

Recent Advances in Bacterial Silage Inoculant Technology

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Introduction

This paper describes the evolution of the purpose of silage inoculant application and how the bacterial composition of inoculants has been modified accordingly. The paper focuses on inoculants containing bacteria that enhance the anaerobic and aerobic phases of silage production. The inclusion of chemicals in inoculants was beyond the scope of this paper.

Traditional Homofermentative Inoculants

The concept of using cultures of lactic acid bacteria to enhance silage fermentation developed near the beginning of the last century but early cultures had insufficient bacteria and little success until freeze drying and encapsulation techniques were developed (Weinberg and Muck, 1996). Traditional silage inoculants were developed to acidify crops during anaerobic storage and thereby minimize losses of dry matter and nutritive value during storage. Such inoculants contained homolactic bacteria to dominate the epiphytic bacteria population, thereby ensuring the fermentation of plant sugars into lactic acid. The lactic acid accumulation reduced pH to levels that inhibited the growth of spoilage organisms, reduced dry matter (DM) losses, preserved the nutritive value of the silage, and in some cases enhanced animal performance. Initially, many inoculants contained only *Lactobacillus plantarum* because of the vigorous growth, acid tolerance, and high lactic acid production potential of this organism. Later, other faster-acting bacteria (*Pediococcus pentosaceus*, *P. acidilacti*, *Enterococcus faecium*, and *L. acidophilus*) were included with *L. plantarum* to ensure that consistent positive results were achieved (Weinberg and Muck, 1996). Additional modifications included direct addition of fermentable substrates to inoculants or inclusion of enzymes to hydrolyze polysaccharides into fermentable sugars. Muck (1993) reviewed trials published between 1985 and 1992 and reported that inoculant application improved fermentation indices (pH, lactic:acetic acid ratio, and ammonia-N) in about 60% of studies. Feed intake and body-weight gain, were increased in about 25% of studies, whereas milk production was increased in 40% of studies. A later review of studies published between 1990 and 1995 reported that inoculation had a similar level of success at improving fermentation indices, but noted that aerobic stability was increased less than a third of the time (Muck and Kung, 1997). In fact, the authors noted that inoculant application had no effect on aerobic stability a third of the

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time and worsened aerobic stability a third of the time, particularly when corn and small-grain cereals were evaluated. Inoculation probably reduced aerobic stability because it shifted the fermentation towards lactic acid rather than better inhibitors of yeasts like acetic acid. Furthermore, the accumulating lactic acid could serve as a substrate for yeasts that degrade lactic acid into carbon-di-oxide and water, and generate heat, leading to nutrient losses. The higher pH caused by lactic acid degradation causes proliferation of bacteria and molds that exacerbate the spoilage. Consequently, the quest for bacteria that would inhibit the growth of yeasts and enhance aerobic stability was initiated.

Heterofermentative inoculants

Cooke (1995) was probably the first to report that *L. buchneri* can inhibit the growth of yeasts and molds. Muck (1996) also discovered that only *L. buchneri* provided substantial and consistent increases in acetate concentration and aerobic stability during an evaluation of the antifungal properties of several microorganisms found in aerobically stable silages. Several subsequent studies have confirmed that *Lactobacillus buchneri* application improves the aerobic stability of silages (Ranjit and Kung, 2000; Driehuis et al., 2001; Adesogan et al., 2003). The mode of action of *L. buchneri* was reported to be through the anaerobic conversion of lactic acid to acetic acid, which inhibits the growth of spoilage-initiating yeasts (Oude Elferink et al., 2001). A by-product of this pathway, 1,2 propanediol can be converted by *L. diolivorans* to propionic acid (Krooneman et al., 2002), which is a more potent antifungal acid than acetic acid. Other approaches to improving aerobic stability by modifying the bacterial composition of inoculants have included using propionic acid-producing bacteria or yeast inhibiting, homofermentative bacteria or other antifungal organisms, but none of these has given consistent improvements in aerobic stability.

