PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 5 (4): 343-347, 2006
ISSN 1680-5194

Deactivation Effects of Polyethylene Glycol (PEG) on *in vitro* Dry Matter Digestibility of *Colophospermum mopane* (*Mophane*) and Acacia Browse Trees in Botswana

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Abstract: This trial evaluated the deactivation effects of PEG 6000 on *in vitro* dry matter digestibility (IVDMD) of *Colophospermum mopane* (*Mophane)* and acacia browse trees. Samples were collected from Sebele Content farm except for *Mophane* and were freezed dried before analysis. PEG was introduced into the digestion tubes at a rate of 160mg/g of sample in the form of a one milli liter of solution containing 80mg PEG before the addition of rumen fluid/buffer medium. It was found that plant species had an effect (P<0.001) on IVDMD. *Viscum verrucosum*, mistletoe that attach on acacia trees, was found to have higher (576.6±27.7g/kg DM) while *Mophane* had the least IVDMD (350.5±31.0g/kg DM). IVDMD between PEG inoculated and control samples was similar (P>0.05). However, IVDMD values of PEG treated samples were numerically higher with those of *Acacia nilotica* approaching significance (P=0.083). Within the PEG treated samples, IVDMD was higher (P<0.05) for *A. nilotica*, *A. tortilis* and *V. verrucosum* and lower for *Mophane* samples. The low digestibility of *Mophane* may not be due to extractable condensed tannins (CT) because fresh *Mophane* leaves have been previously found to contain 27.0g/kg DM condensed tannins. Other anti-nutritional factors may be responsible for its low digestibility. Alternatively, phlobatannins which are a characteristic of heartwood of *Mophane* may be present in fresh leaves and may have formed an insoluble complex with PEG leading to an underestimation of IVDMD. It is concluded that PEG may not be an ideal deactivating agent to improve utilization of fodder trees by resource limited farmers and an alternative such as wood ash should be investigated.

Key words: Acacia, *Mophane*, tannins, digestibility

Introduction

Inadequate amounts and low feed quality result in reduced livestock productivity in tropical countries (Odenyo *et al*., 1997). However, the feed base can be improved by using perennial legume fodders, particularly those with multipurpose function that are more attractive to the resource limited farmers in these countries (Odenyo *et al*., 1999). *Colophospermum mopane* (*Mophane*) stretches from the east of Botswana extending to the northern part into Southern Zimbabwe. Though fallen dry leaves and pods from *Mophane* are important feed resources for domestic and wild animals (Hove and Mpofu, 1996) fresh leaves are rarely consumed by livestock. This may be due to anti-nutritional chemicals contained in fresh leaves of *Mophane* (Macala *et al*., 1992). Acacia species and mistletoes that attach on them in semi-arid environments of Botswana also represent a valuable feed supply to livestock. During drought and extended dry period, livestock are fed on these plants and in some instances, pods are collected and traded to livestock owners. Data from our laboratory Madibela *et al*. (2005) showed that crude protein of fresh leaves of *Mophane* is 166.1g/kg DM. Madibela *et al*. (2004) recorded crude protein levels of acacia browse trees in Botswana, ranging from 114.7 to 213.3 g/kg DM and those of mistletoes were from 156.0 to 163.7 g/kg DM.

According to Odenyo *et al*. (1999) fodder trees often contain anti-nutritional factors (ANFs) and a common ANF in fodder trees is tannin. *Mophane* was observed to contain 27.0g/kg DM condensed tannins (Madibela *et al*. 2005). Condensed tannin (CT) of four mistletoes was found to be 75, 31, 65 and 56 g/kg DM for *Viscum verrucosum*, *V. rotundifolium*, *Erianthenum ngamicum* and *Tapinanthus lugardii*, respectively (Madibela *et al*., 2002). Phale and Madibela (2006) found that *Acacia nilotica* and *A. robusta* had CT levels of 90.0 and 75.9g/kg respectively. Tannins are water-soluble polyphenolics that precipitate proteins from solution (Nelson *et al*., 1995) and this can have both

