INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18F–045/2018/20–8–1846–1852
DOI: 10.17957/IJAB/15.0718
http://www.fspublishers.org

Full Length Article

Meta-Analysis of the Effects of Combined Homo- and Heterofermentative Lactic Acid Bacteria on the Fermentation and Aerobic Stability of Corn Silage

Fanfan Zhang, Xuzhe Wang, Weihua Lu and Chunhui Ma*
Animal Science and Technology, Shihezi University, Xinjiang 832000, China
*For correspondence: zhangfanfan@shzu.edu.cn

Abstract

There is not a definitive dose for the homo- and heterofermentative lactic acid bacteria (LAB) to enhance the fermentation quality and aerobic stability (AS) of corn silage. To address these questions, a meta-analysis was conducted on 24 studies on adding a mixture of homo- and heterofermentative LAB to corn silages. The classification system used was: (1) CON= untreated corn silage with no inoculant applied, (2) HH1= corn silage treated with combined homo- and heterofermentative LAB at $<5 \times 10^5$ cfu/g of fresh forage, (3) HH2= corn silage treated with combined LAB at $\geq 5 \times 10^5$cfu/g. Treating corn silage with HH1, compared with untreated corn silage, significantly influenced the pH, AS, concentration of acetic acid (AA) and ammonia-N (NH_3-N), LAB and yeast counts. Treating corn silage with HH1, compared with untreated corn silage, significantly influenced LAB counts and AS. The pH, concentration of NH_3-N and water-soluble carbohydrates (WSC) were significantly influenced in contrast to HH1 and HH2. However, there is not a significant difference between HH1 and HH2 on the concentrations of AA, AS, LAB and yeast counts. The concentrations of lactic acid (LA) and ethanol, mold counts, DM and DM loss were unaffected ($P > 0.05$) by inoculation. The AS of treated and untreated corn silage showed a weak positive correlation with pH, concentration of AA, and amount of LAB; and a weak negative correlation with concentrations of ethanol, mold and yeast counts. © 2018 Friends Science Publishers

Keywords: Corn silage; Lactic acid bacteria; Homofermentative; Heterofermentative; Aerobic stability; Meta- analysis

Introduction

Currently, corn silage is the most important silage throughout the world. Continued improvement of the quality of corn silage is a necessary part of dairy farming. At present, most research on corn silage has focused on the use of bacterial inoculants to improve its quality (Driehuis *et al*., 2001; Filya, 2003a). Bacterial inoculants for corn silage usually contain one or more species of lactic acid bacteria (LAB), which can usually be divided into two types: homo- and heterofermentative (Mcdonald *et al*., 1991). Homofermentative LAB inoculants, such as *Lactobacillus plantarum*, *Enterococcus faecium*, and *Pediococcus* species, are often used to control ensiling fermentation through rapid and efficient conversion of water-soluble carbohydrates (WSC) into organic acids, mainly lactic acid (LA), under anaerobic conditions (Filya, 2003a; Weinberg *et al*., 2008). The use of this type of inoculant can increase the rate of acidulation and reduce the final pH and protein degradation (Driehuis *et al*., 1997). It can also ensure rapid fermentation during the early stages of ensiling and minimize the loss of nutrients and dry matter (DM) in corn silage (Muck and Kung, 1997). However, such inoculants decrease aerobic stability (AS) when exposed to air (Filya, 2003a; Nkosi *et al*., 2009). This is because homofermentative LAB often have low levels of volatile fatty acids (VFA), which inhibit the growth of yeasts and molds (Kung *et al*., 2003). Muck (1996) was the first to suggest that inoculation with *L. Buchneri* (heterofermentative LAB), might improve the AS of silage when exposed to air. This method had approval from the U.S. Food and Drug Administration (FDA) until 2001. Since then, the unique activity of *L. buchneri* in corn silage has been evaluated (Holzer *et al*., 2003). Research has shown that in a variety of corn silage, inoculation with *L. buchneri* enhances AS via the anaerobic conversion of LA to acetic acid (AA) and 1, 2-propanediol (Elferink *et al*., 2001). Data for whole-crop grain silage indicates that heterofermentative silage does not have negative impacts on cow performance (Taylor *et al*., 2002).

There is a complementary effect in corn silage inoculated with a mixture of homo- and heterofermentative LABs, because each of the LABs has its own benefits (Zuniga *et al*., 1993; Driehuis *et al*., 2001; Filya, 2003b; Bayatkouhsar *et al*., 2012). Therefore, most commercial silage inoculants currently contain a mixture of homo- and heterofermentative LABs to enhance the fermentation

To cite this paper: Zhang, F., X. Wang, W. Lu and C. Ma, 2018. A meta-analysis of the effects of combined homo- and heterofermentative lactic acid bacteria on the fermentation and aerobic stability of corn silage. *Int. J. Agric. Biol*., 20: 1846–1852

process as well as the AS of the silage. However, there are several unclear concerns of this approach. It is not known if corn silage treated with a combination of homo- and heterofermentative LAB has better results than untreated silage, in terms of fermentation characteristics and AS (Weinberg and Muck, 1996; Schmidt and Kung, 2010). Further, there is not a definitive dose for the combined LAB to enhance the fermentation quality and AS. This study summarized the related literature over the past 20 years in order to better explain the effects of combined LABs, at different dose, on the fermentation and the AS of whole-plant corn silage.

