RESEARCH ARTICLE

Efficacy of Different Feed Additives on *In Vitro* Degradability of Maize Hay and Bajra Straw Based Total Mixed Ration

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Abstract

The present study was conducted to investigate the efficacy of different feed additives (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 added 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) on *in vitro* degradability of total mixed ration prepared by taking maize hay, bajra straw and concentrate in the ratio of 30:30:40 and used as substrates for experiment. Rumen liquor was collected from two adult Surti goats 2 h post-feeding. Statistical analysis revealed that IVDMD (%), IVOMD (%), IVNDFD (%), IVADFD (%), TDOMR (%), IVTGP (mL/200 mg), SCFAs (mMol/200 mg), ME (MJ/kg) and NE (MJ/kg) were significantly ($p<0.01$) increased in TMR supplemented with combination of cellulase and xylanase (67.32±1.01, 69.97±1.29, 56.23±0.36, 53.01±1.64, 64.13±0.42, 32.92 ±0.62, 0.75±0.007, 6.16±0.09 and 3.74±0.05 respectively). This was followed by TMR supplemented with cellulase, xylanase and chitosan alone, which also had significantly ($p<0.01$) higher values of all these components as compared to control (52.34±0.31, 54.92±0.50, 41.48±1.63, 38.64±0.53, 49.41±1.20, 22.08±0.56, 0.49±0.01, 4.58±0.08 and 2.70±0.05, respectively). Hence a combination of cellulase and xylanase is recommended as feed additive in maize hay, bajra straw and concentrate based TMR.

Key words: Degradability, Feed additives, *In vitro*, Strained rumen liquor (SRL), Total mixed ration.

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Introduction

Now a day's population of ruminants is increasing day by day which is a major reason behind the feed shortage. Currently there is a net deficit of 35.60% for green fodder, 10.95% for dry fodder and 44.00% for concentrate (Singh *et al.,* 2022). In current circumstances, ruminants are fed with crop residue and other non-traditional feeds. Crop residue contains high fibre which reduces the palatability and digestibility of feeds which ultimately reduces the production and affects the income of farmers. For those different technologies such as physical, chemical, biological, biochemical, engineering and feed additives are used to improve the quality of roughages or crop residues and so on to enhance digestibility.

The use of exogenous fibrolytic enzymes (EFEs) is one of the major methods for improving feed ingredient digestibility. Most commonly used EFEs are cellulase and xylanase (Shekhar *et al.,* 2010; Kadam *et al.,* 2024; Patel *et al.,* 2025). Fibrolytic enzymes break down the complex fibrous feeds into their simpler compounds and improve rumen fermentation (Patel *et al.,* 2025). It was observed that feeding ruminants with EFEs-supplemented diet increases their ability to digest dry matter (DM), organic matter (OM), crude fibre (CF), neutral detergent fibre (NDF), cellulose, and hemicellulose (Morsy *et al.,* 2016; Kadam *et al.,* 2024). Ionophores are naturally occurring carboxylic polyether antibiotics. Most commonly used ionophore is monensin, which ultimately increases the population of Gram-negative bacteria (cellulolytic bacteria), and increases the digestibility and utilization of nutrients (Butaye *et al.,* 2003). Chitosan is a natural biopolymer generated from chitin deacetylation of the exoskeletons of some insects, arthropods, shell debris from crustaceans. The digestibility of dry matter, crude protein, and neutral detergent fibre were increased by chitosan (Dias *et al.,* 2017). Rumen is the natural fermentative chamber of ruminants. For the proper fermentation of feed particles rumen requires suitable population of rumen microbes. Buffering agents such as, sodium bicarbonate and magnesium oxide improved the efficiency of fibre digestion, microbial protein synthesis and OM utilization (Bach *et al.,* 2018). Thus, this study was planned to explore the efficacy of different feed additives on *in vitro* degradability of maize hay and bajra straw based total mixed ration.

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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) was added 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.

Donor Animals and Rumen Liquor Collection

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 feeding standards (2013). 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).