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Table 1: Effects of plant species on *in vitro* dry matter digestibility

Plant species	Digestibility (g/kg DM)
Acacia nilotica	501.93±27.71[ab]
A. tortilis	466.79±27.71[b]
Mophane	350.45±31.00[c]
V. verrucosum	576.61±27.71[a]
Mean	474.83
Significant Level	****** = P<0.001

detrimental and beneficial effects to livestock consuming CT containing fodder trees. At high levels, tannin cause over protection of protein resulting in low utilization of nitrogen (Silanikove *et al*., 1997) while at quantities of less than 50g/kg DM, condensed tannins may increase supply of protein to the small intestine (Miller *et al*., 1995). Despite containing low levels of CT, fresh leaves from *Mophane* are not readily eaten by livestock unless when dry. This may mean that *Mophane* leaves may contain other plant secondary metabolites used against herbivory. Paradoxically, *Mophane* worm (*Imbrasia belina*), which inhabit mophane woodlands feed on fresh leaves. *A. nilotica* is used by livestock but in Botswana *A. tortilis* is most preferred, and this may be an indication of differences in the nature (chemical structure) and quantities of polyphenolic substances between these plants.

PEG preferentially binds with tannins resulting in the reversal of tannin effects (Hove and Mpofu, 1996). The ability of PEG to enhance the utilization of CT containing fodder trees have previously been reported (Pritchard *et al*., 1992; Hove and Mpofu 1996; Miller *et al*., 1997; Palmer and Jones, 2000; Jones *et al*., 2001). However, no information exist about its effects on fodder trees that are used by livestock in Botswana and therefore the aim of this study was to determine the binding effects of PEG on *in vitro* digestibility of *Colophospermum mophane*, *Acacia tortilis*, *A. nilotica* and *Viscum verrucosum* in Botswana.

Materials and Methods

Study site: Sebele Station is situated between latitude of 24°33' S and longitude of 25°57' E at an altitude of 994m asl. The vegetation type is a mixture of Acacia savanna with broaded leaved middle layer trees. The soil type of the area is classified as moderately deep to very deep, imperfectly to moderately well drained dark brown to red sandy clay loams to clays (De Wit and Nachtevgaele, 1990). The station receives an average annual rainfall of about 500 mm. Monthly averages minimum and maximum temperatures are 12.8 and 28.6°C respectively.

Samples and analysis: Samples of *Acacia nilotica*, *A. tortilis* and *Viscum verrucosum*, were obtained from the station farm. *Mophane* woodland exists about 300km north of the station but samples were obtained from trees cultivated as shade trees in the city, a kilometer away from the research station. Samples were handpicked from the terminal shoots. *V. verrucosum* is a mistletoe that attach on Acacia species and it does not have leaves (Madibela *et al*., 2000), but actively growing stems were collected. All samples were placed in a brown paper bags and freeze dried within one hour of collection. They were milled through a 2 mm screen before analysis.

In vitro dry matter digestibility (IVDMD) of a 0.5g sample was determined in triplicates (except the PEG *Mophane* samples which were in duplicate) according to the procedure by Tilly and Terry (1963), by incubating in a thermostatically controlled circulating water bath. The samples were digested in the absence (control) and presence of PEG 6000 (Merk, South Africa; treatment). Assessment of PEG involved adding one milli liter of a solution containing 80mg PEG to each digestion tube (160mg/g sample) before the rumen fluid/buffer medium was added (Jones *et al*., 2001). One milli liter of distilled water was added to control tubes.

