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Effect of Complex Lactic Acid Bacteria on Silage Quality and *In vitro* Dry Matter Digestibility of Corn Straw

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Abstract: The study was carried out to evaluate the effect of complex lactic acid bacteria on the silage fermentation quality of corn straw and the silage *in vitro* dry matter digestibility. In the first experiment, the complex lactic acid bacteria inoculants was applied at 3.0×10^5 cfu g^{-1} consisted of 1.0×10^5 cfu g^{-1} *Enterococcus faecalis* TBT608 and 2.0×10^5 cfu g^{-1} *Lactobacillus plantram* TBT717. Uninoculated silage served as control. After 150 days of storage, the treatment silage had significantly ($p<0.05$) lower pH values, butyric acid, acetic acid, propionic acid, ammonia N concentration and had significantly ($p<0.05$) higher contents of lactic acid than control silage. In the second experiment, *In Vitro* Dry Matter Digestibility (IVDMD) was determined through fermentation, inoculated with rumen fluid of wethers in various combinations with soluble starch. The soluble starch represented a concentrate feed, silage represented feeding sole roughage. The IVDMD was determined at 24 and 48 h after incubation. Silage without inoculated LAB served as control for each feed combination. The results indicate that LAB applied at ensiling increased the IVDMD of the corn straw silage. These results confirmed that application of the complex lactic acid bacteria improved fermentation quality and *in vitro* dry matter digestibility of the corn straw silage.

Key words: Complex lactic acid bacteria, corn straw silage, fermentation quality, *in vitro* dry matter digestibility, quality, rumen fluid, China

INTRODUCTION

Silage is a major component of cattle rations. Feeding high-quality silage may improve cattle performance by the conservation of nutrients and the probiotic effect of lactic acid bacteria (Filya, 2003; Weinberg *et al*., 2004, 2007; Ashiono *et al*., 2006; Vali *et al*., 2005). In china, there are large quantity of corn straw left after maize had harvested. Some of them were used as a component of cattle rations. Therefore, the improvement of corn straw silage quality is of great interest and important. There have been many attempts to use lactic acid bacteria as silage inoculants to improve silage fermentation quality (Kung *et al*., 1993; Cai, 1999; Zahiroddinia *et al*., 2004; Filya *et al*., 2007; Nkosi *et al*., 2009; Schmidt *et al*., 2009; Kristensen *et al*., 2010; Bayatkouhsar *et al*., 2011). Lactic acid bacteria were used to reduce pH and to avoid or decrease, the risk of a clostridial fermentation by the native bacterial population. Lots of studies reported that inoculated silages reduced pH, promoted lactic acid production and reduced ammonia formation (Contreras-Govea *et al*., 2011). *Enterococcus faecalis* TBT608 and *Lactobacillus plantram* TBT717 were isolated from crops had shown great potential to improve silage fermentation quality and performance in previous silage experiments test.

The objective of this study was to evaluate the effect of the complex of *Enterococcus faecalis* TBT608 and *Lactobacillus plantram* TBT7171 on the silage fermentation quality of corn straw and the silage *in vitro* dry matter digestibility.

MATERIALS AND METHODS

Ensiling procedures: Corn straw was harvested (about 42% DM) after the maize harvested at mature stage, chopped into 2-3 cm pieces added adequate water to get the corn strow approximately in 25% DM then ensiled in 200 m^3 silage silos. In treatment group, the chopped

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forages were treated with the complex lactic acid bacteria inoculants which was applied at 3.0×10^5 cfu g^{-1} of forage consisted of 1.0×10^5 cfu g^{-1} *Enterococcus faecalis* TBT608 and 2.0×10^5 cfu g^{-1} *Lactobacillus plantram* TBT717l. Uninoculated silage served as control. At the same time, the sample of corn straw was collected for further analysis. After 150 days of storage, the silages were used to feeding and sampling together.

