The Influences of Addition of Sugar with or without *L. buchneri* on Fermentation and Aerobic Stability of Whole Crop Maize Silage Ensiled in Air-stress Silos

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ABSTRACT : The whole plant of crop maize was chopped and ensiled in double-layered polyethylene bags to determine the influence of residual sugar on the fermentation of lactic acid and aerobic stability by *L. buchneri* in whole crop maize silage made in air-stress condition. There were a total of six treatments used in this experiment as follow: added 25 g de-mineralised water per kg chopped maize serving as control (con), 37.5 g glucose solution containing 12.5 g glucose (g_1), 75 g glucose solution containing 25 g glucose (g_2), 25 g, *L,.buchneri* suspension intended for 10^6 cfu g^{-1} (L.b.), g_1+L.b. and g_2+L.b. All silos were opened at day 91 after ensiling for measuring the pH values, microbiological enumeration, fermentative products and aerobic stability. The dry matter loss increased significantly ($p<0.01$) due to inclusion of sugar or *L. buchneri*. The lower lactic acid concentrations were observed ($p<0.01$) in silages inoculated with *L. buchneri* only or in combination with sugar addition than the correspondent uninoculated silages. Compared with control silage, ethanol production was about 3 or 6-fold higher due to addition 12.5 or 25 g glucose per kg chopped maize at ensiling. The silages added with sugar contained less acetic acid concentration ($p<0.01$) than control, but silages inoculated with *L. buchneri* showed the contrary effects ($p<0.01$) at different sugar levels. No butyric acid was found in uninoculated silages, silages inoculated with *L. buchneri*. producted more propionic acid, 1-propanol and butyric acid. Lactic acid bacteria counts increased markedly ($p<0.01$) due to inoculation with *L. buchneri*, whereas it was reduced ($p<0.01$) by added sugar. No significant difference was observed in count of yeast, but inoculation with *L. buchneri* shows a decreasing trend. Mould count in all silages was less than 2 (log cfu g^{-1}). The added sugar had negative effects on aerobic stability of maize silage made under air-stress conditions, whereas inoculation with *L. buchneri* improves ($p<0.01$) the aerobic stability. *(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 12 : 1738-1742)*

INTRODUCTION

Silages usually serve as main feed in ruminant production. Some oxygen will penetrate the silage during the storage by diffusion through, or physical damage of the protecting cover. Moreover, upon opening the silo for feeding, silage of exposure to oxygen is inevitable. Oxygen stimulates the growth of yeasts that can lead to spoilage of the maize silage, which cause not only loss of nutritional value but also negative effects in the hygienic quality of silages due to the increased risk of proliferation of potentially pathogenic or otherwise undesirable micro-organism (Woolford, 1990). Bacterial inoculants for silage usually contain one or more species of homofermentative LAB that are fast and efficient producers of lactic acid, however, results of different studies indicated that these inoculants can impair aerobic stability (Kung et al., 1991; Sanderson, 1993; Guan et al., 2002a). Therefore attempts have recently been made to develop bacterial inoculants that can improve the aerobic stability (Weinberg and Muck,

1996).

Several studies have shown that inoculation of whole maize with a strain *L. buchneri* resulted in the increase of acetic (and propionic) acid concentration that lead to a reduced survival of yeasts in maize silage (Driehuis et al., 1998,1999; Oude Elferink et al., 1998; Guan et al., 2002b). However, extra sugar can enhance growth of yeast during the whole ensiling in maize silage (Guan et al., 2002b), and normally silages located in silo surface are under air stress condition, the air-stress in this paper was defined as condition under which oxygen can penetrate the silage during the storage by diffusion through. There is still no data available in respect to the effects of extra sugar influencing the fermentation characteristics of whole maize crop silage with *L. buchneri,* therefore the objectives of this study were to determine the influence of residual sugar on the fermentation of lactic acid and aerobic stability by *L. buchneri* in whole crop maize silage made in air-stress condition.

