The effect of the addition of a lactic acid bacterial inoculant to maize at ensiling on silage composition, silage intake, milk production and milk composition

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Abstract

The aim of the study was to determine the effect of the addition of a lactic acid bacterial inoculant to maize at ensiling on the fermentation dynamics during ensiling, aerobic stability of the silage as well as the intake, milk production and milk composition of Jersey cows fed maize silage diets. The inoculant contained *Lactobacillus plantarum* and *Pediococcus acidilactici* as well as amylase. Maize was ensiled in laboratory and bunker silos. The inoculant did not result in a more rapid lowering of the pH or a more rapid lactic acid production compared to untreated maize silage made in laboratory silos. Both the control and inoculated maize silages were well preserved with a pH of 3.57 and 3.62, a lactic acid concentration of 66 and 63 g/kg DM and an ammonia nitrogen concentration of 5.88 and 5.10 g/100 g of total nitrogen respectively. No butyric acid was found in either untreated or inoculated maize silage. The maize silages made in the bunker silos were well preserved with a DM of 283 and 307 g/kg silage, pH of 3.50 and 3.51, lactic acid of 37.0 and 35.3 g/kg DM for the control and inoculated maize silage, respectively. The addition of the inoculant to maize at ensiling improved the palatability, intake and the aerobic stability of maize silage compared to the untreated control maize silage. The intake of untreated and inoculated maize silage by Jersey cows was 7.6 and 8.4 kg DM/day for the control and inoculant treatment, respectively. Milk production, milk composition, live weight and condition score of Jersey cows was not significantly affected by the addition of the inoculant to maize silage.

Keywords: Maize silage, inoculant, lactic acid bacteria, milk production, intake, Jersey cows
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Introduction

Maize (*Zea mays*) is an ideal silage crop with a relative high dry matter (DM) content, low buffering capacity and adequate water-soluble carbohydrates for satisfactory fermentation to lactic acid (McDonald *et al*., 1991). To obtain a high quality well fermented palatable silage, a rapid drop in pH is needed to inhibit the growth of enterobacteria and clostridia (McDonald *et al*., 1991). This happens when homofermentative lactic acid bacteria utilise water-soluble carbohydrates and produce lactic acid. If, however, heterofermentative lactic acid bacteria are dominant on a maize crop prior to ensiling, fermentation will be less efficient and the end products of fermentation will be lactic acid, acetic acid, ethanol and carbon dioxide (McDonald *et al*., 1991). The number of lactic acid bacteria present on maize plants prior to ensiling may be too low to ensure rapid efficient preservation, as was reported by Speckman *et al*. (1981) who surveyed numbers of *lactobacilli* on maize crops in the USA and showed that 69% of samples had counts below 1000 colony forming units per gram of fresh material. Meeske & Basson (1998), however, found that the number of lactic acid bacteria on fresh chopped maize plants prior to ensiling was as high as 10^9 colony forming units per gram of fresh material. This study was done at the Animal Nutrition and Animal Products Institute, Irene (longitude 28° 13 'S: latitude 25° 55 'E, altitude 1524 m). Weise & Wermke (1973) established that lactic acid bacteria prefer moderately warm weather. Lower numbers of lactic acid bacteria may, therefore, be expected on silage crops in more temperate climates as is found in the Southern Cape (longitude 22° 25 'S: latitude 33° 55 'E, altitude 204 m).

The aim of this study was to determine the effect of adding a lactic acid bacterial inoculant to maize at the time of ensiling on fermentation dynamics during ensiling, aerobic stability of the silage, intake, milk production and milk composition of Jersey cows fed maize silage diets.

Materials and methods

Four hectares of maize (PAN 6364) were planted on the 10th of December 1997 at the Outeniqua Experimental Farm in the Southern Cape, South Africa. Maize was harvested at the half to three quarter milk line at a DM content of 30% on the 18th of March 1998. Whole crop maize was chopped with a PZ Zweegers WH90S silage chopper.

