Animal Feed Science and Technology 159 (2010) 105–109

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Effects of combining *Lactobacillus buchneri* 40788 with various lactic acid bacteria on the fermentation and aerobic stability of corn silage

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ARTICLE INFO

Article history:
Received 15 October 2009
Received in revised form 3 June 2010
Accepted 5 June 2010

Keywords:
Silage
Lactic acid bacteria
Lactobacillus buchneri

ABSTRACT

The objective was to compare the effectiveness of combining *Lactobacillus buchneri* 40788 with three lactic acid bacteria on the fermentation, aerobic stability and nutritive value of corn silage. Freshly chopped whole plant corn was untreated or treated with *L. buchneri* 40788 paired with *Lactobacillus plantarum*, *Pediococcus acidilactici*, or *Pediococcus pentosaceus*. *L. buchneri* was added to achieve 4×10^5 CFU/g of fresh forage and the homolactic acid bacteria were added to achieve 1×10^5 CFU/g. Forages were ensiled in laboratory silos for 215 days before opening. Overall, inoculation increased the concentrations of acetic acid and 1,2-propanediol, but it reduced the concentrations of lactic acid, water soluble carbohydrates and ethanol. Effects on acetic acid and 1,2-propanediol were biggest for *L. buchneri* 40788 paired with *P. pentosaceus* > *P. acidilactici* > *L. plantarum*. Combining *L. buchneri* 40788 with *L. plantarum* or *P. acidilactici* increased and tended to increase *in vitro* aNDF digestibility in silage when compared to untreated silage, respectively. Untreated silage had a relatively large proportion of visibly spoiled silage at the surface of the silos, which resulted in poorer recovery of feedable dry matter when compared to silage treated with the combinations of *L. buchneri* 40788 with *L. plantarum* or with *P. acidilactici*. All inoculated silages had fewer numbers of yeasts after ensiling which improved their aerobic stability relative to untreated silage. Addition of *L. buchneri* 40788 to corn silage improves its aerobic stability and combining it with the *P. acidilactici* and *L. plantarum* strains used in this study results in other beneficial effects on silage quality.

1. Introduction

Microbial additives based on classical homolactic acid bacteria have been used to improve the efficiency of silage fermentations (Kung et al., 2003). However, using these types of organisms has sometimes made the silages less stable when they are exposed to air (Muck and Kung, 1997) because there is less production of organic acids with strong antifungal characteristics as a result of the actions of the additive. In contrast, addition of *Lactobacillus buchneri* (a heterolactic acid bacterium) to silage improves aerobic stability via anaerobic production of acetic acid (Oude Elferink et al., 2001). However, treating silages with this organism has led to small dry matter (DM) losses in corn silage (average of 10 g/kg DM lower recovery of DM

Abbreviations: ADF, acid detergent fiber; CFU, colony-forming units; DM, dry matter; aNDF, neutral detergent fiber; aNDF-D, digestibility of neutral detergent fiber; C, control silage; LAB, lactic acid bacteria; LBPA, *Lactobacillus buchneri* 40788 combined with *Pediococcus acidilactici*; LBLP, *Lactobacillus buchneri* 40788 combined with *Lactobacillus plantarum*; LBPP, *Lactobacillus buchneri* 40788 combined with *Pediococcus pentosaceus*; WSC, water soluble carbohydrates; VFA, volatile fatty acids.

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doi:10.1016/j.anifeedsci.2010.06.002

compared to untreated silage) and moderate DM losses in grass and small grain silages (as much as 18 g/kg DM lower DM recovery; Kleinschmit and Kung, 2006a). The undesirable characteristic of each type of microbial additive may be overcome by combining them in a silage inoculant. For example, Filya (2003) combined *L. buchneri* with *Lactobacillus plantarum* and added it to low DM corn silage (about 210 g/kg). He found that DM losses were less for the combination of organisms than with *L. buchneri* alone. However, when *L. buchneri* was combined with *Pediococcus pentosaceus*, this combination did not consistently affect aerobic stability (Kleinschmit and Kung, 2006b; Adesogan et al., 2003).

The objective was to compare the effectiveness of combining *L. buchneri* 40788 with *L. plantarum*, *Pediococcus acidilactici* or *P. pentosaceus* on the fermentation, aerobic stability and nutritive value of corn silage. To our knowledge, this is the first study comparing these three bacteria combined with *L. buchneri* in the same study.

