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Impact of biological and chemical additives on quality of wheat straw and green maize silage

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Abstract

The present investigation was conducted with the objective to study various combinations of biological and chemical additives on silage quality of wheat straw and green maize silage. Different silages were prepared using green maize fodder and wheat straw as such 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), WS (green maize and wheat straw in 7:3 ratio), X (WS added with Xylanase), LP (WS added with *L. plantarum*), LF (WS added with *L. 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, 1×10^6 cfu/g and 2×10^6 cfu/g, respectively. All silages were evaluated in terms of pH, proximate composition and cell wall constituents after 45 days of ensiling. Ensiling significantly ($p < 0.05$) reduced dry matter (DM) content in all additive inoculated silages except for Xylanase treated and WS silage. The pH values of different experimental silages were recorded significantly ($p < 0.01$) lower as compared to control treatment. DM content was significantly ($p < 0.01$) lowered in all additive inoculated silages except for Xylanase inoculated silage as compared to WS silage. OM (Organic matter) content was found to be significantly ($p < 0.01$) higher in LP, LF and LPLF inoculated silages. *L. plantarum* significantly ($p < 0.05$) increases crude protein (CP) content in silage. Xylanase added silage showed significantly ($p < 0.05$) higher EE content. CF content was found to be significantly ($p < 0.05$) lower in *L. fermentum* treated and control silage. Total ash (TA) content was found significantly ($p < 0.01$) lower in LP, LF and LPLF inoculated silages. Nitrogen free extract (NFE) content was found to be significantly ($p < 0.05$) higher in *L. fermentum* inoculated silage. The result showed that Neutral detergent fibre (NDF) content was found to be significantly ($p < 0.05$) lower in Xylanase and *L. fermentum* inoculated silages. Acid detergent fibre (ADF) content was also found to be significantly ($p < 0.05$) lower in Xylanase treated silage. Cellulose content was found to be significantly higher in combination of both bacterial inoculants (LPLF) inoculated silages. Hemicellulose content did not differ significantly ($p > 0.05$). Thus, it is concluded that bacterial inoculants alone and in combination with xylanase improves the nutrient composition among which xylanase significantly increases ether extract content with reduced NDF and ADF content.

Keywords: *L. fermentum*, *L. plantarum*, silage, wheat straw, xylanase

1. Introduction

Agriculture and animal husbandry in India are interwoven with the intricate fabric of the society in cultural, religious and economical ways as mixed farming and livestock rearing forms an integral part of rural living. India has the enormous livestock sector and livestock performs an crucial function in the rural financial system of India. Livestock sector contributes 4.11 % GDP and 25.6 % of total agriculture GDP of India. Also, there is a huge amount of low quality feedstuff is available in India. Wheat straw is one of them and is of poor quality but available locally and inexpensive. Its use in silage along with additives may improve its quality and there by its utilization in animal feeding.

Agro-industrial wastes captured interest owing to its abundant availability, pollution reduction ability, low price and lignocellulosic nature (Aboudi *et al.*, 2016) [1]. Globally, wheat straw, a by-product obtained after harvesting and threshing of wheat grains, is the most important by-product of wheat crop produced in larger quantity (Reddy and Yang, 2005; Hemdane *et al.*, 2016) +. About 529 million tons of wheat straw is generated every year in all over the world (Govumoni *et al.*, 2013) [11] and it is one of the abundant agricultural waste available.

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.

Silage is a fermented feed resulting from the storage of high moisture crop, usually green forages, under controlled anaerobic condition in closed structure (Banerjee, 2019) [4].

Quality of silage can be improved through stimulation of the ensiling process by adding different types of chemical and biological additives at the time of ensiling. Silage additives have been used as a management tool to improve the nutritional value of silage. The important aim of using silage additives is to promote the growth of lactic acid producing bacteria during the fermentation cycle and minimize losses to improve the quality of the silage to avoid other fermentation (Clostridial fermentation) products (Chauhan *et al.*, 2021) [5].

