

## Rumen fermentation patterns in buffalo bulls fed total mixed ration supplemented with exogenous fibrolytic enzyme and/or live yeast culture

Ravikanth Reddy Poonooru<sup>1</sup>, Srinivasa Kumar Dhulipalla<sup>1\*</sup>, Raghava Rao Eleneni<sup>1</sup> and Ananda Rao Kancharana<sup>2</sup>

<sup>1</sup>Department of Animal Nutrition, NTR College of Veterinary Science, Gannavaram, India;

<sup>2</sup>Senior Scientist & Head, Buffalo Research Station, VR Gudem, India.

\*Corresponding author's e-mail: [kumardhulipalla@rediffmail.com](mailto:kumardhulipalla@rediffmail.com)

### ABSTRACT

The objective of this study was to evaluate the effect of exogenous fibrolytic enzyme (EFE) and/or live yeast culture supplementation in total mixed ration (TMR) on rumen fermentation patterns in buffalo. For this, four adult buffalo bulls weighing 377.05±43.36 kg were randomly allotted to four dietary treatments *viz.*, TMR containing R:C ratio of 70:30 (T<sub>1</sub>), T<sub>1</sub> supplemented with EFE at 15 g/animal/day (T<sub>2</sub>), T<sub>1</sub> supplemented with live yeast culture at 10 g/animal/day (T<sub>3</sub>), and T<sub>1</sub> supplemented with EFEs at 15 g/animal/day and live yeast culture at 10 g/animal/day (T<sub>4</sub>). Rumen liquor from the fistulated animals was collected at 0, 1, 2, 4, 6 and 8 h post-feeding, and was analyzed. This study revealed that rumen pH values were highest at 0 h, and were declined to minimum by 4 h post-feeding, while total volatile fatty acids (TVFA), ammonia nitrogen (NH<sub>3</sub>-N), and nitrogen (N) fractions reached to peak at 4 h post-feeding, and later followed a decreasing trend in all the treatments. Supplementation of EFE in TMR (T<sub>2</sub>) had no effect ( $P>0.05$ ) on rumen pH and food and protozoal N concentration, while it influenced to increase ( $P<0.01$ ) the concentration of TVFA, NH<sub>3</sub>-N and other N fractions as compared to the T<sub>1</sub>. Yeast culture supplementation in TMR (T<sub>3</sub>) increased ( $P<0.01$ ) rumen pH, TVFA, NH<sub>3</sub>-N, total N, TCA-insoluble N and residual N. However, no effect ( $P>0.05$ ) on food and protozoal N in buffalo bulls was found. This study indicated that, supplementation of EFE and/or live yeast culture in TMR (T<sub>4</sub>) increased ( $P<0.01$ ) the rumen pH, TVFA, NH<sub>3</sub>-N and N fractions in buffalo bulls as compared to the control group. Therefore, it is concluded that supplementation of EFE and/or live yeast culture in TMR can increase the concentration of rumen metabolites in buffalo bulls.

### Keywords

Buffalo bulls, exogenous fibrolytic enzymes, Live yeast culture, Rumen fermentation pattern, Total mixed ration

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### INTRODUCTION

In India, farmers feed their animals with poor quality crop residues having high fiber content; the fibers prevent the access of ruminal hydrolytic enzymes to cellulose and hemicellulose (Tan et al., 1995). Manipulation of rumen fermentation is possible to increase the nutrient utilization that subsequently improves the efficiency of production by farm animals (Kamra et al., 2002).

In recent years, both yeast culture and exogenous fibrolytic enzymes (EFEs) have been used to improve the utilization efficiency of low-quality roughages. Several studies have shown that enzyme supplementation can positively influence rumen variables, fibrolytic activity of rumen fluid, and microbial populations (Gaafar et al., 2010; Bhasker et al., 2013). Besides, there are reports indicating changes in rumen fermentation and stimulation of ruminal digestion in cattle, sheep and goats when their diets are supplemented with yeast culture (Mahender et al., 2006; Srinivas Kumar et al., 2011). Several attempts have also been made to feed dairy animals with a composite of probiotic preparations (Erasmus et al., 2005), assuming their synergistic effects on the productivity and health of animals. However, only few

comparative studies have been reported describing the effect of addition of live yeast culture and/or EFEs on rumen fermentation. Therefore, this study was designed to assess the effect of EFEs and/or live yeast culture supplementation on rumen fermentation in buffalo bulls fed groundnut haulms based total mixed ration (TMR).