A laboratory study was done to determine the effect of an inoculant on the fermentation dynamics of maize during ensiling. Forty kg of chopped maize was mixed on a polyethylene surface which was cleaned with ethanol. The material was divided into two portions of 20 kg. The lactic acid bacterial (LAB) inoculant Maize-all (Alltech Biotechnology Pty. Ltd.) contained *Lactobacillus plantarum* and *Pediococcus acidilactici* as well as amylase. The inoculant was applied at 5 g/tonne of fresh material to provide 10^6 Colony forming units of lactic acid bacteria per gram of fresh material. Maize was ensiled in 1.5 litre Weck glass jars (J. WECK, GmbH u. Co., Wehr-Oflingen, W. Germany) with glass lids, fastened with metal clamps which enables gas release. Twelve silos were each filled with either inoculated or untreated chopped maize plants. Three silos of each treatment were opened on each of days 2, 10, 50 and 90 of ensiling and representative samples were taken for chemical (stored at –20 °C) and microbiological analysis. At day 90 of ensiling, silage was exposed to air to determine the aerobic stability. Silage was put in three 2-litre polyethylene terephtalate bottles for each treatment, as described by Ashbell *et al*. (1991). Two bottles were filled with wheat straw to monitor the room temperature. All bottles were fitted with a T-type thermocouple and placed in a polystyrene container in a room kept at a temperature between 20 and 25 °C. Temperature changes were measured at hourly intervals using a MC- System 120-02EX, 16 canal data logger for a period of 10 days.

Microbiological analyses were carried out on a representative sample of the three replicates for each of the control and treated silage for each of the test days. Microbial analyses on fresh plant material before ensiling were done after the additive was applied. Forty grams of material was weighed into sterile stomacher bags, 360 ml of sterile saline water added and the samples were homogenized by stomaching for 3 min. The extract was further diluted 10 and 100 fold. Enumeration of *lactobacilli* was done using MRS agar according to Oxoid (1990) and *lactococci* was determined using M17. Colonies were counted directly on the agar plates. Agar plates were incubated at 37 °C for 72 h. Yeasts were enumerated according to the IDF standard 94B procedure, 1990. Plates were incubated at 25 °C for 72 h.

Dry matter of the fresh material and silage was estimated by drying samples in an oven at 60 °C for 72 h. Total non-structural carbohydrates (TNC), pH and lactic acid were determined on filtrates of 40 g of frozen sample added to 360 ml of distilled water, homogenized for 3 min. with a stomacher. The TNC were determined according to Marais (1979) and lactic acid by the colorimetric method of Barker & Summerson (1941). Volatile fatty acids concentrations were determined with a Carlo Erba 4200 gas chromatograph with flame ionisation detector with a 2.35 m x 3 mm stainless steel column packed with 10% SP 1200 containing 1% ortho-phosphoric acid (H_3PO_4). The column was conditioned for 48 h at 165 °C with a nitrogen (N) carrier gas flow of 40 ml/min. *In vitro* organic matter digestibility (IVOMD) was determined according to Tilley & Terry (1963). Total N was determined by the Kjeldahl method (AOAC, 1984). The ammonia nitrogen (NH_3-N) concentration of silage was determined by homogenizing 50 g of silage in 250 ml of a 0.1 N H_2SO_4 solution for three minutes. The homogenate was filtered through Whatman no 4 filter paper and the ammonia concentration in the filtrate was determined by distillation using a Buchi 342 apparatus and a Metröhm 655 Dosimat with an E526 titrator, according to AOAC (1984). This method is based on the method of Pearson & Muslemuddin (1968) to determine volatile N. Least significant differences between treatments in the laboratory study were determined by a one-way ANOVA, using the Statgraphics (1988) statistical computer programme.

The effect of the inoculant on intake and milk production was determined. The inoculant was applied on the silage chopper with an applicator to provide 10^6 colony forming units (CFU) of lactic acid bacteria per g of fresh material. One bunker of 4 m x 1.2 m x 25 m was filled with control and one bunker with inoculated silage within a period of four days (2 d/bunker). Silage was stored for a period of eight months. Aerobic stability of silage was determined four times at 10-day intervals during each of the two feed-out periods, as described for the laboratory study. Representative control and inoculated maize silage samples were taken directly after the silage was removed form the bunker.