2. Materials and methods

2.1. Treatments

Brown midrib corn (F2F797, Mycogen Seeds, Dow AgroSciences LLC, Indianapolis, IN, USA) was harvested and chopped at a DM content of about 320 g/kg. Four silos (20 L capacity) were individually prepared for each of the following treatments: (a) untreated (C), (b) *L. buchneri* 40788 combined with *P. acidilactici* (LBPA), (c) *L. buchneri* 40788 combined with *L. plantarum* (LBLP), and (d) *L. buchneri* 40788 combined with *P. pentosaceus* (LBPP). Bacteria were supplied by Lallemand Animal Nutrition (Milwaukee, WI, USA). Inoculants were enumerated on De Man Rogosa Sharpe agar (Oxoid CM361, Basingstoke, Hampshire, England) to determine the amount of inoculant required to meet the targeted inoculation rate of 4×10^5 CFU/g of fresh forage for *L. buchneri* and 1×10^5 CFU/g of fresh forage for the remaining bacteria. Inoculants were mixed with deionized water and sprayed onto the forage. Untreated forage was sprayed with deionized water so that equal amounts of water were added to all treatments. Each silo was packed with approximately 15 kg of wet forage to achieve a packing density of about 230 kg of DM per m^3. Weights of empty and full silos were recorded. Silos were sealed with oxygen barrier plastic (SlioStop, manufactured by Industria Plastica Monregalese, Mondovi, Italy, exported by Bruno Rimini Corp., London, UK), twine and duct tape, and were stored in a barn at ambient temperature (15–28 °C). After 215 days of ensiling, full silos were weighed. Visible spoilage from the top of each silo was separated and weighed. The weight of visibly spoiled silage was subtracted from the weight of the total silage remaining after fermentation. Recovery of feedable DM was calculated using weights and DM contents of fresh forage and unspoiled silage. The portions of silage free of visible spoilage were mixed thoroughly and samples were collected for analyses.

2.2. Analytical methods

A 25 g sample of fresh forage or silage was blended in 225 ml of sterile 1/4-strength Ringer's solution (Oxoid BR0052G) for 1 min. The pH of each sample was then determined, and a portion of the water extract was filtered through Whatman 54 filter paper (Clifton, NJ, USA). Water extracts (8 ml) were acidified with approximately 50 μl of 50% (vol/vol) H_2SO_4 and frozen prior to analysis for fermentation end products. Water extracts were analyzed for volatile fatty acids (VFA), ethanol and 1,2-propanediol by high-performance liquid chromatography (Dairyland Laboratories, Arcadia, WI) using the method described by Muck and Dickerson (1998). Ammonia-N (Weatherburn, 1967) and water soluble carbohydrates (WSC; Nelson, 1944) were also determined on the water extracts. The DM contents of each sample were determined using a force-draft oven at 60 °C for 48 h. Dried samples were ground through a 1 mm screen using a Cyclone Sample Mill (UDY Corp., Fort Collins, CO, USA). Acid detergent fiber was analyzed (Robertson and Van Soest, 1981) using an Ankom^{200} Fiber Analyzer (Ankom Technology, Macedon, NY, USA) and expressed on a DM basis including residual ash. Neutral detergent fiber was analyzed using the sulfite and amylase method described by Van Soest et al. (1991), but using an Ankom^{200} Fiber Analyzer and expressed on a DM basis including residual ash (aNDF). The digestibility of aNDF (aNDF-D) was determined on corn silage samples using the *in vitro* procedure described by Goering and Van Soest (1970) with some modifications. Those modifications included (a) weighing samples into 100 ml poly carbonate tubes each sealed with a rubber stopper fitted with a glass tube and a rubber policeman (14-105A, Fisher Scientific, Pittsburgh, PA, USA) with a 5 mm slit to allow for venting of gas pressure, (b) incubation in a heated orbital shaker (39 °C, 100 revolutions per min) and (c) incubation for 30 h.

A portion of the water extracts was used for enumeration of lactic acid bacteria by pour plating on De Man Rogosa Sharpe agar and incubated at 30 °C for 48–72 h. Yeasts and molds were determined by pour plating serial 10-fold dilutions of the eater extracts on malt extract agar (Oxoid CM0059) that had been acidified with lactic acid (concentration of 850 g/kg and added at 50 g/kg, vol/vol). Plates were incubated at 32 °C for 48–72 h and those containing a minimum of 30 and a maximum of 300 colony-forming units were enumerated.