## MATERIALS AND METHODS

Four Murrah bulls weighing  $377.05 \pm 43.36$  kg each fitted with a permanent rumen fistula were randomly allotted to four dietary treatments *viz.*, TMR containing R:C ratio of 70:30 (T<sub>1</sub>), T<sub>1</sub> supplemented with EFEs at 15 g/animal/day (T<sub>2</sub>), T<sub>1</sub> supplemented with live yeast culture at 10 g/animal/day (T<sub>3</sub>) and T<sub>1</sub> supplemented with EFEs at 15 g/animal/day and live yeast culture at 10 g/animal/day (T<sub>4</sub>). The necessary ethical approval for the animal experimentation was taken from the NTR College of Veterinary Science, Gannavaram, India.

The EFE used in the present study was a commercial preparation manufactured by M/S Alltech Inc., Nicholasville, USA that contained fermentation extracts of *Aspergillus niger* and *Trichoderma viridae* having cellulases and hemicellulases; 100 IU as Xylanase/g as per claims. These EFEs were supplemented in the diet at 2.5 g/Kg TMR (on DM basis). The live yeast culture used in the present study was manufactured by Lallemand, France and contains *Saccharomyces cerevisiae* 1-1077 at  $4 \times 10^9$  CFU/10 g. The live yeast culture was supplemented at 10 g/animal/day.

The TMR comprised of groundnut haulms 70 parts, maize grain 8.1 parts, De Oiled Rice Bran 10.5 parts, Cotton seed cake 7.5 parts, Sunflower cake 3.0 parts, Mineral mixture 0.6 parts, and Salt 0.3 parts. The buffalo bulls were fed respective TMR at 9.00 AM and 3.00 PM all through the experimental period (ICAR, 1998). Clean and fresh drinking water was supplied to the animals throughout the trial period. The rumen liquor was collected from fistulated bulls at 0, 1, 2, 4, 6 and 8 h post-feeding. The pH of rumen liquor was measured immediately after collection using digital pH meter, and ammonia (NH<sub>3</sub>) nitrogen (N) was estimated by micro-diffusion method (Conway, 1957) using mixed indicator (Livingston et al., 1964). The samples were analyzed for TVFA concentration (Barnett and Reid, 1957), total N (Micro-kjeldahl), TCA-insoluble protein N (Cline et al., 1958), residual N and food and protozoal N (Singh et al., 1968).

The data were analyzed statistically (Snedecor and Cochran, 1994), and were tested for significance by Duncan's multiple range test (Duncan, 1955) using SPSS 17.0 version.

## RESULTS AND DISCUSSION

The particulars of rumen pH, TVFA, NH<sub>3</sub>-N, total nitrogen (N), TCA-insoluble protein N, Residual N, and Food and Protozoal N in buffalo bulls as affected by feeding TMRs containing different R:C ratio supplemented with or without EFEs at 0, 2, 4, 6 and 8 h post-feeding are presented in **Table 1**.

### Rumen pH

The mean rumen pH was lower ( $P < 0.01$ ) in SRL of buffalo bulls fed T<sub>1</sub> when compared to other treatments (**Table 1**). Supplementation of EFEs in TMR (T<sub>2</sub>) increased the mean rumen pH as compared to the control, but the differences were not significant ( $P > 0.05$ ). Similar to these findings, most of the researchers reported that EFE supplementation had no effect on rumen pH (Pinos-Rodriguez et al., 2008; Singh and Das, 2009; Ganai et al., 2011; Bhasker et al., 2013; Lara Bueno et al., 2013; Torres et al., 2013). On the other hand, supplementation of live yeast culture in TMR (T<sub>3</sub>) increased ( $P < 0.01$ ) the rumen pH as compared to the control. It has been reported that by feeding yeast, the number of lactic acid utilizing bacteria increases in the rumen which resulted in low concentration of lactic acid and high pH. These results corroborated with the earlier reports of Garg et al. (2009), Ibrahim et al. (2012), Raj Kiran and Srinivas Kumar (2013) and Nehra et al. (2014). Furthermore, supplementation of both EFEs and live yeast culture in TMR (T<sub>4</sub>) increased ( $P < 0.01$ ) the rumen pH as compared to the control demonstrating the additive effect. These results corroborated with the findings of Can et al. (2007). In contrast, Lopuzanska-Rusek and Bilik (2011) reported that supplementation of both EFEs and live yeast culture in rations of dairy cows had no effect ( $P > 0.05$ ) on rumen pH. The mean pH values were highest ( $P < 0.01$ ) at 0 h and declined to minimum 4 h post-feeding, followed by a gradual increase. A similar trend was observed in all the treatments.