One of the early concerns about *L. buchneri* inoculation was that the increased acetic acid concentration of inoculated silages would reduce feed intake in ruminant livestock. However, no intake reduction has been reported in studies where *L. buchneri*-treated silage has been fed (Driehuis et al., 1999a; Kendall et al., 2002; Ranjit et al., 2002; Taylor et al., 2002; Kung et al., 2003a). Some studies have reported that the heterofermentative pathway of *L. buchneri* increased silage pH or concentrations of water-soluble carbohydrates (WSC) or DM (Muck, 2002; Adesogan and Salawu, 2004). Other studies questioned the value of inoculation with heterofermentative bacteria like *L. buchneri*, because their less efficient fermentations culminate in large losses of DM (Pahlow et al., 2003). Consequently, a meta analysis of effects of *L. buchneri* inoculation was conducted to ascertain the pervasiveness of these problems. Kleinschmit and Kung (2006a) compared 26 corn silage experiments from 15 research studies, and separately compared 17 grass and small-grain silage experiments from 10 research studies. Treatments were classified into the following categories: untreated silage (LB), silage treated with *L. buchneri* at $\leq 1 \times 10^5$ cfu/g of fresh forage (LB1), and silage treated with *L. buchneri* at $> 1 \times 10^5$ cfu/g. Inoculation of corn silages with *L. buchneri* increased pH, acetic acid concentration, and aerobic stability, and reduced

lactic acid concentration, DM recovery and yeast counts. Except for decreasing yeast counts, inoculation of small-grain or grass silages with *L. buchneri* had similar effects but also increased propionic acid and ethanol concentrations, and decreased WSC concentrations. Most of these effects were more pronounced as the rate of *L. buchneri* application increased, particularly in corn silages. The authors concluded that though significant, the reductions in DM recovery were numerically small and their practical importance was surpassed by the benefits of improved aerobic stability. Nevertheless, various research studies were initiated to improve the effects of *L. buchneri* inoculants on fermentation indices, while maintaining their ability to enhance aerobic stability. Such studies led to the development of combo or dual-purpose inoculants.

Mixtures of Heterofermentative and Homofermentative Bacteria

Homolactic bacteria have recently been included in *L. buchneri* inoculants to enhance the anaerobic fermentation phase and thereby prevent increases in pH and DM losses resulting from inoculation with *L. buchneri* alone (Kleinschmit and Kung, 2006a). Table 1 summarizes the results of some such studies. In almost all cases, aerobic stability was increased by inoculation, but pH or DM losses were not increased, indicating that the inoculants did not adversely affect the fermentation. Adesogan et al. (2006) compared the efficacy of a mixture (1.1×10^{11} , cfu/g) of *L. plantarum*, *L. buchneri* and *E. faecium* or a mixture of *P. pentosaceus* (1×10^5 cfu/g) and *L. buchneri* (4×10^5 cfu/g). The inoculants were applied as recommended or at twice the recommended rate. All inoculants reduced lactic to acetic acid ratios and yeast counts and increased aerobic stability, but inoculant type and application rate did not affect these measures. Kleinschmit and Kung, 2006) examined the efficacy of inoculants containing *P. pentosaceus* R1094 (1×10^5 cfu/g) and *L. buchneri* 40788 (4×10^5 cfu/g) in silages ensiled for different 7 durations ranging from 14 to 361 d. They discovered that inoculation increased acetate and 1,2 propanediol concentrations from day 56 to 361 and decreased yeast counts in silages ensiled for 42, 56, 70 and 282 days. However, aerobic stability was only increased in silages ensiled for 14, 56, and 361 d. The authors speculated that the inconsistent improvements in aerobic stability might have been due to the growth of spoilage causing acetic acid bacteria. Future inoculants should have the ability to inhibit the growth of acetic acid bacteria and molds as well as yeasts.