Three kilograms of well mixed silage from the bottom portion of each silo (free of visible spoilage) were placed back (without packing) into clean silos and a thermocouple wire was inserted into the center of each silage mass. The thermocouple wires were connected to a data logger (model number CR10X, Campbell Scientific, Inc., Logan, UT, USA) that recorded the temperature every 10 min and then averaged the temperature over a 2 h period. Aerobic stability was defined as the time it took for the temperature in the silage masses to rise 2 °C above ambient temperature.

Table 1
The microbial populations and chemical composition (±standard deviation) of untreated corn forage prior to ensiling.

Item	
LAB, log CFU/g of fresh forage	6.51 ± 0.089
Yeasts, log CFU/g of fresh forage	5.66 ± 0.127
Molds, log CFU/g of fresh forage	5.01 ± 0.069
DM, g/kg	318 ± 2.0
WSC, g/kg DM	90 ± 6.1
N, g/kg DM	12.4 ± 1.0
NH_3–N, g/kg DM	0.5 ± 0.02
ADF, g/kg DM	210 ± 11.3
aNDF, g/kg DM	389 ± 1.8

LAB = lactic acid bacteria; CFU = colony-forming units; DM = dry matter; WSC = water soluble carbohydrates; N = nitrogen; ADF = acid detergent fiber; aNDF = neutral detergent fiber assayed with a heat stable amylase and expressed inclusive of residual ash.

2.3. Statistical analysis

All chemical analyses are presented on a DM basis (except DM and pH) and microbial data was converted to $\log_{10}$ and is presented on a wet matter basis. The study was conducted as a completely randomized design. Data was subjected to analysis of variance by the General Linear Models procedure of SAS (SAS, 2001) with treatment and residual error included in the model. Means were separated by Tukey's test ($P<0.05$; Snedecor and Cochran, 1980).

3. Results

The nutrient and microbial contents for fresh corn prior to ensiling are given in Table 1. The DM content of whole plant corn was 318 g/kg and values for other nutritive constituents were within normal range (Kleinschmit et al., 2005).

Table 2 shows the recovery of feedable DM and nutrient content of untreated and inoculated corn silages after 215 days of ensiling. Untreated silage had the greatest amount of visibly spoiled silage when compared to other treatments (data not shown), resulting in lower recovery of feedable DM for C compared to silages inoculated with LBPA and LBLP. Recovery of feedable DM did not differ between C and silage treated with LBPP. The DM of silages ranged from 297 to 318 g/kg and was lower in inoculated silages than in C although this difference was small. The N content of LBPP was highest among the treatments whereas the N content was similar among the remaining treatments. Ammonia-N content was highest in LBPA when compared to all other treatments, intermediate in concentration for LBPP and lowest in C and LBLP. Relative to C, inoculation did not affect the content of ADF or aNDF. Residual WSC was highest in C (33 g/kg DM) and lower in inoculated silages than in inoculated silages (average of 19 g/kg DM). The digestibility of aNDF was higher for LBLP and tended to be higher ($P<0.10$) for LBPA than for untreated silage. Treatment with LBPP did not affect aNDF-D relative to C.

Numbers of yeasts were extremely low or not detectable in silages treated with *L. buchneri* 40788, but were 4.33 log CFU/g in C. Molds were also very low in all silages (Table 3). Total numbers of viable lactic acid bacteria determined by pour plating on De Man Rogosa Sharpe agar were higher in inoculated silages than in C.

The concentrations of lactic acid were lower in LBPA and LBPP, with an average of 53 g/kg DM, than in C and LBLP, which averaged 60 g/kg DM (Table 4). Concentrations of acetic acid and 1,2-propanediol were greater in inoculated *versus* C but

Table 2
The DM recovery, nutrient content and aNDF digestion of silages after 215 days of ensiling.