MATERIAL AND METHODS

Experimental procedure

The whole crop maize (variety LG2181) was harvested through a precision-chop forage harvester. The intended

Table 1. Chemical analyses and epiphytic bacteria of the fresh maize at ensiling

Items	Con	g_1	g_2	L.b.	L.b.+g_1	L.b.+g_2
DM, g kg^{-1}	290	299.5	299.8	291.7	303.3	302.2
WSC, g kg^{-1} DM	85.7	116.1	138.8	88	113.2	132.9
N, g kg^{-1} DM	10.3	-	-	10.1	-	-
NDF, g kg^{-1} DM	462.9	-	-	456.5	-	-
ADF, g kg^{-1} DM	222.1	-	-	243.3	-	-
LAB, log$_{10}$ cfu g^{-1}	5.99	-	-	6.2	-	-
Lactobacilli, log$_{10}$ cfu g^{-1}	5.64	-	-	6.2	-	-
Yeast, log$_{10}$ cfu g^{-1}	4.7	-	-	4.3	-	-
Mould, log$_{10}$ cfu g^{-1}	3.7	-	-	3.6	-	-

-: not determinated. Inoculant containing *L. buchneri* supply: 9.5×10^7 cfu on medium of Rogosa 6.2.
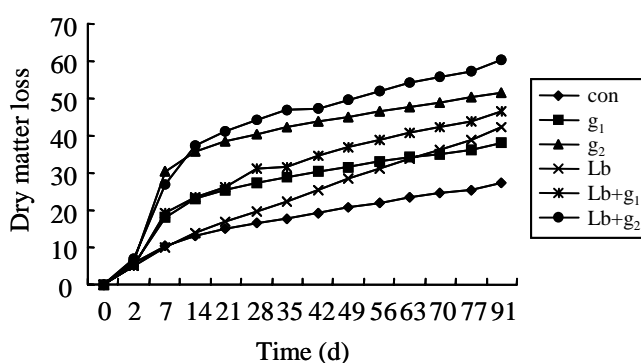

Figure 1. Dry matter loss (g kg^{-1} DM) in maize silages with different treatments during the whole ensiling under air-stressed condition.

inoculation level for *L. buchneri* (which was isolated from maize silage and served as one candidate for commercial inoculants) was 10^6 cfu g^{-1}, the suspension were applied using a pressure sprayer while mixing in a concrete mixer. Laboratory silos were double-layered polyethylene bags, which were stored in the dark at 20°C. There were 3 bags per treatment (*c.* 2 kg per bag) and all bags on day 91 post ensiling were opened, one 30 g silage sample per jar was taken and add 270 g demineralized water, and then blend 5 min with stomacher for measuring the pH, microbiological enumeration and fermentative products.

There were a total of six treatments used in this experiment as follow: added 25 g de-mineralised water per kg chopped maize serving as control (con), 37.5 g glucose solution containing 12.5 g glucose (g_1), 75 g glucose solution containing 25 g glucose (g_2), 25 g *L. buchneri* suspension intended for 10^6 cfu *L. buchneri* per g maize (L.b.), 12.5 g glucose kg^{-1}+*L. buchneri* 10^6 cfu g^{-1} (g_1+L.b.), and 25.0 glucose g kg^{-1}+*L. buchneri* 10^6 cfu g^{-1} (g_2+L.b.)

Analytical procedure

The maize samples taken from control and treated only with *L. buchneri* at ensiling were analyzed for pH, lactobacilli, total lactic acid bacteria (LAB), yeast and mould, but only sample from the control also for measuring DM, crude protein (CP), neutral detergent fibre (NDF),

