Effect of Tannin from Acacia Nilotica Pods on 
in vitro and in sacco Nutrient Degradability 
and Rumen Fermentation Pattern

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The study was carried out on Acacia nilotica extracted tannins on in vitro, in sacco nutrient degradability and rumen fermentation pattern. The results revealed that different extraction processes tried for tannin recovery was comparatively higher in methanol and cold water as compared to others. Two levels of tannins of pure tannic acid, extracted tannin through cold water process and babul pods were compared with different substrates such as GNC alone, concentrate mixture and TMR using 50:50 of roughage and concentrate. The in vitro nutrient digestibility (IVDMD and IVOMD) and rumen fermentation (NH3 and CH4) were reduced in tannin supplemented groups as compared to control (without tannin). However, total gas production was found higher in treated groups over control. In Sacco effective degradability of TMR was decreased in both pure tannic acid and extracted tannin powder where as babul pods did not alter the degradability.

Keywords: Tannin, Acacia nilotica, in vitro, in sacco

INTRODUCTION

Succesful evolution of plants from environment to land was achieved largely by massive formation of plant phenolic compounds accounting for about 40% of the medicinal plants. Of the secondary substances, tannins are the most abundant and widely distributed phenolic polymers found in higher plants and in many habitats including sewage, sludge, forest litter and the rumen (Muller-Harwey et al. 1988). Trees, shrub foliage and agro-industrial by-products are of importance in animal production because they do not compete with human food and can provide good protein supplements, especially in the dry season. The main constraint in using these feed resources is their anti nutritional factors, particularly tannins which are natural compounds widely distributed in the plant kingdom. These compounds are present in roots, leaves, fruits and seeds. Tannin being anti-nutritional, limits the utilization of various agro-industrial by products by inhibiting the nutrient utilization and so the animal productivity (Barman and Rai, 2006a). It is therefore, necessary to have a greater understanding of different aspects of tannin metabolism and its interactions with digestible nutrients in animal system. Beneficial as well as detrimental effects of tannins have been reported (Barman, 2004). Babul tree is used extensively as a traditional/ ayurvedic medicine. Its bark, gum, leaves and pods are used medicinally in India and West Africa. Acacia nilotica leaves contain relatively uncommon catechin gallate tannins while pods and bark contain (-) epigallocatechin gallates (Ayoub, 1985). Tanner et al., (1990) and Barman (2004) also reported catechin gallates in A. nilotica pods. These compounds even being of condense tannin can be degraded in rumen to yield gallic acid, catechin and epicatechin. Keeping the above facts in mind, the present study was done to evaluate the effect of extracted tannins from Acacia nilotica pods on rumen fermentation patterns in vitro.

MATERIALS AND METHODS

Various tannin extraction process were used to prepare crude extract from Acacia nilotica pods using different solvents viz cold water, hot water, methanol and acetone. The sample was dried at 55 10 C and ground to pass through a sieve.
size of 1mm diameter. Tannins extraction was done using 400 mg ground sample in conical flask with 40 ml diethyl ether containing 1 per cent acetic acid (v/v) and mixed to remove the pigment material. Carefully discarded the supernatant after 5 minute and 20 ml of 70 per cent aqueous acetone was added and sealed the flask with cotton plug covering with aluminum foil and kept in electrical shaker for 2 h for extraction. Then it was filtered through Whatman filter paper No. 1 and sample was kept in refrigerator at 4 0C until analysis. Tannin was estimated in all type of extracted powder and Acacia nilotica pods. The tannin rich Acacia nilotica pods powder, pure tannic acid and extracted tannin were added to Groundnut cake, concentrate and TMR at the rate of tannin equivalent to 2 & 4% (w/w) to evaluate the in vitro and in sacco nutrient degradability. Tannin fraction from Acacia nilotica pods were extracted with different methods were quantified. The protein precipitation capacity of tannins from Acacia nilotica pods and extracted powder i.e., formation of soluble tannin-protein complexes were assayed using BSA. TMR (13 % CP, 62 % TDN) was prepared with Concentrate: Roughage in the ratio of 50:50. Three rumen fistulated male crossbred (Holstein Friesian x Sahiwal) cattle were selected as a donor of rumen liquor for in vitro study and same were also used for in sacco study. Animals were maintained under well ventilated house and fed on 2.0 kg concentrate and ad libitum maize green fodder to each animal. Chemical composition of the feed used for in vitro and in sacco studies is presented in Table 1.