Materials and Methods

The experiments were examined in this review included the whole-plant corn silage which were ensiled at least 60 d. Inoculants applied the homo- and heterofermentative LAB in this study. Only studies that reported standard errors of the mean were included, and were mainly culled from peer-reviewed journals and conference proceedings. Table 1 shows the studies summarized in this review. Data that evaluated the effects of combined LAB on fermentation and AS of corn silage were taken from 24 studies (Table 1). For all types of corn silage, the treatments were classified as follows: (1) CON= untreated corn silage with no inoculant applied, (2) HH1= corn silage treated with a combination of homo- and heterofermentative LAB at $<5 \times 10^5$ cfu/g of fresh forage, (3) HH2= corn silage treated with combined LAB at $\geq 5 \times 10^5$cfu/g. The treatments were divided in this way because the standard suggested application rate of microbial inoculants is 10^5cfu/g (Kleinschmit and Kung, 2006a).

This study used meta-analysis techniques to summarize the DM content before ensiling, chemical and microbial composition, DM loss, and AS at the end of fermentation (≥ 60 d). The chemical composition of corn silage was reported on the basis of DM. The amounts of LAB, molds, and yeasts were reported on a $\log_{10}$ basis. The AS from each study was measured as the number of hours the silage was exposed to air before a 2ºC rise in temperature above the ambient temperature, and was converted to $\log_{10}$ prior to statistical analysis, because the variances in the data were not normally distributed (Kleinschmit and Kung, 2006a). One-way ANOVA was used to evaluate the statistical significance among CON, HH1, and HH2 (Cho *et al*., 2014). Any significant differences between the means were identified from the *P*-values of the ANOVA and the effects were considered significant at $P < 0.05$. When the calculated values of *F* were significant, the Duncan range test ($P < 0.05$) was used to interpret any significant differences among the mean values, and $P < 0.01$ was also considered in the meta-analysis. A linear regression investigating the AS in relation to the chemical and microbial properties was performed as described by Schmidt and Kung (2010). All of the above statistical analyses were performed using Version 13 of SPSS for Windows (SPSS Inc., Chicago, IL).

Result

The DM content before ensiling, chemical and microbial composition, DM and DM loss and AS of the corn silage at the end of fermentation is shown in Table 2. The DM content was no significant difference ($P > 0.05$) among CON (33.38%), HH1 (33.88%) and HH2 (33.87%) before ensiling. There was no significant difference among CON (32.61%), HH1 (31.91%) and HH2 (33.00%) in the DM content of the corn silage. Corn silage treated with the lower dose of combined LAB (3.94 for HH1) had higher ($P < 0.01$) pH compared with the untreated (3.75 for CON) and higher dose (3.74 for HH2) corn silage, and there was no significant difference between CON and HH2. The concentrations of AA were further increased ($P < 0.01$) when the corn silage was treated with HH1 (2.63%) than CON (1.67%), and concentrations of AA had no significant difference between the higher dose of combined LAB (2.23% for HH2) than in corn silage treated with lower dose (HH1) and untreated (CON). The concentrations of LA, ethanol, and DM loss were unaffected ($P > 0.05$) by inoculation. Compared with CON (0.272%) and HH2 (0.143%), treated corn silage with HH1 (0.652%) had higher ($P < 0.01$) concentrations of ammonia-N (NH_3-N), and there was no significant difference between CON and HH2. Compared with CON (1.94%), treated corn silage with combined LAB (2.79% for HH1 and 1.16% for HH2) had no significant difference in concentrations of WSC. However, the WSC concentrations were higher ($P < 0.01$) in HH1-treated corn silage than in the corn silage treated with HH2. Compared with CON (6.52 $\log_{10}$ cfu/g), the LAB amounts were further increased ($P < 0.01$) when the corn silage was treated with HH1 (8.28 $\log_{10}$ cfu/g) and HH2 (8.06 $\log_{10}$ cfu/g), and there was no significant difference between HH1 and HH2. The mold counts were unaffected by inoculation. Treating corn silage with HH1 (2.37 $\log_{10}$ cfu/g) and HH2 (2.26 $\log_{10}$ cfu/g) decreased ($P < 0.05$) the yeast count compared with CON (3.76 $\log_{10}$ cfu/g), and there was no significant difference between HH1 and HH2. Inoculation with combined LAB (193 h for HH1 and 124 h for HH2) significantly increased ($P < 0.05$) the AS of the corn silage compared with CON (69 h), and there was no significant difference between HH1 and HH2.

