Cereal crop residues form the staple feed for ruminant livestock in India. Among the various cereal crop residues, paddy straw (Oryza sativa) is the main roughage source for majority of cattle and buffaloes reared in Indian small holdings. Low degradability of paddy straw is due to its high lignin content and shortage of essential nutrients for rumen microbes. This has led to search for appropriate treatments and supplements to improve its nutritive value. There has been substantial attention to treatment, but less to supplementation strategies. The first limitation of straw is the imbalance of nutrients both for rumen microbes and for the host animal. A wide range of agro-industrial byproducts are available in large quantities which have considerable nutritional potential. Brewery waste (Brewer's grains) is a by product of ethanol industry which uses cereal grains as feed stock. When grain is fermented to produce ethanol, primarily the starch is utilized, leaving behind a protein rich residue that can be used in livestock diets. India ranks fifth in the world in ethanol production and produces ethanol from about 278 distilleries, majority of which use agricultural crops as feed stock. One ton of grain on fermentation yields 430 kg of distiller's grain. As the ethanol industry grows, greater quantities of distiller's grain will become available for use as animal feed at potentially reasonable cost. Distiller's grain has a moderate content of protein and high level of crude fibre which make it an attractive ingredient to be used in ruminant feed. In spite of its abundant availability, the important factors which limits to utility as animal feed is their short shelf life (3 to 7 days) and transportation difficulty. Knowledge on degradability pattern of brewery waste incorporated with paddy straw would throw more light on its usefulness and would help to formulate complete feed for dairy cattle. Hence, an attempt was made in the present study to evaluate the in vitro rumen CP degradability pattern of brewery waste incorporated paddy straw using Rumen Simulation Technique (RUSITEC).

**MATERIALS AND METHODS**

The in vitro crude protein degradability of experimental feeds such as control concentrate mixture, paddy straw, brewery waste, experimental concentrate mixture incorporated with fresh brewery waste, experimental concentrate mixture incorporated with dried brewery waste and paddy straw treated with brewery waste for 3 days was determined using the rumen simulation technique (RUSITEC). The following seven treatments were incubated in the eight
Table 1. Per cent ingredient composition of the control and experimental concentrate mixtures used.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentrate mixture (%)</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow Maize</td>
<td>37.00</td>
<td>40.00</td>
<td></td>
</tr>
<tr>
<td>Groundnut Cake</td>
<td>29.00</td>
<td>21.50</td>
<td></td>
</tr>
<tr>
<td>Wheat bran</td>
<td>30.50</td>
<td>10.00</td>
<td></td>
</tr>
<tr>
<td>Brewery waste</td>
<td>0.00</td>
<td>25.00</td>
<td></td>
</tr>
<tr>
<td>Mineral Mixture*</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Salt</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Shelli Gril</td>
<td>1.50</td>
<td>1.50</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Per cent chemical composition of the concentrate mixtures, brewery waste, paddy straw and brewery waste incorporated paddy straw* (on DM basis)

<table>
<thead>
<tr>
<th>Item</th>
<th>Concentrate mixture</th>
<th>Brewery waste</th>
<th>Paddy straw</th>
<th>Brewery waste incorporated paddy straw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Experimental</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry matter</td>
<td>95.15 ± 0.56</td>
<td>94.76 ± 0.26</td>
<td>29.15 ± 0.43</td>
<td>90.35 ± 0.81</td>
</tr>
<tr>
<td>Crude protein</td>
<td>20.06 ± 1.05</td>
<td>20.12 ± 0.63</td>
<td>24.34 ± 0.60</td>
<td>4.42 ± 0.18</td>
</tr>
<tr>
<td>Ether extract</td>
<td>4.84 ± 0.45</td>
<td>4.82 ± 0.28</td>
<td>5.19 ± 0.18</td>
<td>0.86 ± 0.05</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>6.34 ± 0.18</td>
<td>8.88 ± 0.35</td>
<td>19.62 ± 0.31</td>
<td>34.19 ± 0.61</td>
</tr>
<tr>
<td>Total ash</td>
<td>8.32 ± 0.18</td>
<td>8.54 ± 0.16</td>
<td>5.76 ± 0.14</td>
<td>10.64 ± 0.25</td>
</tr>
<tr>
<td>Nitrogen free extract</td>
<td>60.44 ± 0.87</td>
<td>57.64 ± 1.03</td>
<td>45.00 ± 0.79</td>
<td>49.89 ± 0.64</td>
</tr>
<tr>
<td>Organic matter</td>
<td>91.68 ± 0.18</td>
<td>91.47 ± 0.16</td>
<td>94.25 ± 0.14</td>
<td>89.36 ± 0.25</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>2.56 ± 0.21</td>
<td>2.68 ± 0.14</td>
<td>4.42 ± 0.15</td>
<td>8.76 ± 0.20</td>
</tr>
</tbody>
</table>

