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Effect of Homofermentative Inoculant on Fermentation Characteristics and Nutritive Values of Corn Silage

EFFECT OF HOMOFERMENTATIVE INOCULANT ON FERMENTATION CHARACTERISITCS AND NUTRITIVE VALUES OF CORN SILAGE

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ABSTRACT

An *in vitro* study was planned to assess the effects of a homofermentative microbial inoculant on the fermentation parameters and nutritive value of corn silage. The inoculant was applied at concentrations of $5x10^4$cfu/g of forage (T1), $1x10^5$cfu/g of forage (T2) $1.5x10^5$cfu/g of forage (T3) and a negative control group (T0) without bacterial inoculant in three replicates each. At day 3, 7, 45 and 90 of the experiment individual buckets were opened to characterize the material, quick acidification, dry matter recovery, and aerobic stability of silage respectively. The temperature of the trial samples was 32.75±1.92 throughout the trial duration. A rapid and significant reduction in pH even at third day of trial from 6.5 to 3.61 in the treatment (T2 and T3) groups and remained consistent till 90 day of experiment (with non-significant fluctuations) when compared with control group (6.5 to 5.0). The levels of lactic acid, acetic acid and propionic acids were significantly ($P<0.05$) higher for treatment groups (i.e. T2 and T3) than the T1 and T0 groups and almost stabilized till 90 day of the trial. A consistency in dry matter contents were observed at 3rd, 7th and 90th day of trial for T2 and T3 treatment groups. As far as the crude protein contents are concerned, a non-significant reduction was observed in treatment groups. Overall, inoculant shows nutritive stability and consistency of acids produced at $1x10^5$cfu/g and $1.5x10^5$cfu/g inclusion levels of inoculant.

Key words: Silage, inoculants, nutrition, acid profile, corn

INTRODUCTION

Ensiling (silage making) is the process of preservation of green fodder under anaerobic environmental conditions for cattle feeding around the year. The aim of silage making is to achieve within the ensiled mass a sufficient concentration of lactic acid produced as a result of the presence of micro-organisms within the cut crop; to inhibit other forms of bacterial activity and thus, preserving the material (Cheli et al., 2013). This basic concept comprises of the factors essential for lactic acid production such as elimination of air, availability of ample carbohydrates, adequate moisture content and the initiation of an early and rapid fermentation.

Commonly used fodder for silage-making are corn, sorghum, millet, oat, and Sudan grass. Corn (*Zea mays*), as a forage and grain has features of both types of feeds; considered as an important component of dairy rations in the world where corn can be grown (Kowsar et al., 2008). Nutritionally, corn silage is a very heterogeneous material consisting of starch (grain) and fiber (fodder). It is lower in crude protein (CP) and higher in digestible energy (DE) than other forages. It also differs from other forages in terms of quality that does not decline/drop with advancing in maturity. This is because the increasing amount of grain in the crop

offsets the decline in digestibility normally associated with structural tissues (in the case of corn, stem). Comparatively, corn is relatively easy to ensile (Allen et al., 2003).

In Pakistan, three corn crops are harvested annually. To make corn silage, additives/inoculants are usually added. Silage additives aid in increasing rate of fermentation, and/or in some way substantiate the efficiency of the normal fermentation process. Epiphytic populations of lactic acid bacteria (LAB) on plant material are often lower in number (<1 x 10^5 cfu/g) than other phyllosphere communities (produce end products other than lactic acid) (Filya, 2001). Homolactic fermentation is more desirable than other types of fermentations because it results in a theoretical recovery of 100% for DM and 99% for energy in contrast to lower recoveries of DM and energy from other fermentations (note that certain types of heterolactic fermentation are also efficient) (Baytok et al., 2005).

Commonly used homofermentive bacterial strains in silage inoculants include: *Lactobacillus plantarum, L. acidophilus, Pediococcus acidilactici, P. pentosacaceus,* and *Enterococcus faecium* (Kleinschmit and Kung, 2006). With increased commercialization of the dairy industry, for uninterrupted balanced feed supply, corn silage production is in practice since last two decades in Pakistan. This study was planned to assess the effect of graded levels of homofermentative inoculant on fermentation parameters and nutritive values during a period of 90 days.

