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THE EFFECT OF BACTERIAL SILAGE INOCULANT ON THE FERMENTATION, CELL WALL CONTENTS AND AEROBIC STABILITY OF MAIZE SILAGE

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Abstract. This research was carried out to determine the effects of bacterial silage inoculant using as silage additives on the fermentation charakteristics, cell wall contents and aerobic stability of maize silages. Maize silage was harvested in the 89 (BBCH) developmental ripening stage. Biological additive was used as additive which contains *Enterococcus faecium* PCM 1858, *Pediococcus acidilactici* PAL-34, *Lactobacillus plantarum* PCM 493, *Lactobacillus buchneri* DSMZ 5987, *Lactobacillus rhamnosus* PCM 489, *Lactobacillus brevis* PCM 488, *Lactobacillus lactis* PCM 2379. Maize was ensiled in 4 dm^3 special PCV laboratory microsilos with a cover permitting gaseous products. The microsilos were stored at 10–15±2ºC undr laboratory conditions. Microsilos from each group were sampled for microbiological and chemical analyses on the days 3, 14, 21 and 60 after ensiling, whereas aerobic stability was determined after 7 days. As a result, bacterial silage inoculant improved fermentation, decreased cell wall contents, deoxynivalenol (DON) concenration, do not improved aerobic stability of maize silage after 7 days exposure to air.

Keywords: aerobic stability, deoxynivalenol, maize silages

INTRODUCTION

Feeding of animals aims at keeping them in good health and ensuring optimal production. Feeds must be of good quality and adjusted to the animals needs. Any treatments in relation to the health of animals should be guided by a widely understood prophylaxis. Feed should be free of any type of agents that might be harmful to the health of animals. Such agents include mycotoxins, dioxines, mould etc. [Selwet 2008 a]. Maize epiphytic microflora can consist of, to a considerable extent, toxigenic fungi of *Fusarium, Aspergillus, Penicillium* genera which can produce, among others trichothecenes (deoxynivalenol, T2 toxin), [Creppy 2002, Kostulak-Zielińska et al. 2002, Selwet 2008 b]. In poorly prepared silages, undesired bacterial an fungal microflora may constitute a serious threat, e.g. *Enterobacteriaceae, Clostridium* yeasts as well as products of their secondary me-

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tabolism, frequently constittuting toxic compounds [Driehuis et al. 2000]. *Enterobacteriaceae*, during protein decomposition, contribute to the creation of biogenic amines e.g. putrescines (PUT), causing ketonemia [Steidlova and Kalač 2002]. Clostridia are created by endospores which, together with blood, enter milk and reduce the quality of milk products [Giffel et al. 2002]. Yesats can create conditions favourable for the development of mould fungi as well as other undesirable microorganisms, which result in losses of organic matter and worsen the organoleptic properties of silages [Selwet 2005]. It is important to employ special additives in the proces of ensiling, which enchance this process, protect the plant material against the activity of harmful microflora and, at the same time, prolong the aerobic stability of silages after opening of silos [Selwet 2006].

The biological additives are adventageous because they are safe and easy to use, non corrosive to machinery, do not pollute the environment, and are regarded as natural products [Koc et al. 2008]. The objective of the performed experiments was to determine the effect of biological praparation on the chemical composition os maize silage and on the numbers of lactic acid bacteria, mould and yeasts fungi as on the improvement of aerobic stability of silage.

MATERIAL AND METHODS

The plant material consistet of maize (*Zea mays* L.) cultivar SMH (FAO 230) from the Smolice Plant Breeding Ltd, IHAR Group, with dry matter content 230 g kg^{-1} which was harvested in the 89 (BBCH) developmental ripening stage, cut at height of 20 cm and chopped from 3 to 5 cm. The treatments were foolows: CMS-control maize silage (maize without additive) and IMS-inoculant maize silage (the preparation dose was 4 cm^3 kg^{-1} FM). The CMS was treated with an equivalent amount of water.

Fresh material was ensiled in microsilos (n = 50) of 4 dm^3 volume with a cover permitting gaseous products. The microsilos were stored at 10–15±2ºC under laboratory conditions. Microsilos from each group were sampled for microbiological and chemical analyses on the days 3, 14, 21 and 60 after ensiling, whereas aerobic stability was determined after 7 days.