The factors affecting AS in the corn silage are shown in Fig. 1. The AS of the corn silage had a weak positive correlation with pH (R^2=0.17, $P < 0.001$), the AA concentrations (R^2=0.40, $P < 0.001$), and the numbers of LAB (R^2=0.30, $P < 0.001$). The AS also showed a weak negative correlation with concentrations of ethanol (R^2=0.18, $P < 0.001$), the mold counts (R^2=0.13, $P < 0.01$), and the yeast counts (R^2=0.25, $P < 0.001$). The AS of the corn silage did not have a linear relationship ($P > 0.05$) with DM, DM loss, concentrations of LA, WSC and NH_3-N (this data was not shown).

Table 1: Literature summarized in this review of the fermentation and aerobic stability of corn silage treated with combination of homo- and heterofermentative lactic acid bacteria

Study	Cites	Study region	Harvested stage and dry matter content	Storage patterns	lactic acid bacteria		Application rate of fresh forage, cfu/g	Length of ensiling, d
					Homo-	Hetero-		
1	Filya, 2003a	Turkey	Dent stage, 23.5 % dry matter	jar	*L. plantarum*	*L. buchneri*	Untreated Homo- (1.0×10^6) + Hetero- (1.0×10^6)	90
2	Filya, 2003b	Turkey	1/3 milk line stage, 37.8 % dry matter	Glass jar	*L. plantarum*	*L. buchneri*	Untreated Homo- (1.0×10^6) + Hetero- (1.0×10^6)	60
3	Aksu and Baytok, 2004	Turkey	Not described	Plastic container	*L. plantarum, L. rhamnosus, P. pentosaceus*	*L. buchneri L. brevis*	Untreated Homo- + Hetero- (2.5×10^{12})	60
4	Kleinschmit and Kung, 2006b	America	1/2 milk line stage, about 38 % dry matter	pile	*P. pentosaceus*	*L.buchneri*	Untreated Homo- (1.0×10^5)+ Hetero- (4.0×10^5)	70, 282, 361
5*	Huisden *et al*., 2009	America	2/3 milk line stage, About 40 % dry matter	silo	*P. pentosaceus*	*L.buchneri*	Untreated Homo- (1.0×10^5)+ Hetero- (4.0×10^5)	135
6	Huisden *et al*., 2009	America	2/3 milk line stage About 39 % dry matter	silo	*L. plantarum, E. faecium*	*L.buchneri*	Untreated Homo- + Hetero- (1.0×10^5)	135
7	Hu *et al*., 2009	Britain	33.1 % and 40.6 % dry matter period	pile	*L. plantarum*	*L.buchneri*	Untreated Homo- (1.0×10^5)+ Hetero (4.0×10^5)	240
8	Kang *et al.*, 2009	America	Approximately 39 % dry matter period	Plastic mini silo	*L. casei*	*L.buchneri*	Untreated Homo- (1.0×10^4)+ Hetero- (1.0×10^5)	110
9	Reich and Kung Jr, 2010	America	About 32 % dry matter period	silo	*P. pentosaceus*	*L.buchneri*	Untreated Homo- (1.0×10^5)+Hetero- (4.0×10^5)	215
10	Reich and Kung Jr, 2010	America	About 32 % dry matter period	silo	*L. plantarum*	*L.buchneri*	Untreated Homo- (1.0×10^5)+Hetero- (4.0×10^5)	215
11	Reich and Kung Jr, 2010	America	About 32 % dry matter period	silo	*P. acidilactici*	*L.buchneri*	Untreated Homo- (1.0×10^5)+ Hetero- (4.0×10^5)	215
12	Schmidt and Kung, 2010	America	About 31-40 % dry matter period	silo	*P. pentosaceus*	*L.buchneri*	Untreated Homo- (1.0×10^5)+ Hetero- (4.0×10^5)	120
13	Arriola *et al*., 2011	America	35 % dry matter period	silo	*P. pentosaceus*	*L.buchneri*	Untreated Homo- (1.0×10^5)+ Hetero- (4.0×10^5)	575
14	Kleinmans *et al*., 2011	New Zealand	About 34 % dry matter period	silo	*L. plantarum, L. lactis, P. acidilactici, E. faecium*	*L.buchneri*	Untreated Homo- + Hetero- (1.1×10^{11})	60
15	Kleinmans et al., 2011	New Zealand	About 34 % dry matter period	silo	*L. plantarum, E. faecium*	*L.buchneri*	Untreated Homo- + Hetero- (1.1×10^{11})	60
16	Kleinmans *et al*., 2011	New Zealand	About 34 % dry matter period	silo	*L. plantarum, E. faecium*	*L.buchneri*	Untreated Homo- + Hetero- (1.1×10^{11})	60
17	Selwet, 2011	Poland	Dough stage, about 23 % dry matter	microsilo	*P. acidilactici, L. plantarum, L. rhamnosus, L. lactis, E. faecium*	*L.buchneri, L. brevis*	Untreated Homo- + Hetero- (5.0×10^8)	60
18	Tabacco *et al*., 2011	Italy	1/2 milk line stage, about 35 % dry matter	bunker silo	*L. casei*	*L.buchneri*	Untreated Homo- (1.0×10^4)+ Hetero- (1.0×10^5)	90
19	Bayatkouhsar *et al*., 2012	Iran	Medium dough stage, about 30 % dry matter	Mini silo	*L. acidophilus, L. casei, L. plantarum, P. pentosaceus*	*L.buchneri*	Untreated Homo- + Hetero- (1.0×10^{10})	90
20	Mohammadzadeh *et al.*, 2012	Iran	29.8 % dry matter	bunker silo	*E. faecium, L. plantarum, P. pentosaceus, L. casei*	*L.buchneri*	Untreated Homo- + Hetero- (1.5×10^5)	120
21	Queiroz *et al*., 2012	Netherlands	about 40 % dry matter	Plastic bucket silo	*P. pentosaceus*	*L.buchneri*	Untreated Homo- (1.0×10^5)+ Hetero- (4.0×10^5)	97
22	Queiroz *et al*., 2013	America	about 31 % dry matter	pile	*P. pentosaceus*	*L.buchneri*	Untreated Homo- (1.0×10^5)+ Hetero- (4.0×10^5)	120
23**	Jatkauskas *et al.*, 2013	Lithuania	Dough stage, about 30 % dry matter	silo	*L. plantarum, E. faecium*	*L.buchneri*	Untreated Homo- + Hetero- (1.5×10^5)	90
24	Junges *et al*., 2013	Brazil	30.7 % dry matter	silo	*E. faecium, L. plantarum*	*L. brevis*	Untreated Homo- + Hetero- (1.5×10^5)	60, 90, 120

