RESEARCH ARTICLE

Effects of Xylanase and Bacterial Inoculants on *In Vitro* Rumen Fermentation Pattern of Seasonal Pasture Hay and Green Maize Based Silage

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Abstract

The present study was conducted to evaluate the effects of xylanase and bacterial inoculants on *in vitro* rumen fermentation pattern of seasonal pasture hay and green maize based silage. Different silages were prepared by using green maize fodder and seasonal pasture hay in the proportion of 10:0 & 7:3 ratio in plastic jars of 3 kg capacity by adding common salt @ 0.5%, urea @ 1% and molasses @ 1.5% in each silage with seven different treatments, *viz.,* Control (only green maize), PH (green maize and seasonal pasture hay in 7:3 ratio), X (PH added with xylanase), LP (PH added with *L. plantarum*), LF (PH added with *L. fermentum*), LPLF (PH added with both bacterial inoculants) and XLPLF (PH added with xylanase and both bacterial inoculants). Xylanase, *L. plantarum* and *L. fermentum* were used @ 1500 IU/g, 1 x 10^6 cfu/g and 2 x 10^6 cfu/g, respectively. All silages were used for *in vitro* study after 45 days of ensiling. None of the additives affected rumen pH. IVDMD (*in vitro* dry matter degradability) was found significantly ($p<0.05$) higher in X, XLPLF and LPLF silages. All additives significantly ($p<0.01$) increased IVOMD (*in vitro* organic matter degradability) except LF silage as compared to PH silage. Values for total gas production and TVFA (Total volatile fatty acids) production were significantly ($p<0.001$) increased during *in vitro* rumen fermentation and that of PF (partitioning factor) were significantly ($p<0.001$) decreased in all additives inoculated silages as compared to PH silage. Content of NH_3-N (Ammonia nitrogen) was significantly ($p<0.001$) higher in LP silage and that of total N was significantly ($p<0.001$) higher in X, LP, XLPLF silages as compared to PH silage during *in vitro* study. It could be concluded that xylanase and lactic acid bacterial (LAB) inoculants improved rumen fermentation quality of silage. Among all additives, xylanase is the best silage additive to improve *in vitro* rumen fermentation pattern.

Key words: Bacterial inoculants, Green maize, Seasonal pasture hay, Silage, Xylanase.

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Introduction

Livestock plays an important role in the rural economy of India. The health and productivity of livestock are closely related to quantum of quality forage provided to the animals. India faces deficiency of green and dry fodder due to increased animal population and urbanization. According to Roy *et al*. (2019) in India, availability of total green fodder, dry fodder and concentrate was estimated as 734.19, 326.40 and 61.00 metric ton (MT) against the requirements of 827.19, 426.10 and 85.78 MT, respectively. An overall deficit of green fodder, dry fodder and concentrate was 11.24, 23.4 and 28.9%, respectively. Silage making practices is adopted by farmer to overcome green fodder shortage during lean season. A huge amount of low quality feedstuffs are available in India. Seasonal pasture hay is one of them and is of poor quality. Its use in silage with green fodder may improve its quality and thereby its utilization in animal feeding. Maize (*Zea mays*) is the most suitable crop for silage preparation because of its relatively constant nutritive value, high yield and having high concentration of soluble carbohydrates for fermentation to lactic acid (Hundal *et al*., 2019).

The primary goal of making silage is to maximize the preservation of original nutrients in the forage crop for feeding at later stage. Various silage additives are used to control the preservation process, and to retain as many nutrients as present in the original fresh forage at the time of feeding. Molasses and urea are the most commonly used silage additives that serve as source of fermentable carbohydrate and provide fermentable nitrogen for

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microorganisms in the silage and rumen of the animal, respectively. Biological additives like bacterial inoculants have been used to increase the rate of acidification of ensiled forages (Weinberg and Muck, 1996). Inoculation with homofermentive or facultatively heterofermentive lactic acid bacteria (LAB) rapidly decreases pH and increases lactic acid compared to other fermentation products (Carvalho *et al*., 2020).