Mixtures of Esterase-secreting Heterofermentative Bacteria and Homofermentative Bacteria

Several inoculant preparations have included enzymes to increase hydrolysis of forage polysaccharides to sugars that can be used as growth substrates by epiphytic or inoculant bacteria. Cellulase and hemicellulase have been the most widely used enzyme complexes because they have the potential to increase fiber digestion and increase fermentable sugar concentration. However, a review of the efficacy of enzyme addition reveals that silage fermentation quality was improved in less than half of published studies (Kung et al, 2003b). Fiber concentration was frequently reduced by enzyme application but fiber digestibility results were variable. Consequently, the

authors suggested that enzymatic hydrolysis during ensilage may be restricted to easily digestible cell walls, leaving relatively less digestible components in treated silages. Recent research has focused on adding esterase to cellulase-hemicellulase enzyme preparations to increase the potency of fibrolytic enzymes. Unlike cellulase and hemicellulose enzymes, certain ferulic acid esterase enzymes can hydrolyze ester linkages that bind sugars to lignin. Several studies have shown that esterase enzymes can complement cellulase and hemicellulase enzyme effects on plant cell walls, thereby increasing DM or fiber digestibility. (Bartolome et al., 1997; Rodrigues et al., 2001; Yu et al., 2005; Eun and Beauchemin, 2006; Krueger et al., 2008; Krueger and Adesogan, 2008). We recently showed that application of a xylanase–esterase enzyme to the total mixed ration of dairy cows increased the level and efficiency of milk production (Adesogan et al., 2007). Nsereko et al. (2006a) screened 1000 lactic acid bacteria for esterase production, noted that 500 produced ferulic acid esterase, and conducted more detailed studies on 8 of the bacteria (*Lactobacillus buchneri* PTA 6138, NRRL B-30866; *L. crispatus* NRRL B-30868, 30869 and 30870; *L. reuteri* NRRL B-30867, *L. brevis* NRRL B-30865; and an unidentified *Lactobacillus* strain NRRL B-30871). When compared to untreated perennial ryegrass, all inoculated samples had 9 to 11% greater neutral detergent fiber (NDF) digestibility, but only inoculation with *L. buchneri* strain 6138 and NRRL B-30866 increased pH and acetate concentration. In a subsequent trial, inoculation of four corn silage hybrids with a combination of *L. buchneri* 6138 and *L. paracasei tolerans* 6135 extended the aerobic stability by 42 to 128 h and improved 48 h NDF digestibility by 7% (Nsereko et al., 2006b). A one-percentage unit increase in NDF digestibility of corn silage has been proposed to elicit a 0.17 kg increase in DM intake and a 0.25 kg increase in 4% fat-corrected milk yield (Oba and Allen, 1999). Therefore, potential benefits of an inoculant that could improve the fermentation, aerobic stability, and NDF digestibility of corn silage would be considerable.

An experiment was designed to validate the promise of this novel inoculant. The objective was to determine effects of an inoculant containing homolactic bacteria and esterase-producing *L. buchneri* bacteria on the fermentation, aerobic stability, and NDF digestibility of two corn silage hybrids.

Methods

Two corn hybrids (Croplan Genetics 851RR2, CG; Vigoro 61R36, VG) were harvested at approximately 38% DM and ensiled after treatment with nothing (Control) or Pioneer 11CFT inoculant solution (Pioneer Hi-Bred, A DuPont Business, Johnston, IA). The inoculant was applied at 2 ml/kg to supply 6.0×10^4 cfu/g of *Lactobacillus casei* strain PTA6135 and *L. buchneri* strain PTA6138. The inoculant was dissolved in 154 ml of water and sprayed in a fine mist on 70 kg of forage. A similar quantity of deionized water was sprayed on the control forage. Four replicates of each of the untreated or treated forages were weighed (10 kg) into plastic bags and ensiled in 20 l macro silos for 135 d.