* BONSYLAGE® used microbial inoculant usually containing homo- and heterofermentative lactic acid bacteria at 2.5×10^{12} cfu·g^{-1}

** In addition to lactic acid bacteria inoculants, chemical additives were added

Discussion

The combination of homo- and heterofermentative LAB is a complex system, which markedly influences the process of fermentation in corn silage. The DM content of corn silaga was about 33% before ensiling in this study. This indicated the fermentation results were not due to initial DM content. In the studies reviewed in this meta-analysis, inoculation with a lower dose ($<5\times10^5$ cfu/g) of combined LAB increased the pH and concentrations of AA as compared to untreated silage. Adding homofermentative LAB can rapidly reduce pH and accelerate the early stages of the process of fermentation (Muck and Kung, 1997). Meanwhile, treating corn silage with heterofermentative LAB moderately increases the pH (Kleinschmit and Kung, 2006a). The lower dose of combined LAB resulting in moderately increased pH was probably caused by the higher dose of heterofermentative LAB, which was at least 4 times greater compared with the dose of homofermentative LAB in the fermentation of combined LAB (Table 1); this phenomenon was reported by Arriola *et al*. (2011). Further, 4 days after ensiling, heterofermentative LAB was dominant in the process (Mcdonald *et al*., 1991). The increased concentrations of AA in the corn silage at the lower dose of combined LAB, was very close to what was expected (2.53% expected compared to 2.63% actual) in corn silage (Kleinschmit and Kung, 2006a). However, treatment with higher dose ($\geq 5\times10^5$ cfu/g) did not result in higher pH and AA concentrations.

Table 2: The effects of homo- and heterofermentative lactic acid bacteria on the fermentation characteristics, microbial composition, and aerobic stability of corn silage