RESULTS AND DISCUSSION

The in vitro fermentation data of groundnut cake with tannin supplementation has been presented in Table 2, 3 & 4. Acacia nilotica pods supplementation at the rate of 2 and 4% tannin equivalent significantly (P<0.01) reduced the IVTDMD (from 91.84 to 82.95 %), IVTOMD (from 92.41 to 84.14) and IVTCPD (from 95.08 to 91.66 %) in comparison to control. The incubation period ranged from 24-48 hrs. Total gas and methane production was not affected by treatment supplementation. However, ammonia production was significantly reduced (P<0.01) at higher tannin i.e., 4% level of pure tannic acid, extracted tannin powder and babul pods powder and ranged from 18.20 to 10.30 mg/dl. The in sacco digestion kinetics and effective degradability of dry matter, organic matter and crude protein (Table.5) were not affected by tannin supplementation either by pure tannic acid or Acacia pods or extracted
### Table 1: Chemical composition (%DM) of substrates used in *in vitro* and *in sacco* studies

<table>
<thead>
<tr>
<th>Ingredient composition of concentrate mixture</th>
<th>Parameter</th>
<th>GNC</th>
<th>Concentrate</th>
<th>Acacia nilotica pods</th>
<th>Berseem</th>
<th>Wheat straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>95.59</td>
<td>95.67</td>
<td>92.59</td>
<td>83.73</td>
<td>91.27</td>
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</tr>
<tr>
<td>OM</td>
<td>92.60</td>
<td>94.60</td>
<td>94.65</td>
<td>87.44</td>
<td>93.81</td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>38.26</td>
<td>22.70</td>
<td>14.05</td>
<td>17.22</td>
<td>2.20</td>
<td></td>
</tr>
<tr>
<td>EE</td>
<td>6.72</td>
<td>3.42</td>
<td>1.47</td>
<td>7.90</td>
<td>1.17</td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>8.44</td>
<td>8.43</td>
<td>14.90</td>
<td>22.17</td>
<td>42.91</td>
<td></td>
</tr>
<tr>
<td>NFE</td>
<td>33.39</td>
<td>60.23</td>
<td>62.38</td>
<td>39.15</td>
<td>46.84</td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td>7.40</td>
<td>8.26</td>
<td>5.35</td>
<td>12.56</td>
<td>6.19</td>
<td></td>
</tr>
<tr>
<td>NDF*</td>
<td>29.74</td>
<td>22.13</td>
<td>31.28</td>
<td>52.58</td>
<td>75.43</td>
<td></td>
</tr>
<tr>
<td>ADF*</td>
<td>12.98</td>
<td>8.33</td>
<td>19.07</td>
<td>38.11</td>
<td>54.74</td>
<td></td>
</tr>
</tbody>
</table>

#### Feed Ingredients

- **Maize grain**: 33
- **Ground nut cake**: 21
- **Oiled mustard cake**: 12
- **Wheat bran**: 20
- **Rice bran**: 11
- **Mineral mixture**: 02
- **Common salt**: 01

