In Vitro Effect of Probiotic Mix and Fibrolytic Enzyme Mixture on Digestibility of Paddy Straw

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Abstract | The study was undertaken to evaluate the effects of probiotic mix and fibrolytic enzyme mixture on in-vitro fermentation and digestibility using whole rumen flora from sheep. The feed additives viz. probiotic mix and fibrolytic enzyme mixture were incorporated @ 3g, 6g, 9g, 12g, 15g/kg dry matter of complete feed for adult sheep. The rations were subjected to in-vitro analysis to evaluate DM, OM and NDF digestibility for determination of optimum level of incorporation of feed additives in the complete ration. Significant improvement in nutrient degradability of DM, OM and NDF was observed due to probiotics mix and fibrolytic enzyme mixture supplementation with maximum values at 3g and 9g kg-1DM, respectively. Another in vitro study was carried to evaluate the effect of 3g and 9g kg-1 DM of probiotics mix and fibrolytic enzyme mixture alone and in combination on rumen fermentation parameters in complete feed having paddy straw and concentrate mixture in 50:50 ratio. The probiotics mix and fibrolytic enzyme mixture alone and in combination significantly improved nutrient degradability (DM, OM and NDF), total rumen nitrogen and TVFA in comparison to control. Additions of probiotic mix in complete feed decreased rumen pH alone and in combination with enzyme mix with a significant decrease in rumen ammonia nitrogen.

Keywords | Complete feed, Fibrolytic enzyme mix, In vitro degradability, Paddy straw, Probiotics mix.

INTRODUCTION

Feed additives have immense importance in livestock ration because of improvement of nutrient utilization, modify rumen fermentation and optimize performance in animal production systems. Since the use of chemicals hormones and antibiotics as livestock feed additive are banned in many countries, “natural” additives such as probiotics, feed enzymes, herbs etc are gaining tremendous importance. These natural feed additives apart from increasing productivity, also reduce the risk of transfer of potential human pathogens, decrease the antibiotic load and the risk of antibiotic resistance development, and limit excretion of pollutants. Manipulation of rumen microbial ecosystem in favorable direction is still a pertinent goal for animal nutritionist. Natural feed additives show a potential for manipulation of rumen fermentation. The most of important feed additives in this direction are “probiotics and enzymes” which have no residual effects. The “probiotics” or direct fed microbial seems to be more natural, restoring the normal and ideal flora in the digestive tract to its full capacity and results in increased animal production without any risk of human health hazards. Among probiotics, Saccharomyces cerevisiae (brewers and baker’s yeast) and Lactobacillus acidophilus (lactic acid producing bacteria) have got maximum attention among nutritionists throughout world. Yeast in ruminants stabilizes the pH of rumen and therefore, favour the growth of cellulolytic
Usage of exogenous fibrolytic enzymes has been reported to have positive effect on digestion. Many recent studies have reported increased digestion of dry matter and fibre measured in situ and in vitro on usage of fibrolytic enzymes (Khattab et al., 2013; Giraldo et al., 2014). The extent to which level the probiotic mix and enzyme mix can be incorporated in livestock ration to increase nutrient intake and utilization as well as animal performance has not been well established. Therefore, the present investigation was undertaken to study the effect of probiotics mix and fibrolytic enzyme mix at different levels on in vitro nutrient degradability of paddy straw and fermentation pattern in paddy straw-based complete feed using rumen liquor of sheep.

**MATERIAL AND METHOD**

The study was carried at Division of Animal Nutrition and Mountain Research Centre for Sheep and Goat (MRCSG), Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar. The samples of paddy straw and complete feed were oven-dried, ground and passed through 1mm sieve in a Willy mill and stored in plastic bottles for further use. The proximate constituents of paddy straw, probiotic mix, fibrolytic enzyme mix and complete feed was done as per AOAC (2000) and fibre fractions as per Van Soest et al.(1991). The modified in vitro procedure of Tilley and Terry (1963) was used to assess in vitro dry matter degradability (IVDMD), in vitro organic matter degradability (IVOMD) and in vitro nutrient detergent fibre degradability (IVNDFD) at 48 and 72 hr post-incubation and total gas production at 24 hr post-incubation.

