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RESEARCH ARTICLE

CONSEQUENCE OF INOCULANTS ON NUTRITIVE IMPORTANCE OF MAIZE SIALGE

Muhammad Arif[1], Hamza Abid[1], Muhammad Saeed[3*], Servet Yalçin[2], Mohamed E. Abd El-Hack[4], Muhammad Asif Arain[3], Qurban Ali Shah[6], Abbasi I. H. R[3], SayedHaider Abbas Raza[3], Hafiz Muhammad Zakria[3]. Rab Nawaz Soomro[3], Ghulam Hussain Jaffar[5]**

[1]Department of Animal Sciences College of Agriculture University of Sargodha Pakistan.
[2]Department of Animal Science Faculty of Agriculture Ege University,Izmir, Turkey
[3]College of Animal Sciences, Northwest A&F University, Yangling, 712100, China.
[4]Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig, 44111, Egypt
[5]Department of Livestock and Dairy Development, Balochistan, (PAKISTAN).
[6]University of Agriculture, Water & Marine Sciences, Uthal Pakistan.

Abstract:

This investigation was conducted to evaluate the effects of different inoculants on nutritive value and *in-vitro* dry matter (DM) digestibility of different maize crop silages. Three varieties of maize (*Zea mays*) (A) 31P41, (B) 32B33and (C) P1543 were cultivated at University College of Agriculture, University of Sargodha. Two inoculants; pioneer 1132 (blend of *E. faecium, L. plantarum)* and Pioneer11C33 (blend of *L. buchneri, L. plantarum and E. faeciu)* were used to make silage of maize fodder at half kernel milk line stage having moisture 65-70%. Five kilogram maize fodder was ensiled in triplicate lab silo having dimension (3`x1.5`) in a 3x4x4 factorial arrangement under Complete Randomized Design for 28 days. Highest value for DM was observed in silage having Variety C and a_1b_1 levelofinoculants. Lowest value for DM was observed in silage having variety A and $a_0b_{1.4}$ levelof inoculants. Whereas, highest values for NDF and ADF observed in silage having variety C and a_0b_0 level of inoculants. Lowest value for pH was observed in silage having variety A and treatment level a_1b_1 ofinoculants. Whereas, highest value for pH was observed in silage having variety B and a_0b_0 level of inoculation. Inoculation of lactic acid bacteria significantly increased the DM, pH and in-vitro digestibility dry mater(IVDMD) and decreased the Neutral detergent fiber (NDF), Acid detergent fiber (ADF) contents of all varieties silages but crude protein (CP) remained unaffected. Further investigation requires to identification of mode of action as well as other effective or toxic effect on animal.

Key words: Inoculants, nutritive value, *in-vitro* dry matter, silages.

Introduction

Shortage of fodder, low yield per acre and fodder scarcity periods greatly reduce the livestock productivity in tropical areas Sarwar*et al*.(2002). Best alternative to overcome scarcity period could be the preservation of fodder MohdNajib*et al*. (1993). Different techniques have been used for the preservation of surplus fodder. The plant materials are preserved as silage or hay during scarcity period for sustained animal growth and milk production throughout the year (Hall and Martens, 2012). Hay making is mostly dependent on weather condition, due to lack of drying facilities in developing countries. Silage could be used at any time when required, particularly during periods of drought (Koon, 1993).There is a wide range of nutritional and microbial additives, which were added during the ensiling process of grasses (MacDonald, 1981). These additives were divided into four categories i.e. (1) fermentation stimulants; e.g. bacterial inoculants and enzymes (3) aerobic deterioration inhibitors and (4) nutrients; maize grains, molasses urea or anhydrous ammonia (Ferns and mayne, 1994; Kung, 1992 and Fransen and struby, 1998). These additives played an important role in achieving stable pH in a much shorter time, which corresponded to speedy use of lactic acid content (main product of fermentation) and a slower accumulation of acetic acid (Rooke et al., 1985). The stable pH was helpful for achieving desirable fermentation and quality silage with higher nutritive value and minimum ensiling losses (Thomas and Thomas, 1985).The fermentation phase is influenced by pH, availability of bacterial substrate, CP content, moisture content and predominate bacteria during ensiling process (Thomas and Thomas, 1985; Bolsen*et al*., 1996). As soon as the fermentation is completed, silage will preserve more nutrients. For achieving the faster fermentation, lactic acid is the dominanting acid by LAB. Lactic acid was

stronger acid as compared to other organic acids (acetic, propionic and butyric) and it quickly dropped the pH of silage (Schroeder, 2004; Kung and shaver, 2001). This in turn caused the poor fermentation of freshly cut material leading to lower nutritive value (Kung and shaver, 2001).

Chemical fermentation stimulants included enzyme and acid preparations. Enzymes like hemicellulases and cellulases were considered as potential means for provision of energy substrates and improved degradability of silage in rumen (Fredeen and McQueen, 1993). Formic acid and sulphuric acid were the most commonly used acid preparations for achieving low pH and minimizing nutrient losses (Gordon, 1989). Although chemical additives were very useful for ensilation, but could be dangerous to handle, unpleasant and cause corrosiveness of ensiling equipments. On the other hand, biological additives were safe to handle and non-corrosive (Bolsen, *et al*., 1995; Kung, 1996). Bacterial inoculants were most commonly used biological additives for tropical grasses (Bolsen and Heidker 1985; Pahlow and Honig, 1986).

