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# The Effect of Inoculants on Nutrient Losses of Corn Silage and High-moisture Corn Stored in Mini Silos

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## Summary with Implications

*Two experiments were conducted to determine the effects of inoculants (BONSILAGE CORN 200G and BONSILAGE HMC 200G) containing Lactobacillus buchneri, Lactobacillus plantarum and Pediococcus acidilactici on nutrient losses and aerobic stability of corn silage and high moisture corn. Corn silage and high moisture corn were inoculated and stored in mini silos with nutrient loss and spoilage characterizations at 30, 90, and 120 days with multiple inoculation levels. Longer ensiling times led to greater total acid production. The higher levels of inoculation led to lower total acid production and higher pH. Inoculating corn silage and high moisture corn also affected the fermentation process by decreasing lactic acid production and increasing acetic acid production. The increase in acetic acid production may be partially responsible for the increased aerobic stability observed for the inoculated feeds. Previous research would support our finding of greater stability and lower DM losses with L. buchneri inoculants.*

## Introduction

Lactic acid bacteria (LAB) containing inoculants have been developed to enhance fermentation and mitigate aerobic spoilage of ensiled feeds. Homofermentative LAB have the ability to convert one molecule of glucose directly into two molecules of lactic acid, decreasing pH and allowing for better DM and energy recovery in silages. *Lactobacillus buchneri,* a heterofermentative LAB, possess a unique pathway that allows it to degrade two molecules of lactic acid to form 1 molecule of acetic acid. Acetic acid inhibits the growth of yeasts, which are the leading cause of spoilage in silage and high-moisture corn (HMC) exposed to oxygen.

It has become increasingly common to inoculate both silage and HMC with a combination of *L. buchneri* and homofermentative LAB. Inoculating with a mixture of *L. buchneri* and homofermentative LAB has been shown to increase lactic acid production, rapidly drop pH, and improve DM. However, results of aerobic stability have been variable when inoculating with this combination. Thus, the objectives of these experiments were to determine the effects of BONSILAGE CORN 200G and BONSILAGE HMC 200G on nutrient losses and aerobic stability of corn silage and HMC, while stored in mini silos.

## Procedure

In Exp. 1, corn silage was harvested at the Eastern Nebraska Research and Extension Center (ENREC) near Mead, NE on September 14, 2015 at 35% DM. Prior to bunker packing, 120 lb of silage was acquired and brought to the University of Nebraska-Lincoln's metabolism area. Sixty lb of sample was inoculated with *L. buchneri* and *Lactobacillus plantarum* (BONSILAGE CORN 200G; Schaumann Inc. Mendota Heights, MN.) at 400,000 colony forming units (CFU)/g of silage by using a hand held spray bottle and mixed for 7 minutes as inoculate was applied. Twenty lb of inoculated sample was then added to 20 lb of fresh non-inoculated silage and mixed in the feed mixer for 7 minutes to obtain 40 lb of silage inoculated at 200,000 CFU/g. This yielded 40 lb of silage at each inoculate level: 0 CFU/g, 200,000 CFU/g, and 400,000 CFU/g.

Corn silage was packed into mini PVC silos (0.08 $ft^3$), at 14.5 lbs DM/$ft^3$ (which is representative of the corn silage packing density used in the cattle industry). Silos were then sealed using covers fitted with gas release valves to ensure an anaerobic environment. Silos were stored for 30 or 90 days in a temperature controlled room. A total of 24 mini PVC silos of corn silage were made with 4 silos at each time point for each inoculant level. On the designated opening day (30 or 90 days), silos were weighed, emptied, sub-sampled for nutrient analysis and samples were frozen. All nutrient analysis was conducted by Dairy One (Ithaca, New York) while yeast and mold counts were analyzed by Midwest Laboratories (Omaha, NE).

Following the ensiling process, half of the silage sample that had been removed from the mini silos was evaluated for aerobic stability. Silage was removed from the freezer, allowed to thaw, and mixed by hand for thirty seconds. After mixing, silage was added to a 1000 mL plastic bottle. Bottles were filled to 1 inch from the top, weighed, and an initial temperature was recorded. Bottles were then stored in a temperature controlled room for two weeks. To determine aerobic stability bottles were weighed and temperature probed twice per day (0800 and 1500) for two weeks.

High-moisture corn was harvested at the ENREC near Mead, NE on September 26, 2015 at 70% DM. The same procedure as described above (corn silage procedure) was used for HMC, with the exception of inoculant used, packing density, and level of inoculant applied. High moisture corn was inoculated with *L. buchneri, Pediococcus acidilactici, and Lactobacillus plantarum* (BONSILAGE HMC 200G; Schaumann Inc.) A packing density of 45 lbs DM/$ft^3$ was used and the three inoculant levels for HMC were 0, 300,000 and 600,000 CFU/g.

