Effect of applying inoculants with heterolactic or homolactic and heterolactic bacteria on the fermentation and quality of corn silage

K. G. Arriola, S. C. Kim,^{1,2} and A. T. Adesogan

Department of Animal Sciences, Institute of Food and Agricultural Sciences, University of Florida, Gainesville 32608

ABSTRACT

This study examined the effect of applying different bacterial inoculants on the fermentation and quality of corn silage. Corn plants were harvested at 35% DM, chopped, and ensiled in 20-L mini silos after application of (1) deionized water (CON) or inoculants containing (2) 1×10^5 cfu/g of *Pediococcus pentosaceus* 12455 and *Propionibacteria freudenreichii* (B2); (3) 4×10^5 cfu/g of *Lactobacillus buchneri* 40788 (BUC); or (4) 1×10^5 cfu/g of *Pediococcus pentosaceus* 12455 and 4×10^5 cfu/g of *L. buchneri* 40788 (B500). Four replicates of each treatment were weighed into polyethylene bags within 20-L mini silos. Silos were stored for 575 d at ambient temperature (25°C) in a covered barn. After silos were opened, aerobic stability, chemical composition, and yeast and mold counts were determined. The DNA in treated and untreated silages was extracted using lysozyme/sodium dodecyl sulfate lysis and phenol/chloroform and used as a template for a conventional PCR with primers designed on the 16S rRNA gene to detect the presence of *L. buchneri* in all silage samples. Acetic acid concentration was greater in B2 silages versus others (6.46 vs. 4.23% DM). Silages treated with BUC and B500 had lower pH and propionic acid concentration and greater lactic acid concentration than others. The B500 silage had the greatest lactic:acetic acid ratio (1.54 vs. 0.41), and only treatment with BUC reduced DM losses (5.0 vs. 14.3%). Yeast and mold counts were less than the threshold (10^5) typically associated with silage spoilage and did not differ among treatments. Consequently, all silages were very stable (>250 h). Aerobic stability was not improved by any inoculant but was lower in B500 silages versus others (276 vs. 386 h). The conventional PCR confirmed the presence of similar populations of *L. buchneri* in all

silages. This may have contributed to the prolonged aerobic stability of all silages.

Key words: corn silage, inoculant, *Lactobacillus buchneri*, *Propionibacterium*

INTRODUCTION

Inoculants containing selected strains of lactic acid bacteria (**LAB**) have been developed to reduce the influence of epiphytic LAB on the outcome of ensiling forages. The principal function of homofermentative inoculants is to ensure a rapid and efficient fermentation of water-soluble carbohydrates (**WSC**) into lactic acid, a rapid decrease in pH, and improved silage conservation with minimal fermentation losses (Weinberg et al., 1993). However, such inoculants have not increased aerobic stability in many studies (Muck and Kung, 2007). Rather, some have decreased aerobic stability (Moon et al. 1980) by enhancing the growth of spoilage yeasts. Inoculating forages at harvest with *Lactobacillus buchneri* improves the aerobic stability of silages (Muck, 1996; Taylor et al., 2002), likely because this organism converts lactic acid to acetic acid under anaerobic conditions (Oude Elferink et al., 2001). Recently, *L. buchneri* has been combined with homolactic bacteria to improve the aerobic stability and fermentation of silages (Kung et al., 2003). Driehuis et al. (2001) reported that *L. buchneri* alone or in combination with *Pediococcus pentosaceus* and *Lactobacillus plantarum* improved aerobic stability of grass silage, reduced yeast and mold counts, and had lower DM loss compared with the untreated silage. Other studies reported improved aerobic stability when combinations of homolactic and heterolactic inoculants were applied to sorghum (Filya, 2003) and corn (Huisden et al., 2009). However, Adesogan et al. (2004) reported that treating bermudagrass with *P. pentosaceus* and *L. buchneri* improved fermentation but did not improve aerobic stability because their control silage was more stable due to a butyric fermentation. Kleinschmit and Kung, Jr. (2006) stated that an inoculant containing *P. pentosaceus* and *L. buchneri* reduced yeast counts and improved aerobic stability in a manner that varied with the ensiling duration. Kang et al. (2009) demonstrated

Received September 9, 2010.

