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Influence of Varying Levels of Organic Green Culture and Enzose on Silage Characteristics of Mott Grass and its Digestion Kinetics in *Nili-Ravi* Buffalo Bulls

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ABSTRACT

The objective of the study was to assess the effects of organic green culture (multiple probiotic) and enzose (corn dextrose) on pH, lactic acid and chemical composition of mott grass silage (MGS) and its *in situ* digestion kinetics in *Nili-Ravi* buffalo bulls. Mott grass (MG) harvested at 50 days of re-growth was ensiled in small laboratory silos with organic green culture (OGC) and enzose at rate of 0, 1.5 and 3% of MG dry matter (DM) for 10, 20 and 30 days in a 3 x 3 x 3 factorial arrangement. Silage pH decreased and lactic acid contents increased with increasing level of enzose and ensiling time, and remained unchanged by OGC treatment. Dry matter and nitrogen losses in MGS were lower ($P<0.05$) with higher levels of OGC and enzose, and, increased ($P< 0.05$) with increasing fermentation time. The NDF% of MGS increased with addition of OGC and increased ensiling time, however, was unaffected by enzose treatment. The ADF% of MGS was higher than MG, increased with increased ensiling time, however, was unaffected by varying level of enzose and OGC. In situ NDF digestibility, digestion rate and lag time of MG and its silage were similar. The DM digestion rate was higher and lag time was lower in MG than in MGS, whereas DM digestibility remained unchanged. Organic green culture did not reduce the time for completion of ensiling process of mott grass. Enzose can be used as a good source of fermentable carbohydrates for preparing silage of fodders with low fermentable sugars.

Key Words: Mott grass silage; Fermentable carbohydrates; Bacterial inoculants; *In situ* digestion kinetics

INTRODUCTION

Fodders are the cheapest source of livestock feeding the world over. In developing countries, low per acre fodder yield and limited area under fodder cultivation are the major constraints in the provision of fodder to livestock. Fodder scarcity periods further limit fodder supply during extreme summer and winter. Preservation of fodders as hay and silage are the alternatives continuous fodder supply to the livestock round the year. Fodder crops are ensiled by natural fermentation of plant carbohydrates by anaerobic bacteria leading to steady decline in pH. Ensiling process is complete at a pH range of 4.2-4.6. The fermentation process is highly influenced by availability of bacterial substrate (fermentable carbohydrates), crude protein (CP) content, moisture content and predominant bacteria during ensiling process (Khorasani *et al.,* 1993 & Bolsen *et al.,* 1996). Earlier the fermentation is completed; more nutrients are retained in the silage. For achieving early completion of fermentation and rapid decline in pH, lactic acid, produced by lactic acid bacteria, plays the major role (Kung & Shaver, 2001).

The major fodder crops used for ensiling are maize, barley, sorghum, millet, mott, jambo and oat grass (Bolsen *et al*., 1996). Mott dwarf elephant grass (*Pennisetum purpureum*) is a fast growing, highly productive and multi-cut fodder species. However, mott grass (MG) has low concentration of fermentable carbohydrates. Bacterial inoculants, with single or multiple strains of bacteria, can serve as fermentative agents for speedy fermentation of substrate (fodders) during ensiling process (Weinberg & Muck, 1996). However, inoculation response depends upon the availability of fermentable sugars which is the main substrate of lactic acid bacteria and prerequisite for better fermentation (Thomas & Thomas, 1985). The present study was planned to assess the effects of organic green culture (highly cone. & multiple probiotic containing *Lactobacillus Acidophilus, Cerevisiae Saccharomyces, Bacillus subtillus & Aspergillus oryzae*) and Enzose E-001 (as fermentable energy source) on pH and chemical composition of mott grass silage and its digestion kinetics in situ in *Nili-Ravi* buffalo bulls.

MATERIALS AND METHODS

Mott grass. Mott grass was obtained at 50 days of its re-growth from an experimental field adjacent to Animal Nutrition Research Center, University of Agriculture, Faisalabad, Pakistan. Mott grass was chopped using a locally manufactured chopper, dried at 60°C and ground through a willey mill to 2 mm screen. The DM, nitrogen content and ash of the MG were determined using methods described by AOAC. (1990). The ADF, NDF, hemicellulose, cellulose and acid detergent lignin (ADL) of MD were determined using methods described by Van Soest *et al.* (1991).