Item	CON	n	HH1	n	HH2	n	*F*-values	*P*-values
DM, % before ensiling	33.38 ± 1.02	24	33.88 ± 1.59	12	33.87 ± 0.93	21	0.072	0.930
DM, %	32.61 ± 1.47	28	31.91 ± 1.62	14	33.00 ± 1.58	23	0.094	0.910
pH	3.75 ± 0.03^{bB}	34	3.94 ± 0.11^{aA}	14	3.74 ± 0.02^{bB}	29	4.633	0.013
Acetic acid, % DM basis	1.67 ± 0.18^{bB}	33	2.63 ± 0.41^{aA}	14	2.23 ± 0.19^{abAB}	28	4.056	0.021
Lactic acid, % DM basis	5.08 ± 0.39	33	5.02 ± 0.79	14	4.42 ± 0.28	28	0.800	0.454
Ethanol, % DM basis	1.21 ± 0.19	25	0.90 ± 0.21	13	1.10 ± 0.22	19	0.476	0.624
DM loss, %	4.12 ± 0.82	23	3.33 ± 0.64	12	3.71 ± 0.69	18	0.238	0.789
NH_3-N, % DM basis	0.272 ± 0.075^{bB}	19	0.652 ± 0.233^{aA}	5	0.143 ± 0.020^{bB}	19	6.559	0.003
WSC*, % DM basis	1.94 ± 0.26^{abAB}	23	2.79 ± 0.81^{aA}	9	1.16 ± 0.13^{bB}	21	5.153	0.009
LAB**, $\log_{10}$ cfu·g^{-1}	6.52 ± 0.34^{bB}	17	8.28 ± 0.38^{aA}	8	8.06 ± 0.31^{aA}	16	7.990	0.001
Molds, $\log_{10}$ cfu·g^{-1}	3.01 ± 0.29	21	2.61 ± 0.28	10	2.11 ± 0.38	18	2.046	0.141
Yeasts, $\log_{10}$ cfu·g^{-1}	3.76 ± 0.32^{aA}	22	2.37 ± 0.38^{bAB}	10	2.26 ± 0.34^{bB}	21	6.341	0.004
Aerobic stability, $\log_{10}$	1.84 ± 0.06^{bB}	27	2.28 ± 0.09^{aA}	13	2.09 ± 0.08^{aAB}	26	7.257	0.002
Aerobic stability, h	69		193		124			

CON = untreated corn silage with no inoculant applied, HH1 = inoculation with homo- and heterofermentative lactic acid bacteria at <5 × 10^5 cfu·g^{-1} of fresh forage, HH2 = inoculation with homo- and heterofermentative lactic acid bacteria at ≥5 × 10^5 cfu·g^{-1}

*WSC= water-soluble carbohydrates; ** LAB= lactic acid bacteria

$^{a, b and A, B}$ Means within row with unlike superscripts differ ($P < 0.05$ and $P < 0.01$)

Data were represented as mean ± standard error

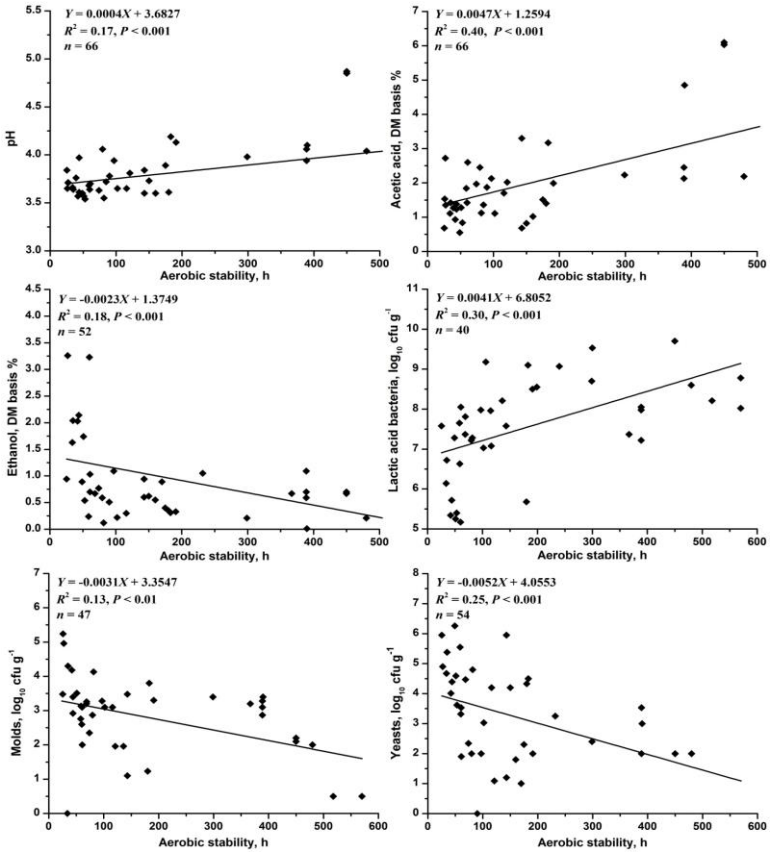

Fig. 1: Relationships between the aerobic stability and pH, acetic acid (DM basis), ethanol (DM basis), lactic acid bacteria, molds, yeasts, respectively. The database was composed of untreated corn silage, treating corn silage with homo- and heterofermentative lactic acid bacteria at < 5 × 10^5 cfu·g^{-1} of fresh forage, and corn silage treated with homo- and heterofermentative lactic acid bacteria at ≥ 5 × 10^5 cfu·g^{-1}

