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The effect of a propionic acid bacterial inoculant applied at ensiling on the aerobic stability of wheat and sorghum silages

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The effect of a new strain of *Propionibacterium shermanii* (PAB), applied at ensiling, on the aerobic stability of wheat and sorghum silages was studied in several experiments under laboratory conditions. In the one experiment with wheat and in those with sorghum a lactic acid bacteria (LAB) inoculant (*Lactobacillus plantarum* and *Pediococcus cerevisiae*) was also included. After treatment, the chopped forages were ensiled in 1.5-L anaerobic jars which were sampled in triplicate on predetermined dates to follow fermentation dynamics. At the end of the experiments, the silages were subjected to an aerobic stability test. The PAB inoculant improved the aerobic stability only in one experiment with wheat, in which the decrease in pH was very slow; the final pH remained relatively high (4.5). The PAB-treated silages contained 19.5 ± 2.0 g of propionic acid per kg of dry matter. In the experiments with sorghum, the control and PAB-inoculated silages were stable, whereas LAB-inoculated silages deteriorated. The results suggest that PAB can survive in and improve the aerobic stability of only slow-fermenting silages which are prone to aerobic deterioration.

Keywords: silage; aerobic stability; propionic acid bacteria

Introduction

Ensiling is a preservation method for moist forage crops. It is based on fermentation with lactic acid bacteria (LAB) which convert water-soluble carbohydrates into lactic acid under anaerobic conditions. As a result the pH decreases and the moist forage is preserved.

Silages might be exposed to air during preparation, storage and especially feedout. Air is detrimental to ensiling which is an anaerobic process [16] and therefore aerobic stability, which affects quality and the extent of losses, is an important characteristic of silages.

In order to improve the ensiling process, various additives, chemical and biological, have been developed. The biological additives are advantageous because they are safe and easy to use, non-corrosive to machinery, do not pollute the environment and are regarded as natural products. Bacterial inoculants are added to silages in order to stimulate lactic acid fermentation and accelerate the decrease in pH, and thus to improve silage preservation. Most of the available inoculants consist of selected strains of homofermentative LAB, such as *Lactobacillus plantarum*, *Pediococcus* and *Enterococcus* species. Many reports have shown the advantages of such inoculants [4, 6, 10, 12]. However, recent studies under laboratory conditions [13, 14] indicated that the addition of LAB inoculants impaired the aerobic stability of silages of mature cereal crops (wheat, sorghum, maize). This was indicated by a rise in pH, visible moulding and intensive production of CO_2 during aerobic exposure. Similar problems caused by the use of LAB inoculants have been observed in other studies [5, 9]. The explanation for this phenomenon is that under anaerobic conditions, the homofermentative LAB inoculants produce mainly lactic acid, which can serve as a substrate for lactate-assimilating yeasts upon exposure to air. In such fermentations, only small amounts of short-chain volatile fatty acids (VFAs) such as acetic, propionic and butyric acids are produced. These short-chain aliphatic acids inhibit yeasts and moulds [7] and therefore LAB-inoculated silages deteriorate faster upon exposure to air.

In order to use biological additives to overcome the problem of aerobic deterioration of silages, it has been suggested that other types of inoculants, such as *Bacillus* species and propionic acid bacteria (PAB) be used [8]. It would be expected that such additives would produce in the silage substances which have antimycotic properties and which would, therefore, inhibit the development of yeasts and moulds upon aerobic exposure. PAB can ferment lactate and sugars to propionate and acetate; they might thus protect silages upon exposure to air.

In a previous study [15], a PAB inoculant was tested in pearl millet and maize silages with and without LAB inoculants. In that study the PAB had only a marginal effect on the aerobic stability of the silages and it was hypothesized that the added PAB did not survive the acidic conditions in the silage. The combination of PAB and LAB inoculants resulted in the least stable silages.

The purpose of the experiments described below was to study further the effect of a PAB inoculant applied at ensiling on wheat and sorghum fermenting at various rates, on the aerobic stability of the respective silages.