MATERIALS AND METHOD

Experimental plan

Fresh whole corn plant (31P41) was shredded to about 1.30 cm size, subdivided into 7 kg each for one negative control group (T_0) and three treatment groups i.e. T_1 – 2 g/ton (5 x 10^4 cfu/g of forage), T_2 – 4 g/ton (1 x 10^5 cfu/g of forage) and T_3 – 6 g/ton (1.5 x 10^5 cfu/g of forage). All these groups were prepared in triplicates. For homogenized inoculant application, designated levels of inoculant for each treatment were mixed in 20 ml lukewarm water and sprayed over the layers of fodder and tightly packed in air tight plastic bags; then in 10 kg capacity plastic buckets as 'mini silos' separately for each replicate and treatment.

Inoculant

In the present study, a mixture of different two homofermentative strains, *Enterococcus faecium* BIO 34 (DSM 3530) and *Lactobacillus plantarum* IFA 96 (DSM19457) was used. Each gram of inoculant contained 25,000 million/cfu of microorganisms. The carrier for microorganisms was inulin.

Sampling

The sampling plan for the experiment is shown in Table 1. To avoid air penetration, mini silos were prepared for each replicate of treatments for planned sampling days. These mini-silos were opened at 3rd, 7th, 45th and 90th day of experiment to characterize the material, acidification and dry matter recovery of silage respectively.

Chemical analyses

Initial temperature was noted by using stainless steel sensor probe. The dry matter (DM) was determined by oven drying at 103-105 °C for about 16 h (AOAC, 2000; Method No. 934.01). However, partially dried (55-60 °C) samples were analyzed for proximate analysis i.e. crude protein (AOAC, 2000; Method No. 954.01), crude fat (AOAC, 2000; Method No. 920.39), crude Fiber (AOAC, 2000; Method No. 962.09) and ash (AOAC, 2000; Method No. 942.05), neutral detergent fiber (NDF) and acid

detergent fiber (ADF) of forage and silages (AOAC, 1990; Method No. 973.18). The energy values were determined by following the NRC prediction equations. For pH, each sample was determined in triplicates by using a 1.20 g sample added to 30 ml of distilled water. After mixing for 2 minutes, pH was determined by using digital pH meter. For Buffering Capacity (BC) determination, a 0.5 g of dry forage was dispensed in 30 ml of distilled water. The initial pH was recorded after allowing 2 min equilibration. For BC, a 30 ml solution was acidified under continuous stirring from its initial pH to pH of 5 with 1 N HCl. Then a similarly prepared solution was titrated from its initial pH to pH of 7 with 1 N NaOH.

Fermentation acids

The organic acids (i.e. acetic acid, propionic acid, lactic acid and formic acid) were determined by using HPLC (Agilent, Model 1100, Germany) with RID detector (Agilent G1362A) and Chem-station software. Mobile phase was used as 0.01 m/l H_2SO_4 with a flow rate of 0.4 ml/min. The detector temperature was 45 °C. The inject volume was 40 µl and run time was 30 min per sample. For sample preparation about 5.0 g of silage sample was taken into a 250 ml flask. Distilled water (100 ml) was added and blended for 1 h in gyratory shaker at room temperature. Then the sample extract was poured into a 1000 ml volumetric flask and volume was made up to the mark and filtered (0.45 µm filter) into sample vials. The analytical range of the method was between 100 mg/L to 2000 mg/L, the optimum concentration for determination was approximately 1000 mg/L.

Statistical analysis

For data analysis SPSS 20 software was used and computed for general linear model one-way ANOVA followed by post HOC, for significance level at $P<0.05$.

RESULTS

Temperature

The effect of inoculation on changes in temperature of corn silage samples is shown in Figure 1. At the start of the experiment, temperature of fresh corn fodder was recorded as 32.75 ºC. For the duration of 90 days ensiling process, temperature of the treated silages was recorded and observed as non-significant ($P>0.05$) with narrow range variation (i.e. up to ±1.92 ºC).

