalis*, respectively. The values of total phenol were higher in freeze dried than that in shade dried samples.

Tannin content of plants may vary from species to species, plant to plant of the same species, tissue to tissue of the same plant, location to location, environment to environment and year to year of production (Mehansho *et al.*, 1987) because synthesis of polyphenolic compound by light intensity varies with all these factors. As a result, concentration of hydrolysable tannin as gallic acid equivalent varied among the foliages of *Morus indica and Erythrina orientalis.* Condensed tannin contents in all foliages were lower in shade dried samples than in freeze dried samples. It occurred due to high temperature stress. The shade dried samples dried very rapidly at high temperature (about 30°C), which might have induced the formation of additional condensed tannins in the leaves of plants (Lees *et al.,* 1994).

Tannins have widespread occurrence in higher plants and not known to have any physiological functions in plants producing tannins. They are found in the wood, bark, leaves and fruits of many species (Greutach, 1973). Condensed tannins have been found in the tissues of several legumes including the leaves of some herbaceous species. A small variation in tannin contents has been found in the foliages used in this study. The values were better correlated to biological activity of tannin i.e. increase of gas production in presence of PEG. The non-significant correlation between condensed tannins and percent gas increase might be due to the variation in extractability of tannins from different species or to the variation in specificity of the tannin measuring reagents. Condensed tannins of different species have different physiological and chemical properties (Mangan, 1988). The higher the concentration of polyphenolic compounds lower is the gas production. Therefore, it may be suggested that the addition of tannin binding agent can increase gas production as well as the utilization of tannin rich foliages.

Table 2. Tannin contents in freeze dried and shade dried *Morus indica and Erythrina orientalis*

Parameter (g/100g)	*Morus indica*		Level of significance	*Erythrina orientalis*		Level of significance
	Freeze dried	Shade dried		Freeze dried	Shade dried	
Total phenol	2.4	1.5	NS	2.4	1.8	NS
Total tannin	1.3	0.8	NS	1.2	0.9	NS
Condensed tannin	0.5	0.2	NS	0.5	0.2	NS
Hydrolysable tannin	0.6	0.7	NS	0.8	0.7	NS

The total amount of gas produced within 24 h of incubation from the foliages immersed in rumen buffer with and without PEG are presented in Table 3. Addition of PEG for inhibition of tannin increased gas production and the mean values were 12.0% vs 15.8% in freeze dried samples and 0.2% vs 2.5% in shade dried samples of *Morus indica and Erythrina orientalis,* respectively. *In vitro* gas production by incubation of *Morus indica* and *Erythrina orientalis* in the presence and absence of PEG with a hay standard in order to achieve accuracy of *in vitro* gas production in controlled rumen environment is given in Table 3. Total gas produced by incubation of hay was 41.7 ml, which was close to 44.4 ml used for standard hay. Therefore, the results obtained for the foliages in the present experiment were under optimal rumen environment. The increased amount of gas produced due to addition of PEG in freeze dried sample was very low and in shade dried sample it was negligible.

Table 3. Effect of polyethylene glycol (PEG) on *in vitro* gas production on freeze dried and shade dried *Morus indica and Erythrina orientalis*

Foliages	In vitro gas production(ml)				Level of significance
	Freeze dried		Shade dried		
	-PEG	+PEG	-PEG	+PEG	
Morus indica	37.4	44.4	41.7	46.9	*
Erythrina orientalis	21.4	27.2	25.6	29.1	*

The increase in gas production by addition of PEG indicates the inhibiting action of tannins on digestion in the rumen which might have resulted increased microbial fermentation similar with the findings of Makkar and Becker (1996). In presence of PEG about 70% of the total gas was produced in the first 12h of incubation and the remaining 30% during the last 12h of incubation from all the foliages. The addition of PEG did not affect gas production of tannin free hay. The rate of gas production was less for the first 3h but increased gradually up to 9h of incubation. It occurred due to the fact that the rumen microbes need some times for adaptation before increasing microbial activity for proper fermentation. Higher the biological activity of tannins on rumen microbes, higher is the gas production (Makkar *et al.,* 1995 b). The extent of protection of proteins from rumen degradation by tannins was quantified using PEG as tannin binding agent. Addition of PEG to tannin containing foliages increased *in vitro* gas production (Table 3).

The nutritive value of organic matter for ruminant is influenced substantially by the protein content of the foliages and 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. Significant effect on gas production was observed for all freeze dried and shade dried samples of *Morus indica and Erythrina orientalis.*

The organic matter digestibility (OMD) of the foliages is shown in Table 4. Inhibitory effects of tannin by addition of PEG resulted increased in organic matter digestibility of the foliages. The increased organic matter digestibility by addition of PEG were 7.1% vs 5.6% in freeze dried and 4.3% vs 3.0% in shade dried samples of *Morus indica* and *Erythrina orientalis,* respectively. The Metabolizable Energy (ME) is shown in Table 5. Addition of PEG increased metabolizable energy content and the mean values were 2.8% vs 1.3% in freeze dried and 1.3% vs 0.9 in shade dried samples for *Morus indica* and *Erythrina orientalis,* respectively.

Table 4. Effect of tannin on Organic Matter Digestibility (OMD) in freeze dried and shade dried *Morus indica and Erythrina orientalis*

Foliages	OMD (%)				Level of significance
	Freeze dried		Shade dried		
	-PEG	+PEG	-PEG	+PEG	
Morus indica	56.6	63.7	60.5	64.8	*
Erythrina orientalis	45.3	50.9	49.5	52.5	*

Significant effect of OMD was observed both in freeze dried and shade dried samples of *Morus indica and Erythrina orientalis.* Much of the earlier work on gas measurement (McBee, 1953; El-Shazly and Hungate, 1965; Czerkawski and Breckenridge, 1969) 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 rather it subjects to wide fluctuations following changes in diet of the animal.

Higher gas volume was achieved in the foliages such as *Erythrina orientlis* having the higher CP content. The organic matter digestibility and metabolizable energy content were also increased. Formation of PEG tannin complexes might ffect on *in vitro* organic matter digestibility of tanniniferous foliages by reducing microbial effect on organic matter digestibility (Palmer and Jones, 1999). The present study further validated the findings that the addition of PEG may increase organic matter digestibility and metabolizable energy content (Table 4 & 5). Differences in organic matter digestibility in presence or absence of PEG between foliages and among foliages may be explained partly by differences in protein and energy consumption, presence of tannins and fibres, which may have either decreased or prevented from ruminal fermentation (Barry and Manley, 1986). The values obtained in presence of PEG provided potential improvement for feeding values of the foliages in absence of tannins. Therefore, it may be suggested that removal of the adverse effects of tannins by PEG may increase organic matter digestibility of tannin rich foliages.

Table 5. Effect of tannin on Metabolozable Energy (ME) content in freeze dried and shade dried *Morus indica and Erythrina orientalis*

Foliages	ME(MJ/kgDM) content				Level of significance
	Freeze dried		Shade dried		
	-PEG	+PEG	-PEG	+PEG	
Morus indica	8.4	11.2	9.0	10.3	NS
Erythrina orientalis	6.5	7.8	7.2	8.1	NS

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