In Exp. 2, HMC was harvested at the ENREC near Mead, NE on September 24, 2016 at 75% DM. The same procedure as described in Exp. 1 was used to inoculate and mix the HMC sample. The same HMC inoculate used in Exp. 1, BONSILAGE HMC 200G, was utilized in Exp. 2. There were two inoculant levels a control, 0 CFU/g, and 600,000 CFU/g HMC. High

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**Table 1. Effect of inoculant containing *L. buchneri* and *Lactobacillus plantarum* (BONSILAGE CORN 200G; Schaumann Inc.) on nutrient recovery of corn silage after ensiling for 30 or 90 days (Exp. 1)**

| | | | | | | | | *P*-Values[2] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30 Days | | | 90 Days | | | SEM | Days | CFU | | Interaction | |
| Item | 0[1] | 200,000[1] | 400,000[1] | 0[1] | 200,000[1] | 400,000[1] | | | L | Q | L | Q |
| Total Acids, % DM | 7.12 | 6.92 | 6.93 | 7.60 | 7.87 | 7.26 | 0.24 | <0.01 | 0.07 | 0.46 | 0.95 | 0.06 |
| pH | 3.45 | 3.93 | 3.95 | 3.95 | 3.93 | 4.03 | 0.09 | <0.01 | 0.05 | 0.60 | 0.20 | 0.21 |
| Dry Matter, % | 41.9 | 32.8 | 42.0 | 32.1 | 31.5 | 32.0 | 5.08 | 0.25 | 0.81 | 0.24 | 0.90 | 0.28 |
| NDF | 38.8 | 36.8 | 37.3 | 41.9 | 40.6 | 39.8 | 1.40 | 0.02 | 0.07 | 0.34 | 0.83 | 0.45 |
| CP, % DM | 9.15 | 9.15 | 9.28 | 9.10 | 9.25 | 9.18 | 0.10 | 0.85 | 0.35 | 0.79 | 0.81 | 0.34 |
| Lactic Acid, % DM | 5.39 | 5.26 | 4.83 | 5.36 | 5.06 | 4.03 | 0.22 | 0.08 | <0.01 | 0.19 | 0.14 | 0.75 |
| Acetic Acid, % DM | 1.72 | 1.66 | 2.09 | 2.01 | 2.76 | 3.19 | 0.18 | <0.01 | <0.01 | 0.78 | 0.15 | 0.40 |

[1]Level of inoculant applied to sample as CFU/g of feed; CFU = colony forming unit
[2]*P*-values were considered significant at ≤ 0.05; Days represents the effect of ensiling time; CFU represents the linear or quadratic effect of inoculant level; Interaction represents the linear or quadratic interaction of days and inoculant level

**Table 2. Effect of inoculant containing *L. buchneri*, *Pediococcus acidilactici*, and *Lactobacillus plantarum* (BONSILAGE HMC 200G; Schaumann Inc.) on nutrient recovery of high moisture corn after ensiling for 30 or 90 days (Exp. 1)**

| | | | | | | | | *P*-Values[2] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30 Days | | | 90 Days | | | SEM | Days | CFU | | Interaction | |
| CFU[1] | 0[1] | 300,000[1] | 600,000[1] | 0[1] | 300,000[1] | 600,000[1] | | | L | Q | L | Q |
| *Variable* | | | | | | | | | | | | |
| Total Acids, % DM | 1.52 | 1.43 | 1.45 | 1.76 | 1.64 | 1.55 | 0.06 | <0.01 | 0.05 | 0.58 | 0.71 | 0.29 |
| pH | 4.13 | 4.17 | 4.18 | 4.08 | 4.23 | 4.35 | 0.07 | 0.52 | 0.03 | 0.81 | 0.09 | 0.97 |
| Dry Matter, % | 68.4 | 68.3 | 68.7 | 68.9 | 68.7 | 68.5 | 0.18 | 0.35 | 0.85 | 0.42 | 0.06 | 0.59 |
| NDF | 8.05 | 7.93 | 7.56 | 7.28 | 7.23 | 6.53 | 0.30 | <0.01 | 0.05 | 0.42 | 0.63 | 0.71 |
| CP, % DM | 9.28 | 9.33 | 9.23 | 8.95 | 8.98 | 8.8 | 0.07 | <0.01 | 0.19 | 0.19 | 0.95 | 0.39 |
| Lactic Acid, % DM | 1.37 | 1.22 | 1.25 | 1.56 | 1.15 | 0.91 | 0.13 | 0.51 | 0.01 | 0.49 | 0.60 | 0.78 |
| Acetic Acid, % DM | 0.15 | 0.20 | 0.20 | 0.20 | 0.47 | 0.60 | 0.07 | <0.01 | <0.01 | 0.44 | 0.28 | 0.65 |

[1]Level of inoculant applied to sample as CFU/g of feed; CFU = colony forming unit
[3]*P*-values were considered significant at ≤ 0.05; Days represents the effect of ensiling time; CFU represents the linear or quadratic effect of inoculant level; Interaction represents the linear or quadratic interaction of days and inoculant level

moisture corn was ensiled for either 90 or 120 days with 4 silos per treatment at each time point, allowing for 16 silos total. All lab analyses were the same as Exp. 1 and aerobic stability was again tested by recording weight and temperature change over a three week period.