***In vitro* dry matter digestibility measurements:** The *in vitro* experiments, the tubes contained control or treatment dried silage powder samples (through a 1 mm sieve) with or without soluble starch according to the 2-stage fermentation *in vitro* technique (Tilley and Terry, 1963; Weinberg *et al*., 2007). The combinations of silage and starch were used as (Table 1):

- C0: Silages only (270 mg silage per tube)
- C1: Silages plus starch at a 2:1 ratio (180 mg of silage + 90 mg of starch per tube)
- C2: Silages plus starch at a 1:2 ratio (90 mg of silage + 180 mg of starch per tube)

Rumen fluid was collected from 4 fistulated wethers. The RF was collected before the morning feeding and strained through 4 layers of cheesecloth and flushed with CO_2 before use in the *in vitro* digestibility tubes, according to the 2 stage fermentation technique (Tilley and Terry 1963; Weinberg *et al*., 2007). The procedure included incubation of silage-starch combinations in 20 mL of buffer and 5 mL of RF in 50 mL sealed tubes for 24 or 48 h at 39°C followed by an additional incubation with 20 mL of 0.2% pepsin in 0.1 N HCl for 48 h at 39°C. At the end of this procedure, the undigested solids were precipitated by centrifugation at 1,200×g for 10 min and dried in oven at 65°C for 48 h and digestibility of the residual dry matter and *in vitro* dry matter digestibility was determined.

Table 1: Preparation of tubes

Groups	Tubes	Ingredients	Periods (h)
Treatment	1-3	Treatment silage only	24
	4-6	Treatment silage only	48
	7-9	Treatment silage plus starch at 2:1 ratio	24
	10-12	Treatment silage plus starch at 2:1 ratio	48
	13-15	Treatment silage plus starch at 1:2 ratio	24
	16-18	Treatment silage plus starch at 1:2 ratio	48
Control	19-21	Control silage only	24
	22-24	Control silage only	48
	25-27	Control silage plus starch at 2:1 ratio	24
	28-30	Control silage plus starch at 2:1 ratio	48
	31-33	Control silage plus starch at 1:2 ratio	24
	34-36	Control silage plus starch at 1:2 ratio	48
Blank	37-39	Blank (Rumen fluid only)	24
	40-42	Blank (Rumen fluid only)	48

Chemical analysis: In laboratory, 35 g of each sample and 70 mL amount of distilled water were added in 100 mL glass conical flask soaked under at 4°C for 24 h. The silage extracts were filtered through 2 layers of gauze and a filter paper (Xinhua Co., China). The filtrate was stored at -20°C prior to chemical analyses (Shao *et al*., 2007). The filtrate was used for determining pH, Lactic Acid (LA), Ammonium Nitrogen (AN) and Volatile Fatty Acids (VFAs).

The pH of silages was measured with a glass electrode pH meter (HANNA pH211, Hanna Instruments Italia Srl, Italy). Lactic acid in water extracts of the silages was determined spectrophotometrically according to Barker and Summerson, NH_3-N content was determined spectrophotometrically according to the Method of Phenol-Hypochlorite Reaction (Broderick and Kang, 1980; Shao *et al*., 2005) and WSC contents were determined by colorimetry after with anthrone-sulfuric acid reaction (Thomas, 1977; Yahaya *et al*., 2002).

Volatile Fatty Acids (VFAs) were detected using gas chromatography (Shimadzu GC-14B, Japan with 30 m ×0.25 mm ×0.25 μm fused silica capillary column; condition: column temperature at 130°C injection temperature at 180°C and detection temperature at 220°C). The DM contents were determined by drying in an oven at 65°C for 48 h (AOAC, 1984).

Total Nitrogen (TN) was determined by the Methods of Kjeldahl (AOAC, 1984), Crude Protein (CP) was calculated with 6.25 multiplied by TN. The contents of ADF, NDF and ADL were determined according to AOAC procedures (AOAC, 1984), hemicellulose concentration was calculated as the difference between NDF and ADF and cellulose concentration was calculated as the difference between ADF and the sum of ADL plus ash concentrations.