Accepted November 20, 2010.

¹Corresponding author: kimsc@gnu.ac.kr

²Current address: Department of Animal Science, Institute of Agriculture and Life Sciences, Gyeongsang National University, Jinju 660-701, South Korea.

that effects of inoculants containing *Lactobacillus casei* and *L. buchneri* on aerobic stability of corn silage varied with the corn hybrid tested. Therefore, more research is needed on effects of combinations of *L. buchneri* and homolactic bacteria.

Propionic acid bacteria can ferment glucose and lactate to acetate and propionic acid (Moon, 1983), and a few studies have shown that such inoculants improved the aerobic stability of silage (Dawson et al., 1998; Filya et al., 2004). Effects of combinations of propionibacteria and homolactic bacteria on silage fermentation and aerobic stability are not well known. The objective of this study was to examine the effect of applying bacterial inoculants containing heterofermentative bacteria (*L. buchneri*) alone or homofermentative (*P. pentosaceus*) and heterofermentative bacteria (*Propionibacteria freudenreichii* or *L. buchneri*) together on the fermentation, quality, and aerobic stability of corn silage.

MATERIALS AND METHODS

Forage and Treatments

A corn hybrid (Vigoro 61R36; Royster Clark Inc., Greeley, CO) was grown in April 2006 at the Dairy Research Unit, University of Florida (latitude 30°N and longitude 82.5°W; elevation: 61 m above sea level). The temperature range, total precipitation, and average humidity during the growing season were 4.2 to 39.3°C, 28 cm, and 71%, respectively. The crop was harvested in July 2006 at 35% DM with a Claas Jaguar 850 (Claas of America, Columbus, IN) forage harvester. Forages were chopped to a theoretical cut length of 1.9 cm and treated with (1) deionized water (**CON**); (2) Biotol Plus II (**B2**), applied at 21.9 mg/kg of fresh forage to supply 1×10^5 cfu/g of *Pediococcus pentosaceus* 12455 and *Propionibacteria freudenreichii*; (3) Buchneri 40788 (**BUC**) applied at 8 mg/kg of fresh forage to supply 4×10^5 cfu/g of *L. buchneri*; (4) a combination inoculant, Buchneri 500 (**B500**), applied at 8 mg/kg of fresh forage to supply 1×10^5 cfu/g of *P. pentosaceus* 12455 and 4×10^5 *L. buchneri* 40788, respectively. Inoculants were dissolved in 950 mL of deionized water and sprayed uniformly onto the forages under constant mixing. All inoculants were supplied by Lallemand Animal Nutrition (Milwaukee, WI). Four replicates of each treatment were weighed (10 kg) into polyethylene bags within 20-L mini silos, sealed, and stored for 575 d at ambient temperature (25°C) in a covered barn. Dry matter recovery was calculated from the initial and final weights and the DM concentrations of the fresh and ensiled forage.