According to Jalc *et al*., (2009) Natural population of lactic acid producing bacteria (LAB) on plant material was comparatively low resulting in less production of lactic acid. Lactic acid bacteria were of both homofermentative (*Lactobacillus plantrum*, *Enterococcus faceium and Pedicoccusspp* and heterofermentaive origin (*Lactobacillus buchneri;* Muck, 2008). Inoculants with chiefly homofermentative LAB frequently reduced the aerobic stability of silage due to the insufficient production of VFA (Rust *et al*., 1989; Weinberg *et al*., 1993; Muck and Kung, 1997). Heterofermentative bacteria produced less lactic acid compared to acetic acid and butyric acid production (Sheperd*et al*., 1995; Aksu*etal*., 2004). Excess production of acetic acid and butyric acid decreased the palatability and digestibility of silage while increased NH_3 and CO_2 production and gave bad odour to silage (Kung, 2001). Higher oxygen concentration in the silage, promotes fungal growth which raised the internal temperature thus deteriorating the quality of silage (McDonald *et al*., 1991).

Heterofermentative (*Lactobacillus buchneri*) bacteria now commercially available produced high concentration of acetic acid in ensiling process that inhibit the growth of fungi and prevented the silage from spoilage upon exposure to air (Weinberg *et al*., 2003; Filya, 2003). Therefore, in order to ensure the efficient fermentation process, different inoculants could be used to produce well preserved silage. Inoculants improved the crude protein (CP), digestibility and reduced acid detergent fiber (ADF), neutral detergent (NDF) of corn silage (Nkosi*et al*., 2011; $p<0.05$). Inoculation of corn, alfalfa and grass silages also improved the DM digestibility of silage (Gordon, 1989; Schaefer *et al*., 1989; Phillip *et al*., 1990; McAllister *et al*., 1998). The use of inoculants could improve the nutritive value of maize silage. Therefore present study was planned to evaluate effect of inoculants on nutritive value and *in-vitro* DM digestibilityof maize silage.

The main objective of this study was to evaluate the effect of inoculants (Pioneer1132 and 11C33) on nutritive value and *in-vitro* digestibility of maize silages (Pioneer1543, 32B33 and 31P4) at half kernel milk line stage having moisture 65-70%.

2. Materials and Methods

The research was conducted at University College of Agriculture, University of Sargodha. Maize fodder was chopped as 1-2cm particle size. After filling, bags were tightly compressed evenly to remove air from bags completely and made them air tight to produce the anaerobic environment. The chemical analysis and *in-vitro* DM digestibility were determined as mentioned beneath.

2.1. Managemental Measures

Managmental measures like (a) Time of sowing (b) Sowing method (c) Harvesting time were followed as according to company recommendation.

2.1.2 Harvesting Time

Irrigation period maintained until $3/4^{th}$ cob cover became dry then irrigation was stopped. At this stage crop was harvested at DM 65-70% and half kernel milk line. For each chemical analysis of maize silage, randomly, 250g sample was collected from each bag and then dried in hot air oven at $65\text{-}70^0$C and stored for further analysis. The oven dried samples were ground through 1mm sieve and were analyzed for DM, CP, NDF and ADF (AOAC 1990; Van Soest*et al*., 1991).

2.1.3 Dry matter determination

Calculation of the % moisture was made by following formula.

$$\%\text{Moisture} = \frac{(W1-W2)}{W1} \times 100$$

DM= 100- %Moisture
W1= Wt. of petridish including sample weight.
W2 = Wt. of petridish after oven drying

2.1.4 Crude protein determination
Calculation of the % CP was made by using following formula.

$$\%CP = \frac{\text{Vol of 0.1N } H_2SO_4 \text{ used} \times \text{Dilute. Of sample solution} \times 0.0014 \text{ X } 6.25}{\text{Wt. of sample} \times \text{Sample solution used}} \times 100$$

2.1.5 Neutral detergent insoluble fiber determination
Calculation of the NDF was made by using following formula.

$$NDF = \frac{(\text{weight of crucible + cell wall contents}) - \text{weight of crucible}}{\text{Wt. of dry sampl}} \times 100$$

2.1.6 Acid detergent insoluble fiber determination
Calculation of the ADF was made by using following formula

$$ADF\% = \frac{W1-W2}{S} \times 100$$

W1= Wt. of oven dried crucible including fiber.
W2= Wt. of empty oven dried crucible.
S= Wt. of oven dried sample.

2.1.7 pH
The pH of samples were determined using 20 g of wet material added to 100 ml of distilled water. The sample was homogenized for 10 minutes in a blender and pH was determined by using a digital pH meter (Waldo and Schultz, 1956).

