EFFECT OF ADDING DIFFERENT LEVELS OF TANNIN POWDER ON TOTAL GAS AND METHANE PRODUCTION AND IN VITRO DIGESTIBILITY

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Abstract
This experiment was conducted to study the effect of adding four different levels of tannin powder (0.2, 0.4, 0.6 and 0.8%) to mixed ration on in vitro total gas and methane in vitro production, some rumen liquor characteristics and in vitro digestibility of dry matter and organic matter after 24, 48, 72 and 96 hr. of incubation periods. The results showed a significant decrease (P <0.01) in total gas and methane production by adding tannin powder at different levels and different incubation periods compared to control treatment. The results showed that nitrogen ammonia concentration decreased significantly (p<0.01) in all treatments after different incubation periods with increased tannin levels. The results showed that the second treatment (0.2% of tannin powder) resulted in a significant increased (p <0.01) in vitro dry matter digestibility, organic matter digestibility and metabolizable energy. This was reflected in increased significantly (p<0.01) volatile fatty acids concentration. While the high levels of tannin powder (0.6 and 0.8%) reduced significantly (p<0.01) in vitro digestibility of dry matter and organic matter, metabolizable energy and volatile fatty acids concentration, increasing tannin levels significantly (p<0.01) decrease total count of protozoa in the rumen liquor, we conclude that the presence of tannin in ruminant diets at low and medium levels reduce methane production and improve the in vitro digestibility.

Key words: Tannin, In vitro gas production, In vitro digestibility, methane, protozoa.

Introduction
Greenhouse gases are a concern because of their negative effects on global warming and climate change (IPCC, 2007). Methane is a component of greenhouse gases, contributing 9 to 4 percent of total gases (Patra et al., 2012). Methane is typically produced from the anaerobic fermentation process of the ruminant digestive system, which produces about 39% of the world’s methane (Gerber et al., 2013). In addition, 2-15% of the total energy consumed from feed to methane is converted during fermentation in the rumen, thus reducing the efficiency of feed energy use (Kennedy and Charmley, 2012). In recent years researchers have sought solutions to reduce the production of hydrogen gas and methane gas without harming the animal by controlling the ecosystem of the rumen through feed additives such as the addition of vegetable oil (Hassan and Irhim, 2016) or essential oils such as castor oil and flax seed (Kutar et al., 2017) or are non-food additives such as the addition of nitrates and urea (Hassan and Ali, 2017) or flavonoids of cranberry leaves (Al-Bayati and Hassan, 2018). Some studies have shown that tannin can be added to the diet to improved rumen fermentation with low concentrations (Jayanegara et al., 2012). Secondary metabolism product of plant such as tannins also have significant potential to discourage methane production with minimal effect passive of food fermentation in the rumen (Bhatta et al., 2013). Tannins are a complex group of phenolic compounds their effect to ruminant may be beneficial or harmful depending on the type of tannin consumed, chemical composition, molecular weight and quantity consumed (Puchala et al., 2012). Condensed tannin extracted from chestnut wood (Castanea sativa) led to a decrease methane production by 5.5% (Bhatt et al., 2009). The production of methane decreases linearly whenever increased amount of tannin in the plants through the effect on rumen microorganisms, especially organisms producing methane. This suggests that tannin is partly responsible for reducing methane production (Huang et al., 2010). The aim of this experiment to investigate the effect of adding different levels of tannin to mixed ration in the in vitro methane production, which is one method for estimating the nutritional value of feed, in addition to
estimating in vitro digestibility of dry matter and organic matter and some characteristics of rumen liquor.

**Materials and Methods**

This study was conducted in the college of Agricultural Engineering Sciences, University of Baghdad, in the animal feeding laboratory, department of animal production, period from 14/10/2018 to 28/11/2018. The experiment use mixed ration (40% roughage + 60% concentrate). Table 1, show the components and chemical composition of concentrate.

