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Effect of Supplementation of Exogenous Fibrolytic Enzymes in Total Mixed Ration on Rumen Fermentation Pattern in Dairy Cows

P.M. Lunagariya*, S.V. Shah, R.S. Gupta, Subhash Parnerkar, Hiren Pansuriya, Kashmira Khaire and G.R. Patel

Department of Animal Nutrition Research

College of Veterinary Science and Animal Husbandry, AAU, Anand-388 001, Gujarat

Corresponding Author: drpravinml@gmail.com

Abstract

The study was planned to evaluate the effect of exogenous fibrolytic enzymes (EFE) supplementation for 56 days @ 240 mg/kg total mixed ration (TMR) on rumen fermentation in dairy cows. For this six dry non-pregnant cows were assigned in each treatment with and without EFE. The rumen metabolites were evaluated on last day of experiment. About 250 ml rumen liquor was collected from each cow at pre-feeding and at 2, 4 and 6 h post-feeding. Strained Rumen Liquor (SRL) pH of cows was 6.78 and 6.70; and total volatile fatty acids (TVFAs) concentration was 8.85 and 11.22 mM/dl, respectively for cows fed TMR without enzymes (control T_1) and TMR with fibrolytic enzymes (T_2) with significant difference ($P<0.05$). The pH was lowest and TVFAs was highest at 4 h post-feeding. The values for ammonia, total, non-protein, protein and soluble nitrogen concentration in SRL were 12.43 and 13.04; 95.74 and 103.74; 28.60 and 26.72; 67.15 and 77.02; and 27.15 and 29.01 mg/dl, respectively for cows fed control T_1 and T_2 TMR. All nitrogen fractions were within normal range and peaked at 4 h post-feeding, lowered at 6 h post-feeding and least at 8 h post-feeding. The rumen liquor total and protein nitrogen were higher ($P<0.05$) for cows fed exogenous fibrolytic enzymes and remained higher up to 8 hour post-feeding, indicating good fermentation activity. These results indicated better utilization of protein sources and carbohydrate release by action of fibrolytic enzymes. The study concluded that feeding exogenous fibrolytic enzymes (240 mg/kg) supplemented TMR improves rumen fermentation in dry non-pregnant Gir and crossbred dairy cows.

Key Words: Cows, Exogenous fibrolytic enzymes, Total mixed ration, Rumen fermentation.

Introduction

In India, the animals are fed mainly hedge grasses, crop residues, agro-industrial by-product and dry fodder, since the green roughage, dry roughage and concentrate are deficit up to 62.76, 23.46 and 63.00% of requirement (Kore, 2014). This deficit suggests the need for efficient and improved use of feed resources. The feeding of fodder directly to farm animals in general has high crude fiber and medium digestibility. Thus to improve the digestibility, it is important to break down the linkage between cellulose, hemicellulose and lignin. In this regard, cellulases and xylanases are respectively amongst the two major enzyme groups that are specified to break β1-4 linkages joining

sugar molecules of cellulose and xylans found in plant cell-wall components (Beauchemin *et al.,* 2003). Again manipulation of rumen fermentation is a way to increase the nutrient utilization that subsequently improves the efficiency of production by farm animals (Kamra *et al*., 2002). Several studies have shown improved rumen fermentation, nutrient digestibility and milk yield on fibrolytic enzymes supplementation (Bassiouni *et al*., 2010; Gaafar *et al*., 2010). Therefore present study was aimed at evaluate the effect of supplementation of exogenous fibrolytic enzymes in total mixed ration on rumen metabolites in dairy cattle.

Materials and Methods

The study was conducted at University Livestock Research Station, Anand, Gujarat on twelve dry non-pregnant cows for 56 days (excluding 7 days pre-experimental feeding). The commercial exogenous fibrolytic enzymes - Roxozyme GT ® (contained endo 1,4-β glucanase 800, 1(3), 4-β glucanase 700, and endo 1,4-β xylanase 2700 IU/g procured from M/S, DSM Nutritional Product Pvt. Ltd., Pune, India) was used @ 240 mg/kg TMR as revealed optimum through *in vitro* study.

Twelve Gir & crossbred dairy cows were divided into two equal groups of six based on their body weight (446.33±19.01 kg, CV=14.76%) having equal numbers (three) of each Gir and crossbred cows in each treatment. The cows were fed individually either T_1 (Control) TMR (60% sorghum hay +40% concentrate) without fibrolytic enzyme or T_2 TMR with exogenous fibrolytic enzymes (240 mg/kg TMR) to meet their nutrient needs as per ICAR (1998) standard. The cows were fed measured quantity of TMR in equal part each in morning and afternoon and left over were weighed next day morning before feeding. The cows were let loose for exercise for two hours in the morning (9:00 to 11:00 am) and one hour in the afternoon (3:00 to 4:00 pm) under controlled conditions, during which they had free access to fresh clean drinking water. Deworming of all the cows was carried out using broad spectrum anthelmintic before initiation of the experiment.