Additives like enzymes may improve digestibility of nutrients via numbers of mechanism that include direct hydrolysis of sugar, change in gut viscosity and change in the site of digestion (Kung Jr, 2010). The main function of the exogenous fibrolytic enzymes is to supply maximum amount of nutrients from the digestible, potentially digestible and indigestible fractions of the cell wall (Mocherla *et al*., 2017). Considering huge availability of seasonal pasture hay at cheaper price in India, and role of biological and chemical additives in silage production, the present work was envisaged to study their effects on quality and *in vitro* rumen fermentation pattern of seasonal pasture hay and green maize based silage.

Materials and Methods

Ethical statement

The present study was conducted at Department of Animal Nutrition, College of Veterinary Science and A. H., Kamdhenu University, Junagadh, Gujarat. All the experimental procedures were approved by Institutional Animal Ethics committee (IAEC) (Protocol No: KU-JVC-IAEC-LA-75/21).

Preparation of Silage

Different silages were prepared by using green maize fodder and seasonal pasture hay in the proportion of 10:0 & 7:3 ratio in plastic jars of 3 kg capacity (3 replicates in each) by adding common salt @ 0.5%, urea @ 1% and molasses @ 1.5% in each silage with seven different treatments, *viz*., Control (only green maize), PH (green maize and seasonal pasture hay in 7:3 ratio), X (PH added with xylanase), LP (PH added with *Lactobacillus plantarum*), LF (PH added with *Limosilactobacillus fermentum*), LPLF (PH added with both bacterial inoculants) and XLPLF (PH added with xylanase and both bacterial inoculants). Xylanase, *L. plantarum* and *L. fermentum* were used @ 1500 IU/g, 1 x 10^6 cfu/g and 2 x 10^6 cfu/g, respectively. All silages were evaluated for *in vitro* rumen fermentation pattern after 45 days of ensiling.

Estimation of Rumen pH, IVDMD, IVOMD, Total Gas Production and PF Value

After 45 days of ensiling, sampling of silage was done. Silage samples were oven dried at 100± 5°C for overnight. The dried samples were ground to pass through a 1 mm screen and used as substrate for determining the IVDMD, *in vitro* organic matter degradability (IVOMD) and total gas production. IVDMD and IVOMD were analyzed as per the standard methods. Approximately 0.5 g finely ground silage sample was taken with 40 mL CO_2 saturated phosphate carbonate buffer solution and 10 mL strained rumen liquor in Erlynmayer flask and incubated at 39 °C for 48 h in CO_2 incubator with periodic shaking. After 48 h of incubation, contents were filtered through sintered crucible and dried at 100 °C overnight and weighed. Dry residues were ashed at 550 °C.

Total gas production was determined by the method of Menke and Steingass (1988). About 200 mg samples were taken into glass syringes with 30 mL buffer solution and rumen fluid. Then glass syringes were placed into incubator at 39 °C for 24 h. After 24 h, total gas production was measured and suitable aliquot was taken from glass syringe for determination of rumen pH, TVFA, NH_3-N and total N. The rumen pH was measured by pen type pH meter. TVFA and NH_3-N were analyzed as per the standard methods and the total N content was measured as per the Kjeldahl method (AOAC, 2005).

Statistical Analysis

The data were analyzed for descriptive statistics (mean and standard error). Treatment effects on different parameters were analyzed by one way analysis of variance according to Snedecor and Cochran (1994). Pair wise mean differences between groups were compared by Duncan's new multiple range test for the significance at $p<0.05$.