### Table 2: Effect of treated Groundnut cake (GNC) with different types and levels of tannin on *in vitro* true nutrient digestibility, gas, methane & NH₃ production (24 h incubation)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GNC (C)</th>
<th>Pure tannic acid</th>
<th>Extracted acacia tannin powder</th>
<th>Acacia nilotica pods</th>
<th>S.E.M.</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVTDMD%</td>
<td>91.84±0.32</td>
<td>89.83±0.35</td>
<td>90.09±0.36</td>
<td>82.95±0.37</td>
<td>0.29**</td>
<td>0.01</td>
</tr>
<tr>
<td>IVTOMD%</td>
<td>92.41±0.30</td>
<td>91.01±0.32</td>
<td>90.33±0.35</td>
<td>83.99±0.59</td>
<td>0.26**</td>
<td>0.01</td>
</tr>
<tr>
<td>IVTCPD%</td>
<td>95.08±0.19</td>
<td>94.60±0.11</td>
<td>94.65±0.12</td>
<td>94.63±0.19</td>
<td>0.37**</td>
<td>0.01</td>
</tr>
<tr>
<td>Total gas (ml/g/h)</td>
<td>5.50±0.55</td>
<td>7.22±0.63</td>
<td>7.28±0.45</td>
<td>7.06±0.22</td>
<td>0.46**</td>
<td>0.01</td>
</tr>
<tr>
<td>Methane (ml/500gm)</td>
<td>7.80±0.83</td>
<td>6.75±0.64</td>
<td>9.37±0.81</td>
<td>8.71±1.18</td>
<td>0.49NS</td>
<td>0.20</td>
</tr>
<tr>
<td>Ammonia (mg/dl)</td>
<td>15.74±0.16</td>
<td>13.65±0.14</td>
<td>18.20±0.39</td>
<td>16.13±0.14</td>
<td>0.37**</td>
<td>0.01</td>
</tr>
</tbody>
</table>


Mean values with different superscripts a, b, c within a column differ significantly (NS-Non significant; **-P<0.01).

### Table 3: Effect of treated concentrate with different types and levels of tannin on *in vitro* true nutrient digestibility, gas, methane & NH₃ production (24 h incubation)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Concentrate (C)</th>
<th>Pure tannic acid</th>
<th>Extracted acacia tannin powder</th>
<th>Acacia nilotica pods</th>
<th>S.E.M.</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVTDMD%</td>
<td>80.30±1.64</td>
<td>75.30±1.39</td>
<td>81.80±0.85</td>
<td>73.90±0.49</td>
<td>0.84**</td>
<td>0.01</td>
</tr>
<tr>
<td>IVTOMD%</td>
<td>85.17±1.26</td>
<td>77.73±0.64</td>
<td>84.67±0.68</td>
<td>74.00±0.49</td>
<td>0.76**</td>
<td>0.01</td>
</tr>
<tr>
<td>IVTCPD%</td>
<td>83.67±1.35</td>
<td>82.43±1.02</td>
<td>88.57±0.68</td>
<td>79.80±0.38</td>
<td>0.75**</td>
<td>0.01</td>
</tr>
<tr>
<td>Total gas (ml/g/h)</td>
<td>8.36±0.40</td>
<td>5.64±0.23</td>
<td>8.32±0.13</td>
<td>7.32±0.16</td>
<td>0.15**</td>
<td>0.01</td>
</tr>
<tr>
<td>Methane (ml/500gm)</td>
<td>19.87±1.45</td>
<td>10.83±0.26</td>
<td>19.73±1.07</td>
<td>20.87±2.28</td>
<td>0.64**</td>
<td>0.01</td>
</tr>
<tr>
<td>Ammonia (mg/dl)</td>
<td>22.67±0.27</td>
<td>15.87±0.93</td>
<td>15.49±0.81</td>
<td>15.87±0.93</td>
<td>0.37**</td>
<td>0.01</td>
</tr>
</tbody>
</table>


Mean values with different superscripts a, b, c within a column differ significantly (NS-Non significant; **-P<0.01).