Preparation of laboratory silos. The MG was chopped with an average particle length of ½ inches. The Organic green culture (composed of *Lactobacillus Acidophilus, Cerevisiae Saccharomyces, Bacillus subtillus & Aspergillus oryzae*) and enozse were mixed with the chopped MG at the rate of 0, 1.5 and 3 g kg^{-1} and 0, 1.5, and 3%, respectively and ensiled for 10, 20 and 30 days. Thus, there were three factors: inoculant (OGC), enzose and ensiling time with three levels each in a 3 x 3 x 3 factorial arrangement with six replicates each. In total, there were nine treatments. Silos were prepared and placed at the room temperature. Two silos from each treatment group were opened at 10, 20 and 30 day for determination of pH and lactic acid contents (Bakers & Summerson, 1961) and other chemical analysis.

Statistical analysis. The data were analyzed using SPSS software. The Duncan's Multiple Range test was used for differentiation of means with significant difference (Steel & Torrie, 1984).

***In situ* digestion kinetics trial.** One-ruminally fistulated *Nili-Ravi* buffalo bull was used to determine the comparative *in-situ* DM and NDF digestion kinetics of MG its silage preserved for 30 days with 3% enzose and 3 g kg^{-1} of OGC. Buffalo bull was housed on a concrete floor in separate pen. Fresh and clean water was made available round the clock. The bull was given ten days adaptation period to the diet followed by four days of incubation period for *in-situ* nylon bags. The bull was fed the same diet as being incubated in their rumen during *in situ* trial, to avoid the effects of diet on rumen fermentation pattern (Clark & David, 1990).

The nylon bags measuring l0 x 23 cm, with an average pore size of 50 micrometer were used for this purpose. Each bag contained l0 g sample (on DM basis) and separate bags were used for MG and its best-screened silage. For each time three bags were used for each sample. Two bags were used to determine DM and NDF digestion, while the third was kept as blank for DM and NDF disappearance determinations. The bags were closed and tied with nylon fishing line and soaked in distilled water (39°C) for 15 minutes just before placing them into the rumen. The bags were then exposed to ruminal fermentation for 1, 2, 4, 6, 10, 16, 24, 36, 48 and 96 hours (Sarwar & Nisa, 1999). The sample bags were placed in rumen in reverse sequence and all the bags were removed at the same time to reduce variation associated with washing procedure (Sarwar *et al.,* 1995). These samples were washed in running tap water until water runs clear and then dried in hot air oven at 60°C. After equilibration, the bags were weighed back and residue was transferred to 100-mL beakers for NDF analysis. Digestion Coefficient of DM and extent of digestion of DM and NDF of MG silage were determined by the methods described by Sarwar *et al.* (1991).

Statistical analysis. The data on *in situ* digestion kinetics parameters were analyzed by t-test using SAS (1988).

RESULTS AND DISCUSSION

The chemical composition of MG is presented in Table I. The CP is higher and fibre fractions are lower in the MG than the previously reported figures by Sarwar and Nisa (1999). The reason for higher CP and lower fibre fractions in MG in the present study than the previous study is its early harvesting. The MG was harvested at 50 and 60-days of re-growth in the present and previously reported study, respectively.

Silage characteristics. Silage pH decreased while lactic acid contents increased with increasing level of enzose and ensiling time (Tables II, III). The low pH level and high lactic acid contents in MGS ensiled with enzose was because of increased availability of fermentable carbohydrates. These soluble-sugars provided substrate to lactic acid bacteria resulting in increased accumulation of lactic acid resulting in low pH. Sarwar *et al.* (2005) reported that ensilation of oat grass with higher levels of cane molasses increased availability of fermentable sugars for better growth of lactic acid bacteria resulted in high lactic acid content and low pH. Leibensperger and Pitt (1988) added molasses to the wilted and un-wilted grasses and reported increased level of soluble carbohydrates with no effect on pH. In the present study, the minimum pH and highest lactic acid contents were observed in the silage ensiled for 30 days probably because of increased population density of epiphytic lactic acid which increased the accumulation of lactic acid with increasing fermentation time and subsequently reduced pH (Table III). The present results also in agreement with the findings of Nisa *et al.* (2005) who reported best stabilized ensiling conditions with minimum pH in MGS ensiled for 30 days.