The rumen fermentation study was carried out at the end of experiment. The progressive changes in pH, total volatile fatty acids (TVFAs) production and various nitrogen fractions were studied before feeding (0 hr), and at 2, 4 and 6 hrs post-feeding in strained rumen liquor (SRL). About 250 ml of rumen liquor was collected each time from each cow using stomach tube and employing negative suction. The rumen liquor was immediately brought to laboratory and strained through four layers of cheese cloth. The pH was determined immediately using a digital pH meter. An ammonia-N and total-N were analyzed immediately. Rest of the SRL was stored in glass bottle by adding mercuric chloride @ 0.5 g/100 ml SRL in deep freeze for further analysis. All nitrogen fractions and total volatile fatty acids (TVFA) were analyzed as per Galyean (2010) and Tiwari *et al.* (2012). The data generated were analyzed as per Snedecor and Cochran (1994) using General Linear Model tests (SPSS 9.00 software).

Results and Discussion

Total mixed ration (TMR) contained 10.27, 2.86, 24.04, 52.06, 10.77, 2.02, 0.96, 0.48, 52.33, 26.66, 16.62 and 25.67% of crude protein, ether extract, crude fibre, NFE, total ash, silica, calcium, phosphorus, neutral detergent fibre, acid detergent fibre, cellulose and hemicellulose, respectively. Further the values for rumen metabolites are presented in Table 1.

SRL pH:

The average SRL pH of cows under T_1 and T_2 groups was 6.78 and 6.70, respectively and differed significantly ($P<0.05$). The SRL pH decreased up to 4h post-feeding and again increased at 6 and 8 h post-feeding ($P<0.05$; Table 1), reached almost to pre-feeding state indicating maximum microbial activity within 6 h of feeding. The SRL pH during different periods also differed significantly ($P<0.01$), while interaction between treatment and period was non-significant. The values of SRL pH observed are in line with Lunagariya and Pande (2016). Rajamma *et al.* (2014) also reported decreased ($Pd\leq0.01$) rumen liquor pH in buffalo bulls when fed two type of TMR (roughage to

concentrate ratio 60:40 and 70:30) supplemented with fibrolytic enzymes @ 15 g/day (6.71 and 6.82) in comparison to buffalo bulls fed TMR without fibrolytic enzymes (6.81 and 6.90). The value, fermentation pattern and effect of EFE observed in present study were in agreement with these researchers. Similar rumen pH (7.04 *vs.* 6.91) were also reported by Bassiouni *et al.* (2010) for multiparous lactating Friesian cows fed fibrolytic enzyme @ 1 g/kg DM. However, others (Arriola *et al.,* 2011; Khanh *et al.,* 2012) observed non-significant effect of fibrolytic enzymes supplementation on ruminal pH in cows. The decrease in rumen pH was probably because of higher energy release due to fibrolytic enzyme supplementation (Dehghani *et al.,* 2011).

Table 1: Average ruminal pH, TVFA and N fractions of cows fed EFE supplemented TMR

