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Effects of Treating Whole-plant or Chopped Rice Straw Silage with Different Levels of Lactic Acid Bacteria on Silage Fermentation and Nutritive Value for Lactating Holsteins

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ABSTRACT : Two experiments were carried out to investigate i) the effects of four levels of lactic acid bacteria inoculants (LAB; 0, 2×10^5, 3×10^5 and 4×10^5 cfu/g fresh forage) and two physical forms of rice straw (whole and chopped rice straw) on silage fermentation quality and nutritive value of rice straw (RS) silage for lactating Holsteins and ii) the effects of the replacement of corn silage (CS) with different inclusion levels (0, 25 and 50%) of LAB treated RS on lactating performance of Holstein dairy cows. Rice straw packed with stretch film was ensiled for 45 d. The results showed that the higher level of LAB inoculants in the silage quadratically decreased pH, NH_3-N and acetic acid concentrations and increased the contents of lactic acid and total organic acids. The CP content and DM losses in the silage declined linearly as the level of LAB addition was increased. Compared with whole-plant rice straw silage (WRS), chopped rice straw silage (CRS) dramatically reduced pH by 0.83. The concentrations of NH_3-N were similar in WRS and CRS and both were less than 50 g/kg of total N. Chopping rice straw before ensiling significantly enhanced the lactic acid concentration and total organic acids content whereas the concentration of acetic acid declined. The CP, NDF and ADF content of CRS was 13.4, 5.9 and 10.2% lower than in WRS, respectively. Except for butyric acid concentration, significant interaction effects of inoculation level and physical form of RS were found on all fermentation end-products. Our findings indicated that milk yield and composition were not affected by different level of RS inclusion. However, because of the lower cost of WRS, cows consuming a ration in which WRS was partially substituted for CS had 3.48 Yuan (75% CS+25% WRS) and 4.56 Yuan (50% CS+50% WRS) more economic benefit over those fed a CS-based ration. It was concluded that the chopping process and LAB addition could improve the silage quality, and that substitution of corn silage with RS silage lowered the cost of the dairy cow ration without impairing lactation performance. (**Key Words :** Whole-plant Rice Straw, Lactic Acid Bacteria, Chopping Process, Silage Quality, Performance, Holstein)

INTRODUCTION

Rice straw (RS) is an abundant by-product of rice production. Statistics shows that about 188 million tons of RS are produced annually in China (Guo et al., 2002). However, only 15% of RS is used in animal rations and most of them are accumulated or burned in the field such that not only considerable RS is wasted but also serious environmental pollution occurs (Liu et al., 2005). Moreover, the rapid development of the dairy industry in northeastern China, along with the shortage of good quality forages, has provoked an urgent need to search for locally available forages to be included in the ration of dairy cows.

Ensiling is regarded as a good forage preservation method which has been widely used for many years, since fermentation by some microbes is an effective way to improve the digestibility, palatability and nutritive value of straws (Nishino et al., 2004; Brusetti et al., 2006; Gao et al., 2008). However, previously RS silages could not be widely used in northeastern China due to two reasons. Firstly, in some rice varieties, stems were too dry to be ensiled successfully at the time of harvesting. Secondly, there is a lack of suitable ensiling facilities. However, introduction of new rice varieties and invention of a rice harvester after threshing has made ensiling possible. The new varieties of paddy rice (e.g. var. Dongnong 03-28, var. Kongyu 131, var. Ken 95-295 and etc.) are being introduced and applied in the cropping system now. These varieties have mid-late maturity and the crop can be harvested at full-ripening stage while the stems contain high moisture and nutrient content which is more favorable for ensiling. Since the viable lactic

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acid bacteria (LAB) population attached to the whole plant is not enough to enhance LAB dominant fermentation (less than 10^2, cfu/g; Yu et al., 2008), it is deemed necessary to supply inoculants to rice straw for successful making of silage (Driehuis et al., 1999a; Weinberg et al., 1999; 2002).