	C	LBPA	LBLP	LBPP	SEM
DM recovery, g/kg	941^{b}	980^{a}	965^{a}	937^{b}	10.3
DM, g/kg	318^{a}	302^{b}	303^{b}	297^{b}	2.6
N, g/kg DM	13^{b}	13^{b}	13^{b}	14^{a}	0.1
NH_3–N, g/kg DM	1.0^{c}	1.6^{a}	1.1^{c}	1.3^{b}	0.04
ADF, g/kg DM	212^{ab}	198^{b}	217^{a}	211^{ab}	4.8
aNDF, g/kg DM	361	340	364	367	8.9
WSC, g/kg DM	33^{a}	20^{b}	20^{b}	18^{b}	1.4
aNDF-D, g/kg aNDF	606^{b}	633^{ab}	647^{a}	607^{b}	10.8

C = untreated silage; LBPA = silage treated with *Lactobacillus buchneri* 40788 (4×10^5 CFU/g of wet forage) and *Pediococcus acidilactici* (1×10^5 CFU/g of wet forage); LBLP = silage treated with *Lactobacillus buchneri* 40788 (4×10^5 CFU/g of wet forage) and *Lactobacillus plantarum* (1×10^5 CFU/g of wet forage); LBPP = silage treated with *Lactobacillus buchneri* 40788 (4×10^5 CFU/g of wet forage) and *Pediococcus pentosaceus* (1×10^5 CFU/g of wet forage); DM recovery = dry matter recovery of feedable silage; DM = dry matter; WSC = water soluble carbohydrates; N = nitrogen; ADF = acid detergent fiber; aNDF = neutral detergent fiber assayed with a heat stable amylase and expressed inclusive of residual ash; WSC = water soluble carbohydrates; aNDF-D = digestibility of neutral detergent fiber assayed with a heat stable amylase and expressed inclusive of residual ash.
[a,b,c]Means in rows with the same superscript do not differ ($P<0.05$).

Table 3
Microbial content (wet basis) corn silages after 215 days of ensiling.

	C	LBPA	LBLP	LBPP	SEM
Yeasts, log CFU/g	4.33[a]	0.00[b]	0.00[b]	0.67[b]	0.367
Molds, log CFU/g	1.23	0.50	0.50	0.00	0.501
LAB, log CFU/g	5.68[c]	8.21[ab]	8.02[b]	8.78[a]	0.223

C = untreated silage; LBPA = silage treated with *Lactobacillus buchneri* 40788 (4×10^5 CFU/g of wet forage) and *Pediococcus acidilactici* (1×10^5 CFU/g of wet forage); LBLP = silage treated with *Lactobacillus buchneri* 40788 (4×10^5 CFU/g of wet forage) and *Lactobacillus plantarum* (1×10^5 CFU/g of wet forage); LBPP = silage treated with *Lactobacillus buchneri* 40788 (4×10^5 CFU/g of wet forage) and *Pediococcus pentosaceus* (1×10^5 CFU/g of wet forage); LAB = lactic acid bacteria.
[a,b,c]Means in rows with the same superscript do not differ ($P<0.05$).

Table 4
The pH, fermentation end products (% DM basis) and aerobic stability from corn silages after 215 days of ensiling.

	C	LBPA	LBLP	LBPP	SEM
pH	3.61[a]	3.61[a]	3.58[b]	3.61[a]	0.009
Lactic acid, g/kg DM	60[a]	54[b]	60[a]	53[b]	1.4
Acetic acid, g/kg DM	14[d]	36[b]	29[c]	44[a]	0.7
1,2-Propanediol, g/kg DM	7[d]	21[b]	12[c]	31[a]	2.3
Ethanol, g/kg DM	3.5[a]	1.4[c]	2.3[b]	1.3[c]	0.13
Aerobic stability[e], h	180[b]	518[a]	570[a]	570[a]	23.5

C = untreated silage; LBPA = silage treated with *Lactobacillus buchneri* 40788 (4×10^5 CFU/g of wet forage) and *Pediococcus acidilactici* (1×10^5 CFU/g of wet forage); LBLP = silage treated with *Lactobacillus buchneri* 40788 (4×10^5 CFU/g of wet forage) and *Lactobacillus plantarum* (1×10^5 CFU/g of wet forage); LBPP = silage treated with *Lactobacillus buchneri* 40788 (4×10^5 CFU/g of wet forage) and *Pediococcus pentosaceus* (1×10^5 CFU/g of wet forage).
[a,b,c,d]Means in rows with the same superscript do not differ ($P<0.05$).
[e] Number of hours before a rise of 2 °C after exposure to air.

the response was greatest from LBPP > LBPA > LBLP. Aerobic stability was similar among inoculated silages (>500 h), which were more stable than C (180 h).