Twenty two multiparous cows, averaging 127 days in milk, were blocked in pairs according to milk production (previous 4 weeks), days in milk, lactation number, live weight and condition score. Within each block, cows were randomly allocated to either control or inoculated silage treatment. The control and inoculated maize silage diets were fed to the two groups of cows for two periods of 30 days in a two by two cross-over design. Each period consisted of a 10 day adaptation and 20 day measurement period. Milk production was recorded daily and milk composition weekly. A composite sample of afternoon and morning milking was taken for determination of protein, butterfat, lactose and milk urea nitrogen. Live weight and condition score of cows were determined on two consecutive days after milking at 09:00 on days 0, 30 and 60 of the experimental period.

Samples of the control silage, inoculated maize silage and concentrates were taken on Mondays, Wednesdays and Fridays and were frozen at –4 °C. Samples were pooled for each week of the experimental period. This resulted in three composite samples for each of the control silage, inoculated silage and concentrate for each period. Samples of maize silage were processed and DM, organic matter (OM), IVOMD, crude protein (CP), pH, lactic acid, acetic acid, propionic acid and butyric acid were determined as described for the laboratory study. Neutral detergent fibre (NDF) was determined according to Van Soest *et al*. (1991), TNC according to Marais (1979) and starch according to Rasmussen & Henry (1990).

Cows were milked twice daily at 06:00 and 15:30 and each cow received 5.5 kg concentrate on a DM basis daily. The concentrate was divided into two equal portions and was fed after each milking. The dairy concentrate consisted of 34.2% maize, 15% wheat, 5% molasses meal, 6% fish meal, 5% wheat bran, 12% cottonseed, 18% cottonseed oilcake, 2% feed lime, 0.5% dicalcium phosphate, 1% salt, 1% urea, 0.3% mineral premix on a DM basis. The concentrate was formulated to contain 26% CP, 12 MJ ME/kg, 1.1% calcium and 0.65% phosphorus on a DM basis. Dry matter intake of silage was determined on a daily basis. Silage was fed individually to cows and they had free access to silage from 8:00 to 12:00 and from 16:30 to 20:30. The cows were kept in a small rest camp with access to water only from 12:00 to 15:30 and from 20:30 to 06:00 the next morning.

Data of the milk production study were analysed using SAS (1996). The GLM procedure was followed for a cross-over design with two treatments and two periods.

Results

The chemical composition and microbial analysis of maize ensiled in laboratory silos are presented in Table 1.

Table 1 Chemical composition (g/kg DM) and microbial analysis of maize ensiled in laboratory silos for 90 days with or without the addition of a lactic acid bacterial inoculant

	Control	Inoculant	SEM
Number	3	3	
Dry matter (DM)	263[a]	253[b]	1.0
Organic matter (OM)	953	952	1.8
In vitro digestible OM	718	724	8.7
Crude protein	82[a]	76[b]	0.5
NH_3-N (% total nitrogen)	5.88[a]	5.10[b]	0.034
Acetic acid	0.08	0.06	0.071
Propionic acid	NF	NF	
n-Butyric acid	NF	NF	
Gas loss g/100 g DM	6.47	6.25	0.183
Microbial analysis (log_{10} CFU/g silage)			
Yeast	1.34	0.82	0.911
Lactobacilli	4.88	5.02	4.034
Lactococci	5.41[a]	7.94[b]	0.632

[ab] Row means with different superscripts do differ at $P < 0.05$;
SEM - Standard error of mean; NF - Not found; CFU – colony forming units

The changes in pH, TNC and lactic acid concentrations in the control and inoculated silages during the ensiling period and after 10 days of aerobic exposure at the end of the ensiling period are

given in Table 2. Untreated and inoculated silages were stable when exposed to air and no increase in temperature above the ambient temperature was recorded over a period of 240 hours. The pH, TNC and lactic acid concentrations of untreated and inoculated maize silage did not change during aerobic exposure.

