Original Research Article

Effect of different levels of corn steep liquor addition on fermentation characteristics and aerobic stability of fresh rice straw silage

Xinxin Li [a,1], Wenbin Xu [a,1], Jinshan Yang [a], Hongbo Zhao [b], Hangshu Xin [a], Yonggen Zhang [a,*]

[a] College of Animal Science and Technology, Northeast Agricultural University, Harbin 150030, China
[b] Institute of New Rural Development, Northeast Agricultural University, Harbin 150030, China

A B S T R A C T

The object of this study was to determine the proper mixing ratio of fresh rice straw to corn steep liquor (CSL) to obtain a high protein content silage feed. The following experimental silages were generated: the control (C1), composed of fresh rice straw without CSL additive, mixed with CSL in the ratios of 4:1 (C4), 3:1 (C3) and 2:1 (C2). Lactic acid bacteria (LAB) inoculant was applied at the rate of 50 mL/kg (fresh basis) of forage to achieve a final application rate of 1×10^6 cfu/g of fresh matter (FM). Duplicate silos for each treatment were opened after 0, 3, 7, 10, 20, 30, 45 and 60 d for microbiological and chemical analysis. The results showed that the addition of CSL significantly increased crude protein (CP) contents, and decreased neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents of treatments after 60 d of ensiling ($P < 0.05$). The lactic acid contents in C4 and C3 were significantly higher than that in C1 ($P < 0.05$). In summary, mixing fresh rice straw with CSL at addition levels of 4:1 (C4) and 3:1 (C3) can improve the fermentation quality and nutrient composition of fresh rice straw silage. However, a large proportion of CSL (C3) had a negative impact on the aerobic stability of fresh rice straw.

1. Introduction

Corn steep liquor (CSL), a viscous liquid mixture consisting entirely of the water-soluble components of corn steeped in water, is a by-product of the wet corn milling industry. It typically contains 525 g/kg dry matter (DM), 205 g crude protein (CP)/kg DM, 10 g fiber/kg DM, 88 g ash/kg DM, 130–220 g carbohydrate/kg DM and a small amount of sulfurous acid (<0.01 g/kg DM) (Chiani et al., 2010). Corn steep liquor has been successfully applied to partially or totally replace yeast extract, which is more expensive, for the production of cellulolytic enzymes (Nascimento et al.,

2009). Corn steep liquor has also been successfully used in the production of succinic acid (Agarwal et al., 2006; Liu et al., 2010), cellulose (Noro et al., 2004) and ethanol (Nascimento et al., 2009; Saxena and Tanner, 2012; Silveira et al., 2001). In addition, CSL is widely used as an inexpensive source of nitrogen resource, vitamins, and minerals for cultivation of microbes such as *Enterococcus faecalis* RKY1, *Lactobacillus rhamnosus* CGMCC1466 and *Lactobacillus* sp. RKY2 for the production of lactic acid (Wee et al., 2006a, b; Yu et al., 2008). Yu et al. (2008) found that CSL in cooperation with other components resulted in 30.4% higher lactic acid concentration than yeast extract in the lab fermenter. Recently, due to the rapid development of the production of corn starch, substantial amounts of CSL have been produced. Many starch manufacturers discharge corn soak water directly into the environment, which has a negative impact on the environment (Silva et al., 2010).

Rice straw accounts for 30.4% of all the straw resources in China (Xie et al., 2010). Low digestibility and low protein content are the two major limitations on the use of rice straw as ruminant feed (Han, 1975). Ensiling can be considered as an efficient way to improve the palatability and nutritive value of the rice straw (Gao

et al., 2008). Fresh rice straw with a DM content of 250 g/kg has high concentrations of water-soluble carbohydrates (WSC; 9.79% DM), which are suitable for ensiling (Wilkinson, 2005). Many studies have reported using agricultural by-products as additives to improve the fermentation quality of forage (Huisden et al., 2009). For example, molasses and potato pulp have been used in the silage of straw (Baytok et al., 2005; Watson and Ferguson, 1937; Zhang et al., 2012). These results showed that adding these inexpensive components could significantly increase the concentrations of CP, WSC and lactic acid, as well as reduce pH and, NDF and ADF contents in rice straw. Furthermore, unpalatable byproducts can be improved and used in feed application after ensiling (Cao et al., 2009). Corn steep liquor may have the potential capability to improve rice straw quality as a nitrogen source for its high content in amino acids and polypeptides (Yu et al., 2008).

However, it is difficult for the lactic acid bacteria (LAB) to attach to rice straw during ensiling fermentation. Furthermore, silage of fresh rice straw spoils fast upon exposure to air. Therefore, dual-purpose inoculants containing homofermentative and heterofermentative LAB have been widely used in forage silage (Ranjit and Kung et al., 2000; Schmidt et al., 2009), which is helpful for the rapid production of lactic acid, preserving nutrients and increasing silage palatability, as well as improving the aerobic stability of silage (Hu et al., 2009; Kristensen et al., 2010; Schmidt et al., 2009; Schmidt and Kung, 2010).