Five levels of fibrolytic enzyme mixture and probiotic mix were selected separately, which were added to fermentation vessels (Jakhmola et al., 2010) containing 500 mg finely ground paddy straw @ 3g (T1), 6g (T2), 9g (T3), 12g (T4), 15g (T5) per kg DM of paddy straw. Three adult male Corriedale sheep were given 20 days adaptation period feeding complete feed containing paddy straw at 50% level to meet nutrient requirements as per ICAR (2013). Rumen fluid was collected from sheep before morning feeding (Solvia and Hess, 2007) by perforated tubing device under negative pressure and late squeezed through four layers of muslin cloth to get inoculums (SRL). The inoculum was transferred to pre-warmed flask (39ºC) and flushed with CO2. SRL (10 ml) was dispensed into pre-warmed (39ºC) fermentation vessels with feed additives. One fermentation vessel with only paddy straw was kept as control (T0).

The fibrolytic enzyme mixture (Allenzimix HP) used in the study contains different enzymes like cellulase with 12,00,000U concentration, amylase with 52,50,000U, xylose with 3,50,000U, Protease with 15,00,000U, Pectinase with 2,50,000U, B-Glucanase with 4,80,000U, Lipase with 120,000U, Phytase with 5,00,000U and Mannase with 100,000U concentration. The probiotic mix contains *Saccharomyces cerevisiae* 2×1010cfu/g and *Lactobacillus acidophilus* 6×1010cfu/g in equal ratio.

40 ml of McDougall buffer is flushed with oxygen free CO2 in each fermentation vessels. The experiment was conducted in completely randomized block design and each treatment run in triplicate with negative controls (SRL + buffer alone). The controls were used to correct for fermentation residues resulting directly from SRL. Each vessel cork fitted with control value was kept in incubator at 39ºC for 24 hr. The level of 48 hr incubation two drops of saturated HgCl2 was added in each vessel to stop microbrial activity. Contents of each vessel were transferred to 1 L spout-less beakers. The vessels were thoroughly washed with neutral detergent solution and final volume made to 150 ml. The contents were refluxed for 1 hr at 100ºC, filtered and washed through preweighed Gooch crucible (Grade 1, 50 ml capacity). This undigested residue (NDF) was oven-dried at 100ºC for 24 hr, cooled in desiccator and weighed. Loss in DM and NDF was digested dry matter and digested NDF. The crucibles containing residue were ignited in muffle furnace at 500ºC and the ash left in crucible after ignition was subtracted from residual dry matter to get the organic matter content. For in vitro studies at 72 hr, incubation of samples was done in same way as for 48 hr incubation. The reaction was stopped by adding 2 ml of 6 N HCl and 0.1 g pepсин powder (1:3000) to each vessel at the end of 48 hr incubation. The vessels were incubated for another 24 hr and procedure repeated as for 48 hr incubation, except the addition of HgCl2 at 48 hr. The optimum levels of feed additives, chosen from experiment I, were mixed with finely ground (1 mm) complete feed containing paddy straw and concentrate mixture in 50:50 ratio. The concentrate mixture contained maize 6, wheat bran 7.6, deoiled rice bran 9, mustard oil cake 10, soyabean meal 15, molasses 0.8, mineral mixture 0.8 and salt 0.4 parts.
The experimental procedures for degradability of nutrients in complete feed were similar to experiment I. The pH in rumen liquor was determined immediately after the termination of incubation at 48 hr using portable digital pH meter. Total volatile fatty acids was determined as per method of Barnett and Reid (1957) using Markham still distillation apparatus, total rumen nitrogen as per AOAC (2000) and ammonia nitrogen by spectrophotometer method (Chaney and Marbach, 1962).

STATISTICAL ANALYSIS

The data were analyzed statistically as per Snedecor and Cochran (1994) to draw the inference.

