Short communication

Evaluation of Bengal gram (Cicer arietinum) husk as a source of tannin and its interference in rumen and post-rumen nutrient digestion in sheep

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Abstract

Bengal gram (Cicer arietinum) husk (BGH) was evaluated for the content of tannin and its interference in digestion. The BGH contained (% DM) 76.0 NDF, 65.2 ADF, 6.1 ADL and 8.4 tannin. The gas production (ml/200 mg DM/24 h) from BGH incubated with rumen inoculum was 45.0, which increased to 61.3 when polyethylene glycol (PEG 6000) was added. The magnitude of the increase in gas production was not explained by the quantity of tannin-bound protein (acid detergent insoluble nitrogen \times 6.25) or the total protein. This suggested that carbohydrate could be the main substrate bound by tannin in BGH. The availability of tannin-bound substrate in the post-rumen digestive tract was assessed through a metabolic trial. Twelve male lambs aged between 6 and 8 months were divided into three groups of four animals in each group. BGH was incorporated in the diet at 0, 6.8 and 14% by replacing deoiled rice bran (DORB). Incorporation of BGH in the diet increased OM digestibility (P<0.002) from 63.3% (Group I) to 68.1% (Group II) and 72.4% (Group III). The observed differences among the diets was quantitatively explainable by the difference in ME content (8.5 MJ) of BGH and DORB after PEG addition. The faecal excretion (g/day) of organic neutral detergent solubles (ONDS) (54.5, 55.3 and 46.7), and neutral detergent soluble nitrogen (NDSN) (4.4, 4.4 and 3.9) for the three groups were similar. Therefore, it is
speculated that the tannin-bound substrate in BGH is carbohydrate, protected from rumen fermentation but digested in the small intestine. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Bengal gram husk; Tannin; Rumen availability; Polyethylene glycol; Sheep

### 1. Introduction

Although the proximate composition of Bengal gram (*Cicer arietinum*) husk (BGH) is comparable to that of cereal straw (Sen et al., 1978), and the cost is equivalent to, or higher than that of maize grain, it is one of the preferred feed ingredients in the diet of crossbred dairy cows in and around Bangalore, India. Therefore, feeding of BGH is rather difficult to justify unless specific advantages are confirmed. Re-evaluation of BGH for its nutritional and antinutritional components revealed that apart from high fibre content, more than 25% of the total nitrogen (N) was in the form of acid detergent insoluble N (ADIN), and tannins ranged from 6 to 8% of the dry matter (DM) (unpublished data). Although the tannins are generally regarded as antinutritional, certain types of tannins naturally present in feedstuffs are reported to benefit the ruminants (Barry, 1989). If BGH incorporation in the diet brings about such beneficial effects due to the presence of tannins, the BGH can be advantageously used to increase efficiency of nutrient utilisation in ruminants. Therefore, this study was conducted to evaluate BGH as a source of tannin and its influence on nutrient digestion using ram lambs.

### 2. Materials and methods

#### 2.1. Animals and diet

Twelve male lambs aged between 6 and 8 months were divided into three groups of four each of comparable age and body weight (24.0±2.2). The lambs were housed in individual stalls and subjected to similar management practices. Before starting the experiment, the lambs were dewormed with Albendazole and fed on identical diet for a period of 15 days, to achieve uniformity in nutrient intake.

The experimental diet consisted of finger millet (*Eleucine coracana*) straw (FMS) offered ad libitum (0.4 kg/day) and a compound feed mixture (CFM) as a supplement to provide adequate energy, protein and other nutrients (NRC, 1975). Three isonitrogenous CFM were formulated for three groups by replacing deoiled rice bran (DORB) with 0% (Group I), 10% (Group II) and 20% (Group III) BGH (Table 1). The daily allowance of CFM for individual lambs for maintenance and a gain of 80 g/day was calculated based on previous weeks body weight and FMS intake. The CFM was fed in the morning at 08:30 hours and chaffed FMS at 12:30 hours daily. Refusals were weighed on the following day morning to obtain an estimate of intake. Lambs were watered twice a day at 10:00 and 15:30 hours.

The lambs were adapted to the test diets for 2 weeks. The metabolism trial lasted for 5 days, during which daily intake of CFM and FMS, and output of faeces and urine were
recorded. Samples of feed offered, feed refusals, faeces and urine were collected daily in
the morning. Dried samples were pooled for analysis of chemical constituents and rumen
in vitro studies.