The objective of this experiment was to investigate the effects of adding different proportion of CSL on the fermentation characteristics and aerobic stability of fresh rice straw silage.

The hypothesis of this study was that there is an appropriate adding ratio of CSL to obtain good quality fresh rice straw silage with higher protein content and long aerobic stability.

2. Materials and methods

2.1. Ensiling

Rice was harvested at the maturity stage. Fresh rice straw was collected from the Xiangfang Experimental Farm of Northeast Agricultural University (Harbin, China) and chopped to theoretical lengths of 2–3 cm by straw chopper. Corn steep liquor obtained from Cargill Biochemistry Co., Ltd (Songyuan, China) was used in the study. The following experimental treatments were generated: control without CSL (C1), C4 with 200 g/kg CSL and 800 g/kg fresh rice straw, fresh basis; C3 with 250 g/kg CSL and 750 g/kg fresh rice straw, fresh basis; C2 with 333 g/kg CSL and 667 g/kg fresh rice straw, fresh basis. Chemical compoisitons of CSL has been published (Li et al., 2016).

The LAB inoculant obtained from Northeast Agricultural University consisted of two strains of homofermentative LAB *Lactobacillus plantarum* and *Lactobacillus casei* in combination with heterofermentative *Lactobacillus buchneri*. The inoculants were applied at a rate of 50 mL/kg (wet basis) of forage with a sprayer, for each treatment, to achieve a final application rate of 1×10^6 cfu/g of fresh matter (FM). Approximately 300 g of fresh rice straw from each treatment was packed into a plastic bag (Polyethylene; 400 mm × 500 mm), and all of the bags were sealed with a vacuum sealer and stored at ambient temperature (18 ± 2°C) for 60 days. Duplicate silos for each treatment were opened after 0, 3, 7, 10, 20, 30, 45 and 60 d. The silages were randomly subsampled from several different positions, and then mixed to generate a composite sample for microbiological and chemical analysis. The rest of the corn silage sample bags were subjected to the aerobic stability test after 60 days.

2.2. Chemical and microbial analyses

The silage samples were dried at 65°C and analyzed for DM according to AOAC (1990) procedures. The CP content was measured by the Kjeldahl method (AOAC, 1990). The ADF and NDF were analyzed according to the procedures of Van Soest et al. (1991) using the Ankom system (Ankom 220 fiber analyzer; Ankom) with heat-stable α-amylase. Water-soluble carbohydrate (WSC) concentration was measured by the colorimetric method (Dubois et al., 1956). Both fresh and silage juice were extracted by blending 10 g forage (fresh basis) in 90 mL of distilled water and stored for 24 h at 4°C in a refrigerator (Nishino and Uchida, 1999). The homogenate mixture was then filtered through 4 layers of cheesecloth (Xing et al., 2009). Then, the filtrate was used for the determination of pH, ammonia-N (NH₃-N), lactic acid and VFA. The pH was directly measured using a pH meter (Sartorius Basic pH Meter, Germany). The NH₃-N concentration was determined by an ammonia-sensing electrode (Expandable Ion Analyzer EA 940, Orion, USA). Samples for VFA analysis were prepared as described by Li and Meng (2006). The concentrations of VFA were analyzed by gas–liquid chromatography (GC 2010, Tokyo, Japan) equipped with a flame-ionization detector and a FFAP capillary column (HP-INNOWAX, 30 m × 0.250 mm × 0.25 μm). The lactic acid content was determined by high-performance liquid chromatograph (Waters 600, Tokyo, Japan) following the procedure of Muck and Dickerson (1987).

Additional portions of fresh and silage juice were extracted by blending 10 g forage (fresh basis) in 90 mL of distilled water for 30 min at ambient temperature, followed by filtering through a 4 layers of cheesecloth. Lactic acid bacteria counts were determined by pour plating on MRS agar and enumeration of yeasts and molds by pouring on malt extract agar (Oxoid CM0059). Plates were incubated at 37°C for 48 h and numbers of colony-forming units were counted.

The aerobic stability test was determined by monitoring the temperature increase of silage samples due to microbial activity during exposed to air. Detailed measurement procedure was described in previous publish paper (Tabacco et al., 2011).

All of the chemical analyses were carried out in triplicate and expressed on a dry matter basis, with the exception of the microbiological data (%FM), DM content (%FM) and NH₃-N (% total nitrogen [TN]).

2.3. Statistical analysis

All of the microbial data were transformed to log units. The data were analyzed as a completely randomized design by using the SAS ANOVA procedure (SAS Institute, 2011). The results were presented as the mean values and standard error of the means. Differences between treatment means were determined by Duncan's multiple range test method. Differences among means with $P < 0.05$ were accepted as representing statistically significant differences.

