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Effects of Bacterial Inoculants and Xylanase on Silage Fermentation Characteristics of Wheat straw and Pasture hay based Green Maize Silage

Saman Y. Belim*[1], Harish H. Savsani[1], Yash G. Kansagara[1], Mitesh R. Chavda[1] and Mulraj D. Odedara[2]

[1]Department of Animal Nutrition, College of Veterinary Science and A.H., Kamdhenu University, Junagadh-362001

[2]Cattle Breeding Farm, Kamdhenu University, Junagadh

* Correspondence: belimsaman1998@gmail.com

ABSTRACT

Different silages were prepared using green maize fodder and wheat straw and pasture hay separately 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 silage with nine different treatments, *viz.,* Control (only green maize), WS (green maize and wheat straw in 7:3 ratio), X (WS added with Xylanase), LF (WS added with *Lactobacillus fermentum*), LPLF (WS added with both bacterial inoculants), PH (green maize and pasture hay in 7:3 ratio), XPH (PH added with Xylanase), LFPH (PH added with *Lactobacillus fermentum*), LPLFPH (PH added with 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 in terms of their proximate composition, cell wall constituents and quality parameters of silage on 45 days of ensiling. Bacterial inoculants significantly reduce DM content of silage. LPLF silage and XPH and LFPH significantly increases crude protein content. X and LPLFPH shows significant improvement in EE content. LF shows significantly improve values of CF and NFE. X and XPH silage significantly lowers NDF and ADF content. LPLF and XPH silage significantly improves cellulose while additives do not have any significant effect on hemicellulose content. Bacterial inoculants and Xylanase significantly improves TVFA, WSC and LA to lower silage pH for very good silage. Xylanase, *Lactobacillus fermentum* and combination of bacterial inoculants with wheat straw and pasture hay silage, respectively significantly reduces ammonia nitrogen for excellent silage quality. Thus, it is concluded that Xylanase and/or *Latobacillus fermentum* significantly improves overall silage fermentation characteristics and nutrient content in green maize based silage along with wheat straw or pasture hay in 7:3 ratio.

KEYWORDS: *Lactobacillus fermentum*, *Lactobacillus plantarum*, Pasture hay, Silage,Wheat straw

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Wheat straw and pasture hay is inexpensive and available locally, its use in silage along with additives may improve its quality and there by its utilization in animal feeding. Silage additives have been used as a management tool to improve the nutritional value of silage. They are natural or industrial products added in smaller quantities during ensiling to control the preservation process so as to retain as many of nutrients as present in the original fresh forage.

Wheat straw and pasture hay are available locally and in expensive. They are generally burnt in the field itself by the farmers and not utilized in the feeding of animals and thus go as a waste. Incorporating wheat straw and pasture hay in silage production along with different feed additives, will judiciously utilize this poor quality roughage in silage production to enhance the productive performance of animals by reducing the shortage of quality fodder in the present scenario.

Inoculation with homofermentive or facultatively heterofermentive lactic acid bacteria during ensiling rapidly decreases pH of silage and increases content of lactic acid than the other fermentation products in silage (Carvalho et al., 2020). Also, treating forages with enzymes may improve their digestibility via number of mechanisms that include direct hydrolysis of sugar, improvement in palatability, change in gut viscosity and change in the site of digestion (Kung Jr, 2010). Keeping the above facts in view, the proposed experiment was planned to study the effect of Lactobacillus bacterial inoculants and xylanase in maize based green silage along with different dry fodders like wheat straw and seasonal pasture hay.

Enzyme xylanase was procured from the standard manufacturer company @ 100 ¹ /kg. The result showed that percent cost saving was found to be higher in xylanase added wheat straw silage as compared to control (maize alone) silage.

The present study was conducted at Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat.