Materials and methods

Experimental

The crops used in these experiments were wheat and sorghum. There were three experiments with wheat (Israeli cultivar 'Ariel' harvested at the milk-soft dough ripening

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stage). Two sorghum varieties were used: forage cultivar FS5 (Dekalb, Plant Genetics, Lubbock, TX, USA) and grain type sorghum 947 (Pioneer, Johnston, IA, USA), harvested at the milk ripening stage.

Whole plants were chopped to *ca* 2 cm (with a Wintersteiger® chopper) 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 650–800 g (wet weight) of chopped forage, without a headspace. The degree of compaction used in the laboratory experiments was around 70% of that used on a farm scale. The jars were stored at ambient temperature (27 ± 2° C). In the experiments with wheat there were 15 jars per treatment, which were sampled in triplicate on days 1, 3, 5, 10 and 90 after ensiling. In the experiments with sorghum there were six jars which were sampled in triplicate on days 2 and 60. At the end of the experiments the final silages were subjected to an aerobic stability test lasting 5 days, in a system described in [1]. In this test, CO_2 produced during aerobic exposure was measured along with chemical and microbiological parameters which serve as spoilage indicators.

The treatments used in the experiments with wheat were: control (no additive) and a new strain of *Propionibacterium shermanii* (PAB). The PAB inoculant (made by Lallemand SA, Saint-Simon, France) contained 10^{11} colony-forming units (CFU) g^{-1} powder, (manufacturer's statement). It was applied by suspending 2.5 g of powder in 950 ml of water, 23 ml of which were sprayed over 12 kg of the chopped forage, spread over a 1 × 3-m area, and was then thoroughly mixed. Thus, 5×10^5 CFU of PAB inoculant were applied per g of fresh crop. Experiment 2 with wheat included also a treatment with LAB: Silo-King (made by Agri-King, Fulton, IL, USA) which contained 5×10^{10} CFU of *L. plantarum* and *P. cerevisiae* g^{-1} powder (manufacturer's statement). It was applied by suspending 500 mg of powder in 250 ml of water, 12.5 ml of which were sprayed over 12 kg of the chopped forage, as described above. Thus 10^5 CFU of the LAB inoculant were applied per g of fresh crop.

The following treatments were used in the experiments with sorghum: control (no additive), LAB, PAB, and a combination of LAB + PAB. The inoculants used were the same as in the wheat experiments.

Analytical procedure

The chemical analysis was carried out on an individual silo basis. Dry matter (DM) was determined by oven-drying the material for 48 h at 60° C. Ash was obtained after 3 h at 550° C. Water-soluble carbohydrates (WSC) were determined by the phenol-sulphuric acid method [3]. Lactic acid (LA) was determined by a spectrophotometric method [2]. The protein removal step was omitted in our laboratory to better reflect LA determination in silages. Volatile fermentation end-products were determined with a gas chromatograph using a Chromosorb 101 column over a temperature range of 140–210° C [11].

The microbiological examination included the enumeration of lactobacilli (on pour-plate Rogosa agar; Oxoid CM627 incubated at 30° C for 3 days), yeasts and moulds (on spread-plate malt extract agar acidified with lactic acid to pH 4.0 and incubated at 30° C for 3 days) and enterobacteria (on Violet Red Bile Glucose agar; Oxoid CM485, using the double-layer technique incubated at 37° C for 24 h).

The statistical analysis included one-way analysis of variance and Duncan's multiple range test; for sorghum which had a 2 × 2 factorial design, a two-way analysis of variance was also included; these were performed on the results obtained with the Statistical Analysis System (SAS, Cary, NC, USA).