Results and Discussion

Chemical composition of green maize, seasonal pasture hay and mixture of green maize and seasonal pasture hay used for ensiling is given in Table 1**.**

The effects of bacterial inoculants and xylanase on *in vitro* rumen fermentation pattern are presented in Table 2. Data revealed that rumen pH was not affected by any additives. Chen *et al.* (2019) and Oskoueian *et al.* (2021) also noticed no any significant difference in bacterial inoculants added silage on rumen pH as compared to control. The IVDMD (%) was found significantly ($p<0.05$) higher in X (57.32 ± 1.93), XLPLF (56.46 ± 1.72) and LPLF (55.46 ± 1.83) silages as compared to PH (49.25 ± 0.64) silage. Dakore (2018), Yadav (2018) and Chen *et al.* (2019) also reported significantly increased IVDMD of silages added with enzyme and bacterial inoculants. Our results concurred well with findings of these studies.

In vitro gas production techniques are usually used to evaluate rumen degradability of organic matter and indicate the metabolizable energy of feed (De Boever *et al*., 2005). Gas production rate is highly positively correlated with a rumen degradation rate of feed (Muck *et al*., 2007). In present study, IVOMD (%) was significantly ($p<0.01$) higher in all additives added silages as compared to PH silage, except LF, however total gas production was found significantly ($p<0.001$) higher in all additives added silages as compared to PH silage. PF value was significantly ($p<0.001$) lower in all additives added silages as compared to PH silage. The findings regarding

Table 1: Chemical composition of green maize, seasonal pasture hay and mixture of green maize and seasonal pasture hay used for ensiling (% DM basis)

Attributes	Green maize	Seasonal pasture hay	Green maize : Seasonal pasture hay (7:3)
DM	33.10 ± 0.05	90.38 ± 0.15	40.95 ± 0.12
OM	91.00 ± 0.60	88.73 ± 0.71	90.9 ± 0.25
CP	9.17 ± 0.06	3.96 ± 0.34	7.23 ± 0.69
EE	1.77 ± 0.23	1.01 ± 0.07	1.44 ± 0.14
CF	32.68 ± 1.08	40.55 ± 0.41	39.52 ± 1.36
NFE	47.38 ± 1.22	43.21 ± 0.70	42.71 ± 2.44
TA	9.00 ± 0.60	11.27 ± 0.71	9.10 ± 0.25
NDF	67.55 ± 0.26	79.64 ± 0.49	71.08 ± 0.56
ADF	44.91 ± 1.61	60.38 ± 0.50	50.9 ± 0.08
HE	22.64 ± 1.34	19.25 ± 0.01	20.18 ± 0.48
CE	34.55 ± 0.53	43.88 ± 0.68	38.57 ± 0.07
ADL	4.36 ± 0.79	10.40 ± 0.03	5.96 ± 0.03

DM- dry matter; OM- organic matter; CP- crude protein; EE- ether extract; CF- crude fibre; NFE- nitrogen free extract; TA- total ash; NDF- neutral detergent fibre; ADF- acid detergent fiber; HE- hemicellulose; ADL- acid detergent lignin; CE- cellulose

Table 2: Effects of bacterial inoculants and xylanase on *in vitro* rumen fermentation pattern of different experimental silages

	Parameters				
Treatments	**pH**	**IVDMD* (%)**	**IVOMD* (%)**	**Total gas** (mL/200 mg DM)**	**PF****
Control	6.77 ± 0.02	51.24^{ab} ± 1.28	54.92^{b} ± 0.50	19.71^{b} ± 0.96	4.13^{de} ± 0.08
PH	6.77 ± 0.03	49.25^{a} ± 0.64	51.27^{a} ± 0.95	16.37^{a} ± 0.84	4.33^{e} ± 0.09
X	6.70 ± 0.02	57.32^{c} ± 1.93	58.28^{b} ± 1.56	24.90^{d} ± 0.93	3.66^{a} ± 0.07
LP	6.78 ± 0.02	53.04^{abc} ± 1.85	55.25^{b} ± 0.93	21.52^{bcd} ± 1.82	3.91^{bcd} ± 0.07
LF	6.76 ± 0.03	54.37^{abc} ± 1.87	54.62^{ab} ± 1.48	21.08^{bc} ± 1.43	4.02^{cd} ± 0.09
LPLF	6.73 ± 0.03	55.46^{bc} ± 1.83	56.34^{b} ± 1.36	22.29^{bcd} ± 0.67	3.82^{abc} ± 0.05
XLPLF	6.75 ± 0.03	56.46^{bc} ± 1.72	57.54^{b} ± 1.13	23.41^{cd} ± 0.64	3.77^{ab} ± 0.06
p value	0.614	0.016	0.006	<0.001	<0.001

IVDMD- *in vitro* dry matter degradability; IVOMD- *in vitro* organic matter degradability; PF- partitioning factor.