4. Discussion

The microbial data from this study shows that when added to chopped whole plant corn, *L. buchneri* dominates the resulting fermentation because the numbers of residual lactic acid bacteria were markedly higher, and yeasts were lower, in inoculated than in untreated silage. Numbers of *L. buchneri* (determined by real-time PCR and reported in a separate study; Schmidt et al., 2008) were markedly higher in inoculated silages. Dominance by *L. buchneri* resulted in higher concentrations of acetic acid and 1,2-propanediol, which supports the pathway of lactic to acetic degradation described by Oude Elferink et al. (2001). Kleinschmit and Kung (2006a) reported that treating corn and small cereal grains with *L. buchneri* 40788 did not affect the N or ammonia-N content of silages and we have no explanation why treatment with LBPA and LBPP resulted in silages with higher ammonia-N than in C. Inoculation resulted in silages with less residual WSC than that found in untreated silage and was probably a reflection of a more extensive fermentation in the former. Inoculation did not affect the ADF or aNDF content of corn silage compared to untreated corn silage, which is in agreement with past findings (Ranjit and Kung, 2000; Kleinschmit et al., 2005).

One criticism of using *L. buchneri* as a silage inoculant has been the concern that a silage fermentation dominated by a heterolactic bacterium would result in higher losses of DM. In a meta-analysis of studies comparing untreated silage with silages treated only with *L. buchneri*, Kleinschmit and Kung (2006a) reported that DM recovery was lower in silages treated with this organism, although the difference was very small. Driehuis et al. (2001) reported that combining *L. buchneri* with a homolactic acid bacterium reduced fermentation losses with treatment of *L. buchneri* alone. Results of our study are in general agreement with this finding.

Besides improving DM recovery and aerobic stability, some silage inoculants are used to improve the digestibility of silages. Weinberg et al. (2007) speculated that some silage inoculants may compete with other microorganisms in the rumen for readily fermentable substrate resulting in less lactic acid causing a higher ruminal pH (Weinberg et al., 2003), which in turn could be favorable for fibrolytic bacteria in the rumen. As a result, fiber digestion in the rumen could be enhanced when cows are fed silage treated with an inoculant. Weinberg et al. (2007) specifically showed that corn ensiled with *L. buchneri* 40788 resulted in improvement of *in vitro* ruminal aNDF digestion. However, this finding has not been consistent as some strains of *L. buchneri* added to the silages have not affected fiber digestion (Filya, 2003; Adesogan et al., 2003). An alternative mechanism to improve fiber digestibility of silages is to treat it with select strains of lactic acid bacteria that express ferulic acid esterase (Nsereko et al., 2008). This enzyme has the potential to increase digestibility of fiber by separating hemicellulose from lignin. Kang et al. (2009) reported that addition of *L. buchneri* PTA6138, with known ferulic acid esterase activity, plus *Lactobacillus casei* PTA6315 to whole plant corn at ensiling improved *in situ* aNDF-D in one of two corn silage hybrids. It is unknown if *L. buchneri* 40788 (used in the current study) possesses this characteristic. Although LBLP and LBPA improved aNDF-D, for unknown reasons, the combination with LBPP

did not. More research studying potential interactions among lactic acid bacteria for the potential to improve NDF-D is warranted.

5. Conclusions

Inoculation with *L. buchneri* 40788 resulted in silage with a higher concentration of acetic acid and lower numbers of yeasts. Although the magnitude of the response was dependent on the bacterium it was paired with, the improvement in aerobic stability was the same for all inoculated silages. Inoculation with LBLP and LBPA, but not LBPP resulted in an improvement in feedable DM and aNDF-D when compared to untreated silage. Addition of *L. buchneri* 40788 to corn silage improves the aerobic stability of silages and, depending on the specific bacterium it is combined with, it may result in other beneficial effects on silage quality.

Acknowledgements

The authors wish to express their appreciation to Renato Schmidt, Megan Hofherr and the farm crew of the University of Delaware Dairy for their assistance throughout this study. L.J. Reich conducted this research as an undergraduate researcher in the Science and Engineering Scholars Program, University of Delaware.

