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Microbial Fermentation of Maize Silage for Enhancement of Nutrients

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Microbial fermentation of silage was carried out to improve the nutritional quality of the fodder maize silage. Lactic acid bacteria (*Lactobacillus acidophilus, Lactobacillus plantarum, Lactococcus lactis*) and *Saccharomyces cerevisiae* (Yeast) were used as silage inoculants. Their efficiency were tested individually and in combination. Application of *Lactobacillus acidophilus* + *Lactobacillus plantarum* + *Lactococcus lactis* + *Saccharomyces cerevisiae* (Yeast) showed significant difference in maize silage quality. The combined application of inoculants has recorded the better nutritional parameters like crude fibre (216.50 g/kg dry matter), crude protein (17.93 g/kg dry matter), neutral detergent fibre (NDF) (468.29 g/kg dry matter), acid detergent fibre (ADF) (257.30 g/kg dry matter), dry matter (DM) (292.39 g/kg dry matter) and Ash content (44.46 g/kg dry matter). The combined application of efficient lactic acid bacterial cultures helps in obtaining a good silage rich in nutrients and palatable for the acceptance by the livestock.

Key words : Lactic acid bacteria, Silage, Ensiling, Palatability, Nutrient.

The health and productivity of livestock are closely linked with the quantum of quality forage provided to the animal. In the past, animals had the accessibility to adequate quantity of forage, crop residues and concentrates. But now the scenario of forage production and utilization envisages a different picture. The gap between the supply and demand for good quality forage continues to be widened due to constraints like land and resource inputs. Increase in the acreage under fodder crops is limited due to competition with grain and cash crops and there is great scope for developing improved practices like forage conservation by silage making and for nutritious forage.

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Ensiling (silage making) is a better way to preserve forage. Silage fermentation is performed by one or more strains of lactic acid bacteria and yeasts. The most common is L*actobacillus plantarum*. Other bacteria involved in silage making are heterofermentative lactic acid bacteria (LAB) which include *Lactobacillus buchneri*, *Enterococcus* species and *Pediococcus* species. These naturally occurring bacteria ferment the carbohydrates (sugar) present in the herbage to produce mainly lactic acid. The major functions of lactic acid bacteria during the fermentation process include antimicrobial activity against spoilage causing organisms, improvement of nutritional value, taste and antirancid factors. The reduced level of antinutritional factors result in improved bioavailability of minerals and starch as well as increase in protein efficiency ratio (Nout and Sarkar, 1999). Keeping these points in view an attempt was made to improve the nutritional quality of silage through lactic acid bacteria and yeast fermentation

MATERIAL AND METHODS

The forage used for making silage was obtained from Fodder Scheme, Main Research Station, Hebbal, Bangalore. Fodder maize (*Zea mays* var African tall) of 250-300 kg was collected and silage was made in closed pits and was kept for fermentation for a period of 90 days and at the end of fermentation nutrient analysis was made. The comparative efficiency of lactic acid bacterial (LAB) cultures in improving the nutritional quality of silage was studied by using three known efficient lactic acid bacterial cultures(*Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactococcus lactis*) obtained from National Dairy Research Institute (NDRI), Southern regional campus, Bangalore. The yeast culture used in study was *Saccharomyces cerevisiae*.

Preparation of silage

Fodder maize was harvested at milk to dough stage. After harvest of the crop, it was left in the field for 5-6 hours to reduce the moisture content by around 20%. After drying under natural conditions the fodder was chopped to 1-3 inches for making better quality silage. The dimension of whole pit was 3m x1m x 1m. 10 kg of chopped substrate was used for each treatment for silage making. The silage pits were filled with maize fodder leaving no air space and after every 3 to 4 layer desired lactic acid bacterial, yeast and silage additives (molasses and urea at 1% each) were added and filling was repeated to create anaerobic condition. After filling the silage pit, it was thoroughly pressed so that no air is left in silo otherwise there will be chances of mould growth which may lead to spoilage of silage. After filling, silos were covered with polythene sheet to prevent the entry of mud and rain water.