3. Results and discussion

3.1. Chemical composition prior to ensiling

The chemical compositions of the treated and untreated fresh rice straw before ensiling are shown in Table 1. Wilkinson (2005) suggested that the DM contents should be in the range of 250–400 g/kg for forage silage. The DM contents of the 4 treatment forages were different due to the different addition levels of CSL, and they were all higher than 250 g/kg DM. In this study, to ensure the successful ensiling with maximal CSL absorption, no extra water was sprayed onto the forages to adjust the DM content of the

Table 1
Chemical compositions of the fresh rice straw with different levels of corn steep liquor (CSL) addition before ensiling.[1]

Item	Treatments[2]				SEM	P-value
	C1	C4	C3	C2		
DM, %	25.9[d]	29.9[c]	30.9[b]	32.6[a]	0.92	**
CP, %DM	4.78[d]	13.3[c]	15.3[b]	17.9[a]	1.86	**
NDF, %DM	62.0[a]	47.5[b]	42.7[c]	37.8[d]	3.43	**
ADF, %DM	38.0[a]	27.1[b]	26.3[c]	23.5[d]	2.09	**
WSC, %DM	9.79[a]	8.35[b]	6.56[c]	6.07[d]	0.56	**
Ash, %DM	16.0[b]	15.7[c]	16.0[b]	16.2[a]	0.06	**

WSC = water-soluble carbohydrate.
[a, b, c, d] Means in the same row with different letters differ ($P < 0.05$).
[1] **$P < 0.01$.
[2] C1: composed of fresh rice straw without CSL additive; C2: fresh rice straw mixed with CSL in the ratio of 2:1; C3: fresh rice straw mixed with CSL in the ratio of 3:1; C4: fresh rice straw mixed with CSL in the ratio of 4:1.

Table 3
Changes of pH value of fresh rice straw with different levels of corn steep liquor (CSL) addition during ensiling.[1]

Day	Treatments[2]				SEM	P-value
	C1	C4	C3	C2		
0	5.48[a]	4.01[b]	4.05[b]	3.99[b]	0.24	**
3	3.95[b]	4.07[a]	4.06[a]	4.02[a]	0.02	*
7	3.80[c]	4.08[a]	4.04[ab]	3.99[b]	0.04	**
10	3.94[b]	4.12[a]	4.08[a]	4.07[a]	0.03	**
20	4.10[b]	4.21[a]	4.18[a]	4.12[b]	0.02	*
30	3.89[b]	4.10[a]	4.09[a]	4.08[ab]	0.03	**
45	4.04	4.17	4.19	4.16	0.03	NS
60	4.05[b]	4.20[a]	4.19[a]	4.17[a]	0.02	**

[a, b,c] Means in the same row with different letters differ ($P < 0.05$).
[1] NS = not significant, *$P < 0.05$ and **$P < 0.01$.
[2] C1: composed of fresh rice straw without CSL additive; C2: fresh rice straw mixed with CSL in the ratio of 2:1; C3: fresh rice straw mixed with CSL in the ratio of 3:1; C4: fresh rice straw mixed with CSL in the ratio of 4:1.

4 groups to the same level. Because of the high CP content (37.00% DM) of CSL, the application of CSL significantly increased the CP content and decreased the NDF and ADF contents of the fresh rice straw silage compared with the content in C1 ($P < 0.05$). The concentration of WSC in 4 forage samples ranged from 6.07% to 9.79% DM, met the requirement for successful ensiling (3.0% of DM) (Haigh and Arker, 1985).

3.2. Changes of population of LAB, pH value, concentration of NH3-N (% of total N) during the ensiling time

Changes in the population of LAB of 4 treatments after 0, 3, 7, 10, 20, 30, 45 and 60 d are shown in Table 2. Before ensiling (0 d), the 4 groups were supplied with the same amount of LAB inoculants, approximately 7.00 $\log_{10}$ cfu/g of FM. In general, LAB reaches at least 5.00 $\log_{10}$ cfu/g of FM could be an important factor for a successful fermentation of silage (Cai et al., 1999). After 3 d of fermentation, the population of LAB in C1 increased rapidly and reached 9.01 $\log_{10}$ cfu/g of FM, which was significantly higher than the other 3 treatments ($P < 0.05$). The C4 and C3 experienced a slight growth trend after 3 and 7 d of fermentation compared with 0 d. After 10 d, the population of LAB decreased to 7.01 and 7.08 $\log_{10}$ cfu/g of FM, respectively. The lower pH value of C4, C3 and C2 (Table 3) might inhibit the growth of LAB during the initial stages of fermentation. The population of LAB in C1 and C2 peaked at 20 d and declined gradually after 30 and 45 d. The decrease of LAB over time was expected because low pH and lack of fermentable substrates result in the death of bacteria (MacDonald et al., 1991;

Table 2
Changes of the population of lactic acid bacteria (LAB) ($\log_{10}$ cfu/g) of fresh rice straw with different levels of corn steep liquor (CSL) addition during ensiling.[1]

