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## Fermentation Quality of Forage Oat (*Avena sativa* L.) Silages Treated with Pre-fermented Juices, Sorbic Acid, Glucose and Encapsulated-Glucose

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This experiment was carried out to evaluate the effects of adding encapsulated-glucose, glucose, sorbic acid or pre-fermented juice of epiphytic lactic acid bacteria (FJLB) on the fermentation characteristics and residual mono- and disaccharides compositions of forage oat silages. The additive treatments were as follows: (1) control (no addition), (2) encapsulated-glucose addition at 0.5% as glucose, (3) glucose addition at 1%, (4) sorbic acid addition at 0.1%, (5) FJLB addition at a theoretical application rate of  $2.67 \times 10^6$  CFU/g, on the fresh weight basis of forage oat, respectively. Based on the results, although control and encapsulated-glucose had higher contents of BA and AN, the fermentation in all silages was clearly dominated by LAB. Glucose addition improved well the forage oat fermentation quality, however, the utilization efficiency of WSC was lower than that of both sorbic acid and FJLB additions. Sorbic acid addition showed the lowest contents of ethanol, AA, VFAs and AN, and the highest contents of residual fructose and total mono- and disaccharides as well as the highest LA/AA value with significantly higher LA content. These indicated that sorbic acid addition not only inhibited the activity of clostridial and other undesirable bacteria but also stimulated homofermentative LAB activity. These decreased the loss of mono- and disaccharides and greatly increased the utilization efficiency of fermentable substrates by epiphytic LAB. FJLB addition had the lowest pH value and the highest LA content among all additive treatments, and showed the most intensive LA fermentation. This also corresponded well with the observation that FJLB had a higher residual mono- and disaccharides than the other additive treatments except for sorbic acid addition.

### INTRODUCTION

The ensilage of forage crops is accompanied by a multitude of microbiological and biochemical changes. It depends on the natural fermentation, in which the epiphytic lactic acid bacteria (LAB) convert water-soluble carbohydrate (WSC) into lactic acid (LA) under anaerobic conditions. When pH decreases to the certain extent (approximately 4.2), the harmful microbiological activity is inhibited and the silage is well preserved for a long period (McDonald *et al.*, 1991). Thus, the success of ensilage is principally dependent on the creation of anaerobic conditions in the silo and the presence at ensilage of both sufficient LAB and adequate WSC in the crop (Rooke, 1990). Anaerobic conditions

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can be obtained by proper ensiling, such as chopping, compacting and sealing and so forth, which also depends on the physical structure of the silage material and it is mainly a technical factor that is essential to any silage material (Zhang *et al.*, 1997). However, these technical factors are usually limited and not perfectible in fact. There can still be considerable activity of aerobic microorganisms during the very early stages of ensiling and it is generally accepted that the activity of aerobic bacteria after ensiling is undesirable. These organisms consumed WSC, which were required by desirable LAB, and caused the fermentable substrate shortage (Alli *et al.*, 1985; Lacey *et al.*, 1981). In addition the population density of epiphytic LAB has been reported to range from  $10^3$  to  $10^7$  colony forming units (CFU)  $g^{-1}$  fresh matter (FM) of chopped herbage entering the silo (Fenton, 1987; McDonald *et al.*, 1991; Moran *et al.*, 1991). A large proportion of these bacteria, being heterofermentative, may not be the most effective organisms for promoting a predominant LA fermentation in the silo (Lindgren *et al.*, 1983; Grazia and Suzzi, 1984; Merry *et al.*, 1995). In order to improve the fermentation quality during ensiling, it is suggested that additives, such as the pre-fermented juice of epiphytic LAB (FJLB), sorbic acid, glucose and encapsulated-glucose, can be applied to the grass material before ensiling.

It was well documented that the added FJLB to silages was effective in improving the fermentation quality of silage, and often resulted in the increase of LA and in the reduction of ammonia-N (AN) even when the addition of commercial LAB was ineffective (Ohshima *et al.*, 1997 a, b, c). Sorbic acid addition was observed to be effective for preventing mould and yeast growth (aerobic bacteria), and it has been used as an ingredient of commercial silage additives (Alli and Baker, 1982; Lacey *et al.*, 1981; Hattori *et al.*, 1996; Woolford, 1975). Ohyama and co-workers (1971, 1973, 1975) demonstrated the advantages of adding glucose to herbage to increase fermentable substrate content and promote LAB growth improving the fermentation quality.

