Effect of Additives on the Fermentation Quality and Residual Mono- and Di-saccharides Compositions of Forage Oats (*Avena sativa* L.) and Italian Ryegrass (*Lolium multiflorum* Lam.) Silages

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ABSTRACT : This study aimed to evaluate the effects of silage additives on the fermentation qualities and residual mono- and di-saccharides composition of silages. Forage Oats (*Avena sativa* L.) and Italian Ryegrass (*Lolium multiflorum* Lam.) were ensiled with glucose, sorbic acid and pre-fermented juice of epiphytic lactic acid bacteria (FJLB) treatments for 30 days. In both species grass silages, although the respective controls had higher contents of butyric acid (20.86, 33.45g kg^{-1} DM) and ammonia-N/total nitrogen (100.07, 114.91 g kg^{-1}) as compared with other treated silages in forage oats and Italian ryegrass, the fermentation was clearly dominated by lactic acid bacteria. This was well indicated by the low pH value (4.27, 4.38), and high lactic acid/acetic acid (6.53, 5.58) and lactic acid content (61.67, 46.85 g kg^{-1} DM). Glucose addition increased significantly ($p<0.05$) lactic acid/acetic acid, and significantly ($p<0.05$) decreased the values of pH and ammonia-N/total nitrogen, and the contents of butyric acid and volatile fatty acids as compared with control, however, there was a slightly but significantly ($p<0.05$) higher butyric acid and lower residual mono- and di-saccharides as compared with sorbic acid and FJLB additions. Sorbic acid addition showed the lowest ethanol, acetic acid and ammonia-N/total nitrogen, and highest contents of residual fructose, total mono- and di-saccharides and dry matter as well as high lactic acid/acetic acid and lactic acid content. FJLB addition had the lowest pH value and the highest lactic acid content, the most intensive lactic acid fermentation occurring in FJLB treated silages. This resulted in the faster accumulation of lactic acid and faster pH reduction. Sorbic acid and FJLB additions depressed clostridia or other undesirable bacterial fermentation, thus this decreased the water-soluble carbohydrates loss and saved the fermentable substrate for lactic acid fermentation. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 11 : 1582-1588)***

Key Words : Forage Oats, Italian Ryegrass, Silage, Additives, Mono- and Di-saccharides

INTRODUCTION

The success of ensilage is principally dependent upon creation of anaerobic conditions in the silo and the presence at ensilage of both sufficient lactic acid bacteria (LAB) and adequate water-soluble carbohydrates (WSC) in the grass (Rooke, 1990). Although anaerobic conditions can be improved by proper ensiling, such as chopping, compacting and sealing and so forth, there can still be considerable activity of aerobic microorganisms during the very early stages of ensiling (aerobic phase) and it is generally accepted that the activity of aerobic microorganisms during the very early stage of ensiling is undesirable. These organisms consume WSC, which is required by desirable LAB, causing fermentable substrate shortage (Lacey et al., 1981; Alli et al., 1985; Guan et al., 2002).

Glucose addition is to compensate the WSC loss caused by the initial undesirable bacteria activity (yeast, mold and aerobic bacteria) and ensure that a sufficint amount of WSC remains at the vigorous stage of LAB growth and produces lactic acid (LA), several researches have demonstrated the advantages of glucose as silage additive to improve the fermentation quality (Ohyama et al., 1971, 1973, 1975). Woolford (1975) had confirmed that sorbic acid had a strong inhibiting effect on the growth of yeasts and moulds, and it was widely used as a preservative in food industry and also as an ingredient of commercial silage additives to prevent aerobic deterioration after opening silos (Alli and Baker, 1982; Hattori et al., 1996; Shao et al., 2004). In this experiment sorbic acid is used as an additive to depress the loss of WSC by undesirable organism (yeast, mould and aerobic bacteria) during the initial phase of ensiling, save the WSC for LAB, and improve the fermentation quality (Shao et al., 2003; Shao et al., 2004). It was well documented that adding the pre-fermented juice of epiphytic lactic acid bacteria (FJLB) to silages was effective for improving fermentation quality, and often resulted in the increase of LA and in the reduction of ammonia-N (AN)

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even when the addition of commercial LAB was ineffective (Ohshima et al., 1997a, b, c). In the present study, it was considered that FJLB would promote the onset of LAB fermentation, and increased the rate of LA production and pH reduction, and decreased the loss of WSC by restricting undesired bacteria activity.