Bacterial additive was used which contains *Enterococcus faecium* PCM 1858, *Pediococcus acidilactici* PAL-34, *Lactobacillus plantarum* PCM 493, *Lactobacillus buchneri* DSMZ 5987, *Lactobacillus rhamnosus* PCM 489, *Lactobacillus brevis* PCM 488, *Lactobacillus lactis* PCM 2379 in the amount of 5 x 10^8 cfu g^{-1} (about 400 000 cfu lactic acid bacteria g^{-1} silage).

Silage extracts were prepared by adding 90 cm^3 of physological solution of sodium chloride to 10 g of silage sample and homogenizing for 10 min in laboratory blender. Microbial counts were determined using a decimal dilution series of silage extracts. Yeasts and mould were counted on OGYE Agar-oxytetracycline-glucose-yeast-extract agar (Oxoid) after incubation for 120 h at 25ºC, and lactic acid bacteria (LAB) on MRS Agar (Oxoid) after incubation for 24 h at 37ºC. Chemical analysis included the determination of dry matter (DM), water soluble carbohydrates (WSC), lactic acid (LA), acetic acid

(AA), crude protei (CP), ammonia-N (NH_3-N), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and pH. The basic composition of feed was determined according to AOAC [1990]. WSC was identified according to the method given by McDonald and Henderson [1964], ammonium-N [Conway 1962], NDF, ADF, ADL [Van Soest et al. 1991]. Values of pH were measured using a pH meter (Hann Instruments) in a suspension prepared from 20 g silage in 180 cm^3 demineralized water homogenized for 10 min.

The concentration of fatty acids was determined using a gas chromatograph equipped with FID detector, glass column 80/100 Chromosorb ® WAN of Supelco Co., 2 m long, I.D. 2 mm filled with GP 10% SP-1200/1% H_3PO_4 and autosampler Varian 8200 CX. Hydrogen was used as the carrier gas (flow 30 cm^3 min^{-1}), stove temperature 120°C, spraying temperature 250°C, detector temperature 300°C. Acid norms of Fiuka Co. were used as standards. Ethanol was determined in an aqueus extracts using a gas chromatograph with FID detector and a semi-capillary FFAP column (Hewlett-Packard, Wardbronn, Germany) over a temperature range of 45–203°C.

Deoxynivalenol (DON) was determined according to methodology given by Wiśniewska-Dmytrow and Kozak [2006]. Deoxynivalenol was extracted from the experimental plant material with water in the presence of polyethylene glycol. The extract was then purified on the immunological affinity column (DONtest^{TM}HPLC, of VICAM Company) containing antibodies specific for this mycotoxin. Deoxynivalenol was eluted from the column with 1.5 ml methyl alcohol of HPLC purity with the speed of 1 drop min^{-1} into a test tube of 5 ml volume. The eluate was evaporated dry in a stream of nitrogen at the temperature of 40ºC. The residue was dissolved in 0.5 ml of standard mobile phase (0.2 μg ml^{-1}) and mixed on a Vortex. After thickening, the eluent was determined qualitatively and quantitatively with the assistance of liquid chromatography (LC) method using the UV-VIS detector.

Forage samples (120 g) were used for aerobic stability test. Samples were thoroughly shaken to ensure air exposure and then packed loosely in 500 ml plastic containers. Samples were covered with double-layered cheesecloth to prevent drying and contaminations, and incubated for 7 days at 10–15±2°C. Four small holes were made on top and bottom of each container to permit air exchange. An additional container filled with water to measure ambient temperature. Thermal insulator was wrapped around the sides of each container to prevent heat loss. Thermocouple probes were placed in the geometric centre of the containers to measure temperature using a Hotmux data logger (DDC Corporation, Pennsauken, NJ, USA) with temperature recorded every 2 min and averaged every 2 h. Aerobic stability was defined as time required to raise silage temperature by 2 C [Levital et al. 2009].

The staistical analysis was a one way analysis of variance with Duncan's multiple range test, performed with the SAS package [1999].