Different silages were prepared using green maize fodder and wheat straw and pasture hay separately 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 silage with nine different treatments, *viz.,* Control (only green maize), WS (green maize and wheat straw in 7:3 ratio), X (WS added with Xylanase), LF (WS added with *Lactobacillus fermentum*), LPLF (WS added with both bacterial inoculants), PH (green maize and pasture hay in 7:3 ratio), XPH (PH added with Xylanase), LFPH (PH added with *Lactobacillus fermentum*), LPLFPH (PH added with 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. Different additives were spread as per their application rate in different treatments and mixed thoroughly. Fodder mass along with different additives were packed in plastic jar having the capacity of 3 kg and designed with valve at the lid of the jar. Air from the jars was removed with the help of vacuum pump. Jars of different treatments were stored at room temperature for 45 days. The store house was disinfected and appropriate measures were taken to avoid the entry of rats, mice and birds.

All silages were evaluated after 45 days of ensiling. Before ensiling samples of green maize fodder, wheat straw, pasture hay, mixture of green maize & wheat straw (7:3) and mixture of green maize & pasture hay (7:3) were analysed for proximate composition and cell wall constituents. Samples from different experimental silage were evaluated in terms of their proximate composition and cell wall constituents according to the methods of AOAC (2023) and Van Soest et al. (1991), respectively.

Organoleptic criteria are the most important way to judge the silage quality. Silage was observed visually for its colour and categorized in four different grades as: Grade 1 - Golden yellow, Grade 2 - Light brown, Grade 3 - Dark brown, Grade 4 – Blackish.

For estimation of silage pH, total volatile fatty acid (TVFA) and ammonia nitrogen (NH3-N), water extracts of silages were prepared by adding 90 mL of distilled water to 10 g fresh silage sample in a beaker and homogenized by mechanical homogenizer. A few drops of 0.1% mercuric chloride were added into the extract and kept in refrigerator at 4 °C. After 48 hours, material was filtered through four layer muslin cloth and stored at 4 °C. The pH of silage was measured by pen type pH meter. TVFA, lactic acid and NH3-N were analyzed as per the method given by (Prasad, 2015). Total nitrogen was estimated by Kjeldahl method (AOAC, 2023). Silage samples were oven dried at 100 ± 5 °C for overnight. The dried samples were ground to pass through a 1 mm screen for analysis of Water soluble carbohydrate (WSC) as per the standard method (Yemm and Willis 1954).

The data were analysed 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 $p<0.05$.

The result regarding proximate composition are presented in Table 1. The dry matter was significantly ($p<0.01$) lower in different silages as compared to WS and PH. Similarly, Jalc et al. (2009), Khota et al. (2017), Dakore (2018) and Yadav (2018). In contrast, Su et al. (2019) reported DM was significantly ($p<0.05$) increase. OM was significantly ($p<0.01$) higher in LF treated silage. It is corroborated with Dakore (2018) while in distinction, Yadav (2018) reported significantly reduced OM. Crude protein content was found to be significantly ($p<0.05$) higher in Xylanase treated pasture hay silage. Similarly, Jalc et al. (2009) noted higher CP in *L. fermentum* added silage. EE was significantly ($p<0.05$) higher in xylanase treated wheat straw silage. Similarly, Khota et al. (2017), Zielinskal and Fabiszewska (2017), Dakore (2018) and Yadav (2018) reported higher EE content. Unlikely, Nkosi et al. (2012) observed significantly lower EE content. CF was significantly ($p<0.05$) lower in LF treated wheat straw silage.

Unlikely, Jalc et al. (2009) obtained significant decrease in the CF. The TA was significantly ($p<0.01$) lower in LF treated wheat straw silage. In contrast, Jalc et al. (2009) reported non significant decrease. NFE was significantly ($p<0.05$) higher in LF treated wheat straw silage.