Forage oats and Italian ryegrass are major silage crops in China, which are very suitable species for planting in Jiangsu of China, and they have been widely used for silage making as an important feed source for dairy cattle. However, there are high moisture content at the vegetative stage and insufficient epiphytic LAB present prior to ensiling, which sometimes resulted in clostridia bacteria activity occurring (Iwasaki et al., 1978; Cai et al., 1999).

The objectives of the present study were to evaluate the effects of addition of glucose, sorbic acid and FJLB on the fermentation qualities and residual mono- and di-saccharides compositions of two species grass.

MATERIALS AND METHODS

Additive preparation

The FJLB was prepared from forage oats and Italian ryegrass according to the following manners; a 100 g sample of freshly cut grass was macerated with 300 ml of distilled water using a blender. The macerated sample was filtered through double layers cheesecloth, and 200 ml of the filtrate was collected into a 500 ml-glass bottle containing 4 g of glucose. The glass bottle was fitted with a gas trap and maintained at 30°C for 3 days (Ohshima et al., 1997a, b, c). After 3 days of anaerobic incubation, the pH value and the population of FJLB were determined just before silage treatment.

Silage making

Forage oats and Italian ryegrass were cultivated in the experimental field of Nanjing Agricultural University. The primary growth of forage oats and Italian ryegrass was hand-harvested with a sickle at the vegetative stage on 13 April 2000. The harvested materials were immediately chopped into about 1 cm length and treated with additives. After thorough mixing, the grasses were ensiled into a laboratory silo (1 liter capacity) in triplicates. The silage treatments were as follows: (1) control (no addition), (2) glucose addition at 1%, (3) sorbic acid addition at 0.1%, (4) FJLB addition at a theoretical application rate of 1.51×10^5 CFU g^{-1} for forage oats, and 3.62×10^5 CFU g^{-1} for Italian ryegrass on fresh weight basis, respectively. The amount of ensiled grass was 700 g and 650 g for forage oats and Italian ryegrass at each treatment, respectively. These were followed by being sealed with a screw top and kept at 25°C. All silos were opened after 30 days of storage.

Chemical analyses

The chopped forage oats and Italian ryegrass were immediately collected for the determination of the contents of dry matter (DM), mono- and di-saccharides compositions (fructose, glucose, sucrose), total nitrogen (TN), crude protein (CP) and the population of epiphytic lactic acid bacteria (LAB) in initial fresh grasses. After the silos were opened and the contents were mixed thoroughly, a 50-g of sample was taken from each silo and a 150-g of distilled water was added before being stored in the refrigerator at 4°C for 24 h. Then, the extracts were filtered through double layered cheesecloth and a filter paper (Toyo No. 5A, Japan) and the filtrates were used for the determination of pH, AN, LA, ethanol, and volatile fatty acids (VFAs). The pH of silage was measured using a glass electrode pH meter (Horiba Co. Japan). The LA content was determined using the method of Barker and Summerson (1941), and the contents of VFAs and ethanol with gas chromatography (Shimadzu, Japan, GC-17A with 12 m capillary column, condition: column temperature 100°C, injection temperature 250°C). Total nitrogen (TN) was determined by macro-Kjeldahl procedures 7.033-7.037 (AOAC, 1984) and AN with an ammonia electrode (Model IM-22P, Toa Electronics Ltd. Japan), crude protein (CP) was determined with 6.25 multiplied by TN. The DM content of the fresh grasses and silage masses was determined by drying in an oven at 60°C for at least 48 hours (AOAC, 1984), whereas that of silages was determined by the removal of water using toluene distillation with ethanol correction (Dewar and McDonald, 1961). Mono- and di-saccharides compositions of the fresh grasses and silages were determined by high performance liquid chromatography (HPLC) as shown in our previous report (Shao et al., 2002). The population of epiphytic LAB in the fresh grasses and the FJLBs was determined by the plate count methods. Grass samples (10 g) were shaken well with 90 ml of sterilized distilled water, and 10^{-1}-10^{-8} serial dilutions were made in 0.85% sodium chloride sterilized solution. LAB were counted on a plate agar containing bromocresol purple and GYP-$CaCO_3$ agar after incubation in an anaerobic box (N_2:H_2:CO_2 = 80:10:10, TE-HER Hard Anaerobox, ANX-1, Hirosawa Ltd, Toky, Japan) at 30°C for 3 days. LAB were detected by a yellowish colonies with a clear zone due to dissolving $CaCO_3$ (Masuko et al., 1992).