The effects of LAB additive on the fermentation characteristics of alfalfa silage (Filya et al., 2007; Tyrolova and Vyborna, 2008), grass silage (Driehuis et al., 2001), corn and sorghum silage (Filya, 2003; 2006) have been extensively studied. However, there is little information on application of LAB additive to RS silage (Gao et al., 2008). The current study was therefore aimed: i) to investigate the effects of LAB on silage fermentation quality and nutritive value of RS; ii) to compare silage quality of whole-plant RS silage (WRS) with chopped RS silage (CRS); and iii) to investigate whether corn silage can be partially substituted with LAB-treated WRS in diets of dairy cows without impairing lactation performance.

MATERIALS AND METHODS

Rice straw and inoculants

Fresh rice straw (*Oryza sativa* var. Dongnong 03-28) was obtained from the Xiangfang Experimental Farm of the Northeast Agricultural University (Harbin, China). The rice was harvested at full-ripening stage while the stems had high moisture content (60-75%) and were still green. Lactic acid bacteria inoculants were provided by Chr. Hansen Biosystems (Milwaukee, WI, USA) which contained 1.8×10^{10} colony-forming units (cfu)/g of *Lactobacillus buchneri* and *Pediococcus pentosaceus*.

Ensiling

Rice straw was ensiled whole or chopped to a theoretical length of 6-cm using a forage harvester (Model Jialian-3086, Jiamusi Jialian Harvest Machinery Co., Ltd, China). Chopped and whole rice straw was treated with four different levels of LAB inoculants i.e. i) no additive, ii) 2×10^5 cfu LAB/g of fresh forage, iii) 3×10^5 cfu LAB/g of fresh forage, and iv) 4×10^5 cfu LAB/g of fresh forage. Microbial inoculants were dissolved in deionized water and sprayed uniformly on the RS constantly and thoroughly. Approximately 30 kg of RS was then packed and sealed with stretch film (Model FSB-1300, Shenyang Fangke Machinery Manufacturing Co., Ltd, China) and stored at ambient temperature (20 to 25°C) in an enclosed barn for 45 d. Each treatment was ensiled in three experimental silos using a total of 24 packs. After completion of ensiling time, silos were opened; the RS silages were randomly sampled by drawing vertical cores from three different positions, and then mixed thoroughly to get a composite sample. Samples were stored on ice until taken back to the laboratory for chemical analyses.

Performance trial

All procedures involving animals were approved by the Animal Care and Use Committee, Animal Science and Technology College, Northeast Agricultural University (Heilongjiang, China). Thirty lactating Holstein dairy cows (153±12 DIM and 20.8±3.1 kg/d daily milk yield) were used in a 10 wk performance trial (1 wk pre-treatment period followed by a 9 wk treatment period). Cows were blocked by lactation stage and randomly assigned to each of three different silage source diets (10 cows per treatment). Silage was obtained from the whole-plant rice straw treated with LAB (the same inoculants as those used in silo study) to achieve a final application rate of 3×10^5 cfu/g of fresh forage. Then 0, 25 and 50% of the corn silage (CS; without any additive) was replaced with WRS in the forage component of the diet. All the diets were formulated to meet National Research Council (NRC, 2001) requirements. Diets contained forage and concentrate mix in a ratio of 53:47 (DM basis) and were fed *ad libitum* to cows as total mixed ration (TMR). Cows were housed in tie stalls and were fed individually for at least 5 to 10% refusal (as-fed basis). The cows had free access to freshwater. Samples of silages and TMR were collected twice weekly and composited for chemical analyses.

Cows were milked twice at the milking parlor and milk yield was recorded daily. Milk was sampled proportionately to milk yield from consecutive morning and evening milkings and analyzed immediately for fat, protein, solids-not-fat and lactose by a mid-infrared spectrophotometer (Model MilkoScan 4000, FOSS NIR Systems, USA) following the manufacturer's instructions.

Laboratory analyses

Silage samples were analyzed for DM, CP, (AOAC, 1997) NDF and ADF (Van Soest et al., 1991). Heat stable α-amylase was applied in NDF analysis.