There is the largest decrease in WSC due to the consumption by undesired microorganisms (yeast, mould or other aerobic bacteria) and respiration, resulting in the insufficiency of fermentable substrate available for LAB in the early stage of ensiling (Shao *et al.*, 2002). However, there is also the largest growth of LAB in this period of ensiling, therefore we have decided to make a medium (encapsulated-glucose), which might be expected to give slower release rates of glucose into silage mass in order to coincide with early growth of LAB by providing additional substrate on time.

The objectives of the present study were to evaluate the effects of addition of encapsulated-glucose, glucose, sorbic acid and FJLB on the fermentation quality of forage oat silages.

## MATERIALS AND METHODS

### Additive preparation

#### (1) Pre-fermented juice preparation using epiphytic LAB (FJLB):

The FJLB was prepared from forage oat according to the following manners; a 100-g sample of freshly cut forage oat 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 to which 4 g of glucose was

added. The glass bottle was fitted with a gas trap and maintained at 30 °C for 3 days (Ohshima *et al.*, 1997 a, b, c). After 3 days of anaerobic incubation, the pH value and the population of LAB of pre-fermented juice were determined just before being added to the silage material.

(2) Encapsulated-glucose preparation:

The encapsulated-glucose was prepared as follows: glucose powder was filled into a commercial capsule; one capsule contains 0.4 g glucose. The total 8 pieces of encapsulated-glucose were mixed into the chopped forage oat just before ensiling into each silo, and thus a theoretical application rate of 0.5% glucose on FM basis.

### Silage making

Forage oat was cultivated in the experimental field of Kyushu University, Hakozaki, Fukuoka, Japan. The first growth of forage oat was hand-harvested with a sickle at the vegetative stage on 13 April 2000. The harvested material was immediately chopped into about 1 cm length prior to treatments. The silage treatments were as follows: (1) control (no addition), (2) encapsulated-glucose addition at equal to 0.5% for glucose, (3) glucose addition at 1%, (4) sorbic acid addition at 0.1%, (5) FJLB addition at a theoretical application rate of  $2.67 \times 10^6$  CFU/g, on FM basis of forage oat, respectively. After thorough mixing, a 630-g of forage oat at each treatment was ensiled into a laboratory silo (1 liter capacity) in triplicates. This was followed by being sealed with a screw top and kept at the ambient temperature of 25 °C. All silos were opened after 30 days of ensilage.

### Chemical analyses

The chopped grass was immediately collected for the determination of contents of dry matter (DM), mono- and disaccharides compositions (fructose, glucose and sucrose), and the population of epiphytic LAB in the initial forage oat. 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 hours. Then, the extracts were filtered through double layers cheesecloth and a filter paper (Toyo No. 5A, Japan), and the filtrate was 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 VFAs and ethanol contents with gas chromatography (Shimadzu, GC-17A with 12 m capillary column, condition: column temperature 100 °C, injection temperature 250 °C), AN content with an ammonia electrode meter (Model IM-22P, Toa Electronics Ltd., Japan). The DM contents of the grass and silages were determined by drying in an oven at 60 °C for at least 48 hours (AOAC, 1984), and DM of silage was recalculated with the contents of volatile components. Mono- and disaccharides compositions of the grass and silages were determined by high performance liquid chromatography (HPLC) as shown in our previous report (Shao *et al.*, 2002). The population of LAB in the fresh grass and the FJLB was determined by counting the CFU with GYP-CaCO<sub>3</sub> agar plate (Masuko *et al.*, 1992).

### Statistical analyses

The statistical analysis included one-way analysis of variance with additive treat-

ments 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), the significance was declared at  $p < 0.05$ .

## RESULTS

Table 1 shows the characteristics of the initial forage oat and FJLB. It contained 139.01 g/kg for DM, 14.82 g/kg for fructose, 13.42 g/kg for glucose, 34.69 g/kg for sucrose and 62.92 g/kg for mono- and disaccharides. The population of epiphytic LAB was  $3.46 \times 10^9$  CFU/g FM for the original fresh grass,  $1.06 \times 10^8$  CFU/ml for the FJLB, respectively, and the pH value of FJLB was 3.88.