Statistical analyses

The experiment was a complete randomized design, with 3 treatment and 3 replicates, the statistical analysis included one-way analysis of variance with additive treatments as a factor and Fisher's least significant difference test; these were performed by ANOVA using the GLM procedure of the Statistical Analysis System (SAS, 1984).

Table 1. Characteristics of two grasses and FJLB before ensiled

Forage species	Dry matter ($g\ kg^{-1}$)	Crude protein ($g\ kg^{-1}$ DM)	Fructose ($g\ kg^{-1}$ DM)	Glucose ($g\ kg^{-1}$ DM)	Sucrose ($g\ kg^{-1}$ DM)	Mono- and di-saccharides ($g\ kg^{-1}$ DM)	LAB[1] (CFU g^{-1} FM)	LAB[2] (CFU ml^{-1})	pH of FJLB
Forage oats	139.01	120.54	14.82	13.42	34.69	62.92	3.46×10^{3}	1.06×10^{8}	3.88
Italian ryegrass	186.49	65.63	54.07	44.83	15.07	113.97	2.91×10^{3}	4.35×10^{8}	3.77

[1] The population of epiphytic lactic acid bacteria in initial two fresh grasses expressed as colony forming unit per g fresh matter (CFU g^{-1} FM).
[2] The population of pre-fermented juice of epiphytic lactic acid bacteria (FJLB) prior to being added to two grasses (CFU ml^{-1}).

Table 2. Fermentation quality of forage oats silage treated with glucose, sorbic acid and FJLB[1]

Item	Treatments			
	Control	Glucose	Sorbic acid	FJLB
pH (SD)	4.27 (0.36)[b, 2]	3.86 (0.10)[a]	3.87 (0.06)[a]	3.63 (0.02)[a]
Dry matter (SD) ($g\ kg^{-1}$)	127.31 (1.80)[a]	128.08 (5.34)[a]	136.78 (0.60)[b]	135.17 (1.08)[b]
Lactic acid (SD) ($g\ kg^{-1}$ DM)	61.67 (12.53)[a]	113.17 (13.68)[b]	116.42 (15.03)[b]	131.27(18.01)[b]
Acetic acid (SD) ($g\ kg^{-1}$ DM)	9.96 (1.68)[b]	6.56 (0.17)[a]	5.87 (1.68)[a]	7.58 (2.38)[ab]
Propionic acid (SD) ($g\ kg^{-1}$ DM)	1.20 (0.62)[b]	0.25 (0.43)[a]	0.00 (0.00)[a]	0.00 (0.00)[a]
Butyric acid (SD) ($g\ kg^{-1}$ DM)	20.86 (6.47)[c]	3.49 (5.86)[b]	0.97 (1.18)[a]	0.04 (0.08)[a]
Volatile fatty acids (SD) ($g\ kg^{-1}$ DM)	32.07 (7.61)[c]	10.34 (6.78)[b]	6.84 (2.10)[a]	7.62 (2.30)[a]
Ethanol (SD) ($g\ kg^{-1}$ DM)	4.73 (1.49)[b]	3.16 (0.16)[ab]	1.46 (1.78)[a]	2.54 (0.39)[a]
AN/TN[2] (SD) (g AN kg^{-1} TN)	100.07 (16.01)[b]	64.76 (22.0)[a]	47.10 (15.07)[a]	62.19 (10.69)[a]
Lactic acid/acetic acid (SD)	6.53 (3.06)[a]	17.28 (2.26)[b]	21.01 (10.37)[b]	18.25 (4.48)[b]

[1] FJLB: Pre-fermented juice of epiphytic lactic acid bacteria, AN: Ammonia-N, TN: Total nitrogen.
[2] Values followed by different letters in the same row show significant differences at $p<0.05$.