RESULTS AND DISCUSSION

CHEMICAL COMPOSITION OF FEED INGREDIENTS, SUPPLEMENTS AND EXPERIMENTAL COMPLETE FEEDS

The chemical composition of paddy straw reported is presented in Table 1. Results for chemical composition were in close agreement with observations of Khattab et al. (2013) and Sheikh et al. (2014). The chemical composition of probiotic mix was in accordance to the existing reports of Rekha et al. (2005) and Ganai et al. (2015). There has been slight improvement in nutrient content (CP, EE, NFE and minerals) of additive supplemented complete feed as also previously reported by Ganai et al. (2015) and Khattab et al. (2013).

Table 1: Chemical composition of paddy straw, complete feed and feed additives.

<table>
<thead>
<tr>
<th>Chemical components (% DM)</th>
<th>Paddy straw</th>
<th>Complete feed</th>
<th>Probiotics mix</th>
<th>Fibrolytic enzyme mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>84.73</td>
<td>89.53</td>
<td>99.50</td>
<td>98.00</td>
</tr>
<tr>
<td>CP</td>
<td>4.85</td>
<td>15.51</td>
<td>40.10</td>
<td>16.20</td>
</tr>
<tr>
<td>EE</td>
<td>1.37</td>
<td>3.15</td>
<td>2.5</td>
<td>1.50</td>
</tr>
<tr>
<td>CF</td>
<td>35.08</td>
<td>21.64</td>
<td>3.10</td>
<td>-</td>
</tr>
<tr>
<td>NFE</td>
<td>48.40</td>
<td>49.06</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TA</td>
<td>10.31</td>
<td>8.64</td>
<td>6.3</td>
<td>-</td>
</tr>
<tr>
<td>AIA</td>
<td>4.18</td>
<td>3.31</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NDF</td>
<td>79.90</td>
<td>68.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ADF</td>
<td>61.11</td>
<td>42.15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HC</td>
<td>18.79</td>
<td>25.88</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cellulose</td>
<td>44.37</td>
<td>34.37</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ADL</td>
<td>10.63</td>
<td>5.49</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ca</td>
<td>0.38</td>
<td>1.93</td>
<td>1.90</td>
<td>-</td>
</tr>
<tr>
<td>P</td>
<td>0.20</td>
<td>0.59</td>
<td>0.80</td>
<td>-</td>
</tr>
</tbody>
</table>

In vitro nutrient degradability and fermentation parameters of paddy straw are presented in Table 2. There was significant (P<0.01) improvement in degradability of dry matter, OM and NDF as an effect of supplementation of enzyme mix and probiotic mix at various levels and on the basis of overall results, 9 g/kg level for enzyme mix and 3 g/kg DM level for probiotic mix were selected as optimal level. These results are comparable with the earlier report of Ganai et al. (2015) reported improved in vitro digestibility of DM, OM, NDF and total gas production in kids supplemented with *Saccharomyces cerevisiae* bájra straw at dose rate of 10⁶ cfu/g. Similar results were found by El-Waziry et al. (2007) recorded improvement in degradability pattern of nutrients either in vitro or in sacco due to supplementation of yeast culture in groundnut haulms and berseem hay, respectively. Similarly Chandrasekharaihet et al. (2016) reported that supplementation of recombinant *Butirribrio fibrisolvens* and yeast mixed culture resulted significantly improved OM and NDF degradability of wheat straw when compared to control. Regarding the effect of fibrolytic enzymes, Lamid et al. (2013) stated that addition of lignocellulolytic enzymes @ 5% into rice straw increase digestibility of nutrients and improve in vitro rumen fermentation with significantly higher production of volatile fatty acid and ammonia. Similarly Vande and Useni (2012), found increased the in vitro DM and NDF disappearances after 36 hours in sheep fed milled substrate consisting of a 50:50 mixture of lucerne hay (LH) and wheat straw (WS). Mao et al. (2013) conducted in vitro gas test to investigate the effects of cellulase (CEL) and xylanase (XYL) on in vitro rumen fermentation and microbial population with rice straw as substrate, concluded that the application of CEL and XYL could improve rumen fermentation, increase rice straw digestion and affect the rumen microbial population. Thammiaha et al. (2016) who studied in vitro efficacy of lignin peroxidases also reported that it enhanced the digestibility of crop residues when feed to ruminants. Other workers (Chopra et al., 2007; Diaz et al., 2013; Khattab et al., 2013) have also reported improvement in degradability pattern of nutrients in different roughages either in vitro or in sacco with supplementation of enzymes possibly due to increased microbial count and activity.