Treat.	Hours post-feeding						Period effect
	0	2	4	6	8	Avg.	
Ruminal pH							
T_1	6.89±0.02	6.67±0.03	6.60±0.01	6.81±0.03	6.92±0.04	**6.78**^{b}±0.03	**
T_2	6.83±0.04	6.59±0.03	6.39±0.06	6.80±0.08	6.89±0.09	**6.70**^{a}±0.06	
Total Volatile Fatty Acids (mM/dl SRL)							
T_1	7.47±0.25	9.01±0.33	10.53±0.15	9.29±0.14	7.93±0.19	**8.85**^{a} ±0.21	**
T_2	8.04±0.36	12.71±0.35	14.93±0.22	12.23±0.25	8.17±0.22	**11.22**^{b}±0.28	
Ammonia – N (mg/dl SRL)							
T_1	10.03±0.65	13.43±0.72	16.70±0.33	13.31±0.62	8.70±0.42	**12.43**±0.55	**
T_2	9.81±0.37	14.19±0.51	18.55±0.42	13.85±0.47	8.79±0.33	**13.04**±0.42	
Total-N (mg/dl SRL)							
T_1	59.15±2.10	106.89±7.89	134.49±4.49	103.76±5.32	74.41±4.34	**95.74**^{a}±4.83	**
T_2	72.17±3.83	109.13±7.69	142.96±4.25	112.47±7.77	81.97±3.00	**103.74**^{b}±5.31	
NPN (mg/dl SRL)							
T_1	28.70±1.07	40.02±3.95	26.48±1.16	24.33±1.16	23.45±0.79	**28.60** ±1.63	**
T_2	28.64±2.09	36.63±3.84	26.43±1.68	22.63±0.87	19.25±1.27	**26.72** ±1.95	
TCA Precipitable-N (mg/dl SRL)							
T_1	30.45±2.18	66.87±9.78	108.01±4.75	79.44±4.89	50.96±3.89	**67.15**^{a} ±5.10	**
T_2	43.53±3.34	72.50±7.08	116.54±3.93	89.83±7.33	62.72±2.13	**77.02**^{b} ±4.76	
Soluble-N (mg/dl SRL)							
T_1	21.05±1.03	29.33±2.39	35.26±4.11	26.65±1.88	23.47±0.82	**27.15** ±2.05	**
T_2	23.47±0.81	29.84±1.19	36.68±1.26	29.05±1.20	26.02±0.58	**29.01** ±1.01	

** Period effect is highly significant (P<0.01)
Means with different superscripts (a,b) within column for a parameter differ significantly (P<0.05).

SRL TVFA:

The average TVFAs concentration was 8.85 and 11.22 mM/dl SRL, respectively, for cows fed TMR without enzymes (control T_1) and with fibrolytic enzymes (T_2). The peak concentration of TVFAs was observed at 4 h post-feeding and declined thereafter at 6 and 8 h post-feeding. The difference in

concentration of TVFAs between treatments, periods as well as interaction between treatments and periods were highly significant (P<0.01). It is likely that supplementation of fibrolytic enzyme might have released more sugar, which might have been utilized by rumen microbes to produce more volatile fatty acids and caused decrease in pH, which is evident from the data. Bassiouni *et al.* (2010) and Gaafar *et al.* (2010) reported significant increased TVFA concentration in lactating Friesian cows and lactating buffaloes, respectively, fed fibrolytic enzyme supplemented TMR in comparison to control TMR. The time study revealed pattern of lower TVFA concentration (13.7 meq/dl) before feeding and increased gradually until 4 h after feeding (16.7 meq/dl) then decreased (14.6 meq/dl) at 6 h (Bassiouni *et al.,* 2010). Similar findings were also reported by Arriola *et al.* (2011) and Rajamma *et al.* (2014) for Holstein dairy cows and buffalo bulls, respectively.

Rumen Ammonia nitrogen (NH_3-N):

The average SRL NH_3-N concentration of cows was 12.43 and 13.04 mg/dl under T_1 (control) and T_2 (enzyme) treatments, respectively, the difference being non-significant. The peak concentration of NH_3-N reached at 4 h post-feeding then declined at 6 and 8 h post-feeding. The changes during different periods of sampling were significant (P<0.01), but the interaction between treatments and periods was non-significant. However, the values for NH_3-N observed under both the treatments are within the normal range. In a study of Chung *et al,* (2012), the supplementation of exogenous fibrolytic enzyme @ 0.5 and 1.0 ml/kg TMR DM had nonsignificant effect on rumen ammonia concentration (6.4 and 5.9 mM) in comparison to TMR without enzymes (5.8 mM) with significant period effect (P<0.01) in Holstein dairy cows. Similarly Khanh *et al*. (2012) revealed unaffected rumen fluid NH_3-N concentration on fibrolytic enzymes supplementation @ 50 mg/kg DM TMR in HF crossbred cows. Our findings are in accordance with these reports on fermentation pattern.

Rumen total nitrogen (total-N):

Average SRL total-N concentration was significantly (P<0.05) higher in cows under T_2 (enzyme) than T_1 (control) group (103.74 vs 95.74 mg/dl). The concentration of total-N increased from pre-feeding and peaked at 4 h post-feeding and then declined at 6 and 8 h post-feeding (Table 1). The periodical changes in concentration of SRL total-N were significant (P<0.01). However, interaction between treatment and period was non-significant. Rajamma *et al.* (2014) reported higher ($P \leq 0.01$) SRL total-N concentration on daily feeding fibrolytic enzymes (15 g) supplemented TMR having roughage to concentrate ratio 60:40 and 70:30 in buffalo bulls with increased ($P \leq 0.01$) total-N up to 4 h post-feeding and decreased at 6 and 8 h. Similarly higher ($P \leq 0.01$) rumen liquor total-N (81.40 mg/100 ml) were also reported by Poonooru *et al.* (2015) on daily feeding exogenous fibrolytic enzyme (15 g) supplemented TMR (roughage to concentrate ratio 70:30) in comparison control TMR. They noted total-N increased at 2 h and further increase was noted at 4 h, decreased at 6 and 8 h post feeding in Murrah bulls. These results are in accordance with findings of present study.