### Total volatile fatty acids (TVFA)

The mean TVFA (meq/L SRL) concentration was lower ( $P < 0.01$ ) in SRL of buffalo bulls fed T<sub>1</sub> when compared to other treatments (**Table 1**). Supplementation of EFE in TMR (T<sub>2</sub>) increased ( $P < 0.01$ ) the TVFA concentration

**Table 1.** Rumen fermentation pattern in buffalo bulls fed TMR supplemented with EFE and/or live yeast culture.

Diet/Hour	pH	TVFA (meq/L)	NH <sub>3</sub> -N (mg/100 mL)	Total N (mg/100 mL)	TCA-IPN (mg/100 mL)	Residual N (mg/100 mL)	Food & Protozoal N (mg/100 mL)
<b>Dietary Treatment</b>							
T <sub>1</sub>	6.81 <sup>a</sup>	81.56 <sup>a</sup>	10.12 <sup>a</sup>	75.75 <sup>a</sup>	25.40 <sup>a</sup>	22.41 <sup>a</sup>	17.81 <sup>a</sup>
T <sub>2</sub>	6.91 <sup>ab</sup>	86.51 <sup>b</sup>	10.97 <sup>b</sup>	81.40 <sup>b</sup>	27.03 <sup>b</sup>	24.60 <sup>b</sup>	18.81 <sup>ab</sup>
T <sub>3</sub>	6.95 <sup>bc</sup>	87.86 <sup>c</sup>	11.15 <sup>bc</sup>	83.25 <sup>c</sup>	27.55 <sup>bc</sup>	25.24 <sup>c</sup>	19.31 <sup>ab</sup>
T <sub>4</sub>	7.01 <sup>c</sup>	89.46 <sup>d</sup>	11.33 <sup>c</sup>	85.00 <sup>d</sup>	28.33 <sup>c</sup>	25.76 <sup>c</sup>	19.59 <sup>b</sup>
	**	**	**	**	**	**	*
SEM	0.042	1.706	0.268	2.006	0.620	0.737	0.391
<b>Time of rumen liquor sampling (h)</b>							
0 h	6.88 <sup>d</sup>	69.75 <sup>a</sup>	8.97 <sup>a</sup>	68.63 <sup>a</sup>	23.69 <sup>b</sup>	22.53 <sup>a</sup>	13.44 <sup>a</sup>
2 h	6.78 <sup>b</sup>	91.02 <sup>c</sup>	12.60 <sup>c</sup>	88.94 <sup>d</sup>	31.03 <sup>d</sup>	25.59 <sup>c</sup>	19.72 <sup>b</sup>
4 h	6.74 <sup>a</sup>	100.39 <sup>d</sup>	13.64 <sup>d</sup>	99.19 <sup>e</sup>	35.97 <sup>e</sup>	27.80 <sup>d</sup>	21.78 <sup>c</sup>
6 h	6.78 <sup>c</sup>	90.50 <sup>c</sup>	10.56 <sup>b</sup>	80.38 <sup>c</sup>	25.75 <sup>c</sup>	23.50 <sup>b</sup>	20.56 <sup>bc</sup>
8 h	6.86 <sup>d</sup>	80.09 <sup>b</sup>	8.69 <sup>a</sup>	69.63 <sup>b</sup>	18.94 <sup>a</sup>	23.09 <sup>ab</sup>	18.91 <sup>b</sup>
	**	*	**	**	**	**	**
SEM	0.035	2.494	0.462	2.786	1.377	0.543	0.682

<sup>a</sup> ( $P \leq 0.05$ ), <sup>\*</sup> ( $P \leq 0.01$ ), N=Nitrogen, TVFA=Total Volatile Fatty Acids, IPN=Insoluble Protein Nitrogen, SEM=Standard Error of the Mean