No clear reason has been identified for this finding. The meta-analysis showed that the yeast count was reduced from 3.76 $\log_{10}$ cfu/g to 2.37 $\log_{10}$ cfu/g (lower dose of combined LAB) and 2.26 $\log_{10}$ cfu/g (higher dose of combined LAB) due to the production of AA, which increased from 1.67% to 2.63% and 2.23%, as reported by Driehuis *et al.* (1999) who

clear that AA is a inhibitor of yeast. The moderately decreased ($P > 0.05$) concentration of ethanol was mainly caused by the yeasts (Driehuis *et al*., 1999), which converted glucose to ethanol. This review was not able to identify the role of combined LAB on ethanol, because the yeast count was influenced by multiple factors (Tabacco *et al*., 2011). In this meta-analysis, corn silage treated with combined LAB could markedly increase ($P < 0.05$) the LAB numbers. Undoubtedly, inoculation with combined LAB enhances the LAB numbers and AA concentrations to ensure the early stages of the fermentation process and also improves AS in aerobic stage (Zuniga *et al*., 1993; Driehuis *et al*., 1997; Arriola *et al*., 2011). However, when fermentation was in the stable stage (≥60 d); the increased LAB and AA had no effect on the mold counts. Unexpectedly, the amount of LAB and yeasts was not determined by the dose of combined LAB in the stable stage.

Kleinschmit and Kung (2006a) reported that treating corn silage with heterofermentative LAB at a lower rate (<1 × 10^5 cfu/g) decreased ($P < 0.01$) the concentration of LA, and the concentration of LA was further reduced ($P < 0.01$) in corn silage treated at the higher rate (> 10^5 cfu/g) of application. However, treatment with one or more species of homofermentative LAB increased ($P < 0.05$) the concentrations of LA as compared with application of heterofermentative LAB (Cho *et al*., 2014). In this meta-analysis, samples treated with combined LAB moderately decreased ($P > 0.05$) the concentration of LA. This might be due to the synergy effect of the combined LAB change on the efficiency of conversion of LA to AA and 1, 2-propanediol in the anaerobic fermentation of combined LAB (Kleinschmit and Kung, 2006b; Schmidt and Kung, 2010). Further, this review did not count the 1, 2-propanediol concentrations because there was minimal data available in the previous studies. Therefore, the cause for the changes of LA concentrations remained unclear. In recent research, the ratio of LA:AA usually exceeded 3:1 when corn silage was treated with homofermentative LAB alone (Kung and Stokes, 2001). However, when heterofermentative LAB dominated the fermentation; the ratio of LA: AA was less than 3:1 (Kleinschmit and Kung, 2006a). This review achieved similar results in adding heterofermentative LAB alone, adding combined LAB resulted in the ratio of LA:AA equaling 1.9:1 (with the lower dose of combined LAB) and 2.0:1 (with the higher dose of combined LAB) (Table 2). This was likely because heterofermentative LAB was dominant in the anaerobic conversion and caused the degradation of LA to AA in the fermentation of combined LAB (Elferink *et al*., 2001).

The concentration of NH_3-N is an important reflection of the degree of protein degradation (Driehuis *et al*., 1997). The meta-analysis showed that adding a lower dose (<5 × 10^5 cfu/g) of combined LAB resulted in a higher concentration of NH_3-N than in untreated corn silages, and this result was similar to Mohammadzadeh *et al*. (2012). Meanwhile, higher pH levels in HH1 (lower dose of combined LAB) treatment may also explain the greater NH_3-N concentration as compared to CON (no inoculant applied) treatment, because the lower pH inhibits proteolysis activity in corn silage (Mcdonald *et al*., 1991). Compared with HH1 treatment, corn silage treated with a higher dose (≥ 5 × 10^5 cfu/g) had a lower ($P < 0.01$) NH_3-N concentration. This is because the HH2 (higher dose of combined LAB) treatment may accelerate the initial LA fermentation rate, resulting in lower protein degradation (Filya, 2003a). Unexpectedly, there was no significant difference between HH2 and CON.

Adding the homofermentative LAB to corn silage can ensure that the loss of DM is minimized, as well as ensuring efficient conversion of WSC into LA under anaerobic conditions (Muck and Kung, 1997; Filya, 2003a; Weinberg *et al*., 2008). However, heterofermentative LAB's efficiency of converting WSC into LA is only 17–50% of the homofermentative LAB's, and this LA is mainly type D-LA, which is difficult for livestock to ingest (Mcdonald *et al*., 1991; Holzer *et al*., 2003). Therefore, large-scale heterofermentation does not aid maximizing the reservation of nutrition in corn silage. The meta-analysis showed that fermentation loss was not affected by adding combined LAB, the result was similar to Driehuis *et al*. (2001), Filya (2003a, b). Moreover, adding combined LAB did not influence the concentration of WSC, Mohammadzadeh *et al*. (2012) also found similar results. However, compared with the lower dose of combined LAB, the higher dose of combined LAB resulted in a lower concentration of WSC. A possible explanation for this was that a higher dose of combined LAB enhanced the efficiency of the conversion of WSC to LA (Filya, 2003a; Weinberg *et al*., 2008). In summary, the interaction between hetero- and homofermentative LAB remains unclear in the combined LAB fermentation system and further research into this area is needed.