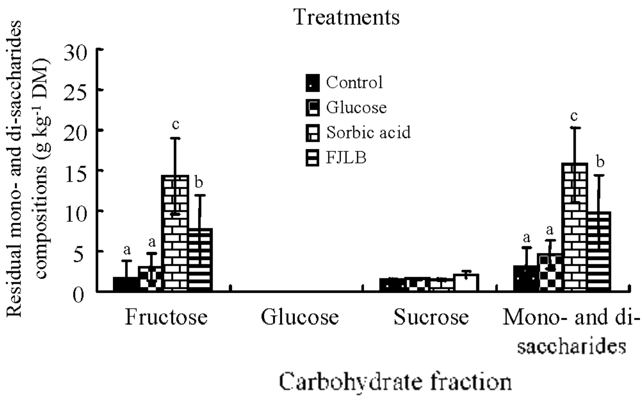

Figure 1. Effect of additives on the residual mono- and di-saccharides in forage oats silage (*Different letters in the same fraction show significant differences at $p<0.05$). FJLB: pre-fermentated juice of epiphytic lactic acid bacteria.

RESULTS

Table 1 shows the characteristics of the initial two grasses and FJLBs. The DM contents of forage oats (*Avena sativa*, L.) and Italian ryegrass (*Lolium multiflorum* Lam) were 139.01 g kg^{-1} and 186.49 g kg^{-1}, respectively. Italian ryegrass had higher DM content than that of forage oats. The two grasses had similar low populations of epiphytic LAB (3.46×10^{3} CFU g^{-1} for forage oats, 2.91×10^{3} CFU g^{-1} for Italian ryegrass). Forage oats had higher contents of CP and sucrose, and lower contents of fructose, glucose and total mono- and di-saccharides when compared with Italian ryegrass. There were similar pH values and LAB numbers in the FJLBs for the two grasses.

Forage oats

The fermentation quality and residual mono- and di-saccharides compositions in forage oats are presented in Table 2 and Figure 1, respectively. The glucose, sorbic acid and FJLB additions significantly ($p<0.05$) decreased the values of pH and AN/TN and the contents of BA and total VFAs, whereas significantly ($p<0.05$) increased LA/AA and LA content as compared with control silage. However, glucose addition showed a slightly but significantly ($p<0.05$) higher BA as compared with both of sorbic acid and FJLB additions. Glucose and sorbic acid additions significantly ($p<0.05$) decreased AA content, and FJLB addition showed an insignificant decrease ($p>0.05$) as compared with control. Propionic acid (PA) was only detected in both control and glucose addition silages. Although all silages had low ethanol content, control silage showed a significantly ($p<0.05$) higher content of ethanol than sorbic acid and FJLB additions. There was a significant ($p<0.05$) increase in DM content in both of sorbic acid and FJLB additions, and glucose addition did not show significant ($p>0.05$) difference as compared with control. The additions of sorbic acid and FJLB showed significantly ($p<0.05$) higher contents of residual fructose and total mono- and di-saccharides (Figure 1), but glucose addition did not show significant ($p>0.05$) differences as compared with control. There were not significant ($p>0.05$) differences in residual sucrose content that showed only a small amount, and no residual glucose was detected in all silages.

Italian ryegrass

The fermentation quality and residual mono- and di-

Table 3. Fermentation quality of Italian rygrass silage treated with glucose, sorbic acid and FJLB[1]