Various bacterial inoculants have been used as silage additives to increase the rate of acidification of ensiled forages (Weinberg and Muck, 1996) [27]. They can be applied early in the process to ensure that fermentation occurs appropriately and to improve the silage quality (Muck *et al.*, 2018) [21]. 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) [19]. The main function of the exogenous fibrolytic enzymes is to release maximum amount of nutrients from the digestible, potentially digestible and indigestible fractions of the cell wall (Mocherla *et al.*, 2017) [20]. Fibrolytic enzymes degrade the cell wall at a faster rate and additionally water soluble carbohydrate are pooled to provide growth fermentation substrate for lactic acid bacteria (LAB) (Ebrahimi *et al.*, 2014) [8]. Considering huge availability of wheat straw at cheapest price in India, and role of biological and chemical additives in silage production, the present study was conducted to study their effects on silage quality of wheat straw and green maize silage.

2. Material and Methods

Different silages were prepared using green maize fodder and wheat straw 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), WS (green maize and wheat straw in 7:3 ratio), X (WS added with xylanase), LP (WS added with *L. plantarum*), LF (WS added with *L. 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, 1×10^6 cfu/g and 2×10^6 cfu/g, respectively. All silages were evaluated for silage fermentation characteristics after 45 days of ensiling. Before ensiling samples of green maize fodder, wheat straw and mixture of green maize & wheat straw (7:3) were analysed for proximate composition and cell wall constituents. Sampling of silages were done on 45th day of ensiling. Samples from different experimental silage were evaluated in terms of their proximate composition and cell wall constituents according to the methods of AOAC (2019) + and Van Soest *et al.* (1991) [26], respectively except for the dry matter content of silage which was analysed as per the method given by Philip and John (1977) [23]. The pH of silage was estimated as per the method of Wilson and Wilkins (1972) [28]. 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) [25]. Pair wise mean difference between groups were compared by Duncan's New Multiple

Range Test as modified by Kramer (1957) [18].

3. Results and Discussion

Fodders used for preparation of different experimental silages were analysed for proximate composition and cell wall constituents (% DM basis) and the results are presented in Table 1. The data regarding the effect of ensiling on DM content of different experimental silages are presented in Table 2. The data on the paired t test revealed that ensiling significantly ($p < 0.01$) reduced the DM content of both control as well as different experimental silages prepared from green maize (GM): wheat straw (WS)-7:3 except WS and Xylanase inoculated silage. Similar results were recorded by Dakore (2018) [6], Yadav (2018) [30] and Chauhan *et al.* (2021) [5], as they observed significantly reduced DM content of different experimental silages as compared to before ensiling. In the same line, Khota *et al.* (2017) [17] noticed significantly lower DM content in all experimental silages as compared to control silage.

The results regarding proximate composition and cell wall constituents of different experimental silages (% DMB) are presented in Table 3 and Table 4, respectively. 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. These results are in agreement with the observations of most of the workers, Jalc *et al.* (2009) [16], Nkosi *et al.* (2012) [22], Govea *et al.* (2013) [10], Guo *et al.* (2014) [12], Khota *et al.* (2017) [17], Zielinska and Fabiszewska (2018) [32], Dakore, (2018) [6], Yadav (2018) [30] and Zhao *et al.* (2021) [31] as they observed significantly lower pH in all inoculated silages as compared to control silage.

The result showed that DM contents of different experimental silages were found to be significantly ($p < 0.01$) higher in all treated silages as compared to control silage. However, that of LP, LF, LPLF and XLPLF were significantly ($p < 0.01$) lower as compared to X and WS silage and values for LPLF and XLPLF were statistically similar. In the same line, Khota *et al.* (2017) [17] noticed significantly lower DM content in all experimental silages as compared to control silage. The findings in the present experiment are in disagreement with Jalc *et al.* (2009) [16], who reported significantly higher DM content of different additives treated experimental silages as compared to control silage. However, they observed significantly higher DM content in *Lactobacillus plantarum* treated silage which is in disparity with current results. The OM was noted significantly ($p < 0.01$) higher in LF, LPLF, LP and X experimental silage as compared to C silage and this was at par with WS treatment. Well corroborated with Dakore (2018) [6] who reported significant increase in the OM content of all additive inoculated silages as compared to control. Yadav (2018) [30] recorded significantly lower OM content in different experimental silages as compared to control silage. CP content of LP (*Lactobacillus plantarum*) inoculated silage was noticed significantly ($p < 0.01$) higher as compared to all other experimental silage, except LPLF which was at par but significantly higher from WS. However, numerical increase in crude protein content was noticed in all additive inoculated silage as compared to WS silage. In support to current findings, Xing *et al.* (2009) [29], Nkosi *et al.* (2012) [22] and Khota *et al.* (2017) [17] recorded significantly higher CP in *L. plantarum* treated silage also, Dong *et al.* (2020) [7] noted numerically higher values of CP content in *Lactobacillus*

