Asian-Aust. J. Anim. Sci.
Vol. 24, No. 5 : 636 - 642
May 2011

www.ajas.info
doi: 10.5713/ajas.2011.10416

Effect of Different Rates of Ethanol Additive on Fermentation Quality of Napiergrass (*Pennisetum purpureum*)*

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ABSTRACT : The effect of different rates of ethanol additive on fermentation quality of napiergrass (*Pennisetum purpureum*) and residual water soluble carbohydrate were studied in the experiment. The addition rate of ethanol was 0%, 1.5%, 2.5%, 3.5%, 4.5% on fresh weight of napiergrass. The laboratory silos were kept in the room, then were opened on 1, 3, 5, 7, 14, 30 days after ensiling and the changes of silage quality were analyzed, respectively. There was a fast and large reduction in pH from the 5th day of ensiling to below 4.2 except for the 4.5% treatment. After five days the pH of silage decreased slowly and the pH of the ethanol additions was lower than the control. Lactic acid content of ethanol treatments increased significantly ($p<0.05$) from the 5th day of ensiling, reaching the highest value on either the 7th day or 14th day. The ethanol additive inhibited the break down of silage protein and the ammonia nitrogen content of ethanol addition silage was significantly ($p<0.05$) lower than the control after 30 days of ensiling. Within the initial first day of ensiling the water soluble carbohydrate content declined quickly. The efficiency of water soluble carbohydrate usage was higher in silage with ethanol than in the control. The acetic acid of ethanol treatment was significantly ($p<0.05$) lower than control on first and 14th day, but there was no significant ($p>0.05$) difference among the ethanol addition silages. The volatile fatty acids content of silage increased gradually from the first day of ensiling and reached the peak on 14th day or 30th day and the content of ethanol addition treatment was significantly ($p<0.05$) lower than the control. The experimental results indicated that adding ethanol inhibited the use of protein and water soluble carbohydrate of aerobic bacteria and reduced the silage losses during the early stage of ensiling and thus supplied more fermentation substrate for lactic acid bacteria and improved the fermentation quality of napiergrass. (**Key Words :** Napiergrass, Ethanol, Ensilage, Fermentation Quality, Residual WSC)

INTRODUCTION

It is only under anaerobic conditions that lactic acid bacteria (LAB) can dominate fermentation, and achieving and maintaining the anaerobic conditions during the ensiling of forage was very important (McDonald, 1991). However, this is not always possible in practice because many crops contain too much moisture and most farm silos are of such a size that they cannot be filled in one day. Previous studies indicated that the fermentation of the initial aerobic phase in the silos was crucial for successful silage (Shao et al., 2002, 2005). The amount of atmospheric oxygen trapped in a sealed silo is used up rapidly by the respiratory system of the plants and the quantity of soluble carbohydrate metabolized is negligible. Studies by Greenhill (1964) indicated that plant cell breakdown and the release of plant juices by plasmolysis is a prerequisite for the development of the LAB during the early stage of ensiling. The infiltration of even small amounts of air is thought to delay both plasmolysis and the onset of pH reduction. As a result, the presence of oxygen in the silo encourages the development of yeasts and causes an increase in the number of Gram-negative bacteria. In most cases, delayed sealing or a mass of atmosphere trapped in the silo resulted in a reduction in the amount of lactic acid produced and frequently a normally dominant lactic acid bacterial fermentation was replaced by a clostridial one

* Financial support for this research was provided by Nature Science Fund of China.

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Received September 27, 2010; Accepted January 28, 2011

(McDonald, 1991). In addition to a reduction in the level of fermentable carbohydrates, other effects of trapped oxygen have been noted. The energy released by the oxygen respiration of carbohydrate metabolism is responsible for a rise in temperature of ensiled herbage, which can bring about extensive chemical changes to the protein (Kim et al., 2006).

Ensiling tropical grass is difficult primarily due to its coarse porosity, stemmy structure and low water soluble carbohydrate (WSC) contents. They are usually less dense and presumably more permeable, and relatively large quantities of air may be trapped in the forage mass than in temperate grasses. In tropical grass silage making, techniques to rapidly consolidate the grass tightly and seal immediately is even more important than with temperate grasses.