The analyzed studies clearly indicate that inoculation with a mixture of homo- and heterofermentative LAB improved the AS time of corn silage (Driehuis *et al*., 2001; Elferink *et al*., 2001; Filya, 2003a, b; Schmidt and Kung, 2010). At the end of the ensiling period, the positive linear correlation between AS time and the concentrations of AA (R^2=0.40, $P < 0.001$) (Fig. 1) was confirmed, as reported by Schmidt and Kung (2010), who demonstrated a positive linear correlation between AS and the concentration of AA (R^2=0.95, $P < 0.05$). Further, Filya (2003b) reported that the AS is mainly affected by heterofermentative LAB by increasing the concentration of AA to decrease yeast growth, and enhances the AS time of the corn silage, in the mixture of LAB. Tabacco *et al*. (2011) also reported that the AS time and yeast count is reflected in the equation: AS (h) = 174.95 × $e^{-0.4073YC}$ (YC= yeast count, $\log_{10}$ cfu/g of fresh forage; R^2=0.63) when heterofermentative LAB was added. In this review, the negative linear correlation between AS time and yeast count was confirmed (R^2=0.25, $P < 0.001$). Because yeast converts glucose to ethanol in the fermentation process of corn silage (Driehuis *et al*., 1999), the concentration of ethanol was also negatively correlated with AS time

(R^2=0.18, $P < 0.001$). The moderately increased pH in the stable stage (≥60 d) of corn silage benefited from the extended AS time, and a linear positive correlation between AS time and pH was identified (R^2=0.17, $P < 0.001$). In summary, the concentration of AA is mainly produced by LAB, in particular the heterofermentative LAB (Elferink *et al*., 2001), and the amount of LAB was positively correlated with AS time (R^2=0.30, $P < 0.001$). Therefore, AA can inhabit the growth of molds (Kung *et al*., 2003) and mold counts and AS time showed negative correlation (R^2=0.13, $P < 0.01$) (Fig. 1). When exposed to the air, the chemical and microbial composition of the corn silage was usually changed. Therefore, these changes were the main factors to affect the AS time in the aerobic stage.

Conclusion

This study found that adding combined LAB had an effect on the chemical and microbial composition of corn silage, with the exception of the concentration of DM, DM loss, LA and ethanol, mold counts. Further, only the pH, WSC and NH_3-N concentration were a dose-dependent. The AA concentration, LAB and yeast counts, and AS time were not a dose-dependent. So we suggest that the added dose of combined LAB should not exceed 5×10^5 cfu/g. In the actual fermentation process of corn silage, the effect of adding combined LAB is not only influenced by the dose, it is also affected by temperature, initial moisture, and bacteria activity, among other factors. Thus, combined LAB should be added relative to the actual fermentation requirements.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (31460637), the National Forage Industry Technical system of China (CARS-34), and the Graduate Research and Innovation Project in Xinjiang Autonomous Region of China (XJGRI2015037).

References

Aksu, T. and E.D. Baytok, 2004. Effects of a bacterial silage inoculant on corn silage fermentation and nutrient digestibility. *Small Rumin. Res.*, 55: 249–252

Arriola, K.G., S.C. Kim and A.T. Adesogan, 2011. Effect of applying inoculants with heterolactic or homolactic and heterolactic bacteria on the fermentation and quality of corn silage. *J. Dairy Sci*., 94: 1511–1516

Bayatkouhsar, J., A.M. Tahmasbi and A.A. Naserian, 2012. Effects of microbial inoculant on composition, aerobic stability, *in situ* ruminal degradability and in vitro gas production of corn silage. *Int. J. Agric. Sci*., 2: 774–786

Cho, S., J.S. Kang, K.J. Cho, H.L. Kang, H.K. Chan, J. Song, K. Lee, S.Y. Kim and E.J. Kim, 2014. Effect of homofermentative and heterofermentative lactic acid bacteria on the quality and aerobic stability of silage: meta-analysis. *J. Kor. Soc. Grassl. Forage Sci*., 34: 247–253

Driehuis, F., S.J.W.H.O. Elferink and P.G.V. Wikselaar, 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

Driehuis, F., S.J.W.H.O. Elferink and S.F. Spoelstra, 1999. Anaerobic lactic acid degradation during ensilage of whole crop maize inoculated with *Lactobacillus buchneri* inhibits yeast growth and improves aerobic stability. *J. Appl. Microbiol*., 87: 583–594

Driehuis, F., P.G.V. Wikselaar, A.M.V. Vuuren and S.F. Spoelstra, 1997. Effect of a bacterial inoculant on rate of fermentation and chemical composition of high dry matter grass silages. *J. Agric. Sci*., 128: 323–329

Elferink, S.J.O., 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

Filya, I., 2003a. 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., 2003b. The effect of *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria, on the fermentation, aerobic stability and ruminal degradability of wheat, sorghum and maize silages. *J. Appl. Microbiol*., 95: 1080–1086