Chemical Analysis

Subsamples of each untreated fresh forage and each treated silage were collected, dried in a forced-air oven at 60°C for 48 h, and ground to pass a 1-mm screen using a Wiley mill (A. H. Thomas, Philadelphia, PA). The chemical composition of samples from each replicate silo was measured in duplicate. Concentrations of NDF and ADF were measured using the method of Van Soest et al. (1991) in an Ankom 200 Fiber Analyzer (Ankom Technologies, Macedon, NY). Starch was determined using the procedure of Holm et al. (1986). The anthrone reaction assay (Ministry of Agriculture, Fisheries and Food, 1986) was used to quantify WSC. After silos were opened, corn silage samples also were analyzed for aerobic stability by placing thermocouple wires at the center of a bag containing 1 kg of silage, within an open-top polystyrene box. The silages were covered with 2 layers of cheesecloth to prevent drying. The thermocouple wires were connected to data loggers (Campbell Scientific Inc., North Logan, UT) that recorded the temperature every 30 min for 14 d. Aerobic stability was denoted by the time (h) before a 2°C rise in silage temperature above ambient temperature (23°C). Silage subsamples from each silo were submitted to Dairyland Laboratories Inc. (Arcadia, WI) for analysis of yeast and mold counts using YM-11 agar (AOAC, 1995), VFA by HPLC (Canale et al., 1984), pH using a pH meter (Orion 710+; Thermo Fisher Scientific Inc., Waltham, MA), and ammonia-N by distillation (AOAC, 1995).

DNA Extraction

Silage samples (50 g) from each treatment were diluted with 100 mL of distilled water and macerated in a blender to obtain silage extract for further analysis. The DNA was extracted from the water extract using the phenol-chloroform method (Giraffa et al., 2000). A 1.5 mL aliquot of each water extract was centrifuged at $12,500 \times g$ for 5 min, the supernatant was discarded and this step was repeated. The pellets were washed twice with Tris-EDTA buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0), resuspended in 500 μ L of Tris-EDTA-saccharose buffer (50 mM Tris-HCl, 1 mM EDTA, 6.7% saccharose, pH 8.0) followed by incubation at 37°C for 30 min with 200 μ L of lysozyme (50 mg/mL). This was followed by a second incubation at 56°C for 30 min with 15 μ L of proteinase K, and a third incubation at 56°C overnight with 125 μ L of SDS (20% wt/vol). The DNA was extracted by adding 200 μ L of phenol and chloroform, centrifuging at $7,000 \times g$ for 5 min

Table 1. Chemical composition of corn forages treated with or without bacterial inoculants before ensiling¹

Item	CON	B2	BUC	B500	SEM
DM, %	35.4	32.9	31.5	33.1	1.46
NDF, % of DM	42.8	43.0	43.0	41.7	1.07
ADF, % of DM	25.1	25.1	25.3	25.7	0.97
Starch, % of DM	25.5	28.4	22.9	24.8	2.72

Means in the same row with different superscripts differed ($P < 0.05$).

¹CON = control, no inoculant; B2 = *Pediococcus pentosaceus* and *Propionibacteria freudenreichii*; BUC = *Lactobacillus buchneri*; B500 = *Pediococcus pentosaceus* and *L. buchneri*.

at 4°C, and transferred to a new tube. This step was repeated 3 times, followed by precipitation with cold (−20°C) isopropanol (600 µL) and centrifugation at 12,500 × *g* for 30 min at 4°C. A 1-mL aliquot of ethanol (70%) was added to the pelleted DNA and centrifuged at 12,500 × *g* for 5 min at 4°C. Purified DNA was dried and resuspended overnight at 4°C in 150 µL of Tris-EDTA buffer. Ribonuclease HIII (1 µL; 5,000 U/mL; New England BioLabs, Ipswich, MA) was added to resuspended DNA, and after incubation at 37°C for 1 h, DNA samples were stored at −20°C until use.