To obtain successful silage various additives are applied in tropical grass ensilage fermentation. Cellulase, hemicellulase, molasses, glucose, previously fermented juice and LAB were often used as silage fermentation stimulants to improve silage fermentation quality (Weinberg et al., 1995; Ohshima et al., 1997; Meeske et al., 1999; Yunus et al., 2000). Another option is to add aerobic bacteria inhibitors to tropical grasses to produce good silage. Formic acid, acetic and propionic acid have been successfully used as inhibitors in silage (Yunus et al., 2001). The research carried out by Kleinschmit et al. (2005) indicated that chemical based additives (sodium benzoate and sodium sorbate added at a rate of 0.1% of fresh weight of grass) improved dry matter recovery and resulted in silages with higher concentrations of residual WSC than controls, suggesting partial inhibition of fermentation during ensiling of whole plant corn. In other experiments formalin and formic, propionic acid mixtures were used as aerobic bacteria inhibitors to restrict undesirable organisms (Haigh et al., 1998; Taylan et al., 2006). Ethanol can be used to kill or restrict microorganism, however, at present the effect of ethanol addition on silage fermentation is not clear.

Napiergrass is one of the important tropical forages and is now widely distributed throughout tropical and subtropical regions of the world. It is also major silage crop in the south of China and widely used either as fresh or as hay and silage. However, there is little information on the early silage fermentation of napiergrass silage, especially treated with different rates of ethanol addition. In the study presented here, the effect of different rates of ethanol on dynamic fermentation of napiergrass silage was examined.

MATERIALS AND METHODS

Silage making

Napiergrass (*Pennisetum purpureum*) was cultivated in spring of 2006 in an experimental field at the Jiangsu Academy of Agricultural Science with the initial growth of grass harvested at the vegetative stage on July 8, 2006. The harvested grass was chopped into about 1cm length with a forage cutter and 100 grams of chopped grass with ethanol additive (except for the controls) immediately packed into a plastic laboratory silo (100 ml capacity) in triplicates then sealed with a screw top and stored in a room kept at 25°C. The silos were opened on 1, 3, 5, 7, 14, 30 days during ensiling.

Chemical analyses

The chopped grasses were immediately collected for the determination of dry matter (DM), total nitrogen (TN), crude protein (CP) and WSC content. After the silos were opened and the contents were mixed thoroughly, 35 g of the sample was taken from each silo. This was followed by adding about 70 g distilled water and macerating at 4°C for 24 h. The extracts were filtered through two layers of cheesecloth and a filter paper, and then filtrate was stored at -20°C prior to chemical analyses. The filtrate was used for determining pH, ammonia nitrogen (AN), lactic acid (LA) and volatile fatty acids (VFAs). Silage pH was measured using a glass electrode pH meter (HI223). TN was analyzed by the Kjeldahl method (Salawu et al., 1999) and CP was determined by multiplying TN by 6.25. The AN content was determined by the phenol-hypochlorite procedure (Kleinschmit et al., 2005). LA content was determined by the method of Josefa (1999). The VFAs content were determined with gas chromatography (Shimizu GC-14B, condition: column temperature at 135°C, injection and detection temperature at 220°C). DM content of the fresh materials and silages were determined by drying in an oven at 65°C for at least 72 h (Filya et al., 2000). The WSC content of fresh materials and silages were determined using the method of Kim (2006). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) was determined using methods described by Van-Soest et al. (1991).

Statistical analysis

Data on chemical composition of the silages was analyzed by ANOVA using the general linear model procedure of the Statistical Analysis System (SAS, 1999). The analysis was conducted within silage treatments and within day of silage fermentation. Silage treatment was the main effect. When silage treatment was significant ($p<0.05$), data means were separated using Fisher's least significant difference test.

RESULTS

Changes in chemical compositions of napiergrass silage are presented in Table 2 and 3. The major fermentation

Table 1. Characteristics of napiergrass before ensiled

Items	DM (g kg^{-1})	CP (g kg^{-1} DM)	WSC (g kg^{-1} DM)	NDF (g kg^{-1} DM)	ADF (g kg^{-1} DM)
Napiergrass	148.59	56.63	134.03	606.67	430.20

Table 2. Effect of ethanol additive on composition of napiergrass silage during ensiling