* Mean of four values ± SE

Figure 1: In vitro effective crude protein degradability of experimental feeds in RUSITEC, %
Table-3. In vitro effective crude protein degradability of experimental feeds in RUSITEC, %

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Soluble 'a', %</th>
<th>Degradable 'b', %</th>
<th>Undegradable, %</th>
<th>Rate of degradation 'c', % / h</th>
<th>Effective degradability, %</th>
<th>Escape, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control feed</td>
<td>10.87</td>
<td>45.19</td>
<td>43.94</td>
<td>0.56</td>
<td>53.97</td>
<td>46.03</td>
</tr>
<tr>
<td>Experimental feed with fresh brewery waste (25%)</td>
<td>9.31</td>
<td>45.18</td>
<td>45.51</td>
<td>0.55</td>
<td>52.37</td>
<td>47.63</td>
</tr>
<tr>
<td>Experimental feed with dried brewery waste (25%)</td>
<td>9.28</td>
<td>43.31</td>
<td>47.41</td>
<td>0.54</td>
<td>50.51</td>
<td>49.49</td>
</tr>
<tr>
<td>Brewery waste (fresh)</td>
<td>8.80</td>
<td>36.66</td>
<td>54.54</td>
<td>0.42</td>
<td>43.25</td>
<td>56.75</td>
</tr>
<tr>
<td>Brewery waste (dried)</td>
<td>7.70</td>
<td>33.30</td>
<td>58.99</td>
<td>0.38</td>
<td>38.79</td>
<td>61.21</td>
</tr>
<tr>
<td>Paddy straw</td>
<td>6.73</td>
<td>24.02</td>
<td>69.25</td>
<td>0.28</td>
<td>28.64</td>
<td>71.36</td>
</tr>
<tr>
<td>Brewery waste incorporated paddy straw</td>
<td>7.95</td>
<td>30.41</td>
<td>61.64</td>
<td>0.38</td>
<td>36.32</td>
<td>63.68</td>
</tr>
</tbody>
</table>

reaction vessels of the RUSITEC.

1. Control feed (CF)
2. Experimental feed (75%) + Fresh brewery waste (25%) (EFFBW)
3. Experimental feed (75%) + Dried brewery waste (25%) (EFDBW)
4. Fresh brewery waste (FBW)
5. Dried brewery waste (DBW)
6. Paddy straw (PS) and
7. Brewery waste incorporated paddy straw (BWIPS)

The experiment was replicated. Per cent ingredient composition of the control and experimental concentrate mixtures used are presented in Table-1.

To every 100 kg of concentrate mixture 20 grams of Nicomix AB$_2$D$_3$K (Nicholas Piramal India Ltd, Mumbai) containing Vitamin A-82500 IU, Vitamin D$_3$-12000 IU, Vitamin B$_2$-50 mg, Vitamin K-10 mg per gram was added.