Statistical analysis: All data were analyzed statistically by one-way Analysis of Variance (ANOVA) and statistical significance among control and treatment was determined by Fisher's least significant difference test; these were performed by ANOVA using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS, 9.0), the significance was declared at $p<0.05$.

RESULTS AND DISCUSSION

Silage fermentation characteristics: The chemical composition of the corn straw material in this experiment is shown in Table 2 while composition of silages used in

Table 2: Chemical compositions of corn straw material

Dry matter (g kg^{-1} FW)	Crude protein (g kg^{-1} DM)	Water soluble carbohydrate (g kg^{-1} DM)	Neutral detergent fiber (g kg^{-1} DM)	Acid detergent fiber (g kg^{-1} DM)
41.73	57.60	54.23	631.48	335.72

FW: Fresh Weight; DM: Dry Matter

Table 3: Chemical compositions of corn straw silages[1]

Items	Control (nature fermented silage)	Treatment (LAB inoculated silage)
Dry matter (g kg^{-1} FW)	205.51^{b}	227.03^{a}
pH value	4.61^{a}	4.15^{b}
Lactic acid (g kg^{-1} DM)	2.93^{b}	35.87^{a}
Acetic acid (g kg^{-1} DM)	43.58^{a}	30.83^{b}
Lactic/acetic ratio	0.07^{b}	1.17^{a}
Propionic acid (g kg^{-1} DM)	3.20^{a}	0.86^{b}
Butyric acid (g kg^{-1} DM)	16.70^{a}	0.00^{b}
Total volatile fatty acids (g kg^{-1} DM)	63.48^{a}	31.69^{b}
CP (g kg^{-1} DM)	37.41^{b}	45.18^{a}
AN/TN (g kg^{-1} TN)	569.90^{a}	299.78^{b}
WSC (g kg^{-1} DM)	131.14^{a}	8.69^{b}
NDF (g kg^{-1} DM)	708.96^{a}	666.22^{b}
ADF (g kg^{-1} DM)	391.44^{a}	371.79^{b}
Hemicellulose (g kg^{-1} DM)	316.52^{a}	294.43^{b}
Cellulose (g kg^{-1} DM)	357.97^{a}	346.01^{b}

[1]Values followed by different small letters in the same row show significant differences at p<0.05

Table 4: pH of *in vitro* dry matter digestibility fluid

	24 h		48 h	
Starch treatment	Control	Treatment	Control	Treatment
C0	6.79^{Ab}	6.80^{Ab}	7.09^{Aa}	7.06^{Aa}
C1	6.63^{Bb}	6.64^{Bb}	6.86^{Ba}	6.87^{Ba}
C2	6.43^{Cb}	6.45^{Cb}	6.61^{Ca}	6.62^{Ca}

Values followed by different small letters in the same row show significant differences at p<0.05. Values followed by different large letters in the same column show significant differences at p<0.05

this experiment for treatment and control were shown in Table 3. After fermentation, the treatment silage had significantly (p<0.05) higher lactic acid and CP contents than control silage and had significantly (p<0.05) lower pH values, butyric acid, acetic acid, propionic acid, AN/TN, WSC, NDF, ADF, hemicelluloses and cellulose. While lactic/acetic ratio was significantly (p<0.05) higher in treatment silage.

The pH of *in vitro* dry matter digestibility fluid is show in Table 4. At 24 and 48 h, pH values of starch treatment C1 were significantly (p<0.05) higher than no-starch C0 while pH values of high dose starch treatment C2 were significantly (p<0.05) higher than starch treatment C1. From 24-48 h, all the pH values increased, pH values at 48 h were higher (p<0.05) than pH values at 24 h. At 24 h, all pH values of treatment were significantly (p<0.05) higher than control. At 48 h, the pH values of treatment C0 were significantly (p<0.05) lower than control C0 while the pH values of treatment C1 and C2 were significantly (p<0.05) higher than control C1 and C2.

