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The effect of a bacterial inoculant on fermentation, microbial status and aerobic stability of whole crop maize silage

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Abstract

Effects of an inoculant with a new combination of lactic acid bacteria strains on fermentation, microbial status and aerobic stability of whole crop maize silage were determined under laboratory conditions. For ensiling were used 2.0 l poly-propylen containers, which were opened on days 3, 7, 15 and 50 for sampling and analysis of pH, dry matter (DM), water-soluble carbohydrates (WSC), lactic acid (LA) and volatile fatty acids (VFA). The microbiological status and aerobic stability of silages was determined on day 50 of ensiling. There was no significant difference in WSC and DM contents between inoculated and the control silage. Addition of inoculant caused higher ($P<0.05$) lactic acid and acetic acid concentrations, with no presence of butyric acid. Total number of bacteria, as well as molds and yeasts was significantly lower in inoculated silage and it was aerobically more stable than the control as indicated by significantly ($P<0.05$) lower CO_2 production.

Keywords: silage, whole crope maize, Bonsilage mais Flussig, fermentation, microbial status, aerobic stability

Introduction

Whole crop maize (*Zea mays*) is the most popular cereal crop conserved as silage in many parts of the world, and is regarded as an ideal crop for silage making because of its high yields, nutritive value, low buffering capacity and high water-soluble carbohydrates (WSC) content (McDONALD [1]). Ensiling is based on conversion of WSC by lactic acid bacteria (LAB) into lactic, acetic and butyric acids. As a result, pH decreases and the forage is preserved (McDONALD & al. [2]).

The genus *Lactobacillus* belongs to the large group of LAB which are all gram-positive organisms and produce lactic acid by fermentation. There are two groups of species depending on the ability to ferment sugars: homo-fermentative species, converting sugars mostly into lactic acid, and hetero-fermentative species, converting sugars into lactic acid, acetic acid, ethanol and CO_2. The number of LAB present on maize plants at harvest may be too low to ensure rapid and efficient preservation, and therefore silage inoculants have been developed to improve the nutritive value of silages and to reduce risks during ensiling (HENDERSON [3]). Most available inoculants consist of selected strains of homo-fermentative LAB, such as *Lactobacillus plantarum, Pediococcus and Enterococcus* species which improve silage fermentation, but often reduce aerobic stability (MUCK & KUNG [4]). In contrast, *Lactobacillus buchneri*, a hetero-fermentative LAB, improves the aerobic stability of silages (MUCK, [5]) by accumulation of acetic acid (DRIEHUIS & al. [6]).

Both homo-fermentative and hetero-fermentative lactobacilli have advantages as silage inoculants. At the beginning of fermentation, production of lactic acid by homo-fermentative lactobacilli is preferred to reduce pH faster, which may inhibit growth of undesirable microorganisms and improve fermentation quality **(**CAI & al. [7]). Good aerobic stability is then controlled by the hetero-fermentative lactobacilli, since the activity of yeast is impaired due to acetic acid produced **(**DRIEHUIS & al. [8], FILYA [9]**).** Combining homo-fermentative and hetero-fermentative inoculants has become popular and has been used for various forages **(**FILYA [9], WEINBERG & al. [10]). Ideally, a silage additive should be safe to handle, reduce dry matter losses, improve aerobic stability, increase the nutritive value of the silage and give an economic return which is greater than its cost (NKOSI & al. [11]).

The aim of this study was to determine effects of adding a silage inoculant with a new combination of LAB strains to whole crop maize, grown under south-eastern European conditions, on fermentation dynamics, microbial status and aerobic stability of silage.