*Mineral mixture supplied by Kerala Feeds Ltd. Kerala, containing Calcium (minimum) 20 per cent, Phosphorus (minimum) 12 per cent, Magnesium (minimum) 5 per cent, Iron (minimum) 0.4 per cent, Copper (minimum) 0.1 per cent, Zinc (minimum) 0.8 per cent, Manganese (minimum) 0.12 per cent, Cobalt (minimum) 0.012 per cent, Iodine (minimum) 0.026 per cent, Sulphur 1.8 - 3 per cent, Arsenic (maximum) 7 ppm, Lead (maximum) 20 ppm and Flourine (maximum) 0.07 per cent.

The proximate analysis of above mentioned treatments were carried out as per standard procedure (AOAC4) and presented in Table-2.

The detailed experimental procedures are as follows: Rumen digesta and rumen liquor were collected from six cows immediately after slaughter from the District Slaughter House, Kuriachira, Thrissur, Kerala. It was thoroughly mixed and transported to the laboratory (within 30 minutes) in a pre heated vacuum flask (39°C). Handling of rumen liquor in the laboratory was carried out by continuous flushing with CO$_2$. The rumen fluid was strained through a double layered muslin cloth into
a CO₂ filled beaker. Each reaction vessel was charged with 500 ml of strained rumen liquor and 200 ml of artificial saliva. One nylon bag (pore size 100 m) containing 80g of rumen solid digesta (fibrous residue from the rumen content straining) and another containing 10 g (dry matter) of feed to be tested were placed into the perforated feed container and the assembly was put into the reaction vessel which was filled up to the brim with distilled water making the total volume of the container to one liter.

The composition of one litre artificial saliva is as follows:
- Sodium hydrogen carbonate (NaHCO₃) - 9.80 g
- Disodium hydrogen ortho phosphate (Na₂HPO₄ · 2H₂O) - 7.00 g
- Potassium chloride (KCl) - 0.57 g
- Sodium chloride (NaCl) - 0.47 g
- Magnesium sulphate (MgSO₄ · 2H₂O) - 0.12 g
- Calcium chloride (CaCl₂) - 0.04 g

The artificial saliva was prepared with one litre distilled water and kept at 39°C and then carbon dioxide was infused into it. Artificial saliva was pumped at a constant ratio of infusion (650 ml/day) into the reaction vessel by a peristaltic pump. The effluent and fermentation gases were collected in effluent collection vessels (containing few drops of saturated HgCl₂ solution) and gas collection bags, respectively. After 24 hours the solid inoculum was removed and a new bag of feed was placed in the feed container. Thus each reaction vessel at a time contained 2 bags introduced each in 2 consecutive days and removed 48 hours later. The bag to be removed was allowed to drain, squeezed and washed in artificial saliva in a polyethylene bag. The washings were returned to the respective reaction vessels. The removed bags were further washed and dried at 60°C for 48 hours. Each experiment totally consisted of 7 days adaptation period followed by collection period.

In Vitro Crude Protein Degradability Studies in RUSITEC:
Loss in weight of nylon bag after 0, 2, 6, 12, 24, 48 and 72 hours of incubation in RUSITEC followed by washing and drying was recorded to calculate dry matter disappearance. The in vitro disappearance of nutrients was calculated using the following formula and expressed as percentage on dry matter basis.

\[
\text{In vitro CP degradability} = \frac{(\text{weight of bag with CP before incubation}) - (\text{weight of bag with CP before incubation})}{\text{weight of CP}} \times 100
\]

The effective degradability of CP was calculated and results of the CP (dry matter basis) degraded at various time intervals and were fitted to exponential equation6 mentioned as below:

\[
P = a + b \left(1 - e^{-ct}\right)
\]

Where, 
- \(P\) = Per cent of degradation at time \(t\), 
- \(a\) = Per cent soluble fraction, 
- \(b\) = Insoluble but potentially degradable as percentage 
- \(a+b\) = The value of potential degradability of the material as percentage 
- \(c\) = The degradation rate, expressed as percentage/hour 

(a, b, c are constant in exponential equation).

The data were analysed statistically as per the standard statistical methods given by Snedecor and Cochran.