In both Exp. 1 and 2 individual mini silos served as the experimental unit. Data from Exp. 1 were analyzed as a 2 × 3 factorial using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) while data from Exp. 2 were analyzed as a 2 × 2 factorial.

## Results

### *Exp. 1–Corn Silage*

No interactions between days ensiled and level of inoculant were observed for corn silage in Exp. 1 ($P \geq 0.06$; Table 1). Silage pH increased linearly ($P = 0.05$) as CFU level increased from 0 to 400,000. Corn silage pH also increased as days ensiled increased ($P < 0.01$). Total acids had a tendency to decrease linearly ($P = 0.07$) as CFUs increased and was greater ($P < 0.01$) for corn silage ensiled for 90 days (7.6) compared to corn silage ensiled for 30 days (7.0).

Level of inoculant and time ensiled did not affect the DM content of the corn silage ($P \geq 0.24$). Organic matter content of corn silage was also not affected by inoculant level or time ensiled ($P \geq 0.28$) and averaged 94.3% across all treatments. There was a tendency for NDF to decrease linearly ($P = 0.07$) as CFU level increased from 0 to 400,000 CFU/g. Days ensiled also affected NDF level ($P = 0.02$), with corn silage ensiled for 30 days having less NDF (averaging 37.7%) compared to corn silage ensiled for 90 days (averaging 40.8%).

As level of inoculant increased from 0 to 400,000 CFU/g lactic acid linearly decreased ($P < 0.01$) and acetic acid concentration linearly increased ($P < 0.01$). Silage ensiled for 90 days had greater acetic acid concentrations ($P < 0.01$) compared to silage ensiled for 30 days. The lactic:acetic acid ratio linearly decreased ($P < 0.01$) as CFU level increased and was greater for silage ensiled for 30 days compared to silage ensiled for 90 days ($P < 0.01$).

During aerobic stability analysis, inoculate and ensiling time both had no effect on % DM lost ($P \geq 0.68$). Temperature was not different between the three inoculant levels

**Table 3. Effect of *inoculant containing* L. buchneri, Pediococcus acidilactici, and Lactobacillus plantarum (BONSILAGE HMC 200G; Schaumann Inc.) on nutrient recovery of *high moisture corn* after ensiling for 90 or 120 days (Exp. 2)**

| | 90 Days | | 120 Days | | | *P*-Values[2] | | |
|---|---|---|---|---|---|---|---|---|
| Item | 0[1] | 600,000[1] | 0[1] | 600,000[1] | SEM | Days | CFU | Days×CFU |
| Total Acids, % DM | 2.19 | 1.98 | 2.41 | 2.16 | 0.05 | <0.01 | <0.01 | 0.65 |
| pH | 4.00 | 4.23 | 3.93 | 4.28 | 0.02 | 0.57 | <0.01 | 0.01 |
| Dry Matter, % | 68.4 | 67.4 | 67.7 | 67.2 | 0.003 | 0.14 | 0.01 | 0.34 |
| DM lost during ensiling, % | 4.49 | 3.15 | 5.98 | 4.93 | 0.003 | <0.01 | <0.01 | 0.68 |
| NDF | 8.40 | 8.15 | 8.05 | 7.23 | 0.48 | 0.21 | 0.28 | 0.56 |
| CP, % DM | 8.60 | 8.78 | 8.75 | 8.60 | 0.08 | 0.88 | 0.88 | 0.06 |
| Lactic Acid, % DM | 1.89 | 0.95 | 2.07 | 0.83 | 0.04 | 0.44 | <0.01 | <0.01 |
| Acetic Acid, % DM | 0.29 | 1.03 | 0.32 | 1.33 | 0.05 | <0.01 | <0.01 | 0.01 |

[1]Level of inoculant applied to sample as CFU/g of feed; CFU = colony forming unit

[2]*P*-values were considered significant at ≤ 0.05; Days represented the effect of ensiling time; CFU represented the effect of inoculant level; Days×CFU represents the interaction of ensiling time and inoculant level

($P = 0.83$) in silage stored for 30 days and increased quadratically ($P < 0.01$) over the 13 day aerobic stability test. Silage ensiled for 90 days and inoculated with 400,000 CFU/g was 1.8°C cooler ($P < 0.01$) than the non-inoculated treatment and temperature of all three treatments increased quadratically ($P <0.01$) over the 13 day aerobic stability test.

