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Effect of Inclusion Level of Commercial Additive on Quality and Digestibility of Silages Made From Cereal Fodders

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Abstract

The present study examined the effect of biological silage additive (Sil-all 4 × 4) on nutritive value, fermentation and physical quality of silages made from maize, sorghum and oat fodders. All the cereal fodders were harvested at 30-35% DM contents and ensiled in laboratory silos as 4 groups; 1) CON without any additive, 2) 8G, the additive was used @ 8 g/ton of fresh forage material, 3) 10G, additive was used @ 10 g/ton, and 4) 12G, additive was used @ 12 g/ton. The fodders were given 35 days of ensilation period. The incorporation of additives increased DM% in Maize (29.0, 30.5, 31.9 and 31.7), Sorghum (28.3, 29.5, 30.2 and 30.8) and Oat (29.6, 30.5, 30.2, and 30.7), respectively. Whereas, a similar pattern was also observed for CP content in all silage types. However, NDF and ADF concentration decreased with increasing additive level. The NDF% decreased in maize (64.3, 57.9, 56.2 and 49.9), Sorghum (64.7, 63.2, 62.9 and 58.5) and Oat (65.3, 63.3, 60.9 and 59.1) silages. A similar trend was also revealed for ADF content in all. However, the pH recorded on day 15, 18, and 22 as well as after 35 days of ensilation was also significant ($P<0.05$) among the treatments in all silages. Lactic acid concentration and In-Vitro Dry Matter Digestibility (IVDMD) were higher in inoculated as compare to control silage. Numerically the highest flieg score regarding to silage quality were recorded in 10G followed by 12G, 8G and CON silages. The results of the current study indicate that cereal fodders ensiled with 10g/ton of sil-all additive could be economical in terms of nutrients recovery.

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Introduction

Considering the actual weather conditions, silage is the best method for preserving fresh forage with minimal losses. The quality and nutritive value are influenced by many biological and technological factors including stage of harvest, sugar content in forage, use of inoculant and type of silos. When appropriate techniques are used, silage will have high nutritional value and quality [1]. The silage quality is often poor or unsatisfactory when the fermentation conditions are not fully met [2]. Factors that influence the degree of fermentation include wilting green forage, cut length, ensiling type of technology, and the amount of an additive used [3]. Silage additives include feedstuffs, urea, inoculants and acids [4]. The major goal in silage making is to preserve silage material with minimum nutrient loss. In order to achieve this goal, growth of acid producing bacteria should be stimulated. Especially, lactic acid producing bacteria is generally used to accomplish this target. Wheat is usually used to deliver readily available carbohydrates, needed for fermentation process during ensiling and commercial bacterial inoculant is used to create a desirable microbial population to convert energy into organic acids ultimately reducing pH in ensiled forage.

Cereal fodders especially maize is an ideal crop for silage making due to comparatively high Dry Matter (DM) content, acceptable crude protein (buffering capacity) and adequate water-soluble carbohydrates (energy) for lactic acid production [5]. To inhibit the growth of enterobacteria and clostridia bacteria, a rapid drop in pH is needed, for achieving high quality well fermented palatable silage [5]. This happens when native homo fermentative acids producing bacteria utilize water-soluble carbohydrates and produce organic acids [5]. However, heterofermentative lactic acid bacteria are dominant on a cereals crop prior to nsiling, fermentation will be less efficient and the end products of fermentation will be lactic acid, acetic acid, ethanol and carbon dioxide [5]. The population of lactic acid bacteria present on maize plants prior to ensiling is too low which is not sufficient for conversion of energy into organic acids. Meeske and Basson [6], found that the number of lactic acid bacteria on fresh chopped maize plants prior to ensiling was as high as 109 colony forming units per gram of fresh material.

Ensiling phenomenon is based on natural anaerobic fermentation of fodder in presence of lactic acid producing bacteria, which converts readily fermentable carbohydrates into organic acids [7]. During this process water soluble carbohydrates are respired and intrinsic plant proteases can convert the protein into ammonia [8]. Early achieved anaerobic conditions and rapid decline in pH can minimize the nutrient losses by reducing respiration and prolonged fermentation [1]. So, a rapid decline in silage pH with the addition of inoculants can improve the fermentation characteristics, nutritive value and utilization of the silage.