Conventional PCR Conditions

The PCR was performed to amplify a region of the bacterial 16S rRNA gene of *L. buchneri* with the forward primer LBR2 that corresponds to the region from base 186 to 215 (5'-GAAACAGGTGCTAATACCGTATAACAACCA-3') and the reverse primer LBR1 that corresponds to the region from base 316 to 345 (5'-CGCCTTGG-TAGGCCGTTACCTTACCAACA-3'; GenoMechanix LLC, Gainesville, FL). The expected product size was 159 bp. A touchdown PCR was done in a PTC-100 programmable thermal controller (MJ Research Inc., Waltham, MA) to reduce the incidence of false product formation, enhance correct annealing, and increase the specificity of the amplification (Kidd and Ruano, 1995). The annealing temperature was set at 61°C for the first cycle and reduced by 0.5°C per cycle for the next 9 cycles to reach a final melting temperature (T_m) of 56°C. The previous melting temperature was used for the remaining 20 cycles. An extension time of 30 s at 72°C was used, with a final extension of 7 min and hold at 4°C. Amplification was done in a standard reaction mixture containing 16.75 µL of H₂O, 2.5 µL of 10 × Taq buffer advanced (5 Prime), 1 µL of each primer, 2.5 µL of 2 mM dNTP (Thermo Fisher Scientific Inc.), 0.25 µL of Taq polymerase (5 U/µL; 5 Prime), and 2 µL of the template. Electrophoresis was conducted on 2.0% agarose gel stained with

ethidium bromide, and DNA was visualized under UV light and photographed (Sambrook et al., 1989).

Statistical Analysis

The data were analyzed as a completely randomized design using the GLM procedure of SAS (v. 9.2 SAS Institute Inc., Cary, NC). The general model was $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} = response variable, μ = overall mean, T = effect of treatment i , and e_{ij} = error term. The F-protected least significant difference test was used to compare least squares means and significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

The chemical composition of the corn forages was normal for silage hybrids produced in Florida (Table 1; Huisden et al., 2009; Kang et al., 2009). The control silage had the greatest DM concentration, but other chemical components did not differ among the forages. Corn forage treated with B2 had a lower DM concentration than B500 and CON silages, whereas CP concentration was greater in CON and B2 silages than in the others (Table 2). As expected, the WSC concentrations of all silages were reduced during the fermentation. The B500 silage had the greatest ($P < 0.05$) residual WSC concentration (1.49 vs. 1.18% DM), suggesting that plant sugars were less extensively fermented by the bacteria in this inoculant compared with those in others. In contrast, Filya (2003) reported that CON and *L. plantarum*-inoculated corn silages had greater ($P < 0.05$) residual WSC than silages inoculated with *L. buchneri* alone or *L. buchneri* and *L. plantarum* together. High residual WSC concentrations in silages are desirable because they reflect a more efficient fermentation in the silo and indicate greater availability of energy-yielding substrates for ruminal microbes, but such concentrations also can induce spoilage yeast growth.

All pH values were within the range of 3.8 to 4.1 (Table 3), which reflects adequate fermentation for restricting the growth of undesirable microorganisms like Clostridia. Dry matter loss was lower in BUC silages compared with CON and B2 silages (5.0 vs. 14.3%). Control and B2 silages had greater pH and lower lactic acid concentration than BUC and B500 silages. The combination of the greater total acid and lactate concentrations and lower pH of BUC silages explain the lower DM loss from this silage. Despite having pH and lactate concentration similar to BUC, the B500 treatment did not reduce DM loss because it had similar total acid concentration to the control. Propionic acid

Table 2. Effect of bacterial inoculants on the chemical composition of corn silages¹

Item	CON	B2	BUC	B500	SEM	P-value
DM, %	31.4	29.9	31.2	31.7	0.84	0.09
CP, % of DM	10.5 ^a	10.3 ^a	9.86 ^b	9.86 ^b	0.31	0.03
NDF, % of DM	44.4 ^a	43.2 ^{ab}	42.3 ^b	42.0 ^b	1.06	0.04
ADF, % of DM	27.2	27.4	26.2	26.6	0.99	0.42
Starch, % of DM	26.1	27.8	28.3	25.2	2.81	0.41
Water-soluble carbohydrate, % of DM	1.22 ^b	1.14 ^b	1.18 ^b	1.49 ^a	0.12	0.01

^{a,b}Means in the same row with different superscripts differed ($P < 0.05$).