The faeces from each lamb were collected from faecal collection bags twice a day at
09:00 and 18:00 hours stored in polythene bags and weighed every day at 09:30
hours. One tenth by weight of the daily faeces voided by each lamb was used for
DM determination by drying at 70°C to constant weight. Dried daily samples were
pooled, ground through 1 mm sieve and preserved for chemical analyses and rumen
in vitro studies. For N determination, the faeces samples (1/100th of the daily voids)
were preserved in 25% sulphuric acid to make a pooled sample for 5 days for individual
lamb.

The urine was collected using collection bags and stored in a 2.5 l bottle containing
20 ml of 25% sulphuric acid. Urine collected over 24 h was measured every day at 08:30
hours. Samples of urine (1/50th of total output) from individual lambs were collected
every day morning in a 500 ml Kjeldahl flask containing 15 ml of concentrated sulphuric
acid and stored at room temperature for N determination. The DM content of feed offered
and orts were also determined by drying at 70°C.

2.2. Chemical analyses and rumen in vitro incubation

2.2.1. Chemical analyses

The feeds, ingredients and faeces were analysed for crude protein, ether extract and ash
(AOAC, 1984). Neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent
lignin (ADL), ADIN and silica were determined according to Van Soest et al. (1991). The
tannin content in BGH was determined by vanillin-HCl method (Burns, 1971).

2.2.2. In vitro studies

A non-lactating cow weighing 400 kg, fitted with a large diameter flexible rumen
canula (Bar Diamond, USA), receiving a diet made up of 35% FMS and 65% CFM of
Group I served as the donor of rumen inoculum for in vitro studies. FMS and CFM were
offered separately. The CFM was offered in two equal portions at 06:00 and 13:00 hours.

Table 1
Ingredient composition (%) of compound feed mixtures

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>39.7</td>
<td>39.5</td>
<td>38.5</td>
</tr>
<tr>
<td>Groundnut meal, s.e.³</td>
<td>30.0</td>
<td>30.5</td>
<td>31.5</td>
</tr>
<tr>
<td>Deoiled rice bran, s.e.</td>
<td>20.0</td>
<td>10.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Bengal gram husk</td>
<td>0.0</td>
<td>10.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Molasses</td>
<td>7.5</td>
<td>7.0</td>
<td>6.7</td>
</tr>
<tr>
<td>Salt</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Urea</td>
<td>0.8</td>
<td>1.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

³ s.e. denotes solvent extracted.
The rumen fluid was collected in the morning between 09:00 and 10:00 hours before offering roughage.

The metabolisable energy (ME) in feed and ingredients were determined according to Menke and Steingass (1988). The interference of BGH tannins in rumen fermentation and the recovery of tannin-bound substrate in faeces were assessed using polyethylene glycol (PEG 6000) in rumen incubations (Makkar et al., 1995) of feeds and faeces. The magnitude of increase in gas production on PEG addition to the substrate at a ratio of 2:1 w/w, was taken as an index of tannin’s interference in rumen fermentation. In incubations containing BGH substrate with and without PEG, total volatile fatty acids (TVFA) were separated by steam distillation in a Markham apparatus and determined by the titrimetric method (Lewis, 1955).

2.3. Statistical analysis

The data were analysed by one way analysis of variance (Snedecor and Cochran, 1968).

3. Results

3.1. Chemical composition and rumen in vitro studies

The chemical composition of BGH, DORB, CFM, and FMS offered are presented in Table 2. The BGH contained 76% NDF and 65.2% ADF. On sequential analysis, ADF → NDF, the residue was 56.4%. The gas production from rumen in vitro incubation was significantly (P<0.001) higher in BGH as compared to DORB and FMS. A low gas production in DORB accompanied by high lignin and ADF ash is suggestive of high content of rice hulls. The BGH contained 8.4% tannin, and rumen incubation in vitro resulted in gas (ml/200 mg DM/24 h) and TVFA (µmoles/200 mg DM/24 h) of 45.0±0.39 and 658±33, respectively, which increased to 61.3±0.63 and 964±23 after PEG addition. The addition of PEG to CFM of Group II and Group III also resulted in increased gas production of 4 and 5.4 ml, respectively. The gas production from faeces without and with PEG for Group I, Group II and Group III was 6.3±0.18 and 6.1±0.35, 5.4±0.16 and 7.0±0.15, 5.1±0.15 and 7.2±0.15 ml, respectively.

3.2. Dry matter intake, digestibility and N balance

The DMI, nutrient digestibility, and N balance for the three groups are presented in Table 3. The mean DMI (g/day) for Group I, Group II and Group III was 824, 842 and 818, respectively, and the differences were not statistically significant (P>0.05). The digestibility of OM, NDF and ADF were significantly higher in Groups II and III than in Group I (P<0.002).