### *Exp. 1–High Moisture Corn*

No interactions between days ensiled and level of inoculant were observed for HMC in Exp. 1 ($P \geq 0.06$; Table 2). As level of inoculant increased from 0 to 600,000 CFU/g pH linearly increased ($P = 0.03$); time ensiled had no effect ($P = 0.52$) on pH. Total acids decreased linearly ($P = 0.07$) as CFUs increased and were greater ($P < 0.01$) for HMC ensiled for 90 days (1.65) compared to HMC ensiled for 30 days (1.47).

As level of inoculant increased from 0 to 600,000 CFU/g, NDF linearly decreased ($P = 0.05$). Corn ensiled for 30 days had significantly greater NDF levels compared to HMC ensiled for 90 days ($P < 0.01$). Lactic acid concentration decreased linearly ($P = 0.01$) as level of inoculant increased from 0 to 600,000 CFU/g but was not affected by days ensiled ($P = 0.51$). Concentration of acetic acid increased linearly ($P < 0.01$) as level of inoculant increased from 0 to 600,000 CFU/g and was greater ($P < 0.01$) for HMC ensiled for 90 days compared to HMC ensiled for 30 days.

There was an interaction of days ensiled and inoculant ($P < 0.01$) on % DM lost during the aerobic stability test. The HMC stored for 30 days had increased DM losses with increasing inoculant level while HMC ensiled for 90 days had decreasing DM losses as level of inoculant increased. There was also an interaction of days ensiled and inoculant ($P < 0.01$) for temperature change during the stability test. Non-inoculated corn ensiled for 30 days had a lower temperature ($P < 0.01$) compared to the inoculated corn while temperature of all three treatments quadratically increased ($P < 0.01$) over the 13 day aerobic stability test. For HMC ensiled for 90 days, the 600,000 CFU/g inoculation treatment was 1.8°C cooler than corn inoculated at 300,000 CFU/g and 5.2°C cooler than non-inoculated corn.

### *Exp. 2–High Moisture Corn*

The pH was greatest ($P < 0.01$; Table 3) for inoculated HMC in both the 90 and 120 day ensiling periods. There was no effect of length of ensiling ($P = 0.57$) on pH. Total acids decreased when HMC was inoculated ($P < 0.01$) and HMC ensiled for 120 days had a greater amount of total acids ($P < 0.01$) compared to HMC ensiled for 90 days. Dry matter was lower for HMC that was inoculated ($P = 0.01$). Percent DM lost during ensiling was less for HMC that was inoculated ($P < 0.01$) compared to the non-inoculated sample. Corn that was ensiled for 120 days had a greater % of DM loss ($P < 0.01$) compared to HMC that was ensiled for 90 days. Inoculated HMC ensiled for 90 or 120 days had less lactic acid and more acetic acid ($P < 0.01$). Lactic acid concentration was not affected by ensiling time ($P = 0.44$) while acetic acid concentration increased with the longer ensiling time ($P < 0.01$).

There was no interaction of days ensiled and inoculant ($P = 0.52$) on % DM lost during the aerobic stability test. The inoculated HMC had lower % DM loss ($P = 0.01$) compared to HMC that was not inoculated. Days ensiled did not affect ($P = 0.46$) % DM loss of HMC during the 21 day aerobic stability test. There was an interaction ($P < 0.01$) for temperature change in HMC samples during the aerobic stability test. After a 90 day ensiling period, the inoculated HMC was 1.1°C cooler ($P < 0.01$) than the non-inoculated corn and temperature of both increased quadratically ($P < 0.01$) over the 21 day aerobic stability test. The HMC inoculated and ensiled for 120 days was 3.1°C cooler ($P < 0.01$) than non-inoculated corn and temperature of both quadratically increased ($P < 0.01$) over the 21 day aerobic stability test.

## Conclusion

The current studies demonstrated that treating corn silage and high moisture corn with *L. buchneri* combined with other lactic acid bacteria affects the fermentation process and nutrient losses by decreasing lactic acid concentration and increasing acetic acid concentration. Ensiling time and level of inoculant applied both play a role in the proportion of lactic acid and acetic acid

produced, total acids produced and pH. The increase in acetic acid in the later stages of ensiling could be partially responsible for the increased aerobic stability observed when using *L. buchneri.* Utilizing a combination inoculant containing *L. buchneri* and lactic acid bacteria on corn silage and high moisture corn may decrease nutrient losses and aerobic spoilage of these feeds.

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