as compared to the control animal. The increased TVFA concentration observed with EFEs supplementation could be a result of higher availability of fermentable soluble carbohydrates due to increased fibrolytic activity in rumen. The present results were in agreement with the findings of previous workers (Gaafar et al., 2010; Bhasker et al., 2013; Kholi and Aziz, 2014; Rajamma et al., 2014). Similarly, supplementation of live yeast culture in TMR (T<sub>3</sub>) increased ( $P < 0.01$ ) the TVFA concentration as compared to the control. The higher concentrations of TVFA in the rumen fluid could be attributed to stimulatory effect of yeast culture on viable and total bacterial population, which in turn enhanced the fermentation in the rumen and resulted in increased production of TVFA (Gurpreet Singh et al., 2008). These observations are very consistent with the findings of Ibrahim et al. (2012), Raj Kiran and Srinivas Kumar (2013), Nehra et al. (2014) and Ganai et al. (2015). Furthermore, supplementation of both EFEs and live yeast culture in TMR (T<sub>4</sub>) increased ( $P < 0.01$ ) the TVFA concentration as compared to the control. These results corroborated with the findings of Can et al. (2007) and Lopuszanska-Rusek and Bilik (2011) who reported that supplementation of both EFEs and live yeast culture had significant effect on TVFA concentration. Peak concentration of TVFA was recorded at 4 h post-feeding irrespective of the treatment, followed by a gradual decrease.

#### Ammonia nitrogen (NH<sub>3</sub>-N)

The mean NH<sub>3</sub>-N (mg/100 mL SRL) concentration was lower ( $P < 0.01$ ) in SRL of buffalo bulls fed T<sub>1</sub> when compared to other treatments (Table 1). The study indicated that supplementation of EFE in TMR (T<sub>2</sub>) increased ( $P < 0.01$ ) the rumen NH<sub>3</sub>-N concentration as compared to T<sub>1</sub>. These results are in corroboration with the findings of Bhasker et al. (2013), Kholi and Aziz

(2014) and Rajamma et al. (2014). In contrast, Gaafar et al. (2010) reported that EFE decreased ( $P < 0.01$ ) NH<sub>3</sub>-N concentration, while Pinos-Rodriguez et al. (2008), Singh and Das (2009) and Ganai et al. (2011) reported no effect of EFE on NH<sub>3</sub>-N concentration. This variation might be due to difference in environmental factors, feed type, feed allocation method, and type of enzyme blend (Sutton et al., 2003). Similarly, supplementation of yeast culture in TMR (T<sub>3</sub>) increased significantly ( $P < 0.01$ ) the concentration of NH<sub>3</sub>-N in SRL of buffalo bulls as compared to the control. This increased NH<sub>3</sub>-N concentration in SRL could be due to active degradation of proteins and hydrolysis of NPN substances in rumen. Similarly, significantly ( $P < 0.01$ ) higher NH<sub>3</sub>-N concentration in SRL on feeding complete diets supplemented with yeast culture were reported earlier (Raj Kiran and Srinivas Kumar, 2013). Furthermore, supplementation of both EFEs and live yeast culture in TMR (T<sub>4</sub>) increased ( $P < 0.01$ ) the NH<sub>3</sub>-N concentration as compared to the control. Similar findings were reported earlier (Can et al., 2007). In contrast, Lopuszanska-Rusek and Bilik (2011) reported that supplementation of both EFEs and live yeast culture in rations of dairy cows had no effect ( $P > 0.05$ ) on NH<sub>3</sub>-N concentration. Time of sampling had a significant ( $P < 0.01$ ) effect on NH<sub>3</sub>-N concentration, which peaked at 4 h post-feeding irrespective of the treatment, and then declined.

#### Total nitrogen

The total N concentration (mg/100 mL SRL) was lower ( $P < 0.01$ ) in SRL of buffalo bulls (Table 1) fed T<sub>1</sub> as compared to T<sub>2</sub>, T<sub>3</sub> or T<sub>4</sub>. Supplementation of EFE in TMR (T<sub>2</sub>) had increased ( $P < 0.01$ ) the total N concentration in SRL of buffalo bulls when compared to the control. These results are in line with the findings of Kholi and Aziz (2014) in goats, and Rajamma et al.

(2014) in buffalo bulls. Further, supplementation of yeast culture in TMR ( $T_3$ ) increased ( $P<0.01$ ) the total N concentration when compared to  $T_1$ . Raj Kiran and Srinivas Kumar (2013), Nehra et al. (2014) and Ganai et al. (2015) also reported similar findings. Furthermore, supplementation of both EFE and live yeast culture in TMR ( $T_4$ ) increased ( $P<0.01$ ) the total N concentration as compared to the control. The higher total N concentration observed on feeding TMR supplemented with EFE and/or live yeast culture might be a result of better degradation of protein in the rumen due to efficient fermentation, as a result of EFE and/or live yeast culture supplementation. However, the peak concentration of total N was recorded at 4 h post-feeding followed by a gradual decrease.