Therefore, the current study was planned to determine the effect of adding three inclusion level of sil-all (a commercial biological silage inoculant) on cereal fodders maize, sorghum and oat at the time of ensiling on fermentation characteristics, chemical composition and physical quality of the silages.

Materials and methods

Fodder crops

The three fodder crops i.e. maize (Zea maize), sorghum (*Sorghum bicolor*) and oats (*Avena sativa*) were used for silage making. The detail of planting and harvesting has been presented in Table 1. The maize, sorghum, and oats were planted during the month of June, July and November 2012, respectively on agriculture field of Dairy Animals Training and Research Center, University of Veterinary and Animal Sciences, Ravi Campus Pattoki, Pakistan (31°1'0" North, 73°50'60" East, 186 meters elevation). The fodder crops were harvested after full bloom with an average dry matter of 30-35% and then chopped by mechanical chopper (Fimax, V-Belt Driven, MC 10X, Turkey) with a chop size of about 2 cm.

Table 1: Date of sowing and harvest for three cereals fodders.

Fodder type	Date of sowing	Date of harvest
Maize	15 July	21 October
Sorghum	15 Jun	19 September
Oats	15 November	28 March

Inoculants and treatment groups

A commercial biological additive (SIL-ALL® 4 X 4, Lallemand Inc. Canada) containing homo and hetero-fermentative lactic acid producing bacteria (*L. plantarum*, *Enterococcus faecium*, *Pediococcus acidilacti*, and *Lactobacillus salivarius*) and 4 enzymes (α-amylase, cellulase, hemicellulose and pentosanase) were used for inoculation. Each fodder crop was divided into 4 groups; 1) CON the silage was made without the addition of inoculants; 2) 8G inoculants were added @ 8 g/ton on fresh forage material; 3) $10G_{inoculants}$ were used @ 10 g/ton on fresh forage; 4) 12G inoculants were used @ 12 g/ton on fresh forage material.

Application of inoculants on fodders and ensiling

The chopped material of maize, sorghum, and oats forages was weighed and spread out on a 5×5-meter plastic sheet for each treatment group separately. For inoculation purpose a fresh inoculant culture of "SIL-ALL® 4 X 4" was first dissolved separately in 200 ml of distilled water according to mentioned dose (8, 10 and 12g/ton) and then sprayed the whole suspension evenly onto each mass of respective forage and one treatment was made as control sprayed with 200ml of distilled water (no-inoculant) then mixed manually by rolling the forage on the plastic sheet. The treated forages of each respective treatments were ensiled in a pre-labeled polyethylene bags silo with loading capacities (35-40 kg), having dimensions 80×40 cm. All the bags were sealed immediately and stored under shed at room temperature for fermentation.

During fermentation period a random sample was taken from each treatment on day 15, 21 and 28, for determination of pH. After 30 days of ensiling period all bags of respective silages were opened and a composite sample was taken for physical quality, chemical composition fermentation characteristics and In-Vitro Dry Matter Digestibility (IVDMD).

Physical quality of silages

For physical analysis, the quality of silages was determined by total flieg score described by Kilic [9]. Flieg score was calculated using a formula (flieg Score = 220 + (2 x Dry Matter% - 15) - 40 x pH) reported by Kilic [9]. The flieg score with value 81-100, 61-80, 41-60, 21-40 and 0-20 represented the silage quality a very good, good, medium, low and poor, respectively.

Chemical composition of silages

For chemical composition, approximately, 250g sample (in triplicate) was taken from each silo type, dried in a hot-air oven (Memmert, Beschickung-Loading Model 100-800, Germany) at 60°C for 72 hours (for DM%), then ground through hammer mill (Wiley laboratory Mill, Standard Model No. 2, Arthur H. Thomas Company, USA) making particle size of about 0.5 to1mm and stored in pre-labeled bottles for further laboratory analyses. Nitrogen (N) contents of samples were determined by procedure AOAC, [10] using Kjeldahl apparatus (ID 984.13), and then multiplying the N concentration by a factor 6.25 to calculate CP. The NDF and ADF contents were determined according to Van Soest et al. [11]. The gross energy of the silage samples was determined through the IKA C-2000 Bomb Calorimeter, while Metabolizable Energy (ME) was calculated as 63% of the gross energy [12].