Rumen non-protein nitrogen (NPN):

The NPN concentrations were 28.60 and 26.72 mg/dl SRL (P>0.05) under T_1 (control) and T_2 (enzyme), respectively. The changes in average values of NPN in both the groups during different periods of sampling were significant (P<0.01) without any interaction effect of period and treatment. Similar non-significant differences for SRL NPN (26.11 and 31.78 mg/dl) for Kankrej x Jersey crossbred cows fed two types of cocktail enzymes diet and control diet (30.84 mg/100 ml) were reported by Naik (2004). They also reported significantly higher value for NPN at 2 h post-feeding, which is in agreement with present study.

Rumen TCA precipitable (protein) nitrogen:

The average values for SRL protein-N for cows fed TMR T_1 (control) and TMR T_2 (enzyme) were 67.14 and 77.02 mg/dl, respectively with significant difference (P<0.05). The average values for both the groups rose significantly till 4 h post-feeding and then declined (Table 1). The differences

for treatments and periods were highly significant; however, the effect of interaction between treatment and period was non-significant. The results indicated better utilization of protein sources and carbohydrate released by action of fibrolytic enzymes. The non-significant but similar values for SRL protein-N (62.26 and 63.38 mg/100 ml) for crossbred cows fed two types of cocktail enzymes diet and control diet (71.80 mg/100 ml) were reported by Naik (2004) with numerically ($P>0.05$) higher value at 2 h post-feeding. Rajamma *et al.* (2014) also reported significantly higher ($P\leq0.01$) effect of feeding exogenous fibrolytic enzymes (15 g/animal/day) on rumen liquor TCA insoluble protein nitrogen (28.10 and 26.33 mg/100 ml) in relation to without enzymes (26.33 and 24.21 mg/100 ml) when buffalo bulls fed TMR having roughage to concentrate ratio 60:40 and 70:30, respectively. The rumen liquor TCA insoluble protein nitrogen increased ($P\leq0.01$) up to 4 h post-feeding and decreased at 6 and 8 h post-feeding. The effects for periodical changes in protein nitrogen of these investigations are in accordance with present study.

Rumen soluble nitrogen (soluble-N):

The average value for soluble-N was 27.15 and 29.01 mg/dl SRL ($P>0.05$) under T_1 TMR (control) and T_2 TMR (enzyme), respectively. The concentration of soluble-N increased significantly ($P<0.01$) up to 4 h post-feeding and decreased thereafter at 6 and 8 h post-feeding. An interaction between times of sampling and treatments were found to be non-significant. Non-significant values for SRL soluble-N (19.41, 23.19 mg/100 ml) on feeding two cocktail enzymes supplemented diet and control diet (24.29 mg/100 ml) in lactating crossbred cows were reported by Naik (2004), while effect of period of sample was significant ($P<0.05$) and peaked at 2 h post-feeding.

From the overall results of rumen fermentation pattern, it can be concluded that the feeding of exogenous fibrolytic enzyme improved total-N and protein-N fermentation, which might be utilized effectively by dairy animals (Bhanderi, 2012).

Conclusion

Average rumen liquor pH was near neutral in both treatments and least at 4 h post-feeding with higher rumen TVFAs concentrations, indicating higher sugar release by exogenous fibrolytic enzymes activity with conversion to volatile fatty acids by rumen microbiota. The rumen liquor pH was lower ($P<0.05$) and TVFAs were higher ($P<0.05$) for cows fed exogenous fibrolytic enzymes, indicating an inverse relation. All nitrogen fractions were within normal range and peaked at 4 h post-feeding, lowered at 6 h and least at 8 h post-feeding. The rumen liquor total and protein nitrogen were higher ($P<0.05$) for cows fed exogenous fibrolytic enzymes and remained higher up to 8 h post-feeding, indicating good fermentation activity. These results indicated better utilization of protein sources and carbohydrate released by action of fibrolytic enzymes, thus the feeding exogenous fibrolytic enzymes (240 mg/kg) supplemented TMR improves rumen fermentation in dry non-pregnant Gir and crossbred dairy cows.

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