2.1.8*In-Vitro* dry matter digestibility
The maize silage samples were analyzed for *in-vitro* DM digestibility according to the method as described by Tilley and Terry (1963). The fresh rumen contents were brought from local slaughter house in insulated bottles and transported immediately to the experimental site. The rumen contents were squeezed through four layer of cheese cloth kept in water bath having temperature 39^{o}C degree until incubation took place. 2.5g of each sample was taken in a separate bottle having 50ml rumen liquor 200ml buffer solution (Buffer solution : KH_2PO_4 2g/L, $MgSO_4.7H_2O$ 0.1 g/L, NaCl 2.8 g/L, $CaCl_2$ 0.1g/L, Na_2HPO_4 6g/L; Elmenofy et al., (2012). The bottles were kept in water bath having fix temperature 39^{0}C degree. The samples were run for *in-vitro* DM digestibility at 6, 12, 24 and 36 hours of incubation.

2.1.9 Statistical analysis
The data recorded was subjected to statistical analysis using analysis of variance under Completely Randomized Design in 3x4x4 factorial arrangement. The difference among treatments was studied as described by Steel *et al*., (1996).

3. Results and Discussions
Interaction was found ($p<0.05$) between varieties and inoculants for DM. Inoculants had individual and cumulative effect on DM. The DM was highest (13.84%) in silage having Variety C at a_1b_1 levelsofinoculants and DM was lowest (12.16%) in silage having variety A at $a_0b_{1.4}$ levelof inoculants. Highest DM was observed (7.04% and 6.74%) at a_1 and b_1 levels of inoculants in silage having variety C and DM was lowest (6.58% and 6.32%) at a_0 and $b_{1.4}$ levels of inoculants in silage having variety A. Variety C remained best in term of DM. The best levels of inoculants were a_1 and b_1 and the poor levels of inoculants were $a_{1.4}$ and $b_{1.4}$(Table-1, Table -3 and Table -4).
The results of the present study are in agreement with Bilal, (2009); Dabo*et al*. (1988); Lee *et al*. (1991) and Ruiz *et al*. (1992) who reported that inoculation of silage increased the DM of silage. The results of the present study were not in agreement with the studies of Junges*et al*. (2013) and Borreani*et al*. (2007) who reported that DM was not affected by inoculation. Contradiction in results might be related to the various factors like type and maturity stage

of the crop and ensiling technique used (Henderson and MacDonald, 1984; MacDonald *et al*. 1991; Ghazali*et al*. 2013 and Haghparvar*et al*. 2012).

No individual or combined effects of inoculant and variety were observed ($p>0.05$) for CP (Table-1, Table -3 and Table -4).

The results of the present study are in agreement with Mohammadzadeh*et al*. (2011); Filya (2003a); Kleinschmit and Kung, (2006a) who reported that CP was unaffected by inoculation. The results of the present study are in agreement with the Junges*et al*. (2013) and Borreani*et al*. (2007) who reported that inoculation did not significantly increase the CP content of silage.Unaltered CP observed in present study was probably due to ideal pH (< 4.2) for almost all silos because at this pH proteolytic activity is ceased and so CP losses did not take place in any of the silos (Gupta *et al*. 1981; Etman*et al*.1994 and MacDonald *et al*. 2010).

Interacion was observed ($p<0.05$) between inoculants and different varieties silages for NDF and ADF. Neutral detergent fiber and ADF significantly decreased by independent and combined inoculation. Lowest NDF and ADF were observed (5.61%, 9.75%) in silage having variety A and B at a_1b_1 level of inoculants and highest NDF and ADF were observed (5.95%, 10.81%) in silage having variety C at a_0b_0 level of inoculants. Lowest NDF and ADF were observed (3.46%, 2.79%, 3% and 2.69% respectively) at a_1 and b_1 levels of inoculants in the silage having variety A and C and highest NDF and ADF were observed (3.59%, 2.87%, 3.09%, 2.69%) at a_0, b_1 and b_0 levels of inoculants in the silage having variety A, B and C. Variety A and C significantly reduced the NDF and ADF contents. The best levels of inoculants were $a_{1.4}$, a_1, $b_{1.4}$, b_1 and the poor levels were a_0 and b_0(Table-1, Table -3 and Table -4).

The results of the present study are in agreement with Hafez *et al*. (2012); Gaafer, (2004); Etman*et al*. (1994); Mohammad *et al*. (1999); Junges*et al*. (2013) ; Bendary*et al*. (2001); El shinnawy*et al*. (2003) and El-Ashary*et al*. (2003) who reported that inoculation significantly decreased the NDF and ADF. Reduction in fiber contents might be attributed to convertion of fiber into WSC by LAB and fiber fraction remained unaffected in control silage due to low microbial activity (Nkosi*et al*. 2011).

There was significant interaction ($p<0.05$) between varieties and inoculants for pH. pH significantly decreased by individual and cumulative effect of inoculants. Lowest pH was observed (13.3%) in silage having variety A and treatment level a_1b_1 ofinoculants and highest pH was observed (15.36%) in silage having variety B and a_0b_0 level of inoculation. The best levels of inoculants were a_1 and b_1 and the poor levels were a_0 and b_0 (Table-1, Table -3 and Table -4).