Items	Storage period	Treatments				
		0	1.5%	2.5%	3.5%	4.5%
pH	1	5.28±0.17^{cB}	4.24±0.01^{aD}	4.31±0.08^{aE}	4.35±0.05^{aC}	4.70±0.01^{bD}
	3	4.12±0.04^{aA}	4.02±0.05^{aC}	4.07±0.01^{aD}	4.13±0.18^{aBC}	4.51±0.07^{bD}
	5	4.04±0.06^{bA}	3.89±0.03^{aBC}	3.94±0.02^{abC}	4.01±0.06^{abABC}	4.07±0.14^{bC}
	7	4.11±0.09^{aA}	3.79±0.02^{aA}	3.86±0.03^{aBC}	4.07±0.34^{aABC}	4.10±0.02^{aC}
	14	4.10±0.36^{aA}	3.87±0.04^{aB}	3.80±0.06^{aAB}	3.80±0.02^{aA}	3.79±0.01^{aA}
	30	3.98±0.22^{aA}	3.95±0.05^{aC}	3.74±0.02^{aA}	3.91±0.01^{aAB}	3.85±0.20^{aAB}
LA (g kg^{-1} DM)	1	15.01±6.79^{aA}	31.93±12.79^{aA}	22.56±11.64^{aA}	21.29±1.93^{aA}	16.49±5.35^{aA}
	3	25.49±2.49^{bA}	43.60±1.29^{aA}	39.91±2.64^{bB}	28.40±13.17^{bA}	22.40±4.11^{bA}
	5	43.55±9.70^{abA}	72.00±6.09^{bB}	67.03±5.67^{cC}	54.08±2.86^{bB}	71.01±6.02^{cB}
	7	72.48±8.24^{abB}	76.95±10.31^{bB}	79.11±1.06^{bC}	78.01±8.03^{bC}	67.54±10.95^{aB}
	14	58.23±26.97^{aA}	76.68±15.74^{bB}	77.89±7.35^{bC}	84.92±10.38^{bC}	61.54±5.96^{abB}
	30	52.21±41.31^{aA}	74.78±6.79^{bB}	76.48±4.39^{bC}	72.00±1.46^{bC}	61.71±8.78^{abB}
AA (g kg^{-1} DM)	1	12.27±4.52^{bA}	1.80±0.59^{aA}	3.82±2.17^{aA}	4.37±1.89^{aA}	3.80±0.71^{aA}
	3	9.39±5.94^{aA}	7.04±4.13^{aAB}	6.66±2.36^{aAB}	7.37±0.72^{aAB}	9.02±1.33^{aBC}
	5	8.74±4.96^{aA}	6.73±0.94^{aAB}	10.62±1.79^{aBC}	5.95±2.47^{aAB}	8.96±1.77^{aBC}
	7	13.42±9.51^{aA}	9.13±6.38^{aB}	8.62±1.02^{aB}	13.84±4.93^{aC}	7.91±0.58^{aBC}
	14	26.30±16.74^{bB}	9.39±2.56^{aB}	10.56±4.51^{aBC}	7.28±1.98^{aAB}	10.08±1.59^{aC}
	30	16.49±0.27^{bA}	10.49±2.14^{abB}	13.10±1.09^{abC}	10.95±3.15^{abBC}	6.05±4.07^{aAB}
PA (g kg^{-1} DM)	1	0.13±0.22^{a}	0.12±0.11^{aB}	0.01±0.12^{a}	0.03±0.05^{a}	0.03±0.05^{a}
	3	0.01±0.01^{a}	0.01±0.02^{aA}	0.03±0.05^{a}	0.03±0.05^{a}	0.03±0.05^{a}
	5	0.04±0.04^{a}	0.03±0.04^{aA}	0.00±0.00^{a}	0.00±0.00^{a}	0.03±0.05^{a}
	7	0.02±0.02^{a}	0.00±0.00^{aA}	0.19±0.32^{a}	0.00±0.00^{a}	0.02±0.04^{a}
	14	0.00±0.00^{a}	0.01±0.01^{aA}	0.01±0.01^{a}	0.03±0.05^{a}	0.04±0.12^{a}
	30	0.00±0.00^{a}	0.01±0.01^{aA}	0.00±0.00^{a}	0.03±0.05^{a}	0.00±0.00^{a}
BA (g kg^{-1} DM)	1	0.00±0.00^{a}	0.00±0.00^{aA}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
	3	0.00±0.00^{a}	0.23±0.40^{aAB}	0.24±0.41^{a}	0.00±0.00^{a}	0.01±0.01^{a}
	5	0.02±0.04^{a}	0.13±0.16^{aAB}	0.08±0.13^{a}	0.00±0.00^{a}	0.00±0.00^{a}
	7	0.13±0.23^{a}	0.35±0.60^{aAB}	0.04±0.07^{a}	0.08±0.11^{a}	0.00±0.00^{a}
	14	0.09±0.16^{a}	0.65±0.30^{aB}	0.00±0.00^{a}	0.00±0.00^{a}	0.01±0.02^{a}
	30	0.00±0.00^{a}	0.09±0.12^{aAB}	0.00±0.00^{a}	0.06±0.10^{a}	0.04±0.08^{a}
DM (g kg^{-1})	1	152.93±2.83^{aB}	145.53±4.37^{aA}	153.71±5.24^{aB}	155.85±14.04^{aC}	156.89±5.42^{aAB}
	3	156.32±9.01^{bcB}	141.28±7.88^{aA}	145.07±0.88^{abB}	160.23±3.18^{bcC}	161.34±0.97^{cB}
	5	158.34±2.15^{bB}	148.69±4.81^{aA}	142.34±3.93^{aB}	158.76±4.89^{bC}	167.90±4.04^{bC}
	7	137.96±9.09^{aA}	149.50±2.40^{abA}	147.23±3.40^{abB}	152.05±6.80^{abBC}	160.42±10.27^{bB}
	14	131.95±4.31^{aA}	140.48±2.11^{aA}	146.82±1.07^{aB}	145.89±3.20^{aAB}	151.65±3.51^{bAB}
	30	131.69±3.29^{aA}	139.84±3.32^{aA}	140.47±11.85^{aA}	142.33±4.74^{aA}	144.64±7.93^{aA}