The tannin powder was obtained from local markets (100% tannin) extracted from oak and shark, added to the mixed ration with four levels (0.2, 0.4, 0.6, 0.8%) to study the effect of tannin on total gas and methane production, some rumen liquid characteristics and in vitro dry matter and organic matter digestibility, table 2 shows the chemical composition of mixed ration with different levels of tannin.

**In vitro total gas and methane production**

In vitro total gas production was estimated by taking 6 replicates per sample according to the Menke and Steingass (1988). 200 mg of experimental mixed ration, adding 20 ml of artificial saliva and 10 ml of rumen liquor extracted from freshly slaughtered lamb. The samples were placed in 100 mL glass syringe, carbon dioxide was added only once time to each syringe, immediately before incubation, the piston was closed to completely remove the air, then incubated in a water bath at 39°C for 24, 48, 72 and 96 hr., with planck for each period of incubation, with moving the syringe twice daily. The syringes was withdrawn to estimate the total gas production and 4 ml of 4% NaOH added to 3 samples only to estimate methane production according to Fievez et al., (2005).

Metabolizable energy (ME), in vitro organic matter digestibility (IVOMD), short chain fatty acids (SCFA) and net energy for lactation (NEL) estimate by using total volume of gas production after 24 hr., of incubation period using the following equations:

\[
\text{ME (MJ/kg DM)} = 0.012 \times \text{CP} + 0.031 \times \text{EE} + 0.005 \times \text{CF} + 0.014 \times \text{NFE (MAFF, 1975)}.
\]

\[
\text{IVOMD (\%)} = 14.88 + 0.889 \times \text{GV} + 0.45 \times \text{CP} + 0.651 \times A \times (\text{ASH})
\]

\[
\text{SCFA (m mol /100 ml)} = 0.0239 \times \text{GV} - 0.061 \text{ according to Menke and Steingass, (1988)}
\]

\[
\text{NEL (MJ/Kg DM)} = 0.096 \times \text{GV} + 0.0038 \times \text{CP} + 0.000173 \times \text{EE}^2 + 0.54 \text{ according to Getachew et al., (1999)}.
\]

**Rumen liquor characteristics**

After each incubation periods, estimates the pH value, NH₃-N, TVFA (Filipek and Dvorak, 2009) and total count of protozoa after 24 hr. of incubation period (Warner, 1962).

**Chemical analysis**

Experimental diets were determent dry matter, organic matter, crude protein, crude fiber, ether extract and nitrogen free extract according to A.O.A.C., (2005). In vitro dry matter and organic matter digestibility estimate according to Tilley and Terry, (1963).

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**Table 1:** Chemical composition and percentages of ingredients of concentrate (% dry matter).

<table>
<thead>
<tr>
<th>Metabolizable Energy (MJ/Kg DM)</th>
<th>Nitrogen free extract %</th>
<th>Ash %</th>
<th>Ether Extract %</th>
<th>Crude fiber %</th>
<th>Crude protein %</th>
<th>Organic matter %</th>
<th>Dry matter %</th>
<th>%</th>
<th>Items</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.72</td>
<td>64.89</td>
<td>5.56</td>
<td>4.53</td>
<td>11.00</td>
<td>14.02</td>
<td>84.55</td>
<td>90.12</td>
<td>39</td>
<td>Wheat bran</td>
</tr>
<tr>
<td>13.38</td>
<td>77.14</td>
<td>6.43</td>
<td>4.37</td>
<td>3.21</td>
<td>8.85</td>
<td>82.67</td>
<td>89.10</td>
<td>11</td>
<td>yellow corn</td>
</tr>
<tr>
<td>11.55</td>
<td>61.28</td>
<td>7.35</td>
<td>1.93</td>
<td>16.57</td>
<td>12.88</td>
<td>80.62</td>
<td>87.97</td>
<td>23</td>
<td>Alfalfa hay</td>
</tr>
<tr>
<td>10.11</td>
<td>53.88</td>
<td>6.27</td>
<td>1.32</td>
<td>35.17</td>
<td>3.37</td>
<td>85.10</td>
<td>91.37</td>
<td>17</td>
<td>Barley straw</td>
</tr>
<tr>
<td>12.54</td>
<td>42.17</td>
<td>5.01</td>
<td>3.57</td>
<td>5.49</td>
<td>43.77</td>
<td>85.47</td>
<td>90.48</td>
<td>8</td>
<td>Soybean meal</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>Salt</td>
</tr>
</tbody>
</table>