acid detergent fibber (ADF) and water soluble carbohydrate (WSC). All silage samples were subject to analyze for pH, DM, ethanol and volatile fatty acids, lactic acid, sugar and the numbers of LAB, *lactobacilli*, yeast and mould. The dry matter, CP, ash, NDF, ADF and WSC were determined as described by van Vuuren et al. (1993). Bacteria counts, pH and concentrations of lactic acid, VFA and ethanol were determined in extracts of samples of maize or silage, prepared as described by Spoelstra (1983). Lactic acid was determined as described by Spoelstra (1983). Ethanol and VFA were determined by gas chromatography, using Hewlett Packard 5730A equipment, a 25 m medium bore capillary column (Chrompack CP-Sil-5CB) and helium as carrier gas. *Lactobacilli* were enumerated on double layered poured plates of Rogosa SL Agar (Difco) acidified with glacial acetic acid to pH 5.4, incubated 3 days at 30°C. Lactic acid bacteria (LAB) were enumerated on doubled layered pour plates of Rogosa SL Agar (Difco) adjusted with sodium hydroxide to pH 6.2 containing 100 mg L^{-1} cycloheximide, incubated 3 days at 30°C. Yeast and mould were enumerated on double layered pour plates of Malt Extract Agar (MEA) acidified by lactic acid to pH 3.5, incubated 3 days at 30°C. Aerobic stability of silage were determined by incubation at 20°C of 0.3 kg lots in insulated containers with holes lids and bottoms to allow air to enter and carbon dioxide to escape. Temperature was measured continually by a thermocouple placed in the center of material, coupled to data taker. Aerobic stability was defined as the time needed to increase the temperature 1°C above ambient temperature.

Statistical analysis

The statistical analysis included one-way analysis of variance and Duncan's multiple range test; these were performed by ANOVA using the GLM procedure of the SAS as a randomized complete block design.

RESULTS

Composition of maize before ensiling

The chemical analysis and the epiphytic bacteria

number of LAB, *Lactobacilli*, yeast and mould of the pre-ensiled maize crop are given in Table 1. The treatment with inoculant showed a slight higher the LAB and *Lactobacilli*, lower yeast than control maize.

Dry matter loss

The dry matter loss increased markedly (p<0.01) due to inclusion of the added sugar (as shown in Figure 1), and it intensified with the more sugar addition on the basis with or without *L. buchneri*, *L. buchneri* enhanced (p<0.01) DM loss further at different sugar existence. The similar amount of sugar left in all the final silages suggested that all added sugars were nearly consumed, in line with the changes of DM loss.

Microbiological composition in final silages

Microbiological composition in final silages is shown in Table 2. *Lactobacilli* counts in silages of 91 days varied from 7.92 (log cfu g^{-1}) to 9.07, which were reduced (p<0.01) by added sugar, but increased significantly (p<0.01) by inoculation with *L. buchneri*. No significant difference was observed in count of yeast, but inoculation with *L. buchneri* showed a decreasing trend. Mould count in all silages was less than 2 (log cfu g^{-1}).

Chemical composition of silages after 91 days

The fermentative products concentrations in silages after 91 days ensiled in double layers polyethylene silos are shown in Table 2. The concentration of lactic acid varied from 16.69 to 88.48 g kg^{-1} DM, which increased markedly (p<0.01) with inclusion of extra sugar at ensiling. Silages inoculated with *L. buchneri* or with sugar addition together contained less (p<0.01) lactic acid than the correspondent silages without inoculation with *L. buchneri*. The ethanol

production was improved significantly (p<0.01) due to extra sugar addition both with and without *L. buchneri*. The added sugar resulted in lower acetic acid concentration (p<0.05) than control, but inoculation with *L. buchneri* increased (p<0.01) acetic acid than correspondent uninoculated silages at each sugar levels at ensiling. Silages inoculated with *L. buchneri* contained more propionic acid (p<0.01), 1-propanol (p<0.01) and butyric acid (p<0.01) than those in uninoculated silages, but added sugar reduced the formation of propionic acid, and 1-propanol (p<0.01) in those silages. No butyric acid was detected in control or only sugar treated silages, but it was found in all inoculated silages. No 1, 2-propandiol can be found in silages.