Values with different superscripts^{a-e} within a column significantly varied at $p<0.05$ and $p<0.001$.

Table 3: Effects of bacterial inoculants and xylanase on TVFA, NH_3-N and total N content in rumen liquor during *in vitro* rumen fermentation of different experimental silages

	Parameters		
Treatments	**TVFA** (mmol/100 mL)**	**NH_3-N** (mg/dL)**	**TN**(mg/dL)**
Control	7.03^{b} ± 0.65	43.49^{a} ± 1.66	86.52^{cd} ± 1.31
PH	5.36^{a} ± 0.21	41.79^{a} ± 0.95	82.68^{ab} ± 1.00
X	9.02^{d} ± 0.39	43.77^{a} ± 1.56	89.12^{d} ± 1.25
LP	7.94^{bcd} ± 0.22	50.50^{b} ± 1.04	95.31^{e} ± 1.05
LF	7.10^{bc} ± 0.25	43.52^{a} ± 0.92	85.45^{bc} ± 1.29
LPLF	8.25^{bcd} ± 0.41	41.21^{a} ± 0.96	80.21^{a} ± 1.19
XLPLF	8.42^{cd} ± 0.63	43.96^{a} ± 1.49	90.03^{d} ± 1.27
p value	<0.001	<0.001	<0.001

TVFA- total volatile fatty acid; NH_3-N – ammonia nitrogen; TN- total nitrogen

Values with different superscripts^{a-e} within a column significantly varied at $p<0.001$.

significant increase in IVOMD (%) and total gas production (mL/200 mg DM) in enzyme and bacterial inoculants added silages corroborated well with Dakore (2018), Yadav (2018) and Chen *et al.* (2019), who also reported significant increase in IVOMD and total gas production in enzyme and bacterial inoculants added silage.

All bacterial inoculants, xylanase and their combination produced significantly ($p<0.001$) higher TVFA (Table 3) during *in vitro* rumen fermentation as compared to PH silage (5.36 ± 0.21 mmol/100 mL), being highest in X silage (9.02 ± 0.39 mmol/100 mL). Significant higher TVFA content (mmol/100 mL) in rumen liquor of enzyme and bacterial inoculants added silages was also reported by Chen *et al.* (2019). The higher *in vitro* ruminal TVFA concentration of the silages produced in all additives added silages was in parallel to their IVDMD, as the concentration of TVFA has a positive correlation with the amount of substrate fermentation in the rumen (McDonald *et al*., 2011). The content of NH_3-N in rumen liquor during *in vitro* rumen fermentation was significantly higher in *L. plantarum* inoculated silage (50.50 ± 1.04 mg/dL) as compared to other experimental silages. The present finding are in agreement with Marbun *et al.* (2020), who reported higher concentration of ammonia-N in *L. plantarum* inoculated silage as compared to the rest of the treatments. Total N (mg/dL) was significantly ($p<0.001$) higher in LP (95.31 ± 1.05) followed by XLPLF (90.03 ± 1.27) and X (89.12 ± 1.25) silages as compared to PH (82.68 ± 1.00) silage.

Conclusions

The results indicated that all the additives significantly improved *in vitro* rumen fermentation. Among all additives, xylanase alone gives best result during *in vitro* rumen fermentation. *L. plantarum* significantly increased NH_3-N and total N in rumen liquor during *in vitro* rumen fermentation.

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