Table 1 Chemical analysis of the fresh forages

Forage type	Values for				
	DM	pH	WSC	Ash	CP
Wheat (exp 1)	465 ± 6	6.2	42 ± 4	72 ± 1	74 ± 1
Wheat (exp 2)	324 ± 1	6.4	71 ± 4	97 ± 1	73 ± 0
Wheat (exp 3)	478 ± 5	6.3	37 ± 1	78 ± 2	65 ± 2
Sorghum (FS5)	264 ± 4	5.7	127 ± 13	62 ± 1	55 ± 1
Sorghum (947)	233 ± 5	5.9	159 ± 6	68 ± 1	60 ± 0

DM, dry matter; WSC, water-soluble carbohydrates; CP, crude protein
Values for DM, WSC, Ash and CP are g kg^{-1} ± standard deviation; $n = 3$

Results

Tables 1 and 2 give the chemical and microbiological compositions of the forages used. With these forages a wide range of chemical compositions and ensiling characteristics was obtained. In general, sorghum had a higher content of water-soluble carbohydrates and lower content of crude protein than wheat. In experiment 3 with wheat the numbers of LAB were extremely low, but they increased markedly after 1 day of ensiling.

Figure 1 shows the changes in pH in the control wheat silages of the three experiments during the first 10 days of ensiling. The pH of the wheat silages of experiment 3 remained the highest during the first 10 days of ensiling, and it was still relatively high (4.5) on day 90. The LAB (experiment 2) and PAB inoculants had only a minor effect on the rate of pH change in the experiments with wheat. In the experiments with sorghum the pH decreased very quickly: on day 2 the pH was around 4.5 in the controls and PAB-treated silages, and 3.9 in the LAB-inoculated silages. The final pH values were below 4 for all treatments.

Tables 3 and 4 give the results of chemical and microbiological analyses of the final silages. The major fermentation product in all silages was lactic acid (LA), and the major

Table 2 Microbiological analysis of the fresh forages

Forage type	CFU (log_{10} g^{-1}) of:		
	Lactobacilli	Yeasts	Moulds
Wheat (exp 1)	5.3 (9.8)	5.1	4.3
Wheat (exp 2)	5.5 (9.8)	4.4	2.8
Wheat (exp 3)	1.3 (7.4)	4.1	2.7
Sorghum (FS5)	3.0 (9.5)	4.6	3.7
Sorghum (947)	2.7 (10.3)	5.8	5.4

Numbers in parentheses indicate the log_{10} number of lactobacilli after 1 (wheat) or 2 (sorghum) days of ensiling

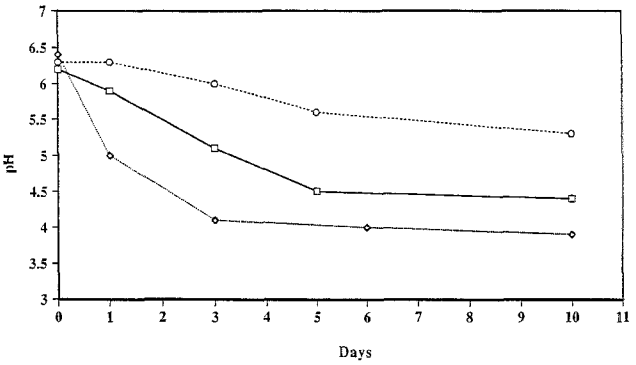

Figure 1 Change in pH during ensiling of wheat from different harvests: —□— exp 1, ---◇--- exp 2, ---○--- exp 3

those in experiment 1, the LAB-treated silages being the least stable, as indicated by CO_2 production and the extensive development of yeasts and moulds. The result that LAB inoculants impair the aerobic stability of wheat silages is in agreement with previous findings [14]. In experiment 3, one of the three control samples spoiled completely during the aerobic stability test ($CO_2 = 30.7$ g kg^{-1} DM, pH = 5.9), whereas all the PAB-treated samples remained stable. In this experiment, the stable PAB-treated silages had markedly fewer yeasts than the controls at the end of the aerobic stability test. Although the statistical analysis did not indicate significant differences with regard to CO_2, all the results described here indicate greater aerobic stability in the PAB-treated silages than in the controls.

In experiments with the sorghum cultivars, the controls and PAB-treated silages were stable, whereas those treated with LAB deteriorated upon aerobic exposure.