**In vitro nutrient degradability of experimental complete feeds**

Digestibility of nutrients in *in–vitro* system was more pronounced when these optimal levels of feed additives (9g/kg DM of substrate for enzyme mix and 3g/kg DM levels for probiotic mix) alone and in combinations were incorporated in complete feed containing paddy straw and concentrate mixture at 50:50 ratio was used as substrate (Table 3). Significant increase in degradability of DM, OM and
Table 2: In vitro DM, OM and NDF digestibility of paddy straw at 48 and 72h of incubation (%DM basis).

<table>
<thead>
<tr>
<th>Feed additive</th>
<th>Hour of Incubation</th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVOMD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enzyme mix</td>
<td>48**</td>
<td>39.72±0.90a</td>
<td>42.34±1.04ab</td>
<td>42.03±0.30bc</td>
<td>45.06±0.53c</td>
<td>40.62±1.66e</td>
<td>41.01±1.00f</td>
</tr>
<tr>
<td>Probiotic mix</td>
<td>48**</td>
<td>39.72±0.90a</td>
<td>40.00±0.17d</td>
<td>41.02±0.30b</td>
<td>42.55±0.42e</td>
<td>41.00±0.72e</td>
<td>41.07±0.38f</td>
</tr>
<tr>
<td>Enzyme mix</td>
<td>72**</td>
<td>40.39±0.73c</td>
<td>44.03±0.67c</td>
<td>43.03±0.48c</td>
<td>46.15±0.07d</td>
<td>42.28±1.38h</td>
<td>42.04±0.39h</td>
</tr>
<tr>
<td>Probiotic mix</td>
<td>72**</td>
<td>40.95±0.82c</td>
<td>45.16±0.33c</td>
<td>41.87±0.57c</td>
<td>42.53±0.78e</td>
<td>42.47±0.30e</td>
<td>42.10±1.00f</td>
</tr>
</tbody>
</table>

abcd Means superscripted with different letters in a row for a particular data differ significantly from each other* (P<0.05), ** (P<0.01)

NDF and rumen fermentation parameters were reported in supplemented feeds (C1 and C2) than the control (C0) and the effect was more pronounced in combination group (C3). These results are in line with the earlier report of Ganai et al. (2015) where in higher digestibility of DM, OM, NDF and total gas production values at supplementation of yeast to bajra straw based complete feed using goat rumen liquor in in vitro study. Other workers (Malik and Singh, 2009) have also reported improvement in degradability pattern of nutrients either in vitro or in sacco due to supplementation of yeast culture. Regarding the supplementation of fibrolytic enzymes, Thakur et al. (2008) reported an increase in DM and NDF degradability in total mixed rations having different roughage and concentrate ratios (75:25, 60:40 and 50:50). Ganai (2011) also reported improved digestibility of DM, OM, NDF and total gas production showing maximum values at supplementation of exogenous fibrolytic enzyme at dose rate of 2 g/kg DM. An increase in IVOMD, IVNDFD and TGP were reported as an associative effect of enzyme and yeast when supplemented together in cereal straws by Tang et al. (2008). The results of experiment undertaken in phase-I, taking into account the digestibility at 48 and 72 h incubation revealed that in vitro DM, OM and NDF utilization from complete feed could be improved by supplementation of feed additives at optimal level alone and...
There has been significant effect of supplementation of feed additives on the fermentative metabolites (Table 3). The supplementation of probiotic mix alone or in combination with enzyme mix, to paddy straw based complete feed had significant effect on pH of in vitro medium. The results reported in vitro studies suggested that addition of probiotic mix alone as well as in combination with fibrolytic enzymes modify rumen pH. However, fibrolytic enzymes had no effect on the pH of in vitro medium. These results are in accordance with reports of Kamara et al. (2002) and Garg et al. (2009) who suggested that supplementation of yeast causes an elevation in pH possibly due to utilization of lactic acid from ruminal contents, thereby stabilizing pH (Dawson and Tricarica, 2002).