Day	Treatments[2]				SEM	P-value
	C1	C4	C3	C2		
0	7.07	7.02	7.06	7.02	0.01	NS
3	9.00[a]	7.32[bc]	7.37[b]	7.08[c]	0.29	**
7	9.01[a]	7.27[b]	7.33[b]	6.88[c]	0.31	**
10	8.60[a]	7.01[bc]	7.08[b]	6.83[c]	0.27	**
20	9.24[a]	8.38[b]	7.55[c]	7.49[c]	0.27	**
30	8.79[a]	8.27[b]	7.25[c]	6.85[d]	0.29	**
45	7.93[b]	8.66[a]	8.03[b]	6.18[d]	0.35	**
60	8.06[b]	8.67[a]	8.22[b]	6.32[c]	0.34	**

[a, b, c, d] Means in the same row with different letters differ ($P < 0.05$).
[1] NS = not significant, **$P < 0.01$.
[2] C1: composed of fresh rice straw without CSL additive; C2: fresh rice straw mixed with CSL in the ratio of 2:1; C3: fresh rice straw mixed with CSL in the ratio of 3:1; C4: fresh rice straw mixed with CSL in the ratio of 4:1.

Pahlow et al., 2003). In contrast, the population of LAB in C4 and C3 began to increase again at 30 d. Heterofermentative (*L. buchneri*) LAB appeared to be the predominant bacterium in silages during this phase. The population of LAB in all 4 groups was stabled after 45 d. The number of LAB in C4 was significantly higher than that in C1, C2 and C3 ($P < 0.05$). After 60 d of ensiling, C2 had the lowest population of LAB. In the experiment of using CSL as a culture media for the production of succinic acid, when the CSL concentration increased to 20%, cell growth was poor due to high viscosity in the medium (Agarwal et al., 2006). Saxena and Tanner (2012) also reported that high concentrations of CSL remarkably inhibited cellular growth due to the presence of higher concentrations of some inhibitory components in CSL. High concentrations of dissolved sulfur are produced during wet milling processes when corn is soaked in dilute sulfurous acid to be softened for subsequent grinding and facilitation of starch liberation (Hull et al., 1996). Higher additive ratio of CSL increases the sulfur content in the fermentation medium, which inhabits the bacteria growth.

In Table 3, because of the low pH value of CSL (pH = 3.95), its mixture with fresh rice straw results in a low pH value of approximately 4.00, which may repress the growth of LAB during the initial stages of fermentation. Zheng et al. (2003) reported that the concentration of H^+ had an indirect effect on the growth of LAB and the productivity of lactic acid. The optimum initial pH of the silage material for the growth of *L. plantarum* was 5.5–6.5 (Kemp et al., 1989), as the decrease of pH suppresses the growth of LAB. At day 0, the pH of the mixture in three experimental groups all presented a low value due to the low pH of CSL. It is an acid based storage. After 3 d, the pH value of C1 decreased dramatically to 3.95, which is significantly lower than that in C2, C3 and C4 ($P < 0.05$). After 60 d of ensiling, the CSL treatment groups stayed at a low pH level, which was still higher than that of C1. In a study by Noro et al. (2004), CSL was used to maintain the pH within the optimal range during the production of bacterial cellulose, because of its buffering capacity, which could inhibit the decrease in pH. The pH of C1 began to grow at 30 d and stabilized at approximately 4.04 after 45 d. Higher values of pH were detected in C4, C3 and C2 than in C1 ($P < 0.05$) at 60 d. However, all of the values were lower than 4.20, which could be enough for the good preservation of silage feed. Liu et al. (1999) suggested that the optimum pH of silage was 4.0–4.3. Kung et al. (2000) reported that silages treated with ammonia had a higher final pH than did untreated silages or silages treated with buffered propionic-based preservative.

The C1 had a higher concentration of NH3-N (% of total N) compared with C4, C3 and C2 from 3 d to 45 d ensiling (Table 4).

Table 4
Changes of NH_3-N (% of total N) content of the fresh rice straw with different levels of corn steep liquor (CSL) addition during ensiling.[1]

Day	Treatments[2]				SEM	P-value
	C1	C4	C3	C2		
0	0.87[b]	1.85[a]	1.53[a]	1.50[a]	0.14	*
3	1.90	2.00	1.97	2.02	0.02	NS
7	2.87[a]	2.12[b]	2.03[b]	2.06[b]	0.13	**
10	2.90[a]	1.86[b]	1.94[b]	1.93[b]	0.16	**
20	3.40[a]	2.47[b]	2.47[b]	2.07[c]	0.19	**
30	3.99[a]	2.99[b]	2.42[c]	2.15[d]	0.27	**
45	4.32[a]	4.52[a]	2.98[b]	2.53[c]	0.33	**
60	4.98[a]	4.84[a]	3.22[b]	2.55[c]	0.40	**

[a, b, c, d] Means in the same row with different letters differ ($P < 0.05$).
[1] NS = not significant, *$P < 0.05$ and **$P < 0.01$.
[2] C1: composed of fresh rice straw without CSL additive; C2: fresh rice straw mixed with CSL in the ratio of 2:1; C3: fresh rice straw mixed with CSL in the ratio of 3:1; C4: fresh rice straw mixed with CSL in the ratio of 4:1.

