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Research Article

The Effects of Bacterial Inoculants on the Fermentation, Aerobic Stability and Rumen Degradability Characteristics of Wheat Silages*

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Abstract: This study was carried out to determine the effects of lactic acid bacteria (LAB) inoculants on the fermentation, aerobic stability and in situ rumen degradability characteristics of wheat silages.

Wheat was harvested at the early dough stage. Inoculant-1188 (Pioneer®, USA) and Sil-All (Alltech, UK) were used as LAB inoculants. Inoculants were applied to silages at 1.5 x 10^6 cfu/g levels. Wheat material was ensiled in 1.5-l special anaerobic jars, equipped with a lid enabling gas release only. Three jars from each group were sampled for chemical and microbiological analysis 2, 4, 8, 15 and 50 days after ensiling. At the end of the ensiling period all silages were subjected to an aerobic stability test for 5 days. In addition, in situ rumen degradability characteristics were determined in the silages.

Both homofermentative LAB inoculants improved the fermentation characteristics of wheat silages. At the end of the ensiling period inoculants increased the lactobacilli and decreased yeast and mold numbers of the silages. However, both LAB inoculants led to higher CO_2 production and impaired the aerobic stability of the silages. The in situ degradability characteristics of the wheat silages were not affected by the inoculant treatment.

Key Words: Aerobic stability, fermentation, inoculant, rumen degradability, silage, wheat

Bakteriyal İnokulantların Buğday Silajlarının Fermantasyon, Aerobik Stabilite ve Rumen Parçalanabilirlik Özellikleri Üzerine Etkileri

Özet: Bu çalışma laktik asit bakteri (LAB) inokulantlarının buğday silajlarının fermantasyon, aerobik stabilite ve in situ rumen parçalanabilirlik özelliklerini saptamak amacıyla düzenlenmiştir.

Araştırmada, buğday hamur olum döneminde hasat edilmiştir. LAB inokulantı olarak İnoculant-1188 (Pioneer®, USA) ve Sil-All (Alltech, UK) kullanılmıştır. İnokulantlar silajlara 1,5 x 10^6 cfu/g düzeyinde katılmışlardır. Buğday materyali yalnızca gaz çıkışına olanak tanıyan 1,5-litrelik özel anaerobik kavanozlara silolanmıştır. Silolamadan sonraki 2, 4, 8, 15 ve 50. günlerde her gruptan 3'er kavanoz açılarak kimyasal ve mikrobiyolojik analizler yapılmıştır. Silolama döneminin sonunda açılan tüm silajlara 5 gün süre ile aerobik stabilite testi uygulanmıştır. Ayrıca silajların in situ rumen parçalanabilirlik özellikleri saptanmıştır.

Her iki homofermantatif LAB inokulantı da buğday silajlarının fermantasyon özelliklerini geliştirmiştir. Silolama dönemi sonunda inokulantlar buğday silajlarının lactobacilli sayılarını artırırlarken, maya ve küf sayılarını düşürmüşlerdir. Ayrıca her iki LAB inokulantı da, yüksek bir CO_2 üretimine yol açarak buğday silajlarının aerobik stabilitesini düşürmüştür. Buğday silajlarının in situ rumen parçalanabilirlik özellikleri inokulant uygulamasından etkilenmemiştir.

Anahtar Sözcükler: Aerobik stabilite, fermantasyon, inokulant, rumen parçalanabilirliği, silaj, buğday

Introduction

Silage is the feedstuff produced by the fermentation of a crop, forage or agricultural by-product of generally greater than 50% moisture content. In the ensiling process lactic acid bacteria (LAB) convert water-soluble carbohydrates (WSCs) into organic acids, mainly lactic acid, under anaerobic conditions. As a result, pH decreases and the moist forage is protected from spoilage microorganisms (1). In order to improve the ensiling process various chemical and biological additives have