In contrast, some researchers have observed either no effect or even reduction in rumen pH upon yeast supplementation (Brossard et al., 2006; Ganai et al., 2015). Ganai (2011) had also reported no effect of enzyme supplementation on in vitro rumen pH. The variation may be attributed to different experimental conditions related to animal (species, physiological stage and level of intake), diet (composition and mode of distribution), experimental design (group or Latin square) and probiotic mix (strain, dose, combination and viability).

The TVFA concentration was significantly (P<0.01) higher in feed additive supplemented complete feed as compared to un-supplemented feed, which indicated the stimulatory effects of probiotic mix and fibrolytic enzymes on fibre-degrading micro-organisms, facilitating availability of more energy. An increase in TVFA concentration upon supplementation of yeast has been reported (Garg et al., 2009; Ganai et al., 2015). Chadema and Offer (1990) reported that \textit{S. cerevisiae} had no effect on total or individual VFAs, but others found stimulation in the proportion of propionate at the expense of acetate (Newbold et al., 1990) or even an increase in the proportion of acetate (Mustsvangwa et al., 1992). The highly variable nature of the responses in VFA proportions to probiotic mix supplementation lead to the suggestion that the effect of yeast on microbial numbers in the rumen rather than a direct effect, which might explain the gains observed in productivity (Wallace and Newbold, 1993). Regarding fibrolytic enzymes Lamid et al. (2013) reported that addition of lignocellulolytic enzymes @ 5% into rice straw improves in vitro rumen fermentation with significantly higher production of volatile fatty acid and ammonia. Mao et al. (2013) also reported that addition of cellulase and xylanase increased in vitro gas production and total volatile fatty acids with reduced ammonia nitrogen. The higher (P<0.01) level of total N upon probiotic mix and enzyme supplementation alone and in combination may be attributed to possibly higher proteolytic activity. Yoon and Stern (1996) reported similar proteolytic activity. The present results are consistent with earlier reports of Garg et al. (2009). The concentration of rumen ammonia nitrogen was numerically lower in feed additive supplemented groups as compared to un-supplemented group but the difference was statistically non significant. The lower concentration of NH$_3$-N in feed additive supplemented animals may be associated with increased uptake and assimilation of NH$_3$-N by rumen microbes due to stimulation of bacterial growth. There have been variable earlier reports of different researchers (El-Ghani et al., 2004; Garg et al., 2009) reports reduced NH$_3$-N concentration (Oeztuerk, 2009) reports no effect on NH$_3$-N concentration and Sales (2011) and Ozsoy et al. (2013) reports increased NH$_3$-N concentration due to yeast supplementation. Torres et al. (2013), Mao et al. (2013) and Tang et al. (2013) reported that enzymatic treatment tends to linearly increase N-NH$_3$ concentration.

The study revealed a significant improvement in digestibility of nutrients and rumenfermentation in in–vivo rumen fluid of sheep as an effect of supplementation of probiotics mix and fibrolytic enzyme mixture supplementation with maximum values at 3g and 9g kg$^{-1}$ DM respectively. However, in vivo trial is required before advocating these findings to sheep raisers.

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**CONFLICT OF INTEREST**

There is no conflict of interest among the authors and has no financial repercussions.

**AUTHORS CONTRIBUTION**

All the authors contributed significantly to the paper. Author along with Ahmad and Afzal carried the trial and compiled the results. AM Ganai and HA Ahmed designed the protocol and helped in statistical analysis.

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