The N intake (g/day) in Group I, Group II and Group III were 21.2, 21.4 and 20.9, respectively. There was no difference among the groups in N voided through dung and urine. The N balance (g/day) for Group I, Group II and Group III were 6.9, 6.8 and 6.7,
respectively ($P>0.05$). The faecal N excretion (g/day) discounted for neutral detergent insoluble N (4.4, 4.4 and 3.9) and the total metabolic matter (organic ND solubles) (54.5, 55.3 and 46.7) were also similar for the three groups.

4. Discussion

A difference of 8.8 units between ADF and NDF in the sequential analysis (ADF $\rightarrow$ NDF) of BGH, agreed closely with the tannins determined by vanilline-HCl method (8.4%). An increased gas volume of 16.3 ml on addition of PEG is suggestive of either 43.2 mg of carbohydrate or 89.7 mg of protein made available for fermentation by PEG binding of tannin (1 ml gas from 2.5 to 2.8 mg fermented carbohydrate or 5.5 mg fermented protein) (Steingass, 1983). However, since the quantity of total protein in 200 mg of incubated BGH was 10.2 mg (Table 2), this could contribute to only 1.9 ml of gas. Therefore, increase in gas production on addition of PEG may be regarded as suggestive of BGH tannin’s interference mainly in carbohydrate fermentation in the rumen.

Contrary to this observation, the inclusion of BGH in the diet at 6.8 and 14% in Groups II and III resulted in higher OM digestibility (Table 3). Although the difference in OM

### Table 2
Chemical composition (g/kg DM), in vitro gas production and ME (MJ/kg DM) content predicted from in vitro incubations with and without polyethylene glycol for Bengal gram husk, deoiled rice bran, compound feed mixture and finger millet straw used in metabolism trial

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Bengal gram husk</th>
<th>Deoiled rice bran</th>
<th>Compound feed mixture</th>
<th>Finger millet straw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
<td>Group II</td>
<td>Group III</td>
<td>Group I</td>
</tr>
<tr>
<td>Organic matter</td>
<td>962</td>
<td>793</td>
<td>901</td>
<td>913</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>51</td>
<td>97</td>
<td>210</td>
<td>213</td>
</tr>
<tr>
<td>Ether extract</td>
<td>11</td>
<td>1</td>
<td>33</td>
<td>28</td>
</tr>
<tr>
<td>Neutral detergent fibre</td>
<td>760</td>
<td>809</td>
<td>405</td>
<td>393</td>
</tr>
<tr>
<td>Acid detergent fibre</td>
<td>652</td>
<td>578</td>
<td>199</td>
<td>222</td>
</tr>
<tr>
<td>Acid detergent lignin</td>
<td>61</td>
<td>133</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>Acid detergent fibre ash</td>
<td>2</td>
<td>156</td>
<td>28</td>
<td>26</td>
</tr>
<tr>
<td>ADINb $\times 6.25$</td>
<td>17</td>
<td>19</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Tanninsc</td>
<td>84</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Gas**

Without PEG 45.0±0.39 13.7±0.18 52.9±0.51 54.8±0.53 57.9±0.48 27.3±0.45 27.3±0.40

With PEG 61.3±0.63 13.6±0.20 52.5±0.56 58.8±0.52 63.3±0.61 27.3±0.40 27.3±0.40

**ME**

Without PEG 8.6 2.4 11.0 11.4 11.7 6.5

With PEG 10.9 2.4 11.0 12.0 12.5 6.5

a Group I=0% BGH; Group II=10% BGH and Group III=20% BGH.
b Acid detergent insoluble nitrogen.
c Vanillin-HCl method.
d ml/200 mg DM/24 h.
e Polyethylene glycol 6000.
digestibility among diets was partly explainable by the difference in ME content (6.2 MJ) between DORB and BGH, the observed variation among diets could not be quantitatively justified unless the improvement in ME content of BGH after PEG addition was taken into account (Table 2). This could mean that the tannin-bound energy component in BGH, although not fermented in the rumen, was digested post-ruminally as suggested by Barry et al., (1986) and Waghorn et al., (1987) with tannin containing forages. The response of gas production to PEG addition in the in vitro assays with CFM (Table 2) and faeces (Section 3.1) also tended to support this inference. If all the tannin bound substrate in CFM would remain in faeces as a component of indigestible DM (36 and 31.5%, Table 3), a response in gas (ml/200 mg DM/24 h) production to PEG close to 7.6\(\hat{\times}\)\(\hat{4}\)\(\hat{0}\).\(\hat{68}/0.36\) and 12\(\hat{\times}\)\(\hat{5.4}\)\(\hat{0}\).\(\hat{7}/0.315\) should be expected if it is assumed to be proportional to that of the CFM (4 and 5.4 ml, Table 2) and its contribution to the total DMI (0.68 and 0.7, Table 3). However, the increase in gas production in the incubated faeces of Group II and Group III after PEG addition was 1.6 and 2.1 ml, respectively, indicating that 79 (Group I) and 83% (Group II) of the tannin bound substrate in CFM was digested post-ruminally. This can be expected to increase the OMD of the total diet by, respectively, 7.5 and 14.6% in Group II and Group III over Group I, compared to the observed increase in OMD by 7.6 and 14.4%. Since carbohydrate fermentation in the large intestine increases metabolic matter and faecal N excretion (Van Soest, 1982), tannin-bound substrate in BGH, if carbohydrate and fermented in the large intestine, an increase in metabolic matter and faecal N excretion can be expected. Similar faecal