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been developed. Biological additives are advantageous because they are safe and easy to use, are non-corrosive to machinery, do not pollute the environment, and are natural products. Bacterial inoculants are added to silage in order to stimulate lactic acid fermentation, accelerating the decrease in pH, and thus improving silage preservation (2). Most available inoculants consist of selected strains of homofermentative LAB, such as *Lactobacillus plantarum*, and *Pediococcus* and *Enterococcus* species (3). Many studies have shown the advantages of such LAB inoculants (4-6). However, some studies under laboratory conditions (7-10) indicated that the addition of homofermentative LAB inoculants impaired the aerobic stability of silages of mature cereal crops (wheat, sorghum, corn). This was suggested by a rise in pH, visible mold growth and intensive production of CO_2 during aerobic exposure. Similar problems caused by the use of homofermentative LAB inoculants have also been observed in other studies (11-13). Earlier observations had suggested the opposite, that homofermentative LAB inoculants improved the aerobic stability of silages (14,15). A possible explanation for the instability of inoculated silages upon aeration is that under strict anaerobic conditions homofermentative LAB inoculants produce solely lactic acid, whereas in natural fermentation various volatile fatty acids (VFAs) are produced, e.g., as acetic, propionic and butyric acids. These short-chain fatty acids possess antimycotic activity, and thus inhibit yeasts and molds upon aerobic exposure (16).

The purpose of the present work was to study the effects of homofermentative LAB inoculants on the fermentation, aerobic stability and in situ rumen degradability characteristics of wheat silages.

Material and Methods

Materials and silage preparation

Wheat (*Triticum aestivum* L.) was harvested at the early dough of maturity. After harvest the wheat was wilted for 2 h in the field (35.3 ± 0.24% dry matter (DM)) and chopped to about 1.5 cm and ensiled in 1.5-l special anaerobic jars (Le Parfait, France), equipped with a lid enabling gas release only. Three jars from each group were sampled for chemical and microbiological analysis 2, 4, 8, 15 and 50 days after ensiling. At the end of the ensiling period the silages were subjected to an aerobic stability test for 5 days in a system developed by Ashbell et al. (17). In this system, numbers of yeasts and molds, change in pH and amount of CO_2 produced during the test are used as aerobic deterioration indicators.

The following treatments were used in the experiment:

Control (no additive)

Inoculum A (IA): Inoculant-1188 (Pioneer®, USA) containing *Lactobacillus plantarum* and *Enterococcus faecium*. Final application rate of 1.5×10^6 cfu/g of fresh forage.

Inoculum B (IB): Sil-All (Alltech, UK) containing *Pediococcus acidilactici*, *Lactobacillus plantarum*, S*treptococcus faecium* and cellulase, hemicellulase, pentosanase and amylase enzymes. Final application rate of 1.5×10^6 cfu/g of fresh forage.

The application rate determined by the manufacturers stated the level of LAB in the products. On the day of the experiment, inoculants were suspended in 20 ml of tap water and the whole suspension was sprayed over 10 kg (wet weight) of chopped forage spread over a 1 x 4 m area. All inoculants were applied to the forages in a uniform manner with constant mixing.

Analytical procedures

Chemical analyses were performed in triplicate. The DM, crude ash and crude protein contents of the fresh material and silages were determined by the Weende method (18). Wet samples stored at –20 °C were extracted for 3 min in a Stomacher® blender in water (1:9) for WSC analyses. WSCs were determined by the phenol sulfuric acid method (19). Lactic acid and VFAs were determined by the Lepper method (18). Ammonia-N was determined by the Kjeldahl method without a digestion step but with the addition of base (20). Fermentation losses were evaluated according to weight loss (8).

Microbiological analysis was performed on pooled samples of the 3 replicate silos per treatment per time point except for replicate samples that differed considerably in appearance. The microbiological evaluation included enumeration of lactobacilli on pour-plate Rogosa agar (Oxoid CM627, Oxoid, Basingstoke, UK), and yeast and molds on spread-plate malt extract agar (Difco, Detroit, MI, USA) acidified with lactic acid to pH 4.0. Plates were incubated for 3 days at 30 °C. Since

microbiological analysis was performed on a single sample per time point, no statistical analysis was possible. All microbiological data were transformed to log_{10}. Visual appraisal was performed as described by Filya et al. (2).

Rumen degradability characteristics of the silages were measured by the in situ method reported by Mehrez and Ørskov (21). Air-dried forage samples were ground through a 2.5 mm screen using a laboratory-type mill. The milled samples were placed in 9 x 14 cm Dacron bags (pore size 40-60 µm), which were inserted into rumen of 3 cannulated male merino sheep fed a concentrate and alfalfa hay diet. The Dacron bags were incubated in the rumen for 48 h.