Estimation of Proximate Composition and Fibre Fractions

Samples of maize hay, bajra straw, concentrate and prepared different TMRs were analysed for proximate composition as per the AOAC (2005) and fibre fraction according to Geoering and Van Soest (1970).

Estimation of *In Vitro* Degradability

TMRs with different feed additives were analysed for *in vitro* degradability as per the Tilley and Terry method (1963).

Estimation of Truly Degradable Organic Matter in Rumen (TDOMR):

TMRs with different feed additives were analysed for truly degradable organic matter in rumen as per the Geoering and Van Soest (1970).

Estimation of *In Vitro* Total Gas Production

TMRs with different feed additives were analysed for *in vitro* total gas production as per the Menke and Steingass (1988). The gas production was measured after 24 h of incubation.

Estimation of Partitioning Factor (PF):

$$PF = \frac{\text{TDOMR (mg)}}{\text{Net gas production (mL)}}$$

Estimation of Short Chain Fatty Acids (SCFAs):

SCFAs (mMol/200 mg) = 0.0222* (mL gas at 24 h) – 0.00425

Estimation of Microbial Biomass Production (MBP):

MBP (mg) = TDOMR (mg) – (2.25 × net gas production), where constant 2.25 is the stoichiometric factor.

Estimation of Efficiency of Microbial Biomass Production (EMBP):

$$\text{EMBP (mg)} = \frac{\text{TDOMR (mg)} - (2.25 \times \text{net gas production})}{\text{TDOMR (mg)}} \times 100$$

Estimation of Metabolizable Energy (ME) and Net Energy (NE):

ME (MJ/kg DM) = 0.146 × GP + 0.007 × CP + 0.0224 × EE + 1.24

NE (MJ/kg DM) = 0.096 × GP + 0.0038 × CP + 0.000173 × EE^2 + 0.54

Where, GP = Net gas production (mL/200 mg DM)

Statistical Analysis

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

Results and Discussion

Proximate composition and fibre fractions (% DM basis) of bajra straw, maize hay, concentrate and TMR is presented in Table 1. The DM, OM, NFE and hemi-cellulose did not differ much between different ingredients and formulated TMR, however, the other components varied greatly, with mean values (%) of CP, EE, CF, TA, NDF, ADF, cellulose and lignin in TMR as 11.26, 1.64, 26.98, 9.32, 58.30, 32.70, 22.53 and 7.26, respectively.

Efficacy of different feed additives on IVDMD, IVOMD, IVNDFD and IVADFD are presented in Table 2. Results revealed that IVDMD (%) and IVOMD (%) were significantly ($p<0.01$) increased in T_8 as compared to control. This was followed by T_2, T_3, T_4, T_7 and T_1 which also had significantly ($p<0.01$) higher IVDMD (%) and IVOMD (%) as compared to control. In the present study cellulase, xylanase and its combination significantly increased IVDMD (%) and IVOMD (%) that might be because of their possible fibrolytic activity, which promotes the breakdown of lignocellulose bond of fibre particles and enhance the degradability of feed particles. These results were consistent with the reports of Rajamma *et al.* (2015), Vallejo *et al.* (2016) and Lunagariya *et al.* (2018), who observed significantly increased IVDMD (%) and IVOMD (%) by supplementing EFEs in diets.

In the present experiment, adding chitosan to a diet significantly increased IVDMD (%) and IVOMD (%). This enhancement in IVDMD and IVOMD may be attributed to

Table 1: Proximate composition and fibre fractions (% DMB) of feed ingredients and TMR