Holzer, M., E. Mayrhuber, H. Danner and R. Braun, 2003. The role of *Lactobacillus buchneri* in forage preservation. *Trends Biotechnol*., 21: 282–287

Hu, W., R.J. Schmidt, E.E. McDonell, C.M. Klingerman and L. Kung, 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

Jatkauskas, J., V. Vrotniakiene, C. Ohlsson and B. Lund, 2013. The effects of three silage inoculants on aerobic stability in grass, clover-grass, lucerne and maize silages. *Agric. Food Sci*., 11: 1–76

Junges, D., P. Schmidt, C.O. Novinski and J.L.P. Daniel, 2013. Additive containing homo and heterolactic bacteria on the fermentation quality of maize silage. *Acta Scientiarum. Anim. Sci*., 35: 371–377

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

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

Kleinmans, J.J., W.R. Dewar, H.J.H. Erasmus and R.J. Densley, 2011. Using silage inoculants to improve the quality of pasture and maize silage in New Zealand. *N.Z. Grassl. Assoc*., 73: 75–80

Kung, L. and M.R. Stokes, 2001. Analyzing silages for fermentation and products. Http: ag. udel. edu/departments/anfs/faculty/kung/articles/analyzing silages for fermentati.htm

Kung, L., M.R. Stokes and C.J. Lin, 2003. Silage additives. *Agronomy*, 42: 305–360

Mcdonald, P., A.R. Henderson and S.J.E. Heron, 1991. *The Biochemistry of Silage*. Chalcombe Publications, UK

Mohammadzadeh, H., M. Khorvash, G.R. Ghorbani and W.Z. Yang, 2012. Frosted corn silage with or without bacterial inoculants in dairy cattle ration. *Livest. Sci*., 145: 153–159

Muck, R.E., 1996. *A Lactic Acid Bacteria Strain to Improve Aerobic Stability of Silages*, pp: 42–43. U.S. Dairy Forage Research Center, Madison, Wisconsin, USA

Muck, R.E. and L. Kung, 1997. *Effects of Silage Additives on Ensiling*. Silage: Field to feedbunk. NRAES–99

Nkosi, B.D., R. Meeske, D. Palic, T. Langa, K.J. Leeuw and I.B. Groenewald, 2009. Effects of ensiling whole crop maize with bacterial inoculants on the fermentation, aerobic stability, and growth performance of lambs. *Anim. Feed Sci. Technol*., 154: 193–203

Queiroz, O.C.M., K.G. Arriola, J.L.P. Daniel and A.T. Adesogan, 2013. Effects of 8 chemical and bacterial additives on the quality of corn silage. *J. Dairy Sci*., 96: 5836–5843

Queiroz, O.C.M., S.C. Kim and A.T. Adesogan, 2012. Effect of treatment with a mixture of bacteria and fibrolytic enzymes on the quality and safety of corn silage infested with different levels of rust. *J. Dairy Sci.*, 95: 5285–5291

Reich, L.J. and L. Kung Jr, 2010. Effects of combining *Lactobacillus buchneri* 40788 with various lactic acid bacteria on the fermentation and aerobic stability of corn silage. *Anim. Feed Sci. Technol*., 159: 105–109

Schmidt, R.J. and L. Kung, 2010. The effects of *Lactobacillus buchneri* with or without a homolactic bacterium on the fermentation and aerobic stability of corn silages made at different locations. *J. Dairy Sci*., 93: 1616–1624

Selwet, M., 2011. The effect of bacterial silage inoculant on the fermentation, cell wall contents and aerobic stability of maize silage. *Acta Sci. Pol. Zootech.*, 10: 83–92

Tabacco, E., S. Piano, A. Revello-Chion and G. Borreani, 2011. Effect of *Lactobacillus buchneri* LN4637 and *Lactobacillus buchneri* LN40177 on the aerobic stability, fermentation products, and microbial populations of corn silage under farm conditions. *J. Dairy Sci*., 94: 5589–5598

Taylor, C.C., N.J. Ranjit, J.A. Mills, J.M. Neylon and J.L. Kung, 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

Weinberg, Z.G. and R.E. Muck, 1996. New trends and opportunities in the development and use of inoculants for silage. *FEMS Microbiol. Rev*., 19: 53–68

Weinberg, Z.G., G. Ashbell, Y. Hen and A. Azrieli, 2008. The effect of applying lactic acid bacteria at ensiling on the aerobic stability of silages. *J. Appl. Microbiol*., 75: 512–518

Zuniga, M., I. Pardo and S. Ferrer, 1993. An improved medium for distinguishing between homofermentative and heterofermentative lactic acid bacteria. *Int. J. Food Microbiol*., 18: 37–42

(Received 26 March 2018; Accepted 14 April 2018)