The results of the present study are supported by the finding of various scientists Ghazali*et al*. (2013); Adesogan (2008); Gao *et al*. (2008); Jalc*et al*. (2009) Kung *et al*. (2003) and Huisden*et al*. (2009) who reported that pH of the silage was significantly affected by inoculation of LAB. The results of the present study are in line with the finding of Jalc*et al*. (2009); Kung and shaver, (2001) and Koc*et al*. (2008) as they reported bacterial inoculation significantly lowered the pH of corn silage. The reason behind this phenomenon was dominating homolactic fermentation leading to the increased lactic acic content consequently, decreased pH of silages (Bolsen*et al*. 1996 and MacDonald *et al*. 2010).

The results of the present study are contradictory to the findings of Coskutuna*et al*. (2009); MacDdonald*et al*. (1991); Sucu and filya, (2006); junges*et al*. (2013) and Ozduven*et al*. (2009) as they reported that inoculants had no effect on pH of maize silages. It might be attributed to lower availability of WSC for lactic acid production by acid producing bacteria and competition between different microbes that resisted the change in pH (Seale, 1986 and Juunges*et al*. (2013).

The interaction was found ($p<0.05$) between inoculants and different varieties for IVDMD. The highest value for IVDMD was observed (9.76%) in silages having variety C and a_1b_1 levelof inoculants. The lowest IVDMD was observed (8.89%) in silage having variety B and a_0b_0 levels of inoculation. The highest IVDMD was observed (5.04%, 4.92%) at a_1 and b_1 levels of inoculants in the silage having variety B and lowest IVDMD was observed (4.79%, 4.69%) at a_0 and b_0 levels of inoculants in the silage having variety C. The IVDMD remained best for variety C. The best levels of inoculants were a_1 and b_1 and poor levels were a_0 and b_0 (Table -2, Table -5 and Table -6).

The results of the present study are in agreement with Elmenofy*et al*. (2012); Mandebvu*et al*. (1999); Weinberg *et al*. (2007) Filya, (2007); Kilic and Saricicek, (2011) who reported that inoculation significantly improved the IVDMD of silages.It was probably due to the inoculation of LAB which permoted conversion of fiber into WSC resulting in decreased lag time and thus increased IVDMD (Kilic and Saricicek, 2011 and Elemenofy*et al*. 2012).The results of the present study are not in line with Saricicek, (2013); Hristov and McAllister, (2002); Weinberg and Muck, (1996) who reported that inoculation significantly affected the IVDMD of silages. The results of the present study are not in agreement with Kaldmae*et al*. (2009) who reported that IVDMD was not significantly affected by inoculation. It might be attributed to the lower WSC and higher content of undegradeable fiber fraction

(Sadeghi*et al*. (2012; Rinne and Nykanen, 2009 and Hunt *et al*. 1992, 1993). Contradiciton in results was also possibley because of the variations in method used for IVDMD determination (Saricicek, 2013). In the present study Tilley and Terry method (Tilley and Terry, 1963) was used while in other studies pepsin cellulase method (Saricicek, 2013) was used to determine the digestibility.

Table -1: Effect of inoculants on chemical composition of maize silages

Variety	Inoculant a (mg/Kg)	Inoculant b (mg/Kg)	Dry matter (%)	Crude protein (%)	Neutral detergent insoluble fiber (%)	Acid detergent insoluble fiber (%)	pH
		b_0	30.09^{de}	7.10	45.74^{cde}	24.04^{abc}	4.07^{ab}
	a_0	$b_{0.6}$	29.95^{de}	7.91	44.62^{klm}	23.23^{cde}	3.88^{bc}
		b_1	30.22^{de}	7.85	43.62^{jklm}	23.63^{bcd}	3.79^{bc}
		$b_{1.4}$	29.46^{e}	8.73	$44.72^{fghijklm}$	23.54^{bcd}	3.73^{bc}
	$a_{0.6}$	b_0	30.06^{de}	8.18	44.65^{jklm}	23.34^{bcd}	3.87^{bc}
		$b_{0.6}$	30.01^{de}	7.93	44.60^{klm}	23.18^{cde}	3.90^{bc}
		b_1	30.15^{de}	8.73	44.67^{ijklm}	23.65^{bcd}	3.86^{bc}
A		$b_{1.4}$	30.32^{de}	8.14	$44.72^{ghilklm}$	23.63^{bcd}	3.93^{bc}
	a_1	b_0	31.04^{abcde}	8.14	44.66^{jklm}	23.58^{bcd}	3.84^{bc}
		$b_{0.6}$	29.97^{de}	8.13	44.67^{jklm}	23.55^{bcd}	3.82^{c}
		b_1	33.41^{ab}	8.33	44.20^{m}	22.69^{def}	3.71^{c}
		$b_{1.4}$	29.74^{de}	8.15	44.57^{lm}	23.60^{bcd}	3.82^{c}
	$a_{1.4}$	b_0	30.49^{cde}	8.15	44.54^{lm}	23.54^{bcd}	3.92^{bc}