The fermentation qualities with additive treatments are presented in Table 2. There was not a large difference in the fermentation quality between control and encapsulated-glucose silage. The addition of encapsulated-glucose had only a slight decrease in pH, and a small increase in the contents of total VFAs and LA. The glucose, sorbic acid and FJLB additions significantly ( $p < 0.05$ ) decreased pH and the contents of butyric acid (BA) and total VFAs, whereas significantly ( $p < 0.05$ ) increased LA content as compared with control silage. There were not significant differences in acetic acid (AA) content among all silages, however, there was a decrease with larger extent for glucose, sorbic acid and FJLB additions than for encapsulated-glucose addition. Thus glucose, sorbic acid and

**Table 1.** Characteristics of forage oat and FJLB before being ensiled (g kg<sup>-1</sup> DM)

Dry matter (g kg <sup>-1</sup> )	Fructose	Glucose	Sucrose	Mono-and disaccharides	LAB of fresh oat <sup>1)</sup>	LAB of FJLB <sup>2)</sup>	pH of FJLB
139.01	14.82	13.42	34.69	62.92	$3.46 \times 10^9$	$1.06 \times 10^8$	3.88

1) The population of epiphytic LAB in the initial forage oat (CFU g<sup>-1</sup> FM).

2) The population of epiphytic LAB in FJLB (CFU ml<sup>-1</sup>).

**Table 2.** Fermentation quality of forage oat silage treated with encapsulated-glucose, glucose, sorbic acid and FJLB

Item	Treatments				
	Control	Encapsulated-glucose	Glucose	Sorbic acid	FJLB
pH (SD)	4.27 (0.36) c <sup>1)</sup>	4.15 (0.19) bc	3.86 (0.10) ab	3.87 (0.06) ab	3.63 (0.02) a
DM (SD) (g kg <sup>-1</sup> )	127.31 (1.80) b	117.09 (3.60) a	128.08 (5.34) b	136.78 (0.60) c	135.17 (1.08) c
LA (SD) (g kg <sup>-1</sup> DM)	61.67 (22.53) a	76.97 (17.84) a	113.17 (13.68) b	116.42 (50.03) b	131.27 (18.01) b
AA (SD) (g kg <sup>-1</sup> DM)	9.96 (1.68) a	9.56 (6.83) a	6.56 (0.17) a	5.87 (1.68) a	7.58 (2.38) a
PA (SD) (g kg <sup>-1</sup> DM)	1.20 (0.62) ab	3.22 (2.85) b	0.25 (0.43) a	0.00 (0.00) a	0.00 (0.00) a
BA (SD) (g kg <sup>-1</sup> DM)	20.86 (18.47) c	21.73 (11.38) c	3.49 (5.86) b	0.97 (1.18) a	0.04 (0.08) a
VA (SD) (g kg <sup>-1</sup> DM)	0.05 (0.09) a	0.00 (0.00) a	0.04 (0.08) a	0.00 (0.00) a	0.00 (0.00) a
VFAs (SD) (g kg <sup>-1</sup> DM)	31.47 (21.61) c	34.52 (18.79) c	12.05 (6.78) b	6.83 (2.10) a	7.62 (2.30) a
Ethanol (SD) (g kg <sup>-1</sup> DM)	4.73 (1.49) b	2.99 (0.40) ab	3.16 (0.16) ab	1.46 (1.78) a	2.54 (0.39) a
AN (SD) (g kg <sup>-1</sup> DM)	2.12 (0.33) b	2.32 (0.51) b	1.36 (0.47) a	0.93 (0.29) a	1.24 (0.23) a
LA/AA(SD)	6.53 (3.06) a	11.32 (6.86) ab	17.28 (2.26) b	21.01 (10.37) b	18.25 (4.48) b

1) Values followed by different letters in the same row show significant differences at  $p < 0.05$ .

**Table 3.** The residual mono- and disaccharides compositions of forage oat silage treated with encapsulated-glucose, glucose, sorbic acid and FJLB (g kg<sup>-1</sup> DM)

Item	Treatments				
	Control	Encapsulated-glucose	Glucose	Sorbic acid	FJLB
Fructose (SD)	1.63 (2.18)a <sup>1)</sup>	0.67 (0.25)a	2.95 (1.70)a	14.26 (5.64)c	7.64 (4.33)b
Glucose (SD)	0.00 (0.00)a	0.22 (0.38)a	0.00 (0.00)a	0.00 (0.00)a	0.00 (0.00)a
Sucrose (SD)	1.45 (0.21)a	1.35 (0.51)a	1.57 (0.07)a	1.43 (0.14)a	2.06 (0.45)a
Mono- and disaccharides (SD)	3.08 (2.29)a	2.23 (0.07)a	4.52 (1.76)ab	15.69 (5.65)c	9.70 (4.74)bc

1) Values followed by different letters in the same row show significant differences at  $p < 0.05$ .