Item	Treatment			
	Control	Glucose	Sorbic acid	FJLB
pH (SD)	4.38 (0.06)[c, 2]	4.00 (0.20)[b]	4.05 (0.15)[b]	3.59 (0.01)[a]
Dry matter (SD) (g kg^{-1})	154.90 (0.35)[a]	177.46 (4.64)[b]	183.15 (2.33)[c]	182.06 (1.90)[bc]
Lactic acid (SD) (g kg^{-1} DM)	46.85 (10.80)[a]	49.11 (5.77)[a]	49.78 (8.03)[a]	121.76 (5.67)[b]
Acetic acid (SD) (g kg^{-1} DM)	10.70 (3.16)[b]	4.94 (0.49)[a]	4.60 (1.28)[a]	5.30 (2.07)[a]
Propionic acid (SD) (g kg^{-1} DM)	2.53 (1.26)[b]	0.56 (0.28)[a]	0.50 (0.70)[a]	0.12 (0.11)[a]
Butyric acid (SD) (g kg^{-1} DM)	33.45 (3.03)[c]	7.94 (2.43)[b]	2.72 (2.69)[b]	0.34 (0.22)[a]
Volatile fatty acids (SD) (g kg^{-1} DM)	46.68 (8.67)[c]	13.44 (2.01)[b]	7.82 (3.49)[ab]	5.76 (1.77)[a]
Ethanol (SD) (g kg^{-1} DM)	2.42 (0.40)[a]	1.84 (0.10)[a]	1.26 (0.97)[a]	1.67 (0.10)[a]
AN/TN[1] (SD) (g AN kg^{-1} TN)	114.91(3.97)[b]	65.91 (12.99)[a]	55.12 (16.37)[a]	65.58 (4.64)[a]
Lactic acid/acetic acid (SD)	5.58 (3.18)[a]	9.94 (0.81)[b]	10.82 (1.16)[b]	25.93 (3.52)[c]

[1] FJLB: Pre-fermented juice of epiphytic lactic acid bacteria, AN: Ammonia-N. TN: Total nitrogen.

[2] Values followed by different letters in the same row show significant differences at $p<0.05$.

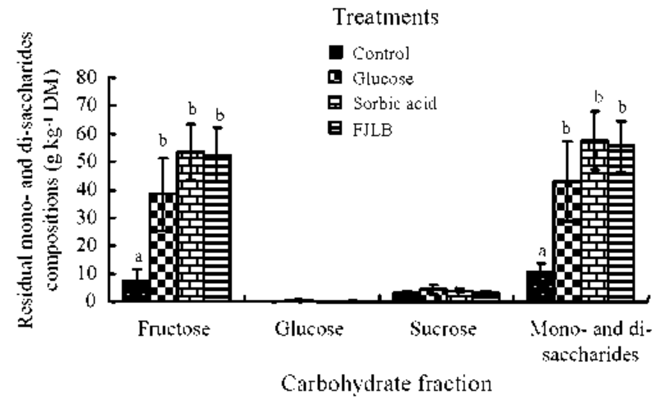

Figure 2. Effect of additives on the residual mono- and di-saccharides in Italian ryegrass silage (* Different letters in the same fraction show significant differences at $p<0.05$). FJLB: pre-fermentated juice of epiphytic lactic acid bacteria.

saccharides compositions in Italian ryegrass are presented in Table 3 and Figure 2, respectively. The glucose, sorbic acid and FJLB additions significantly ($p<0.05$) decreased the values of pH and AN/TN and the contents of AA, BA, PA and total VFAs, whereas significantly ($p<0.05$) increased LA/AA and DM content. The decrease in pH value and BA content was significantly ($p<0.05$) larger in FJLB addition than in glucose and sorbic acid additions. The FJLB addition showed the lowest VFAs content among all silages. The glucose and sorbic acid additions had a slightly but significantly ($p<0.05$) higher BA content as compared with FJLB. FJLB addition significantly ($p<0.05$) increased LA, however, glucose and sorbic acid additions showed only a slight ($p>0.05$) increase as compared with control. There were not significant ($p>0.05$) differences in ethanol content among all silages. The higher residual fructose and total mono- and di-saccharides were found in all silages as compared with forage oats silages. Glucose, sorbic acid and FJLB additions showed significantly ($p<0.05$) higher contents of residual fructose and total mono- and di-saccharides than control. The amounts of residual fructose and total mono- and disaccharides were in the following order: sorbic acid >FJLB>glucose>control. The content of sucrose, which was small in amounts, did not show significant ($p>0.05$) differences among all silages, and also no residual glucose was detected in all silages.