After 60 d, higher concentrations of NH_3-N (% of total N) were detected in C1 and C4 compared with C2 and C3 ($P < 0.05$). Thus, more deamination occurred in C1 and C4. This result is in agreement with the results of the addition of urea or other Non-protein nitrogen sources to corn silages (Lopez et al., 1970; Schaadt and Johnson, 1969). The growing of heterofermentative *L. buchneri* in C4 promoted protein degradation, which resulted in the high concentration of NH_3-N. Previous studies have reported that the addition of *L. buchneri* could increase the concentration of NH_3-N in corn and sorghum silage (Filya, 2003). McDonald et al. (2002) reported that well-preserved silages should contain less than 100 g NH_3-N/kg TN. The NH_3-N (% of total N) in the trial groups and in C1 were all less than 100 g NH_3-N/kg TN. The CP in the silage was well preserved.

3.3. Chemical composition of fresh rice straw and CSL silages after 60 d fermentation

In Table 5, the DM loss (DML) in C3 and C2 were significantly higher than those in C1 and C4 ($P < 0.05$), which were 3.76% and 4.09%, respectively. Jatkauskas et al. (2013) reported that the growth of LAB inoculant in silage could significantly reduce DM loss. The CP content of C2, C3 and C4 ranged from 14.99% to 21.20%, which was significantly higher than the CP content of C1 ($P < 0.05$). The highest NDF and ADF content were found in C1 ($P < 0.05$). These results indicated that the addition of CSL significantly improved the quality of the rice straw silage. Concentration of WSC was lower in C1 and C4 compared with C3 ($P < 0.05$), indicating that WSC was better utilized by the fermentation bacteria in C1 and C4 to produce sufficient lactic acid to decrease pH and inhibit the growth of harmful bacteria. This result is in agreement with Cao et al. (2011). In addition, C1 and C4 had higher water-soluble carbohydrate loss (WSCL) than C3 and C2 ($P < 0.05$). The large population of LAB especially the strain of heterofermentative (*L. buchneri*) contributed to higher consumption of WSC (Kung and Ranjit, 2001).

3.4. Organic acid concentration of fresh rice straw and CSL silages after 60 d fermentation

The effects of different addition level of CSL on the fermentation characteristics of fresh rice straw silage are shown in Table 5. Kılıc (1986) reported that the concentration of lactic acid in good quality silage should be greater than 2%. The lactic acid content in C4 and C3 were 5.32% and 5.52% DM, respectively, which were significantly

Table 5
Effect of different levels of corn steep liquor (CSL) addition on the chemical compositions, concentrations of organic acid and aerobic stability (hours for the feed temperature to increase 2°C above ambient temperature) of fresh rice straw silage after 60 d fermentation.[1]

Item	Treatments[2]				SEM	P-value
	C1	C4	C3	C2		
DM, %	25.5[d]	29.3[c]	29.7[b]	31.2[a]	0.79	**
DML, %	1.53[c]	2.13[b]	3.76[a]	4.09[a]	0.41	**
CP, %DM	5.65[d]	14.99[c]	17.32[b]	21.20[a]	2.17	**
NDF, %DM	63.5[a]	50.0[b]	44.4[c]	38.4[d]	2.80	**
ADF, %DM	41.5[a]	32.0[b]	27.7[c]	23.6[d]	2.00	**
WSC, %DM	0.69[c]	0.77[bc]	1.22[a]	1.02[ab]	0.08	**
WSCL, %	93.0[a]	90.8[a]	81.4[b]	83.3[b]	1.89	**
Ash, %DM	17.5[b]	18.0[a]	17.2[c]	17.2[c]	0.12	**
Concentration of organic acid						
Lactic acid, % DM	3.89[c]	5.32[ab]	5.52[a]	4.68[b]	0.25	*
Acetic acid, % DM	2.57[a]	2.72[a]	1.60[b]	1.24[c]	0.24	**
LA:AA, % DM	1.52[b]	1.96[b]	3.46[a]	3.80[a]	0.37	**
Butyric acid, %DM	0.89[b]	1.06[a]	–	–	0.19	**
Propionic acid, %DM	–	–	–	–	–	–
Aerobic stability, h	>168[a]	63[b]	37[c]	34[c]	3.76	**

DML = dry matter loss; WSCL = water-soluble carbohydrate (WSC) loss. LA: AA = lactic acid: acetic acid.
[a, b, c, d] Means in the same row with different letters differ ($P < 0.05$).
[1] *$P < 0.05$ and **$P < 0.01$.
[2] C1: composed of fresh rice straw without CSL additive; C2: fresh rice straw mixed with CSL in the ratio of 2:1; C3: fresh rice straw mixed with CSL in the ratio of 3:1; C4: fresh rice straw mixed with CSL in the ratio of 4:1.

