RESEARCH ARTICLE

In Vitro Rumen Fermentation of Maize Hay and Bajra Straw Based Total Mixed Ration Containing Different Feed Additives

Sneh D. Patel[1*], Deendayal Garg[1], Harish H. Savsani[1], Jignesh A. Chavda[1], Arun K. Sharma[2], Sanket M. Kalam[1]

Abstract

The present study was conducted to investigate the efficacy of different feed additives on rumen fermentation of total mixed ration. TMR was prepared by taking maize hay, bajra straw and concentrate in the ratio of 30:30:40, and additives monensin 30 mg/kg (T_1), cellulase 0.5%, (10,00,000 IU/g) (T_2), xylanase 0.5% (1,50,000 IU/g) (T_3), chitosan 1% (T_4), sodium bicarbonate 1% (T_5), magnesium oxide 1% (T_6), combination of sodium bicarbonate + magnesium oxide 0.5% each (T_7) and combination of cellulose + xylanase 0.5% each (T_8) were added as eight treatments. Rumen liquor was collected from two adult Surti goats 2 h of post-feeding. All post-fermentation parameters were measured after 24 h of incubation. pH was significantly ($p<0.01$) increased in T_7 (6.87±0.03) as compared to control and it was at par with T_5 (6.83±0.03) and T_6 (6.83±0.03). TVFAs (mMol/dL) was significantly ($p<0.01$) increased in T_8 (11.64±0.24) as compared to other treatments and control. This was followed by T_2 (9.87±0.48), T_3 (9.16±0.16) and T_4 (9.47±0.61) which also had significantly ($p<0.01$) higher TVFAs. NH_3-N (mg/dL) was significantly ($p<0.01$) increased in T_8 (48.67±3.52) as compared to control and it was at par with T_2 (43.33±2.40). Total-N was significantly ($p<0.01$) increased in T_8 (99.87±5.19) as compared to control and it was at par with T_2 (96.13±5.19), T_3 (91.47±4.06) and T_4 (92.40±4.27). TCA-N was significantly ($p<0.01$) increased in T_8 (70.93±4.93) as compared to control and it was at par with T_2 (65.33±3.36) and T_4 (60.67±4.06). Looking to the overall results it could be inferred that combination of cellulase and xylanase is the best feed additives among all and can be incorporated in the feed at the level of 0.5% each to alter the rumen fermentation & efficient nutrient utilization.

Key words: Feed additives, Goat, Rumen fermentation, Total mixed ration.

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Introduction

Feed additives (probiotics, prebiotics, ionophores, antioxidants, enzymes and hormones) are non-nutritive substances that in small quantities stabilize feed and improve growth and performance of individual. Feeding additives alter rumen fermentation by utilizing more nutrients and improve livestock productivity. Exogenous fibrolytic enzymes (EFE) enhance the microbial activities in gut which ultimately increase the production of TVFAs and NH_3-N (Rajamma *et al.,* 2014). Monensin is a selective antimicrobial agent that increases feed efficiency by suppressing the majority of ruminal bacteria that produce lactate. Monensin increases ruminal pH (McGuffey *et al.,* 2001). Chitosan is a natural biopolymer generated from chitin deacetylation. Chitosan, which has mechanical similarities to monensin, alters the profiles of TVFAs by enhancing propionate and decrease acetate and methane emissions (Haryati *et al.,* 2019). A proper population of rumen microorganisms is necessary for the proper fermentation of feed particles in rumen. The ideal growth of rumen bacteria depends on a number of physiochemical characteristics of rumen including pH, anaerobic conditions, temperature and buffering capacity. Sodium bicarbonate and magnesium oxide are commonly used as buffering agents which increase rumen pH. Thus, this study was planned to explore the efficacy of different feed additives on rumen fermentation of maize hay and bajra straw based TMR.

[1]Department of Animal Nutrition, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Junagadh-362001, Gujarat, India.

[2]Department of Veterinary Physiology and Biochemistry, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Junagadh-362001, Gujarat, India.

Corresponding Author: Sneh D. Patel, Department of Animal Nutrition, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Junagadh-362001, Gujarat, India. e-mail: patelsneh0702@gmail.com

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Materials and Methods

Preparation of TMR

TMR was prepared by taking maize hay, bajra straw and concentrate in the ratio of 30:30:40 and was used as substrate for experiment. Monensin (T_1), cellulase (10,00,000 IU/g) (T_2), xylanase (1,50,000 IU/g) (T_3), chitosan (T_4), sodium bicarbonate (T_5), magnesium oxide (T_6), combination of sodium bicarbonate+magnesium oxide (T_7) and combination of cellulase+xylanase (T_8) were added in TMR at the level of 30 mg/kg, 0.5%, 0.5%, 1%, 1%, 1%, 0.5% (Each) and 0.5% (Each) on DM basis, respectively.