Average means±standard deviations.
Values followed by different small letters show significant differences among treatments with the same ensiling day at $p<0.05$.
Values followed by different capital letters show significant differences among ensiling days with the same treatment at $p<0.05$.

Table 3. Effect of ethanol additive on composition of napiergrass silage during ensiling

Items	Storage period	Treatments				
		0	1.5%	2.5%	3.5%	4.5%
LA/AA	1	1.58±0.83^{aA}	12.42±2.94^{cB}	6.00±0.27^{bA}	7.53±2.46^{bA}	4.39±1.42^{abA}
	3	5.11±4.19^{aA}	4.29±0.68^{aA}	6.50±2.11^{aA}	4.14±2.43^{aA}	2.51±0.51^{aA}
	5	6.17±3.35^{aA}	6.40±1.79^{aA}	6.42±1.01^{aA}	10.04±3.67^{aBC}	8.04±0.89^{aAB}
	7	7.89±5.65^{aA}	10.96±5.43^{aAB}	9.25±0.98^{aA}	6.07±2.06^{aAB}	8.59±1.17^{aAB}
	14	2.00±2.92^{aA}	5.26±2.09^{abA}	8.40±3.70^{bcA}	12.02±2.14^{cC}	6.24±1.44^{abA}
	30	4.18±6.71^{aA}	5.37±7.22^{aB}	5.89±0.83^{aA}	7.03±2.42^{abAB}	13.61±8.09^{bB}
Total VFAs (g kg^{-1} DM)	1	12.40±4.44^{bA}	1.93±0.48^{aA}	3.83±2.16^{aA}	4.40±1.86^{aA}	3.82±0.71^{aA}
	3	9.39±5.94^{aA}	7.29±1.05^{aAB}	6.88±1.74^{aAB}	7.41±0.67^{aA}	9.06±1.38^{aBC}
	5	8.80±4.90^{aA}	6.89±0.82^{aAB}	10.69±1.89^{aB}	5.95±2.47^{aAB}	8.99±1.73^{aBC}
	7	13.57±3.45^{aA}	9.47±6.97^{aB}	8.85±1.31^{aB}	13.93±4.88^{aC}	7.93±0.62^{aBC}
	14	26.43±12.54^{bB}	10.05±2.26^{aB}	10.57±4.52^{aB}	7.31±1.94^{aAB}	10.13±1.54^{aC}
	30	16.62±0.22^{bA}	10.59±2.27^{abB}	13.10±1.09^{abB}	11.03±3.01^{abBC}	6.09±4.42^{aAB}
AN/TN (g AN kg^{-1} TN)	1	68.49±14.86^{bA}	47.21±7.28^{abA}	43.48±2.24^{abA}	21.28±2.86^{aA}	17.75±8.62^{aA}
	3	78.79±34.41^{bA}	55.25±5.72^{abA}	50.68±3.28^{abA}	51.99±5.15^{abB}	32.76±6.45^{aB}
	5	80.76±6.34^{bA}	77.09±12.41^{abAB}	70.95±10.24^{abAB}	64.44±7.24^{abBC}	48.26±10.76^{aB}
	7	80.53±33.49^{aA}	79.25±5.23^{aAB}	76.42±5.92^{aAB}	61.43±3.35^{aBC}	54.38±1.25^{aBC}
	14	96.75±11.35^{bB}	90.04±35.51^{abB}	85.63±4.21^{aB}	76.65±18.62^{aC}	69.12±7.15^{aC}
	30	82.70±25.05^{aA}	79.38±1.51^{aAB}	76.32±8.91^{aAB}	76.79±43.22^{aC}	70.74±5.01^{aC}
WSC (g kg^{-1} DM)	1	27.32±2.27^{aC}	38.68±5.39^{bD}	39.81±5.63^{bB}	63.48±4.22^{cC}	68.58±8.61^{cC}
	3	17.24±4.30^{aB}	26.32±14.39^{abC}	27.45±11.66^{abAB}	40.72±6.48^{bB}	42.75±10.14^{bB}
	5	13.35±1.94^{aAB}	16.47±1.90^{abB}	16.90±2.92^{abA}	27.32±7.56^{bAB}	32.52±2.38^{bB}
	7	11.92±2.11^{aAB}	12.37±2.96^{abAB}	13.22±2.05^{abA}	20.25±10.28^{bAB}	21.27±4.89^{bA}