¹CON = control, no inoculant; B2 = *Pediococcus pentosaceus* and *Propionibacteria freudenreichii*; BUC = *Lactobacillus buchneri*; B500 = *Pediococcus pentosaceus* and *L. buchneri*.

concentration was greater in CON and B2 silages than BUC and B500 silages. Acetic acid concentration was greatest in the B2 silage (6.46 vs. 4.23% DM), lowest in the B500 silage, and intermediate in CON and BUC silages. The B500 silage had the greatest lactic:acetic acid ratio (1.54 vs. 0.41).

The fermentation results of the CON, BUC, and B500 silages were unusual. Normally, untreated corn silage undergoes a homolactic fermentation resulting in relatively low pH values (<4.0) due to high concentrations of lactate and low concentrations of acetate and propionate (Rooke and Hatfield, 2003). In contrast, the CON silage had among the highest pH values, lowest lactate concentrations, and highest acetate and propionate concentrations. These results suggest that the fermentation was dominated by heterolactic bacteria. Furthermore, the homolactic bacteria in B2 and B500 did not dominate the fermentation sufficiently to result in greater lactate concentrations and lower DM loss relative to those in the CON silage. Results of inoculation with BUC and B500 were atypical in that application of *L. buchneri* to corn forage usually increases the concentration of acetate and reduces the lactate concentration

(Ranjit and Kung, 2000; Filya, 2003; Hu et al., 2009). This could be due to the presence of high concentrations of epiphytic *L. buchneri* that naturally occurred in the control silages. The conventional PCR analysis was performed to examine this theory. It revealed the presence of similarly sized bands representing epiphytic populations of *L. buchneri* in all silages irrespective of the fact that inoculant-sourced *L. buchneri* was only added in 2 (B500 and BUC) of the 5 treatments (Figure 1). This may explain why inoculants containing *L. buchneri* did not have normal effects on the fermentation. Factors predisposing to high natural growth of epiphytic populations of *L. buchneri* on corn forages or silages are unknown and warrant further research.

Propionic acid bacteria can ferment glucose and lactate to acetate and propionate (Moon, 1983); therefore, the presence of *Propionibacteria* in B2 partly explains the greater acetate concentration and numerically greater propionate concentration of B2 silages relative to CON silages. However, inoculation with *Propionibacteria* has had equivocal effects on the propionic acid concentration of silages, with few studies showing positive effects (Dawson et al., 1998) and many others showing little to

Table 3. Effect of bacterial inoculants on DM losses, fermentation indices, yeast and mold counts, and aerobic stability of corn silages¹

Item	CON	B2	BUC	B500	SEM	P-value
DM loss, %	14.9 ^a	13.6 ^{ab}	5.0 ^c	8.2 ^{ab}	3.92	0.016
pH	4.10 ^a	4.06 ^a	3.90 ^b	3.80 ^b	0.05	<0.001
NH ₃ -N, % of CP	8.25	9.68	8.92	8.01	1.15	0.28
Total acids, % of DM	7.15 ^b	8.88 ^a	8.40 ^a	7.30 ^b	0.58	0.005
Lactic acid, % of DM	1.56 ^b	1.31 ^b	3.34 ^a	4.17 ^a	0.72	0.001
Acetic acid, % of DM	4.85 ^b	6.46 ^a	4.88 ^b	2.97 ^c	0.59	<0.001
Propionic acid, % of DM	0.72 ^a	1.09 ^a	0.17 ^b	0.08 ^b	0.16	<0.001
Butyric acid, % of DM	0.01	0.01	0.01	0.07	0.06	0.47
Isobutyric acid, % of DM	0.01	0.01	0.01	0.01	0.00	—
Ethanol, % of DM	0.01	0.10	0.01	0.01	0.07	0.28
Lactic:acetic acid ratio	0.33 ^b	0.21 ^b	0.69 ^b	1.54 ^a	0.35	0.001
Yeasts, log cfu/g	<3.00	<3.00	<3.00	<3.00	0.00	—
Molds, log cfu/g	3.4	3.67	4.15	3.75	0.71	0.54
Aerobic stability, h	390 ^a	390 ^a	381 ^a	276 ^b	53	0.07

^{a-c}Means in the same row with different superscripts differed ($P < 0.05$).

