The effect of applying *Lactobacillus buchneri* at ensiling on wheat silage studied in laboratory and farm experiments

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Introduction

Lactobacillus buchneri (LB) is a heterofermentative lactobacillus that produces high levels of acetic acid in the silage which inhibits yeasts and molds. Therefore, it is used to enhance the aerobic stability of silages during storage and feed out. Previous experiments under laboratory conditions with purified strains from a culture collection proved its high efficiency to stabilize wheat and maize silages during aerobic exposure (Weinberg et al. 1999, 2001).

The purpose of the present experiments was to test a commercial additive comprising LB in wheat silage in on farm scale ensiling operation. A laboratory experiment was performed parallel to the farm experiment to allow time-course study.

Experimental

1.Farm experiment

The experiment was performed on a commercial farm (Nir Galim), which allocated two adjacent bunker silos that were filled simultaneously: control and treated. The silos (40x12x3 m) were filled with 1200 ton wheat during 4 days (26.3-29.3.01). The wheat was harvested at the milk stage of maturity and was wilted shortly in the field. Trucks with 8 tons (fresh weight) of chopped wheat arrived at the silos alternately.

On the second day of ensiling two groups of 5 Dacron bags were buried at two sites along each silo. Each bag contained about 1400 g of chopped wheat, untreated or treated, respectively. A thermocouple wire was connected to each group of bags for temperature measurements. When the unloading front reached a group of bags they were retrieved and brought to the laboratory for analysis. In addition, after opening of the silos, random samples were brought from the face of the silages for analysis.

The treatment comprised 50 bags containing 400 g of LB that were obtained from Biotal USA. Two inoculant bags were suspended in 160 liters of tap water in a sprayer. The rate of supply of the sprayer was 2.9 liters per minutes. Each truckload of wheat (8 tons fresh weight) that arrived at the treated silo was sprayed from all sides for 2.5 minutes with 7.3 liters of the inoculant suspension, after which the pile was spread and compacted in the silo. Hence, one ton of fresh wheat was treated with 4.5 g of inoculant. The top of the bunker was sprayed with additional 60 liters before sealing.

2.Laboratory experiment

The laboratory experiment was performed in 1.5 liter glass jars which are equipped with a lid which enables gas release only. There were 15 jars per treatment which were used for sampling in triplicate on days 1, 3, 7, 14 and 66 after ensiling.

The following treatments were used: control (no additives), laboratory spraying with LB and on farm spraying. The laboratory spraying was performed by suspending 440 mg of the inoculant in 100 ml of tap water. Twenty five ml of the suspension were diluted to 60 ml and sprayed over 10 kg of chopped wheat. The on farm treatment included inoculated wheat that was taken from the treated silo immediately after spraying and spreading.

At the end of the ensiling period the final silages (day 66) were subjected to an aerobic stability test lasting 5 days, in a system developed by Ashbell et al. (1991). In this system, CO_2 produced during aerobic exposure is measured along with change in pH and yeast and mold counts serve as spoilage indicators.

Analysis

The chemical analysis was carried out on an individual bag or jar basis. Dry matter was determined by oven drying for 48 h at 60^0C. Water-soluble carbohydrates (WSC) were determined by the phenol-sulfuric acid method according to Dubois et al. (1956). Lactic acid was determined by a spectrophotometric method, according to Barker and Summerson (1941). Volatile fermentation end-products were determined with a gas chromatograph with a semi-capillary FFAP column (Hewlett Packard) over a temperature range of 45-230^0C.

The microbiological evaluation included the enumeration of lactobacilli (on pour plate Rogosa agar, Oxoid), and yeast and mold (on spread plate malt extract agar acidified with lactic acid to pH 4.0). All plates were incubated for 3 days at 30^0C. This analysis was performed on a single representative sample.

Results

The composition of the fresh wheat used in the experiments is given in Table 1. Because the filling of the silos lasted for 4 days and the wheat came from various fields, there was a large variability on all parameters that had been measured. The DM

content varied between 35 and 46% and the WSC content was sufficient to ensure proper ensiling fermentation.

1. Laboratory experiment

The change in pH during ensiling is given in Figure 1. Both inoculant treatments resulted in faster and steeper decrease in pH as compared with the control. There was no marked difference between the laboratory and on farm treatments with regard to pH change.

The results of the chemical analysis are given in Table 2. Weight losses were higher in the treated silages than in the control silages, which is typical to a heterolactic fermentation. As expected, the treated silages contained significantly higher levels of acetic acid.

The results of the microbiological analysis are given in Table 3. The number of lactobacilli increased dramatically after one day and remained high for at least two weeks. At the end of the storage period their numbers slightly decreased. The treated silages had higher numbers of lactobacilli than the control. The number of yeasts and molds were low in all silages, and slightly lower in the treated ones.

The results of the aerobic stability test are given in Table 4. The control silages spoiled after 5 days of aerobic exposure as indicated by the visual appraisal, rise in pH and intensive CO_2 production. The treated silages remained stable. We cannot explain the high number of yeasts found in the laboratory treated silages after aerobic exposure.