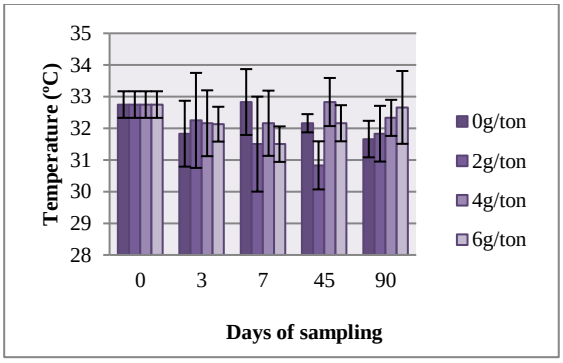

Figure 1. Temperatures variability during the 90-day course of ensiling process.

pH

Decline in pH during silage formation is an indicator of its quality (Moharrery, 1997). Good quality silage has a pH value of 4.2 or lower (Bolen et al., 1992). A significant ($P<0.05$) reduction in pH at the 3rd day of experiment from 6.50 to 3.56 was observed in corn silage sample treated with graded levels of inoculant. Furthermore, the stability of pH levels was found maintained significantly ($P<0.05$) till 90th day of experiment in samples treated with graded levels of inoculant i.e. 2, 4 and 6 g/ton when compared with T_0 (5.5) Figure 2.

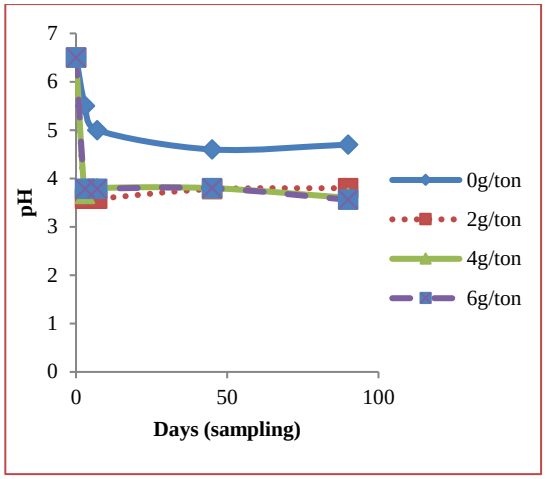

Figure 2. pH decline during the 90-day course of ensiling process.

Buffering capacity (BC)

Buffering capacity of forages can be defined as the degree to which forage material resists changes in pH (Yunus et al., 2000). Samples with inoculant (i.e. T_2 and T_3) significantly ($P<0.05$) showed maintained BC when compared with negative control group (T_0). Furthermore, as compared to the treatment groups, samples without inoculant, observed for decline in BC from day 45 onwards (Figure 3) was too late to preserve.

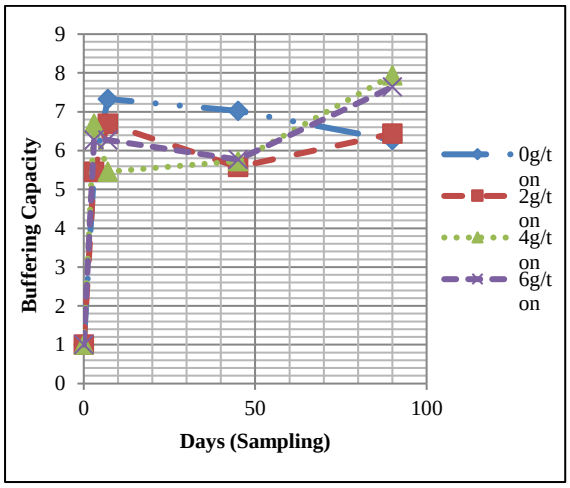

Figure 3. Buffering Capacity (BC) of corn silage with different inoculant inclusion levels at 0, 3, 7, 45 and 90 day of ensiling.

Fermentation acid profile

During present study, silage samples were analyzed for 4 acids i.e. acetic acid, lactic acid, propionic acid and formic acid as demonstrated in Table 2. Fermentation acid production was affected by graded levels of inoculants. The concentration of acetic acid was significantly ($P<0.05$) higher for treatment groups than the group without inoculants (T_0). Similarly, concentrations of lactic acid and propionic acid were significantly ($P<0.05$) higher and almost stabilized till 90th day of opening at inoculant inclusion level 4 (T_2) and 6 g/ton (T_3). Formic acid was not detected at all during various sampling days. In absence of inoculant, the rate and efficiency of the natural fermentation process was highly variable, depending on the number of lactic acid bacteria on the crop, the particular strains of lactic acid bacteria, temperature and sugar contents of the crop. This was further confirmed by the inconsistent findings of various acids during present study over a period of 90 days. In treatment groups i.e. T_1, T_2 and T_3, lactic acid to acetic acid ratio was observed greater than 3:1, which is an indicator of good preservation.