According to the method of Nishino and Uchida (1999), a 20 g silage sample was homogenized with 180 ml of distilled water and stored overnight at 4°C in a refrigerator. Then the mixture was filtered through Whatman 54 filter paper (Sinopharm Chemical Reagent Beijing Co., Ltd, China) and the filtrate was used for pH, ammonia-N, lactic acid and VFA determination. The pH was directly measured by pH meter (Thermo Orion, USA). The NH_3-N concentration was measured by ammonia-sensing electrode (Expandable Ion Analyzer EA 940, Orion, USA). Lactic acid concentration was analyzed using high performance liquid chromatography (Waters 600E, USA) following the procedure of Muck and Dickerson (1988). Samples for VFA analysis were prepared as described by Li and Meng (2006), and VFA concentrations were determined using gas chromatography (GC-2010, Shimadzu,Japan) with a 30 m FFAP capillary column (0.25 mm i.d. and 0.30 μm film thickness). Each analysis was performed in triplicate.

Statistical analysis

Data were subjected to statistical analysis using the general linear model (GLM) procedure of SAS (1999) for a completely randomized design to test the main and interactive effects of physical processing (whole-plant or chopped) and LAB addition on fermentation pattern and nutritive value of ensiled rice straw. Orthogonal polynomial contrast was used to examine the linear and quadratic responses of increasing the LAB additive supplement. The data for lactation performance was analyzed with repeated measures, using the PROC MIXED procedure of SAS. The model used was

$$Y_{ijk} = \mu+T_i+S_j+C\ (S)_{j(k)}+e_{ijk}$$

where Y_{ijk} is the observation, μ is the overall mean, T_i is fixed effect of treatment, S_j is fixed effect of stage of lactation, $C\ (S)_{j(k)}$ is random effect of cows nested in the stage of lactation, and e_{ijk} is the residual error term. The interaction of stage of lactation with treatment was excluded from the model because it was not significant for the primary response variables. Least squares means are reported throughout, and significance was declared at $p<0.05$ unless otherwise noted.

RESULTS AND DISCUSSION

The pH and ammonia nitrogen

The data on silage fermentation quality of different LAB addition in WRS or CRS are presented in Table 1. The pH is often considered as one of the crucial factors in evaluating the fermentation quality of silage (Muck, 1988). Chopping RS dramatically decreased pH by 17.8% so that pH fell below 4.0. Chopping the RS before storage made the compaction of materials easier and therefore provided an optimum anaerobic condition for the activity of lactic acid producing bacteria and fermentation of substrates in RS. The close correlation between chopping length and fermentation quality of grass silage found by Panditharatne et al. (1988) could partly explain the pH changes in our study. The results showed that pH declined quadratically as the inclusion level of LAB increased (p = 0.002) suggesting the higher the inclusion level of LAB the better the quality of RS.

Increasing the level of LAB quadratically decreased NH_3-N concentration (p = 0.01), which was consistent with the findings of Carpintero et al. (1979) and Heron et al. (1989) who found that proteolysis in silage increased with increasing pH within the range of 3 to 7. Many researchers reported an increase in NH_3-N when adding *L. buchneri* to alfalfa (Kung et al., 2003; Schmidt et al., 2009), grass (Driehuis et al., 2001) and corn and sorghum (Filya, 2003), but in our research, combined addition of lactic acid bacterium *L. buchneri* with *P. pentousaceus* did not increase

Table 1. The effect of different levels of lactic acid bacteria addition and physical treatments on fermentation quality of rice straw silages