2. Farm experiment.

The maximal temperature in the control silage was measured on March 30, and it was 44.3^{0}C; the maximal temperature in the treated silage was 41^{0}C and it was measured on April 3. After one month of ensiling the temperature decreased gradually; On May 30 the temperature in both silages was 37-38^{0}C.

The results of the chemical analysis are given in Table 5. The period during which the bags stayed in the silos varied considerably according to the unloading schedule of the farm. The first series of bags from the control silo were retrieved after 3 months; then the farm used the treated silage and therefore, the second series of control bags were obtained after almost a year. Dry matter losses could be calculated properly only for the first control and treated bags: unfortunately the second treated bags were lost and

the analysis was performed on silage samples which were taken at the approximate site of the bags. For the second series of control bags the calculations resulted in addition of 1-4% DM which we cannot explain (similar results were obtained in previous farm experiments). For the samples that could be calculated, the treated bags had slightly higher losses as compared with the controls, which is typical to hetero-lactic fermentation.

In general, the chemical analysis of the control and treated silages did not differ markedly. Residual WSC in the treated silage were lower than in the control silage and the treated silage contained also butyric acid. Surprisingly, there was no difference in the acetic acid content between the silages. The second series of the control samples contained more lactic and acetic acid than the rest of the samples. This may be attributed to the fact that the last bags were close to the end of the silage and air might have penetrated and changed the fermentation patterns of the LAB.

The results of the microbiological analysis are given in Table 6. The first control and second treated samples had LAB populations, the other samples had neither LAB nor yeasts and molds.

Table 7 gives the result of the aerobic stability test. In general, all silages were stable upon aerobic exposure. The second samples series of the treated silages had slight signs of deterioration (some CO_2 build-up and yeast counts).

3. Random samples from the face of the silages

Table 8 gives the results of the analysis of samples brought from the face of the silages. Both control and treated silages were of good quality with typical color, odor and texture. The shoulders and top of the control silage seemed to be moldy whereas the treated silage was clean. The level of yeasts and molds in these samples was low.

Table 9 gives the result of the aerobic stability test of these samples. One sample from the control silage and the sample taken from the face of the treated silage were unstable in the aerobic stability test.

Discussion

The inoculant which has been tested in the present experiment contained Lactobacillus buchneri. This is a hetero-fermentative LAB which is supposed to produce high levels of acetic acid in the silage which inhibits yeasts and molds, and so preserve the silage upon aerobic exposure.

The laboratory experiment clearly indicated the advantage of this silage additive in protecting the wheat silage upon aerobic exposure. Wheat which was treated on the farm and ensiled in the mini-silos behaved like wheat which was treated under controlled laboratory conditions. This indicated that the spraying of the inoculant on the farm silage was effective.

However, the results from the farm experiment do not show much advantage to the application of the inoculant. The LB inoculant did not produce much more acetic acid in the treated silage as compared with the control silage (Table 5). In addition, as happens often, the control silage was of good quality and stable upon aerobic exposure due to careful management. The bags have been buried in the center of the silos where preservation is always the best. Therefore, the advantage of any silage additive could not be demonstrated.

The results of the random sample brought from the treated silage on October 29 are of concern. However, it is impossible to estimate for how long such random samples were exposed to air before taken for analysis and therefore these results should be considered carefully.

Since fermentation losses are higher in hetero-fermentative ensiling we recommend to consider an inoculant which contains both L. plantarum and L. buchneri. The former results in faster fermentation and reduced losses and the latter protects the silage upon aerobic exposure. Previous experiments indicated the advantage of such combination (references 2, 3).

For the time being we think that more tests are needed before it is possible to fully recommend L. buchneri as an additive for protecting wheat silage upon aerobic exposure.

Acknowledgements

We wish to thank the feed center personnel of Nir Galim farm for their excellent cooperation and help during the experiment. We thank Mr. Musa Avital from the Volcani Center for his help with the sprayer used on the farm.

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Table 1. Composition of the fresh wheat.

	Date	**26.3.01**	**27.3.01**	**28.3.01**	**29.3.01**
Parameter	**Treatment**				
% DM	**Control**	45.8±0.2	38.6±0.2	35.1±0.7	37.7±1.2
	LB	39.5±0.3	40.7±0.1	34.5±0.1	38.3±0.1
PH	**Control**		6.2	6.0	6.3
	LB		6.3	6.1	6.3
WSC	**Control**		6.2	8.8	8.2
	LB		7.6	7.2	8.6
Lactobacilli	**Control**	4.1	7.1	9.4	
	LB		8.2	9.4	
Yeasts	**Control**	5.3	3.6	5.3	
	LB		5.0	4.1	
Molds	**Control**	4.9	4.3	3.2	
	LB		5.5	4.4	

WSC = water-soluble carbohydrates (% in DM). The microbiological results are given as $\log_{10}$ number of colony forming units g^{-1} DM.