		$b_{0.6}$	30.41^{de}	8.63	44.56^{lm}	23.59^{bcd}	3.82^{c}
		b_1	30.92^{bcde}	8.07	44.67^{ijklm}	23.56^{bcd}	3.80^{c}
		$b_{1.4}$	29.61^{e}	8.12	44.71^{hijklm}	23.49^{bcd}	3.82^{c}
	a_0	b_0	30.23^{de}	7.24	40.70^{abc}	24.26^{ab}	4.28^{a}
		$b_{0.6}$	31.08^{abcde}	8.05	45.71^{cde}	23.25^{cde}	3.94^{bc}
		b1	31.66^{abcde}	8.43	45.64^{cdefgh}	23.36^{bcd}	3.83^{c}
		$b_{1.4}$	29.93^{de}	8.53	45.69^{cde}	23.40^{bcd}	3.77^{c}
	$a_{0.6}$	b_0	30.86^{cde}	8.15	$45.40^{cdefghijk}$	23.22^{cde}	3.82^{c}
		$b_{0.6}$	31.36^{abcde}	8.82	45.67^{cdefg}	23.46^{bcd}	3.85^{bc}
		b_1	30.62^{cde}	7.91	45.67^{cdefg}	23.35^{bcd}	3.75^{c}
B		$b_{1.4}$	29.87^{de}	8.10	45.78^{cde}	23.35^{bcd}	3.94^{bc}
	a_1	b_0	31.18^{abcde}	8.90	45.83^{bcde}	23.56^{bcd}	3.77^{c}
		$b_{0.6}$	30.42^{cde}	8.15	45.68^{cdef}	23.20^{cde}	3.74^{c}
		b_1	32.93^{abc}	8.85	46.77^{ab}	22.20^{f}	3.72^{c}
		$b_{1.4}$	30.37^{de}	8.17	$44.90^{efghijklm}$	23.35^{bcd}	3.88^{bc}
	$a_{1.4}$	b_0	29.47^{e}	8.19	$45.54^{cdefghijk}$	23.31^{bcde}	3.83^{bc}
		$b_{0.6}$	30.43^{cde}	8.17	45.73^{cde}	23.32^{bcde}	3.84^{bc}
		b_1	30.47^{cde}	8.11	$45.64^{cdefghi}$	23.44^{bcd}	3.87^{bc}

		$b_{1.4}$	29.83^{de}	8.10	45.76^{cde}	23.46^{bcd}	3.83^{bc}
	a_0	b_0	31.49^{abcde}	7.16	46.83^{a}	24.66^{a}	3.81^{c}
		$b_{0.6}$	30.95^{bcde}	8.16	45.89^{abcd}	23.13^{cdef}	3.80^{c}
		b1	30.89^{cde}	8.15	45.85^{bcde}	23.11^{cdef}	3.79^{c}
		$b_{1.4}$	30.18^{de}	8.18	45.91^{abcd}	23.15^{cdef}	3.73^{c}
	$a_{0.6}$	b_0	30.62^{cde}	8.79	45.20^{defghijkl}	23.18^{cde}	3.78^{c}
		$b_{0.6}$	30.70^{cde}	8.16	45.49^{cdefghijkl}	23.16^{cdef}	3.74^{c}
		b_1	31.07^{abcde}	8.2	45.80^{cde}	23.26^{cde}	3.77^{c}
C		$b_{1.4}$	31.42^{abcde}	8.19	45.60^{cdefghij}	23.20^{cde}	3.82^{c}
	a_1	b_0	31.12^{abcde}	7.94	45.94^{abcd}	23.12^{cdef}	3.79^{c}
		$b_{0.6}$	32.20^{abcd}	8.19	45.92^{abcd}	23.17^{cde}	3.87^{bc}
		b_1	33.54^{a}	8.82	45.77^{defghijklm}	22.36^{ef}	3.75^{c}
		$b_{1.4}$	31.30^{abcde}	8.20	45.13^{defghijklm}	23.16^{cdef}	3.81^{c}
	$a_{1.4}$	b_0	30.47^{cde}	8.31	45.23^{defghijklm}	23.21^{cde}	3.79^{c}
		$b_{0.6}$	31.39^{abcde}	8.20	45.63^{cdefgh}	23.30^{bcde}	3.78^{c}
		b_1	31.36^{abcde}	8.30	45.47^{cdefgh}	23.27^{cde}	3.86^{bc}
		$b_{1.4}$	29.92^{de}	8.24	45.43^{cdefghijkl}	23.20^{cde}	3.76^{c}
SEM			0.43	0.31	0.16	0.16	0.04
Significance		Variet	*	NS	*	*	*

	y					
	I_a	*	*	*	*	*
	I_b	*	NS	*	*	*
	Variety×I_a	NS	NS	*	NS	*
	Variety×I_b	NS	NS	*	NS	*
	Variety× $I_{a×}I_b$	*	NS	*	NS	*

Means in the same coloums with different (a……m) superscripts are significantly different ($p<0.05$) SEM stand for standard error mean.

Variety A= Pioneer 31P41. B= pioneer 32B33.C= Pioneer1543. Inoculant a=1132 (blend of *E. faecium, L. plantarum.)*

Inoculant b=11C33 (blend of *L. buchneri, L. plantarum and E. faeciu)*

Table -2: Effect of inoculants on *in-vitro* dry matter digestibility of maize silages.