RESULTS AND DISCUSSION

The performed chemical analysis of maize silages (Table 1) showed a significant ($P \leq 0.01$) influence of the biological preparation on the reduction of dry matter losses on days 14 and

60 of ensiling. In silages with biological additives, a significantly (P≤0.01) higher WSC concentration on the 21st and 60th days of ensiling was observed. After opening of microsilos on day 60 of the experiment, control silages were characterised by a significantly (P<0.0) higher NH_3-N content in comparison with silages containing biological additives. No impact of the differentiating factor on changes in protein quantities in silages was found. The applied biological additive caused a significant (P≤0.01) increase of lactic acid concentration on days 3 and 21 of ensiling. No effect of the applied preparation on changes in quantities of acetic acid in the examined samples was recorded. Prior to ensiling, on fresh plant material, DON presence at the level of 61.01 ppb was determined. The ensiling process decreased concentrations of this mycotoxin. After opening microsilos on the 60th day of ensiling, silages with the employed biological additive contained significantly (P≤0.01) less DON in comparison with the control. Concentrations of NDF, ADF and ADL were found decreased as a result of ensiling (Table 2). Silages treated with the biological preparation contained smaller (P≤0.01) quantities of fibre and lignin in comparison with control combinations. Cutting off oxygen access to plant material caused an increase in LAB counts and reduced numbers of moulds and yeasts (Table 3). The highest LAB counts were determined on the 21st day of ensiling, although no significant (P≤0.01) differences were demonstrated between control samples and those ensiled with the biological preparation. The lowest number of fungal cells was determined on day 60 of the ensiling process but differences in their numbers in individual experimental combinations were not significant (P≤0.01).

Table 1. Chemical analysis of the maize silage
Tabela 1. Skład chemiczny kiszonki z kukurydzy

Days Dni	Treatment Kombinacja	pH	DM Sucha masa g · kg^{-1}	WSC Cukry proste g · kg^{-1} DM	NH_3-N g · kg^{-1} DM	CP Białko surowe g · kg^{-1} DM	LA Kwas mlekowy g · kg^{-1} DM	AA Kwas octowy g · kg^{-1} DM	DON Deoksyniwalenol ppb
0	FM	4.51	230.30	70.21	–	60.11	–	–	61.01
3	CMS	3.61	221.10	63.16	–	58.23	11.05a	6.12	61.00
	IMS	3.47	228.21	60.17	–	60.03	16.21b	7.01	60.01
14	CMS	3.71	211.11a	33.71	–	57.12	14.25	7.11	60.13
	IMS	3.55	220.24b	36.45	–	59.81	17.11	6.22	57.02
21	CMS	3.81	210.24	23.11a	–	56.44	13.12a	7.31	60.10
	IMS	3.74	217.12	29.71b	–	57.23	18.21b	6.46	56.09
60	CMS	4.11	205,12a	8.11a	0.51a	54.12	17.12	7.61	58.00a
	IMS	4.08	214.33b	14.87b	0.25b	56.01	20.11	7.00	48.18b

a, b – means in columns designated with the same letters do not differ significantly at the level P≤0.01.
a, b – średnie w kolumnach oznaczone tymi samymi literami nie różnią się istotnie na poziomie P≤0,01.
FM – fresh matter (FM – świeża masa); CMS – control maize silage (maize without additive); (CMS – kontrola (kukurydza bez dodatku); and IMS – inoculant maize silage (the preparation dose was 4 cm^3 kg^{-1} FM) (IMS – kukurydza z dodatkiem, dawka 4 cm^3 kg^{-1} FM); DON – deoxynivalenol; DM – dry matter.

In the course of the 7-day long aerobic exposure (Table 4), pH value of silages, fungal cell numbers as well as DON concentration were found increased and dry matter losses were determined in all experimental combinations. In comparison with numerical values obtained on the 60th day of ensiling, dry matter losses amounted to, respectively 1.95% for CMS and 6.63% for IMS. Numbers of fungi increased by 98.04% in CMS and by 98.90% in IMS; however, silages treated with biological additives contained significantly ($P \leq 0.01$) higher numbers of fungi in comparison with the control. Concentrations of DON increased by 60.03% in CMS and by 66.74% in IMS. During oxygen stability tests, silage temperatures were found to increase both in control samples as well as in samples supplemented with the biological preparation (Fig. 1). The addition to silages of the biological preparation did not reduce heating of silages during aerobic exposure.

Table 2. Effect of adding inoculant on the structural composition of the maize silage
Tabela 2. Wpływ inokulanta na skład włókna w kiszonkach z kukurydzy

Days Dni	Treatment Kombinacja	NDF g · kg^{-1} DM	ADF g · kg^{-1} DM	ADL g · kg^{-1} DM
0	FM	570.10	340.21	85.12
60	CMS	531.26a	284.11a	72.23a
	IMS	493.43b	230.71b	61.11b

a, b – means in columns designated with the same letters do not differ significantly at the level $P \leq 0.01$.
a, b – średnie w kolumnach oznaczone tymi samymi literami nie różnią się istotnie na poziomie $P \leq 0,01$.
NDF – neutral detergent fiber – włókno neutralne detergentowe;
ADF – acid detergentt fiber – włókno kwaśne detergentowe;
ADL – acid detergent lignin – lignina kwaśna detergentowa.