The OGC did not show any significant effect on pH and lactic acid concentration (Table IV). This indicates that the inoculant species present in organic green culture were not effective in providing bacteria active for fermentation and, therefore, not capable of reducing time for completion of ensiling process. These results are contrary to the study of Higginbotham *et al.* (1998) and Gordon *et al.* (1961) who reported that lactic acid contents of the silages were significantly influenced by the inoculant treatment.

Chemical composition. The DM and N recovery was higher in inoculant treated silages as compared to control (Table V). The increased DM recovery may have been due to homolactic fermentation (Weinberg *et al*., 1993; Sharp *et al.,* 1994; Cai *et al*., 1999), which decreased the fermentation losses, and higher N contents were probably because of decreased proteolysis by inoculant treatment. Rooke *et al.* (1988) and Anderson *et al.* (1989) reported that application of inoculant that contained lactic acid bacteria reduced proteolysis during ensiling process. The NDF% of MGS increased with increasing level of OGC. However,

Table I. Chemical Composition of mott grass harvested at 50 Days re-growth

Parameters	Percentage
Dry matter	12.5
Crude protein	12.0
NDF	68.4
ADF	33.7
Hemicellulose	34.7
Cellulose	29.6
Acid detergent lignin	1.0
Ash	14.3

Table II. Effect of varying levels of enzose on silage characteristics of mott grass harvested at 50 days of re-growth

Parameters	Enzose (percentage)			
	0	1.5	3	SEM
pH	4.7[a]	4. 7[b]	4.1[c]	0.06
Lactic acid (%)	4.1[b]	4.1[b]	4.5[a]	0.0572

[a-c]Rows bearing different superscripts differ significantly (P≤0.01)

Table III. Effect of various time intervals on silage characteristics of mott grass harvested at 50 days re-growth

Parameters	Ensiling time (days)			
	10	20	30	SEM
pH	5.3[a]	4.3[b]	3.9[c]	0.06
Lactic acid (%)	3.3[a]	4.5[a]	4.9[b]	0.05

[a-c]Rows bearing different superscripts differ significantly (P<0.01)

Table IV. Effect of varying levels of organic green culture on silage characteristics of mott grass harvested at 50 days of re-growth

Parameters	TREATMENT Organic green culture (g/kg)			
	0	1.5	3	SEM
pH	4.6	4.5	4.4	0.06
Lactic acid (%)	4.2	4.2	4.3	0.05

All results were non-significant (P>0.05)

Table V. Effect of varying levels of organic green culture on chemical composition (percentages) of mott grass harvested at 50 days re-growth

Parameters	organic green culture (g/kg)			
	0	1.5	3	SEM
Dry matter	11.1[b]	11.41[a]	11.31[a]	0.08
Crude protein	10.0[b]	10.0[ab]	10.6[a]	0.02
NDF	69.0[c]	69.6[b]	70.9[a]	0.19
ADF	43.4	43.1	43.3	0.19
Hemicellulose	26.0[c]	26.5[b]	27.6[a]	0.25
Cellulose	40.2	39.7	39.7	0.42
Acid detergent lignin	1.2	1.2	1.1	0.05
Ash	12.4	13.1	12.6	0.18

[a-c]Rows bearing different superscripts differ significantly (P≤0.05)

concentrations of ADF, cellulose, ADL and ash remained unchanged by OGC addition (Table V). The increase in cell wall proportion was due DM and N losses that have resulted in increased proportion of NDF in the MGS. Contrary to the present findings, Rooke *et al.* (1988) and Gordon (1989) reported that microbial inoculation reduced the NH_3-N

Table VI. Effect of varying levels of enzose on chemical composition (percentage) of mott grass harvested at 50 days of re-growth