**Table 2:** Chemical composition and percentages of ingredients of concentrate (% dry matter).

<table>
<thead>
<tr>
<th>Metabolizable Energy (MJ/Kg DM)</th>
<th>Nitrogen free extract %</th>
<th>Ash %</th>
<th>Ether Extract %</th>
<th>Crude fiber %</th>
<th>Crude protein %</th>
<th>Organic matter %</th>
<th>Dry matter %</th>
<th>%</th>
<th>Items</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.13</td>
<td>41.17</td>
<td>10.51</td>
<td>2.31</td>
<td>26.78</td>
<td>19.25</td>
<td>82.45</td>
<td>92.95</td>
<td></td>
<td>T1 Tannin 0%</td>
</tr>
<tr>
<td>10.38</td>
<td>44.62</td>
<td>10.35</td>
<td>2.01</td>
<td>24.13</td>
<td>19.25</td>
<td>82.35</td>
<td>92.35</td>
<td></td>
<td>T2 Tannin 0.2%</td>
</tr>
<tr>
<td>10.23</td>
<td>42.29</td>
<td>9.53</td>
<td>2.42</td>
<td>26.57</td>
<td>19.21</td>
<td>84.20</td>
<td>93.73</td>
<td></td>
<td>T3 Tannin 0.4%</td>
</tr>
<tr>
<td>10.16</td>
<td>42.54</td>
<td>10.33</td>
<td>1.93</td>
<td>25.93</td>
<td>19.30</td>
<td>83.37</td>
<td>93.37</td>
<td></td>
<td>T4 Tannin 0.6%</td>
</tr>
<tr>
<td>10.14</td>
<td>43.18</td>
<td>10.54</td>
<td>1.72</td>
<td>25.55</td>
<td>19.07</td>
<td>83.67</td>
<td>94.17</td>
<td></td>
<td>T5 Tannin 0.8%</td>
</tr>
</tbody>
</table>

ME (MJ / kg Dm) = 0.012 × CP + 0.031 × EE + 0.005 × CF + 0.014 × NFE (MAFF, 1975).
Table 5: Effect of adding different levels of tannin powder on metabolizable energy (MJ/kg DM), in vitro organic matter digestibility (%), short chain fatty acids (mmol/100 ml) and net energy for lactation (MJ/kg DM) (mean + standard error).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Metabolizable energy (MJ/Kg DM)</th>
<th>In vitro organic matter digestibility %</th>
<th>Short chain fatty acids (mmol/100 ml)</th>
<th>Net energy for lactation (MJ/kg DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Control</td>
<td>13.38 ± 0.01a</td>
<td>62.68 ± 0.29a</td>
<td>0.83 ± 0.01a</td>
<td>4.08 ± 0.01a</td>
</tr>
<tr>
<td>T2 0.2 %</td>
<td>12.44 ± 0.02b</td>
<td>59.37 ± 0.46b</td>
<td>0.70 ± 0.01b</td>
<td>3.57 ± 0.01b</td>
</tr>
<tr>
<td>T3 0.4 %</td>
<td>12.21 ± 0.01c</td>
<td>56.07 ± 0.27c</td>
<td>0.66 ± 0.03c</td>
<td>3.16 ± 0.01c</td>
</tr>
<tr>
<td>T4 0.6 %</td>
<td>10.96 ± 0.04d</td>
<td>48.42 ± 0.35d</td>
<td>0.42 ± 0.02d</td>
<td>2.55 ± 0.02d</td>
</tr>
<tr>
<td>T5 0.8 %</td>
<td>10.28 ± 0.02e</td>
<td>44.81 ± 0.26e</td>
<td>0.33 ± 0.01e</td>
<td>2.17 ± 0.02e</td>
</tr>
</tbody>
</table>

T1: control, T2: added 0.2% Tannin powder, T3: added 0.4% tannin powder, T4: added 0.6% tannin powder, T5: added 0.8% tannin powder; ** means that there are significant differences at the probability level (P < 0.01).