Variety	Inoculant a (mg/Kg)	Inoculant b (mg/Kg)	DMD at 6hr (%)	DMD at 12hr (%)	DMD at 24hr (%)	DMD at 36hr (%)
	a_0	b_0	30.97^{jkl}	40.91^{b}	46.15^{b}	52.63^{nopqr}
		$b_{0.6}$	33.21^{abc}	42.35^{b}	47.50^{b}	$53.88^{cdefghijk}$
		b1	32.33^{bcd}	42.07^{b}	47.20^{b}	$53.67^{efghijkl}$
		$b_{1.4}$	$31.91^{defghij}$	42.09^{b}	47.76^{b}	$53.22^{jklmnopq}$
	$a_{0.6}$	b_0	32.40^{cdef}	42.35^{b}	47.40^{b}	$53.75^{defghijkl}$
		$b_{0.6}$	$31.94^{defghij}$	42.27^{b}	47.36^{b}	$53.63^{fghijklm}$
		b_1	32.05^{defghi}	42.24^{b}	47.22^{b}	$53.59^{ghijklm}$
A		$b_{1.4}$	$31.93^{defghij}$	42.05^{b}	47.43^{b}	$53.78^{cdefghijk}$
	a_1	b_0	$31.64^{efghijk}$	42.12^{b}	56.50^{a}	$53.69^{efghijkl}$
		$b_{0.6}$	31.99^{defghi}	41.98^{b}	47.83^{b}	$53.68^{efghijkl}$
		b_1	33.79^{ab}	42.96^{b}	48.29^{b}	54.66^{bc}
		$b_{1.4}$	32.06^{defghi}	41.58^{b}	47.38^{b}	$53.49^{hijklmn}$
	$a_{1.4}$	b_0	$31.75^{efghijk}$	42.10^{b}	47.74^{b}	$53.45^{hijklmno}$
		$b_{0.6}$	$31.62^{efghijk}$	41.99^{b}	47.60^{b}	$53.52^{ghijklmn}$

		b_1	$31.84^{defghij}$	42.20^{b}	47.52^{b}	$53.38^{ijklmnop}$
		$b_{1.4}$	31.18^{ijkl}	42.51^{b}	47.28^{b}	53.50^{hijklm}
	a_0	b_0	30.42^{l}	41.05^{b}	45.53^{b}	45.53^{s}
		$b_{0.6}$	$31.50^{efghijk}$	42.69^{b}	45.96^{b}	$45.96^{lmnopqr}$
		b_1	$31.43^{fghijkl}$	42.75^{b}	46.37^{b}	46.37^{opqr}
		$b_{1.4}$	$31.40^{fghijkl}$	42.50^{b}	46.43^{b}	46.43^{nopqr}
	$a_{0.6}$	b_0	31.32^{hijkl}	42.43^{b}	46.31^{b}	$46.31^{klmnopqr}$
		$b_{0.6}$	31.47^{fghijk}	42.07^{b}	46.60^{b}	46.60^{pqr}
		b_1	$31.61^{efghijk}$	42.15^{b}	46.62^{b}	46.62^{qr}
B		$b_{1.4}$	$31.45f^{ghi}$	42.76^{b}	46.67^{b}	46.67^{mnopqr}
	a_1	b_0	31.44^{fghijk}	42.82^{b}	46.60^{b}	46.60^{nopqr}
		$b_{0.6}$	31.35^{ghijkl}	42.78^{b}	46.25^{b}	46.25^{opqr}
		b_1	34.00^{a}	43.53^{b}	47.74^{b}	$47.74^{cdefghijk}$
		$b_{1.4}$	$31.55^{efghijk}$	42.46^{b}	46.19^{b}	4619^{r}
	$a_{1.4}$	b_0	$31.50^{efghijk}$	42.83^{b}	46.29^{b}	46.29^{qr}
		$b_{0.6}$	$31.55^{efghijk}$	42.53^{b}	46.43^{b}	46.43^{opqr}
		b_1	$31.52^{efghijk}$	41.97^{b}	46.29^{b}	46.29^{pqr}
		$b_{1.4}$	31.34^{ghijkl}	42.53^{b}	46.23^{b}	46.23^{qr}
	a_0	b_0	30.78^{kl}	40.99^{b}	47.10^{b}	$53.05^{klmnopqr}$
		$b_{0.6}$	32.51^{cde}	42.90^{b}	48.00^{b}	54.39^{bcdefg}
		b1	32.08^{defghi}	42.95^{b}	48.23^{b}	54.80^{ab}
		$b_{1.4}$	32.35^{cdefg}	42.82^{b}	48.00^{b}	54.54^{bcde}
	$a_{0.6}$	b_0	32.27^{cdefgh}	42.73^{b}	48.13^{b}	$54.25^{bcdefghi}$
		$b_{0.6}$	32.09^{defghi}	42.74^{b}	48.12^{b}	$54.27^{bcdefghi}$
		b_1	32.27^{cdefgh}	42.85^{b}	48.47^{b}	$54.23^{bcdefghi}$
C		$b_{1.4}$	32.31^{cdefgh}	42.84^{b}	48.24^{b}	$54.31^{bcdefgh}$
	a_1	b_0	32.25^{cdefgh}	42.76^{b}	48.19^{b}	54.59^{bcd}
		$b_{0.6}$	32.22^{cdefgh}	42.89^{b}	48.49^{b}	54.51^{bcdef}
		b_1	34.02^{a}	44.51^{ab}	49.51^{ab}	55.62^{a}