The *In Vitro* Dry Matter Digestibility of silages (IVDMD) are shown in Table 5. All the IVDMD values in 48 h is significantly higher (p<0.05) than 24 h,

Table 5: *In Vitro* Dry Matter Digestibility (IVDMD) of silages (g kg^{-1})

Time	Silages	C0	C1	C2
24 h	Control	478.12^{Ba}	469.47^{Ba}	357.46^{Bb}
	Treatment	514.42^{Aa}	492.43^{Ab}	394.73^{Ac}
48 h	Control	587.33^{Ba}	583.24^{Ba}	537.37^{Ab}
	Treatment	612.75^{Aa}	604.94^{Ab}	545.90^{Ac}

C0 = Without starch; C1 = Starch:Silage 1:2; C2 = Starch:Silage 2:1. Values followed by different small letters in the same row show significant differences at p<0.05. Values followed by different large letters in the same column show significant differences at p<0.05

respectively. In 24 h, all the IVDMD values of treatment silages in different doses of starch were significantly higher (p<0.05) than control in 48 h, the IVDMD values of silages treatment in starch dose C0 and C1 were significantly higher (p<0.05) than control, treatment silage in starch dose C2 were higher than control but the difference was not significantly (p>0.05).

The starch effect could be get as: the IVDMD values trended to decrease as the soluble starch dose increased at 24 and 48 h. At 24 h, treatment silage IVDMD value in starch dose C0 was significantly higher (p<0.05) than that of C1; the treatment silage IVDMD value in starch dose C1 was also significantly higher (p<0.05) than that of C2. While control silage IVDMD value in starch dose C0 was also higher than that of C1 but not significantly (p>0.05). At 48 h, the trend of silage IVDMD value is same to that at 24 h.

In this investigation, two lactic acid bacteria strains, *Enterococcus faecalis* TBT608 and *Lactobacillus plantram* TBT7171 composed into the complex lactic acid bacteria inoculation used as the dose of 1.0×10^{5} cfu g^{-1} *Enterococcus faecalis* TBT608 and 2.0×10^{5} cfu g^{-1} *Lactobacillus plantram* TBT7171 of fresh forage in ensiling of corn straw silage. There is large quantity corn straw in china every year at the season of maize harvesting. Its use to produce silage for cattle is beneficial to environmental protection and economic development. However, corn straw is not sufficiently suitable for ensiling after maize harvested because of low forage quality and low quantity of epiphytic lactic acid bacteria. Therefore, researchers can not get good quality corn straw silage from nature ensiling without additive such as the control silage in this experiment. The preservation of forage in ensiling depends on the production of sufficient organic acid to inhibit activity of undesirable microorganisms under anaerobic conditions. The epiphytic Lactic Acid Bacteria (LAB) that are present on forage crops convert sugar into lactic acid in the ensiling process. As a result, the pH is reduced and the forage is

preserved (Cai *et al*., 1999). So, applying suitable Lactic Acid Bacteria (LAB) inoculation at ensiling to those low quality roughage such as corn straw is very important. Sufficient lactic acid bacteria could ensure rapid and vigorous fermentation that results in faster accumulation of lactic acid, lower pH values at earlier stages of ensiling and improved forage conservation.

Enterococcus faecalis TBT608 and *Lactobacillus plantram* TBT7171 are all homofermentative lactic acid bacteria, *Enterococcus faecalis* TBT608 is species of *coccus*, *Lactobacillus plantram* TBT7171 is bacillus. Researchers have the hypothesis as: in the process of ensiling, enterococcus and lactobacillus act a different function, in the early stage of ensiling the enterococcus grew vigorously, promote adequate fermentation pattern, rapidly produce lactic acid and consequent silage pH decrease. As the accumulation of lactic acid, enterococcus' activity was restrained and lactobacillus starts to act the function of produce lactic acid. When the amount of lactic acid is enough, all the activities of microorganism were inhibited including of enterococcus and lactobacillus. Thus, the forage was preserved under anaerobic condition.

