

## The Effects of Two Inoculants Applied to Forage Sorghum at Ensiling on Silage Characteristics

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**ABSTRACT :** Whole forage sorghum (*saccharatum*) cultivar FS5 was harvested at the soft dough ( $303 \pm 9$  g kg<sup>-1</sup> DM) stage of maturity. The sorghum was chopped into approximately 20 mm pieces and ensiled under laboratory conditions in 1.5 L Weck glass jars. At ensiling, it was treated with two commercial silage inoculants: Pioneer 1188 (Inoculant A) and Eco-corn (Inoculant B). The inoculant A and B was applied at *ca*  $2 \times 10^5$  or  $2 \times 10^4$  colony forming units g<sup>-1</sup> DM., respectively. Silage with no additives served as a control. Three jars per treatment were opened on days 2, 4, 8, 15 and 60 post-ensiling to study fermentation dynamics. After 60 days of ensiling the silages were analyzed and subjected to an aerobic stability test lasting 5 days. Results showed that both inoculants caused a more rapid rate of pH decrease and a higher amount of lactic acid production. All the silages were well preserved and were stable upon exposure to air. Inoculants did not influence ( $p > 0.05$ ) the ash and total N contents, but tended to reduce acetic acid ( $p < 0.05$ ), butyric acid ( $p < 0.01$ ) and propionic acid ( $p < 0.01$ ) contents, and to increase the lactic acid content ( $p < 0.01$ ). The lower DM content of silages treated with Inoculant A agrees with the greater gas loss resulting from the DM loss, which was in good agreement with the higher yeast counts upon aerobic exposure. Silage treated with inoculant B had the highest DM ( $p < 0.05$ ) and lactic acid contents ( $p < 0.01$ ), and the lowest acetic acid content ( $p < 0.05$ ), which agrees with the rapid reduction of pH and smaller gas loss. Inoculant B reduced the ADF ( $p < 0.01$ ), ADL and NDF ( $p < 0.05$ ) contents, which also indicates smaller losses of organic soluble material. The control silages contained the highest levels of volatile fatty acids but no lactic acid, indicating secondary fermentation. It was concluded that both inoculants may improve the fermentation process, since silages from all treatments were stable upon aerobic exposure, no advantage could be attributed to any of the inoculants used. (*Asian-Aust. J. Anim. Sci.* 2002. Vol 15, No. 2 : 218-221)

**Key Words :** Sorghum, Silage, Inoculants

### INTRODUCTION

Sorghum (*saccharatum*) is a summer crop which has many advantages, because it is less sensitive than corn to drought and saline condition, and requires less fertilization. These characteristics are especially important in semi-arid areas. The nutritional value of various sorghum cultivars is comparable to that of corn (Dickerson, 1986), as confirmed by the finding that steers fed sorghum silage rations attained an excellent average daily gain (Dalke et al., 1993). Suazo et al. (1993) found that irrigated and dryland grain sorghum silages were of similar nutritive quality to the corn silages. Because of its thick stem, sorghum cannot be dried to hay, and ensiling is the only possibility for preservation. Ensiling is a widely used method of preservation of moist forage crops. It is based on natural fermentation whereby lactic acid bacteria (LAB) ferment water-soluble carbohydrates (WSC) to organic acids, mainly lactic acid (LA), under anaerobic conditions. As a result the pH decreases, inhibiting detrimental anaerobes, and so the forage is preserved.

Forage sorghum for silage is usually harvested at the

dough stage of maturity, but even at this late stage it is associated with low dry matter (DM) and high WSC contents, which might result in undesirable secondary fermentation and spoilage upon aerobic exposure. Addition of lactic acid bacterial inoculants to sorghum silages has been reported to improve fermentation, but to impair its aerobic stability (Meeske et al., 1993; Weinberg et al., 1993). The latter is due to the fact that such inoculants produce less volatile fatty acids (VFAs), which inhibit yeasts and moulds upon aerobic exposure.

The current experiment was conducted to study the effect of two commercial inoculants applied to forage sorghum at ensiling under laboratory conditions: one comprising lactic acid bacteria, and the other contained in addition, other strains as well, which might inhibit aerobic yeast.