Table 2 The pH, total non-structural carbohydrate and lactic acid concentrations of untreated and inoculated maize silage after 2, 10, 50, 90 days of ensiling and after 10 days of aerobic exposure

	Day 0	Day 2	Day 10	Day 50	Day 90	Day 10 Aerobic
Number	3	3	3	3	3	3
pH						
Control	5.75	3.90	3.71	3.71	3.57	3.63
Inoculant	5.75	3.85	3.68	3.69	3.61	3.62
SEM	0.063	0.024	0.011	0.003	0.014	0.022
Total non-structural carbohydrates (g/kg dry matter, DM)						
Control	159	100	64	19[a]	35	41
Inoculant	159	106	55	25[b]	32	34
SEM	11.4	14.1	12.3	1.52	5.6	3.8
Lactic acid (g/kg DM)						
Control	3.4	34	50	51	71	66
Inoculant	3.4	36	52	61	68	63
SEM	1.3	1.1	2.9	3.2	2.3	3.0

[ab] Column means with different superscripts differ at $P < 0.05$; SEM - Standard error of mean.

No butyric acid was found in either the control or in the inoculated silage and the NH_3-N as percentage of total N was low in both silages. The chemical composition of maize silage made in bunker silos is given in Table 3. Silages were well preserved as indicated by the low pH and absence of butyric acid. The IVOMD of the concentrate was 80.9±1.2%, the CP 262±13 g/kg DM, the NDF 132±22 g/kg DM, the calcium 20.1±9 g/kg DM and the phosphorus 6.6±2 g/kg DM. The intake, milk production and milk composition of the Jersey cows fed the control or inoculated maize silage diets are presented given in Table 4.

Table 3 Chemical composition (g/kg DM) and aerobic stability (hours before temperature increased 2 °C above ambient temperature) of bunker maize silage with or without the adding of a lactic acid bacterial inoculant

	Control	Inoculant	P-value	SEM
Number	6	6		
Dry matter	283[a]	307[b]	0.01	4.6
Organic matter (OM)	957	959	0.25	0.13
In vitro OM digestibility	705	710	0.72	10.4
Crude protein	78.4[a]	83.0[b]	< 0.01	0.82
Ammonia-N/100 g of total nitrogen	8.16[a]	5.29[b]	< 0.01	0.231
Total non-structural carbohydrates	40[a]	54[b]	< 0.01	1.7
Neutral detergent fibre	467	439	0.12	12.4
Starch	233	259	0.14	11.7
pH	3.50	3.51	0.78	0.043
Lactic acid	37.9	35.3	0.47	2.64
Acetic acid	27.0[a]	20.1[b]	0.01	1.42
Propionic acid	1.4	1.7	0.74	0.83
n-Butyric acid	NF	NF		
Aerobic stable (Time in hours)	11.7[a]	23.3[b]	0.045	3.06

[ab] Row means with different superscripts differ at $P < 0.05$;
SEM - Standard error of mean; NF - Not found

Table 4 Intake, milk production and milk composition of Jersey cows fed control or inoculated maize silage diets

	Control	Inoculant	P-value	SEM
Milk production (kg/day)	15.6	15.9	0.47	0.36
Fat corrected milk production (kg/day)	17.4	17.5	0.75	0.40
Butterfat %	4.79	4.74	0.55	0.081
Protein %	3.52	3.55	0.36	0.023
Lactose %	4.89	4.86	0.22	0.022
Milk urea nitrogen (mg/dl)	11.4	11.8	0.40	0.32
Dry matter intake (kg/day)				
Silage	7.6[a]	8.4[b]	< 0.01	0.13
Concentrate	5.5	5.5		
Total	13.1[a]	13.9[b]	< 0.01	0.13
% of live weight	3.78[a]	3.99[b]	< 0.01	0.041
Change in live weight (g/day)	0.31	0.34	0.61	0.043
Change in condition score	0.17	0.13	0.56	0.114

[ab] Row means with different superscripts differ at $P < 0.05$; SEM - Standard error of mean.

During period 2 one cow that was fed the control silage developed mastitis, resulting in a very low intake and milk production. Data of the affected cow, as well as that of the cow with which she was blocked, were removed for both periods.