Dry matter (DM)

In nutritional profile, dry matter of silage has a prime importance and position. The main purpose of silage making is to avoid the degradation of DM. Present study revealed the improvement in DM of samples with inoculants (T_2 and T_3) as compared with the control (T_0) during the experimental duration of 90 days (Table 3). Overall, with all three inclusion levels of inoculant, 2.40-7.19 % improvement in dry matter recovery was observed when compared with the control (T_0).

Crude protein (CP)

The corn silage samples inoculated with microbial inoculants showed slower CP degradation during 3rd, 7th, 45th and 90th day of opening for T_2 and T_3 groups when compared with the control (T_0). However, CP contents were non-significantly different among treatment groups (i.e. T_1, T_2 and T_3). The other proximate parameters i.e. ash, fat and fiber were non-significantly affected by inoculant treatments.

Neutral detergent fiber (NDF) and Acid detergent fiber (ADF)

NDF is an indicator of voluntary intake because it provides bulk to the rumen. In present study, the contents of NDF were not affected significantly. However, the NDF contents of corn silage were lowered when compared with the fresh forage. This may be due to fibrolytic activity of bacteria in the inoculant or due to partial acid hydrolysis of hemicellulose (Bujnak et al., 2011). As can be seen in Table 3, during the course of study, NDF remained unaffected with or without the inoculants with non-significant variation. However, ADF was significantly ($P<0.05$) reduced in treatment groups T_1 and T_2 at 3rd and 7th day of opening when compared with the T_0.

Energies

Energy contents of the corn silage estimated were digestible energy (DE), metabolizable energy (ME), net energy for lactation (NE_L), net energy for maintenance (NE_M) and net energy for growth (NE_G). Various forms of energy were estimated in the present study to assess an overall impact of inoculant on silage quality. In present findings, a significant improvement was observed in almost all forms of energies in T_2 and T_3 group when compared with T_0 (Table 4). However, reduction was observed in ME of treatment groups when compared with fresh forage. This reduction can be explained through unavoidable losses of DM during lag phase of fermentation.

Table 1. Experimental plan and preliminary information.

STUDY PLAN	
Experimental Duration	90 days
Sampling Area	Central Punjab
Types of plant/fodder to be ensiled	Corn whole plant (31P41)
Inclusion level of BioStabil Wraps	T_0: 0 g/ton (negative control group)
	T_1: 2 g/ton (5×10^4 cfu/g of forage)
	T_2: 4 g/ton (1×10^5 cfu/g of forage)
	T_3: 6 g/ton (1.5×10^5 cfu/g of forage)
Days of opening	0 Day of Fermentation
	3rd Day of Fermentation
	7th Day of Fermentation
	45th Day of Fermentation
	90th Day of Fermentation

Table 2. The fermentation characteristics of the corn silage supplemented with graded levels of inoculant .