Item	Whole-plant or Chopped rice straw		LAB addition level, cfu/g fresh forage				SEM	p			
	WRS[1]	CRS[2]	0	2×10^5	3×10^5	4×10^5		Whole-plant or Chopped rice straw	LAB addition level: Linear	LAB addition level: Quadratic	Whole-plant or Chopped rice straw × LAB addition level
pH	4.66[a]	3.83[b]	4.34	4.42	4.24	3.99	0.07	<0.0001	0.001	0.002	0.01
NH_3-N (g kg^{-1} TN[3])	5.14	4.98	6.38	5.62	4.66	3.58	0.20	0.29	<0.0001	0.01	0.004
Organic acids (% DM)											
Lactic acid	1.06[b]	2.45[a]	1.50	1.66	1.77	2.09	0.02	<0.0001	<0.0001	<0.0001	0.002
Acetic acid	0.50[a]	0.45[b]	0.45	0.51	0.48	0.46	0.004	<0.0001	0.01	<0.0001	<0.0001
Propionic acid	0.27	0.27	0.27	0.28	0.27	0.27	0.0001	0.24	<0.0001	<0.0001	<0.0001
Butyric acid	0.33[b]	0.34[a]	0.32	0.35	0.33	0.35	0.01	0.01	0.01	0.34	0.07
Total acid	2.16[b]	3.52[a]	2.54	2.80	2.85	3.17	0.02	<0.0001	<0.0001	0.001	<0.0001
Individual organic acid (%)											
Lactic acid	48.61[b]	69.66[a]	56.52	56.60	59.48	63.94	0.26	<0.0001	<0.0001	<0.0001	<0.0001
Acetic acid	23.39[a]	12.79[b]	19.04	19.77	18.14	15.42	0.22	<0.0001	<0.0001	<0.0001	<0.0001
Propionic acid	12.67[a]	7.80[b]	11.03	10.56	10.14	9.20	0.07	<0.0001	<0.0001	<0.0001	0.01
Butyric acid	15.33[a]	9.75[b]	13.42	13.07	12.24	11.44	0.18	<0.0001	<0.0001	0.004	0.01
L:A[4]	2.14[b]	5.46[a]	3.55	3.47	3.69	4.49	0.04	<0.0001	<0.0001	<0.0001	0.0003

[1] WRS = Whole-plant rice straw silage. [2] CRS = Chopped rice straw silage. [3] TN = Total nitrogen. [4] L:A = Lactic acid:acetic acid.
[a, b] Means within the same row denoted by different letters differ from each other (p<0.05).

the NH_3-N concentration in RS silage. Jatkauskas and Vrotniakiene (2004) also found that inoculating grass silage with homolactic inoculants of LAB lowered NH_3-N content, which was attributed to the fact that pH declined to a lower level faster, resulting in inhibition of proteolytic bacteria (Whiter and Kung, 2001). Although WRS had a numerically higher NH_3-N concentration than CRS, the chopping process did not affect NH_3-N concentration. However, the absolute values indicated that WRS could be considered as acceptable silage according to the report of Wilkinson (1990) who documented that optimally-preserved silage must have a NH_3-N concentration of less than 50 g/kg of total N.

Organic acids

The concentration of lactic acid and its percentage in total organic acids was significantly higher in CRS than in WRS ($p<0.0001$). This phenomenon might be easily explained by the fact that the chopping process tended to increase the epiphytic microflora populations as opposed to those on the standing crops. Moreover, chopping enhances the group of bacteria containing *Lactobacilli, Pediococci* and *Leuconostocs* (Lin et al., 1992). The ratio of lactic acid to acetic acid is a good indicator of the type of fermentation which the silage has undergone. A ratio of at least 2:1 indicates strong homolactic fermentation, suggesting homolactic acid bacterium *P. pentousaceus* is the predominant bacterium during ensiling which is supported by our findings. The LAB addition quadratically increased lactic acid concentration in RS silage.

Acetic acid has been considered as a good antifungal component in naturally fermented silages and shown to improve the aerobic stability (Danner et al., 2003; Holzer et al., 2003) due to its capacity to inhibit yeast and mold growth (Moon, 1983). Despite the yeast and mold numbers not being measured in the present experiment, higher acetic acid concentration in WRS indicated that CRS had less potential for better aerobic stability of silage. The 2×10^5 cfu LAB per gram of fresh forage had the highest acetic acid concentration compared with the other treatments. A similar observation was reported previously by Driehuis et al. (2001) who found significantly lower acetic acid concentration with the bacterial additive containing *L. buchneri* and *P. pentousaceu* compared with the control (no additive) after 90 days of grass ensilage in jars.

Propionic acid concentration did not differ among treatments, while its percentage in total organic acids differed significantly. Quadratically lower percentages of propionic acid were observed in RS treated with LAB which was consistent with findings of Driehuis et al. (2001). However, inconsistent effects of lactic acid bacteria on increasing propionic acid concentration were observed in the study of Kung et al. (2003). The reason for such a discrepancy was not clear.