Proximate components and fibre fractions	Ingredients			TMR (30:30:40)
	Bajra straw	Maize hay	Concentrate	
Dry matter (DM)	90.84	88.55	88.40	89.18
Organic matter (OM)	93.27	91.20	88.35	90.68
Crude protein (CP)	2.53	7.98	20.26	11.26
Ether Extract (EE)	1.13	0.83	2.64	1.64
Crude fibre (CF)	39.70	34.53	11.77	26.98
Nitrogen free extract (NFE)	49.91	47.86	53.68	50.80
Total ash (TA)	6.73	8.80	11.65	9.32
Neutral detergent fibre (NDF)	73.84	63.69	42.60	58.30
Acid detergent fibre (ADF)	46.18	36.92	19.44	32.70
Cellulose	34.89	30.33	7.40	22.53
Hemicellulose	27.66	26.77	23.16	25.60
Lignin	8.63	4.75	8.12	7.26

Table 2: IVDMD, IVOMD, IVNDFD and IVADFD (%) of different treatments

Treatments	Parameters			
	IVDMD** (%)	IVOMD** (%)	IVNDFD** (%)	IVADFD** (%)
T_0 (Control)	$52.34^{f} \pm 0.31$	$54.92^{f} \pm 0.50$	$41.48^{d} \pm 1.63$	$38.64^{c} \pm 0.53$
T_1	$57.71^{de} \pm 1.77$	$60.03^{de} \pm 1.79$	$44.89^{c} \pm 1.59$	$40.04^{c} \pm 0.61$
T_2	$64.26^{b} \pm 0.80$	$67.05^{ab} \pm 0.73$	$53.43^{a} \pm 0.50$	$48.63^{b} \pm 1.28$
T_3	$62.09^{bc} \pm 0.59$	$64.67^{bc} \pm 0.54$	$47.09^{bc} \pm 0.87$	$45.03^{b} \pm 1.27$
T_4	$60.29^{cd} \pm 1.17$	$63.10^{cd} \pm 1.20$	$49.32^{b} \pm 0.55$	$45.38^{b} \pm 0.77$
T_5	$54.26^{f} \pm 0.67$	$56.49^{f} \pm 0.84$	$38.89^{d} \pm 1.23$	$36.79^{c} \pm 1.98$
T_6	$55.03^{ef} \pm 0.60$	$57.53^{ef} \pm 0.52$	$38.54^{d} \pm 1.36$	$36.66^{c} \pm 0.67$
T_7	$57.86^{de} \pm 1.04$	$60.69^{de} \pm 1.49$	$41.37^{d} \pm 0.85$	$38.76^{c} \pm 0.55$
T_8	$67.32^{a} \pm 1.01$	$69.97^{a} \pm 1.29$	$56.23^{a} \pm 0.36$	$53.01^{a} \pm 1.64$
p value	<0.01	<0.01	<0.01	<0.01

^{abcdef} Means with different superscripts within column differ significantly from each other (**p<0.01). IVDMD- *in vitro* dry matter degradability, IVOMD- *in vitro* organic matter degradability, IVNDFD- *in vitro* neutral detergent fibre degradability, IVADFD- *in vitro* acid detergent fibre degradability

increased microbial population in rumen with chitosan, which ultimately increased the degradation of feed particles. On the contrary various workers (Goiri *et al.*, 2010; De Paiva *et al.*, 2016 and Dias *et al.*, 2017) reported non-significant (p>0.005) increase in IVDMD (%) and IVOMD (%) by supplementing chitosan. The addition of monensin significantly increased IVDMD (%) and IVOMD (%) in the current investigation. The results of the current study contradicted the earlier significantly decreased IVDMD (%) and IVOMD (%) with monensin supplementation in the diet reported by Anassori *et al.* (2012) and Besharati *et al.* (2021).