Aerobic stability

The added sugar decreased the aerobic stability and this reduction intensified with increasing level of sugar inclusion, which was not statistically significant (p>0.05). Inoculation with *L. buchneri* improved the aerobic stability (p<0.01), but the added extra sugar reduced the improvement of *L. buchneri*.

DISCUSSION

Previous studies have shown that inoculation of whole maize with a strain *L. buchneri* does not influence the primary fermentation (conversion of sugars into acids), but induce a secondary fermentation in which part of lactic acid present in silage is converted to acetic acid and 1, 2-propanediol (Oude Elferink et al., 1998; Driehuis et al., 1996, 1999; Guan et al., 2002b). There are indications that 1, 2 propanediol can be further converted to 1-propanol and propionic acid (Driehuis et al., 1998; Guan et al., 2002b). The increase in acetic (and propionic) acid lead to a reduced

Table 2. Composition of the maize silages under air-stress conditions in 2-kg polyethylene bags at day 91 [1,2,3]

Items	Con	g_1	g_2	L.b	L.b+g_1	L.b+g_2	SEM
pH	3.82 [c-C]	3.73 [d-D]	3.71 [d-D]	4.31 [a-A]	4.03 [b-B]	4.03 [b-B]	0.02
DM, g kg^{-1}	268.3 [abc-A]	268.7 [abc-A]	274.6 [ab-A]	259.2 [bc-A]	264.5 [abc-A]	256.9 [c-A]	7.32
Sugar, g kg^{-1} DM	4.46 [b-A]	4.56 [ab-A]	4.84 [a-A]	4.56 [ab-A]	4.58 [ab-A]	4.79 [a-A]	0.16
DM loss, g kg^{-1} DM	27.37 [f-F]	37.99 [e-E]	51.55 [b-B]	42.35 [d-D]	46.7 [c-C]	60.51 [a-A]	1.19
Ethanol, g kg^{-1} DM	7.58 [d-D]	23.42 [b-B]	38.22 [a-A]	11.78 [c-C]	22.11 [b-B]	37.09 [a-A]	0.87
Acetic acid, g kg^{-1} DM	43.63 [c-CD]	38.43 [d-D]	37.83 [d-D]	68.17 [a-A]	54.72 [b-B]	56.94 [b-B]	2.57
Lactic acid, g kg^{-1} DM	74.4 [b-B]	88.48 [a-A]	86.21 [a-A]	16.69 [e-D]	48.26 [c-C]	39.51 [d-C]	3.85
Propionic acid, g kg^{-1} DM	1.73 [c-C]	0 [d-D]	0 [d-D]	8.04 [a-A]	4.34 [b-B]	4.3 [b-B]	0.17
1, 2-propandiol, g kg^{-1} DM	0 [a]	0 [a]	0 [a]	0 [a]	0 [a]	0 [a]	0.0
1-propanol, g kg^{-1} DM	5.7 [c-C]	4.75 [cd-CD]	3.79 [d-D]	14.8 [a-A]	10.72 [b-B]	10.77 [b-B]	0.58
Butyric acid, g kg^{-1} DM	0 [c-C]	0 [c-C]	0 [c-C]	0.79 [a-A]	0.78 [a-A]	0.7 [b-B]	0.02
LA/AA	1.71 [b-B]	2.31 [a-A]	2.31 [a-A]	0.25 [d-D]	0.88 [c-C]	0.70 [c-C]	0.15
LAB, log cfu g^{-1}	8.31 [b-B]	7.92 [c-C]	7.97 [c-C]	9.07 [a-A]	8.84 [a-A]	8.83 [a-A]	0.11
Yeast, log cfu g^{-1}	3.23 [a]	3.16 [a]	3.22 [a]	2.46 [a]	2.59 [a]	2.56 [a]	0.57
Mould, log cfu g^{-1}	<2 [a]	<2 [a]	<2 [a]	<2 [a]	<2 [a]	<2 [a]	0.04
Aerobic stability (h)	135 [cd-BC]	107 [d-C]	92 [d-C]	>260 [a-A]	209 [ab-AB]	196 [bc-AB]	29.6