### Table 4: Effect of treated total mixed ration (TMR) with different types and levels of tannin on *in vitro* true nutrient digestibility, gas, methane & NH₃ production (48 h incubation)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TMR (C)</th>
<th>Pure tannic acid</th>
<th>Extracted acacia tannin powder</th>
<th>Acacia nilotica pods</th>
<th>S.E.M.</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVTDMD%</td>
<td>85.05±0.42</td>
<td>76.97±1.18</td>
<td>76.57±0.31</td>
<td>81.54±0.38</td>
<td>0.32**</td>
<td>0.01</td>
</tr>
<tr>
<td>IVTOMD%</td>
<td>77.73±0.62</td>
<td>73.70±0.36</td>
<td>76.58±0.31</td>
<td>70.94±0.74</td>
<td>0.38**</td>
<td>0.01</td>
</tr>
<tr>
<td>IVTCPD%</td>
<td>93.85±0.17</td>
<td>94.04±0.37</td>
<td>90.16±0.14</td>
<td>91.72±0.80</td>
<td>0.61**</td>
<td>0.01</td>
</tr>
<tr>
<td>Total gas (ml/g/h)</td>
<td>6.45±0.23</td>
<td>4.04±0.16</td>
<td>5.14±0.37</td>
<td>5.05±0.33</td>
<td>0.16**</td>
<td>0.01</td>
</tr>
<tr>
<td>Methane (ml/500gm)</td>
<td>18.77±0.36</td>
<td>13.05±0.00</td>
<td>19.72±0.11</td>
<td>12.96±0.42</td>
<td>0.76**</td>
<td>0.01</td>
</tr>
<tr>
<td>Ammonia (mg/dl)</td>
<td>34.53±3.73</td>
<td>28.93±2.47</td>
<td>35.47±0.93</td>
<td>29.87±2.47</td>
<td>1.57NS</td>
<td>0.23</td>
</tr>
</tbody>
</table>


Mean values with different superscripts a, b, c within a column differ significantly (NS-Non significant; **-P<0.01).
tannin powder from Acacia pods or as such Acacia pods at the level of 2 and 4 % tannin level in groundnut cake and it may be due to methods of tannin extraction used. However, the values of effective degradability of DM (60.08 to 39.69 %), OM (60.50 to 37.62 %) and CP (57.95 to 48.80 %) were reduced in tannic acid and tannin extracted powder from Acacia pods. It was non significantly affected by treatments. Bhargava et al. (1973) also reported that tannic acid (HT) @ 6% (w/w) with GNC reduced protein solubility by 76%. Sengar and Mudgal, (1982) found that treatment of tannic acid (HT) @ 10% (w/w) with GNC reduced in vitro NH₃ production by 91%. Dey et al. (2006) concluded that tropical tree leaves (Artocarpus heterophyllus, Ficus glomerata, Ficus bengalensis, and Ficus infectoria) supplementation (at 1-2% condensed tannins equivalent levels) significantly reduced the in vitro nitrogen degradability of groundnut cake. Dentinho et al. (2007) observed that although the phenolic crude extract from Cistus ladanifer L.(0, 12.5, 25, 50, 100 and 150 g/ kg levels) effectively reduced the in sacco soybean protein degradation in the rumen, and increased flux of feed protein to post rumen, it also had a negative effect on in vitro intestinal digestibility. The effective DM and CP degradability reduced from 69 to 52 and 69 to 40 %, respectively as total phenolic concentrations increased from 0 to 150g/kg DM of soybean meal. Martinez et al., (2005) reported that use of graded levels of tannic acid (0, 10, 25 and 50 g/kg DM) decreased the effective protein degradability of barley grain. The IVTDMD and IVTOMD of concentrate (Table 5) was reduced (P<0.01) by supplementation of 2 and 4% tannin equivalent of babul pods, pure tannic acid and 4% extracted powder. However, IVTCPD was significantly (P<0.01) reduced from 83.67 to 71.73 % only at 4% tannin equivalent babul pod. Total gas and methane production was reduced (P<0.01) only at 2 and 4 % pure tannic acid supplementation. However, ammonia level did not influence in TMR by tannin supplementation. In TMR (Table 7) the in sacco effective degradability of dry matter (35.29 to 28.45 %), organic matter (41.14 to 30.29 %) and crude protein (48.84 to 31.37 %) were reduced.