The results further revealed that IVNDFD (%) was significantly (p<0.01) increased in T_8 and T_2 as compared to other treatments and control. This was followed by T_4, T_3 and T_1, which also had significantly (p<0.01) higher IVNDFD (%) as compared to control. The results also revealed that IVADFD (%) was significantly (p<0.01) increased in T_8 as compared to other treatments and control. This was followed by T_2, T_4 and T_3, which also had significantly (p<0.01) higher IVADFD (%) as compared to control (Table 2). In the present study cellulase, xylanase and its combination significantly increased both IVNDFD (%) and IVADFD (%). This might be due to their possible fibrolytic activity, which promotes the breakdown of lignocellulose bond of fibre particles and enhance the degradability of feed particles. The current results of IVNDFD (%) were consistent with those of earlier research by Gemeda *et al.* (2014) and Rajamma *et al.* (2015), who recorded significantly increased IVNDFD (%) with supplementing EFEs in the diet. while Rajamma *et al.* (2015) also recorded significantly increased IVADFD (%) with supplementing EFEs. Kadam *et al.* (2024) recorded significantly improved *in vivo* digestibility of all nutrients from TMR supplemented with a combination of cellulose and xylanase (1:1) @ 0.025 and 0.05% as compared to control in Gir calves.

Efficacy of different feed additives on IVTGP, PF, MBP and EMBP are presented in Table 3. The IVTGP (mL/200 mg) was significantly ($p<0.01$) increased in T_8 as compared to other treatments and control. This was followed by T_2, T_3 and T_4, which also had significantly ($p<0.01$) higher IVTGP (mL/200 mg) as compared to control. The study revealed that cellulase, xylanase and its combination significantly increased IVTGP. This might be due to EFEs that enhance the degradation of lignocellulose bond of the feed particles. Because of higher degradability it increases the total gas production. The results of the study were consistent with those of earlier researchers (Vallejo *et al.,* 2016; Lunagariya *et al.,* 2018), who documented significantly increased IVTGP (mL/200 mg) with EFEs supplementation. In the present experiment, adding chitosan to a diet also significantly increased IVTGP. This might have occurred due to increased microbial population in rumen with its treatment, which ultimately increased the degradation of feed particles. Because of higher degradability of feed stuff, it increases the total gas production. The results of the study are in contrast with those of earlier research by Li *et al.* (2013) and Wencelova *et al.* (2014), who recorded significantly decreased IVTGP with chitosan supplementation in the diet. However, none of the feed additives showed significant ($p>0.05$) effect on partitioning factor (PF), microbial biomass production (MBP) and efficiency of microbial biomass production (EMBP**,** Table 3).

Efficacy of different feed additives on TDOMR, SCFAs, ME and NE are presented in Table 4. The TDOMR (%) was significantly ($p<0.01$) increased in T_8 as compared to other treatments and control. This was followed by T_2, T_4, T_3 and T_1, which also had significantly ($p<0.01$) higher TDOMR (%) as compared to control. In the present study cellulase, xylanase and its combination as well as monensin significantly increased TDOMR (%). This might be because of their possible fibrolytic activity. The results were consistent

Table 3: IVTGP, PF, MBP and EMBP of different treatments

Treatments	Parameters			
	IVTGP** (mL/200mg)	PF (mg/mL)	MBP (mg)	EMBP (mg)
T_0 (Control)	$22.08^{cd} \pm 0.56$	4.24 ± 0.02	44.99 ± 0.87	46.87 ± 0.35
T_1	$23.87^{c} \pm 1.38$	4.18 ± 0.14	47.16 ± 1.58	45.97 ± 1.95
T_2	$28.39^{b} \pm 0.51$	4.00 ± 0.07	51.91 ± 1.30	43.74 ± 1.03
T_3	$27.33^{b} \pm 0.54$	3.82 ± 0.12	44.24 ± 2.75	40.91 ± 1.83
T_4	$26.82^{b} \pm 0.99$	4.08 ± 0.11	50.41 ± 1.58	44.76 ± 1.66
T_5	$21.69^{cd} \pm 0.93$	4.29 ± 0.25	45.08 ± 3.72	47.17 ± 2.97
T_6	$20.66^{d} \pm 0.58$	4.39 ± 0.18	45.66 ± 3.34	48.62 ± 2.17
T_7	$22.45^{cd} \pm 1.45$	4.03 ± 0.34	40.49 ± 5.26	43.33 ± 4.74
T_8	$32.92^{a} \pm 0.62$	3.72 ± 0.08	49.83 ± 2.41	39.40 ± 1.41
p value	<0.01	0.2067	0.7154	0.1764

abcd Means with different superscripts within column differ significantly from each other (**p<0.01). IVTGP- *in vitro* total gas production, PF- partitioning factor, MBP- microbial biomass production, EMBP- efficiency of microbial biomass production