To measure forage *in situ* ruminal degradation, forage samples from each mini-silo were dried at 60°C for 48 h and ground through a 6-mm screen. Six replicates of each forage treatment were weighed (1.8 g) into ANKOM *in situ* dacron bags (5.5 x 5.5

cm; 40±15 microns, ANKOM Technology, Macedon, NY). Bags were incubated for 0, 4, 8, 16, 24, or 48 h in each of two lactating ruminally-fistulated dairy cows given free choice access to a ration consisting of 49% corn silage, 14% alfalfa hay, and 37% concentrate (DM basis). At each incubation period, six replicates per mini silo (2 cows x 3 replicates/cow) were incubated. After incubation, bags were washed in a washing machine using a cool rinse cycle, dried at 60°C for 48 h, and weighed. Bag contents were removed, composited by cow and incubation period, ground through a 1-mm screen with a cyclone mill, and analyzed for DM and NDF concentration. Subsequently, 24- and 48-h DM digestibility (DMD) and NDF digestibility (NDFD) were calculated. The experimental design was a 2 (hybrids) x 2 (inoculant vs. no inoculant) factorial.

Chemical Composition

The CG hybrid had greater residual WSC concentration but lower concentrations of DM, CP, ash, NDF, and hemicellulose than the VG hybrid (Table 3). Differences between pre-ensiled and ensiled NDF concentrations of these silages suggests that greater cell wall hydrolysis had occurred during fermentation of the CG hybrid (loss of 7.6 vs. 3.4 percentage points in NDF due primarily to loss of 7.7 versus 2.7 percentage points in hemicellulose for CG and VG respectively).

Inoculant treatment effects on fermentation indices depended on the hybrid (Table 4). Silage pH tended to be greater in control versus inoculated (3.7 vs. 3.6) and CG than VG silages (3.7 vs. 3.6, $P = 0.096$). However, these differences were relatively small, indicating that all silages had fermented adequately. Inoculation reduced concentrations of total fermentation acids and lactate, and lactate to acetate ratio (2.3 vs. 1.9) in CG reflecting the typical shift in the fermentation away from lactate production when *L. buchneri* is applied. However, inoculation did not affect these measures in VG, and typical increases in acetate concentration were not evident. Acetate concentration was not increased by inoculation with a mixture of homolactic bacteria and *L. buchneri* in a few of the studies listed in Table 1, yet aerobic stability was increased. Improved stability in such instances is often due to production of propionic acid from 1, 2 propanediol by bacteria such as *L. diolivorans* (Krooneman et al., 2002). Why inoculation did not affect the fermentation of VG is not clear.

Fungal Counts and Aerobic Stability

Effects of inoculation on fungal counts and aerobic stability varied with hybrid (Table 5). Inoculation decreased yeast counts and increased stability of CG by 57.3 h (98%) but surprisingly increased yeast counts and decreased stability of VG by 20.5 h (20%). When aerobic stability measurements were repeated using representative subsamples of forage that had been frozen when the silos were opened, inoculation improved aerobic stability by 103 h (47%; 323 vs. 220 h, $P < 0.001$), and the improvement was consistent across hybrids. This improved stability agrees with the across-hybrid increase observed for original samples but the reanalyzed samples were considerably more stable. Most spoilage-causing yeasts and molds grow at temperatures between 0 and 37°C and 10 - 40°C, respectively (McDonald et al., 1991). Freezing of reanalyzed samples probably inhibited fungal growth and may be responsible for the increased aerobic stability. Consequently, the reanalyzed stability

results probably reflect the ability of the inoculants to enhance the aerobic stability of silages that have been subjected to freezing temperatures.

In Situ DM and NDF Degradation

Across hybrids, inoculant application increased the potentially and total degradable fractions (Table 6). Inoculation increased 24 h and 48 h DMD of VG but not CG. Inoculation also increased 48-h NDFD across forages. The 7% across-hybrid increase in NDFD is similar to those (7% for corn silage and 9 to 11% for ryegrass silage) reported for forages inoculated with ferulic-acid esterase-producing *L. buchneri* (Nsereko et al., 2006a, b).

