RESEARCH ARTICLE

Effect of Different Feed Additives on *In Vitro* Total Gas and Methane Production of Maize Hay and Bajra Straw based TMR

Sneh D. Patel[1]*, Deendayal Garg[1], Harish H. Savsani[1], Arun K. Sharma[2], Kalpesh K. Sorathiya[3], Sanket M. Kalam[1]

Abstract

The present study was conducted to investigate the efficacy of different feed additives in TMR on *in vitro* total gas production and methane production. Total mixed ration was prepared by mixing of bajra straw, maize hay and concentrate in the ratio of 30:30:40. Feed additives included in TMR on DM basis were Monensin 30 mg/kg (T_1), cellulase (10,00,000 IU/g) 0.5% (T_2), xylanase (1,50,000 IU/g) 0.5% (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 cellulase + xylanase 0.5% each (T_8). Rumen liquor was collected from two adult Surti goats each 2 h post-feeding of TMR with above additives. Result revealed that *in vitro* total gas production (mL/200 mg) was significantly ($p<0.01$) increased in TMRs supplemented with cellulase (28.39±0.51), xylanase (27.33±0.54) and its combination (32.92±0.62) as well as chitosan (26.82±0.99) as compared to control (22.08±0.56). *In vitro* methane production (%) was significantly ($p<0.01$) increased in TMRs supplemented with magnesium oxide (18.15±1.02) and its combination with sodium bicarbonate (17.91±0.15) as compared to control (16.59±0.19). Whereas, *in vitro* methane production (mL/200 mg & mL/100 mg) was significantly ($p<0.05$) increased in TMR supplemented with combination of cellulase and xylanase (5.13±0.13 & 2.56±0.06) as compared to control (3.66±0.05 & 1.83±0.02). While, ratios of *in vitro* methane production to fermentation parameters were significantly ($p<0.01$) improved in combination of cellulase and xylanase as compared to control.

Key words: Feed additives, Methane production, Total gas production, Total mixed ration.

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Introduction

In current circumstances, ruminants are fed mainly with roughages and concentrates in which roughages like crop residue, pasture grass are the primary source of nourishment for ruminants. Agricultural and agro-industrial activities produce thousands of tonnes by-products per year in which crop residues are major by-products (Graminha *et al.,* 2008). Population of ruminants is increasing day by day which lead to feed shortage, so that farmers feed agro-industrial by-products which interfere with feed utilization, reduce feed intake and digestibility of feed. For better utilization of feed, different feed additives are used by farmers. Feed additives in ruminant diet may increase the digestibility of low-quality fibrous feed and ultimately enhance the productivity of ruminant (Naidu and Reddy, 2003).

The cellulose and hemicellulose are typically the targets of the fibrolytic enzymes, which hydrolyse the structural polysaccharides of the plant cell wall. Supplementations of exogenous fibrolytic enzymes (EFEs) once enhance the fibre degradation, ultimately increases the total gas and methane production (Selzer *et al.,* 2021). Monensin exhibits selective antimicrobial activity and improves feed efficiency by inhibiting most lactate-producing ruminal bacteria. Monensin is frequently employed as a growth promoter to inhibit ruminal methanogenesis (Duffield *et al.,* 2008). Chitosan is a natural biopolymer generated from chitin deacetylation which decreases methanogenic bacterial growth and leads to increase propionate and decrease acetate as well as CH_4 emissions (Haryati *et al.,* 2019).

[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.

[3]Animal Nutrition Research Station, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Anand-388001, 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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Buffering agents (sodium bicarbonate and magnesium oxide) increase the ruminal pH that effectively neutralizes the rumen acidity to improve the efficiency of fibre digestion (Rogers *et al.,* 1982). Thus, this study was planned to explore the efficacy of different feed additives on *in vitro* total gas and methane production of maize hay and bajra straw based total mixed ration.

Materials and Methods

Preparation of TMR

Maize hay, bajra straw and concentrate were ground to pass through 1 mm screen using a willey mill. 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 30 mg/kg, cellulase (10,00,000 IU/g) 0.5%, xylanase (1,50,000 IU/g) 0.5%, chitosan 1%, sodium bicarbonate 1%, magnesium oxide 1%, combination of sodium bicarbonate + magnesium oxide 0.5% each and combination of cellulase + xylanase 0.5% each was added on DM basis in TMR and were designated as T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, respectively.

Donor Animals and Collection of Rumen Liquor

Rumen liquor was collected from two adult Surti goats each of same age and uniform confirmation. Nutrient requirements of donor animals were met by feeding as per ICAR (2013) feeding standard. Nutrient Requirements of Sheep, Goat and Rabbit. 3rd edition Indian council of Agricultural Research, New Delhi. Rumen liquor was collected at 2 h post-feeding and strained through four-layered muslin cloth which was referred as Strained Rumen Liquor (SRL). The experiment was approved by IAEC (Protocol no.: KU-JVC-IAEC-LA-103-23).