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>SEM</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMS Dry matter intake</td>
<td>243.5</td>
<td>270.0</td>
<td>247.0</td>
<td>52.9</td>
<td>0.75</td>
</tr>
<tr>
<td>CFM Dry matter intake</td>
<td>580.5</td>
<td>572.0</td>
<td>571.0</td>
<td>47.4</td>
<td>0.95</td>
</tr>
<tr>
<td>Total Dry matter intake</td>
<td>824.0</td>
<td>842.0</td>
<td>818.0</td>
<td>91.7</td>
<td>0.93</td>
</tr>
<tr>
<td>Dry matter Digestibility</td>
<td>58.5 a</td>
<td>64.0 b</td>
<td>68.5 c</td>
<td>2.8</td>
<td>0.002</td>
</tr>
<tr>
<td>Organic matter</td>
<td>63.3 a</td>
<td>68.1 b</td>
<td>72.4 c</td>
<td>2.5</td>
<td>0.002</td>
</tr>
<tr>
<td>Crude protein</td>
<td>70.2</td>
<td>69.4</td>
<td>69.8</td>
<td>4.6</td>
<td>0.97</td>
</tr>
<tr>
<td>Neutral detergent fibre</td>
<td>41.2 a</td>
<td>49.4 b</td>
<td>55.7 c</td>
<td>3.8</td>
<td>0.002</td>
</tr>
<tr>
<td>Acid detergent fibre</td>
<td>22.1 a</td>
<td>39.0 b</td>
<td>48.4 c</td>
<td>5.3</td>
<td>0.0012</td>
</tr>
<tr>
<td>TDN</td>
<td>60.6 a</td>
<td>64.5 b</td>
<td>68.7 c</td>
<td>2.4</td>
<td>0.004</td>
</tr>
<tr>
<td>ME</td>
<td>9.1</td>
<td>9.7</td>
<td>10.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(a\) Group I=0% BGH; Group II=10% BGH; Group III=20% BGH; TDN=total digestible nutrient and ME=metabolisable energy (MJ/kg DM)=TDN (%)\(\times\)0.15. Means within the same row with different letters differ significantly.
excretion of ONDS and NDSN by the three groups (Section 3.2) is thus suggestive of the tannin-bound BGH substrate undergoing digestion in the post-ruminal digestive tract before reaching the hind gut.

Although the animals used in this experiment had the potential for a gain of 150 g/day (Rangappa, 1993), the dietary N supplied was just adequate to support a gain of 80 g/day. Further, most of the dietary N was supplied from a highly degradable protein source. In spite of this, the level of tannin in the diet had no influence on N balance. This is perhaps attributable to the nature of the tannins. Incorporation of tamarind seed hulls in dairy cattle ration (1.6 and 4.7%) to provide a tannin content of 0.2 and 0.7%, respectively, resulted in an increase in faecal N excretion, decrease in urinary N excretion and increase in weight gain (Bhatta et al., 2000).

5. Conclusions

The BGH contained 8.4% tannin and was responsible for preventing nearly 21% of potentially digestible substrate from rumen fermentation. BGH incorporation in the diet at 6.8 and 14% in Group II and Group III, resulted in tannin concentration of 0.6 and 1.2%, respectively. These levels of tannin did not influence N utilisation. Therefore, incorporation of BGH upto 14% in cereal grain containing diets or in feeding systems assured to supply adequate energy and protein is unlikely to have any beneficial effect. The tannin-protected substrate in BGH is equivalent to 2.3 MJ/kg, and the evidences indicate that most of this substrate is likely to be carbohydrate and digested in the small intestine.

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