Materials and methods

Maize (hybrid AS41) was harvest during September 2009 in province Vojvodina, Serbia using a precision silage chopper to obtain a theoretical 5 mm chop length. *Bonsilage mais Flussig* (Schaumann, Agri Austria GmbH, Brunn am Gebirge, Austria) contains homo-fermentative strains of *Pediococcus pentosaceus* (DSM 12834), *Lactobacillus plantarum* (DSM 12837) and hetero-fermentative *Lactobacillus buchneri* (DSM 12856). It was used as an inoculant by mixing 0.15 g with 0.1 l of water (to provide 2.5×10^5 colony forming units of LAB per gram of fresh maize), and spraying over 30 kg of whole crop maize. The application rate was in accordance with the level of LAB in the inoculant as determined by the manufacturer. In order to add the same amount of moisture as in the treated maize, the control was treated by spraying 0.1 l of water on 30 kg whole crop maize. The whole crop maize (382 g/kg DM) was compacted into 2.0 l poly-propylene containers equipped with a water valve to enable gas release, as proposed by COLOVIC & al. [12]. Each container was filled with approximately 1.4 kg (wet mass) of chopped maize without a headspace, and a packing density of 700 kg/m^3 was obtained. Silage treatments included control (with no additive) and experimental, with added *Bonsilage mais Flussig* (BMF). A total of 24 containers (12 per treatment) were filled and stored at a temperature of 24–28^{0}C.

On day 0, fresh whole crop maize was collected for subsequent chemical analysis. Three silage containers were opened for each treatment on each of days 3, 7, 14 and 50 for determination of pH, dry matter (DM), water-soluble carbohydrates (WSC), lactic acid (LA), and volatile fatty acids (VFA).

The pH and DM were determined following the procedures of AOAC [13]. The WSC were determined by the phenol–sulphuric acid method according to DUBOIS & al. [14]. The lactic acid, VFA were determined according to standard method [15]. Microbial status of silages on day 50 was defined by total number of bacteria, as well as moulds and yeasts. Total number of bacteria was determined aerobically using Plate Count Agar (PCA), after incubation for 3 days at 30 °C [16]. Total number of yeasts and moulds was determined using Rose Bengal Chloramphenicol agar (RBC) after aerobic incubation at 25 °C for 5 days in the dark [17]. At day 50 silages were subjected to an aerobic stability test conducted according to the procedure of ASHBELL & al. [18].

Data was analysed for effects of treatment on the fermentation and aerobic stability of the silages in a completely randomized design by ANOVA using GENSTAT [19]. Data was also tested for normality and homogeneous treatment variances, and significance was declared at the 5% probability level.

Results and discussion

Effects of BMF on pH changes in silages during 50 days are shown in Figure 1.

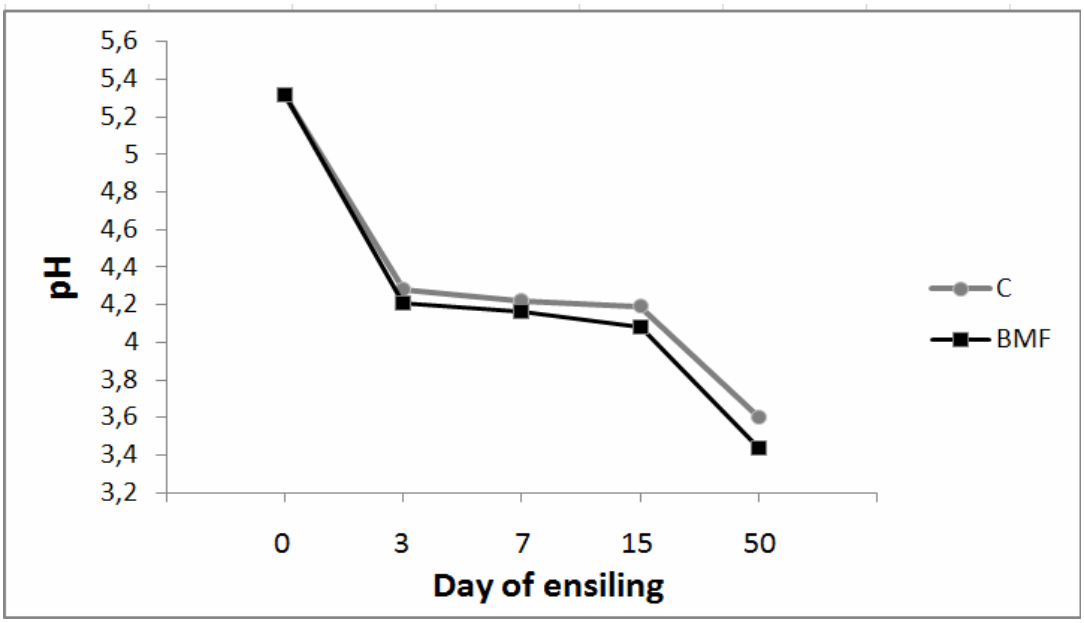

Figure 1. Evolution of pH in fresh whole crop maize (day 0) and experimental silages (C = without inoculant; BMF = with inoculant)

Results of DM and WSC determination in fresh whole crop maize and silages are presented in Table 1. Results indicated that, with respect to DM and WSC content, there were no significant differences between BMF and control silages on all sampling days.