[1,2,3] Con: control; g_1 (add 12.5 g glucose per kg maize); g_2 (add 25 g glucose per kg maize); L.b (intended for 10^6 cfu *L.buchneri* per g maize); DM: dry matter. [a,b,c,d,e,f] Means within a row with no common superscripts differ (p<0.05). [A,B,C,D,E,F] Mean within a row with no common superscripts differ (p<0.01).

survival of yeasts during the anaerobic ensilage phase and growth inhibition of yeasts and moulds during the aerobic phase (Driehuis et al., 1998, 1999; Oude Elferink et al., 1998). The results of this trial demonstrated that use of *L. buchberi* in silage made in air stress condition can enhance substantially the aerobic stability of maize silage. The explanation for this effect is that the survival of yeasts during the fermentation was impaired due to the increase in acetic (and propionic) acid in silages inoculated with *L. buchneri*, this was confirmed by the results found in another trial in which the maize silages were made in anaerobic silos (Guan et al., 2002b). Therefore the less yeast counts were found in inoculated silages than the correspond silages. Furthermore, growth of yeasts during exposure of silage to O_2 was almost completely inhibited (Dreihuis et al., 1999).

Results obtained in maize silages made under anaerobic condition confirmed the micro-organism responsible for more ethanol production in silages is yeast (Guan et al., 2002b). As observed in silages made in anaerobic silos, the inclusion of extra sugar at ensiling produced the much higher ethanol, this is mainly due to conversion of sugar into ethanol by yeast during the fermentation. Both *L. buchneri* and inclusion of extra sugar enhanced the ethanol production.

All the inoculated silages with *L. buchneri* were characteristiced by increased concentration of acetic acid, propionic aicd, 1-propanol, DM loss, and higher final pH, which is in agreement with data obtained in anaerobic silos (Guan et al., 2002b). Data from the present trial indicated that inoculation with *L. buchneri* reduced yeast counts and increased LAB counts, the similar results were reported in other experiments (Driehuis et al., 1996; Driehuis et al., 1998; Oude Elferink et al., 1998; Guan et al., 2002b). Previous studies with pure cultures of *L. buchneri* have shown that *L. buchneri* is capable of converting lactic acid into acetic acid and 1, 2-propandiol (Oude Elferink et al., 1998), however, sometimes 1-propanol and propionic acid instead of 1, 2-propanediol was detected (Driehuis et al., 1996; 1998). The curves of developments of different fermentative products during the whole process under anaerobic condition (data not shown) suggested that there are two degradation pathways of lactic acid by *L. buchneri*, both of them begin to function after 14 days at the same time. The first metabolism way of lactic acid is that, after 2 weeks post-ensiling, *L. buchneri* started to ferment lactic acid into acetic acid and carbon dioxide, slight more DM loss in inoculated silage with *L. buchneri* attribute to loss of carbon dioxide. The second pathway is that lactic acid was firstly converted into 1, 2-propandiol, then 1, 2-propandiol into 1-propanol and (or) propionic acid further in silages. No 1, 2-propandiol can be found in silages, suggesting that it finished the conversion into 1-propanol and (or) propionic acid.

Another finding in this study is that there is an antagonism between the added sugar and function of *L. buchneri*, confirmed by the fermentative product concentration in final silages, similar results were found in silage made in anaerobic silos (Guan et al., 2002b). Data from aerobic stability testing also supports the finding. As respects to aerobic stability, the added sugar had a negative effect on the aerobic stability under air-stress conditions. In contrary, inoculation with *L. buchneri* caused a pronounced improvement in the aerobic stability, but functions of *L. buchneri* were reduced by added sugar at ensiling. In conclusion, the present study demonstrated that: 1) inoculation with *L. buchneri* improves the aerobic stability of maize silage made in air-stress condition, whereas the added sugar had negative effects on aerobic stability; 2) there is an antagonism between added sugar and *L. buchneri* within air-stressed silages.

ACKNOWLEGEMENTS

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