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Effects of bacterial inoculants and xylanase on silage quality of seasonal pasture hay and green maize based silage

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Abstract
The present study was envisaged to study the effect of bacterial inoculants and xylanase on silage quality 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 jar of 3 kg capacity by adding common salt @ 0.5%, urea @ 1% and molasses @ 1.5% in each silages 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 evaluated for proximate and chemical composition after 45 days of ensiling. Ensiling significantly ($p<0.01$) reduces DM content in all experimental silages except PH. Silage pH was significantly ($p<0.01$) reduced in all inoculated silages except X silage. The contents of DM was significantly ($p<0.001$) lower in all inoculated silages, while that of CF was significantly lower in XLPLF silage as compared to PH silage. The CP content was significantly higher in LP silage while, NFE and EE were significantly higher in LP, LPLF and in LPLF, XLPLF silages, respectively as compared to PH silage. CF, CP and EE contents of all inoculated silages were comparable with control. NDF was significantly ($p<0.01$) decreased in X silage while, ADF content was significantly ($p<0.01$) decreased in in X and XLPLF silages as compared to PH silage. Cellulose and hemicellulose contents were not affected by any treatments. Thus, it could be concluded that among all additives bacterial inoculants significantly improves silage quality and nutritional value.

Keywords: Bacterial inoculants, green maize, seasonal pasture hay, silage, xylanase

Introduction
With the rapid development of animal husbandry and requirements for higher milk production from ruminant systems, feed shortage has become a constraint on ruminant production. In India, total green fodder availability was estimated 734.2 MT, against requirement 827.19 MT. So, an overall deficit of green fodder was 11.24% (Roy *et al*., 2019) [13]. To overcome the acute shortage of green fodder during lean period forage preservation by silage making is an alternative method. 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) [4]. Seasonal pasture hay is an agricultural product used as dry fodder in animal feeding but it is poor in quality. For improving the feed quality of seasonal pasture hay, ensiling is one of important methods.
Silage additives had been used to improve the silage quality (Cai *et al*., 1998) [2]. Bacterial inoculants and enzymes are the most popular silage additives. The addition of water-soluble carbohydrates (i.e., molasses), fiber-degrading enzymes (i.e., cellulase, xylanase) and lactic acid bacteria (LAB) to induce rapid initial fermentation (Muck *et al*., 2018) [10]. Fibrolytic enzymes are often used in silage making to enhance the degradation of plant cell walls carbohydrates to fermentable sugars, which could be used by LAB during ensiling (Zhang *et al*. 2010) [18]. Large number of forage crops and grasses are found that most of the epiphytic LAB are heterofermentative, while the number of homofermentative LAB are very small, but which play a more important role in promoting lactic acid fermentation process than heterofermentative LAB during ensiling. Lactobacillus plantarum, one of homofermentative LAB, has been widely used to improve silage quality, which is ascribed to its wide fermentation substrate and lactic acid production efficiency (Li and Nishino 2011) [9].

Considering huge availability of seasonal pasture hay in India, role of fibrolytic enzyme and bacterial inoculants in silage production, the present study was envisaged to study the effects of bacterial inoculants and xylanase on silage quality of seasonal pasture hay and green maize based silage.

Materials and Methods

Different silages were prepared using green maize fodder and seasonal pasture hay in the proportion of 10:0 & 7:3 ratio in plastic jar of 3 kg capacity (3 replication in each) by adding common salt @ 0.5%, urea @ 1% and molasses @ 1.5% in each silages 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 evaluated for silage fermentation characteristics and *in vitro* rumen fermentation pattern after 45 days of ensiling.

Before ensiling samples of green maize fodder, seasonal pasture hay and mixture of green maize & seasonal pasture hay (7:3) were analysed for proximate composition and cell wall fractions. Sampling of silages were done on 45^{th} day of ensiling. Samples from different experimental silage were analysed for proximate composition and cell wall fractions according to the method described by AOAC (2005) [1] and Van Soest *et al*. (1991) [16], respectively except for the dry matter content of silage which was analysed as per the method given by Philip and John (1977) [12].