higher than those in C1 (3.89% DM) ($P < 0.05$). Muck and Kung (1997) reported that moderate concentrations of nitrogen source additives such as ammonia could increase the concentrations of lactic and acetic acids. However, Britt and Huber (1976) showed that high concentrations of ammonia depressed lactic acid formation throughout the ensiling period because of the delayed growth of LAB. This finding was in agreement with the population of LAB (Table 2) in this study. The C4 and C1 had significantly higher concentrations of acetic acid compared with C3 and C2 ($P < 0.05$). Acetic acid has been regarded as a potent inhibitor of fungi, which plays an active role in aerobic deterioration (MacDonald et al., 1991; Woolford, 1975); thus, its high concentrations were more likely the primary reason for improvements in the aerobic stability of silages treated with *L. buchneri*. Kung and Stokes (2001) suggested that a desirable lactic acid to acetic acid ratio of greater than 3:1 would be an indication of more dominant homolactic fermentation. In Table 5, LA: AA in C4 and C1 were 1.96 and 1.52, indicating that heterofermentative LAB dominates the ensiling process in these two groups. Heterofermentative LAB (*L. buchneri*) could improve aerobic stability by fermenting lactic acid to acetic acid and 1,2 propanediol (Oude Elferink et al., 1999). Kung et al. (2000) showed that treatment with ammonia could decrease the ratio of lactic acid to acetic acid and increase the NH_3-N concentration in silage. Theoretically, silages treated with *L. buchneri* also contain higher concentrations of propionic acid (Kung and Ranjit, 2001), but the amounts of it in this study were less than 0.01% DM (data not shown).

3.5. Aerobic stability

The aerobic stability of different types of silage after 60 days of ensiling is presented in Table 5. The C1 remained unheated throughout 168 h of monitoring, which was significantly longer than the other three treated silages ($P < 0.05$). As the addition level of CSL increased, the silages were inclined to spoil upon exposure to air. The C3 and C2 only remained unspoiled for 37 and 34 h,

respectively. The aerobic deterioration of silage is undesirable and reduces the nutritional value; in particular, residual WSC and residue lactic acid increase the risk of the proliferation of undesirable microorganisms (MacDonald et al., 1991). Higher concentrations of residual WSC in C3 and C2 could affect aerobic stability at feed-out, owing to more rapid fungal growth on WSC than that on fermentation products (Muck and Bolsen, 1991). Additionally, higher concentrations of lactic acid in C3 and C2 can be used as substrates for growth by yeast during aerobic exposure (Kung and Ranjit, 2001). Li et al. (2013) showed that ensiled dry rice straw with CSL and LAB inoculants had an aerobic stability of more than 372 h. High moisture content also contributed to the growth of yeasts and mold, which were associated with aerobic spoilage (Guo et al., 2013). Acetic acid has strong antifungal properties (Woolford, 1975), which could very well explain the long preservation of C1 (>168 h) and C4 (63 h).

4. Conclusions

The adding proportion of CSL at 250 g/kg (C3) could obtain high protein content silage, but the aerobic stability of fresh rice straw is significantly decreased. In conclusion, mixing fresh rice straw with CSL at addition level of 4:1 (C4) and 3:1 (C3) can improve the fermentation quality and nutrient composition of fresh rice straw silage.

Acknowledgements

References

Agarwal L, Isar J, Meghwanshi GK, Saxena RK. A cost effective fermentative production of succinic acid from cane molasses and corn steep liquor by Escherichia coli. J Appl Microbiol 2006;100:1348–54.

AOAC. Official methods of analysis of AOAC international. 15th ed. Arlington: Association of official Analytical Chemists; 1990.

Baytok E, Aksu T, Karsli MA, Muruz H. The effects of formic acid, molasses and inoculant as silage additives on corn silage composition and ruminal fermentation characteristics in sheep. Turk J Vet Anim Sci 2005;29:469–74.

Britt DG, Huber JT. Preservation of and animal performance on high moisture corn treated with ammonia or propionic acid 1, 2. J Dairy Sci 1976;59:668–74.

Cai Y, Benno Y, Ogawa M, Kumai S. Effect of applying lactic acid bacteria isolated from forage crops on fermentation characteristics and aerobic deterioration of silage. J Dairy Sci 1999;82:520–6.

Cao Y, Cai Y, Takahashi T, Yoshida N, Tohno M, Uegaki R, et al. Effect of lactic acid bacteria inoculant and beet pulp addition on fermentation characteristics and in vitro ruminal digestion of vegetable residue silage. J Dairy Sci 2011;94:3902–12.

Cao Y, Takahashi T, Horiguchi K-i. Effects of addition of food by-products on the fermentation quality of a total mixed ration with whole crop rice and its digestibility, preference, and rumen fermentation in sheep. Anim Feed Sci Technol 2009;151:1–11.

Chiani M, Akbarzadeh A, Farhangi A, Mehrabi MR. Production of desferrioxamine B (Desferal) using corn steep liquor in Streptomyces pilosus. Pak J Biol Sci 2010;13:1151–5.