3rd Day						
Parameters	**Treatment Groups (BioStabil)**					
	T_0	**T_1**	**T_2**	**T_3**	**SD**	**P value**
Lactic Acid (%)	3.57[a]	3.60[a]	4.24[b]	4.34[a]	0.253	0.24
Acetic Acid (%)	0.96[a]	1.11[a]	1.10[a]	1.08[a]	5.47	0.86
Propionic Acid (%)	0.36[a]	0.35[a]	0.48[b]	0.36[a]	4.93	0.00
Total Acids (%)	5.01[a]	5.06[a]	5.82[b]	5.98[a]	0.50	0.48
Lactic Acid: Acetic Acid	3.73[a]	3.27[a]	3.84[a]	4.01[a]	0.31	0.21
7th Day						
Parameters	**Treatment Groups (BioStabil)**					
	T_0	**T_1**	**T_2**	**T_3**	**SD**	**P value**
Lactic Acid (%)	3.74[a]	4.38[ab]	5.38[b]	4.61[ab]	0.20	0.43
Acetic Acid (%)	1.37[a]	1.33[a]	1.30[a]	1.60[a]	9.36	0.02
Propionic Acid (%)	0.44[a]	0.51[a]	0.48[a]	0.49[a]	3.68	0.00
Total Acids (%)	5.55	6.22[ab]	7.17[b]	6.71[ab]	0.61	0.10
Lactic Acid: Acetic Acid	2.76[a]	3.41[ab]	4.14[b]	2.93[a]	0.69	1.16
45th Day						
Parameters	**Treatment Groups (BioStabil)**					
	T_0	**T_1**	**T_2**	**T_3**	**SD**	**P value**
Lactic Acid (%)	5.93 [a]	6.53 [ab]	5.62[ab]	6.13 [ab]	0.19	0.002
Acetic Acid (%)	1.65[a]	0.99[a]	0.76[ab]	1.35[a]	7.14	0.25
Propionic Acid (%)	0.53 [a]	0.46 [a]	0.40 [a]	0.45 [a]	4.54	0.00
Total Acids (%)	8.11 [a]	7.99 [ab]	6.79 [b]	7.92[ab]	0.61	0.16
Lactic Acid: Acetic Acid	3.59[a]	6.55[bc]	7.39[c]	4.54[ab]	0.69	1.17
90th Day						
Parameters	**Treatment Groups (BioStabil)**					
	T_0	**T_1**	**T_2**	**T_3**	**SD**	**P value**
Lactic Acid (%)	3.74[b]	4.04[a]	4.95[b]	5.45[a]	0.79	0.01
Acetic Acid (%)	0.98[b]	0.64[a]	0.60[a]	0.54[a]	0.19	0.00
Propionic Acid (%)	0.42[a]	0.32[a]	0.41[a]	0.42[a]	0.04	0.06
Total Acids (%)	4.36[a]	4.74[a]	5.83[b]	6.41[b]	0.94	0.23
Lactic Acid: Acetic Acid	3.82[a]	6.31[b]	8.25[c]	10.09[c]	2.68	0.44

[a-c]*Means within row with different superscript are significantly different (Duncans test; P <0.05)*

Table 3. Nutritive composition in corn silage (n=3).