Parameters	Enzose (percentage)			
	0	1.5	3	SEM
Dry matter	11.0[b]	11.4[a]	11.6[a]	0.08
Crude protein	9.8[b]	10.0[b]	10.6[a]	0.01
NDF	69.5	69.5	69.9	0.19
ADF	43.5	42.8	43.3	0.19
Hemicellulose	26.0	26.6	26.6	0.25
Cellulose	40.1	39.5	40.0	0.42
Acid detergent lignin	1.2	1.1	1.1	0.05
Ash	12.6	12.8	12.7	0.18

[a-c]Rows bearing different superscripts differ significantly (P≤0.01)

Table VII. Effect of various time intervals on chemical composition of mott grass silage harvested at 50 days re-growth

Parameters	Ensiling time (Days)			
	10	20	30	SEM
Dry matter	11.8[a]	11.2[b]	10.8[c]	0.08
Crude protein	10.6[a]	10.0[b]	9.4[c]	0.02
NDF	64.6[c]	70.1[b]	74.4[a]	0.20
ADF	38.3[c]	44.2[b]	47.2[a]	0.20
Hemicellulose	26.3[a]	25.9[c]	27.1[b]	0.25
Cellulose	34.4[b]	40.9[a]	44.2[a]	0.42
Acid detergent lignin	1.1[b]	1.3[a]	1.1[b]	0.05
Ash	13.2[a]	13.0[a]	11.8[b]	0.18

[a-c]Rows bearing different superscripts differ significantly (P≤0.01)

Table VIII. In situ digestion kinetics of mott grass and its silage*

Parameters	mott grass	mott grass silage	SEM
	Dry matter		
Lag (h)	2.5[a]	3.5[b]	0.10
Rate (%/h)	5[a]	4.5[b]	0.2
	Extent. (%)[A]		
At 48 h	63	59	9
At 96 h	65	63	9
	NDF		
Lag (h)	3.4	3.8	0.15
Rate (%/h)	5.1	5	0.2
	Extent. (%)[A]		
At 48 h	61	57	8
At 96 h	64	61.5	9

*Silage was prepared from mott grass harvested at 50 days of re-growth and ensiled with 3% enzose and 3 grams of organic green culture per kg and ensiled for 30 days in small laboratory silos.
[a-b]Rows bearing different superscripts differ significantly (P≤0.05).

contents and subsequently the fibrous fraction of the silage. However, Nadeau *et al.* (1995) applied bacterial inoculant to oat grass silage and reported no effect on cell wall concentration.

Enzose affected the chemical composition of silage. Dry matter and Nitrogen losses were less with increased level of enzose in the silage (Table VI). The higher concentration of soluble sugars of enzose provided substrate for lactic acid bacteria during ensiling which increased the rate of lactic acid production. Lactic acid production reduces the carbon loss which results more DM recovery. Lactic acid production also reduces proteolysis (Heron *et al.,* 1989; Williams *et al.* 1992; Cussen *et al.,* 1995).

Dry matter contents of MGS decreased with increasing ensiling time (Table VII) Rees (1997) ensiled grasses and reported that WSC were constantly degraded during ensiling process into organic acids predominantly lactic acid due to the action of microorganisms. This constant degradation of WSC might lose many useful nutrients and reduced DM contents. Nitrogen contents of the MGS decreased, however, NDF% increased with ensiling time. This may have been due to extensive proteolysis of CP into NH_3-N of MGS. Eun *et al.* (2004) reported that the cell contents decreased and the cell wall components increased with increased ensiling time.

In situ digestion kinetics. *In situ* rate of DM disappearance was significantly higher and lag time (h) was lowered in MG than MGS (Table VIII). These results are in line with Sarwar *et al.* (1999) who observed faster rate of DM disappearance and reduction in lag time for mott grass than MGS. In another study, Nisa *et al.* (2005) compared *in situ* digestion kinetics of MG and MGS ensiled with 2% molasses (DM basis) in *Nili-Ravi* buffalo bulls and reported that ruminal rate of DM disappearance and lag time were similar in MG and MGS.

The NDF rate and extent of degradation and lag time were similar in MGS and MG (Table VIII) Nisa *et al.* (2005) reported that degradabilities of MGS were significantly higher than that of MG (at 48 h). Tauqir and Sarwar (2004) reported that DM and NDF degradabilities of jambo and mott grasses were significantly higher, but extent of digestion was similar across their respective silages.

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