The data were analyzed for descriptive statistics (mean and standard error). Treatment effects on different parameters were analyzed by one way analysis of variance (ANOVA) according to Snedecor and Cochran (1994) [15]. Pair wise mean difference between groups were compared by Duncan's New Multiple Range Test (DNMRT) as modified by Kramer (1957) [8].

Results and Discussions

Table 1. is showing chemical composition of silage material used for silage preparation. Table 2. is showing effects of ensiling on DM content of different experimental silages. Data on paired t-test revealed DM content was significantly (p<0.01) reduced in all experimental silages except PH silage during ensiling process. DM content decreases consequently as ensiling progress could be attributed to nutrient breakdown and fermentation shifted from homofermentative to heterofermentative direction due to shortage of substrate (Nishino *et al*., 2004) [19].

Data on chemical composition of different experimental silages are presented in Table 3. Data on chemical composition revealed that DM content was significantly (p<0.001) reduced in all inoculated silages as compared to PH silage. Highest reduction in DM content was observed in LPLF followed by XLPLF, LF, X and LP. Similar results were found by Jalc *et al.* (2010) [6], Yadav (2018) [17] and Oskoueian (2021) [12], they reported that DM content was significantly (p<0.05) reduce in inoculants treated silage, while Jalc *et al.* (2009) [5], Dakore (2018) [3] and Su *et al.* (2019) [15] reported that DM content was significantly (p<0.05) increase in additives treated silage as compared to control which is contrary to present findings. The CF content was significantly (p<0.05) lower in XLPLF silage as compared to control, however it was numerically lower in all additive added silages. The CF contents of all silages were comparable with control. Present findings are consistent with the findings of Jalc *et al.* (2009) [5], who reported that CF content was significantly (p<0.05) reduced in inoculants treated silages. The reduction in CF content of enzyme and bacterial inoculants treated silages is attributed to fibrolytic activity of xylanase enzyme and bacterial inoculants.

Lactobacillus plantarum has significant improved effect on CP content of silage as compared to all other additives. Similarly various scientist (Xing *et al.*, (2009) [20]; Nkosi *et al* (2012) [21]; Khota *et al.* (2017) [7], Dakore (2018) [3] and Oskoueian *et al.* (2021)) [11] reported that CP content was significantly (p<0.05) increase in *L. plantarum* added silages. Increasing CP content in *L. plantarum* added silage, it might be due to reduction of proteolysis by *L. plantarum* during ensiling. The EE content was significantly highest in XLPLF and LPLF followed by LF, LP and X added silages as compared to PH silage. Similar results were observed by Dakore (2018) [3] and Yadav (2018) [17] in sugargraze and maize silage, respectively. The NFE content was significantly higher in LPLF as compared to PH.

Statistical data shows that NDF and ADF contents were significantly (p<0.01) decreased in xylanase added silages as compared to PH, while other treatments were numerically lower but at par with PH. Findings of Xing *et al*. (2009) [20] and Dakore (2018) [3] were similar to the present findings. Reduction in NDF and ADF contents in xylanase added silages is due to fibrolytic activity of xylanase enzyme. While, no any additives had significant effect on cellulose and hemicellulose content of silage. Similarly, Yadav (2018) [17] reported that no significant difference (p>0.05) in hemicellulose content between additives treated silages and control.

Table 1: Proximate composition and cell wall fractions 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
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
Hemicellulose	22.64±1.34	19.25±0.01	20.18±0.48
Cellulose	34.55±0.53	43.88±0.68	38.57±0.07

Table 2: Effect of ensiling on dry matter content (%) of differential experimental silages

Treatments	Before ensiling	After ensiling	p value
Control	$33.10^{b}\pm0.05$	$32.08^{a\pm}0.10$	0.01
PH	40.95 ± 0.12	$40.52^{\pm}0.38$	0.33
X	$40.95^{b}\pm0.12$	$38.74^{a\pm}0.28$	<0.001
LP	$40.95^{a}b\pm0.12$	$39.43^{a\pm}0.26$	0.001
LF	$40.95^{b}\pm0.12$	$38.55^{a\pm}0.25$	<0.001
LPLF	$40.95^{b}\pm0.12$	$36.12^{a\pm}0.22$	<0.001
XLPLF	$40.95^{b}\pm0.12$	$36.95^{a\pm}0.31$	<0.001

Values with different superscripts^{a-b} within a row significantly varied ($p<0.01$) in paired t-test.