During the ensiling period, acetic and lactic acids are fermentation end-products which improve silage quality (Gao et al., 2005), while butyric acid often deteriorates silage quality (McDonald et al., 1991). The low concentration of butyric acid has been identified as one of the factors that inhibit the growth of fungi (Muck, 1988). The relatively low percentage of butyric acid in total organic acids when chopping RS and increasing the level of LAB addition to silage was an indicator of good fermentation quality and stability observed in the present study. The trend of total organic acid content among treatments was in accordance with the pH changes in

Table 2. The effect of different levels of lactic acid bacteria addition and physical treatments on chemical composition and DM loss of rice straw silages

Item	Whole-plant or Chopped rice straw		LAB addition level, cfu/g fresh forage				SEM	p			
								Whole-plant or Chopped rice straw	LAB addition level		Whole-plant or Chopped rice straw × LAB addition level
	WRS[1]	CRS[2]	0	2×10^5	3×10^5	4×10^5			Linear	Quadratic	
Chemical composition											
DM	28.91[b]	30.10[a]	29.30	29.74	29.75	29.23	0.67	0.04	0.95	0.36	0.05
CP (% DM)	5.01[a]	4.34[b]	4.84	4.82	4.75	4.30	0.17	0.001	0.02	0.06	0.08
NDF (% DM)	60.68[a]	57.10[b]	59.07	60.77	56.41	59.32	2.02	0.04	0.65	0.95	0.54
ADF (% DM)	38.71[a]	34.77[b]	36.25	38.11	36.45	36.15	0.84	0.0002	0.79	0.06	0.87
DM Loss (%)	4.69	3.28	7.99	4.44	2.25	1.26	0.96	0.07	<0.0001	0.63	0.17

[1] WRS = Whole-plant rice straw silage. [2] CRS = Chopped rice straw silage.

[a, b] Means within the same row denoted by different letters differ from each other ($p<0.05$).

silages. Higher content of total organic acids was found in CRS ($p<0.0001$), which could be due to the greater packing density and more substrates released from carbohydrate being available for the better microbial anaerobic fermentation.

Chemical composition and DM losses

The level of LAB and the chopping process affected chemical composition of silage including CP, NDF, ADF and DM losses, with a significant interaction on DM content (Table 2; $p = 0.05$). Chopped rice straw silage had significantly higher DM content than WRS, while there were no statistical differences in DM loss between the silages. However, increasing level of LAB addition in silage linearly decreased DM loss ($p<0.0001$). Oude Elferick et al. (1999a) reported that extra DM loss would result from CO_2 emission, which was formed during the conversion of lactic acid to acetic acid and 1, 2-propanediol. Therefore, the DM loss could be considered as a good reflection of the metabolism of lactic acid by *L. buchneri* during the fermentation processes. The DM loss data in our research implied that inoculation with LAB might significantly reduce the activity of *L. buchneri*. However, previous studies have shown that increasing inoculation level of *L. buchneri* in maize silage resulted in extra DM losses (Driehuis et al., 1999a). This is presumably due to the different ensiled materials used in these experiments. As chopping exposes more surface of ensiled material to the microbes, it also promotes nutrient losses; and CRS decreased the content of CP, NDF and ADF by 13.4, 5.9 and 10.2%, respectively, as compared to WRS ($p<0.05$). As mentioned earlier, homolactic acid addition causes a rapid fall in pH and reduces the NH_3-N content. Surprisingly, the CP content of RS silages linearly decreased ($p = 0.02$) as the level of LAB addition increased. This contradiction could be partly explained by variation in sampling; however, the exact reason was not quite clear. The NDF and ADF contents were not influenced by addition of LAB additive.

