RESEASRCH ARTICLE

Influence of Bacterial Inoculants and Xylanase on Silage Fermentation Characteristics of Wheat Straw and Green Maize Silage

Saman Y. Belim*[1], Harish H. Savsani[2], Mitesh R. Chavda[3], Mulraj D. Odedra[4], Yash G. Kansagara[5], Priyam H. Agravat[6]

Abstract

The present investigation was conducted with the objective to study various combinations of biological and chemical additives on fermentation characteristics of wheat straw and green maize silage. Different silages were prepared using green maize fodder and wheat straw in the proportion of 10:0 & 7:3 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 seven different treatments, *viz.,* Control (only green maize), WS (green maize and wheat straw in 7:3 ratio), X (WS added with Xylanase), LP (WS added with *Lactobacillus plantarum*), LF (WS added with *Lactobacillus fermentum*), LPLF (WS added with both bacterial inoculants) and XLPLF (WS 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 after 45 days of ensiling. The results revealed that the pH values of different experimental silages were recorded significantly ($p<0.01$) lower as compared to control treatment. TVFA content was significantly ($p<0.01$) increased in all additives inoculated silage as compared to control and WS silage. NH_3-N content was significantly ($p<0.01$) lower in X, LF and XLPLF inoculated silage and total N content was significantly ($p<0.01$) higher in LP inoculated silage. Water soluble carbohydrate (WSC) content was significantly ($p<0.05$) lower in LP, XLPLF and LF as compared to WS silage and all additives inoculated experimental silage had significantly ($p<0.01$) higher content of lactic acid as compared to control and WS silage being highest in LP inoculated silage. Thus, it is concluded that all additives improve fermentation characteristics of silage and among all additives; *L. plantarum* is the best silage additive.

Key words: *L. fermentum, L. plantarum,* Silage, Wheat straw, Xylanase

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Introduction

India is facing an acute shortage of animal feed due to urbanization and increased animal population. According to 20th livestock census, the total livestock population in India is 536.76 million showing an increase of 4.8 % over previous census (Anonymous, 2019). Alternatives to overcome the shortage in fodder requirement are to increase the area under fodder cultivation or the judicious use of existing fodder resources or preservation of forage when in surplus. In India, surplus green fodders are available in rainy season, which can be preserved in the form of silage for its utilization as green fodder during lean periods. Fodder can also be preserved as hay, but silage has some known advantages over hay. Silage can be prepared in silage bags, plastic drums or in silo pit. Use of plastic bags and drums is relatively inexpensive and with few workers ensiling can be done manually. Also, agro-industrial wastes captured interest owing to its abundant availability, pollution reduction ability, low price as well as lignocellulosic nature (Aboudi *et al.*, 2016). Wheat straw, a by-product obtained after harvesting and threshing of wheat grains, is relatively inexpensive and available locally, its use in silage along with additives may improve its quality and thereby its utilization for animal feeding.

[1-3,5]Department of Animal Nutrition, College of Veterinary Science and A.H., Kamdhenu University, Junagadh-362001

[4,6]Department of Livestock Production Management, College of Veterinary Science and A.H., Kamdhenu University, Junagadh-362001

Corresponding Author: Saman Y. Belim, Department of Animal Nutrition, College of Veterinary Science and A.H., Kamdhenu University, Junagadh-362001, E-mail: belimsaman1998@gmail.com

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Silage additives have been used as an important managemental 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. They increase aerobic stability, reduce storage losses by promoting rapid fermentation, reduce fermentation losses by limiting the extent of fermentation and improve nutrient composition of silage (Yitbarek and Tamir, 2014). Various bacterial inoculants have been used as silage additives to increase the rate of acidification of ensiled forages (Weinberg and Muck, 1996). 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). Bacterial inoculants have many advantages over chemical additives as they are easy to use, mostly safe and do not pollute the environment. Roughages are the main source of feed for ruminants, which are rich in fibrous portion that cannot be hydrolyzed by the endogenous enzymes. Fibrolytic enzymes degrade the cell wall at a faster rate that provides additional water-soluble carbohydrate and fermentation substrate for lactic acid bacteria (LAB) growth (Ebrahimi *et al*., 2014). The objective of this study was to evaluate bacterial inoculants and enzyme on fermentation characteristics of wheat straw 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 Animal Husbandry, Kamdhenu University, Junagadh, Gujarat. All the experimental procedures were approved by Institutional Animal Ethics committee (IAEC) (Protocol no: KU-JVC-IAEC-LA-76/21), College of Veterinary Science and A.H., Kamdhenu University, Junagadh, Gujarat.

Preparation of Silage

Different silages were prepared using green maize fodder and wheat straw in the proportion of 10:0 & 7:3 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 seven different treatments, *viz.,* Control (only green maize), WS (green maize and wheat straw in 7:3 ratio), X (WS added with Xylanase), LP (WS added with *Lactobacillus plantarum*), LF (WS added with *Lactobacillus fermentum*), LPLF (WS added with both bacterial inoculants) and XLPLF (WS 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 after 45 days of ensiling.

Estimation of Silage Characteristics

Organoleptic criteria are the most important way to judge the silage quality. For this colour of silages was observed visually. For estimation of silage pH, total volatile fatty acid (TVFA) and ammonia nitrogen (NH_3-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 h, 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 NH_3-N were analysed as per the method given by Prasad (2015). Total nitrogen was estimated by Kjeldahl method (AOAC, 2005). 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).

Statistical Analysis

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 $p<0.05$.