Table 2. Chemical analysis of the laboratory silages. Results are in % in DM.
DM content of the fresh wheat was 45.8% for the control and laboratory sprayed inoculum, and 39.5% for the on farm sprayed treatment.

Treatment	%DM	% Weight loss	WSC*	Lactic acid	Acetic acid	Butyric acid
Control	43.7±0.7	0.8±0.2	11.5±1.0	4.3±0.2	1.5±0.4	0.2±0.2
Laboratory	42.4±0.3	1.5±0.0	1.7±0.5	3.8±0.3	3.1±0.3	0.8±0.1
On farm	36.5±0.2	1.3±0.0	2.3±2.6	3.7±0.3	3.1±0.3	0.9±0.1

Ethanol was also found in the silages at 0.2-0.3%, with no differences between treatment.
*WSC = water-soluble carbohydrates.

Table 3. Microbiological analysis of the laboratory silages. Results are given as $\log_{10}$ number of colony forming units g^{-1} DM.

Treatment	Type of microorganism	Fresh crop	Day 1	Day 3	Day 7	Day 14	Day 66
Control	Lactobacilli	4.1	8.5	9.1	9.0	9.7	7.4
	Yeasts	5.3	3.3	3.4	2.5	<2.0	<2.0
	Molds	4.9	4.1	3.5	2.7	2.0	2.4
Laboratory	Lactobacilli	4.1	9.4	9.7	10.5	10.3	8.1
	Yeasts	5.3	2.6	2.3	<2.0	<2.0	<2.0
	Molds	4.9	4.2	2.5	2.8	3.2	3.6
On farm	Lactobacilli	5.2	8.8	9.6	10.7	10.4	8.1
	Yeasts	4.2	3.3	<2.0	<2.0	<2.0	<2.0
	Molds	4.3	3.5	3.4	2.8	2.1	<2.0

Table 4. Results of the aerobic stability test after 5 days.

Treatment	**Visual appraisal**	**pH**	**CO_2 (g kg^{-1} DM)**	**Yeasts**	**Molds**
Control	Moldy	4.5±0.0	14.4±8.3	5.1	4.9
Laboratory	Clean	4.1±0.0	1.1±2.0	8.3	3.8
On farm	Clean	4.1±0.0	0.7±1.3	3.5	2.1

Table 5. Chemical analysis of the farm silages. Results are in % in DM.

Treatment	Date	%DM	% DM loss	pH	WSC	Lactic acid	Acetic acid	Butyric acid
Control 1	10.7.01	38.6±0.3	2.2±0.9	4.1±0.1	3.3±0.2	3.2±0.2	1.7±0.4	0
Control 2	10.3.02	34.1±0.9	-	3.9±0.0	3.0±0.1	5.1±0.3	2.9±0.1	0
LB 1	17.9.01	38.1±0.4	4.0±1.0	4.1±0.0	0.3±0.5	2.9±0.1	1.7±0.4	0
LB 2	27.11.01	34.0±2.3	-	3.9±0.2	0.2±0.5	3.9±0.8	2.0±0.4	0.3±0.1

In addition, the samples of all silages contained 0.1-0.3% in DM ethanol.

Table 6. Microbiological analysis of the farm silages. Results are given as $\log_{10}$ number of colony forming units g^{-1} DM.

Treatment	Date	Lactobacilli	Yeasts	Molds
Control 1	10.7.01	6.4, 5.1*	<2.0	<2.0
Control 2	10.3.02	<2.0	<2.0	<2.0
LB 1	17.9.01	<2.0	<2.0	<2.0
LB 2	27.11.01	4.5, 3.3*	<2.0	<2.0

* Results from 2 samples.

Table 7. Results of the aerobic stability test of the farm silages after 5 days.

Treatment	Visual Appraisal	CO_2 (g kg^{-1} DM)	pH	Yeasts	Molds
Control 1	Clean	0	4.1±0.1	<2.0, 3.4**	<2.0, 3.8**
Control 2	Clean	0	3.9±0.1	<2.0	<2.0
LB 1	Clean	2.4±1.2	4.1±0.0	2.6	<2.0
LB 2	Clean*	6.7±3.0	3.9±0.1	6.6, 8.6**	<2.0

*some yeast dots. ** Values from 2 samples.

Table 8. Analysis of random samples taken from the face of the silages

Treatment	Date	pH	Lactic acid	Acetic acid	Butyric acid	Yeasts	Molds
Control	30.4.01	4.0	4.7	2.4	0.5	4.3	<2.0
Control	31.5.01	4.0	4.4	2.4	0.7	2.8	<2.0
LB	29.10.01	4.1	4.8	1.7	0	4.1	3.6

Table 9. Results of the aerobic stability test of the random samples.

Treatment	Date	CO_2 (g kg^{-1} DM)	pH	Yeasts	Molds
Control	31.5.01	10.8±8.6	4.0	8.4	<2.0
LB	29.10.01	57.8±5.7	6.1±0.8	8.7	<2.0

שינוי ה- pH בתחמיצי חיטה בתנאי מעבדה
pH change during ensiling of wheat in glass jars