The present experiment is a perfect exemplification of this hypothesis. The treatment silage inoculated sufficient lactic acid bacteria, the complex lactic acid bacteria controlled the fermentation pattern of silage to lactic acid and also to effectively inhibit the activity of harmful microorganisms and finally improve the fermentation quality of corn straw silage. Result as: high lactic acid content, low pH level, low content of acetic acid, propionic acid and butyric acid, high crude protein, high lactic/acetic ratio low AN/TN. Whereas the silage of control did not inoculate lactic acid bacteria, the pattern was not controlled in lactic acid fermentation, result in high pH, high content of acetic acid, propionic acid and butyric acid, total volatile fatty acids, crude protein; high AN/TN, low lactic/acetic ratio. This contributed in the lack of suitable epiphytic lactic acid bacteria and undesirable microorganisms' activity such as clostridium butyricum.

In the second experiment, *In Vitro* Dry Matter Digestibility (IVDMD) was determined through fermentation incubated with rumen fluid of wethers in various combinations with soluble starch was carried out. The soluble starch represented a concentrate feed, silage represented feeding sole roughage. The IVDMD was determined at 24 and 48 h after incubation. For each feed combination, silage without inoculated LAB served as control. The results show that, LAB applied at ensiling increased the IVDMD of the corn straw silage at either 24 and 48 h. This is agree with the result of Weinberg *et al*. (2007). The occurrence of digestibility is mainly at 24 h, this maybe because after 24 h fermentation, the accumulation of lactic acid and other productions such as volatile fatty acids and the pH of fermentation fluid had changed, all this could change the micro-ecosystem resulted in decrease of rumen microorganisms' function on dry matter digestibility.

Either in 24 and 48 h as the increased of soluble starch dose, the IVDMD of the corn straw silage decreased, not agree with the report of Weinberg *et al*. (2007). This should because the rumen population of cellulolytic microorganisms prefers to consume the available soluble starch prior to development of mechanisms and enzymes needed for other materals degradation which is agree to the theory of time-consuming process (Miron *et al*., 1996).

The pH of *in vitro* dry matter digestibility fermentation fluid indicate that, either in 24 and 48 h, the inoculated silage had a effect in stabilize the pH of fermentation fluid compare to un-inoculated silage *in vitro* digestibility fermentation inoculated with rumen fluid experiment. This could be shown in Table 3. The reason need further research to discover.

At 24 and 48 h, for each feed combination, the IVDMD of the treatment increased as compare to control. This maybe result in two reason:

- The treatment silage was inoculated with LAB had better quality than control silage
- The treatment silage had ability to stabilize the pH of fermentation fluid as compare to control thus could improve the function of rumen microorganisms, lead to increase *In Vitro* Dry Matter Digestibility (IVDMD)

All these results confirmed that applying of the complex lactic acid bacteria improved fermentation quality and *in vitro* dry matter digestibility of the corn straw silage. These results also agree with in practice enhanced animal performance.

CONCLUSION

The complex lactic acid bacteria inoculants constituted of *Enterococcus faecalis* TBT608 and *Lactobacillus plantram* TBT7171 applying in ensiling of corn straw could improve the fermentation quality of corn straw silage significantly. The inoculated corn straw silage had the effect of increase dry matter digestibility and stabilize the pH of fermentation fluid compare to un-inoculated silage in *in vitro* digestibility fermentation inoculated with rumen fluid experiment. The LAB species and there characteristics in the silage environment and the inoculated silage's interaction with rumen microorganism require further research.

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