Statistical Analysis

Data were analyzed statistically using complete randomized design, treatment means were separated using Duncan, (1955), using the SAS, (2012) statistical package, the model as following:

\[ Y_{ij} = \mu + t_i + \delta_j \]

As:

\[ Y_{ij} = \text{the value of viewing studied} \]
\[ \mu = \text{general average of the studied recipe} \]
\[ t_i = \text{treatment effect i} \]
\[ \delta_j = \text{random error which is distributed normal} \]
\[ \text{distribution is equal to an average of zero and variance of } \sigma^2. \]

Results and Discussion

In vitro total gas and methane production (ml/200 mg Dm)

Table 4, shows decreased significantly (p<0.01) in total gas and methane production with increased tannin levels in the rations during different incubation period compared to control treatment. This was due to the direct effect of tannin on the effectiveness of methane producing microorganisms (Zmora et al., 2012). The results of the current study agreed with reported by Soltan et al., (2013), which indicated that the tannin prevents the production of methane both in vitro or in vivo. It is also consistent with the linear reduction of methane production indicated by Hong et al., (2010) due to the addition of 50 mg CT/Kg Dm. It is also consistent with the linear decrease in total gas and methane production due to the addition of tannin Achieved in study of Rira et al., (2015). Contrary to the above, Bhatia et al., (2012) noted that tannin is not effective in reducing methane production due to the addition of low tannin levels, which did not reduce the methane production.

Metabolizable energy, in vitro organic matter digestibility, short chain fatty acids and net energy for lactation

The results of the statistical analysis in table 5, show that there were a significantly decreased (p<0.01) in values of the metabolizable energy (from 13.38 to 10.28 MJ/Kg DM), in vitro organic matter digestibility (from 62.68 to 44.81%), short chain fatty acids (from 0.83 to 0.33 mmol/100 ml) and net energy for lactation (from 4.08 to 2.17 MJ/Kg DM) with increase tannin levels. This decline can be explained as a result of estimating the values from total gas production after 24 hr., of incubation periods which decrease as the tannin levels increases and similar results have been achieved from other studies using different types and levels of tannin sources such as the results of Kaplan, (2011) who found a negative correlation between in vitro organic matter digestibility and tannin presence in rumen liquor, on the contrary, Theodoridou et al., (2011) explained that intensive tannin did not affect in vitro digestibility. The increase tannin also resulted in decrease (p<0.01) short chain fatty acids concentration. This is consistent with Hassanat and Benchaar, (2013), their results confirmed a linear decrease in vitro short chain fatty acids when the tannin levels increased from 20 to 200 mg / Kg DM.

Table 6: Effect of addition tannin powder on in vitro dry matter and organic matter digestibility (%) and metabolizable energy (MJ/kg DM) (mean + standard error).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Metabolizable energy (MJ/Kg DM)</th>
<th>In vitro organic matter digestibility %</th>
<th>In vitro dry matter digestibility %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Control</td>
<td>10.76 ± 0.01b</td>
<td>70.61 ± 0.54c</td>
<td>68.19 ± 0.14c</td>
</tr>
<tr>
<td>T2 0.2 %</td>
<td>11.82 ± 0.13a</td>
<td>78.95 ± 0.05a</td>
<td>77.54 ± 0.46a</td>
</tr>
<tr>
<td>T3 0.4 %</td>
<td>10.96 ± 0.05b</td>
<td>72.06 ± 0.32b</td>
<td>70.03 ± 0.04b</td>
</tr>
<tr>
<td>T4 0.6 %</td>
<td>8.52 ± 0.02c</td>
<td>56.10 ± 0.21d</td>
<td>55.04 ± 0.16d</td>
</tr>
<tr>
<td>T5 0.8 %</td>
<td>7.88 ± 0.09d</td>
<td>51.89 ± 0.09e</td>
<td>50.09 ± 0.24e</td>
</tr>
</tbody>
</table>