### MATERIALS AND METHODS

#### Experimental procedure

The sorghum used in this experiment was forage sorghum FS5 (Dekalb, Plant Genetics, Lubbock, TX, USA) harvested at the soft dough stage. Whole plants were chopped to *ca* 2 cm and ensiled in 1.5 L glass jars (Weck, Wehr-Oflingen, Germany) equipped with a lid that enables gas release only. Each jar was filled with 650 g (wet weight) of chopped forage, without a headspace. The degree of compaction used in the laboratory experiment was *ca* 70%

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Received March 16, 2001; Accepted October 9, 2001

of that used on a farm sale. The jars were stored at ambient temperature (27±2°C). There were 15 jars per treatment, which were sampled in triplicate on days 2, 4, 8, 15 and 60 after ensiling. At the end of experiment the final silages were subjected to an aerobic stability test lasting 5 days, in a system described by Ashbell et al. (1991). In this test, CO<sub>2</sub> produced during aerobic exposure was measured along with chemical and microbiological parameters which serve as spoilage indicators.

Inoculant A, Pioneer 1188 inoculant (Pioneer Hi-Bred International, Johnston, Iowa, USA) contained 3×10<sup>10</sup> colony-forming units (CFU) g<sup>-1</sup> powder, (manufacturer's statement), which included *Lactobacillus plantarum* and *Streptococcus (Enterococcus) faecium* (manufacturer's statement).

Inoculant B, EcoCorn (Zeneca Bio Products, Billingham, UK) contained 3.5×10<sup>9</sup> colony-forming units (CFU) g<sup>-1</sup> powder, (manufacturer's statement), which included *L. plantarum*, *Serratia rubidaea*, *Pedococcus pentosaceus* and *Bacillus subtilis*.

The viability of the inoculants was confirmed by growing them in Rogosa agar at 30°C.

There were three treatments in this trial as follows:

- 1) Control (no additive);
- 2) Inoculant A. It was applied by suspending 100 mg of powder in 15 ml of tap water which were sprayed over 15 kg of the chopped forage, spread over a 1×3 m area, and then mixed thoroughly. Thus, ca 2×10<sup>5</sup> CFU of Pioneer 1188 inoculant was applied per g of fresh crop.
- 3) Inoculant B. It was applied by suspending 100 mg of powder in 15 ml of water which were sprayed over 15 kg of the chopped forage, spread over a 1×3 m area, and was then mixed thoroughly. Thus, ca. 2×10<sup>4</sup> CFU of Ecocorn inoculant were applied per g of fresh crop.

**Analytical procedure**

The chemical analysis was carried out on an individual silo basis. DM was determined after oven-drying the material for 48 h at 60°C. Ash was obtained after 3 h at 550°C. WSC was determined by the phenol-sulphuric acid method (Dubois et al., 1956). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to Goering and Van Soest (1970). Total nitrogen was determined by the Kjeldahl method. VFAs and lactic acid were determined by extraction with ethyl acetate followed by a gas chromatograph analysis using a FFAP mega-board column (Hewlett Packard) over a temperature range of 60-230°C.

The microbiological examination included the enumeration of the following population: lactobacilli, on pour-plate Rogosa agar (Oxoid CM627), incubated at 30°C for 3 days; yeasts and moulds, on spread plate malt extract agar (Difco) acidified to pH 3.5 with lactic acid, incubated at 30°C for 3 days.

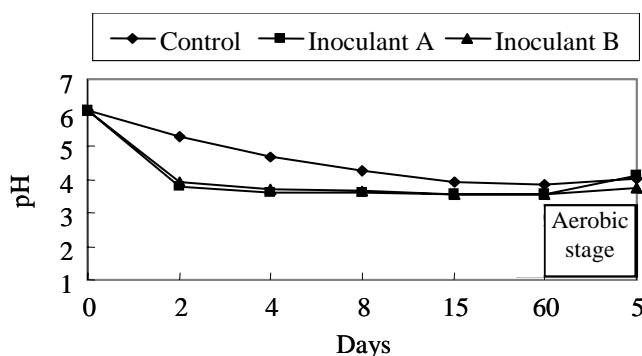
The statistical analysis included one-way analysis of variance and Duncan's multiple range test; these were performed by ANOVA using the GLM procedure of the Statistical Analysis System (SAS, Cary, NC, USA, 1985) as a randomized complete block design.

**RESULTS**

The chemical analysis of the pre-ensiled sorghum harvested at the soft dough stages of maturity is given in table 1.

Figure 1 shows the change in pH during the ensiling of forage sorghum. Both inoculants resulted in a faster drop in pH as compared with the control, and the pH of silages with two kinds of inoculants was below 4.0 already after 2 days of ensiling. The pH of the control and inoculant A silages increased during the aerobic stability test, whereas the pH of inoculant B remained low.