Days	Treatment mg/kg	DM (%)	OM (%)	CP (%)	EE (%)	CA (%)	CF (%)	NDF (%)	ADF (%)	HC (%)	NSC (%)
3	**FF**	**42.20^{c}**	**95.87^{b}**	**8.35^{c}**	**1.80^{a}**	**4.13^{a}**	**30.13^{a}**	**50.35^{a}**	**30.09^{ab}**	**20.26^{a}**	**35.71^{c}**
	T_0	31.63^{a}	95.98^{b}	7.21^{ab}	2.50^{ab}	4.02^{a}	33.79^{bc}	52.04^{a}	34.15^{c}	22.88^{ab}	34.23^{bc}
	T_1	33.03^{ab}	94.45^{a}	6.68^{a}	1.97^{a}	5.88^{b}	34.31^{bc}	52.56^{a}	32.45^{bc}	25.11^{bc}	33.06^{ab}
	T_2	35.5^{ab}	93.73^{a}	8.85^{c}	2.10^{a}	6.26^{b}	35.77^{c}	50.76^{a}	29.25^{a}	26.51^{c}	32.12^{ab}
	T_3	33.19^{b}	94.78^{a}	7.88^{bc}	2.63^{ab}	5.21^{b}	33.20^{b}	53.52^{a}	28.14^{a}	30.67^{d}	30.54^{c}
Std. Dev.		4.15	0.90	0.96	0.54	1.12	2.48	8.67	3.15	3.29	1.59
P value		0.00	0.041	0.007	0.004	0.006	0.007	0.00	0.001	0.01	0.002
7	**FF**	**42.20^{c}**	**95.87^{b}**	**8.35^{b}**	**1.80^{a}**	**4.13^{a}**	**30.13^{a}**	**50.35^{a}**	**30.09^{ab}**	**20.26^{a}**	**35.71^{b}**
	T_0	31.93^{ab}	94.61^{a}	6.95^{a}	1.96^{a}	5.38^{b}	31.49^{a}	51.46^{a}	31.07^{ab}	25.38^{b}	35.50^{b}
	T_1	32.13^{ab}	95.24^{ab}	7.92^{ab}	2.00^{a}	4.76^{ab}	30.38^{a}	50.92^{a}	28.84^{a}	27.08^{bc}	30.74^{b}
	T_2	30.83^{a}	94.77^{a}	7.60^{ab}	1.93^{a}	5.22^{b}	31.10^{a}	56.92^{b}	31.31^{b}	30.60^{d}	27.16^{a}
	T_3	33.01^{b}	94.60^{a}	8.13^{b}	2.10^{a}	5.40^{b}	32.12^{a}	56.08^{b}	32.21^{b}	28.86^{d}	34.40^{a}
Std. Dev.		4.73	0.86	0.76	0.41	0.73	3.62	10.16	3.02	1.91	1.41
P value		0.00	0.02	0.08	0.048	0.31	0.004	0.00	0.00	0.065	0.00
45	**FF**	**42.20^{c}**	**95.87^{c}**	**8.35^{b}**	**1.80^{a}**	**4.13^{a}**	**30.13^{a}**	**50.35^{a}**	**30.09^{a}**	**20.26^{a}**	**35.71^{d}**
	T_0	32.18^{ab}	94.91^{b}	7.04^{a}	2.07^{a}	5.09^{b}	33.22^{b}	52.33^{b}	31.42^{b}	25.91^{b}	33.47^{c}
	T_1	31.92^{ab}	94.71^{ab}	7.01^{a}	1.63^{a}	5.28^{bc}	36.09^{c}	54.25^{c}	32.58^{c}	26.67^{b}	31.82^{b}
	T_2	33.25^{b}	94.60^{ab}	7.22^{a}	2.00^{a}	5.40^{bc}	33.93^{b}	54.32^{c}	32.40^{b}	26.92^{b}	31.04^{b}
	T_3	30.61^{a}	94.46^{a}	7.59^{a}	2.07^{a}	5.54^{c}	38.62^{d}	57.91^{d}	31.80^{bc}	31.10^{b}	26.96^{a}
Std. Dev.		3.44	0.99	1.54	0.43	0.98	4.69	8.45	4.10	2.25	6.18
P value		0.00	0.00	0.01	1.05	0.03	0.00	0.00	0.00	0.12	0.00
90	**FF**	**42.20^{c}**	**95.87^{a}**	**8.35^{d}**	**1.80^{b}**	**4.13^{a}**	**30.13^{a}**	**50.35^{a}**	**30.09^{a}**	**20.26^{a}**	**35.71^{c}**
	T_0	31.85^{b}	94.83^{a}	7.56^{c}	1.05^{a}	5.16^{a}	34.93^{c}	50.76^{ab}	33.02^{b}	22.74^{ab}	35.50^{c}
	T_1	29.66^{a}	94.03^{a}	6.82^{b}	2.11^{b}	5.96^{a}	35.79^{c}	54.47^{c}	32.46^{b}	27.0^{c}	30.74^{ab}
	T_2	32.78^{b}	95.24^{a}	6.57^{ab}	1.33^{ab}	4.76^{a}	33.16^{bc}	55.17^{c}	33.73^{b}	27.43^{c}	27.16^{a}
	T_3	33.38^{b}	95.18^{a}	6.07^{a}	1.50^{ab}	4.81^{a}	31.88^{ab}	53.21^{bc}	32.72^{b}	25.49^{bc}	34.40^{bc}
Std. Dev.		*4.94*	*0.76*	*0.98*	*0.40*	*1.00*	*0.78*	*5.92*	*3.04*	*5.01*	*1.60*
P value		*0.00*	*0.00*	*0.01*	*0.002*	*0.44*	*0.02*	*0.00*	*0.00*	*0.03*	*0.00*

$^{a-c}$Means within row with different superscript are significantly different (Duncans test; P <0.05)

Table 4. Comparative analysis of Energy parameters of corn silage (n=3) during various opening days with graded inoculant levels.