Table 1. DM and WSC content in fresh whole crop maize and experimental silages

Analysis/Sample		Sampling day		
		0	15	50
***DM** (g/kg DM)*				
Fresh maize		382.5		
Silage	BMF	-	$350.2^{a} \pm 7.5$	$350.7^{a} \pm 9.4$
	C	-	$363.7^{a} \pm 12.9$	$341.2^{a} \pm 14.9$
***WSC**(g/kg DM)*				
Fresh maize		74.5		
Silage	BMF	-	$7.2^{a} \pm 0.4$	$5.4^{a} \pm 0.7$
	C	-	$7.4^{a} \pm 0.5$	$6.6^{a} \pm 1.0$

BMF = with inoculant
C = without inoculant
^{ab}Means with different superscripts in the same column are significantly different ($P<0.05$)

Results of determination of lactic, acetic and butyric acid in fresh whole crop maize and experimental silages are shown in Table 2.

Table 2. Fermentation traits of experimental silages

Analysis/Sample		Sampling day		
		0	15	50
***Lactic acid** (g/kg DM)*				
Fresh maize		11.2		
Silage	BMF	-	$37.1^{a} \pm 2.3$	$34.9^{a} \pm 2.0$
	C	-	$34.3^{a} \pm 1.8$	$28.8^{a} \pm 1.0$
***Acetic acid** (g/kg DM)*				
Fresh maize		1.8		
Silage	BMF	-	$14.0^{a} \pm 0.3$	$23.7^{a} \pm 0.8$
	C	-	$13.4^{a} \pm 0.1$	$16.3^{b} \pm 0.3$
***Butyric** (g/kg DM)*				
Fresh maize		0		
Silage	BMF	-	0	0
	C	-	0	0

BMF = with inoculant
C = without inoculant
[ab]Means with different superscripts in the same column are significantly different (P<0.05)

There were no significant difference (P<0.05) in lactic acid concentration between silages on both sampling days. On day 50 concentration of acetic acid in the BMF treated silage were significantly higher (P<0.05) than in the control silage.

Microbial status of silages at day 50 is shown in Table 3.

Table 3. Total number of aerobic bacteria, molds and yeasts in 1 gram of experimental silages on day 50

	Total number of aerobic bacteria	Total number of molds and yeasts
BMF	1,33E+08	7,30E+06
C	2,43E+08	3,70E+07

BMF = with inoculant
C = without inoculant

The total number of aerobic bacteria was about 2 times lower in the samples with bacterial inoculant than in the control silage samples. Total number of moulds and yeasts was about 5 times lower in inoculated than in the control samples.

Aerobic stability test of silages on day 50 (Figure 2), showed a significant difference (P<0.05) of released CO_2 between the BFM (110,3 g CO_2/kg DM) and the control silage (136,8 g CO_2/kg DM), pointing to the positive effect of BMF addition on aerobic stability of whole crop maize silage.