Table 2 gives the chemical analysis of the silages on day 60. The fresh sorghum had high levels of WSC (>120 g kg<sup>-1</sup> DM, table 1), and residual WSC in the final silages was also relatively high, especially in the silages treated with inoculant B (ca 90 g kg<sup>-1</sup> DM). Inoculants did not influence (p>0.05) the ash or CP contents, but tended to reduce acetic acid (p<0.05), butyric acid (p<0.01) and propionic acid (p<0.01) contents, and to increase the lactic acid content (p<0.01). The lower DM content of silages treated with inoculant A is in good agreement with the greater gas loss



**Figure 1.** pH change during ensiling of forage sorghum with or without inoculant

**Table 1.** Chemical analysis of the fresh sorghum (g kg<sup>-1</sup> DM)

Items	DM	CP	WSC	Ash	NDF	ADF	ADL
Content	303±9	48±1	153±2	59±1	457±2	299±3	77±2

**Table 2.** Chemical analysis of sorghum silages (day 60) (g kg<sup>-1</sup>DM)

Analysis	Control	Treatment		SEM
		Inoculant A	Inoculant B	
DM	279 <sup>b</sup>	272 <sup>b</sup>	286 <sup>a</sup>	3.2
WSC	47 <sup>b</sup>	38 <sup>c</sup>	91 <sup>a</sup>	2.5
Ash	68 <sup>a</sup>	66 <sup>a</sup>	63 <sup>a</sup>	1.8
NDF	512 <sup>a</sup>	515 <sup>a</sup>	472 <sup>b</sup>	4.5
ADF	321 <sup>b</sup>	338 <sup>a</sup>	299 <sup>c</sup>	3.6
ADL	67 <sup>ab</sup>	75 <sup>a</sup>	62 <sup>b</sup>	2.7
CP	45 <sup>a</sup>	51 <sup>a</sup>	49 <sup>a</sup>	2.0
Acetic acid	4.3 <sup>a</sup>	3.4 <sup>a</sup>	1.9 <sup>b</sup>	0.3
Propionic acid	2.5 <sup>a</sup>	0.4 <sup>c</sup>	0 <sup>c</sup>	0.1
Butyric acid	2.2 <sup>a</sup>	0 <sup>c</sup>	0 <sup>c</sup>	0.3
Lactic acid	0 <sup>c</sup>	30.0 <sup>a</sup>	31.2 <sup>a</sup>	4.6
Gas loss	49 <sup>a</sup>	69 <sup>a</sup>	9 <sup>b</sup>	17

Means with different letters within rows differ significantly ( $p < 0.05$  or  $p < 0.01$ ). DM=dry matter; WSC=water-soluble carbohydrates; NDF=neutral detergent fiber; ADF=acid detergent fiber; ADL=acid detergent lignin; CP=crude protein; SEM=standard error of the mean.

during fermentation. Silage treated with inoculant B had the highest DM ( $p < 0.05$ ) and lactic acid content ( $p < 0.01$ ), and the lowest acetic acid content ( $p < 0.05$ ), which agrees with the rapid reduction of pH and smaller gas loss. Inoculant B also resulted in reduced the ADF ( $p < 0.01$ ), ADL and NDF ( $p < 0.05$ ) contents, which was due to smaller fermentation losses with inoculant B. The control silages contained higher concentrations of VFAs as compared with the inoculated silages, but did not have any lactic acid. This might indicate secondary fermentation in the control silages, induced by the low DM content.

Table 3 presents the microbiological analysis of the fresh sorghum and of the silages. The fresh sorghum possessed more yeast than lactobacilli, which may attribute to its high WSC content. In all silages the number of lactobacilli increased already after 2 days of ensiling, whereas the numbers of yeasts and moulds in silages, especially those treated with inoculant B were low; table 4 gives the results of the aerobic stability test. All silages produced only little CO<sub>2</sub> upon aerobic exposure; however, silages treated with both inoculants tended to have more CO<sub>2</sub> and resulted in larger glucose loss as compared with the control, which indicates slightly more intensive spoilage; these results were not significant ( $p > 0.05$ ).