The statistical analysis of the results included one-way analysis of variance and Duncan's multiple range test, which were applied to the results using the Statistical Analysis System (22).

Results

The chemical composition of the fresh and ensiled whole crop wheat is given in Table 1. All silages were well preserved. LAB inoculants improved the fermentation parameters of wheat silages, with increasing lactic acid levels, and decreasing acetic acid and pH values ($P < 0.05$). The pH of all inoculated silages decreased faster and to a greater extent compared to the control silage. The WSCs in all silages increased with the decrease in pH. Both LAB inoculants decreased significantly the ammonia-N concentrations of the silages ($P < 0.05$). No butyric acid was present in any of the silages.

The microbiological composition of the silages is given in Table 2. Lactobacilli numbers increased during the fermentation period. At the end of the ensiling period both LAB inoculants decreased yeast and mold numbers.

Table 3 gives the results of the aerobic exposure test. Silage deterioration indicators are pH change, CO_2 production and an increase in yeast and mold numbers. Both inoculants increased significantly pH and CO_2 production in the wheat silages compared to the control silage ($P < 0.05$).

Values for in situ rumen DM and organic matter (OM) degradability after 48 h of incubation are given in Table 4. Inoculation with the homofermentative LAB did not affect in situ rumen DM or OM degradability ($P > 0.05$).

Discussion

To date, bacterial inoculants have been added to silage in order to stimulate lactic acid fermentation, accelerating the decrease in pH and thus improving silage preservation. The same trend was shown in this experiment. Both homofermentative LAB inoculants ensured rapid and vigorous fermentation that resulted in faster accumulation of lactic acid, lower pH values at an earlier stage of ensiling, and improved forage preservation. Many studies have shown the efficacy of such inoculants (4-6). In the present study, both LAB inoculants decreased concentrations of ammonia-N and acetic acid and weight losses of wheat silages compared with the control silage. However, at the end of the ensiling period both inoculants improved microbiological composition of wheat silages compared with the control silage. These findings are agreement with those reported by Spoelstra (4), Williams et al. (5), Saarisalo et al. (6) and Filya and Sucu (23).

The results in the current study clearly indicated that both homofermentative LAB inoculants impaired the aerobic stability of wheat silages. Both inoculated silages were more susceptible to aerobic exposure than the control silage. In this regard, there were no differences between the effects of the 2 inoculants used. This was evident from intensive CO_2 production and development of yeasts. A high level of lactic acid and yeasts impaired the aerobic stability of homofermentative LAB inoculated wheat silages. These findings are agreement with those published by Rust et al. (9), Kennedy (10), Weinberg et al. (12) and Weinberg et al. (13), and our previous experiments (7,8,11,23,24). Weinberg et al. (12) hypothesized that high levels of residual WSCs, combined with high lactic acid concentrations and a lack of protective VFAs in the silage inoculated with homofermentative LAB were associated with aerobic spoilage. This is because both WSCs and lactic acid are substrates for fungi and yeasts, and VFAs often inhibits these organisms. The use of homofermentative LAB inoculants might lead to such conditions in some sugar-rich silages. This is because the homolactic fermentation is more efficient and utilizes less WSCs than heterolactic fermentation, which results in a higher content of WSCs and lactic acid in the silage. When the crop contains less sugar, not much sugar will be left in the silage to encourage yeasts and molds upon aerobic exposure. This difference between our results and those reported by

Table 1. Chemical analyses of the wheat silages (DM %)*.