Table 3: Effects of bacterial inoculants and xylanase on proximate composition (% on DM) of different experimental silages

	Control	PH	X	LP	LF	LPLF	XLPLF	p value
pH	$4.41^{d}\pm0.13$	$4.31^{cd}\pm0.04$	$4.08^{bc}\pm0.07$	$3.75^{a}\pm0.16$	$3.95^{ab}\pm0.04$	$3.98^{ab}\pm0.03$	$3.87^{ab}\pm0.07$	<0.001
DM**	$32.08^{a}\pm0.10$	$40.52^{f}\pm0.38$	$38.74^{de}\pm0.28$	$39.43^{e}\pm0.26$	$38.55^{d}\pm0.25$	$36.12^{b}\pm0.22$	$36.95^{c}\pm0.31$	<0.001
CF*	$37.99^{abc}\pm0.29$	$39.09^{bc}\pm0.60$	$37.86^{abc}\pm0.34$	$39.23^{c}\pm0.51$	$38.13^{abc}\pm0.60$	$37.61^{ab}\pm0.46$	$37.08^{a}\pm0.45$	0.032
CP**	$8.69^{a}\pm0.71$	$8.26^{a}\pm0.55$	$9.01^{a}\pm0.89$	$11.54^{b}\pm0.73$	$8.97^{a}\pm0.74$	$7.61^{a}\pm0.27$	$9.04^{a}\pm0.59$	0.009
EE$^{©}$	$1.16^{ab}\pm0.17$	$0.95^{a}\pm0.08$	$1.07^{ab}\pm0.05$	$1.26^{ab}\pm0.17$	$1.32^{ab}\pm0.18$	$1.39^{b}\pm0.13$	$1.42^{b}\pm0.06$	0.143
NFE**	$36.07^{ab}\pm0.52$	$36.48^{ab}\pm0.84$	$38.45^{bc}\pm0.92$	$35.23^{a}\pm1.00$	$39.02^{bc}\pm1.42$	$40.43^{c}\pm0.68$	$38.89^{bc}\pm0.90$	0.003
NDF	$66.86^{ab}\pm0.10$	$70.37^{c}\pm0.39$	$65.77^{a}\pm0.37$	$69.90^{c}\pm0.33$	$68.99^{bc}\pm0.26$	$69.41^{bc}\pm0.36$	$67.61^{abc}\pm2.32$	0.009
ADF	$47.85^{b}\pm0.31$	$50.49^{cd}\pm0.51$	$45.99^{a}\pm0.41$	$49.52^{c}\pm0.29$	$49.77^{cd}\pm0.37$	$50.84^{d}\pm0.34$	$47.58^{b}\pm0.30$	<0.001
CE	35.91±0.36	33.99±0.95	36.85±0.56	32.04±0.65	34.55±3.16	34.74±0.64	33.88±3.56	0.675
HE	19.01±0.29	19.88±1.75	19.78±0.67	20.38±0.32	19.22±0.54	18.57±0.21	20.03±2.41	0.917

DM-Dry matter; CF- crude fibre; CP- crude protein; EE- ether extract; NFE- nitrogen free extract; NDF- neutral detergent fibre; ADF- acid detergent fibre; CE- cellulose; HE- hemicellulose

Values with different superscripts^{a-f} within a row significantly varied (*- $p<0.05$ and **-$p<0.01$).

$^{©}$ -Though p value in ANOVA shows non-significant difference but on DNMRT analysis significance difference was observed.

Conclusions

The result confirmed that all silage additives alone or their combination improve quality of silage when seasonal pasture hay is used with green maize in silage making at 3:7 ration. However, among all additives, bacterial inoculants are best to improve silage quality and nutritional quality as compared to other.

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