Rumen Liquor Collection from Donor Animal

Two adult male Surti goats of same age and uniform conformation were selected as donor of rumen liquor for *in vitro* study. The animals were given prophylactic dosage of broad spectrum anthelmintics. The nutrient requirements of the donor animals were met by feeding as per ICAR (2013) feeding standards. Rumen liquor was collected at 2 h post-feeding and strained through a four-layered muslin cloth and referred as Strained Rumen Liquor (SRL). The experiment was approved by IAEC (Protocol no.: KU-JVC-IAEC-LA-103-23).

Proximate Composition and Fibre Fractions of TMR

Samples of TMR prepared were analysed for proximate composition as per the AOAC (2005) and fibre fraction according to Goering and Van Soest (1970).

Estimation of Rumen Fermentation Parameters

TMRs with different feed additives were also analysed for post-fermentation parameters such as pH was recorded by using pen-type pH meter, TVFAs were determined by method of Barnett and Reid (1957) by using Markham's steam distillation apparatus and ammonia nitrogen was estimated by Conway's micro-diffusion method (Conway, 1957).

Statistical Analysis

The data were collected and statistically analyzed by one-way analysis of variance and significance of mean differences was tested by Duncan's New Multiple Range Test (DNMRT) as per procedures suggested by Snedecor and Cochran (1994).

Results and Discussion

Proximate composition of TMR contained 89.18, 90.68, 11.26, 1.64, 26.98, 50.80 and 9.32% of DM, OM, CP, EE, CF, NFE and TA, respectively. Fibre fractions of TMR contained 58.30, 32.70, 22.53, 25.60 and 7.26% of NDF, ADF, cellulose, hemicellulose and lignin, respectively.

Efficacies of different feed additives on pH, TVFAs and NH_3-N are presented in Table 1. Significantly ($p<0.01$) higher pH was seen in T_7 (6.87±0.03) as compared to control (6.73±0.03), while, rest of the treatments had no significant effect. The addition of monensin, cellulase, xylanase and their combination as well as chitosan had no significant impact on pH in the current investigation. These results were in contrast with earlier research by Elghandour *et al.* (2015), e Silva *et al.* (2021) and Seankamson *et al.* (2021), wherein EFEs, monensin and chitosan significantly decreased pH. These results were also in contrast with earlier research by Bach *et al.* (2018), Farghaly *et al.* (2019) and Wang *et al.* (2019), who reported that sodium bicarbonate and magnesium oxide supplementation significantly increased pH.

TVFAs (mMol/dL) was significantly ($p<0.01$) increased in T_8 (11.64±0.24) as compared to other treatments and control. This was followed by T_2 (9.87±0.48), T_3 (9.16±0.16) and T_4 (9.47±0.61) which also had significantly ($p<0.01$) higher TVFAs. Cellulase, xylanase and its combination as well as chitosan significantly increased TVFAs. This might have occurred due to higher microbial activities of rumen microbes, which alter the rumen fermentation pattern. These results are consistent with earlier research by Dey *et al.* (2014), Gemeda *et al.* (2014) and Abd-Elkader *et al.* (2019). They stated that Exogenous fibrolytic enzymes and chitosan significantly increased TVFAs.

NH_3-N (mg/dL) was significantly ($p<0.01$) increased in T_8 (48.67±3.52) as compared to control (32.67±1.76) and it was at par with T_2 (43.33±2.40). Cellulase and its combination with xylanase significantly increased NH_3-N. This might be due to higher microbial activities of rumen microbes that alter the rumen fermentation pattern. These results were consistent with earlier research by Rajamma *et al.* (2014), Sujani *et al.* (2016) and Sujani *et al.* (2017). They stated that Exogenous fibrolytic enzymes significantly increased NH_3-N. Chitosan in the present study significantly increased NH_3-N, which may be due to increased microbial population in rumen and alter rumen fermentation pattern. These results were in contrast with earlier research by Dias *et al.* (2017), Pereira *et al.* (2019) and Seankamson *et al.* (2021), who stated that chitosan non-significantly ($p>0.05$) increased NH_3-N.