Conclusion

This study examined the efficacy of an inoculant containing homofermentative bacteria and esterase-producing *L. buchneri* on improving the fermentation, fiber digestion, and aerobic stability of corn silage. Inoculation shifted the fermentation away from lactic acid production and improved the aerobic stability of CG but not VG. Inoculation increased the 24 and 48 h DMD of VG but not CG, but increased the 48-h NDF digestibility of both hybrids as well as the potential and total degradable fractions.

Summary

Early silage inoculants contained homolactic bacteria selected for their ability to rapidly acidify forages through fermentation of plant sugars, and thereby minimize losses of DM and nutritive value. Many of such inoculants improved the fermentation but did not improve or even worsened aerobic stability. Later, *Lactobacillus buchneri* inoculants were used to prevent aerobic stability problems in forages, but their heterolactic fermentative pathway led to small losses of DM and increased pH. Lately, inoculants containing combinations of homolactic bacteria and *L. buchneri* have successfully improved the aerobic stability of forages without increasing DM losses or pH. Recently inoculants containing homolactic bacteria and novel esterase-producing *L. buchneri* were developed to enhance the fermentation, aerobic stability, and digestion of forages. Our study supported preliminary reports that such inoculants improved 48-h NDFD and suggested that their effects on fermentation and aerobic stability may be affected by the hybrid. More research on the efficacy of these inoculants at improving silage quality and animal performance is warranted. Because yeast counts were increased before acetate concentration was increased in some studies, future research should investigate the presence and role of other antifungal compounds and bacteriocins in inoculants. Research should also investigate the potential for developing inoculants that inhibit the growth of pathogenic organisms and acetic acid bacteria in addition to enhancing fermentation, aerobic stability, and nutritive value.

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Table 1. Effect¹ of inoculants containing a mixture of homofermentative and heterofermentative bacteria on fermentation and aerobic stability indices

	Bacteria	Crop	Silo type	pH	Lactic acid	DM Recovery	Acetic acid	Yeasts	Aerobic stability
Weinberg et al. (1999)	<i>L. buchneri</i> <i>L. plantarum</i>	Sorghum	1.5-l jars	ns	ns	nm	ns	-	+
Weinberg et al. (2002)	<i>L. buchneri</i> <i>L. plantarum</i>	Wheat	1.5-l jars	ns	ns	++	++	-	+
			50-l tubs	ns	ns	++	++	-	+
		Corn	1.5-l jars	ns	--	--	++	-	+
			50-l tubs	ns	ns	ns	++	-	+
Filya (2003a)	<i>L. buchneri</i> <i>L. plantarum</i>	Wheat	1.5-l jars	ns	ns	++	++	-	++
		Sorghum	1.5-l jars	ns	--	++	++	-	++
		Corn	1.5-l jars	ns	ns	++	++	-	++
Filya (2003b)	<i>L. buchneri</i> <i>L. plantarum</i>	Corn	1.5-l jars	ns	++	ns	++	-	+
		Sorghum	1.5-l jars	ns	++	ns	++	-	+
Driehuis et al. (2001)	<i>P. pentosaceus</i> <i>L. plantarum</i> <i>L. buchneri</i>	Ryegrass	1-l jars	--	++	++	--	--	++
			1-l bags	--	++	++	ns	--	++
Kleinschmidt and kung (2006b)	<i>P. pentosaceus</i> <i>L. buchneri</i>	Corn	20-l silos	--	ns	-	++	ns	++
Adesogan et al. (2004)	<i>P. pentosaceus</i> <i>L. buchneri</i>	Bermuda-grass	2.8-l silos	--	ns	++	++	ns	ns
Adesogan et al. (2006)	<i>P. pentosaceus</i> <i>L. buchneri</i>	Corn	20-l silos	ns	ns	++	-	--	++
	<i>L. plantarum</i> <i>L. buchneri</i> <i>E. faecium</i>	Corn	20-l silos	++	ns	++	-	--	++

¹ In studies where several ensiling durations were reported, only data from the longest duration is reported.

ns = Not significant.

nm = not measured.