Fermentation characteristics

For fermentation characteristics the pH and lactic acid content was measured in silages. Approximately 25g composite sample from different points of was taken from each silo type immediately after opening. The sample silage was mixed with 100 ml of distilled water [13]. After hydration for 10 min using blender, the diluted material was then filtered through cheese cloth and then pH was determined by using a digital pH meter. The liquid obtained was further filtrated through Whatman 54 filter paper, centrifuged and kept at 20°C for lactic acid determination by high pressure liquid chromatography [14].

In vitro dry matter digestibility of silages

The in-vitro dry matter digestibility trials were conducted at University of Sydney, Camden. The dried samples were taken from Pakistan to Camden by air cargo. For IVDMD study, rumen liquor (inoculant) was collected from rumen of cannulated lactating Holstein cows managed on pasture and cereal-based concentrate (9kg DM/cow/day), at Corstorphine farm, University of Sydney. The collected rumen liquor was filtered through various layers of cheese cloth and mixed with buffered minerals solution in 1:2 ratio and placed at 39° C under O2 free environment. Dry Matter Digestibility (DMD) was determined in vitro by batch incubation of samples in rumen liquor [15]. All the dried samples from respective cereal silages were incubated in duplicate using ANKOM filter bags (F57 filter bags; 128 pore size 25µm, 55 mm long and 50 mm wide, New York, USA). The open side of the bag (having 0.5g ground sample) was sealed with heat sealer impulse, and then put into a 50ml dark bottle. The bottle contained 25 ml of a 2: 1 buffer: rumen fluid saturated with gas N (O_2-free) with 0.5 ml cysteine sulphide reducing agent. Bottles were fitted with rubber plugs placed in an incubator (Forma Scientific, model 39419-1, Marietta, OH, USA). Incubator temperature was 39 Co and bottles were placed at a rotary shaker with 90 oscillations / min (Lab-Line Instruments Inc., Melrose Park, IL, USA). Eight bottles containing only inoculum also included in each series as a blank control. After 48 h of incubation the bags having digested sample were removed from the flasks, washed under running tap water then dried in oven at 60°C for 48 hours. The IVDMD% was calculated from the difference of the dry weight of sample and residues remained in the bag after 48 h of digestion divided by weight of sample×100 [15].

Statistical analysis

The collected data on different factors were analyzed under completely randomized design with one-way analysis of variance, using SAS 9.1.3 portable software. The comparison of means was done by DMR test [16].

Results

Physical quality and fermentation characteristics of silages

Flieg score for maize (108, 114, 119 and 116), sorghum (93, 113, 113 and 113) and oats silages (112, 117, 115 and 116) were presented in Figure 1. Numerically the treated groups had higher flied score compared to CON for all silages. The addition of biological additives at ensiling enhanced the fermentation characteristics including pH and lactic acid of cereal silages. The pH values significantly (P<0.05) decreased and lactic acid concentration increased in inoculated silages compared to control silages. A significantly (P<0.05) lower pH was detected in inoculated one on days 15, 21 and 28 during fermentation, as well as after 30 days of fermentation period as compare to control silages (Table 2). However, non-significant (P>0.05) difference was found between 10G and 12G, while CON was significantly (P<0.05) different with 8G, 10G and 12G in maize, sorghum and oats silages after 30 days of incubation. Lactic acid concentration was also significantly (P<0.05) different between control and inoculated cereals silages, but non-significant (P>0.05) results was observed between 10G and 12G but significant with 8G in all observation (Figure 2).

Table 2: Effects of additive on pH during fermentation kinetics of silage.