The experiment had 14 treatments with 3 replications laid out in Completely Randomised Design (CRD).

Treatments details

T1: Control
T2: Yeast (*Saccharomyces cerevisiae*) (3x103 cfu ml-1)
T3: *Lactobacillus acidophilus* (Homofermentative) (2x106 cfu ml-1)
T4: *Lactobacillus plantarum* (Heterofermentative) (2x106 cfu ml-1)
T5: *Lactococcus lactis* (Heterofermentative) (3x106 cfu ml-1)
T6: Yeast + *Lactobacillus acidophilus*
T7: Yeast + *Lactobacillus plantarum*
T8: Yeast + *Lactococcus lactis*
T9: *Lactobacillus acidophilus* + *Lactobacillus plantarum*
T10: *Lactobacillus plantarum* + *Lactococcus lactis*
T11: *Lactobacillus acidophilus* + *Lactococcus lactis*
T12: *Lactobacillus plantarum* + *Lactococcus lactis* + Yeast
T13: *Lactobacillus acidophilus* + *Lactobacillus plantarum* + Yeast
T14: *Lactobacillus acidophilus* + *Lactobacillus plantarum* + *Lactococcus lactis* + Yeast

Nutrient analysis of silage

Samples of silage were collected and dried in an hot air oven at 60^0 C. Dried samples were powdered and the powdered silage samples were used for the analysis of crude protein (Banerjee,1978), crude fibre (Mahadevan,1965), ash (AOAC,1980), neural detergent fibre (NDF) and acid detergent fibre (ADF) (Van Soest,1991). Mineral components such as nitrogen, phosphorous and potassium content was estimated by using standard analytical methods (Jackson, 1973). Values obtained were expressed on dry weight basis.

Palatability was studied by feeding a known fresh weight of fermented silage to four cows of five years age in the morning hours. The left over sample was weighed and palatability was calculated.

$$\text{Palatability (\%)} = \frac{\text{(Weight of fresh sample offered) - (Weight of sample left over)}}{\text{Weight of fresh sample offered}} \times 100$$

RESULTS AND DISCUSSION

Significant differences between the treatments were observed with respect to crude fibre, crude protein and ash content in maize silage (Table 1). After ensiling for period of 90 days, silage treatment (T14) having *Lactobacillus acidophilus* + *Lactobacillus plantarum* + *Lactococcus lactis* + Yeast recorded highest crude fibre (216.50 g/kg dry matter), highest crude protein (17.93 g/kg dry matter) and highest ash content (44.46 g/kg dry

matter) which was statistically on par with the treatment (T12) having *Lactobacillus plantarum* + *Lactococcus lactis* + Yeast (Crude fibre- 212.37 g/kg dry matter, crude protein - 17.77 g/kg dry matter and Ash content of 39.43 g/kg dry matter). The lowest crude fibre, crude protein and ash content were recorded in untreated silage (T1) (159.45 g/kg dry matter, 10.31 g/kg dry matter and 29.07 g/kg dry matter respectively). Silage with highest neutral detergent fibre (NDF) (468.29 g/kg dry matter) and acid detergent fibre (ADF) (257.30 g/kg dry matter) content was recorded in the treatment combination of (T14) *Lactobacillus acidophilus* + *Lactobacillus plantarum* + *Lactococcus lactis* + Yeast (468.29 g/kg dry matter).

The lactic acid bacterial inoculants have increased the neutral detergent fibre (NDF) and acid detergent fibre (ADF) content in maize silage (Table 2). Increased neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents can be attributed to hydrolysis of celluloses and hemicelluloses to monosaccharides that provide additional sugars for lactic acid production during fermentation. The findings of this study up hold the views of Keady and Steen (1996) who reported such increased NDF and ADF in silage due to fermentation. The increase in crude protein could be attributed to the degradation of protein during ensiling which resulted in higher non-protein nitrogen in the silage than in the herbage before ensiling reported by Haigh (1987). Lactic acid bacterial inoculants utilizes water-soluble carbohydrates to produce lactic acid which is responsible for decreasing the pH in silage. Reduction in pH helped in breakdown of proteins in the silos (Driehuis and Oude Elferink, 2000). Addition of lactic acid bacterial inoculants resulted in less degradation of fibre to fermentable water soluble carbohydrates may be due to partial acid hydrolysis of hemicelluloses as reported earlier by