Discussion

Both the control and inoculated maize silages were well preserved as indicated by the low pH, high lactic acid content, low level of NH_3-N and absence of butyric acid. The pH drop in maize silage was much more rapid in our study with the pH at 3.9 and 3.85 for control and inoculated silage after only two days of ensiling compared to the pH of 4.5 after 5 days of ensiling found by Rust *et al*. (1989). Meeske & Basson (1998) also found that the pH of maize silage dropped to 3.99 after two days of ensiling. The inoculated maize silage in our study had a lower pH than the control silage on days 10 and 50 of ensiling (Table 2), but no differences were found after 90 days of ensiling. The lactic acid concentration of maize silage was not affected by the addition of the inoculant. The level of lactic acid in maize silage (Table 2) compared well with the 63 to 120 g lactic acid/kg DM found by Spoelstra & Van Wikselaar (1992) in maize silage made in laboratory silos.

The CP concentration was lower in the inoculated silage compared to the control silage, suggesting a higher rate of protein breakdown or N loss in this treatment. This is not supported by the NH_3-N concentration (per 100 g of total N) data, which showed less protein breakdown in the inoculated maize silage compared to the control maize silage. The NH_3-N concentration was, however, at an acceptable low level in both treatments, and the difference between the treatments is of no practical importance. The lower CP concentration found in the inoculated silage compared to the control silage is difficult to explain and heterogeneity of chopped maize plants and sample size may have contributed to this. The addition of the inoculant to maize did not affect the IVOMD.

The number of *Lactococci* was significantly higher in the inoculated maize silage prepared in laboratory silos compared to the untreated control silage. This may be due to the added *Pediococcus acidilactici* bacteria present in the inoculant, since *Pediococci* is often present in very low numbers on cereal crops (Woolford, 1984). The number of lactobacilli did not differ between control and inoculated maize silage after 90 days of ensiling. The yeast counts on both the control and inoculated maize silage were low compared to the 2.6 log CFU/g of fresh material found by Meeske & Basson (1998) on maize silage after 90 days of ensiling. The low yeast counts may be a result of the rapid lowering of the pH in the silage as well as the rapid exclusion of air that occurs in laboratory silos (McDonald *et al*., 1991).

The TNC concentration of maize prior to ensiling was high at 159 g/kg DM. The residual TNC after 90 days of ensiling would indicate that sufficient nutrients were available for the lactic acid bacteria to grow. Addition of the inoculant did not have any affect on the TNC levels during fermentation, indicating that TNC was utilized at the same rate in the untreated and inoculated maize

silages. This is in contrast with the more rapid water soluble carbohydrate utilization found by Meeske *et al*. (1999) when adding an inoculant to tropical grass silage.

The inoculated and control maize silages made in bunker silos were both well preserved (Table 3). The DM content of the maize silage of 28 to 30% (Table 3) was optimal and would ensure maximum DM intake (Phipps & Wilkinson, 1985). The inoculated silage had higher DM, NSC and CP concentrations and a lower acetic acid concentration than the control silage. This may indicate a more efficient fermentation. The levels of acetic acid of 27 g/kg DM and 20 g/kg DM found in the control and inoculated maize silage respectively were high compared to the 6 g/kg DM found by Scheafer *et al*. (1989) in bunker maize silage. Ashbell & Lisker (1988), however, reported acetic acid levels of 15 to 21 g/kg DM in maize silage made under farm conditions in a subtropical climate, while Spoelstra & Van Wikselaar (1992) found levels of 10 to 21 g acetic acid/kg DM in maize silage made in laboratory silos.

The starch content of the inoculated and enzyme treated maize silage did not differ from that of the control maize silage (Table 3). This indicates that the amylase in the inoculant did not break down starch, as was found by Spoelstra & Van Wikselaar (1992). They found that the starch content of maize silage was reduced by up to 50% when enzymes with amylolitic activity were added to maize at ensiling. The TNC concentration of the inoculated maize silage was higher and acetic acid concentration lower than that of the control maize silage. This indicates that inoculation did result in more efficient homofermentative fermentation. The NDF concentration of the inoculated maize silage tended to be lower ($P = 0.12$) than that of the control maize silage. This may be as a result of more hydrolysis of hemicellulose in the inoculated maize silage (McDonald *et al*., 1991). Hemicellulose may be broken down during ensiling by hemicellulases present in the original herbage, bacterial hemicellulases and hydrolysis by organic acids produced during fermentation (McDonald *et al*., 1991).