FJLB additions significantly ( $p < 0.05$ ) increased the ratio of lactic to acetic acid (LA/AA), but encapsulated-glucose addition showed an insignificant ( $p > 0.05$ ) increase. Valeric acid (VA) was hardly detected in all treatments, and propionic acid (PA) was detected in both control and encapsulated-glucose treatments. Sorbic acid and FJLB additions significantly ( $p < 0.05$ ) decreased the ethanol content, but glucose and encapsulated-glucose additions showed a slight decrease ( $p > 0.05$ ). Glucose, sorbic acid and FJLB additions significantly ( $p < 0.05$ ) decreased AN content, whereas encapsulated-glucose addition did not show a significant difference ( $p > 0.05$ ) from the control silage. There was a significant ( $p < 0.05$ ) increase in DM content in the additions of sorbic acid and FJLB, however, encapsulated-glucose addition significantly ( $p < 0.05$ ) decreased the DM content as compared with control.

The residual mono- and disaccharides compositions of silages are presented in Table 3. There were not significant ( $P > 0.05$ ) differences in mono- and disaccharides composition between glucose addition and encapsulated-glucose addition. The additions of sorbic acid and FJLB showed significantly ( $p < 0.05$ ) higher contents of residual fructose and total mono- and disaccharides than other additive treatments, and glucose addition showed only a slight increase ( $p > 0.05$ ) in total mono- and disaccharides. There were not significant differences in residual sucrose content that showed only small amounts, and almost no residual glucose was detected in all silages.

## DISCUSSION

Although control and encapsulated-glucose had higher contents of BA and AN, all silages were clearly dominated by LAB as judged by their low pH values (3.63–4.27), low contents of AA (5.87–9.96 g/kg) and total VFAs (6.83–34.52 g/kg), no or very small amounts of PA and VA, whereas high LA/AA (6.53–21.01) and LA content (61.67–131.27 g/kg) (Catchpoole and Henzell, 1971). Except for control and encapsulated-glucose treatments, the good fermentation in these silages was also shown by very low contents of BA (0.04–3.49 g/kg) and AN (0.93–1.36 g/kg). Sufficient fermentable substrates of mono- and disaccharides in forage oat used in this study might lead to LA fermentation silages (Ridla and Uchida, 1998a, b). The potential availability of sufficient fermentable substrates can be further explained as follows: although the mono- and disaccharides in the original forage oat was not very high (62.92 g/kg), forage oat is one of the temperate grasses in which fructans are the most abundant source of WSC. The

fructans can be hydrolyzed into glucose and fructose by plant enzymes during very early stage of ensiling, therefore there might be the fact that considerable amounts of LA were produced from some other substrate such as fructans than the initial mono- and disaccharides during ensiling (Shao *et al.*, 2002).

### **Encapsulated-glucose addition**

There were not marked differences in fermentation products and residual mono- and disaccharides compositions between the control and encapsulated-glucose treatment (Tables 2 and 3). The absence of effect of encapsulated-glucose addition on the fermentation quality in this experiment was probably because the ratio of glucose addition (0.5% glucose on FM basis) was not enough to promote epiphytic LAB activity causing further LA build-up and pH decrease. The activities of clostridial bacteria or other undesirable bacteria in encapsulated-glucose silages could still not be inhibited effectively. This was also reflected by high contents of BA, VFAs and AN in both control and encapsulated-glucose treatment. Only a detected level of residual mono- and disaccharides content suggested that a part of mono- and disaccharides had been consumed by clostridia or other aerobic bacteria during ensiling, being agreement with the results of Tamada *et al.* (1996). In addition the number of epiphytic LAB in the original grass might have also affected the fermentation patterns of the silages. The insufficient number of viable epiphytic LAB ( $<10^5$  CFU/g FM) could decrease the rate and extent of pH reduction and LA production (Hellings *et al.*, 1985; Woolford and Sawcyc, 1984), resulting in a low utilization efficiency of WSC by LAB in the early stage of ensiling. Both control and encapsulated-glucose treatment showed significantly ( $p < 0.05$ ) higher contents of BA and AN than other additive treatments. This was also attributed to high contents of moisture and the low number of epiphytic LAB ( $3.46 \times 10^3$  CFU/g FM) in the original forage oat, which conditions were adequate for clostridial bacteria development (McDonald *et al.*, 1991).