Table 1. Proximate composition of different experimental silages (% DMB)

Treatments	Parameters						
	DM**	OM**	CP*	EE*	CF*	TA**	NFE*©
C	31.39^{a} ±0.32	84.59^{a} ±0.23	8.06^{ab} ±0.17	1.08^{ab} ±0.05	36.06^{a} ±2.39	15.40^{e} ±0.23	39.40^{abc} ±2.61
WS	41.02^{e} ±0.22	87.57^{cde} ±0.64	7.15^{a} ±0.40	0.90^{a} ±0.03	45.11^{c} ±3.69	12.42^{abc} ±0.64	33.37^{a} ±4.65
X	41.63^{f} ±0.30	86.74^{bc} ±0.52	7.54^{ab} ±0.32	1.48^{b} ±0.27	41.15^{ab} ±0.74	13.25^{cd} ±0.52	37.89^{abc} ±1.08
LF	37.51^{d} ±0.19	88.54^{e} ±0.28	7.72^{ab} ±0.32	1.18^{ab} ±0.05	36.09^{a} ±0.96	11.47^{a} ±0.28	43.54^{c} ±1.00
LPLF	35.17^{b} ±0.48	88.21^{de} ±0.10	9.00^{b} ±0.63	1.15^{ab} ±0.06	42.06^{bc} ±1.53	11.79^{ab} ±0.10	36.00^{ab} ±1.90
PH	40.52^{e} ±0.37	84.76^{a} ±0.32	8.25^{ab} ±0.54	0.94^{a} ±0.08	39.08^{ab} ±0.60	15.23^{e} ±0.32	36.47^{ab} ±0.84
XPH	38.73^{d} ±0.27	86.39^{b} ±0.25	9.01^{b} ±0.89	1.07^{ab} ±0.04	37.85^{ab} ±0.33	13.60^{d} ±0.25	38.45^{abc} ±0.91
LFPH	38.55^{d} ±0.25	87.43^{cd} ±0.11	8.97^{b} ±0.73	1.31^{ab} ±0.18	38.13^{ab} ±0.60	12.56^{bc} ±0.11	39.02^{abc} ±1.42
LPLFPH	36.12^{c} ±0.22	87.04^{bc} ±0.04	7.60^{ab} ±0.26	1.39^{b} ±0.12	37.60^{ab} ±0.46	12.95^{cd} ±0.43	40.43^{bc} ±0.68
p value	<0.001	<0.001	0.017	0.036	0.004	<0.001	0.075

[abcde]Means with different superscript within a column differ significantly (**$p<0.01$, *$p<0.05$)

Cell wall constituents

Significantly ($p<0.05$) lower NDF was found in Xylanase treated wheat straw and pasture hay silage as compared to WS and PH silage. Similarly, Govea et al. (2013) Khota et al. (2017), Yadav (2018) and Agarrusi et al. (2019) recorded non-significant decrease in NDF content. Also, Jalc et al. (2010) observed significantly lower NDF in LF inoculated silage. The ADF was significantly ($p<0.01$) lower in Xylanase inoculated wheat straw silage as compared to other all inoculated silage. It might be due to the effect of xylanase to release maximum amount of nutrients from indigestible and potential digestible fraction of cell wall. In agreement, Xing et al. (2009) and Dakore (2018) reported significantly lower ADF content. The cellulose was significantly ($p<0.05$) higher in combination of both bacterial inoculants added wheat straw silage. In accordance, Zhao et al. (2021) reported significant increase in the cellulose content of lactic acid bacteria inoculated silage. The hemi cellulose was non-significant ($p>0.05$) among all experimental silage. In the same line, Yadav (2018) noticed numerically higher value of hemicellulose in enzyme xylanase inoculated silage.

Table 2. Cell wall constituents of different experimental silages (% DMB)