plantarum + molasses inoculated experimental silage. EE content was noticed significantly ($p<0.05$) higher in X (Xylanase) treatment as compared to C, WS, LP and XLPLF treatment. EE content of different experimental inoculated silage were comparable with control (maize silage). The present findings are supported with the results of Khota *et al.* (2017) [17] and Yadav (2018) [30] as they reported non significantly higher EE content of different additives inoculated silages. CF content was reported significantly ($p<0.05$) lower in *Lactobacillus fermentum* treatment as compared to WS and it was at par with C silage. Unlike the present investigation, results obtained from the study of Jalc *et al.* (2009) [16] revealed significant decrease in the CF content of all experimental silages. TA contents of LP, LF and LPLF silage were significantly ($p<0.01$) at par with WS silage. However, TA contents of X and XLPLF were found to be significantly ($p<0.01$) highest among additives inoculated experimental silages which might be due to increase in mineral content because of xylanase. In contrary with current result, Jalc *et al.* (2009) [16] reported non significant decrease in TA content in all additives inoculated silage. The current result was supported by the work of Nkosi *et al.* (2012) [22] that ash content was significantly reduced in *Lactobacillus plantarum* treated whole crop sweet sorghum silage. NFE content was significantly ($p<0.05$) higher in *Lactobacillus fermentum* treatment as compared to all other experimental silage except X and control silage which were at par with all other treatments. NFE contents of different experimental inoculated silage were comparable with control (maize silage). Literature pertaining to NFE content of silages was not available yet, hence it is not possible to discuss NFE content.

The statistical analysis of the data regarding NDF content revealed that X (Xylanase) and LF (*Lactobacillus fermentum*) silage shows significantly ($p<0.05$) lower value of NDF content as compared to WS silage however, it was at par with C, LP, LPLF and XLPLF inoculated silage. NDF contents were comparable with control (maize silage). In the same line, the present findings are corroborated with Govea *et al.* (2013) [10], Khota *et al.* (2017) [17], Yadav (2018) [30] and Agarrusi *et al.* (2019) [2] as they recorded non significant decrease in NDF content of different additives inoculated experimental silages. The result of the present work was also supported with findings of Xing *et al.* (2009) [29], Nkosi *et al.* (2012) [22], Guo *et al.* (2014) [12], Dakore (2018) [6], Dong *et al.* (2020) [7] and Zhao *et al.* (2021) [31] as they observed significant decrease in the NDF content of various additives inoculated experimental silages as compared to control silage. Corresponding to current findings, Jalc *et al.* (2010) [15] observed significantly lower NDF content in *L. fermentum* inoculated silage.

ADF (Acid detergent fibre) content was recorded significantly ($p<0.01$) lower in X (Xylanase) inoculated experimental silage as compared to WS silage however, it was at par with LP, LF and C silage. ADF contents were comparable with control (maize silage). Lower values for NDF and ADF content might be due to the effect of Xylanase to release maximum amount of nutrients from potentially digestible and indigestible fractions of cell wall. In corresponding to current findings, Khota *et al.* (2017) [17] reported non significant increase in ADF content of Acremonium cellulase and *Lactobacillus plantarum* inoculated silage and Yadav, (2018) [30] and Agarrusi *et al.* (2019) [2] reported non significant

decrease in ADF content of various additives inoculated experimental silages however, in present study it differs significantly. The findings of the present study was also supported with results of Jalc *et al.* (2009) [16], Nkosi *et al.* (2012) [22], Guo *et al.* (2014) [12], Dakore, (2018) [6] and Dong *et al.* (2020) [7] as they observed significant reduction in the ADF content of different experimental silages as compared to control silage while, in current study it was numerical reduction in all additive inoculated experimental silages.