Table 3 Chemical analysis of the final silages before exposure to air

Forage type	Treatment	pH	Content of (g kg^{-1} ± SD):			
			WSC	LA	Ethanol	Acetic acid
Wheat (exp 1)	Control	4.2	62 ± 10	35 ± 8	3.0 ± 0.1	6.4 ± 1.1
	PAB	4.3	69 ± 11	31 ± 3	2.4 ± 0.5	6.2 ± 0.9
Wheat (exp 2)	Control	3.7	34 ± 2	72 ± 5	2.0 ± 1.0[a]	8.0 ± 2.0
	LAB	3.7	35 ± 3	70 ± 2	0.8 ± 0.1[b]	4.4 ± 4.5
	PAB	3.7	33 ± 3	73 ± 10	3.0 ± 0.2[a]	10.6 ± 1.2
Wheat (exp 3)	Control*	4.5	66 ± 1	26 ± 4[a]	15.3 ± 3.3[a]	3.6 ± 6.2
	PAB*	4.6	79 ± 8	13 ± 1[b]	7.1 ± 1.3[b]	9.4 ± 1.9
Sorghum (FS5)	Control	3.5	101 ± 9	50 ± 8	12.9 ± 3.3	1.4 ± 1.4
	LAB	3.5	105 ± 9	58 ± 4	8.7 ± 0.3	2.5 ± 2.1
	PAB	3.6	96 ± 5	59 ± 5	13.3 ± 1.4	2.6 ± 1.5
	LAB + PAB	3.5	106 ± 18	56 ± 9	11.8 ± 1.5	1.9 ± 1.7
Sorghum (947)	Control	3.7	90 ± 4	54 ± 2	14.7± 0.7	3.0 ± 0.2
	LAB	3.5	112 ± 47	69 ± 6	24.5 ± 24.3	3.9± 3.4
	PAB	3.6	101 ± 26	64 ± 11	15.3 ± 0.3	2.3 ± 0.8
	LAB + PAB	3.5	95 ± 4	57 ± 3	15.4 ± 3.6	0.5 ± 0.7

DM, dry matter; WSC, water-soluble carbohydrates; LA, lactic acid; LAB, *Lactobacillus plantarum* + *Pediococcus cerevisiae* inoculant; PAB, *Propionibacterium shermanii* inoculant
*The control silages also contained 6.7 ± 4.0 butyric acid and the PAB silages contained 19.5 ± 2.0 and 8.5 ± 1.0 g kg^{-1} propionic and butyric acid, respectively
Within a column and experiment (wheat), means followed by different letters differ significantly ($P<0.05$)

volatile fermentation products were ethanol and acetic acid. In experiment 3 the silages of the PAB treatment had significantly ($P<0.05$) lower levels of LA and ethanol than the controls. Butyric acid was detected only in the control and PAB-treated wheat silages of experiment 3 (6.7 and 8.5 g kg^{-1} DM, respectively) and propionic acid was detected in substantial amounts (19.5 ± 2.0 g kg^{-1} DM) only with the PAB treatment in this experiment. The factorial analysis for sorghum did not reveal significance for any effect, except with regard to ethanol in the LAB treatment in the experiment with Cultivar FS5.

Table 5 gives the results of the aerobic stability test. The large standard deviations of the CO_2 means reflect the fact that the samples of this test did not always yield consistent results. In the wheat silages of experiment 1, one control sample out of three spoiled and became mouldy ($CO_2 = 5.7$ g kg^{-1} DM) while the other two were stable upon aerobic exposure; all PAB-treated samples were stable. In experiment 2 all treatments were less stable than

Discussion

The PAB inoculant was tested as a means to improve the aerobic stability of silages. The LAB inoculant was used for comparison purposes. It was hypothesized that if the former bacterium produced propionic acid in silages, it would suppress the yeasts and moulds which spoil silages under aerobic conditions. In a previous study with pearl millet and maize silages this inoculant had only a marginal effect on aerobic stability [15], probably because the pH in these silages decreased very rapidly to below 4.5, and under such conditions no propionic acid is produced by this bacterium [8]; a PAB additive was efficient in grass silages only if decrease in pH was retarded, eg by delayed filling [8]. Therefore, we speculate that this type of additive would be effective in improving the aerobic stability only in silages of slow-fermenting forage crops. Examples of such crops are legumes, but they yielded aerobically-stable silages in our laboratory. In order to show the effect of PAB