T1: control, T2: added 0.2% Tannin powder, T3: added 0.4% tannin powder, T4: added 0.6% tannin powder, T5: added 0.8% tannin powder; ** means that there are significant differences at the probability level (P < 0.01).
Table 7: Effect of adding different levels of tannin powder on concentration of rumen ammonia nitrogen (mg /100 ml) after different incubation periods (mean + standard error).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>96 hr.</th>
<th>72 hr.</th>
<th>48 hr.</th>
<th>24 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Control</td>
<td>26.53±0.34 a</td>
<td>30.13±0.30 a</td>
<td>32.53±0.24 a</td>
<td>34.79±0.23a</td>
</tr>
<tr>
<td>T2 0.2 %</td>
<td>19.20±0.18 b</td>
<td>20.95±0.22 b</td>
<td>24.63±0.30 b</td>
<td>28.19±0.18 b</td>
</tr>
<tr>
<td>T3 0.4 %</td>
<td>16.85±0.30 c</td>
<td>17.84±0.15 c</td>
<td>20.92±0.21 c</td>
<td>24.17±0.28 c</td>
</tr>
<tr>
<td>T4 0.6 %</td>
<td>11.18±0.21 d</td>
<td>13.09±0.25 d</td>
<td>16.04±0.16 d</td>
<td>18.85±0.17 d</td>
</tr>
<tr>
<td>T5 0.8 %</td>
<td>10.38±0.16 e</td>
<td>10.74±0.22 e</td>
<td>11.95±0.19 e</td>
<td>14.87±0.12 e</td>
</tr>
<tr>
<td>Moral level</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

T1: control, T2: added 0.2% Tannin powder, T3: added 0.4% tannin powder, T4: added 0.6% tannin powder, T5: added 0.8% tannin powder; ** means that there are significant differences at the probability level (P < 0.01).

In vitro dry matter and organic matter digestibility (%) and metabolizable energy (MJ/ kg DM)

The results of table 6, indicate a significant increased (P<0.01) in vitro dry matter and organic matter digestibility and metabolizable energy in the second treatment (0.2% tannin powder) compared to the control treatment (without tannin) (77.54, 78.95% and 11.82 MJ/Kg DM respectively) and the lowest value was recorded for in vitro dry matter and organic matter digestibility and metabolizable energy in the fifth treatment (0.8% tannin powder) (50.09, 51.89% and 7.88 MJ/Kg DM respectively). This improvement (0.2% tannin powder) can be explained as the best among the treatments may be as a result of low protein degradation by microorganisms in the first phase of in vitro digestibility leading to exposure to enzymatic digestion in the second phase of in vitro digestion. Thus increasing the utilization of amino acids and this corresponds to the amount of increase achieved in the study of Widiawati et al., (2013) when the addition of different levels of tannin which led to high in vitro organic matter digestibility, Plaizier et al., (2000) also noted that tannin increases in vitro digestibility when added by 3.0-5.0%. However, the rate of improvement in vitro digestibility and energy was reduced by increasing levels of tannin powder because high levels of tannin are complex with both protein and carbohydrates.