Treatments	Parameters			
	NDF*	ADF**	Cellulose*	Hemicellulose
C	67.41^{abc} ±1.74	47.41^{bc} ±1.70	33.49^{ab} ±0.63	20.00 ±0.70
WS	69.89^{c} ±0.84	49.95^{de} ±0.47	33.58^{ab} ±0.42	19.94 ±0.90
X	65.96^{ab} ±0.72	44.55^{a} ±0.99	33.37^{ab} ±0.43	21.41 ±0.74
LF	66.13^{ab} ±1.45	45.79^{ab} ±0.77	32.23^{a} ±0.57	20.34 ±2.10
LPLF	68.85^{abc} ±1.37	48.21^{bcd} ±0.82	36.50^{b} ±0.68	20.64 ±1.64
PH	70.37^{c} ±0.38	50.49^{de} ±0.50	33.99^{ab} ±0.94	19.87 ±0.71
XPH	65.77^{a} ±0.37	45.99^{ab} ±0.41	36.85^{b} ±0.56	19.77 ±0.67
LFPH	68.98^{bc} ±0.25	49.76^{cde} ±0.37	34.55^{ab} ±3.15	19.21 ±0.53
LPLFPH	69.41^{c} ±0.35	50.83^{e} ±0.34	34.74^{ab} ±0.64	18.73 ±0.20
p value	0.005	<0.001	0.013	0.830

[abcd]Means with different superscript within a column differ significantly (**p<0.01, *p<0.05).

Quality parameters of silage

The results of quality parameter of silage are presented in Table 3. The colour of silage was observed golden yellow in all experimental silage. The pH values of different experimental silage were found to be significantly (p<0.01) lower in all the additives inoculated silage as compared to Control, WS and PH silage. It might be due to the addition of enzyme and bacterial inoculants as they decreased the silage pH rapidly as compared to control. These results are in agreement with the observations of most of the workers, Jalc et al. (2009), Nkosi et al. (2012), Govea et al. (2013), Guo et al. (2014), Khota et al. (2017), Zielinska and Fabiszewska (2018), Dakore, (2018), Yadav (2018) and Zhao et al. (2021) as they observed significantly lower pH in all inoculated silages as compared to control silage. Whereas, Xing et al. (2009) and Marbun et al. (2020) noticed contrary to present finding that pH of silage was not affected significantly by inoculants.

The results of TVFA contents were found be significantly (p<0.01) higher in all additives inoculated silage as compared to Control, WS and PH silage. However, significantly (p<0.01) higher TVFA content was found in *Lactobacillus fermentum* treated wheat straw and pasture hay silage which might be due to better quality fermentation. Corresponding to present findings, Dakore (2018) and Yadav (2018) reported significant higher TVFA content in all additives inoculated silages as compared to control silage. Increase in TVFA content of inoculated silages might be due to better microbial fermentation of carbohydrates.

Ammonia nitrogen content was found to be significantly (p<0.01) lower in all the additives inoculated silage as compared to Control and WS silage which is due to better quality silage. However, numerically lower ammonia nitrogen content were found in Xylanase and *lactobacillus fermentum* treated wheat straw and pasture hay silage, respectively. The results of present finding is well corroborated with findings of Filya (2003), Jalc et al. (2009),Nkosi et al. (2012), Govea et al.(2013), Chen et al. (2019) and Zhao et al. (2021), they reported that ammonia nitrogen content was significantly (p<0.05) reduce in inoculants and enzyme added silages. In distinction to current findings, Agarussi et al. (2019) noted that ammonia nitrogen content was numerically increased in inoculated silages.

Total nitrogen content was found to be significantly ($p<0.05$) higher in Xylanase, *Lactobacillus fermentum* treated PH silage and combination of both bacterial inoculants LPLF treated wheat straw silage which is due to higher crude protein content in the same silages. The present trend regarding the results of Total N content of this study was in corroborated with the findings of Dakore (2018) and Yadav (2018) as they recorded significantly higher Total N content in additives inoculated silage.

WSC content was found significantly ($p<0.01$) lower in *Lactobacillus fermentun* and combination of both bacterial inoculant inoculated pasture hay silage as compared to WS and PH silage. However, numerically lower values were obtained in all the additives inoculated silage as compared to control, WS and PH silage. WSC contents of all inoculated silage were comparable with control (maize silage). In support to current results, Filya (2003), Xing et al. (2009) and Yadav (2018) recorded significantly lower WSC content in all additives inoculated silage as compared to control whereas, findings of Zhao et al. (2021), noticed non significantly lower WSC content in all additives treated silage as compared to control group. In disparity to present results , Nkosi et al. (2012) and Dakore, (2018) observed significantly higher WSC content in Lactic acid bacteria and enzyme inoculated silage.