Cellulose content was noticed to be significantly ($p<0.01$) higher in LPLF experimental silage as compared to other experimental silages, which did not differ significantly with each other. In accordance with present results, Zhao *et al.* (2021) [31] reported significant increase in the cellulose content of lactic acid bacteria inoculated silage. In divergence with the present result Gang *et al.* (2020) [9] noted non significant reduction in additives inoculated experimental silages as compared to control. Hemicellulose content did not differ significantly ($p>0.05$) from each other however, numerically lowest hemicellulose content was recorded in XLPLF inoculated silage. Similar findings were perceived by Gang *et al.* (2020) [9] as they reported non significant increase of hemicellulose in all inoculated experimental silage as compared to control. Findings of Zhao *et al.* (2021) [31] also supported the results of present study but they found significantly higher hemicellulose content in lactic acid bacteria inoculated silage whereas, in present study it was recorded non significant. In dissimilarity with the present result, Dakore (2018) [6] reported significant decrease in hemicellulose content of all additive treated silage while, Yadav (2018) [30] noticed numerically higher value of hemicellulose in enzyme xylanase and both bacterial inoculants inoculated silage which was in disparity with present results.

Table 1: Proximate composition and cell wall constituents of experimental fodders before ensiling (% DM basis)

Parameters	Green maize	Wheat straw	Green maize: Wheat straw -7:3
DM	33.29±0.38	90.89±0.18	42.01±1.45
OM	90.90±0.20	86.95±0.15	88.70±0.19
CP	9.10±0.55	4.22±0.05	6.33±0.83
EE	1.64±0.05	1.04±0.05	1.35±0.07
CF	32.90±1.02	40.26±0.70	38.72±0.42
TA	9.10±0.20	13.05±0.15	11.30±0.19
NFE	47.26±1.42	41.43±0.86	42.30±0.67
NDF	68.08±0.27	79.05±0.14	71.79±0.59
ADF	41.94±1.05	56.82±0.64	48.11±0.09
Cellulose	33.95±1.54	40.30±1.20	36.64±0.35
Hemicellulose	26.14±1.32	22.22±0.79	23.68±0.50

Table 2: Effect of ensiling on DM content of different experimental silages

Treatments	DM before ensiling	DM after ensiling	p value
C**	33.29±0.38 ^b	31.39±0.32 ^a	0.010
WS	42.01±1.45	41.02±0.22	0.236
X	42.01±1.45	41.63±0.30	0.686
LP**	42.01±1.45 ^b	38.51±0.19 ^a	0.006
LF**	42.01±1.45 ^b	37.51±0.19 ^a	<0.001
LPLF**	42.01±1.45 ^b	35.17±0.48 ^a	<0.001
XLPLF**	42.01±1.45 ^b	34.50±0.27 ^a	<0.001

Note: ^{ab}Means with different superscript within a row differ significantly (** $p<0.01$) in paired t test

Table 3: Proximate composition of different experimental silages (% DMB)