RESULT AND DISCUSSION
The data on the chemical composition of concentrate mixtures, brewery waste, paddy straw and brewery waste incorporated paddy straw are given in Table-2. The dry matter content of paddy straw, brewery waste and brewery waste incorporated paddy straw was 90.35 ± 0.81, 29.15 ± 0.43 and 78.11 ± 0.63 per cent, respectively. The crude protein (CP) content of brewery waste and brewery waste incorporated paddy straw was 24.34 ± 0.60 and 9.41 ± 0.44 per cent, respectively on dry matter basis. The CP content of control concentrate mixture and experimental concentrate mixture with brewery waste was
20.06 ± 1.05 and 20.12 ± 0.63 per cent, respectively. The crude fibre (CF), total ash and nitrogen free extract (NFE) of brewery waste were 19.62 ± 0.31, 5.76 ± 0.14 and 45.07 ± 0.79 per cent, respectively, on dry matter basis. Data on soluble fraction ‘a’, degradable fraction ‘b’, rate of degradation ‘c’ and the percentage in vitro effective degradability of CP of control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS in RUSITEC are presented in Table-3 and illustrated in Fig.-1. 

Soluble ‘a’ Fraction: The control feed had the highest ‘a’ value (10.87 per cent), followed by EFFBW, EFDBW and FBW (9.31, 9.28 and 8.80 per cent, respectively) indicating its higher level of rapidly soluble CP percentage as compared to DBW, PS and BWIPS (7.70, 6.73 and 7.95 per cent, respectively). Lowest ‘a’ value was observed for paddy straw when compared to other experimental feeds. The BWIPS showed slightly higher ‘a’ value than paddy straw. Higher in situ and in vitro CP ‘a’ fraction for wet brewer’s grain (51.0 and 27.0 per cent, respectively) and dried brewer’s grain (19.0 and 23.0 per cent, respectively) than those obtained for brewery waste in the present RUSITEC experiment. Higher CP ‘a’ fraction for wet brewer’s grain (26.5 per cent) and wet distiller’s grain (37.1 per cent) than those observed in the present study. Higher CP ‘a’ fraction for barley based distiller’s grain (19.8 per cent) and wheat based distiller’s grain (34.1 per cent) than those values obtained for brewery waste in the present study. The CP ‘a’ fraction was similar for brewer’s dried grain and distiller’s dried grain (17 per cent)12, but the values were higher than the values obtained for brewery waste in the present RUSITEC experiment. 

Degradable ‘b’ Fraction: The higher ‘b’ values of 45.19, 45.18 and 43.31 per cent for control feed, EFFBW, EFDBW respectively, obtained in the present study indicate that these three experimental feeds are high in potentially degradable CP and are low in undegradable dietary CP contents compared to FBW, DBW, paddy straw and BWIPS, which had lower ‘b’ values of 36.66, 33.30, 24.02 and 30.41 per cent, respectively. Among the experimental feeds, paddy straw showed lowest ‘b’ value for crude protein. The BWIPS showed the CP ‘b’ value greater than paddy straw alone. The FBW showed slightly higher CP ‘b’ value than DBW. Higher in situ and in vitro CP ‘b’ fraction for wet brewer’s grain (39.0 and 50.0 per cent, respectively) and dried brewer’s grain (51.0 and 30.0 per cent, respectively) than those obtained for brewery waste in the present RUSITEC experiment. Higher CP ‘b’ fraction for wet brewer’s grain (46.0 per cent) and wet distiller’s grain (50.2 per cent)11 than those obtained for brewery waste in the present study. Similarly, CP ‘b’ fraction for barley based distiller’s grain (68.4 per cent) and wheat based distiller’s grain (59.4 per cent)11 were higher than those obtained for brewery waste in the present study (36.66 per cent). Higher CP ‘b’ fraction for brewer’s dried grain (64.3 per cent) and distiller’s dried grain (56.1 per cent)12 than values obtained for brewery waste in the present RUSITEC experiment. 