### Table 5. Effect of treated groundnut cake with different types and levels of tannin on in sacco degradability

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GNC(C)</th>
<th>Pure tannic acid</th>
<th>Extracted acacia tannin powder</th>
<th>Acacia nilotica pods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry Matter (% of DM)</td>
<td>2% tannin</td>
<td>4% tannin</td>
<td>2% tannin</td>
</tr>
<tr>
<td>DM</td>
<td>a</td>
<td>17.0±1.6</td>
<td>13.2±4.7</td>
<td>15.5±8.9</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>67.2±3.7</td>
<td>63.0±0.4</td>
<td>57.0±3.9</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>0.07±0.2</td>
<td>0.06±0.2</td>
<td>0.06±0.3</td>
</tr>
<tr>
<td>EDMD</td>
<td>52.3±4.4</td>
<td>43.8±11.9</td>
<td>39.6±12.6</td>
<td>47.4±11.9</td>
</tr>
<tr>
<td>R²</td>
<td>0.86</td>
<td>0.94</td>
<td>0.94</td>
<td>0.95</td>
</tr>
<tr>
<td>OM</td>
<td>a</td>
<td>12.4±1.6</td>
<td>6.9±4.6</td>
<td>11.1±1.8</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>71.5±4.6</td>
<td>70.2±0.9</td>
<td>62.2±2.0</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>0.07±0.2</td>
<td>0.06±0.2</td>
<td>0.06±0.3</td>
</tr>
<tr>
<td>EOMD</td>
<td>51.1±4.5</td>
<td>43.5±11.9</td>
<td>37.6±13.4</td>
<td>46.3±12.2</td>
</tr>
<tr>
<td>R²</td>
<td>0.87</td>
<td>0.95</td>
<td>0.94</td>
<td>0.95</td>
</tr>
<tr>
<td>CP</td>
<td>a</td>
<td>15.1±1.2</td>
<td>11.8±4.8</td>
<td>8.4±1.8</td>
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<td></td>
<td>b</td>
<td>67.8±0.1</td>
<td>80.7±8.6</td>
<td>75.2±2.1</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>0.09±0.2</td>
<td>0.08±0.2</td>
<td>0.07±0.2</td>
</tr>
<tr>
<td>ECPD</td>
<td>57.9±3.9</td>
<td>57.7±5.2</td>
<td>48.8±8.6</td>
<td>54.5±10.1</td>
</tr>
<tr>
<td>R²</td>
<td>0.89</td>
<td>0.96</td>
<td>0.97</td>
<td>0.97</td>
</tr>
</tbody>
</table>

a-rapidly degradable, b-slowly degradable, c-rate of disappearance, R2- better adjustment to the non-linear model, S.E.M.: standard error of the mean, ED-Effective degradability, k=0.05, Mean values with different superscripts a,b,c,d within a column differ significantly (NS=Non significant; *=P<0.05).
Table 6. Effect of treated concentrate with different type and level of tannin on in sacco digestion kinetics and nutrient digestibility

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GNC(C)</th>
<th>Pure tannic acid</th>
<th>Exected acacia tannin powder</th>
<th>Acacia nilotica pods</th>
<th>SEm</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Matter (% of DM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>10.28±1.29</td>
<td>8.69±1.12</td>
<td>12.17±1.06</td>
<td>8.22±0.83</td>
<td>8.41±2.59</td>
<td>10.84±1.49</td>
</tr>
<tr>
<td>b</td>
<td>68.43±1.37</td>
<td>63.48±1.75</td>
<td>72.98±2.77</td>
<td>59.41±1.57</td>
<td>71.82±8.53</td>
<td>63.38±9.07</td>
</tr>
<tr>
<td>c</td>
<td>0.06±0.02</td>
<td>0.07±0.01</td>
<td>0.04±0.01</td>
<td>0.05±0.01</td>
<td>0.05±0.02</td>
<td>0.07±0.01</td>
</tr>
<tr>
<td>EDMD</td>
<td>45.38±4.23</td>
<td>44.75±1.54</td>
<td>43.56±1.40</td>
<td>37.89±1.04</td>
<td>44.08±1.62</td>
<td>44.97±0.19</td>
</tr>
<tr>
<td>R²</td>
<td>0.92</td>
<td>0.97</td>
<td>0.93</td>
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<td>0.97</td>
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<tr>
<td>Organic Matter (% of OM)</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>a</td>
<td>8.95±2.25</td>
<td>5.52±0.55</td>
<td>9.15±2.23</td>
<td>7.24±2.70</td>
<td>9.54±1.43</td>
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</tr>
<tr>
<td>b</td>
<td>70.73±1.78</td>
<td>59.46±4.04</td>
<td>68.49±3.87</td>
<td>65.57±8.96</td>
<td>66.31±5.41</td>
<td>65.52±1.92</td>
</tr>
<tr>
<td>c</td>
<td>0.06±0.02</td>
<td>0.07±0.01</td>
<td>0.06±0.02</td>
<td>0.07±0.01</td>
<td>0.07±0.01</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td>EOMD</td>
<td>45.13±4.23</td>
<td>38.47±1.12</td>
<td>44.34±1.62</td>
<td>44.86±1.16</td>
<td>46.44±3.07</td>
<td>44.20±1.54</td>
</tr>
<tr>
<td>R²</td>
<td>0.92</td>
<td>0.95</td>
<td>0.97</td>
<td></td>
<td>0.97</td>
<td>0.97</td>
</tr>
</tbody>
</table>