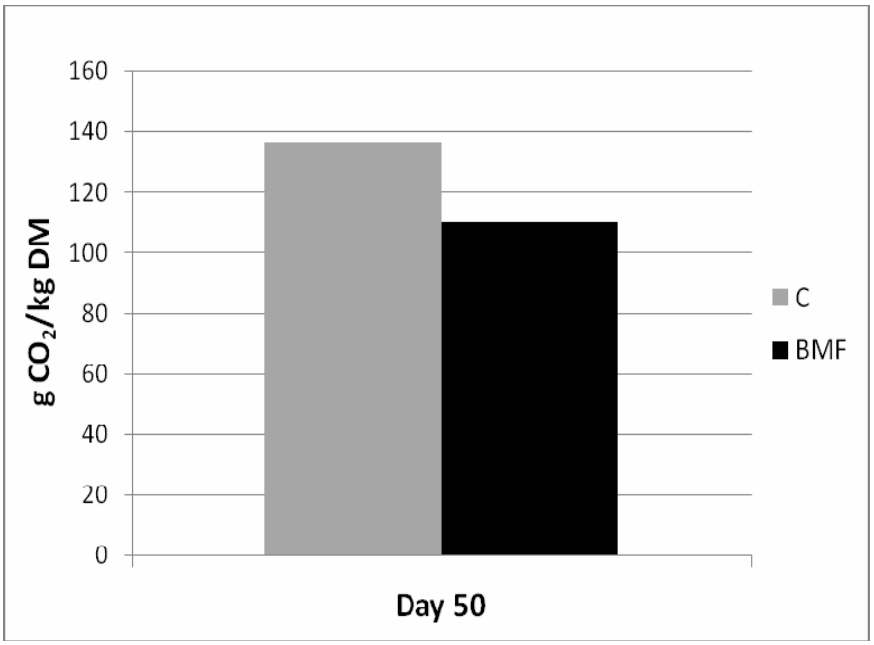

Figure 2. Aerobic stability of experimental silages on day 50 (C = without inoculant; BMF = with inoculant)

The extent of the decline in pH reflects the concentration of LAB which are responsible for the fermentation process (McDONALD & al. [2]). In the present study, a rapid drop in pH occurred on day 3 when it fell to 4.2, which is considered desirable for the successful preservation of forage crops, and is within the range of 3.7–4.2 which is generally observed in maize silages of 300–400 g/kg DM (KUNG & SHAVER [20]).

Addition of BMF resulted in higher lactic acid concentration than in the control at day 50 of ensiling, which is consistent with that reported by KUNG & SHAVER [20] in a 300–400 g/kg DM maize silage. Also, it has been reported that inoculation of silage with a hetero-fermentative LAB resulted in a decrease in lactic acid, while increasing acetic acid concentration (RANJIT & al. [21]), which corresponds with the results obtained in our study.

Water-soluble carbohydrates are essential substrate for the growth of LAB (McDONALD & al. [2]). More than 60 g/kg of WSC in the fresh cut crop is regarded as sufficient quanty for conversion by LAB into lactic and acetic acid (JAAKKOLA & al. [22]), which was fullfiled in the present study (74.5 g/kg of WSC).

Hetero-fermentative inoculants produce higher levels of acetic acid than untreated silages, which results in inhibition of growth of moulds and yeasts (DANNER & al. [23]). This is in line with the results of silage microbial status obtained in this study (Table 3).

According to RANJIT & al. [21], microbial inoculants that contain strains of *L. buchneri* are designed to improve the aerobic stability of silages by producing higher concentrations of acetic acid. In their study, addition of *L. buchneri* 40788 markedly increased concentrations of acetic acid and improved the aerobic stability of maize silage. In our study, the BMF treated silage had significantly lower CO_2 value, indicating that it was aerobically more stable than non-inoculated silage. Aerobic stability of the silage in this study was determined using the original method of ASHBELL & al. [18]. In the similar study conducted by NKOSI & al. [11] a modified method was used, which could explain why they reported lower CO_2 values. Irrespective of the differences in CO_2 values, in both mentioned studies the significant improvement in silage aerobic stability with added BMF has been found.

Conclusions

There was no significant difference in content of DM between silages with and without addition of inoculant (BMF), with a new combination of LAB strains, during the 50-day study. Together with absence of butyric acid, that was indication that proper anaerobic conditions were acheived and maintained during the experiment.

Total number of molds and yeast was significantly lower in the silage inoculated with BMF, thus contributing to lowering the secondary fermentation and related losses of silage nutritive value. Also, total number of aerobic bacteria was significantly lower with addition of BMF.

The aerobic stability of silage, as measured by the release of CO_2 after exposure to air, was significantly improved by addition of BMF, which correlates with significant increase of acetic acid in inoculated silage.

Acknowledgement

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