Rate of Degradation ‘c’: The rate of degradation ‘c’ of CP was higher in control feed, EFFBW and EFDBW (0.56, 0.55 and 0.54 per cent / hour, respectively) in RUSITEC. Among the feeds experimented in RUSITEC, the paddy straw showed minimum rate of degradation ‘c’ value (0.28 per cent / hour) for CP. The BWIPS showed slightly higher rate of degradation of CP (0.38 per cent / hour) when compared to paddy straw. The FBW and DBW showed, intermediary rate of degradation (0.42 and 0.38 per cent / hour, respectively) among the feeds experimented and FBW showed higher rate of degradation of CP than the DBW. In situ and in vitro rate of degradation of CP ‘c’ fraction for wet brewer’s grain were 0.066 and 0.055 per cent / hour, respectively and that of dried brewer’s grain were 0.042 and 0.052 per cent / hour, respectively9. Similar CP degradation rate of 0.049 per hour for distiller’s dried grain11, but slightly faster CP degradation rate of 0.068 /h was reported by many workers12,14 for brewe’s dried grain. Higher rate of degradation of CP for wet brewer’s grain (5.9 per cent / hour) and wet distiller’s grain (8.8 per cent / hour)11 than observed in the present experiment. Similarly, Higher rate of degradation of CP for barley based distiller’s grain (7.8 per cent / hour) and wheat distiller’s grain (6.3 per cent / hour)11 than those obtained for brewery waste in the present RUSITEC experiment. 

Effective Degradability: The control feed, EFFBW and EFDBW had higher in vitro effective CP degradability (53.97, 52.37 and 50.51 per cent, respectively) than other experimental feeds such as FBW, DBW, PS and BWIPS (43.25, 38.79, 28.64 and 36.32 per cent, respectively) in RUSITEC. In the present study, paddy straw showed lowest effective degradability of CP (28.64 per cent) compared to other feeds experimented in RUSITEC. The BWIPS showed higher effective CP degradability (36.32 per cent) than the paddy straw (28.64 per cent). The FBW showed higher CP degradability (43.25 per cent) than DBW (38.79 per cent). Lower in situ undegraded
intake protein value of barley distiller's grain and corn distiller's grain in lactating Holstein cows, which ranged from 37 to 44 and 42 to 44 per cent of CP, respectively than that of FBW and DBW obtained (56.75 and 61.21 per cent, respectively) in the present study. Higher in situ effective degradability of CP for wet brewher's grain (54.9 per cent) and wet distiller's grain (69.1 per cent) than the values for brewery waste obtained (56.75 and 61.21 per cent) in the present study. CP degradation of corn distiller's grain in ruminally cannulated beef steers was 57.6 per cent at 48 hours. Lower effective CP degradability (24.76 per cent) of wet distiller's grain than those obtained for brewery waste in the present RUSITEC experiment (43.25 per cent). The over all results of in vitro degradability studies using RUSITEC indicates that the control feed, EFFBW and EFDBW had higher 'a' and 'b' fractions, rate of degradation 'c' and in vitro effective degradability of CP than other experimental feeds such as FBW, DBW, PS and BWIPS and paddy straw showed the lowest. The BWIPS showed higher in vitro effective CP degradability compared to paddy straw. The FBW showed higher CP 'a' and 'b' fractions, rate of degradation 'c' and effective degradability than DBW. A low in vitro CP disappearance of 30.75 per cent was recorded for paddy straw at 72 hours of incubation (effective CP degradability of 28.64 per cent), whereas the BWIPS had improved in vitro CP disappearance of 38.36 per cent at 72 hours of incubation (effective CP degradability of 36.32 per cent).

ACKNOWLEDGEMENT

The Authors are thankful to the Dean, College of Veterinary and Animal Sciences, Mannuthy, Kerala Agricultural University, Thrissur, Kerala, India for the facilities and financial support provided to carry out this research.

REFERENCES