Table 4: TDOMR, SCFAs, ME and NE of different treatments

Treatments	Parameters			
	TDOMR** (%)	SCFAs** mMol/200mg)	ME** (MJ/kg)	NE** (MJ/kg)
T_0 (Control)	$49.41^{d} \pm 1.20$	$0.49^{cd} \pm 0.01$	$4.58^{cd} \pm 0.08$	$2.70^{cd} \pm 0.05$
T_1	$52.84^{c} \pm 0.99$	$0.54^{c} \pm 0.02$	$4.84^{c} \pm 0.20$	$2.87^{c} \pm 0.13$
T_2	$59.64^{b} \pm 0.40$	$0.65^{b} \pm 0.01$	$5.50^{b} \pm 0.07$	$3.31^{b} \pm 0.04$
T_3	$54.81^{c} \pm 0.74$	$0.62^{b} \pm 0.01$	$5.35^{b} \pm 0.07$	$3.21^{b} \pm 0.05$
T_4	$57.49^{b} \pm 0.65$	$0.61^{b} \pm 0.02$	$5.27^{b} \pm 0.14$	$3.16^{b} \pm 0.09$
T_5	$49.05^{d} \pm 0.73$	$0.49^{cd} \pm 0.01$	$4.52^{cd} \pm 0.13$	$2.67^{cd} \pm 0.08$
T_6	$47.62^{d} \pm 0.71$	$0.46^{d} \pm 0.007$	$4.37^{d} \pm 0.08$	$2.57^{d} \pm 0.05$
T_7	$47.20^{d} \pm 0.90$	$0.51^{cd} \pm 0.03$	$4.63^{cd} \pm 0.21$	$2.74^{cd} \pm 0.13$
T_8	$64.13^{a} \pm 0.42$	$0.75^{a} \pm 0.007$	$6.16^{a} \pm 0.09$	$3.74^{a} \pm 0.05$
p value	<0.01	<0.01	<0.01	<0.01

abcd Means with different superscripts within column differ significantly from each other (**p<0.01). TDOMR- truly degradable organic matter in rumen, SCFAs- short chain fatty acids, ME- metabolizable energy, NE- net energy with earlier research by Besharati *et al.* (2021) and Karmakar (2021), who reported significantly increased TDOMR (%) by supplementing diet with EFEs and monensin.

The SCFAs (mMol/200 mg) was significantly ($p<0.01$) increased in T_8 as compared to other treatments and control. This was followed by T_2, T_3 and T_4 which also had significantly ($p<0.01$) higher SCFAs (mMol/200 mg) as compared to control. The cellulase, xylanase and its combination significantly increased SCFAs. This might be due to higher microbial activity. The results were consistent with those of Lopez *et al.* (2016). Further, the ME (MJ/kg) and NE (MJ/kg) were significantly ($p<0.01$) increased in T_8 as compared to other treatments and control. This was followed by T_2, T_3 and T_4 which also had significantly ($p<0.01$) higher ME (MJ/kg) and NE (MJ/kg) as compared to control (Table 4).

Conclusion

The cellulase, xylanase alone and combination of cellulase and xylanase when added to the TMR at the level of 0.5% each and chitosan at the level of 1% significantly enhance *in vitro* degradability, gas production and nutrient utilization as judged by improved IVDMD, IVOMD, IVNDFD, IVADFD, IVTGP, TDOMR, SCFAs, ME and NE as compared to control. Looking to the overall results it could be inferred that a combination of cellulase and xylanase is the best feed additive among all and can be incorporated in the feed at the level of 0.5% each to alter the rumen fermentation and efficient nutrient utilization.

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