Dubois M, Gilles KA, Hamilton JK, Rebers P t, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem 1956;28:350–6.

Filya I. 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 2003;86:3575–81.

Gao L, Yang H, Wang X, Huang Z, Ishii M, Igarashi Y, et al. Rice straw fermentation using lactic acid bacteria. Bioresour Technol 2008;99:2742–8.

Guo X, Undersander D, Combs D. Effect of Lactobacillus inoculants and forage dry matter on the fermentation and aerobic stability of ensiled mixed-crop tall fescue and meadow fescue. J Dairy Sci 2013;96:1735–44.

Haigh P, Arker J. Effect of silage additives and wilting on silage fermentation, digestibility and intake, and on liveweight change of young cattle. Grass Forage Sci 1985;40:429–36.

Han YW. Microbial fermentation of rice straw: nutritive composition and in vitro digestibility of the fermentation products. Appl Microbiol 1975;29:510–4.

Hu W, Schmidt RJ, McDonell EE, Klingerman CM, Kung Jr L. 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 2009;92:3907–14.

Huisden C, Adesogan A, Kim S, Ososanya T. 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 2009;92:690–7.

Hull SR, Yang BY, Venzke D, Kulhavy K, Montgomery R. Composition of corn steep water during steeping. J Agr Food Chem 1996;44:1857–63.

Jatkauskas J, Vrotniakiene V, Ohlsson C, Lund B, Helgadóttir Á, Hopkins A. Effects of combined lactic acid bacteria strains on fermentation, aerobic deterioration and mould growth in lucerne big bale silage. In: The role of grasslands in a green future: threats and perspectives in less favoured areas. Proceedings of the 17th Symposium of the European Grassland Federation, Akureyri, Iceland; 23–26 June 2013. p. 237–9.

Kemp TL, Karim MN, Linden JC, Tengerdy RP. Response surface optimization of Lactobacillus plantarum batch growth. Biotechnol Lett 1989;11:817–20.

Kılıç A. Silage. Izmir: Bilgehan Publishing; 1986. p. 327.

Kristensen NB, Sloth KH, Højberg O, Spliid NH, Jensen C, Thøgersen R. Effects of microbial inoculants on corn silage fermentation, microbial contents, aerobic stability, and milk production under field conditions. J Dairy Sci 2010;93:3764–74.

Kung L, Robinson JR, Ranjit NK, Chen JH, Golt CM, Pesek JD. Microbial populations, fermentation end-products, and aerobic stability of corn silage treated with ammonia or a propionic acid-based preservative. J Dairy Sci 2000;83:1479–86.

Kung Jr L, Stokes MR. Analyzing silages for fermentation end products. University of Delaware College of Agriculture & Natural Resources; 2001.

Kung L, Ranjit NK. The effect of Lactobacillus buchneri and other additives on the fermentation and aerobic stability of barley silagel. J Dairy Sci 2001;84:1149–55.

Liu B, Yang M, Qi B, Chen X, Su Z, Wan Y. Optimizing L-(+)-lactic acid production by thermophile Lactobacillus plantarum As. 1.3 using alternative nitrogen sources with response surface method. Biochem Eng J 2010;52:212–9.

Liu YX, Yang ZH, Ye JA, Shi ZQ, Wu YM. Reasonable silage modulation and quality evaluation standards. Feed Ind Mag 1999;20(3):4–7 [in chinese].

Li XX, Zhang YG, Zhang WW, Zhang N, Liu KY, Li CL, et al. Effects of mixing corn steep liquor with dry rice straw in different proportions on fermentation quality and nutrient composition of yellow rice straw silage feed. Chin J Anim Sci 2013;25(11):2682–8 [in chinese].

Li X, Xu W, Yang J, Zhao H, Pan C, Ding X, et al. Effects of applying lactic acid bacteria to the fermentation on a mixture of corn steep liquor and air-dried rice straw. Anim Nutr 2016;3(2):229–33.

Li Y, Meng Q. Effect of different types of fibre supplemented with sunflower oil on ruminal fermentation and production of conjugated linoleic acids in vitro. Arch Anim Nutr 2006;60(5):402–11.

Lopez J, Jorgensen N, Niedermeier R, Larsen H. Redistribution of nitrogen in urea-treated and soybean meal—treated corn silage. J Dairy Sci 1970;53:1215–24.

MacDonald P, Henderson A, Heron S. The biochemistry of silage. Chapter 2: Crops for Silage. Great Britain: Chalcome Publications; 1991.

Muck R, Dickerson J. Storage temperature effects on proteolysis in alfalfa silage. Am Soc Agric Eng 1987.

Muck R, Kung Jr L. Effects of silage additives on ensiling. Silage Field Feed 1997. NRAES-99.

Muck RE, Bolsen KK. Silage preservation and silage additive products. Hay Silage Manag N. Am 1991;105.

Nascimento RP, Junior NA, Pereira Jr N, Bon EPS, Coelho RRR. Brewer's spent grain and corn steep liquor as substrates for cellulolytic enzymes production by Streptomyces malaysiensis. Lett Appl Microbiol 2009;48:529–35.