Inoculants level					
Silages	Days of ensiling	0.0(control) (T)	8g/ton (8G)	10g/ton (10G)	12g/ton (10G)
		pH			
Maize	15	4.2 ± 0.02^{a}	3.9 ± 0.03^{c}	4.0 ± 0.04^{b}	4.0 ± 0.01^{b}
	21	3.9 ± 0.08^{a}	3.8 ± 0.01^{b}	3.9 ± 0.02^{a}	3.8 ± 0.01^{b}
	28	3.8 ± 0.01^{a}	3.8 ± 0.08^{ba}	3.7 ± 0.01^{bc}	3.7 ± 0.02^{c}
	After 30 days	3.9 ± 0.02^{a}	3.8 ± 0.08^{b}	3.8 ± 0.08^{b}	3.8 ± 0.01^{b}
Sorghum	15	4.2 ± 0.02^{a}	4.0 ± 0.01^{b}	4.0 ± 0.01^{b}	4.0 ± 0.03^{b}
	21	4.0 ± 0.03^{a}	3.8 ± 0.01^{bc}	3.8 ± 0.02^{c}	3.9 ± 0.01^{b}
	28	3.9 ± 0.01^{a}	3.8 ± 0.02^{b}	3.8 ± 0.02^{b}	3.8 ± 0.01^{ab}
	After 30 days	4.2 ± 0.01^{a}	3.8 ± 0.01^{b}	3.8 ± 0.005^{b}	3.8 ± 0.01^{b}
Oats	15	3.9 ± 0.008^{a}	3.8 ± 0.01^{b}	3.8 ± 0.005^{b}	3.8 ± 0.01^{b}
	21	3.7 ± 0.01^{a}	3.5 ± 0.01^{c}	3.6 ± 0.01^{b}	3.6 ± 0.02^{b}
	28	3.7 ± 0.08^{a}	3.5 ± 0.08^{c}	3.6 ± 0.06^{b}	3.5 ± 0.01^{bc}
	After 30 days	3.8 ± 0.05^{a}	3.7 ± 0.01^{c}	3.8 ± 0.08^{b}	3.8 ± 0.01^{b}

Means within each row followed by different superscripts are significantly different (P<0.05)

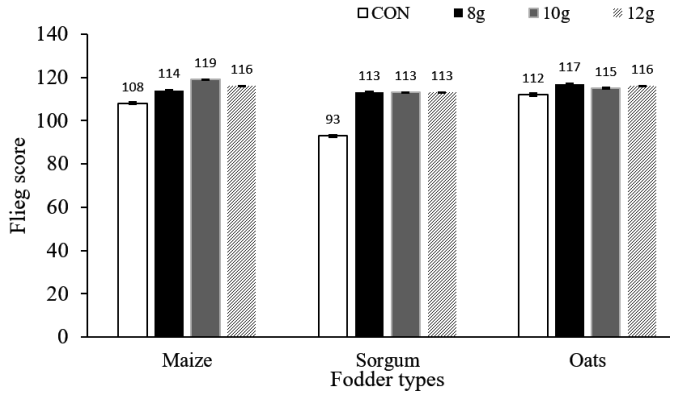

Figure 1: Effect of commercial silage additive on flieg score of three silages. CON group did not have any silage additive; 8g, 10g and 12g had silage additive @ 8 g, 10 g and 12 g /ton of fodder.

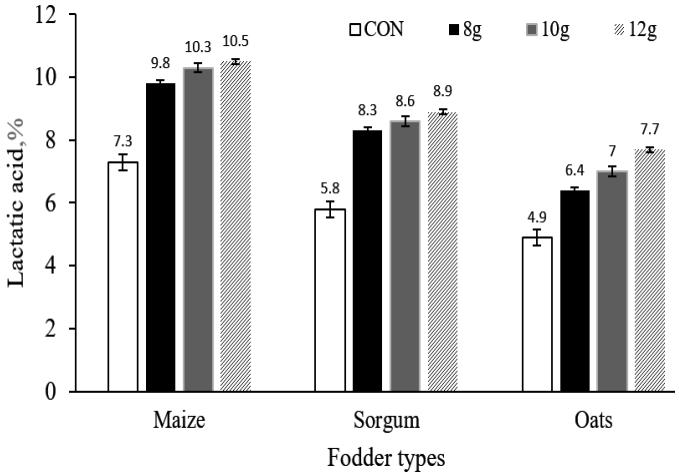

Figure 2: Effect of commercial silage additive on lactic acid concentration of three silages. CON group did not have any silage additive; 8g, 10g and 12g had silage additive @ 8 g, 10 g and 12 g /ton of fodder.