	14	10.54±2.36^{aAB}	12.08±1.73^{aAB}	13.10±1.47^{aA}	14.53±1.26^{aA}	18.44±0.56^{aA}
	30	6.51±1.73^{aA}	6.44±1.17^{aA}	10.09±2.62^{abA}	11.70±4.75^{abA}	15.26±2.78^{bA}

Average means±standard deviations.
Values followed by different small letters show significant differences among treatments with the same ensiling day at $p<0.05$.
Values followed by different capital letters show significant differences among ensiling days with the same treatment at $p<0.05$.

production of silage was lactic acid. There was a quick and large reduction in pH from the 5th day of ensiling to below 4.2 except for the treatment of 4.5%. After five days the pH of silage decreased slowly and the pH of the ethanol additions was lower than the control. The pH of 3.74 in the 2.5% ethanol additive was the lowest among all treatments after 30 days ensiling, but the difference was not significant ($p>0.05$) between treatments. The LA content of the control on the first day of ensiling was 15.01 g kg^{-1} DM and was not different from the ethanol treatments. The LA content of the ethanol treatments increased significantly ($p<0.05$) from the 5th day of ensiling, reaching the highest value on either the 7th day or 14th day. On the 7th day the LA content of the 4.5% addition was 67.54 g kg^{-1} DM and significantly ($p<0.05$) lower than the other ethanol additions. The LA content of ethanol addition (except for 4.5% addition) was significantly ($p<0.05$) higher than the control on 14th day and 30th day.

The acetic acid (AA) content of ethanol addition was significantly lower ($p<0.05$) than control. The AA content of all treatments increased with ensiling days and reached the highest on 14th day or 30th day. The AA content in the ethanol additions was significantly ($p<0.05$) lower than the control on 14th day. Propionic acid (PA) and butyric acid (BA) were absent or detected in small amounts over the ensiling period. The total VFAs content of silage increased gradually from the first day of ensiling and reached the peak on 30th day with the total VFAs content in the control significantly ($p<0.05$) higher than ethanol treatments. The value of LA/AA increased during ensiling and the value of control, 1.5%, 4.5% ethanol treatment reaching a the peak on 30th day, however, the 2.5% and 3.5% treatment reached a maximum on 7th day and 14th day, respectively, and then tended to decrease.