Table 3. Microbiological analysis of the maize silage
Tabela 3. Analiza mikrobiologiczna kiszonki z kukurydzy

Days Dni	Treatment Kombinacja	LAB Bakterie kwasu mlekowego $\log_{10}$ · cfu · g^{-1} FM	Mould and yeasts Pleśnie i drożdże $\log_{10}$ · cfu · g^{-1} FM
0	FM	2.30	1.01
3	CMS	2.70a	0.45a
	IMS	2.87b	0.15b
14	CMS	3.91	0.21
	IMS	4.00	0.10
21	CMS	4.17	0.11
	IMS	4.68	0.07
60	CMS	3.82	0.10
	IMS	4.01	0.07

a, b – means in columns designated with the same letters do not differ significantly at the level $P<0.01$.
a, b – średnie w kolumnach oznaczone tymi samymi literami nie różnią się istotnie na poziomie $P \leq 0,01$.

Table 4. Results of the aerobic stability of the maize silage
Tabela 4. Jakość kiszonki po teście stabilności tlenowej

Treatment Kombinacja	pH	DM Sucha masa g · kg^{-1}	Mould and yeasts Pleśnie i drożdże $\log_{10}$ · cfu · g^{-1} FM	DON Deoksyniwalenol ppb
CMS	6.82	201.13	5.12a	145.12
IMS	6.59	200.11	6.32b	144.89

a, b – means in columns designated with the same letters do not differ significantly at the level P<0.01.
a, b – średnie w kolumnach oznaczone tymi samymi literami nie różnią się istotnie na poziomie P≤0,01.

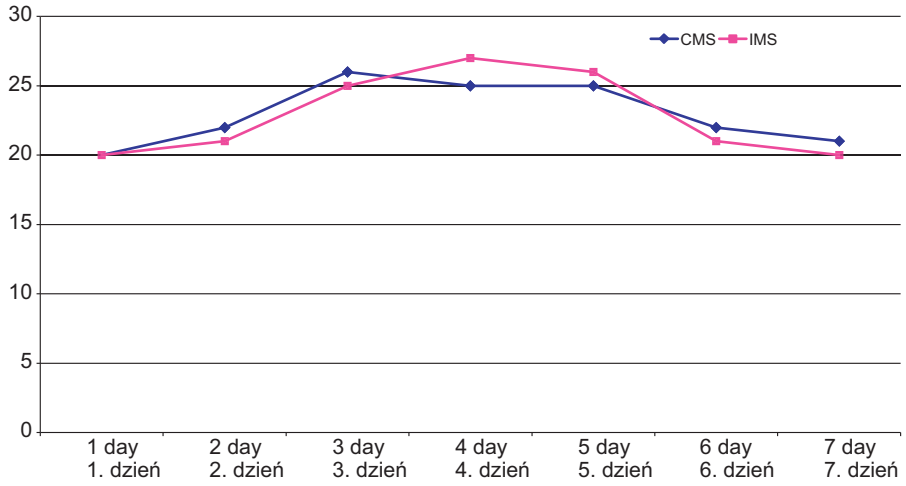

Fig. 1. Changes of temperature of maize silage during the aerobic stability test
Rys. 1. Zmiany temperatury w kiszonce podczas testu stabilności tlenowej

The application of biological additives to maize silages can reduce risks of occurrence of undesirable processes in the course of ensiling and, consequently, may help obtain silage of stable and good quality. This means that such treatment may contribute to possibilities of their longer storage and utilisation in commercial turnover [Weinberg and Ashbell 2003]. In order to take better care of natural environment, more attention should be paid to activities of biological additives characterised by fermentation-stimulating properties. The introduction of lactic bacteria strains together with the inoculum could have resulted in their rapid domination in the environment, reduction of pH and displacement of epiphytic species which lost competition for WSC utilisation. Tylor and Kung [2002] demonstrated an impact of inoculants containing *Lactobacillus buchnerii* in their composition on the increase of dry matter in maize silages which was also confirmed by our studies. Different results, however, were reported by Hassanat et al. [2007] and Potkański et al. [2010]. Protein levels in silages can be associated with the intensity of the proteolysis process during

