

## CHEMICAL COMPOSITION AND AEROBIC STABILITY OF HIGH MOISTURE MAIZE GRAIN SILAGE MADE WITH BACTERIAL OR CHEMICAL ADDITIVES

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*The experiment was carried out to determine the effect of bacterial inoculants or chemical preservatives on fermentation quality, nutritive value and aerobic stability of high-moisture maize grain silages. The lowest ( $P < 0,05$ ) degradation of crude and true protein, water-soluble carbohydrates and starch was characteristic of MK and ME silages. The highest lactic acid in total organic acids ratio was found in M0 silages, what corresponds with the lowest resistance to aerobic deterioration of these silages. The lowest ( $P > 0,05$ ) ethanol and ammonia content was obtained by ensiling high-moisture maize grain with formic and propionic acids and ammonium formate. There was no butyric acid in the silages made. The longest period of aerobic stability (163 h) was observed for ME silages.*

**Key words:** HIGH-MOISTURE MAIZE GRAIN SILAGES, ADDITIVES, CHEMICAL COMPOSITION, AEROBIC STABILITY

### Introduction

In the climatic conditions of Poland, maize is the only cereal whose grain has to be preserved after harvest due to high moisture. In Poland, the preservation of high-moisture grain by mechanical drying is expensive and uneconomic. An alternative and much cheaper method is to ensile grain. The greater nutritive value and digestibility of ensiled grain (mainly starch) compared to dry grain is also important [Knowlton, 2001; Korniewicz, 2005; Owens et al., 2005].

It is possible to produce high-moisture maize grain silages that is of high quality and safe for animals after supplementing the ensiled grain with preparations that stimulate fermentation and improve quality and aerobic stability. Chemical preservatives are considered the most effective. These preparations, containing low-molecular organic acids and their salts, are rapid and strong acidifiers of the ensiled grain environment, which limit or suppress the growth of undesirable epiphytic flora (mainly yeasts and moulds), thus producing silages that are more resistant to aerobic spoilage [Adesogan et al., 2003; Kung et al., 2004].

An alternative method to produce aerobically stable silages is ensiling high-moisture maize grain with bacterial inoculants containing heterofermentative lactic acid bacteria *L. buchneri* or propionic acid bacteria *P. acidipropionici* [Dawson et al., 1998; Adesogan et al., 2003]. When breaking down lactic acid, *L. buchneri* bacteria produce large amounts of acetic acid as well as 1,2-propanediol, propanol and propionic acid. *P. acidipropionici* bacteria increase the content of propionic acid and acetic acid in the silages. The above metabolic products of these bacteria limit the growth of yeasts and moulds, which increases the aerobic stability and nutritive value of the silages [Adesogan et al., 2003; Holzer et al., 2003].

The aim of the present study was to determine the effect of ensiling high-moisture maize grain with bacterial inoculants containing hetero- or homofermentative lactic acid bacteria and propionic acid bacteria as well chemical preservatives containing organic acids and their salts, on the fermentation quality, chemical composition and aerobic stability of the silages obtained.

## Materials and methods

High-moisture maize grain (*Zea mays* cv. Eurostar, FAO 250) was ensiled. Directly after harvesting the grain was grounded into 1,5–2,0 mm particles. The nutrient content of the grain was as follows: dry matter — 659,9 g·kg<sup>-1</sup>, crude ash — 15,1 g·kg<sup>-1</sup> DM, crude protein — 100,8, true protein — 88,9, crude fat — 43,1, crude fiber — 22,8, NDF — 96,8, ADF — 52,6, ADL — 13,0, WSC — 79,8, starch — 693,6 g·kg<sup>-1</sup> DM, buffering capacity — 5,38 meq·100 g<sup>-1</sup> DM.

High-moisture grain was ensiled without additives — M0 and with addition of bacterial inoculants: Lalsil Fresh LB (*L. buchneri* — 3,0×10<sup>5</sup> cfu·g<sup>-1</sup> of grain) — MLF or Lalsil MS01 (*L. plantarum* — 1,5×10<sup>5</sup> cfu·g<sup>-1</sup> of grain, *P. acidipropionici* — 1,5×10<sup>5</sup> cfu·g<sup>-1</sup> of grain) — MLM and chemical preservatives: KemiSile<sup>TM</sup>2S (formic acid — 55 %, propionic acid — 20 %, ammonium formate — 4,3 %, potassium sorbate — 2,5%; 4 ml·kg<sup>-1</sup> of grain) — MK or KemiSile 2000 (formic acid — 55 %, propionic acid — 5 %, ammonium formate — 24 %; 4 ml·kg<sup>-1</sup> of grain) — ME. High-moisture maize grain was ensiled in 120-l polyethylene silos. An average of 132–135 kg of grain was compacted in the silos. The ensiled biomass were stored for 60 days in an enclosed room at 15 ± 2° C.