The value of AN/TN increased gradually during ensiling and the AN/TN of ethanol addition silage was significantly ($p<0.05$) lower than the control. The greatest decrease in WSC content of silage occurred within initial first day of

ensiling as compared with fresh napiergrass. After five days, the WSC content decreased gradually until the end of ensiling. There was no significant ($p>0.05$) difference between the ethanol addition treatments and control from third to 7th day. The WSC content of 4.5% ethanol treatment was significantly ($p<0.05$) higher than the others on 14th day.

DISCUSSION

After 30 days ensiling napiergrass silage in the present study showed a good fermentation quality as judged from the pH and lactic acid content. The pH of ethanol treatment silage was significantly ($p<0.05$) lower than the control on first day. At the start of ensiling there was some residual oxygen in the silo and plant tissue, so, at first, aerobic bacteria dominated the fermentation. Ethanol additive could inhibit the aerobic bacteria and reduced their use of fermentation substrate. It was shown in the present study of napiergrass silage (Table 2) that the rate and extent of reduction in pH was largely within the initial five days of ensiling. The reduction was mainly caused by a rapid and intensive production of LA between first and 5th days. During the initial seven days of ensiling, LA content continued to increase with a small production of AA, LA/AA increasing. These results suggested that homofermentative LAB dominated the early lactic acid fermentation and are in agreement with previous reports by McDonald et al. (1991) and Shao et al. (2005). However, the LA and LA/AA content decreased slowly after 7 days, indicating that there was a shift from homofermentative to heterofermentative LAB. This response also agreed with the report by Shao et al. (2005). Ethanol addition apparently inhibited the deterioration of silage caused by aerobic bacteria during the early stage of ensiling and supplied more WSC for LAB so that the LA content of the ethanol treatments was higher than the control during ensiling. The effect of sorbic acid on fermentation quality of orange peels silage was studied by Weinberg et al. (1989) and the results indicated that higher sorbic acid addition slowed down the lactic acid fermentation rate during 5 days of ensiling, but after 30 days of ensiling the lactic acid content was higher than control and lower in the sorbic acid addition treatment. Their results suggested that sorbic acid also inhibit the deterioration caused by aerobic bacteria during early stage of ensiling and saved more WSC for LA fermentation.

The increase in AA during the ensiling indicated the activity of some heterofermentative LAB and acetic acid bacteria. The total VFAs of silage tended to increase from the initial fermentation and reached the highest on 14th day. This indicated that the greatest fermentation activity occurred within the initial 14 days of ensiling. It has been well known that cell breakdown and the resultant release of plant juices are prerequisite for the production of significant amounts of LA during ensiling (Greenhill, 1964). In the present study, the napiergrass was harvested at vegetative stage and cut into 1 to 2 cm and crushed to facilitate cell breakdown and the release of plant juices. Consequently, the abundant plant juices stimulated the LAB growth, especially homofermentative LAB at the early ensiling stage and the ensiled grass produced LA immediately after ensiling. Gibson et al. (1961) and Shao et al. (2005) also found that there was abundant LA in Italian ryegrass silage during the early stage of ensiling. The ethanol additive effectively inhibited the activity of aerobic bacterial during the early stage of ensiling, so the LA content of ethanol treatment silage was higher than in the control. However, higher amounts of ethanol restricted the activity of LAB as indicated by the lower LA content in the 4.5% treatment during the early stage of ensiling. Therefore, the effect of 1.5%, 2.5% ethanol addition on LA fermentation was better than higher addition. However, for both forage oats and Italian ryegrass, Ohba et al. (2002) demonstrated that the addition of ethanol did not give significant changes in the content of lactic acid.

The AA content of control (12.27g kg^{-1} DM) was significantly ($p<0.05$) higher on the first day as compared to ethanol addition and there was no significant ($p>0.05$) difference between control and ethanol treatment on third, 5th and 7th day. These results indicated that the inhibition of ethanol on aerobic bacteria was remarkable at early stage of ensiling. It may have been that although the laboratory silo was sealed completely, there was still a sufficient amount of O_2 in the silos and plant juices available for acetic acid and other aerobic bacteria to maintain their metabolism. However, as silage fermentation continued, more LA was produced and pH decreased, with the activity of aerobic microorganisms being inhibited with the increasing acidification. Increased ethanol addition decreased the AA content and the LA produced tended to increase. The effect of sorbic acid, formic acid and a combination of the two additions on aerobic bacteria was studied and showed similar results (Haigh et al., 1998; Shao et al., 2005). The present study, PA, BA were absent or detected in only small amounts over the ensiling period. This was attributed to a rapid reduction in pH due to the production of LA inhibiting the activity of clostridia and other bacteria as described by McDonald et al. (1991) and Shao et al. (2005).