Table 1. Influence of Lactic acid bacteria and yeast on crude fiber, crude protein

Treatments	g/kg dry matter		
	Crude fiber	Crude protein	Ash
T_1 = Control	159.45	10.31	29.07
T_2 = Yeast	165.74	11.87	31.54
T_3 = *Lactobacillus acidophilus*	183.11	16.28	36.83
T_4 = *Lactobacillus plantarum*	188.42	17.50	37.79
T_5 = *Lactococcus lactis*	179.16	16.19	36.50
T_6 = Y+ L a	186.75	16.37	36.59
T_7 = Y + L p	189.49	16.89	37.92
T_8 = Y + L l	185.72	16.33	35.25
T_9 = L a + L p	210.58	17.38	37.93
T_{10} = L p + L l	206.59	17.25	37.46
T_{11} = L a + L l	203.76	17.07	37.04
T_{12} = L p + L l + Y	212.37	17.77	39.43
T_{13} = L a + L l + Y	209.83	17.68	38.15
T_{14} = L a + L p + L l + Y	216.50	17.93	44.46
SEM ±	0.90	0.46	0.43
CD at 5%	2.61	1.34	1.24

content of Maize silage (African tall)

Legend:

T1 = Control ;
T2 = Yeast (Saccharomyces cerevisiae) ;
T3 = Lactobacillus acidophilus (Homofermentative);
T4 = Lactobacillus plantarum (Heterofermentative);
T5 = Lactococcus lactis (Heterofermentative);
T6 = Yeast + Lactobacillus acidophilus;
T7 = Yeast + Lactobacillus plantarum ;
T8 = Yeast + Lactococcus lactis
T9 = Lactobacillus acidophilus + Lactobacillus plantarum
T10 =Lactobacillus plantarum + Lactococcus lactis
T11 = Lactobacillus acidophilus + Lactococcus lactis
T12 = Lactobacillus plantarum + Lactococcus lactis + Yeast
T13 = Lactobacillus acidophilus + Lactobacillus plantarum + Yeast
T14 = Lactobacillus acidophilus + Lactobacillus plantarum +Lactococcus lactis + Yeast

Rice (1990). Neutral detergent fibre contain large amounts of lignin which help in the digestibility of the silage and silage treated with lactic acid bacterial inoculants resulted in higher crude protein and ash content. The results confirm the earlier results of Nkosi (2009).

The percent nitrogen, phosphorus and potassium was significantly increased with addition of microbial cultures after 90 days of fermentation which in turn increased the protein availability in silage (Table 3). The higher NPK content was observed in silage when treated with combined application lactic acid bacterial inoculants compared to control. The maximum nitrogen (2.87%), phosphorus (0.40%) and potassium (1.56%) were recorded in lactic acid bacterial treatments compared to control. Increased availability of NPK in silage can be attributed to

Table 2. Effect of Lactic acid bacteria and yeast on Dry matter, Neutral detergent fibre and Acid detergent fibre content of Maize silage (African tall)

Treatments	g/kg dry matter		
	Dry matter	NDF	ADF
T_1 = Control	241.42	377.32	176.98
T_2 = Yeast	248.27	408.47	183.61
T_3 = *Lactobacillus acidophilus*	255.35	425.94	204.98
T_4 = *Lactobacillus plantarum*	260.48	430.05	215.40
T_5 = *Lactococcus lactis*	254.35	423.86	197.08
T_6 = Y+ L a	266.19	434.72	230.85
T_7 = Y + L p	268.72	436.33	235.97
T_8 = Y + L l	263.54	431.08	227.01
T_9 = L a + L p	278.33	447.72	251.79
T_{10} = L p + L l	273.40	444.17	248.81
T_{11} = L a + L l	270.62	440.62	243.09
T_{12} = L p + L l + Y	281.83	461.47	255.48
T_{13} = L a + L l + Y	275.48	454.46	252.57
T_{14} = L a + L p + L l + Y	292.39	468.29	257.30
SEM ±	3.44	2.29	1.18
CD at 5%	9.95	6.64	3.42