Table 8: Effect of adding different levels of tannin powder on concentration of volatile fatty acids (mmol/100 ml) after different incubation periods (mean + standard error).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>96 hr.</th>
<th>72 hr.</th>
<th>48 hr.</th>
<th>24 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Control</td>
<td>59.83±0.11 b</td>
<td>63.02±0.29 c</td>
<td>70.94±0.10 b</td>
<td>80.87±0.08a</td>
</tr>
<tr>
<td>T2 0.2 %</td>
<td>60.54±0.18 a</td>
<td>64.84±0.26 b</td>
<td>72.77±0.47 a</td>
<td>81.26±0.58 a</td>
</tr>
<tr>
<td>T3 0.4 %</td>
<td>61.54±0.64 a</td>
<td>65.86±0.18 a</td>
<td>72.16±0.19 a</td>
<td>81.08±0.16 a</td>
</tr>
<tr>
<td>T4 0.6 %</td>
<td>59.66±0.69 b</td>
<td>62.29±0.21 c</td>
<td>70.87±0.15 b</td>
<td>75.84±0.12 b</td>
</tr>
<tr>
<td>T5 0.8 %</td>
<td>55.29±0.16 c</td>
<td>60.77±0.22 d</td>
<td>64.19±0.20 c</td>
<td>71.01±0.49 c</td>
</tr>
<tr>
<td>Moral level</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

T1: control, T2: added 0.2% Tannin powder, T3: added 0.4% tannin powder, T4: added 0.6% tannin powder, T5: added 0.8% tannin powder; ** means that there are significant differences at the probability level (P < 0.01).

Volatile fatty acids Concentration (mmol /100 ml)

Table 8, shows the effect of adding different levels of tannin powder on short chain fatty acids concentration after different incubation period. There were no significant differences in volatile fatty acids concentration after 24 hr. of in vitro incubation in the first, second and third treatment. This is consistent with Silanikove et al., (2006) and Getachew et al., (2008). Where found that low levels of intensive tannin did not affect the concentration of short chain fatty acids on the contrary, T4 and T5 decreased significantly (p<0.01) compared with others treatment, this decline agree with Martin et al., (2010) who found a decrease in short chain fatty acids concentration with increased tannin level because of the complexity that prevent the breakdown of carbohydrates in the rumen by microorganisms lead to the availability of short chain fatty acids to longer hours to increase their use in manufacturing microbial protein (Beauchemin et al., 2014; Silanikove et al., 2006).

pH

The results of the statistical analysis of pH data in the present study showed that the effect of adding different levels of tannin powder on the pH value of the rumen fluid had the highest value (p<0.01) after 24 hr., of in vitro incubation were 7.0 in the third treatment (0.4% tannin powder) and the
lowest value in the control treatment without the addition of tannin powder amounted to 6.5, also table 9, shows that the pH value after 48, 72 and 96 hr. of in vitro incubation decreased in all treatments compared to the control treatment free of additive (6.9, 7.3 and 7.5, respectively) and the lowest pH value was recorded in the fifth treatment (0.8% tannin powder) 6.7, 6.6 and 6.9, respectively. As shown in the data in table 9, the addition of different levels of tannin powder after different in vitro incubation hr., it did not have a significant impact on the fluctuation in the pH value which ranged between 7.5-6.5 which are suitable for the activity of microorganisms that analyze the fibers and protein in the rumen (Hungate, 1966); Carrasco et al., (2017) recorded pH values approximate the values shown by the current study results where it recorded 6.5-7.0 when adding 0.4% Tannin, while Zawadzki et al., (2010) note the low value pH when using 3 and 4 g tannin condensed / kg dry matter.

**Protozoa**

Table 9, shows that addition of tannin powder to the mixed ration resulted in a significantly decreased (P<0.01) in protozoa number with increased tannin level after 24 hr. of in vitro incubation (from 3.83 to 2.06 cells×10^5/ ml). This is consistent with the results of both Animut et al., (2013); Sallam et al., (2010); Longo et al., (2013). Where the researchers pointed to a linear decline in the protozoa population when adding tannin in different levels, May be one of the reasons for the decline protozoa population after 24 hr. of incubation period the effect of tannin on the unavailability of iron and calcium for protozoa utilizing, which effect on inability to multiply and growth (Ri carde-da Silva et al., 1991).

**Conclusion**

We concluded that low levels of tannin (0.2%) in the diet improved the in vitro dry and organic matter digestibility due to conservation of feed energy with decreased methane production as a result of organic fermentation processes by inhibiting the growth of methanogenesis organisms.

**References**


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