Nishino N, Uchida S. Laboratory evaluation of previously fermented juice as a fermentation stimulant for lucerne silage. J Sci Food Agric 1999;79:1285–8.

Noro N, Sugano Y, Shoda M. Utilization of the buffering capacity of corn steep liquor in bacterial cellulose production by Acetobacter xylinum. Appl Microbiol Biotechnol 2004;64:199–205.

Oude Elferink SJWH, Driehuis F, Gottschal JC, Spoelstra SF. Anaerobic degradation of lactic acid to acetic acid and 1,2-propanediol, a novel fermentation path way in Lactobacillus buchneri, helps to improve the aerobic stability of maize silage. In: Proc. XIIIInt. Silage conf. Swedish Univ. of Agric.Sci., Uppsala, Sweden; 1999. p. 266–7.

Pahlow G, Muck RE, Driehuis F, Elferink SJ, Spoelstra SF. Microbiology of ensiling. Silage Sci Technol 2003:31–93.

Ranjit NK, Kung L. The effect of Lactobacillus buchneri, Lactobacillus plantarum, or a chemical preservative on the fermentation and aerobic stability of corn silage. J Dairy Sci 2000;83(3):526–35.

SAS Institute. SAS User's Guide, Version 9.3. Cary, NC: SAS Institute Inc.; 2011.

Saxena J, Tanner R. Optimization of a corn steep medium for production of ethanol from synthesis gas fermentation by Clostridium ragsdalei. World J Microbiol Biotechnol 2012;28:1553–61.

Schaadt H, Johnson R. Effect of ensiling time on distribution and losses of nitrogen in corn silage treated with limestone and NPN. J Anim Sci 1969;29:57–61.

Schmidt RJ, Hu W, Mills JA, Kung Jr L. The development of lactic acid bacteria and Lactobacillus buchneri and their effects on the fermentation of alfalfa silage. J Dairy Sci 2009;92:5005–10.

Schmidt R, Kung Jr L. 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 2010;93:1616—24.

Silveira M, Wisbeck E, Hoch I, Jonas R. Production of glucose—fructose oxidoreductase and ethanol by Zymomonas mobilis ATCC 29191 in medium containing corn steep liquor as a source of vitamins. Appl Microbiol Biotechnol 2001;55: 442—5.

Silva AC, Guimaraes PMR, Teixeira JA, Domingues Lucília. Fermentation of deproteinized cheese whey powder solutions to ethanol by engineered Saccharomyces cerevisiae: effect of supplementation with corn steep liquor and repeated-batch operation with biomass recycling by flocculation. J Ind Microbiol Biotechnol 2010;37(9):973—82.

Tabacco E, Piano S, Revello-Chion A, Borreari G. 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 2011;94(11):5589—98.

Van Soest P v, Robertson J, Lewis B. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 1991;74:3583—97.

Watson S, Ferguson W. The losses of dry matter and digestible nutrients in low-temperature silage, with and without added molasses or mineral acids. J Agric Sci 1937;27:67—107.

Wee Y, Kim H, Yun J, Ryu H. Pilot-scale lactic acid production via batch culturing of Lactobacillus sp. RKY2 using corn steep liquor as a nitrogen source. Food Technol Biotechnol 2006a;44:293.

Wee YJ, Yun JS, Kim D, Ryu HW. Batch and repeated batch production of l(+)-lactic acid by Enterococcus faecalis RKY1 using wood hydrolyzate and corn steep liquor. J Ind Microbiol Biotechnol 2006b;33:431—5.

Wilkinson JM. In: Wilkinson JM, editor. Silage. Part 6: assessing silage quality. Chapter 19: analysis and clinical assessment of silage. UK: Chalcombe Publications; 2005. p. 198—208.

Woolford MK. Microbiological screening of the straight chain fatty acids (c1-c12) as potential silage additives. J Sci Food Agric 1975;26:219—28.

Xie G, Wang X, Ren L. China's crop residues resources evaluation. Sheng wu gong cheng xue bao = Chin J Biotechnol 2010;26(7):855—63 [in chinese].

Xing L, Chen LJ, Han LJ. The effect of an inoculant and enzymes on fermentation and nutritive value of sorghum straw silages. Bioresour Technol 2009;100(1):488—91.

Yu L, Lei T, Ren X, Pei X, Feng Y. Response surface optimization of l-(+)-lactic acid production using corn steep liquor as an alternative nitrogen source by Lactobacillus rhamnosus CGMCC 1466. Biochem Eng J 2008;39:496—502.

Zhang W, Zhang Y, Liu Z. Effect of different absorbents on fermentation quality of wet potato pulp. J Anim Vet Adv 2012;11:4230—5.

Zheng Y, Wang CL, Gu XB. Influence of pH on the growth of Lactobacillus casei G-7 and lactic acid production. Food Ferment Ind 2003;29(6):35—8 [in chinese].