DISCUSSION

Control (forage oats, Italian ryegrass)

Although respective controls had higher contents of BA (20.86, 33.45 g kg^{-1} DM) and AN/TN (100.07, 114.91 g kg^{-1} DM) as compared with the other treated silages in forage oats and Italian ryegrass, the fermentation was clearly dominated by LAB. This was well indicated by the low pH value (4.27, 4.38), high LA/AA (6.53, 5.58) and LA content (61.67, 46.85 g kg^{-1} DM). This is probably due to the fact that medium or high mono- and di-saccharides content in forage oat (62.92 g kg^{-1} DM) and Italian ryegrass (113.97 g kg^{-1} DM) leads to the LA fermentation. This can be further explained as follows: although mono- and di-saccharides in the original forage oats was not very high (62.92 g kg^{-1} DM), forage oats is one of the temperate grasses in which fructans are the most abundant source of WSC and can be hydrolyzed into glucose and fructose by plant enzymes during very early stage of ensiling for LAB fermentation (Henderson and McDonald, 1971).

On the other hand, the respective control silages showed significantly ($p<0.05$) higher contents of BA, total VFAs and AN/TN (Tables 2 and 3), as well as significantly ($p<0.05$) small amounts of residual mono- and di-saccharides (Figures 1 and 2) than the other treated silages. These indicated that a part of mono- and di-saccharides had been consumed by clostridial or other undesired bacteria during the fermentation period, being agreement with the results of Tamada et al. (1996). This was attributed to high moisture content, medium or abundance of fermentable substrates and low population of epiphytic LAB in the two original grasses, which conditions were adequate for clostridial bacteria and other undesired bacteria development during the period of ensiling (McDonald et al., 1991).

Glucose addition

Glucose addition significantly ($p<0.05$) decreased the values of pH and AN/TN and the contents of BA and total VFAs, whereas significantly ($p<0.05$) increased LA/AA in forage oats and Italian ryegrass silages (Tables 2 and 3) and improved well the fermentation quality. These results are similar to the findings of Ohyama et al. (1971, 1973, 1975). The forage oats silage increased significantly ($p<0.05$) LA content, but Italian ryegrass silage did not show a significant ($p>0.05$) increase. This discrepancy can be explained as follows: In forage oats silage, it might be due to the supplying of glucose (the first fermentation substrate) stimulating the homofermentative LAB activity to produce more LA and decrease pH further. The failure of glucose addition in increasing the LA content in Italian ryegrass silage might be attributed to the relatively higher mono- and di-saccharides content in the original material than forage oats, therefore glucose addition is not very effective. This suggested that the development of LAB could not be fortified in some silage when a lot of fermentable substrate was added, or the original plant material with high content of mono- and di-saccharides. A slightly but significantly ($p<0.05$) higher BA content with glucose addition silages and a slightly higher AN/TN was found as compared with sorbic acid and FJLB additions in the two forage silages. This may be attributed to the inadequate population of epiphytic LAB in the initial forage oats and Italian ryegrass, where the rate of LA production and pH reduction was not fast enough to inhibit the activity of clostridia during fermentation process (Weinberg et al., 1988). This was well reflected in lower amounts of residual fructose and total mono- and di-saccharides than sorbic acid and FJLB additions in the two forage silages, and indicating that glucose addition was lower in the utilization efficiency of WSC by epiphytic LAB than the sorbic acid and FJLB additions. This can be attributed partially to the consumption for clostridia activity, and suggested that the adding fermentable substrate (glucose) to grass mass such as the plant material with low population of epiphytic LAB and high moisture content was not the most efficient in improving the fermentation qualities.