Chemical composition and in-vitro dry matter digestibility of silages

The chemical composition of inoculated and un-inoculated maize, sorghum and oat silages has been shown in Table 3. Inoculated silage had significantly (P<0.05) higher DM Content then Control (CON) for all cereal silages. Also, DM content significantly (P<0.05) differed with the varying inoculants. Higher DM content was observed in 10G followed by 12G and 8G inoculated treatments in all cases. A similar pattern for CP content was observed between control and inoculated silage in all cases. While, in contrast to DM and CP content, a significantly (P<0.05) lowest concentration of NDF and ADF were observed in inoculated as compare to control, and also decreased significantly (P<0.05) with increasing level of inoculants at ensiling of the forages. IVDMD was significantly higher (P<0.05) in inoculated treatments compared to control, nonetheless the difference was non-significant between 8G, 10G and 12G in maize silage. Similarly, in sorghum a significant difference was observed between control and inoculated, but non-significant difference between 10G and 12G while both are significant with 8G treatment. In oats increasing inoculants level also increased IVDMD. The highest IVDMD was recorded in 12G followed by 10G, 8G and CON respectively (Figure 3).

Table 3: Effects of inclusion level of additive on chemical composition of silages.

Additive Level					
Silages	Parameters	0.0(control)	8g/ton	10g/ton	12g/ton
		CON	8G	10G	12G
Maize	DM%	29.0 ± 0.06^{c}	30.5 ± 0.02^{b}	31.9 ± 0.05^{a}	31.7 ± 0.008^{a}
	CP%	6.1 ± 0.06^{c}	6.5 ± 0.11^{a}	6.2 ± 0.05^{b}	6.3 ± 0.01b^{b}
	NDF%	64.3 ± 0.01^{a}	57.9 ± 0.01^{b}	56.2 ± 0.02^{c}	49.9 ± 0.02^{d}
	ADF%	24.8 ± 0.08^{a}	23.2 ± 0.05^{b}	22.4 ± 0.05^{b}	23.5 ± 0.08^{b}
	ME (Mcal/kg)	2.85 ± 0.01^{a}	2.85 ± 0.01^{a}	2.85 ± 0.01^{a}	2.84 ± 0.01^{a}
Sorghum	DM%	28.3 ± 0.01^{d}	29.5 ± 0.06^{c}	30.2 ± 0.03^{b}	30.8 ± 0.02^{a}
	CP%	5.1 ± 0.01^{c}	5.6 ± 0.02^{ab}	5.8 ± 0.02^{a}	5.6 ± 0.09^{b}
	NDF%	64.7 ± 0.03^{a}	63.2 ± 0.03^{b}	62.9 ± 0.03^{b}	58.5 ± 0.02^{c}
	ADF%	33.1 ± 0.68^{a}	33.0 ± 0.26^{a}	32.5 ± 0.65^{a}	32.3 ± 0.90^{a}
	ME (Mcal/kg)	2.80 ± 0.02^{b}	2.80 ± 0.01^{ab}	2.8 ± 0.01^{a}	2.80 ± 0.02^{a}
Oats	DM%	29.6 ± 0.06^{d}	30.5 ± 0.04^{b}	30.2 ± 0.02^{c}	30.7 ± 0.03^{a}
	CP%	5.6 ± 0.08b	6.3 ± 0.06^{a}	6.4 ± 0.02^{a}	6.2 ± 0.11^{a}
	NDF%	65.3 ± 0.08^{a}	63.3 ± 0.10^{b}	60.9 ± 0.32^{c}	59.1 ± 0.19^{d}
	ADF%	34.6 ± 1.02^{a}	35.4 ± 1.52^{a}	35.0 ± 0.92^{a}	35.4 ± 0.90^{a}
	ME (Mcal/kg)	2.82 ± 0.04^{a}	2.82 ± 0.07^{a}	2.83 ± 0.01^{a}	2.83 ± 0.05^{a}