Rumen fluid donors: Rumen fluid was obtained from 5 castrates Tswana goats (2 years). These animals had previously grazed vegetation consisting of tanninferous fodder shrubs and trees. Three months before commencement of the experiment they were confined in a pen. The pen was large enough to allow animals to walk about and had shade on one side to reduce heat. The animals were fed a sorghum stover/concentrate diet (40:60) consisting of 11.4% CP, 0.3% calcium and 0.4% phosphorous on an *ad lib* basis. Water was available at all times. On the day of the analysis, rumen liquor was collected from the five animals using an intra-ruminal tube and evacuation pump into thermo flasks. The liquor was pooled together and at the laboratory it was sieved through cheesecloth and used immediately.

Statistical analysis: Analysis for effects of plant species, treatment and their interaction was performed using the General Linear Models (GLM) procedure (SAS, 1990). Differences between plant species and between treatments (PEG vs Control) were tested for significance by least significant difference (LSD). Means are reported as least square means ±standard error.

Results

Table 1 and 2 shows effects of plant species and of PEG on digestibility of fodder trees. There was a significant (P<0.001) effect of plant species on *in vitro* dry matter digestibility (IVDMD). *Viscum verrucosum* had a high IVDMD value and *Mophane* had the least (Table 1). PEG treated samples and control were similar (P>0.05) though digestibility values of PEG samples were numerically higher than the controls; 489.60±20.79 vs 458.29±19.60 g/kg DM. Among the PEG inoculated samples, *A. nilotica A. tortilis* and *V. verrucosum* had significantly higher and similar IVDMD (Table 2). For samples without PEG, *A. tortilis* and *V. verrucosum* had

higher and similar digestibility but *Mophane* was observed to have a low value, while the Acacia species had similar values.

Table 2: Effects of PEG on *in vitro* dry matter digestibility (g/kg DM) of the different fodder plants

Plant Species	Treatment		
	PEG	Control	P level
Acacia nilotica	553.41±39.19^{a}	450.45±39.19^{b}	0.083
A. tortilis	429.29±39.19^{a}	501.90±39.19^{ab}	0.196
Mophane	377.91±48.00^{b}	323.00±39.19^{c}	0.390
V. verrucosum	597.79±39.19^{a}	575.05±39.19^{a}	0.457

Different superscripts within a column are different at P<0.05

Discussion

The plant species differences in dry matter digestibility in the present experiment indicate the differences in the anti-nutritional chemicals and the proportion of fibre in the different plants. Different tannins in different plant samples may vary not only in total content, but also in their ability to affect degradability, and to bind to proteins or fibre (Silanikove *et al*., 2001). It is not surprising that *Mophane* had a lower IVDMD, because in the fresh form, it is not readily consumed by livestock indicating high anti-nutritional factors. In so far as tannins are concerned, they may not be responsible for this low digestibility because analysis of extractable condensed tannins in our laboratory has indicated a low value of 27.0g/kg DM. Alternatively, *Mophane* leaves may contain high level of bound tannins since those were not analyzed. When compared, *A. nilotica* and *A. tortilis* were found to have the similar digestibility. This is despite the fact that *A. nolitica* has high content of condensed tannins (Phale and Madibela, 2006). IVDMD of *A. tortilis* in the present experiment is higher than previous results observed by Madibela *et al.* (2004). But those of *V. verrucosum* were found to be similar to the values reported by Madibela *et al.* (2004).

The addition of PEG 6000 at the rate of 160mg PEG/g sample was based on the recommendation by Palmer and Jones (2000) who found that at this level, PEG is appropriate for studies with tropical tanniniferous shrub legumes to estimate any deleterious tannin effects. There was no significant difference between addition of PEG and control samples on IVDMD. Numerically, PEG-inoculated samples were higher while those of *A. nilotica* were approaching significance (P=0.083). This is in contrast with other studies which found an increase of *in vitro* true degradability dry matter (Singh *et al.* (2005) gas production (Baba *et al*., 2002; Yildiz *et al.* 2005; Singh *et al*., 2005) rumen degradability (Getachew *et al.,* 2001; Baba *et al*., 2002) and crude protein degradability (Moujahed *et al.,* 2000). The lack of difference in the present study may be due to the fact that PEG bind with some component of the feed to produce an insoluble complex which remain in the residue resulting in an underestimation of IVDMD (Palmer and Jones, 2000). It seems that the insoluble complex in the residue did not dissociate in the subsequent acid-pepsin stage, a factor suggested by Palmer and Jones (2000) when evaluating *Calliandra calothyrsus* and *Leucaena leucocephala*.