Days	Treatment mg/kg	TDN	DE	ME	NE_L	NE_M	NE_G
3	**FF**	**61.04^{c}**	**2.74^{b}**	**2.20^{c}**	**1.37^{b}**	**1.34^{b}**	**0.68^{c}**
	T_0	57.40^{b}	2.43^{a}	1.99^{a}	1.28^{a}	1.18^{a}	0.56^{b}
	T_1	57.16^{b}	2.49^{a}	2.03^{ab}	1.26^{a}	1.15^{a}	0.52^{ab}
	T_2	55.08^{a}	2.54^{a}	2.08^{b}	1.25^{a}	1.23^{a}	0.47^{a}
	T_3	57.31^{b}	2.52^{a}	2.10^{b}	1.29^{a}	1.25^{a}	0.56^{b}
Std. Dev.		*1.109*	*0.01*	*0.04*	*0.018*	*0.04*	*0.04*
P-Value		*0.002*	*0.00*	*0.002*	*0.012*	*0.249*	*0.149*
7	**FF**	**61.04^{b}**	**2.74^{a}**	**2.20^{a}**	**1.37^{a}**	**1.34^{b}**	**0.68^{a}**
	T_0	57.31^{ab}	2.55^{a}	2.13^{a}	1.28a	1.26^{a}	0.52^{a}
	T_1	59.42^{ab}	2.56^{a}	2.03^{a}	1.26^{a}	1.15^{a}	0.59^{a}
	T_2	56.36^{a}	2.57^{a}	2.12^{a}	1.25^{a}	1.18^{a}	0.47^{a}
	T_3	58.45^{ab}	2.52^{ab}	2.10b^{a}	1.29^{a}	1.25	0.56^{a}
Std. Dev.		*2.10*	*0.07*	*0.069*	*0.07*	*0.08*	*0.09*
P-Value		*0.16*	*0.21*	*0.41*	*0.31*	*0.70*	*0.30*
45	**FF**	**61.04^{d}**	**2.74^{b}**	**2.20^{b}**	**1.37^{c}**	**1.34^{b}**	**0.68^{d}**
	T_0	52.96^{a}	2.48^{a}	1.93^{a}	1.18^{a}	1.17^{a}	0.40^{a}
	T_1	54.71^{b}	2.40^{a}	1.97^{a}	1.22^{a}	1.18^{a}	0.46^{ab}
	T_2	54.67^{b}	2.41^{a}	1.98^{a}	1.22^{a}	1.20^{a}	0.48^{b}
	T_3	57.45^{c}	2.37^{a}	2.16^{b}	1.31^{b}	1.27^{b}	0.59^{c}
Std. Dev.		*1.59*	*2.48*	*2.02*	*1.25*	*1.13*	*0.51*
P-Value		*0.00*	*0.01*	*0.00*	*0.00*	*0.83*	*0.00*
90	**FF**	**61.04^{c}**	**2.74^{c}**	**2.20^{b}**	**1.37^{b}**	**1.34^{b}**	**0.68^{c}**
	T_0	54.75^{a}	2.43^{a}	1.98^{a}	1.23^{a}	1.17^{a}	0.47^{a}
	T_1	55.12^{a}	2.40^{a}	1.98^{a}	1.19^{a}	1.23^{a}	0.47^{a}
	T_2	57.21^{ab}	2.43^{a}	2.03^{ab}	1.28^{ab}	1.33^{ab}	0.54^{ab}
	T_3	58.96^{bc}	2.52^{ab}	2.18^{ab}	1.36^{b}	1.38^{b}	0.61^{bc}
Std. Dev.		*1.95*	*0.05*	*0.09*	*0.07*	*0.095*	*0.06*
P-Value		*0.01*	*0.02*	*0.11*	*0.02*	*0.62*	*0.01*

FF – fresh fodde: $^{a-d}$Means within row with different superscript are significantly different (P <0.05)

DISCUSSION

In present laboratory trial, inoculant was applied with graded dosage levels to assess the quality maintenance of silage over a period of 90 days. Although, fermenting epiphytic population exists on plant and performs once maintained under anaerobic environment. The success of a microbial inoculant depends upon type and characteristics of forage used for ensiling, climatic conditions, epiphytic microflora and type of microbial inoculant (Kung and Muck, 1997). The prime importance of inoculant is to support the epiphytic bacteria with additional population for quick acidification process and instant decline in pH for nutrient preservation (Henderson and McDonald, 1984).