		$b_{1.4}$	32.28^{cdefgh}	42.99^{b}	48.35^{b}	54.55^{bcde}
	$a_{1.4}$	b_0	32.22^{cdefgh}	42.93^{b}	48.07^{b}	54.50^{bcdef}
		$b_{0.6}$	32.22^{cdefgh}	42.73^{b}	48.23^{b}	54.50^{bcdef}
		b_1	$31.69^{efghijk}$	49.88^{a}	48.46^{b}	54.39^{bcdefg}
		$b_{1.4}$	$31.67^{efghijk}$	42.80^{b}	48.59^{b}	$54.09^{bcdefghij}$
SEM			0.17	0.98	1.33	0.15
Significance		Variety	*	*	*	*
		I_a	*	NS	NS	*
		I_b	*	*	NS	*
		Variety× I_a	*	NS	NS	*
		Variety× I_b	*	NS	NS	NS
		Variety× $I_{a\times}I_b$	*	NS	NS	*

IVDMD stand for *in-vitro* dry matter digestibility Means in the same coloums with different (a……s) superscripts are significantly different ($p<0.05$).

SEM stand for standard error mean. Variety A= Pioneer 31P41. B= pioneer 32B33.C= Pioneer1543. Inoculant a=1132 (blend of *E. faecium, L. plantarum).*

Inoculant b=11C33 (blend of *L. buchneri, L. plantarum and E. faeciu.)*

Table -3: Effect of inoculant a on chemical composition of maize silages

Variety	Inoculant a (mg/Kg)	Dry matter (%)	Crude protein (%)	Neutral detergent insoluble fiber (%)	Acid detergent insoluble fiber (%)	pH
	a_0	29.93^{d}	7.73	44.93^{d}	23.61^{a}	3.92^{ab}
	$a_{0.6}$	30.13^{cd}	8.08	44.66^{de}	23.45^{abc}	3.85^{abc}
A	a_1	31.04^{abc}	8.19	44.52^{e}	23.36^{abc}	3.80^{cd}
	$a_{1.4}$	30.35^{bcd}	8.10	44.62^{de}	23.55^{ab}	3.86^{bcd}
	a_0	30.72^{bcd}	7.94	45.85^{ab}	23.57^{ab}	3.92^{a}
	$a_{0.6}$	30.68^{bcd}	8.24	45.76^{bc}	23.34^{abcd}	3.84^{b}

						cd
B	a_1	31.22^{ab}	8.31	45.79^{abc}	34.08^{cd}	3.79^{d}
	$a_{1.4}$	30.05^{cd}	8.14	45.66^{bc}	23.38^{abc}	3.85^{b} cd
	a_0	30.88^{bcd}	7.91	46.12^{a}	23.51^{ab}	3.79^{d}
	$a_{0.6}$	30.95^{bcd}	8.35	45.52^{bc}	23.20^{bcd}	3.80^{d}
C	a_1	32.04^{a}	8.29	45.51^{bc}	22.95^{d}	3.79^{c} d
	$a_{1.4}$	30.78^{bcd}	8.26	45.44^{c}	23.24^{abcd}	3.78^{c} d
SEM		0.21	0.15	0.16	0.16	0.02
Significance	Variety	*	NS	*	*	*
	I_a	*	*	*	*	*
	Variety×I_a	NS	NS	*	NS	*

Means in the same coloums with different (a……d) superscripts are significantly different ($p<0.05$). SEM stand for standard error mean. Variety A= Pioneer 31P41.
B= pioneer 32B33.C= Pioneer1543. Inoculant a=1132 (blended of *E. faecium, L. plantarum).* Inoculant b=11C33 (blended of *L. buchneri, L. plantarum and E. faeciu)*

Table -4: Effect of inoculant b on chemical composition of maize silages.

Variety	Inoculant b (mg/Kg)	Dry matter (%)	Crude protein (%)	Neutral detergent insoluble fiber (%)	Acid detergent insoluble fiber (%)	pH
	b_0	30.42^{bcd}	7.89	44.90^{c}	23.62^{a}	3.91^{a}
	$b_{0.6}$	30.08^{cd}	8.01	44.61^{c}	23.39^{abc}	3.89^{ab}
A	b_1	31.17^{ab}	8.08	44.55^{c}	23.38^{abc}	3.79^{b}
	$b_{1.4}$	29.78^{d}	8.12	44.68^{c}	23.57^{ab}	3.84^{ab}
	b_0	30.43^{bcd}	7.92	45.82^{ab}	23.59^{a}	3.95^{a}

	$b_{0.6}$	30.82^{abc}	8.30	45.70^{ab}	23.31^{abc}	3.84^{ab}
B	b_1	31.42^{ab}	8.32	45.93^{a}	23.08^{c}	3.78^{b}
	$b_{1.4}$	30.00^{cd}	8.11	45.53^{b}	23.39^{abc}	3.84^{ab}
	b_0	30.93^{abc}	8.05	45.75^{ab}	23.54^{ab}	3.78^{b}
	$b_{0.6}$	31.31^{ab}	8.18	45.74^{ab}	23.19^{bc}	3.78^{b}
C	b_1	31.71^{a}	8.38	45.59^{ab}	23.00^{c}	3.80^{b}
	$b_{1.4}$	30.70^{abcd}	8.20	45.52^{b}	23.18^{bc}	3.80^{b}
SEM		0.21	0.15	0.09	0.16	0.02
	Variety	*	NS	*	*	*
Significance	I_b	*	NS	*	*	*
	Variety×I_b	NS	NS	*	NS	*

Means in the same coloums with different (a……d) superscripts are significantly different ($p<0.05$). SEM stand for standard error mean.
Variety A= Pioneer 31P4. B= pioneer 32B33

Table -5: Effect of inoculant a on *in-vitro* dry matter digestibility of maize silages.