### **Glucose addition**

Glucose addition decreased remarkably the contents of BA, AN and total VFAs and pH value, whereas there were remarkable increases in LA content and LA/AA, and improved well the forage oat fermentation quality. These results are similar to the findings of Ohyama *et al.* (1971, 1973, 1975). It may be due to the supplying of more amount of fermentable substrate (1% glucose addition) compared with encapsulated-glucose addition, stimulating the homofermentive LAB to produce more LA and decrease pH further. This suggests that, although there is an inadequate population of epiphytic LAB in the initial forage oat, the larger amount of fermentable substrate addition (the first fermentation substrate glucose) may improve the fermentation quality of silage (Ohyama *et al.*, 1975). However, there were very low residual mono- and disaccharides and higher BA content as compared with both sorbic acid and FJLB additions. This may be due to the inadequate population of epiphytic LAB of the initial forage oat ( $3.46 \times 10^3$  CFU/g FM), thus the rate of LA production and pH decrease were still not enough fast to inhibit the clostridial growth completely. These indicated that glucose addition was lower in the utilization efficiency of WSC by epiphytic LAB than the sorbic acid and FJLB additions, where adding fermentable substrate to silage did not seem most efficient in improving the

fermentation quality in the present experiment.

### **Sorbic acid addition**

Sorbic acid addition showed the lowest contents of ethanol, AA, total VFAs and AN, and the highest contents of DM and residual mono- and disaccharides, and the highest LA/AA, with no or only very small amounts of PA, VA and BA. These results are in agreement with those of other workers (Alli *et al.*, 1985; Weinberg *et al.*, 1988, 1989). This indicated that sorbic acid addition not only was very effective in the inhibition of clostridial and other aerobic bacterial activity but also stimulated homofermentative LAB activity to show the most efficient LA fermentation during the early stage of ensiling. There were significant decreases in the loss of mono- and disaccharides and DM by the undesirable bacteria, resulting in the most efficient utilization of fermentable substrate by epiphytic LAB. Therefore, it was suggested that 0.1% sorbic acid addition is very effective in improving the silage quality, even when there were inadequate epiphytic LAB population and low DM in the original forage oat.

### **FJLB addition**

FJLB addition showed the lowest pH value and the highest LA content, indicating that FJLB addition had the most intensive LA fermentation among all treatments silages. This was also reflected by lower residual mono- and disaccharides content in FJLB addition than in sorbic acid addition, because more amounts of mono- and disaccharides might be utilized by LAB in the FJLB addition silage. Moreover, FJLB addition significantly decreased the contents of AN, ethanol and total VFAs with an absence of PA, BA, and VA contents, and significantly increased LA/AA, resulting in a large improvement in the fermentation quality of the silage. These indicated that FJLB addition to ensure the rapid and vigorous LA fermentation resulted in faster accumulation of LA and reduction of pH values at earlier stages of ensiling, thus depressing the proteolytic activity and avoiding the risk of clostridial or other undesirable bacteria fermentation of the silage. The high efficacy of FJLB addition also demonstrated an evidence of the shortage of epiphytic LAB in the original grass ( $3.46 \times 10^3$  CFU/g FM). In addition FJLB still showed significantly higher residual mono- and disaccharides than the other additive treatments except for sorbic acid addition, indicating that FJLB addition also increased the utilization efficiency of mono- and disaccharides. These results are consistent with the reports of Ohshima *et al.* (1997 a, b, c). This can be explained as follows. First, adding FJLB to silage supplied abundant species and larger number of LAB, where these strains of LAB were more likely that some of them could adapt to the specific environment and enhance the LA production (Ohshima *et al.*, 1997 a, b, c). Second, the FJLB prepared from forage oat, the same plant with the silage material, might be more efficient in improving the quality of silage than that from other crops, because the number and the kinds of epiphytic LAB on the different crops were considered to be different. Ohshima and co-workers (1997 a, b, c) also found that silages of good fermentation quality could be made at any ensiling temperature from direct cut alfalfa harvested in different stages of maturity by adding FJLB, a novel additive, even when the alfalfa ensiled without any additives or with a conventional LAB additive results in poor quality silage. Furthermore, whether FJLB prepared from one crop might be more effective in improving the quality of

silage from other crops was studied, and it was suggested that FJLB from other plants are available as silage additives but it is more effective when made from the same plant to be ensiled (Ohshima *et al.*, 1997 a, b, c).

Based on the present study, the improvement in the fermentation quality with additives was ranked in the following order: the treatment with sorbic acid or FJLB > glucose > encapsulated-glucose > control. These suggested that adding a number of species of domestic LAB (FJLB) and aerobic bacteria inhibitor (sorbic acid) to plant materials such as forage oat, which contain intermediate amount of WSC and low population of epiphytic LAB with low level of DM content, were more important and efficient than adding fermentable substrates (glucose and encapsulated-glucose) for improving the fermentation quality of the silage. Therefore, 0.1% sorbic acid and FJLB ( $2.67 \times 10^5$  CFU/g FM) additive treatments were recommended for improving the silage quality in the present study, and especially FJLB will be attractive for farmers not only from its efficacy but also from the economical view point.

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