The AN/TN of silage increased gradually and reached the highest value on either the 14th or 30th day. During ensiling, protein was degraded into amino acids by microorganisms and plant enzymes, and was further broken down into ammonia or amines resulting in loss of silage protein. The AN/TN of ethanol treatment silage was significantly ($p<0.05$) lower than control from the start of

ensiling to 14th day. The results indicated that ethanol inhibited the aerobic bacteria degradation of protein in the early stages. The value of AN/TN decreased with increasing ethanol addition at the end of ensiling. In other studies the volatile nitrogen content of maize silage treated with sorbic acid was significantly ($p<0.05$) lower than in the controls (Alli et al., 1985). Guo et al. (2007) demonstrated that formic acid, formaldehyde and their mixture significantly ($p<0.05$) depressed the ammonia nitrogen content of alfalfa silage. The results of the present study indicated that ethanol could also act as a silage fermentation inhibitor to effectively reduce the ammonia nitrogen content of silage.

WSC is the fermentation substrate for LAB or aerobic bacteria during ensiling. The research carried out by Weinberg et al. (1989) suggested that the higher the sobic acid adding, the more residual WSC was found in orange peel silage, moreover, the fermentation pattern of higher sorbic acid addition treatment was more efficient. The results of Alli et al. (1985) also indicated that potassium sorbate adding at the rate of 0.90 g kg^{-1} fresh grass led to the retention of large quantities of WSC in maize silage. Others also got the similar results from silage with aerobic bacteria inhibitors (Nsereko et al., 1998; Shao et al., 2007). In our study, the WSC content of control silage decreased greatly from 134.03 g kg^{-1} DM to 27.32 g kg^{-1} DM on the first day with the WSC content of ethanol treatment significantly ($p<0.05$) higher than the control during early stages of ensiling. These results suggested that ethanol inhibited aerobic bacteria from making use of the available WSC and saved the substrate for LAB, as indicated by the lactic acid content of silage during the early stage of ensiling. The WSC content in the over 2.5% ethanol treatments was significantly higher ($p<0.05$) than the control on the 14th day and could be an indication of an increase in the utilization of fermentable carbohydrates which resulted from inhibiting the activity of aerobes by ethanol. The present results indicate the ethanol treatment also effectively increases the available WSC content in silage.

CONCLUSION

These results suggested that adding over 2.5% ethanol effectively inhibited the activity of aerobic bacteria during early stage of ensiling, improved fermentation quality of silage and reducing silage losses.

REFERENCES

Alli, I., S. Pabari, R. Fairbairn and B. E. Baker. 1985. The effect of sorbates on the ensilage of chopped whole-plant maize and lucerne. J. Sci. Food Agric. 36:63-70.

Filya, I., G. Ashbell, Y. Hen and Z. G. Weinberg. 2000. The effect of bacterial inoculants on the fermentation and aerobic stability of whole crop wheat silage. Anim. Feed Sci. Technol. 88:39-46.

Gibson, T., A. C. Stirling, R. M. Keddie and R. F. Rosenberger. 1961. Bacteriological changes in silage as affected by laceration of the fresh grass. J. Appl. Bacteriol. 24:60-70.

Greenhill, W. L. 1964. Plant juices in relation to silage fermentation. I. The role of juice. J. Br. Grassl. Soc. 19:30-37.

Guo, X. S., W. R. Ding, J. G. Han and H. Zhou. 2007. Characterization of protein fractions and amino acids in ensiled alfalfa treated with different chemical additives. Anim. Feed Sci. Technol. 142:89-98.

Haigh, P. M. and O. D. Davies. 1998. Effect of formic acid with formalin or barley incorporation into grass silage on silage fermentation and the performance of dairy cows. J. Agric. Engng Res. 69:261-265.

Josefa, M. and M. T. Antonio. 1999. A comparative study on the determination of lactic acid in silage juice by colorimetric, high-performance liquid choromatography and enzymatic methods. J. Sci. Food Agric. 79:1722-1726.