Sorbic acid addition

Sorbic acid addition significantly ($p<0.05$) decreased pH and significantly ($p<0.05$) increased LA/AA, and no or small amounts of PA and BA in two grass silages as compared with control. These indicated that sorbic acid was very effective in the inhibition of clostridia activity and stimulated the homofermentative LAB growth. There were higher contents of residual mono- and di-saccharides and DM and similar LA contents in sorbic acid additive silages as compared with glucose additive silages in the two grasses. These indicated that sorbic acid additions significantly ($p<0.05$) decreased the losses of mono- and di-saccharides and DM during the fermentation periods, and resulted in the high utilization efficiency of WSC by epiphytic LAB. From the facts mentioned above, although microbial analyses (yeast, mould and other aerobic bacteria) were not carried out on this study, it could still be suggested that sorbic acid might effectively depress the losses of WSC by aerobic microorganisms during the early stage of ensiling, and save WSC for LAB in the two grass silages. This was well reflected in sorbic acid treated silages with the lowest AN/TN, and the highest residual mono- and di-saccharides, where there was an inhibition of the activity of certain aerobic microorganisms causing the higher levels of AN/TN and larger amounts of WSC loss. These results are in agreement with other workers (Alli et al., 1985; Weinberg et al., 1988, 1989), who also found the sorbic acid addition increased the residual WSC and DM contents, and inhibited the activity of aerobic microorganisms. Sorbic acid addition to forage oats showed higher efficiency in LA fermentation than Italian ryegrass, where sorbic acid addition significantly ($p<0.05$) increased LA content and almost no BA was found in forage oats silage (Table 2). However, there were not significant ($p<0.05$) increases in LA content in Italian ryegrass silage and there was a slightly but significantly ($p<0.05$) higher BA content as compared with FJLB addition (Table 3). This indicated that there was a different effect of sorbic acid treatment in silages of different species and characteristics of forages (Table 1). It is suggested that 0.1% sorbic acid addition is effective in improving the silage quality, even when there are inadequate epiphytic LAB population and low DM content in original grasses.

FJLB addition

FJLB addition showed the lowest pH value and the highest LA content in both forage oats and Italian ryegrass silages, and the most intensive LA fermentation in FJLB treated silages. This was also reflected by lower residual mono- and disaccharides content in FJLB addition than that in sorbic acid additions of forage oats and Italian ryegrass, because more amounts of mono- and disaccharides may be utilized by LAB in FJLB addition silage. Moreover, FJLB addition significantly ($p<0.05$) decreased AN/TN and total VFAs content, with an absence of PA and BA, and increased significantly ($p<0.05$) LA/AA, which largely improved the fermentation quality of the silage. These indicated that FJLB addition to ensure rapid and vigorous LA fermentation resulted in faster accumulation of LA and faster pH reduction at earlier stages of ensiling, thus depressing proteolytic activity of the two grass silages and avoiding the risk of clostridial or other undesirable bacteria activity. The high efficacy of FJLB addition for improving the fermentation also demonstrated the shortage of epiphytic LAB in the original forage oats and Italian ryegrass (Table 1). In addition, FJLB showed significantly ($p<0.05$) higher residual mono- and di-saccharides than glucose addition, indicating that FJLB addition increased the utilization efficiency of mono- and di-saccharides. These results are consistent with the reports of Ohshima et al. (1997a, b, c). This can be explained as follows: Firstly, adding FJLB to silage supplied more abundant species and larger numbers of LAB, and these strains of LAB are more likely that some of them could adapt to the specific environment and enhance LA production. Secondly, the FJLBs prepared from forage oats and Italian ryegrass respectively, the same material as that for the silage making, might be more efficient in improving the quality of silage than that from other grasses, because the numbers and kinds of epiphytic LAB on the different grasses were considered to be different (Ohshima et al., 1997a, b, c). Thirdly, some substances stimulating LAB proliferation in silages might be produced during 3 days incubation (during 3 days of FJLB preparation).

Comparing the fermentation qualities among all silages in the present study, the improvement in the fermentation quality was ranked as follows: FJLB and sorbic acid >glucose>control. This suggests that adding a number of species of domestic LABs (FJLB) and aerobic bacteria inhibitor (sorbic acid) to plant materials, which contain almost sufficient WSC but low DM content and low population of epiphytic LAB such as forage oats and Italian ryegrass in the present case, are more important and efficient than adding fermentable substrates (glucose) for improving the fermentation quality of the silage. In conclusion, sorbic acid and FJLB additive treatments, which decrease the WSC loss for LAB during the early phase of ensiling. Ensiling is recommended for the reliable improvement of silage quality in the present study, but FJLB additive is more economical than sorbic acid.

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