References

Adesogan, A.T., Salawu, M.B., Ross, A.B., Davies, D.R., Brooks, A.E., 2003. Effect of *Lactobacillus buchneri*, *Lactobacillus fermentum*, *Leuconostoc mesenteroides* inoculants, or a chemical additive on the fermentation, aerobic stability, and nutritive value of crimped wheat grains. J. Dairy Sci. 86, 1789–1796.

Driehuis, F., Oude Elferink, S.J.W.H., Van Wikselaar, P.G., 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.

Goering, H.K., Van Soest, P.J., 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications). Agricultural Handbook No. 379. ARS-USDA, Washington, DC, USA.

Kang, T.W., Adesogan, A.T., Kim, S.C., Lee, S.S., 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.

Kleinschmit, D.H., Kung Jr., L., 2006a. A meta-analysis of the effects of *Lactobacillus buchneri* on the fermentation and aerobic stability of corn and grass and small-grain silages. J. Dairy Sci. 89, 4005–4013.

Kleinschmit, D.H., Kung Jr., L., 2006b. The effects of *Lactobacillus buchneri* 40788 and *Pediococcus pentosaceus* R1094 on the fermentation of corn silage. J. Dairy Sci. 89, 3999–4004.

Kleinschmit, D.H., Schmidt, R.J., Kung Jr., L., 2005. The effects of various antifungal additives on the fermentation and aerobic stability of corn silage. J. Dairy Sci. 88, 2130–2139.

Kung Jr., L., Taylor, C.C., Lynch, M.P., Neylon, J.M., 2003. The effect of treating alfalfa with *Lactobacillus buchneri* 40788 on silage fermentation, aerobic stability, and nutritive value for lactating dairy cows. J. Dairy Sci. 86, 336–343.

Muck, R.E., Dickerson, J.T., 1998. Storage temperature effects on proteolysis in alfalfa silage. Trans. ASAE 31, 1005–1009.

Muck, R.E., Kung Jr., L, 1997. Effects of silage additives ensiling. In: Silage: Field to Feedbunk. NRAES-99. NRAES, Ithaca, NY, USA, pp. 187–199.

Nelson, N., 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153, 375–380.

Nsereko, V.L., Smiley, B.K., Rutherford, W.M., Spielbauer, A., Forrester, K.J., Hettinger, G.H., Harman, E.K., Harman, B., 2008. Influence of inoculating forage with lactic acid bacterial strains that produce ferulate esterase on ensilage and ruminal degradation of fiber. Anim. Feed Sci. Technol. 145, 122–135.

Oude Elferink, S.J.W.H., Krooneman, J., Gottschal, J.C., Spoelstra, S.F., Faber, F., Driehuis, F., 2001. Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by *Lactobacillus buchneri*. Appl. Environ. Microbiol. 67, 125–132.

Ranjit, N.K., Kung Jr., L., 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.

Robertson, J.B., Van Soest, P.J., 1981. The detergent system of analysis and its application to human foods. In: James, W.P.T., Theander, O. (Eds.), The Analysis of Dietary Fiber in Food. Marcel Dekker, NY, NY, USA, pp. 123–158.

SAS Institute, 2001. SAS User's Guide. Statistics, Version, 8th ed. SAS Institute, Inc., Cary, NC.

Schmidt, R.J., Emara, M.G., Kung Jr., L., 2008. The use of a quantitative real-time polymerase chain reaction assay for identification and enumeration of *Lactobacillus buchneri* in silage. J. Appl. Microbiol. 105, 920–929.

Snedecor, G.W., Cochran, W.G., 1980. Statistical Methods, 6th ed. Iowa State University Press, Ames, IA, USA.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.

Weatherburn, M.W., 1967. Phenol–hypochlorite reaction for determinations of ammonia. Anal. Chem. 39, 971–974.

Weinberg, Z.G., Shatz, O., Chen, Y., Yosef, E., Nikbahat, M., Ben-Ghedalia, D., Miron, J., 2007. Effect of lactic acid bacteria inoculants on *in vitro* digestibility of wheat and corn silages. J. Dairy Sci. 90, 4754–4762.

Weinberg, Z.G., Muck, R.E., Weimer, P.J., 2003. The survival of silage inoculant lactic acid bacteria in rumen fluid. J. Appl. Microbiol. 94, 1066–1071.