the first few days of ensiling when over 75% of protein compounds are transformed into non-protein nitrogen compounds as a result of the activity of plant proteases. Ammonia is among the main products of proteolysis whose concentration depends on the rate of amino acid degradation. Apart from the application of stimulators, drying up and the content of dry matter in the plant raw material constitute important factors limiting protein degradation [Winters et al. 2001]. Feed fibres are made up of cellulose and hemicellulose referred to as structural fibres and pectins. Lignin constitutes an important barrier for bacteria as it confines their access to cell wall constituents and, consequently, limits lignin digestion in the rumen. The more NDF is in feed, the smaller can be its intake. Feed digestibility can decline with the increase of the ADF fraction [Huidsen et al. 2009]. In our studies, we observed a decline in NDF, ADF and ADL concentrations following the application of the biological preparation. Similar results were reported in studies carried out by Huidsen et al. [2009] and Kang et al. [2009]. The content of lactic acids in silages could have been influenced by WSC concentration, buffer capacity and breakup of the plant material. In their experiments, Nishino et al. [2004] recorded lower concentrations of lactic acid and higher of acetic acid in silages supplemented with biological additives containing *Lactobacillus buchnerii* in their composition, which contradicts results obtained in our studies. This could be connected with the ability of these bacteria to metabolise, in anaerobic conditions, of lactic acid into acetic acid and 1.2-propanediol [Oude Elfering et al. 2001]. Our investigations failed to demonstrate a significant impact of the applied biological preparation on reduction in fungal cell numbers. Kleinschmit et al. [2005] recorded a distinct decrease in numbers of fungal cells following the application of inoculant containing *Lactobacillus buchnerii* from the level of 4.43 $\log_{10}$ cfu g^{-1} to 2 $\log_{10}$ cfu g^{-1}. After the aerobic stability test, silages supplemented with biological additives may contain higher quantities of fungi which can be associated with a smaller content of escaping acetic acid which could inhibit the development of fungi [Weinberg and Ashbell 2003]. DON levels in silages were not high and did not exceed the acceptable standard of 1 750 ppb in accordance with the EU Directive 1881/2006.

Farmers frequently ask if and when it is worth using preparations facilitating ensiling forages. Additives can guarantee considerably higher silage stability which is important in modern systems of dairy cattle feeding. However, the results of our experiment failed to confirm the effectiveness of the applied biological preparation in improving the aerobic stability of maize silage. This was confirmed by a significantly higher fungal attack of inoculated samples as well as higher temperature increase in these silages during the stability test.

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WPŁYW INOKULANTA BAKTERYJNEGO NA PRZEBIEG FERMENTACJI, SKŁADNIKI ŚCIANY KOMÓRKOWEJ I STABILNOŚĆ TLENOWĄ KISZONEK Z KUKURYDZY

Streszczenie. Celem pracy było określenie wpływu inokulanta bakteryjnego na przebieg fermentacji, skład ścian komórkowych oraz stabilność tlenową kiszonek z kukurydzy. Kukurydzę zbierano w fazie 89. (BBCH). Zastosowany dodatek biologiczny zawierał w swoim składzie: *Enterococcus faecium* PCM 1858, *Pediococcus acidilactici* PAL-34, *Lactobacillus plantarum* PCM 493, *Lactobacillus buchneri* DSMZ 5987, *Lactobacillus rhamnosus* PCM 489, *Lactobacillus brevis* PCM 488, *Lactobacillus lactis* PCM 2379. Kukurydzę zakiszano w mikrosilosach laboratoryjnych wykonanych z PCV o pojemności 4 dm^3 z możliwością odprowadzenia produktów gazowych. Mikrosilosy były składowane w warunkach laboratoryjnych w temperaturze 10–15±2ºC. Próbki kiszonek do analiz mikrobiologicznych i chemicznych pobierano w 3., 14., 21. i 60. dniu po otwarciu mikrosilosów, a stabilność tlenową określano po 7 dniach napowietrzania. Uzyskane wyniki wskazują wpływ dodatku biologicznego na poprawę przebiegu fermentacji, obniżenie składników ścian komórkowych i poziomu deoksyniwalenolu (DON). Zastosowany dodatek biologiczny nie wpływał na poprawę stabilności tlenowej kiszonek podczas 7-dniowego napowietrzania.

Słowa kluczowe: deoksyniwalenol, kiszonki z kukurydzy, stabilność tlenowa

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