Results and Discussion

Data pertaining to pH, TVFA, NH_3-N, total nitrogen, WSC and lactic acid content of silages after 45 days of ensiling are presented in Table 1. The colour of all experimental silages was observed as golden yellow. The pH values of different experimental silages were recorded significantly ($p<0.01$) lower as compared to control treatment. It might be due to the addition of enzyme and bacterial inoculants as they decreased the silage pH rapidly as compared to control. The pH values of LP and XLPLF were noticed significantly ($p<0.01$) lowest as compared to other experimental silage, however, they were at par with LF treated silage. These results are in agreement with the observations of many of the earlier workers (Jalc *et al*., 2009; Nkosi *et al*., 2012; Guo *et al*., 2014; Khota *et al*., 2017; Zhao *et al*., 2021). Filya (2003) also observed significantly lower pH in *L. plantarum* inoculated silage.

Analysis of variance revealed that TVFA content was significantly ($p<0.01$) increased in all additives inoculated silage as compared to control and WS silage, being significantly highest in XLPLF followed by LF, LP, X and LPLF silage (Table 1), which concurred well with the findings of Dakore (2018) and Yadav (2018).

NH_3-N content was significantly ($p<0.01$) lower in X (Xylanase), LF (*Lactobacillus fermentum*) and XLPLF inoculated silage as compared to Control and WS silage. Govea *et al*. (2013), Dakore (2018) and Zhao *et al.* (2021) also reported significantly lower ammonia nitrogen content in all additives inoculated silage as compared to control. Total N content was significantly ($p<0.01$) higher in LP *(Lactobacillus plantarum*) inoculated silage as compared to all other silages, except LPLF which was statistically similar. However, numerically higher Total N content was observed in all additives inoculated silage as compared to WS silage.

Table 1: Fermentation characteristics of different experimental silages

Treatments	Parameters					
	pH**	TVFA** (mMol/100 g DM)	NH_3-N** (g/kg silage)	Total N** (% DM)	WSC* (g/100g DM)	LA** (g/100g fresh silage)
C	4.41^{c} ±0.06	20.25^{a} ±1.84	4.40^{bc} ±0.20	1.28^{a} ±0.02	1.91^{ab} ±0.26	4.70^{b} ±0.31
WS	4.15^{b} ±0.07	21.19^{a} ±1.95	4.66^{c} ±0.29	1.14^{a} ±0.06	2.48^{b} ±0.26	3.36^{a} ±0.22
X	4.11^{b} ±0.04	42.41^{b} ±3.33	3.03^{a} ±0.43	1.20^{a} ±0.05	1.82^{ab} ±0.04	5.82^{c} ±0.28
LP	3.86^{a} ±0.04	49.78^{c} ±2.66	3.96^{abc} ±0.56	1.55^{c} ±0.09	1.38^{a} ±0.25	6.97^{d} ±0.31
LF	4.00^{ab} ±0.07	50.49^{c} ±1.48	3.03^{a} ±0.23	1.23^{ab} ±0.05	1.77^{a} ±0.31	6.19^{cd} ±0.31
LPLF	4.13^{b} ±0.04	42.36^{b} ±3.49	3.50^{ab} ±0.31	1.44^{bc} ±0.10	1.84^{ab} ±0.05	5.67^{c} ±0.15
XLPLF	3.91^{a} ±0.05	57.51^{d} ±1.24	3.26^{a} ±0.29	1.31^{ab} ±0.06	1.41^{a} ±0.05	6.21^{cd} ±0.32
p value	0.002	<0.001	0.008	0.001	0.019	<0.001

TVFA- total volatile fatty acid; NH_3-N ammonia nitrogen;Total N- total nitrogen; WSC- water soluble carbohydrate; LA- lactic acid

Means with different superscript in a column differ significantly from each other at $^{**}p<0.01$, $^{*}p<0.05$.

Water soluble carbohydrate (WSC, g/100 g silage DM) content was significantly ($p<0.05$) lower in LP, XLPLF and LF as compared to WS silage, however, it was statistically similar with control silage (Table 1). Lower WSC content in all additive inoculated silage indicates its greater utilization for lactic acid production. WSC contents of all inoculated silage was comparable with control (maize silage). These observations supported the earlier findings of Filya (2003), Xing *et al.* (2009), Yadav (2018) and Zhao *et al.* (2021). Lactic acid content (g/100g fresh silage) of all additives inoculated silage was significantly ($p<0.01$) higher as compared to control and WS silage, which also differed significantly. LP (*Lactobacillus plantarum*) inoculated silage showed highest value of lactic acid followed by XLPLF, LF, X and LPLF as compared to WS silage (Table 1). Xing *et al.* (2009), Govea *et al.* (2013), Yadav (2018) and Zhao *et al.* (2021) also recorded findings in accordance with current investigation. WSC were significantly ($p<0.05$) reduced in all additives inoculated silages which might designate that more WSC are utilized by lactic acid bacteria (LAB) as they are indicated as crucial substrates for the growth of LAB for ensuring good fermentation. Also, excellent quality silage is probably to be attained when the lactic acid is chief acid produced which is likely to reduce the silage pH more capably.

Conclusions

It is concluded that all additives (Xylanase, *L. plantarum* and *L. fermentum* either single or in combination) improve fermentation characteristics of green maize and wheat straw based silage. Among all additives, *L. plantarum* is the best silage additive which improves silage fermentation characteristics when green maize and wheat straw are used in silage making at 7:3 ratio.

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