Estimation of *In Vitro* Total Gas Production (IVTGP)

The *in vitro* gas production was estimated as per the method of Menke and Steingass (1988). About 0.2 g of the substrates were taken in calibrated glass syringes and incubated in the syringes at 39°C for overnight. *In vitro* medium was prepared by mixing 400 mL distilled water, 200 mL macro-mineral solution, 200 mL buffer solution, 1 mL micro-mineral solution, 1 mL resazurin solution and 2.40 mL reduction solution which was prepared fresh prior to incubation. The medium was pre-warmed to 39°C and saturated with CO_2 through the solution until the blue color of the medium became pink or clear. The rumen liquor buffer medium was prepared by mixing of SRL (Strained rumen liquor) and medium mixture at the ratio of 1:2. Again CO_2 was passed through the solution for about 15 min. About 30 mL of rumen liquor buffer medium was injected to each calibrated glass syringe. The syringes were shaken gently, residual air or air bubbles were removed and then outlet was closed. Then initial level of piston was recorded and the syringes were placed in an incubator at 39°C and periodically shaken for 24 h. Syringes containing rumen liquor buffer solution without samples were also run as blank. The gas production was measured after 24 h of incubation.

Estimation of *In Vitro* Methane Production (IVMP)

The *in vitro* methane production was estimated as per the method of Johnson and Johnson (1995). After 24 h of incubation of calibrated glass syringes, gas samples were collected in vacuumed glass vials and were analysed for CH_4 using Gas Chromatography by injecting sample from each vial and methane concentration was determined against standard. Column temperature was maintained at 50°C, in which nitrogen was used as a carrier gas, with flow rate of 30 mL/min. Calibration was completed using standard. Peak area was integrated using Thermo Fisher integrator.

Statistical Analysis

The data were collected and statistically analyzed by one-way analysis of variance (Snedecor and Cochran, 1994). Significance of mean differences was tested by Duncan's New Multiple Range Test.

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.

Efficacy of different feed additives on IVTGP and IVMP are presented in Table 1. Results revealed that IVTGP (mL/200 mg) was significantly ($p<0.01$) increased in T_8 (32.92±0.62) as compared to other treatments and control (22.08±0.56). This was followed by T_2 (28.39±0.51), T_3 (27.33±0.54) and T_4 (26.82±0.99), which also showed significantly ($p<0.01$) higher values than the control, whereas T_1, T_5, T_6 and T_7 had no significant effect as compared to control. In the present study cellulase, xylanase and its combination significantly increased IVTGP (mL/200 mg). This might have occurred due to EFEs which enhance the degradation of lignocellulose bond of the feed particles. Because of higher degradability it increases the total gas production. These results were consistent with earlier research by Lopez *et al.* (2016), Vallejo *et al.* (2016), Lunagariya *et al.* (2018) and Selzer *et al.* (2021). Chitosan also significantly increased IVTGP (mL/200 mg). This might be due to increment of microbial population in rumen, which ultimately increases the degradation of feed particles and thereby the total gas production. These results were however in contrast with earlier researchers (Li *et al.*, 2013; Wencelova *et al.,* 2014), who stated that chitosan significantly decreased IVTGP. The IVMP (%) was significantly ($p<0.01$) increased in T_6 (18.15±1.02) and T_7 (17.91±0.15) as compared to other treatments and control (16.59±0.19).

IVMP (mL/200 mg & mL/100 mg) was significantly ($p<0.05$) increased in T_8 as compared to control and it was at par with T_2, T_3 and T_4. Results revealed that combination of cellulase and xylanase significantly increased IVMP (mL/200 mg &

Table 1: *In vitro* gas production (IVTGP) and *in vitro* methane production (IVMP) of different treatments

Treatments	Parameters			
	IVTGP** (mL/200 mg)	IVMP** (%)	IVMP* (mL/200 mg)	IVMP* (mL/100 mg)
T_0 (Control)	$22.08^{cd} \pm 0.56$	$16.59^{b} \pm 0.19$	$3.66^{b} \pm 0.05$	$1.83^{b} \pm 0.02$
T_1	$23.87^{c} \pm 1.38$	$15.25^{b} \pm 0.01$	$3.64^{b} \pm 0.21$	$1.82^{b} \pm 0.10$
T_2	$28.39^{b} \pm 0.51$	$15.68^{b} \pm 0.50$	$4.46^{ab} \pm 0.34$	$2.23^{ab} \pm 0.17$
T_3	$27.33^{b} \pm 0.54$	$16.04^{b} \pm 0.15$	$4.39^{ab} \pm 0.29$	$2.19^{ab} \pm 0.14$
T_4	$26.82^{b} \pm 0.99$	$15.69^{b} \pm 0.12$	$4.21^{ab} \pm 0.14$	$2.10^{ab} \pm 0.07$
T_5	$21.69^{cd} \pm 0.93$	$16.45^{b} \pm 0.29$	$3.56^{b} \pm 0.41$	$1.78^{b} \pm 0.20$
T_6	$20.66^{d} \pm 0.58$	$18.15^{a} \pm 1.02$	$3.77^{b} \pm 0.49$	$1.89^{b} \pm 0.24$
T_7	$22.45^{cd} \pm 1.45$	$17.91^{a} \pm 0.15$	$4.02^{b} \pm 0.25$	$2.01^{b} \pm 0.12$
T_8	$32.92^{a} \pm 0.62$	$15.59^{b} \pm 0.21$	$5.13^{a} \pm 0.13$	$2.56^{a} \pm 0.06$
p value	<0.01	<0.01	0.0244	0.0244