Variety	Inoculant a (mg/Kg)	IVDMD at 6hr (%)	IVDMD at 12hr (%)	IVDMD at 24hr (%)	IVDMD at 36hr (%)
	a_0	32.23^{bc}	31.85^{b}	47.15^{ab}	53.35^{e}
	$a_{0.6}$	32.08^{bc}	42.25^{ab}	47.35^{ab}	53.69^{de}
A	a_1	32.37^{ab}	42.16^{ab}	50.00^{a}	53.88^{cd}
	$a_{1.4}$	31.60^{de}	42.20^{ab}	47.51^{ab}	53.46^{e}
	a_0	31.19^{e}	42.25^{ab}	46.07^{b}	52.18^{h}
	$a_{0.6}$	31.46^{e}	42.35^{ab}	46.55^{b}	52.71^{fg}
B	a_1	32.09^{bc}	42.89^{ab}	46.69^{b}	52.86^{f}
	$a_{1.4}$	31.47^{e}	42.47^{ab}	46.31^{b}	52.47^{gh}
	a_0	31.93^{cd}	42.41^{ab}	47.83^{ab}	54.19^{bc}
	$a_{0.6}$	32.23^{bc}	42.79^{ab}	48.24^{ab}	54.27^{b}
C	a_1	32.69^{a}	43.29^{ab}	48.63^{ab}	54.81^{a}
	$a_{1.4}$	31.95^{cd}	44.46^{a}	48.34^{ab}	54.37^{b}
SEM		0.08	0.49	0.66	0.7
Significance	Variety	*	*	*	*

	I_a	*	NS	NS	*
	Variety×I_a	*	NS	NS	*

IVDMD stand for *in-vitro* dry matter digestibility. Means in the same coloums with different (a……h) superscripts are significantly different ($p<0.05$).

SEM stand for standard error mean. Variety A= Pioneer 31P41. B= pioneer 32B33. C= Pioneer1543. Inoculant a=1132 (blend of *E. faecium, L. plantarum).*

Inoculant b=11C33 (blend of *L. buchneri, L. plantarum and E. faecium*).

Table -6: Effect of inoculant b on *in-vitro* dry matter digestibility of maize silages.

Variety	Inoculant b (mg/Kg)	IVDMD at 6hr (%)	IVDMD at 12hr (%)	IVDMD at 24hr (%)	IVDMD at 36hr (%)
	b_0	31.69^{ef}	41.89^{b}	49.42^{a}	53.38^{e}
	$b_{0.6}$	32.19^{bc}	42.15^{b}	47.57^{ab}	53.68^{de}
A	b_1	32.63^{a}	42.37^{b}	47.55^{ab}	53.82^{cd}
	$b_{1.4}$	31.77^{def}	42.05^{b}	47.46^{ab}	53.49^{de}
	b_0	31.17^{g}	42.28^{b}	46.18^{b}	52.19^{g}
	$b_{0.6}$	31.47^{efg}	42.52^{b}	46.31^{ab}	52.65^{f}
B	b_1	32.14^{bcd}	42.60^{ab}	46.75^{ab}	52.87^{f}
	$b_{1.4}$	$31.43f^{g}$	42.56^{b}	46.38^{ab}	52.52^{fg}
	b_0	31.88^{cde}	42.35^{b}	47.87^{ab}	54.10^{bc}
	$b_{0.6}$	32.26^{abc}	42.81^{ab}	48.21^{ab}	54.42^{ab}
C	b_1	32.51^{ab}	44.92^{a}	48.67^{ab}	54.76^{a}
	$b_{1.4}$	32.15^{bcd}	42.86^{ab}	48.19^{ab}	54.37^{b}
SEM		0.08	0.49	0.66	0.07
	Variety	*	*	*	*
Significance	I_b	*	*	NS	*
	Variety×I_b	*	NS	NS	NS

IVDMD stand for *in-vitro* dry matter digestibility. Means in the same coloums with different (a……g) superscripts are significantly different ($p<0.05$).

SEM stand for standard error mean. Variety A= Pioneer 31P41. B= pioneer 32B33.C= Pioneer1543.

Inoculant b=11C33 (blend of *L. buchneri, L. plantarum and E. faeciu).*

Conclusion

Inoculation of lactic acid bacteria significantly increased the DM, pH and IVDMD and decreased the NDF, ADF contents of all verities silages but CP remained unaffected. Further investigation entails to identification of mechanisms of action as well as other effective or toxic effect on animal.

Conflict of Interest

All author affirmed no conflict of interest.

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