a-rapidly degradable, b-slowly degradable, c-rate of disappearance, R²- better adjustment to the non-linear model, S.E.M.: standard error of the mean, ED- Effective degradability, k=0.05, Mean values with different superscripts a,b,c,d within a column differ significantly (NS=Non significant; *=P<0.05 and **= P<0.01).

(P<0.01) by supplementation of pure tannic acid and extracted tannin powder at the level of 2 and 4 % tannin level (% DM). The Acacia pods as such supplementation at 2 and 4% tannin level did not influence in sacco degradability of total mixed ration. The effective crude protein degradability of TMR was reduced from 43.42 to 31.37 % in treatment group. Barman and Rai, (2006a) found that increasing levels of babul pods from 0 to 65 per cent in total mixed ration (TMR) significantly reduced IVDMD, IVOMD and IVCPD from 66.64 to 52.75; 67.19 to 54.71 and from 77.15 to 46.65 per cent, respectively. The in vitro gas production was also significantly reduced from 174.07 to 148.0 ml/ g substrate in TMRs ranging from 0 to 12 per cent tannin. Tannin concentration was negatively correlated with IVDMD, IVOMD, IVCPD and IVGP. Dubey (2007) replaced maize grain up to 66 percent with babul pods of 4 per cent tannin level in TMR along with Calcium hydroxide and PEG-4000 treatment and did not find reduction for in vitro digestibility of TMRs.
This shows that maize grain can be replaced with babul pods up to 66 per cent, without affecting digestibility if, tannin is chemically treated. Tandon (2009) found in vitro dry matter, organic matter and crude protein digestibility equal to control at Acacia pods tannin concentration of 3.1 to 3.5 per cent in TMR. The gas production was statistically non significant and was in accordance to IVOMD. Rioux et al., (1995) reported that higher tannin (3.8%) cultivar of birdsfoot trefoil silage (Lotus corniculatus L.) did not influenced the effective degradability of DM (82%) and CP (94%) at k=0.5%/h. The efficiency of absorption from small intestine decreased from 58 to 50% when PEG was added to the diet, suggesting that tannins in some way enhanced absorption and utilization of nitrogen in small intestine. Herves et al. (1999) reported that quabrocho tannins at a rate of 15g/100g of Soy bean meal (SBM) can be used as chemical additive to decrease rumen degradation. This enables proteins to by-pass degradation in the rumen and undergoes enzymatic hydrolysis in the abomasums and absorbed from small intestine and undergoes enzymatic hydrolysis in the abomasums and absorbed from small intestine (Min et al. 2001, 2002, 2003). Furthermore tannins form complexes with surfaces of bacterial cell wall and with bacterial enzymes which alter the bacterial growth and reduce proteolytic enzymes activities (Jones et al. 1994).

REFERENCES


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