¹CON = control, no inoculant; B2 = *Pediococcus pentosaceus* and *Propionibacteria freudenreichii*; BUC = *Lactobacillus buchneri*; B500 = *P. pentosaceus* and *L. buchneri*.

Figure 1. Gel electrophoresis analysis after PCR amplification of DNA from silages treated with or without inoculants: lanes 1 and 8 = DNA ladder/marker (Promega Corp., Madison, WI); lane 2 = pure culture of *Lactobacillus buchneri*; lane 3 = CON, untreated control; lane 4 = B2, *Pediococcus pentosaceus* and *Propionibacteria freudenreichii*; lane 5 = BUC, *L. buchneri*; lane 6 = B500, *P. pentosaceus* plus *L. buchneri*; lane 7 = negative control. The expected product size was 159 bp. Color version available in the online PDF.

no effect (Higginbotham et al., 1998; Kung and Ranjit, 2001; Pedroso et al., 2010). Consequently, such bacteria have not usually increased aerobic stability (Weinberg et al., 1995; Higginbotham et al., 1998; Pedroso et al., 2010).

Yeast and mold counts were less than the threshold (10^5) typically associated with silage spoilage (Pahlow and Zimmer, 1985; O'Kiely et al., 1987) and did not differ among treatments (Table 3). Consequently, all silages were stable for long periods (>250 h) even though B500 silages were less stable than the others. That application of *L. buchneri* inoculants did not increase the aerobic stability or decrease the yeast counts relative to those of untreated silages contradicts various reports (Kleinschmit and Kung, 2006; Hu et al., 2009; Huisden et al., 2009; Pedroso et al., 2010). This is likely because of the high population of *L. buchneri* in all silages. The greater deterioration of the B500 silage, which had one of the highest lactate concentrations, reflects the relatively low antifungal property of lactate. In fact, lactate serves as a substrate for several spoilage yeasts such as those of the genera *Candida* and *Pichia* spp. (Pahlow et al., 2003). Acetate, butyrate, and propionate are the

main antifungal acids in silages (Moon, 1983) and the total concentration of these acids was lower in the B500 silage than in others. Weinberg et al. (1993) reported that high levels of residual WSC, combined with high lactate concentrations and insufficient concentrations of antifungal VFA in silages inoculated with homofermentative LAB were associated with aerobic spoilage. The greater residual WSC concentration and lower total antifungal acid concentration may have made the B500 silage more susceptible to deterioration than others in this study.

CONCLUSIONS

The BUC and B500 silages had a more homolactic fermentation evidenced by greater lactate concentration and lower pH than other silages. However, only BUC application reduced DM losses relative to the control treatment. Aerobic stability was generally high (>250 h) and was not improved by inoculant application. Therefore, the BUC inoculant was the best treatment under the conditions of this study. The conventional PCR analysis confirmed the presence of similarly sized

bands representing populations of *L. buchneri* in all treatments and this likely explains the prolonged aerobic stability of all silages.