### TCA insoluble nitrogen

The concentration of TCA insoluble nitrogen (mg/100 mL SRL) (Table 1) was lower ( $P<0.01$ ) in SRL of buffalo bulls fed  $T_1$  as compared to other treatments. The patterns of TCA insoluble nitrogen was similar to that of total N recorded in the present study. It is observed that supplementation of EFes in the TMR ( $T_2$ ) had increased significantly ( $P<0.01$ ) the concentration of TCA insoluble N as compared to  $T_1$ . These results are very consistent with the findings reported earlier (Rajamma et al., 2014). Further, it is observed that supplementation of yeast culture in TMR ( $T_3$ ) increased significantly ( $P<0.01$ ) the concentration of TCA insoluble N as compared to the control. Similar to these findings, Bhima et al. (2009) in Murrah buffalo steers, Garg et al. (2009) in sheep, Nehra et al. (2014) in kids reported significantly higher TCA insoluble N on yeast culture based complete diets. Further, higher levels of TCA insoluble N observed in SRL of buffalo bulls fed yeast culture supplemented TMR might be due to higher incorporation of  $NH_3$ -N into microbial protein yield due to yeast supplementation (Carro et al., 1992), which was confirmed by greater microbial protein yield and microbial true protein reaching the duodenum (Erasmus, 1991). Similarly, supplementation of both EFes and live yeast culture in TMR ( $T_4$ ) resulted in increased ( $P<0.01$ ) TCA insoluble N concentration as compared to the control. Time of sampling had a significant ( $P<0.01$ ) effect on TCA insoluble N concentration, and it reached peak at 4 h post-feeding irrespective of the treatment.

### Residual nitrogen

The residual N concentration (Table 1) was lower ( $P<0.01$ ) in SRL of buffalo bulls fed  $T_1$  as compared to other treatments. Supplementation of EFes in TMR ( $T_2$ )

increased ( $P<0.01$ ) the residual N concentration in SRL of buffalo bulls as compared to  $T_1$ . Similar to these findings, Rajamma et al. (2014) reported that supplementation of EFE in TMR irrespective of the R:C ratio had increased ( $P<0.01$ ) the residual N concentration in buffalo bulls. Further, it is observed that supplementation of yeast culture in TMR ( $T_3$ ) increased ( $P<0.01$ ) the residual N concentration in buffalo bulls as compared to the control. These results are in line with the findings of Raj Kiran and Srinivas Kumar (2013). Furthermore, supplementation of both EFE and live yeast culture in TMR ( $T_4$ ) increased ( $P<0.01$ ) the residual N concentration as compared to the control. Higher concentration of total N in the rumen fluid might have influenced the residual N concentration in bulls. Time of sampling had a significant ( $P<0.01$ ) effect on residual N concentration. Peak concentration of residual N was recorded at 4 h post-feeding, and declined slowly thereafter, following the trend similar to total N and TCA insoluble nitrogen.

### Food and Protozoal nitrogen

The food and protozoal N concentration (Table 1) in buffalo bulls fed TMRs supplemented with EFes and/or live yeast culture indicated that the mean food and protozoal N (mg/100 mL SRL) concentration was lower ( $P<0.01$ ) in SRL of buffalo bulls fed  $T_1$  as compared to those fed in  $T_4$ . Supplementation of EFes in TMR ( $T_2$ ) had increased the concentration of food and protozoal N as compared to  $T_1$ , but the difference was not statistically significant ( $P>0.05$ ). In contrast, Rajamma et al. (2014) reported that supplementation of EFes in TMRs containing different R:C ratios increased ( $P<0.01$ ) the food and protozoal N concentration in buffalo bulls. Similarly, supplementation of yeast culture in TMR ( $T_3$ ) had no effect ( $P>0.05$ ) on food and protozoal N concentration, which was supported by Srinivas Kumar et al. (2011). Supplementation of both EFes and live yeast culture in TMR ( $T_4$ ) increased ( $P<0.05$ ) the residual N concentration as compared to the control animal. Time of sampling had a significant ( $P<0.01$ ) effect on residual N concentration, and the peak concentration was recorded at 4 h post-feeding, followed by a gradual decrease.

## CONCLUSION

Groundnut haulms can be incorporated in TMR up to 70%, and can be fed to buffalo bulls. Further, supplementation of EFes and/or live yeast culture in TMR has a positive effect as evidenced by increasing concentration of rumen metabolites in SRL of bulls.

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