+ = Numerical increase, treatments were not replicated.

- = Numerical decrease, treatments were not replicated

++ = Significant increase at $P < 0.05$.

-- = Significant decrease at $P < 0.05$.

Table 2. Chemical composition of two unensiled corn hybrids with or without addition of an inoculant¹

	Croplan 851RR2		Vigoro 61R36		SEM	<i>Effects, P =</i>		
	Control	Inoculant	Control	Inoculant		Inoculant	Hybrid	Inoculant x Hybrid
DM, %	38.2 ^b	40.6 ^a	40.0 ^{ab}	38.5 ^{ab}	0.70	0.581	0.853	0.019
CP, % of DM	7.5 ^b	7.7 ^b	8.4 ^a	8.2 ^a	0.15	0.914	0.001	0.347
Ash, % of DM	3.5 ^b	3.4 ^b	4.2 ^a	4.0 ^a	0.10	0.177	<0.001	0.874
NDF, % of DM	45.7	46.2	44.4	46.5	0.99	0.238	0.651	0.444
ADF, % of DM	22.2 ^b	22.7 ^b	24.9 ^a	24.4 ^a	0.57	1.000	0.004	0.406
Hemicellulose ² , % of DM	23.5	23.5	19.5	22.3	1.29	0.360	0.068	0.348
WSC, % of DM	6.28 ^a	6.18 ^a	5.23 ^b	5.11 ^b	0.35	0.759	0.017	0.967
pH	5.40	5.50	5.40	5.40	0.09	0.580	0.580	0.580

^{a, b, c}Means within a row with different superscripts differ ($P < 0.05$).

¹ Pioneer 11CFT inoculant (Pioneer Hi-Bred, a DuPont Business, Johnston, IA) at 1.2×10^5 colony forming units/g of *Lactobacillus casei* and *L. buchneri*.

²Hemicellulose = NDF – ADF.

Table 3. Effect of inoculant¹ application on the chemical composition of two corn silages

	Croplan 851RR2		Vigoro 61R36VG		SEM	Effects, <i>P</i> =		
	Control	Inoculant	Control	Inoculant		Inoculant	Hybrid	Inoculant x Hybrid
DM, %	36.0	37.9	39.0	39.4	0.62	0.088	0.003	0.297
CP, % of DM	8.25	8.33	8.98	9.00	0.13	0.713	<0.001	0.854
Ash, % of DM	3.63	3.35	4.30	4.13	0.123	0.092	<0.001	0.691
NDF, % of DM	39.0	37.8	42.6	41.5	1.30	0.392	0.017	0.993
ADF, % of DM	23.0	22.2	23.9	23.7	0.96	0.605	0.233	0.771
Hemicellulose ² , % of DM	16.0	15.6	18.7	17.8	0.62	0.300	0.002	0.695
WSC ³ , % of DM	3.60	3.35	3.08	2.53	0.275	0.171	0.030	0.595

a, b, c Means within a row with different superscripts differ (*P* < 0.05).

¹Pioneer 11CFT inoculant (Pioneer Hi-Bred, a DuPont Business, Johnston, IA) at 6.0×10^4 cfu/g of *Lactobacillus casei* and *L. buchneri*

²Hemicellulose = NDF – ADF.

³WSC = water-soluble carbohydrates.