Table 3. Chemical analysis of Maize silage (African tall)

Treatments	Nitrogen content (%)	Phosphorus content (%)	Potassium content (%)
T1 = Control	2.15	0.19	0.80
T2 = Yeast	2.54	0.23	0.92
T3 = Lactobacillus acidophilus	2.63	0.26	1.19
T4 = Lactobacillus plantarum	2.59	0.28	1.25
T5 = Lactococcus lactis	2.56	0.25	1.18
T6 = Y+ L a	2.69	0.31	1.31
T7 = Y + L p	2.70	0.33	1.34
T8 = Y + L l	2.66	0.29	1.28
T9 = L a + L p	2.78	0.37	1.39
T10 = L p + L l	2.75	0.35	1.37
T11 = L a + L l	2.73	0.34	1.35
T12 = L p + L l + Y	2.84	0.39	1.49
T13 = L a + L l + Y	2.81	0.38	1.43
T14 = L a + L p + L l + Y	2.87	0.40	1.56
SEM ±	0.06	0.03	0.03
CD at 5%	0.18	0.09	0.10

Table 3. Chemical analysis of Maize silage (African tall)

Treatments	Nitrogen content (%)	Phosphorus content (%)	Potassium content (%)
T1 = Control	2.15	0.19	0.80
T2 = Yeast	2.54	0.23	0.92
T3 = Lactobacillus acidophilus	2.63	0.26	1.19
T4 = Lactobacillus plantarum	2.59	0.28	1.25
T5 = Lactococcus lactis	2.56	0.25	1.18
T6 = Y+ L a	2.69	0.31	1.31
T7 = Y + L p	2.70	0.33	1.34
T8 = Y + L l	2.66	0.29	1.28
T9 = L a + L p	2.78	0.37	1.39
T10 = L p + L l	2.75	0.35	1.37
T11 = L a + L l	2.73	0.34	1.35
T12 = L p + L l + Y	2.84	0.39	1.49
T13 = L a + L l + Y	2.81	0.38	1.43
T14 = L a + L p + L l + Y	2.87	0.40	1.56
SEM ±	0.06	0.03	0.03
CD at 5%	0.18	0.09	0.10

decomposition of protein and other organic substances present in the substrate during fermentation. Such increased availability of major crop nutrients was also earlier reported by Stekar *et al.*, (1991) and Filya *et al.*, (2004).

The palatability test has showed that maize silage was more acceptable and preferred by livestock (Table 4). Maize silage treated with (T14) *Lactobacillus acidophilus* + *Lactobacillus plantarum* + *Lactococcus lactis* + Yeast (75.67%) recorded highest acceptability compared to other lactic acid treatments. The lowest palatability percentage was recorded in control T1 (52.12%). The quality of very good silage is determined by its colour, odour and pH of the conserved material. Good silage should be greenish golden yellow in colour with pleasant odour and should possess high acid content irrespective of fodder crops used. Adding microbial inoculants to silage resulted in positive effects on fermentation because of decreasing pH and production of acetic, butyric and propionic acid which resulted in inhibition of undesirable microorganisms such as *Enterobacter*, *Clostridium*, *Listeria*, *Bacilli*, and yeasts. Such kind of beneficial effects by production of organic acids by lactic acid bacterial inoculants inhibiting undesirable microorganism in silage and was reported earlier by Driehuis *et al.*, (2001). Results of this study have showed that the palatability of silage and acceptability of livestock was found good when silage was made with combined *Lactobacillus* cultures than individual *Lactobacillus* inoculants. However among the three lactic acid bacterial cultures used in the study *Lactobacillus plantarum* was found as more efficient and useful in silage preparation.

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