Table 4 Microbial counts of the silages before exposure to air

Forage type	Treatment	CFU (log_{10} g^{-1}) of:		
		Lactobacilli	Yeasts	Moulds
Wheat (exp 1)	Control	5.9	2.8	NF
	PAB	5.8	2.7	NF
Wheat (exp 2)	Control	3.6	NF	NF
	LAB	4.6	3.9	NF
	PAB	3.7	NF	NF
Wheat (exp 3)	Control	7.7	3.4	NF
	PAB	7.3	NF	NF
Sorghum (FS5)	Control	4.8	NF	2.3
	LAB	4.3	NF	2.3
	PAB	6.5	3.2	2.6
	LAB + PAB	5.5	5.1	NF
Sorghum (947)	Control	5.3	2.9	2.6
	LAB	4.5	2.8	2.3
	PAB	4.6	3.2	2.3
	LAB + PAB	5.4	4.8	2.3

DM, dry matter; NF, not found; LAB, *Lactobacillus plantarum* + *Pediococcus cerevisiae* inoculant; PAB, *Propionibacterium shermanii* inoculant

Table 5 Results of the aerobic stability test (5 days)

Forage type	Treatment	pH	CO_2 (g kg^{-1} ± SD)	Yeasts (log_{10} g^{-1})	Moulds (log_{10} g^{-1})
Wheat (exp 1)	Control	4.3 ± 0.1	1.9 ± 3.3	6.4	6.7
	PAB	4.3 ± 0.1	0	6.6	5.1
Wheat (exp 2)	Control	3.8	5.0 ± 4.8	6.5	6.1
	LAB	3.7	11.7 ± 11.8	7.8	6.5
	PAB	3.6	4.1 ± 4.0	5.9	5.9
Wheat (exp 3)	Control	5.0 ± 0.8	10.2 ± 17.7	7.9	NF
	PAB	4.6 ± 0.0	0	3.3	NF
Sorghum (FS5)	Control	3.7 ± 0.0	0.3 ± 0.6	6.8	5.1
	LAB	3.6 ± 0.0	6.4 ± 10.1	6.6	6.6
	PAB	3.6 ± 0.0	3.6 ± 1.6	8.4	6.6
	LAB + PAB	3.6 ± 0.0	4.5 ± 0.6	9.0	4.6
Sorghum (947)	Control	3.8 ± 0.0	1.4 ± 1.2	8.8	4.3
	LAB	4.2 ± 0.9	24.3 ± 25.3	8.8	2.6
	PAB	3.6 ± 0.0	2.4 ± 3.3	8.9	6.6
	LAB + PAB	3.6 ± 0.0	6.0 ± 2.9	9.0	4.3

NF, not found; LAB, *Lactobacillus plantarum* + *Pediococcus cerevisiae* inoculant; PAB, *Propionibacterium shermanii* inoculant

on the aerobic stability of silages we had to find silages which are aerobically unstable and ferment slowly. Wheat was tried because various wheat silages ferment at different rates. In the current series of experiments two of the wheat harvests (experiments 1 and 3) fermented slowly and the uninoculated control samples were aerobically unstable, as compared with completely stable samples from the PAB treatment. Experiment 3 was the only case in which propionic acid was detected in the PAB-treated silages; in the aerobically-exposed silages of this treatment, yeast and mould numbers remained low. In the sorghum silages, which fermented very quickly to low pH values, the PAB did not have any effect.

In conclusion, the addition of a PAB might improve the aerobic stability only of silages with slow acidification rates which are prone to aerobic deterioration. Such silages are mainly of mature, dry cereals.

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