^{a,b,c,d} Means with different superscripts within column differ significantly (*p<0.05) (**p<0.01).

Table 2: Ratios of methane production to fermentation parameters of different treatments

Treatments	Parameters			
	IVMP: IVTGP**	IVMP: TVFAs**	IVMP: ME**	IVMP: IVNDFD**
T_0 (Control)	$0.75^{b} \pm 0.02$	$2.51^{bc} \pm 0.06$	$3.63^{b} \pm 0.10$	$0.40^{b} \pm 0.011$
T_1	$0.64^{c} \pm 0.03$	$2.17^{cd} \pm 0.10$	$3.16^{c} \pm 0.12$	$0.34^{c} \pm 0.011$
T_2	$0.55^{cd} \pm 0.01$	$1.60^{e} \pm 0.13$	$2.85^{cd} \pm 0.09$	$0.29^{de} \pm 0.008$
T_3	$0.58^{c} \pm 0.06$	$1.75^{de} \pm 0.04$	$3.00^{c} \pm 0.02$	$0.34^{c} \pm 0.003$
T_4	$0.58^{c} \pm 0.02$	$1.67^{e} \pm 0.10$	$2.98^{c} \pm 0.09$	$0.31^{cd} \pm 0.003$
T_5	$0.76^{b} \pm 0.04$	$2.27^{c} \pm 0.22$	$3.65^{b} \pm 0.16$	$0.42^{b} \pm 0.017$
T_6	$0.87^{a} \pm 0.03$	$3.00^{a} \pm 0.18$	$4.15^{a} \pm 0.16$	$0.47^{a} \pm 0.014$
T_7	$0.80^{ab} \pm 0.05$	$2.75^{ab} \pm 0.22$	$3.88^{ab} \pm 0.18$	$0.43^{b} \pm 0.011$
T_8	$0.47^{d} \pm 0.01$	$1.34^{e} \pm 0.03$	$2.53^{d} \pm 0.05$	$0.27^{e} \pm 0.003$
p value	<0.01	<0.01	<0.01	<0.01

^{abcde} Means with different superscripts within column differ significantly from each other (**p<0.01).
IVMP- *in vitro* methane production, IVTGP- *in vitro* total gas production
TVFAs- total volatile fatty acids, ME- metabolizable energy, IVNDFD - *in vitro* neutral detergent fibre degradability.

mL/100 mg). This might have occurred due to enhanced degradation of lignocellulose bond of the feed particles by EFEs, and thereby increase in the methane production. These results were consistent with earlier research by Giraldo *et al.* (2007), Chung *et al.* (2012) and Selzer *et al.* (2021), who reported significantly increased IVMP with a combination of cellulase and xylanase in the diet.

Ratios of methane production to fermentation parameters are presented in Table 2. Ratios of IVMP to IVTGP, ME and IVNDFD were significantly (p<0.01) improved in T_8 as compared to other treatments and control. This was followed by T_2, T_3 and T_4 which also showed significant (p<0.01) improvement as compared to control. IVMP: TVFAs was significantly (p<0.01) improved in T_8 as compared to control. Ratio of methane production to fermentation parameters described the rate of methane production. Low ratio of IVMP to IVTGP and TVFAs indicated less methane production from total gas production & total volatile fatty acids. Low ratio of IVMP to ME indicated lesser loss of energy due to methane production, hence more ME was capable for transformation into body. Also, low ratio of IVMP to IVNDFD indicated that the digestibility of poor-quality fibre increased but simultaneously methane production decreased. Result revealed that T_8 has low ratio of methane production to fermentation parameters. This might be due effect of EFEs that alter the microbial population which ultimately reduce the methane production.