Kim, S. C. and A. T. Adesogan. 2006. Influence of ensiling temperature, simulated rainfall, and delayed sealing on fermentation characteristics and aerobic stability of corn silage. J. Dairy Sci. 89:3122-3132.

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

Mcdonald, P., A. R.Henderson and S. J. Heron. 1991. The biochemistry of silage. 2nd edn. Cambrian Printers Ltd. Aberystwyth. 184-236.

Meeske, R., H. M. Basson and C. W. Cruywagen. 1999. The effect of a lactic acid bacterial inoculant with enzymes on the fermentation dynamics, intake and digestibility of *Digitaria eriantha* silage. Anim. Feed Sci. Technol. 81:237-248.

Nsereko, V. L., J. A. Rooke and C. J. Newbold. 1998. Influence of protease inhibitors on nitrogen distribution in ensiled perennial ryegrass and the utilisation of silage nitrogen for growth by rumen bacteria *in vitro*. Anim. Feed Sci. Technol. 76:51-63.

Ohba, N., M. Tobisa, M. Shimojo and Y. Musuda. 2002. Effect of ethanol addition on ensiling of forage Oats and Italian ryegrass. Sci. Bull Faculty Agric. Kyushu Univ. 57:11-15.

Ohshima, M., E. Kimura and H. Yokota. 1997. A method of making good quality silage from direct alfalfa by spraying previously fermented juice. Anim. Feed Sci. Technol. 66:129-137.

Salawu, M. B., T. Acamovica, C. S. Stewart, T. Hvelplund and M. R. Weisbjerg. 1999. The use of tannins as silage additives: effects on silage composition and mobile bag disappearance of dry matter and protein. Anim. Feed Sci. Technol. 82:243-259.

SAS Institute Inc. 1999. SAS/STAT User's Guide:Version 8.2th edn. SAS Institute Inc., Cary, North Carolina.

Shao, T., N. Ohba, M. Shimojo and Y. Musuda. 2002. Dynamics of early fermentation of Italian Ryegrass (*Lolium multiflorum* Lam.) silage. Asian-Aust. J. Anim. Sci. 15:1606-1610.

Shao, T., Z. Zhang, M. Shimojo and Y. Musuda. 2005. Comparison of fermentation characteristics of Italian ryegrass (*Lolium multiflorum* Lam.) and Guineagrass (*Panicum maximum* Jacq.) during the early stage of ensiling. Asian-Aust. J. Anim. Sci. 18: 1727-1734.

Shao, T., L. Zhang, M. Shimojo and Y. Musuda. 2007. Fermentation quality of Italian ryegrass (*Lolium multiflorum*

Lam.) silages treated with encapsulated-glucose, glucose, sorbic acid and pre-fermented juices. Asian-Aust. J. Anim. Sci. 20:1699-1704.

Taylan, A., B. Erol, M. K. Akif and H. Muruz. 2006. Effects of formic acid, molasses and inoculant additives on corn silage composition, organic matter digestibility and microbial protein synthesis in sheep. Small Rumin. Res. 61:29-33.

Van-Soest, P. J., H. B. Robertson and B. A. Lewis. 1991. Methods of dietary fiber, NDF, and non-starch polysaccharides determination in relation to animal material. J. Dairy Sci. 74: 3583-3591.

Weinberg, Z. G., G. Ashbell and B. Horev. 1989. The effect of sorbic acid on loss reduction during storage of orange peels. J. Sci. Food Agric. 46:253-258.

Weinberg, Z. G., G. Ashbell, Y. Hen and A. Azrieli. 1995. The effect of cellulase and hernicellulase plus pectinase on the aerobic stability and fibre analysis of peas and wheat silages. Anim. Feed Sci. Technol. 55:287-293.

Yoshinobu, O. and P. McDonald. 1975. The effect of some additives on aerobic deterioration of silages. J. Sci. Food Agric. 26: 941-948.

Yunus, M., N. Ohba, M. Shimojo and Y. Musuda. 2000. Effects of adding urea and molasses on Napiergrass silage quality. Asian-Aust. J. Anim. Sci. 13:1542-1547.

Yunus, M., N.Ohba, M. Tobisa, M. Shimojo and Y. Musuda. 2001. Effect of glucose and formic acid on the quality of Napiergrass silage after treatment with urea. Asian-Aust. J. Anim. Sci. 14: 211-215.