Table 4. Effect of inoculant¹ application on fermentation indices of two corn silages

	Croplan 851RR2		Vigoro 61R36		SEM	<i>Effects, P =</i>		
	Control	Inoculant	Control	Inoculant		Inoculant	Hybrid	Inoculant x Hybrid
pH	3.68	3.65	3.65	3.55	0.03	0.096	0.096	0.300
NH ₃ -N, % of DM	0.82	0.77	0.80	0.76	0.05	0.377	0.753	0.981
NH ₃ -N, % total N	9.97	9.28	8.92	8.41	0.64	0.368	0.162	0.885
Total								
Fermentation	5.65	4.01	3.92	3.84	0.25	0.005	0.003	0.009
acids, % of DM								
Lactate, % of DM	3.81	2.31	2.81	2.62	0.17	<0.001	0.061	0.002
Acetate, % of DM	1.84	1.70	1.11	1.12	0.11	0.560	<0.001	0.532
Ethanol, % of DM	0.24	0.30	0.22	0.12	0.04	0.580	0.021	0.057
La:Ac	2.11	1.38	2.54	2.35	0.12	0.002	<0.001	0.042

^{a, b, c} Means within a row with different superscripts differ ($P < 0.05$).

¹ Pioneer 11CFT inoculant (Pioneer Hi-Bred, a DuPont Business, Johnston, IA) at 6.0×10^4 colony forming units/g of *Lactobacillus casei* and *L. buchneri*.

²La:Ac = lactic acid to acetic acid ratio.

Table 5. Effect of inoculant¹ application on microbial counts and aerobic stability of two corn silages

	Croplan 851RR2		Vigoro 61R36		SEM	<i>Effects, P =</i>		
	Control	Inoculant	Control	Inoculant		Inoculant	Hybrid	Inoculant x Hybrid
Lactic acid bacteria, log cfu/g	6.63	7.08	7.03	7.28	0.32	0.299	0.371	0.762
Yeasts, log cfu/g	5.55	4.20	3.03	4.80	0.37	0.577	0.023	0.001
Molds, log cfu/g	3.13	3.10	3.10	4.13	0.27	0.088	0.088	0.075
Aerobic stability, h	58.7	116.0	102.0	81.5	6.30	0.014	0.477	<0.001
Aerobic stability, h (Repeated) ²	239.4	348.5	201.0	297.6	12.7	<0.001	0.004	0.631

a, b, c Means within a row with different superscripts differ ($P < 0.05$).

¹ Pioneer 11CFT inoculant (Pioneer Hi-Bred, a DuPont Business, Johnston, Durant, IA) at 6.0×10^4 colony forming units/g of *Lactobacillus casei* and *L. buchneri*.

² Results of reanalyzed aerobic stability of samples frozen (-20°C) at silo opening and thawed 25 days later.

Table 6. Effect of inoculant¹ application on kinetics of *in situ* ruminal DM degradability and 24 and 48 h DM and NDF degradability (%) of two corn silages

	Croplan 851RR2		Vigoro 61R36		SEM	<i>Effects, P =</i>		
	Control	Inoculant	Control	Inoculant		Inoculant	Hybrid	Inoculant x Hybrid
DM degradation parameters ²								
A, %	16.5	15.0	11.2	13.5	2.32	0.863	0.153	0.438
B, %	60.4	65.8	62.4	68.1	2.28	0.021	0.364	0.966
A+B, %	76.8	80.9	73.6	81.5	2.32	0.015	0.577	0.413
C, %/h	7.6	6.3	6.3	6.0	1.16	0.495	0.470	0.667
DM degradation, % of DM								
24 h	64.5	63.6	59.7	63.9	1.14	0.157	0.060	0.031
48 h	77.6	78.0	74.4	78.5	0.88	0.016	0.129	0.048
NDF degradation, % of DM								
24 h	28.3	28.7	22.2	27.6	1.97	0.157	0.081	0.212
48 h	56.5	58.1	52.3	58.0	1.68	0.038	0.215	0.228

a, b, c Means within a row with different superscripts differ ($P < 0.05$).

¹ Pioneer 11CFT inoculant (Pioneer Hi-Bred, a DuPont Business, Johnston, IA) at 6.0×10^4 colony forming units/g of *Lactobacillus casei* and *L. buchneri*.

²A = wash fraction representing immediately degradable fraction; B = potentially degradable fraction; A+B = total degradable fraction; C = fractional fermentation rate.

SESSION NOTES