Lactation study

The experimental diets are shown in Table 3. For the forage component, the chemical composition of corn silages and RS silage fed during the dairy trial were different. Corn silage had much higher content of CP (7.5 vs. 5.8% DM) and less concentration of NDF (57.8 vs. 63.1% DM) and ADF (36.4 vs. 45.3% DM) compared with RS silage. Our

Table 3. Ingredient and chemical composition of the diets

	100% CS[1]	75% CS+25% WRS[2]	50% CS+50% WRS
Ingredient (% DM)			
Corn	24.30	24.26	24.18
Soybean meal	3.06	3.06	3.05
Cottonseed meal	5.65	5.64	5.62
DDG	8.95	8.93	8.90
Brewers grains (dry)	1.18	1.18	1.17
Molasses	1.88	1.88	1.87
Dicalcium phosphate	0.80	0.80	0.80
Calcium carbonate	0.66	0.66	0.66
Salt	0.33	0.33	0.33
Sodium bicarbonate	0.19	0.19	0.19
Mineral and vitamin premix[3]	0.09	0.09	0.09
Corn silage	52.90	39.74	26.57
Rice straw silage	-	13.25	26.57
Chemical composition			
NE_L (Mcal/kg)[4]	1.49	1.45	1.41
CP (g/kg)	138.02	136.08	134.20
NDF (g/kg)	371.42	383.35	395.56
ADF (g/kg)	232.33	244.69	257.23
Ca (g/kg)	5.71	5.69	5.68
P (g/kg)	4.58	4.48	4.39

[1] CS = Corn silage. [2] WRS = Whole-plant rice straw silage.

[3] Contained 35 g/kg Mg, 340 mg/kg Cu, 1,500 mg/kg Mn, 2,010 mg/kg Zn, 15 mg/kg Se, 3.5 mg/kg Co, 25 mg/ kg I, 80,000 IU/kg vitamin A, 7,000 IU/kg vitamin D, 780 IU/kg vitamin E (Beijing Sanyuan Feed Co., China).

[4] Calculated from the tabular value (NRC, 2001).

Table 4. The effect of different level of rice straw silage on milk production, milk composition and market benefit

Item	Treatment diet			SEM	p
	100% CS[1]	75% CS+25% WRS[2]	50% CS+50% WRS		
Yield/day (kg)					
Milk	19.92	20.74	20.31	1.69	0.29
Fat	0.78	0.82	0.80	0.15	0.56
Protein	0.63	0.67	0.64	0.08	0.61
Lactose	0.91	0.96	0.94	0.06	0.45
Solids-not-fat	2.47	2.60	2.53	0.12	0.51
Milk composition (%)					
Fat	3.91	3.96	3.94	0.25	0.78
Protein	3.14	3.21	3.17	0.07	0.82
Lactose	4.59	4.62	4.62	0.13	0.91
Solids-not-fat	12.42	12.56	12.44	0.10	0.75
Market benefit [3] (Yuan/cow/d)					
Diet cost	29.70	27.78	25.88	-	-
Milk income	37.85	39.41	38.59	-	-
Net income	8.15	11.63	12.71	-	-
Benefit increase	0	3.48	4.56	-	-

[1] CS = Corn silage. [2] WRS = Whole-plant rice straw silage.
[3] Assuming the price of concentrate, rice straw silage, corn silage and milk are 2.4 Yuan/kg, 0.1 Yuan/kg, 0.35 Yuan/kg and 1.9 Yuan/kg, respectively.

lactation study was designed to evaluate whether substitution of corn silage by RS had negative effects on performance of dairy cows in the middle stage of lactation (20 kg/d daily milk yield). The results (Table 4) from the present study showed that lactation performance was similar among treatments. However, cows fed a ration with partial RS silage substituted for corn silage produced more milk, and tended to produce more milk fat, milk protein and lactose than those cows fed ration with corn silage alone. When considering the market value of milk produced by cows from the different treatments, those cows consuming the RS silage ration realized 3.48 Yuan (75% CS+25% WRS) and 4.56 Yuan (50% CS+50% WRS) more economic benefit than those fed the ration with only corn silage in the forage component.

CONCLUSIONS

Inoculating RS with LAB additive (combination of *Lactobacillus buchneri* and *Pediococcus pentosaceus*) could significantly improve the silage quality. Chopped rice straw silage had better fermentative quality than whole-plant rice straw silage. Furthermore, corn silage in the diet could be partially substituted by RS silage treated with LAB without adversely affecting milk production for medium-producing dairy cattle in developing countries.

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