The use of fast-growing bacterial strains is the principal factor affecting the fermentation process during ensiling that will in turn, influence livestock performance (Bayatkouhsar et al., 2012;

Weinberg and Muck, 1996). For pH maintenance, various factors are involved, including water soluble carbohydrates (WSC) concentration of fresh fodder, BC, DM content and type of epiphytic bacteria on fresh fodder (McAllister and Hristov, 2000).

In present study, the pH of fresh corn fodder was 6.5 and after good fermentation it was reduced to 3.56-4.0. Immediate decline in pH is regarded as an imperative step in minimizing the nutrient loss during ensiling (Bolsen et al., 1992). The rapid drop of pH is basically supported by lactic acid and other organic acids production. The quick drop in pH is desirable because, this drop in pH causes a reduction in pathogenic bacteria i.e. coliform and clostridia etc. Pathogenic bacteria are avoided due to their tendency to ferment water soluble carbohydrates and lactic acid to undesirable end products like butyrate, acetate and ethanol etc. (McAllister and Hristov, 2000).

Lactic acid is considered as a good indicator for good fermentation. Lactic acid concentration in this trial was observed as 3.74-6.53 % and above, which indicates that silage was of high quality with good preservation. Lactic acid content between 4-6 % of DM and total N less than 11 % indicated that the silage was of good quality and preserved well as is supported by Bolsen et al. (1992). In present study, highest levels were observed during 45th day of opening, ranging from 5.62 to 6.53% and remained almost the same till 90th day of the experiment.

High levels of acetic acids of < 3 % in any type of silage is an indicator of less than desired silage fermentation. Interestingly, present findings showed ~1 to ≤ 2 % of acetic acid for treatment groups. Silage quality was further assessed by considering the lactic acid to acetic acid ratio. Ideally, 3:1 or higher is better. Results showed significantly ($P<0.05$) higher lactic acid to acetic acid ratios in all treatment groups when compared with T_0 (Kung and Shaver, 2001; Rawghani and Zamiri, 2009).

The nutritional composition of silage depends upon the crop type and the moisture content. The rate of fermentation is inversely correlated to the DM content (Jalc, 2009). During present trial, improvement in the DM content was observed with inclusion level of 4 and 6 g/ton. However, a drop in DM was observed in T_0. Data of present study indicated the enhanced recovery of DM and energy parameters in homofermentative bacteria treated silages. Homofermentative bacteria are responsible for rapid decline in pH. This reduction helps in improving the fermentation process by rapid production of lactic acid and provides shorter time for growth to spoilage organisms. Furthermore, Polan et al. (1998) explained that homolactic acid bacteria (LAB) are responsible for <1% loss of gross energy and DM. However, heterofermentative fermentation produces CO_2 that is lost to the environment resulting in a decrease in DM contents.

Among nutritional parameters, ash, protein, fat and neutral detergent fiber (or structural carbohydrates) are generally analyzed directly, while the level of non-structural carbohydrate (NSC) is calculated by difference. In terms of energy contribution, ash has no value while fat, NSC and proteins are generally almost fully digestible somewhere in the digestive tract. Therefore, the energy value of corn silage, exclusive of the NDF, can be accurately calculated. However, it is the NDF portion of the corn silage, due to its relatively high contribution to the overall weight of the silage and its variable digestibility that makes it a key variable in estimating the energy value of corn silage.

In Present study, an improvement/enhanced recovery were observed in TDN, DE, ME and NE for treatment groups, especially T_3 followed by T_2. This is due to reduction in DM losses which was achieved by using the

inoculant. However, a significant reduction in energies is observed in treatment groups when compared with fresh forage. This is due to proteolysis and cellular respiration during initial hours of lag phase of anaerobic fermentation.

CONCLUSION

The findings of this trial, using homofermentive inoculant, indicated that the inclusion of inoculant affected the nutritive and fermentation characteristics of corn silage. A significant improvement in DM was observed even at inoculant dosage level 4 and 6 g/ton of silage. However, 6 g/ton gave significant improvement in TDN, DE, ME and NE. The findings of this trial imply a greater advantage for making silage. It is proposed that further research is required to eliminate the parameters not covered in this study as with respect to environment of Pakistan.

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CONFLICT OF INTEREST

The authors dont have any conflict of interest.

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