Conclusion

The results of the present *in vitro* study indicated that supplementation of cellulase, xylanase alone and in combination at the level of 0.5% each, and chitosan at the level of 1% in TMR significantly improved *in vitro* total gas production as compared to control. The results indicated that supplementation of TMR in combination of cellulase and xylanase at the level of 0.5% each significantly increased *in vitro* methane production also. Cellulase, xylanase and combination of cellulase and xylanase at the level of 0.5% each and chitosan at the level of 1% significantly decreased ratios of methane production to fermentation parameters.

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References

Chung, Y.H., Zhou, M., Holtshausen, L., Alexander, T.W., McAllister, T.A., Guan, L.L., & Beauchemin, K.A. (2012). A fibrolytic enzyme additive for lactating Holstein cow diets: ruminal fermentation, rumen microbial populations, and enteric methane emissions. *Journal of Dairy Science, 95*(3), 1419-1427.

Duffield, T.F., Rabiee, A.R., & Lean, I.J. (2008). A meta-analysis of the impact of monensin in lactating dairy cattle. *Journal of Dairy Science, 91*(6), 2328-2341.

Giraldo, L.A., Ranilla, M.J., Tejido, M.L., & Carro, M.D. (2007). Influence of exogenous fibrolytic enzymes and fumarate on methane production, microbial growth and fermentation in Rusitec fermenters. *British Journal of Nutrition, 98*(4), 753-761.

Graminha, E.B.N., Gonçalves, A.Z.L., Pirota, R.D.P.B., Balsalobre, M.A.A., Da Silva, R., & Gomes, E. (2008). Enzyme production by solid-state fermentation: Application to animal nutrition. *Animal Feed Science and Technology, 144*(1-2), 1-22.

Haryati, R.P., Jayanegara, A., Laconi, E.B., Ridla, M., & Suptijah, P. (2019). Evaluation of chitin and chitosan from insect as feed additives to mitigate ruminal methane emission. In: *AIP Conference Proceedings* (Vol. 2120, No. 1, p. 040008), AIP Publishing LLC.

Johnson, K.A., & Johnson, D.E. (1995). Methane emissions from cattle. *Journal of Animal Science, 73*(8), 2483-2492.

Li, C., Zhao, X., Cao, Y., Lei, Y., Liu, C., Wang, H., & Yao, J. (2013). Effects of chitosan on *in vitro* ruminal fermentation in diets with different forage to concentrate ratios. *Journal of Animal and Veterinary Advances, 12*(7), 839-845.

Lopez, D., Vázquez-Armijo, J.F., López-Villalobos, N., Lee-Rangel, H.A., Salem, A.Z.M., Borquez-Gastelum, J.L., & Rojo-Rubio, R. (2016). *In vitro* gas production of foliage from three browse tree species treated with different dose levels of exogenous fibrolytic enzymes. *Journal of Animal Physiology and Animal Nutrition, 100*(5), 920-928.

Lunagariya, P.M., Shah, S.V., Devalia, B.R., Patel, A.C., & Pandya, P.R. (2018). An *in vitro* dose optimization of exogenous fibrolytic enzymes in total mixed ration for crossbred cows. *International Journal of Current Microbiology and Applied Science, 7*(10), 330-338.

Menke, K.H., & Steingass, (1988). Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research and Development, 28,* 7-55.

Naidu, M.M., & Reddy Ramana, Y. (2003). Improving nutritive value of poor-quality roughages and agro-industrial by products. In: *Feed Processing Technology* (Eds. GVN Reddy, N. Krishna, VLK Prasad, J. Reddy and YR Reddy). Acharaya NG Ranga Agricultural University, Hyderabad Andhra Pradesh, India, p. 79-89.

Rogers, J.A., Davis, C.L., & Clark, J.H. (1982). Alteration of rumen fermentation, milk fat synthesis, and nutrient utilization with mineral salts in dairy cows. *Journal of Dairy Science, 65*(4), 577-586.

Selzer, K., Hassen, A., Akanmu, A.M., & Salem, A.Z.M. (2021). Digestibility and rumen fermentation of a high forage diet pre-treated with a mixture of cellulase and xylanase enzymes. *South African Journal of Animal Science, 51*(3), 399-406.

Snedecor, G.W., & Cochran, W.G. (1994). *Statistical Methods*. 8th edition. Oxford and IBH. New Delhi, India, pp. 503.

Vallejo, L.H., Salem, A.Z.M., Kholif, A.E., Elghangour, M.M.Y., Fajardo, R.C., Rivero, N., & Mariezcurrena, M.D. (2016). Influence of cellulase or xylanase on the *in vitro* rumen gas production and fermentation of corn stover. *Indian Journal Animal Science, 86(*1), 70-74.

Wencelova, M., Varadyova, Z., Mihalikova, K., Kisidayova, S., & Jalc, D. (2014). Evaluating the effects of chitosan, plant oils, and different diets on rumen metabolism and protozoan population in sheep. *Turkish Journal of Veterinary & Animal Sciences, 38*(1), 26-33.