Days of ensiling	Treatment	DM	pH	WSCs	NH_3-N	Crude protein	Crude ash	Lactic acid	Acetic acid	Butyric acid	Weight losses
	Fresh										
0	Wheat	35.3 ± 0.24	6.4 ± 0.02	5.1 ± 0.03	4.0 ± 0.25	8.8 ± 0.25	7.3 ± 0.03	1.2 ± 0.03	0	0	0
	Silage										
2	Control	34.5 ± 0.22	6.3 ± 0.04^{a}	3.0 ± 0.03^{b}	4.3 ± 0.42^{a}	8.5 ± 0.03^{b}	7.0 ± 0.03	1.3 ± 0.06^{c}	0	0	2.4 ± 0.02^{a}
	IA	34.6 ± 0.23	4.3 ± 0.01^{b}	3.4 ± 0.06^{a}	3.2 ± 0.53^{b}	8.9 ± 0.12^{a}	7.0 ± 0.66	1.8 ± 0.03^{b}	0	0	0.9 ± 0.01^{b}
	IB	34.7 ± 0.21	4.0 ± 0.09^{b}	3.3 ± 0.0^{a}	3.5 ± 0.06^{b}	8.9 ± 0.12^{a}	7.2 ± 0.03	2.3 ± 0.03^{a}	0	0	0.7 ± 0.01^{c}
4	Control	34.3 ± 0.43	5.2 ± 0.05^{a}	2.3 ± 0.09^{b}	7.0 ± 0.89^{a}	7.9 ± 0.09^{b}	7.4 ± 0.03	1.5 ± 0.03^{c}	0.3 ± 0.34	0	6.7 ± 0.02^{a}
	IA	34.0 ± 0.13	4.0 ± 0.02^{b}	2.8 ± 0.09^{a}	2.8 ± 0.52^{b}	8.8 ± 0.09^{a}	7.5 ± 0.06	2.0 ± 0.03^{b}	0	0	0.9 ± 0.02^{b}
	IB	34.9 ± 0.08	3.8 ± 0.03^{c}	2.9 ± 0.12^{a}	2.7 ± 0.57^{b}	8.4 ± 0.12^{a}	7.5 ± 0.06	2.4 ± 0.03^{a}	0	0	0.6 ± 0.01^{c}
8	Control	34.5 ± 0.21	4.7 ± 0.01^{a}	1.1 ± 0.03^{b}	7.0 ± 0.73^{a}	7.3 ± 0.15^{b}	7.4 ± 0.06	2.0 ± 0.06^{c}	0.4 ± 0.36	0	9.6 ± 0.21^{a}
	IA	34.4 ± 0.25	3.8 ± 0.02^{b}	1.9 ± 0.0^{a}	2.7 ± 0.10^{b}	8.6 ± 0.12^{a}	7.5 ± 0.03	2.9 ± 0.03^{b}	0	0	1.0 ± 0.01^{b}
	IB	34.2 ± 0.21	3.7 ± 0.03^{c}	2.1 ± 0.06^{a}	2.7 ± 0.17^{b}	8.4 ± 0.12^{a}	7.5 ± 0.09	3.2 ± 0.13^{a}	0	0	0.7 ± 0.05^{c}
15	Control	35.6 ± 0.77	4.5 ± 0.00^{a}	1.0 ± 0.06^{b}	9.4 ± 0.44^{a}	6.9 ± 0.15^{b}	7.6 ± 0.03	2.0 ± 0.06^{c}	0.6 ± 0.03	0	6.9 ± 0.17^{a}
	IA	35.3 ± 0.80	3.7 ± 0.01^{b}	1.8 ± 0.03^{a}	2.1 ± 0.24^{b}	8.2 ± 0.15^{a}	7.6 ± 0.06	3.3 ± 0.07^{b}	0	0	1.1 ± 0.03^{b}
	IB	35.4 ± 0.36	3.7 ± 0.01^{b}	2.1 ± 0.03^{a}	2.2 ± 0.55^{b}	8.1 ± 0.12^{a}	7.7 ± 0.09	3.5 ± 0.03^{a}	0	0	0.9 ± 0.01^{c}
50	Control	35.2 ± 0.31	4.4 ± 0.03^{a}	0.9 ± 0.03^{b}	11.5 ± 1.44^{a}	6.4 ± 0.09^{b}	7.7 ± 0.09	3.0 ± 0.06^{c}	1.1 ± 0.06^{a}	0	9.5 ± 0.24^{a}
	IA	35.5 ± 0.07	3.7 ± 0.01^{b}	1.8 ± 0.06^{a}	1.2 ± 0.67^{b}	8.1 ± 0.12^{a}	7.8 ± 0.06	3.9 ± 0.30^{b}	0.3 ± 0.10^{b}	0	4.2 ± 0.02^{b}
	IB	35.5 ± 0.17	3.7 ± 0.01^{b}	2.0 ± 0.0^{a}	1.5 ± 0.77^{b}	7.9 ± 0.90^{a}	7.9 ± 0.09	4.3 ± 0.03^{a}	0.3 ± 0.10^{b}	0	4.2 ± 0.23^{b}

* Except DM, pH and weight losses, all the chemical analyses' results are given in DM.