## DISCUSSION

Silages made from forage sorghum of recently developed cultivars can serve as excellent fodder. However, because of low DM and high WSC contents, fermentation and seepage losses may be high. Such a composition might also result in poor aerobic stability. Various means were

**Table 3.** Microbiological enumeration of the fresh sorghum and the sorghum silages (log<sub>10</sub> CFU g<sup>-1</sup> DM)

Treatment	Time	LAB	Yeast	Mould
Fresh material	Day 0	4.48	5.45	NF
Control				
Silage	Day 2	7.95	3.87	1.5
	Day 4	7.89	-	-
	Day 8	7.90	-	-
	Day 15	8.37	-	-
	Day 60	4.56	5.57	NF
Aerobic Inoculant A	Day 5	-	8.73	NF
Silage	Day 2	8.98	1.50	NF
	Day 4	9.27	-	-
	Day 8	8.69	-	-
	Day 15	8.08	-	-
	Day 60	5.58	5.89	NF
Aerobic Inoculant B	Day 5	-	9.13	NF
Silage	Day 2	9.14	1.5	NF
	Day 4	8.91	-	-
	Day 8	8.50	-	-
	Day 15	8.17	-	-
	Day 60	4.37	3.01	NF
Aerobic	Day 5	-	8.99	NF

NF, not found; -, not determined; LAB=lactobacilli.

**Table 4.** Results of the aerobic stability test

Treatment	CO <sub>2</sub> production g kg <sup>-1</sup> DM	Glucose loss g kg <sup>-1</sup> DM	Visual Appraisal
Control	1.27 <sup>a</sup>	0.86 <sup>a</sup>	Alcoholic smell
Inoculant A	1.67 <sup>a</sup>	1.14 <sup>a</sup>	Alcoholic smell
Inoculant B	1.86 <sup>a</sup>	1.26 <sup>a</sup>	Alcoholic smell
SEM	0.30	0.20	

Means with the same letter within the same columns do not differ ( $p > 0.05$ ).

tried to improve the ensiling process of forage sorghum. Ashbell et al. (1998) mixed moist forage sorghum with drier grain sorghum, which resulted in improved silages. Lactic acid inoculants enhanced the ensiling fermentation of sorghum by faster production of lactic acid and a more rapid decrease in pH. However, such inoculants enhanced spoilage upon aerobic exposure because lack of enough VFAs in the silage, which inhibit lactate-assimilating yeasts (Meeske et al., 1993; Weinberg et al., 1993). A propionic acid bacterium which might have inhibited yeasts did not survive under the acidic conditions of the silage (Weinberg et al., 1995).

In the current experiment the forage sorghum had low DM and high WSC contents. Two types of inoculants were tried in order to improve the ensiling process of the sorghum: A, comprising homofermentative LAB only, and B, containing in addition also strains of *B. subtilis* and *S. rubidaea*. Both inoculants improved the ensiling fermentation as compared with the control, as apparent from a faster decrease in pH. Silage treated with inoculant B had smaller gas losses and relatively lower cell wall content in the silage, indicating less losses of organic soluble material during fermentation. However, silage treated with inoculant A produced greater gas losses, which is due to itself composition of inoculant A (comprising homofermentative LAB only) that resulted in less VFAs concentration (table 2) and higher yeast numbers in the silage on day 60 (table 3). Because WSC encourages growth of yeast (Schmidt et al., 1997), therefore, under this lower VFAs concentration, yeast converted sugars (high WSC content in the fresh forage sorghum) into ethanol and CO<sub>2</sub> and finally resulted in greater gas losses in silage treated with inoculant A. The control silage had high concentrations of butyric acid, which indicates undesirable secondary fermentation, whereas the inoculated silages did not contain butyric acid. The latter two strains were included in inoculant B because of their ability to inhibit yeasts and mould, presumably by secretion of a peptide which is toxic to them. The silages treated with this inoculant contained lower yeast numbers on day 60 as compared with the silages of the control and of inoculant A (table 3). The pH of the silages of inoculant B was maintained at a low value also after exposure to air. However, the number of yeasts increased after 5 days of aerobic exposure, regardless of treatment. We expected to observe a difference between the two inoculants with regard to aerobic stability, and the control silages to be the most stable, as found in previous experiments (Meeske et al., 1993; Weinberg et al., 1993; Zhang et al., 2000). Since all treatments had a high number of yeasts and produced little CO<sub>2</sub>, it is not possible from the current results to attribute an advantage to either of the inoculants used with regard to aerobic stability.

Inoculants for silage should be efficient throughout the entire ensiling process: all stages of fermentation and aerobic exposure. Since crop composition as well as ensiling conditions vary, inoculants should meet specific requirements of the ensiling operation. We believe that strains isolated from local crops for local silages will serve as best inoculants.

## ACKNOWLEDGMENTS

The authors wish to express their thanks to contribution from the Agricultural Research Organization, The Volcani Center, The Institute for Technology and Storage of Agricultural Products, Bet Dagan, Israel, No0402-E 1998 series.

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