Lactic acid was found significantly($p<0.01$) higher in all the additives inoculated silage as compared to control, WS and PH silage. However, significantly ($p<0.01$) higher lactic acid content was found in *lactobacillus fermentum* followed by xylanase added WS silage, LPLF and LF added pasture hay silage. Lower water soluble carbohydrates content and higher lactic acid content in all the additives inoculated silage indicates that more water soluble carbohydrates are utilized for producing higher amount of lactic acid which results in very good silage quality. In accordance with current investigation, Jalc et al. (2009), Xing et al. (2009), Nkosi et al. (2012), Govea et al. (2013), Guo et al. (2014), Zielinskal and Fabiszewska (2017), Dakore (2018), Yadav (2018), Su et al. (2019), Oskoueian et al. (2021) and Zhao et al. (2021) reported that lactic acid content was significantly higher in bacterial inoculants and enzymes added silages as compared to control silage.

Table 3. Quality parameters of different experimental silages

Treatments	Parameters						
	Colour of silage	pH**	TVFA** (mMol/100g DM)	NH3-N** (g/kg silage)	Total N* (%	WSC** (g/100 g DM)	LA** (g/100g fresh
C	Golden yellow	4.41^{c} ±0.06	20.25^{a} ±1.84	4.40^{b} ±0.20	1.28^{ab} ±0.02	1.91^{ab} ±0.26	4.70^{b} ±0.31
WS	Golden yellow	4.15^{b} ±0.07	21.19^{a} ±1.95	4.66^{b} ±0.29	1.14^{a} ±0.06	2.48^{c} ±0.26	3.36^{a} ±0.22
X	Golden yellow	4.11^{ab} ±0.04	42.41^{b} ±3.33	3.03^{a} ±0.43	1.20ab ±0.05	1.82^{ab} ±0.04	5.82^{cd} ±0.28
LF	Golden yellow	4.00^{ab} ±0.07	50.49^{d} ±1.48	3.03^{a} ±0.23	1.23^{ab} ±0.05	1.77^{ab} ±0.31	6.19^{d} ±0.31
LPLF	Golden yellow	4.13^{ab} ±0.04	42.36^{b} ±3.49	3.50^{a} ±0.31	1.44^{b} ±0.10	1.84^{ab} ±0.05	5.67^{cd} ±0.15
PH	Golden yellow	4.31^{c} ±0.04	21.61^{a} ±0.85	3.13^{a} ±0.04	1.32^{ab} ±0.08	2.14^{bc} ±0.05	4.33^{b} ±0.07
XPH	Golden yellow	4.08^{ab} ± 0.07	43.24^{bc} ±1.79	3.08^{a} ±0.03	1.44^{b} ±0.13	1.59^{ab} ±0.07	5.41^{c} ±0.13
LFPH	Golden yellow	3.95^{a} ±0.04	49.43^{cd} ±2.40	3.16^{a} ±0.08	1.43^{b} ±0.11	1.49^{a} ±0.10	5.71^{cd} ±0.07
LPLFPH	Golden yellow	3.98^{ab} ±0.03	41.56^{b} ±1.95	3.21^{a} ±0.10	1.21^{ab} ±0.04	1.46^{a} ±0.19	5.80^{cd} ±0.12
p value	-	<0.001	<0.001	<0.001	0.04	0.007	<0.001

abcdMeans with different superscript in a column differ significantly from each other (**$p<0.01$, *$p<0.05$)

CONCLUSIONS

Thus, based on present findings of proximate composition, cell wall constituents and quality parameter of silage, it is concluded that Xylanase and *Latobacillus fermentum,* either alone or in combination significantly improves overall silage fermentation characteristics and nutrient content in wheat straw or pasture hay (3%) based green maize (7%) silage.

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