DM, dry matter; WSC, water-soluble carbohydrates; NH_3-N, ammonia-nitrogen; IA, inoculum A; IB, inoculum B.

$^{a\text{-}b\text{-}c}$Within a column means followed by a different letter differ significantly ($P < 0.05$).

Table 2. Microbiological analysis of the wheat silages (log cfu/g DM).

Days of ensiling	Treatment	Lactobacilli	Yeast	Mold
	Fresh			
0	Wheat	3.5	5.7	4.2
	Silage			
2	Control	3.6	6.3	0
	IA	4.0	6.1	0
	IB	4.0	5.9	0
4	Control	4.1	6.1	0
	IA	4.9	5.9	0
	IB	5.0	6.4	0
8	Control	4.8	7.0	0
	IA	5.8	7.2	0
	IB	5.7	6.5	0
15	Control	5.3	6.8	3.6
	IA	6.5	6.6	1.3
	IB	6.5	6.6	1.1
50	Control	5.5	7.7	2.8
	IA	7.4	7.3	0.8
	IB	7.2	7.0	1.0

Log cfu, logarithmic colony forming unit; DM, dry matter; IA, inoculum A; IB, inoculum B.
Microbiological analysis was performed on a single sample each time.

Table 3. Results of the aerobic stability test of the wheat silages.

Treatment	pH	CO_2 (g/kg DM)	Yeasts*	Molds*	Visual appraisal**
Control	3.7 ± 0.03[b]	1.1 ± 0.22[b]	5.0	4.5	1
IA	4.2 ± 0.01[a]	27.8 ± 1.04[a]	9.8	8.6	2
IB	4.1 ± 0.01[a]	25.3 ± 0.61[a]	9.5	7.9	3

DM, dry matter; IA, inoculum A; IB, inoculum B.
* Microbiological analysis was performed on a single sample each time.
** Visual appraisal is expressed using a scale of 1 to 5 where 1: good quality silage with no visible molding, 2: a few small mold spots, 3: scattered mold spots, 4: silage with partially covered molds, lumpy silage, 5: completely mold-covered samples, unpleasant odor and silage particles sticking together.
[a- b]Within a column means followed by a different letter differ significantly ($P < 0.05$).

Table 4. In situ rumen degradability characteristics of the wheat silages (%).

Treatment	DM	OM
Control	56.8 ± 2.23	54.0 ± 2.24
IA	56.6 ± 3.50	54.3 ± 3.33
IB	57.8 ± 2.65	56.7 ± 2.45

DM, dry matter; OM, organic matter; IA, inoculum A; IB, inoculum B.
[a-b]Within a column means followed by a different letter differ significantly ($P < 0.05$).

Ohyama et al. (14) and Pahlow (15) is probably that these researchers infiltrated air into the silage during the ensiling period from the beginning. However, Filya et al. (2) reported that the presence of low concentrations of oxygen in silage results in a shift of homolactic fermentation to heterolactic metabolism, leading to production of organic acids such as acetic and butyric acids, which possess antimycotic activity and inhibit the development of yeasts and molds.

Neither homofermentative LAB affected the in situ rumen DM or OM degradability of wheat silages. These results show that when wheat silages are inoculated with homofermentative LAB containing high levels of lactic and low levels of acetic acid DM and OM degradability are not depressed. Meeske et al. (25) showed that homofermentative LAB inoculants did not affect the in vitro OM degradability of sorghum silages ensiled at 2 stages of maturity. These findings agree with our previous experiments (8,11,23,26).

In conclusion, the results of this study show that homofermentative LAB inoculants accelerate the initial lactic acid fermentation rate, reducing pH and giving lower protein degradation and fermentation losses. The results also show that homofermentative LAB inoculants impair the aerobic stability of wheat silages.

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