According to Silanikove *et al.* (2001) the use of *in vitro* schemes to evaluate the effects of tannin are simple yet information generated is qualitative and does not give insights about *in vivo* effects of these tannins. *In vivo* studies using PEG showed that intake (Pritchard *et al.* 1992; Miller *et al*., 1997; Silanikove *et al.,* 2001; Bhatta *et al*., 2002; Yildiz *et al*., 2005), CP digestion (Pritchard *et al.,* 1992; Moujahed *et al*., 2000; Bhatta *et al.,* 2002; Yildiz *et al*., 2005) and N-retention (Yildiz *et al*., 2005) were improved. The high CP digestibility and NH_3-N concentration was partly attributed to tannin deactivity of PEG (Moujahed *et al*., 2000). In contrast, Smith *et al.* (2005) reported reduced N-retention with PEG treatment. This was also observed by Yildiz *et al.* (2005) when high level (370 verses 185g Oak leaves) of feeding was introduced to lambs. This was attributed either to inadequate fermentable energy in the rumen leading to high urinary N (Smith *et al.* 2005) or reduced ruminally undegraded protein reaching the small intestine (Smith *et al.,* 2005; Yildiz *et al*., 2005).

In the present study, within the PEG treated samples, *V. verrucosum*, *A. tortilis* and *A. nilotica* had higher and similar digestibility (Table 2). *Mophane* had a lower value and this shows that in spite of PEG treatment, compared to other fodder trees, *Mophane* was not readily digestible. PEG is beneficial at tannin level of 50 to 100g/kg DM and not effective at 10g/kg DM (Yildiz *et al.,* 2005). Condensed tannin content of fresh *Mophane* leaves was found to be 27g/kg DM (Madibela *et al.,* 2005) and this may explain the results observed in the present study. However, the heartwood of *Mophane* tree contain novel class of C-ring isomerized condensed tannins called phlobatannins (Ferreira *et al.,* 2003) with extensive structural and stereochemical diversity at a trimetric level (Bonnet *et al*., 1996). It is possible that the leaves may also have this type of tannins and their insoluble complex with PEG lead to underestimation of IVDMD in the present study. Since *Mophane* woodland is widely distributed in Angola, Botswana, Malawi, Mozambique, Namibia, South Africa and Zimbabwe (Mojeremane and Lumbile, 2005) and that its many uses include feed for livestock, it would be important to improve its utilization in animal feeding. Its leaves are consumed when dry, after having fallen to the ground (Hove and Mpofu, 1996; Mojeremane and Lumbile, 2005) indicating that drying may have some beneficial effect on intake and utilization of the leaves. According to Ferreira *et al.* (2003) aerial parts of *Mophane* are rich in essential oils that comprise of α-pinene and limonene and these chemicals are responsible for strong turpentine odour associated with *Mophane*. Previously Macala *et al.* (1992) had associated turpentine with low

feed intake of fresh *Mophane* leaves. This calls for chemical characterization of this resource in so far as far animal nutrition is concerned, to allow for drawing of strategies which would be effective in using it in animal feeding. Smith *et al.* (2005) reported encouraging results when wood ash was used to deactivate fodder material, and this needs to be evaluated on *Mophane*, more especially that ash is readily available in most households. This is supported by the factor that for resource-limited farmers, PEG may not be a cost-effective method to deactivate tanniniferous fodder material.

Acknowledgements

The authors would like to thank Mr Molatlhegi Seboko for looking after the animals. This study was supported by Botswana's Ministry of Agriculture.

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