REFERENCES

- Adesogan, A. T., N. Krueger, M. B. Salawu, D. B. Dean, and C. R. Staples. 2004. The influence of treatment with dual purpose bacterial inoculants or soluble carbohydrates on the fermentation and aerobic stability of bermudagrass. *J. Dairy Sci.* 87:3407–3416.
- AOAC. 1995. Official Methods of Analysis. Vol. I. 16th ed. Association of Official Analytical Chemists, Washington, DC.
- Canale, A., M. E. Valente, and A. Ciotti. 1984. Determination of volatile carboxylic acids (C1–C5i) and lactic acid in aqueous acid extracts of silage by high performance liquid chromatography. *J. Sci. Food Agric.* 35:1178–1182.
- Dawson, T. E., S. R. Rust, and M. T. Yokoyama. 1998. Improved fermentation and aerobic stability of ensiled high moisture corn with the use of *Propionibacterium acidipropionici*. *J. Dairy Sci.* 81:1015–1021.
- Driehuis, F., S. J. W. H. Oude Elferink, and P. G. Van Wijkelaar. 2001. Fermentation characteristics and aerobic stability of grass silage inoculated with *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria. *Grass Forage Sci.* 56:330–343.
- Filya, I. 2003. The effect of *Lactobacillus buchneri* and *Lactobacillus plantarum* on the fermentation, aerobic stability, and ruminal degradability of low dry matter corn and sorghum silages. *J. Dairy Sci.* 86:3575–3581.
- Filya, I., E. Sucu, and A. Karabulut. 2004. The effect of *Propionibacterium acidipropionici*, with or without *Lactobacillus plantarum*, on the fermentation and aerobic stability of wheat, sorghum and maize silages. *J. Appl. Microbiol.* 97:818–826.
- Giraffa, G., L. Rossetti, and E. Neviani. 2000. An evaluation of chelex-based DNA purification protocols for the typing of lactic acid bacteria. *J. Microbiol. Methods* 42:175–184.
- Higginbotham, G. E., S. C. Mueller, K. K. Bolsen, and E. J. DePeters. 1998. Effects of inoculants containing propionic acid bacteria on fermentation and aerobic stability of corn silage. *J. Dairy Sci.* 81:2185–2192.
- Holm, J. I., I. Bjorck, A. Drews, and N. G. Asp. 1986. A rapid method for the analysis of starch. *Starch/Die Stärke* 7:224–226.
- Hu, W., R. J. Schmidt, E. E. McDonell, C. M. Klingerman, and L. Kung Jr.. 2009. The effect of *Lactobacillus buchneri* 40788 or *Lactobacillus plantarum* MTD-1 on the fermentation and aerobic stability of corn silages ensiled at two dry matter contents. *J. Dairy Sci.* 92:3907–3914.
- Huisden, C. M., A. T. Adesogan, S. C. Kim, and T. Ososanya. 2009. Effect of applying molasses or inoculants containing homofermentative or heterofermentative bacteria at two rates on the fermentation and aerobic stability of corn silage. *J. Dairy Sci.* 92:690–697.
- Kang, T. W., A. T. Adesogan, S. C. Kim, and S. S. Lee. 2009. Effects of an esterase-producing inoculant on fermentation, aerobic stability, and neutral detergent fiber digestibility of corn silage. *J. Dairy Sci.* 92:732–738.
- Kidd, K. K., and G. Ruano. 1995. Optimizing PCR. Pages 1–22 in *PCR, A Practical Approach*. M. J. McPherson, B. D. Hames, and G. R. Taylor, ed. Oxford University Press, Oxford, UK.
- Kleinschmit, D. H., and L. Kung, Jr. 2006. The effects of *Lactobacillus buchneri* 40788 and *Pediococcus pentosaceus* R1094 on the fermentation of corn silage. *J. Dairy Sci.* 89:3999–4004.
- Kung, L. Jr., and N. K. Ranjit. 2001. The effect of *Lactobacillus buchneri* and other additives on the fermentation and aerobic stability of barley silage. *J. Dairy Sci.* 84:1149–1155.