Treatments	Parameters							
	pH**	DM**	OM**	CP**	EE*	CF*	TA**	NFE* [©]
C	4.41 ^c ±0.06	31.39 ^a ±0.32	84.59 ^a ±0.23	8.06 ^{ab} ±0.17	1.08 ^a ±0.05	36.06 ^a ±2.39	15.40 ^d ±0.23	39.40 ^{ab} ±2.61
WS	4.15 ^b ±0.07	41.02 ^c ±0.22	87.57 ^{cd} ±0.64	7.15 ^a ±0.40	0.90 ^a ±0.03	45.11 ^b ±3.69	12.42 ^{ab} ±0.64	33.37 ^a ±4.65
X	4.11 ^b ±0.04	41.63 ^c ±0.30	86.74 ^c ±0.52	7.54 ^a ±0.32	1.48 ^b ±0.27	41.15 ^{ab} ±0.74	13.25 ^c ±0.52	37.89 ^{ab} ±1.08
LP	3.86 ^a ±0.04	38.51 ^d ±0.19	88.01 ^d ±0.12	9.73 ^c ±0.57	1.03 ^a ±0.02	41.03 ^{ab} ±1.07	11.98 ^a ±0.12	36.23 ^a ±0.94
LF	4.00 ^{ab} ±0.07	37.51 ^c ±0.19	88.54 ^d ±0.28	7.72 ^{ab} ±0.32	1.18 ^{ab} ±0.05	36.09 ^a ±0.96	11.47 ^a ±0.28	43.54 ^b ±1.00
LPLF	4.13 ^b ±0.04	35.17 ^b ±0.48	88.21 ^d ±0.10	9.00 ^{bc} ±0.63	1.15 ^{ab} ±0.06	42.06 ^{ab} ±1.53	11.79 ^a ±0.10	36.00 ^a ±1.90
XLPLF	3.91 ^a ±0.05	34.50 ^b ±0.27	85.72 ^b ±0.13	8.25 ^{ab} ±0.40	1.05 ^a ±0.02	43.13 ^b ±2.03	14.28 ^c ±0.13	33.29 ^a ±1.74
p value	0.002	<0.001	<0.001	0.003	0.039	0.021	<0.001	0.051

DM-Dry matter; OM- organic matter; CP- crude protein; EE- ether extract; CF- crude fibre; TA- Total ash; NFE- nitrogen free extract; Note: ^{abcd}Means with different superscript within a column differ significantly (***p*<0.01, **p*<0.05).

© - Though p value in ANOVA shows non significant difference but when significance tested by DNMRT it revealed significant difference.

Table 4: Cell wall constituents of different experimental silages (% DMB)

Treatments	Treatments			
	NDF* [©]	ADF**	Cellulose**	Hemicellulose
C	67.41 ^{ab} ±1.74	47.41 ^{abc} ±1.70	33.49 ^a ±0.63	20.00±0.70
WS	69.89 ^b ±0.84	49.95 ^c ±0.47	33.58 ^a ±0.42	19.94±0.90
X	65.96 ^a ±0.72	44.55 ^a ±0.99	33.37 ^a ±0.43	21.41±0.74
LP	66.92 ^{ab} ±0.72	45.61 ^{ab} ±0.82	32.69 ^a ±0.63	21.31±1.29
LF	66.13 ^a ±1.45	45.79 ^{ab} ±0.77	32.23 ^a ±0.57	20.34±2.10
LPLF	68.85 ^{ab} ±1.37	48.21 ^{bc} ±0.82	36.50 ^b ±0.68	20.64±1.64
XLPLF	66.93 ^{ab} ±0.73	48.97 ^c ±0.97	33.27 ^a ±1.00	17.96±1.57
p value	0.190	0.004	0.002	0.575

NDF- neutral detergent fibre; ADF- acid detergent fibre;

Note: ^{abcd}Means with different superscript within a column differ significantly (***p*<0.01, **p*<0.05).

© - Though p value in ANOVA shows non significant difference but when significance tested by DNMRT it revealed significant difference.

4. Conclusions

Ensiling significantly reduces DM content except Xylanase and wheat straw inoculated silage. Bacterial inoculants significantly reduce DM content of silage. *Lactobacillus plantarum* significantly increases CP content in silage. Xylanase inoculated silage shows significant improvement in EE content. *Lactobacillus fermentum* inoculated silage shows significantly improve values of CF and NFE. Xylanase significantly reduce NDF and ADF content. Combination of bacterial inoculants (LPLF) significantly improves cellulose while additives do not have any significant effect on hemicellulose content. Thus, it is concluded that bacterial inoculants alone and in combination with xylanase improves the nutrient composition among which xylanase significantly increases ether extract content with reduced NDF and ADF content.

5. Conflict of interest statement

None of the major conflicts of interest has reported by authors and all authors are agreed for the final statement.

6. Acknowledgement

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