Means within each row followed by different superscripts are significantly different (P<0.05)

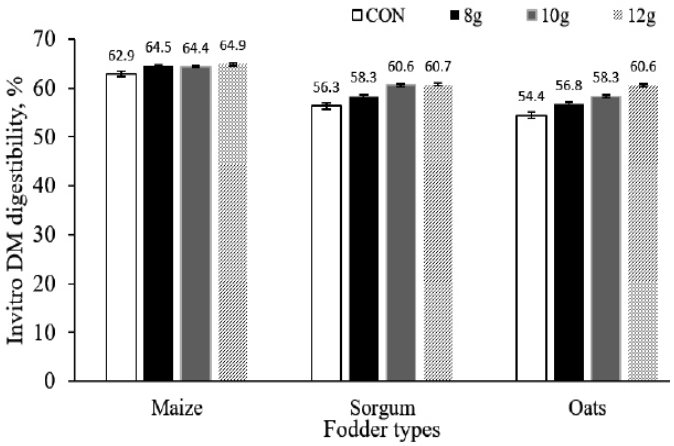

Figure 3: Effect of commercial silage additive on in vitro DM digestibility of three silages. CON group did not have any silage additive; 8g, 10g and 12g had silage additive @ 8 g, 10 g and 12 g /ton of fodder.

Discussion

Physical quality and fermentation characteristics of silages

The findings of lower pH and increased lactic acid concentration in inoculated silages in present study were in agreement to Nkosi et al., [17] who studied the application of bacterial inoculant and cellulase enzyme on fermentation quality of silage made from sorghum forage in laboratory jars. They concluded that inoculation reduced pH, and increased lactic acid content in inoculated silage compared with control silage. Similarly, Sucu and Filya. [18] reported that higher lactic acid concentration and lower pH value was recorded in inoculated corn silage.

Likewise, Aragon et al. [19] reported that inoculation of whole crop maize fodder at ensiling with commercial additive (blend of homo- and hetero-fermentative lactic acid bacteria, BSM) increased the fermentation rate with a significantly deeper pH and increased concentration of lactic acid compared to untreated. The bacterial inoculants stimulate lactic acid fermentation, increasing speed of pH decrease and improving silage preservation.

Chemical composition and In-vitro dry matter digestibility of silages

The results of our study were in agreement with the findings of Aragon et al. [19] who found that DM recovery and digestible protein was significantly ($P<0.05$) higher in maize silage treated with commercial inoculant having bacteria "*Enterococcus faecium*, *Lactobacillus plantarum*, and *Lactobacillus brevis*" in comparison with control silage (without additives). Similarly, Iqbal et al. [20] measured the effects of multiple probiotic (organic green culture) and enzose (corn dextrose) on chemical composition of mott grass silage and reported that DM and CP losses were decreased with increasing levels of multiple probiotic and enzose concentration. The sharp decline in pH of inoculated silages was the major reason in reducing the protein degradation during fermentation process [21] and thereby increasing DM in inoculated silages. Contrary to current findings, Meeske et al. [22] reported that CP concentration was higher in control silage compared to the inoculated silage, and suggested that protein breakdown or N loss was more in laboratory treated maize silage. This contradiction could be due to the variation ensiling temperature as it could significantly affect fermentation process and thereby CP and DM of silages.

In agreement to our findings Ozduven et al. [23] investigated the application of enzymes or lactic acid or mixture of both additives and reported the decrease neutral in NDF content and increased in-vitro dry matter digestibility of triticale silages. However, Ozduven et al. [23] also reported that application of the above additive treatments did not affect ADF concentration in triticale silage, contrary to our ADF results. The inoculants or application of enzymes (cellulases and hemicellulases) degraded the cell wall content of the ensiled crops and subsequently improved the organic matter and fiber digestibility [24]. The results of the current study indicate that cereal fodders ensiled with 10g/ton of sil-all additive could be economical in terms of nutrients recovery.

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