- Kung, L. Jr., C. C. Taylor, M. Lynch, and J. M. Neylon. 2003. The effect of treating alfalfa with *Lactobacillus buchneri* 40788 on silage fermentation, aerobic stability, and nutritive value for dairy cows. *J. Dairy Sci.* 86:336–343.
- Ministry of Agriculture, Fisheries, and Food. 1986. The Analysis of Agricultural Materials. Reference Book 427. HMSO, London, UK.
- Moon, N. J. 1983. Inhibition of the growth of acid tolerant yeasts by acetate, lactate and propionate and their synergistic mixtures. *J. Appl. Bacteriol.* 55:454–460.
- Moon, N. J., L. O. Ely, and E. M. Sudweeks. 1980. Aerobic deterioration of wheat, lucerne and maize silage prepared with *Lactobacillus acidophilus* and a *Candida* spp. *J. Appl. Bacteriol.* 49:75–87.
- Muck, R. E. 1996. A lactic acid bacteria strain to improve aerobic stability of silages. Pages 42–43 in 1996 Research Summaries. US Dairy Forage Research Center, Madison, WI.
- Muck, R. E., and L. Kung, Jr. 2007. Silage production. Pages 617–633 in *Forages: The Science of Grassland Agriculture*. Vol. II. 6th ed. R. F. Barnes, C. J. Nelson, K. J. Moore, and M. Collins, ed. Blackwell Publishing, Ames, IA.
- O'Kiely, P., R. E. Muck, and P. L. O'Connor. 1987. Aerobic deterioration of alfalfa and maize silage. *J. Irish Grassl. Anim. Prod. Assoc.* 21:145.
- Oude Elferink, S. J. W. H., J. Krooneman, J. C. Gottschal, S. F. Spoelstra, F. Faber, and F. Driehuis. 2001. Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by *Lactobacillus buchneri*. *Appl. Environ. Microbiol.* 67:125–132.
- Pahlow, G., R. E. Muck, F. Driehuis, S. J. W. H. Oude Elferink, and S. F. Spoelstra. 2003. Microbiology of ensiling. Pages 31–93 in *Silage Science and Technology* (Agronomy Series No. 42). D. R. Buxton, R. E. Muck, and H. J. Harrison, ed. American Society of Agronomy, Madison, WI.
- Pahlow, G., and E. Zimmer. 1985. Effect of a lactobacillus inoculant on fermentation and aerobic stability of grass silage. Pages 24–31 in *Proc. 15th Intl. Grassld. Congress*, Kyoto, Japan. Institute of Grassland and Forage Research, FAL, Braunschweig, Fed. Rep. Germany.
- Pedroso, A. F., A. T. Adesogan, O. C. M. Queiroz, and S. K. Williams. 2010. Control of *Escherichia coli* O157:H7 in corn silage with or without various inoculants: Efficacy and mode of action. *J. Dairy Sci.* 93:1098–1104.
- Ranjit, N. K., and L. Kung Jr.. 2000. The effect of *Lactobacillus buchneri*, *Lactobacillus plantarum*, or a chemical preservative on the fermentation and aerobic stability of corn silage. *J. Dairy Sci.* 83:526–535.
- Rooke, J. A., and R. D. Hatfield. 2003. Biochemistry of Ensiling. Pages 95–139 in *Silage Science and Technology* (Agronomy Series No. 42). D. R. Buxton, R. E. Muck, and H. J. Harrison, ed. American Society of Agronomy, Madison, WI.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Taylor, C. C., N. J. Ranjit, J. A. Mills, J. M. Neylon, and L. Kung, Jr.. 2002. The effect of treating whole-plant barley with *Lactobacillus buchneri* 40788 on silage fermentation, aerobic stability, and nutritive value for dairy cows. *J. Dairy Sci.* 85:1793–1800.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Weinberg, Z. G., G. Ashbell, K. K. Bolsen, G. Pahlow, Y. Hen, and A. Azrieli. 1995. The effect of a propionic acid bacterial inoculants applied at ensiling, with or without lactic acid bacteria, on the aerobic stability of pearl millet and maize silages. *J. Appl. Bacteriol.* 78:430–436.
- Weinberg, Z. G., G. Ashbell, Y. Hen, and A. Azrieli. 1993. The effect of applying lactic acid bacteria at ensiling on the aerobic stability of silages. *J. Appl. Bacteriol.* 75:512–518.