Table 1: pH, TVFAs and NH_3-N of different treatments

Treatments	Parameters		
	pH**	NH3-N** (mg/dL)	TVFAs** (mMol/dL)
T_0 (Control)	$6.73^{bc} \pm 0.03$	$32.67^{cde} \pm 1.76$	$6.62^{c} \pm 0.23$
T_1	$6.63^{c} \pm 0.03$	$36.67^{bcd} \pm 2.90$	$7.07^{c} \pm 0.35$
T_2	$6.63^{c} \pm 0.03$	$43.33^{ab} \pm 2.40$	$9.87^{b} \pm 0.48$
T_3	$6.67^{c} \pm 0.03$	$38.67^{bc} \pm 1.76$	$9.16^{b} \pm 0.16$
T_4	$6.63^{c} \pm 0.03$	$40.00^{b} \pm 3.05$	$9.47^{b} \pm 0.61$
T_5	$6.83^{ab} \pm 0.03$	$31.33^{de} \pm 1.33$	$7.38^{c} \pm 0.65$
T_6	$6.83^{ab} \pm 0.03$	$28.67^{e} \pm 0.66$	$6.09^{c} \pm 0.49$
T_7	$6.87^{a} \pm 0.03$	$40.67^{b} \pm 0.66$	$6.62^{c} \pm 0.62$
T_8	$6.63^{c} \pm 0.03$	$48.67^{a} \pm 3.52$	$11.64^{a} \pm 0.24$
p value	<0.01	<0.01	<0.01

^{abcde}Means with different superscripts within column differ significantly from each other (**p<0.01).

pH- potential of hydrogen, TVFAs- total volatile fatty acids, NH_3-N- ammonia nitrogen.

Table 2: Total-N, TCA-N and NPN of different treatments

Treatments	Parameters		
	Total-N (mg/dL)	TCA-N (mg/dL)	NPN (mg/dL)
T_0 (Control)	$70.93^{e} \pm 2.46$	$48.53^{c} \pm 1.86$	22.40 ± 1.61
T_1	$83.07^{bcde} \pm 6.53$	$55.07^{bc} \pm 2.46$	28.00 ± 5.82
T_2	$96.13^{ab} \pm 5.19$	$65.33^{ab} \pm 3.36$	30.80 ± 3.23
T_3	$91.47^{abcd} \pm 4.06$	$55.07^{bc} \pm 3.36$	36.40 ± 1.61
T_4	$92.40^{abc} \pm 4.27$	$60.67^{abc} \pm 4.06$	31.73 ± 5.67
T_5	$76.53^{de} \pm 5.67$	$50.40^{c} \pm 4.27$	26.13 ± 1.86
T_6	$75.60^{e} \pm 5.82$	$48.53^{c} \pm 5.67$	27.07 ± 6.53
T_7	$80.27^{cde} \pm 2.46$	$52.27^{bc} \pm 4.93$	28.00 ± 4.27
T_8	$99.87^{a} \pm 5.19$	$70.93^{a} \pm 4.93$	28.93 ± 2.46
p value	0.0042	0.0098	0.5329

[abcde]Means with different superscripts within column differ significantly from each other ($p<0.01$).

NPN- non protein nitrogen.

The efficacies of different feed additives on Total-N, TCA-N and NPN are presented in Table 2. Total-N (mg/dL) was significantly ($p<0.01$) higher in T_8 (99.87±5.19) as compared to control and it was at pat with T_2 (96.13±5.19), T_3 (91.47±4.06) and T_4 (92.40±4.27), while, T_1, T_5, T_6 and T_7 were statistically similar with control. Present study revealed that cellulase, xylanase and its combination significantly increased Total-N. This might have occurred due to higher microbial activities of rumen microbes that alter the rumen fermentation pattern as was seen with other parameters too. These results were consistent with earlier research by Rajamma *et al.* (2014), who stated that EFEs significantly increased Total-N.

Significantly ($p<0.01$) higher TCA-N (mg/dL) was seen in T_8 (70.93±4.93) as compared to control (48.53±1.86) and it was at par with T_2 (65.33±3.36) and T_4 (55.07±3.36). Present findings revealed cellulase and its combination with xylanase significantly increased TCA-N. This might be due to higher microbial activities of rumen microbes that alter the rumen fermentation pattern. These results are consistent with earlier research by Rajamma *et al.* (2014), who stated that supplementing EFEs significantly increased TCA-N. Statistical analysis revealed that none of the feed additives had significant ($p>0.05$) effect on NPN as compared to control.

Conclusions

Cellulase alone and combination of cellulase and xylanase at the level of 0.5% each significantly increased NH_3-N, TVFAs, Total-N and TCA-N as compared to control when added to maize hay and bajra straw based total mixed ration. Xylanase at the level of 0.5% in TMR significantly increased TVFAs and Total-N, and chitosan at the level of 1% significantly increased NH_3-N, TVFAs and Total-N as compared to control. Looking to the overall results it could be inferred that combination of cellulase and xylanase is the best feed additives among all, and can be incorporated in the feed at the level of 0.5% each to alter the rumen fermentation & efficient nutrient utilization.

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