The CP concentration of the inoculated silage was higher ($P < 0.05$) than that of the control silage. The lower percentage of NH_3-N per total N (Table 3) in the inoculated maize silage compared to the control maize silage suggests that less protein breakdown occurred in the inoculated maize silage compared to the control maize silage. This is in contrast with the results found in the laboratory study. The protein concentration of the maize silage was similar to that reported by Cilliers *et al*. (1998) and was higher than the 69±4 g/kg DM reported by Meeske *et al*. (2000) on 21 maize hybrids.

The milk production, fat corrected milk production and milk composition of cows did not differ between the control and inoculated silage diets (Table 4). The intake of inoculated silage was higher ($P < 0.05$) than that of the control silage (Table 4). Silage intake of cows receiving the inoculated silage in Period 1 was 8.68 kg DM/day and this decreased to 7.49 kg DM/day during Period 2 when control silage was fed. The inoculated silage appeared to be more palatable than the control silage. This may have been caused by the lower acetic acid content and improved aerobic stability of the inoculated maize silage compared to that of the control maize silage. Meeske *et al*. (1999) also found that inoculated tropical grass silage had a lower acetic acid content and a higher intake than that of untreated silage. Meeske & Basson (1998) found that, although the chemical composition of untreated and inoculated maize silage was similar, lambs tended to ingest more ($P = 0.07$) of the inoculated silage than of the control silage. Honig & Daenicke (1993) found that Simmental bulls consumed more of an inoculated maize silage compared to untreated control silage, although no marked improvement in silage fermentation was found. Rust *et al*. (1989) added a lactic acid bacterial inoculant at 2×10^{-5} CFU per gram of fresh material to maize and increased the *lactobacillus* organisms on the crop by 15% at the time of ensiling. This resulted in an increased lactic acid concentration in the inoculated maize silage, but had no effect on intake and weight gains by crossbred steers. The inoculant had no effect on changes in live weight and condition score of cows over a 60 day period.

Aerobic deterioration of maize silage is initiated by yeasts or acetic acid bacteria (Driehuis & Van Wikselaar, 1996). The inoculated maize silage in our study was more stable than the control maize silage. This is in agreement with the study of Woolford (1975) and differs from the work of Moon *et al*. (1980) and Rust *et al*. (1989) who found that inoculated maize silage was less stable than untreated maize silage when exposed to air. Spoelstra & Van Wikselaar (1992) showed that the starch in maize silage is degraded relatively easily by amylase. The liberated sugars are then fermented to ethanol by yeasts, resulting in higher yeast counts and lowered aerobic stability of enzyme treated maize silage. Scheafer *et al*. (1989) and Sanderson (1993) found that resistance to aerobic deterioration was not affected by the use of a lactic acid bacterial inoculant on maize silage.

The number of yeast present on the crop at ensiling as well as the time from harvesting until anaerobic conditions prevail has a major impact on the aerobic stability of maize silage (McDonald *et al*., 1991). Conditions when making the laboratory maize silage were ideal, resulting in both the control and inoculated silages being stable when exposed to air. Bunker maize silage, on the other hand, which was made under less favourable conditions was more susceptible to aerobic deterioration. Therefore, when evaluating the effect of additives on the aerobic stability of silage, laboratory studies should be followed up with large scale studies where silage is made on a commercial scale.

Conclusions

Maize ensiled in laboratory silos differed markedly from maize ensiled in bunker silos. The laboratory silos created optimal ensiling conditions. This resulted in maize silage with a high lactic acid concentration, a low acetic acid concentration and a high aerobic stability. Maize silage made in bunker silos had a lower lactic acid concentration and a higher acetic acid concentration than that of maize silage made in laboratory silos. The inoculant improved the aerobic stability of maize silage made in a bunker compared to untreated maize silage. Studies to determine the effect of additives on the aerobic stability of silages done with laboratory silos should be followed up with studies on large scale commercial silos.

The addition of the inoculant did not affect fermentation dynamics of maize substantially during the ensiling period or the chemical composition of maize silage. The addition of the lactic acid bacterial inoculant did improve the palatability of maize silage and resulted in a higher ($P < 0.05$) intake of silage by Jersey cows. Milk production, milk composition, live weight change and condition score of cows fed inoculated maize silage were not significantly different from those of cows fed control maize silage.

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