Grain and silages samples were analysed for dry matter content using the drying method at 105 °C for 12 h. Grain buffering capacity was determined according to Playne and McDonald [1966]. In silages pH level was determined using an pH/Ion Analyser MA 235 [Mettler Toledo, Switzerland]. The level of lactic, acetic, propionic, butyric and formic acids in the silages was determined using liquid chromatography. The analysis was performed using an LC 5000 liquid chromatograph with a UV/VIS detector [INGOS, Czech Republic] and an Ostion LG KS 0800 H<sup>+</sup> column [Tessek, Czech Republic]. Column operating temperature was 50 °C, with mobile phase of 0.005 M H<sub>2</sub>SO<sub>4</sub>. Ethanol content of the silages was determined using liquid chromatography. The analysis was performed using a Varian Star 3400 CX gas chromatograph [Varian, USA] with an FID detector and a DB-FFAP capillary column (30 m long, 0.53 mm in diameter), using argon as the carrier gas. Operating temperature was 90–205° C for the column, 200° C for the sample injector, and 240 °C for the detector. The NH<sub>3</sub>-N content of the silages was determined using the Conway method described by Skulmowski [1974].

Grain and silage samples intended for further chemical analyses were dried in a drier at 50 °C for 48 h and ground [Fritsch Pulversette 15 mill, Germany] into 1.0 mm sized particles. These samples were analysed for the level of crude ash [AOAC, 2007], crude fat [AOAC, 2007] using an Ankom XT15 extractor [Ankom, USA], and crude fiber [AOAC, 2007] using an Ankom<sup>220</sup> Fiber Analyser [Ankom, USA]. The crude protein [% total-N ×6,25] content was determined according to Kjeldahl [AOAC, 2007] and the true protein [% protein×6,25] content according to Licitra et al. [1996] using a Kjeltac 2200 unit [Foss, Denmark], NDF, ADF and ADL [Goering and Van Soest, 1970] using an Ankom<sup>220</sup> Fiber Analyser [Ankom, USA], starch according to Faisant et al. [1995] and WSC using the colorimetric method [Dubois et al., 1956].

The aerobic stability of silages from high-moisture maize grain was tested for 7 days in an air-conditioned room in the ambient temperature of 20 °C, according to the method of Honig [1985]. Aerobic stability was measured by the number of hours during which the temperature of the silages subjected to aerobic exposure did not exceed the ambient temperature in the air-conditioned room by 2 °C [Honig, 1985].

The results of experiment were analysed statistically using one-way analysis of variance and Tukey's test [SAS, ver. 9.1, 2001–2003].

## Results and discussion

The nutrients content of high-moisture maize grain silages is given in Table 1. Compared to the control variant M0, the lowest dry matter content was found in the silages MLF and MLM and the highest in the silages MK and ME.

Ensiling high-moisture maize grain with bacterial inoculants or chemical preservatives did not cause a significant ( $P > 0,05$ ) changes in the content of crude ash, crude fat, starch, crude fiber, NDF, ADF and ADL in silages.

Ensiling high-moisture grain with the each silage additives increased the crude protein and true protein content of the silages made. The highest amount of these protein fractions was found in the MK and ME silages and differed significantly ( $P < 0,05$ ) compared to their content in the M0 silages.

The lowest ( $P < 0,05$ ) amount of WSC (4,1 and 5,2 g·kg<sup>-1</sup> DM) was found in the MLF and MLM silages. WSC content was two-fold higher in the M0 silages and 4–5-fold higher in the MK and ME silages on average.

Table 1

The nutrients content of high-moisture maize grain silages

Item	Type of silage					Mean (n=25)	SD
	M0	MLF	MLM	MK	ME		
Dry matter (g·kg <sup>-1</sup> )	642,7	636,5	635,0	647,2	648,6	642,1	5,48
	(g·kg <sup>-1</sup> DM)						
Crude ash	15,5	15,6	15,0	18,3	19,1	16,7	1,66
Crude fat	44,1	43,9	44,2	44,9	44,5	44,3	0,35
Crude protein	90,2 b	97,1 ab	96,2 ab	98,7 a	99,1 a	96,3	3,21
True protein	79,2 b	82,5 ab	83,8 ab	86,1 a	87,5 a	83,8	2,90
Crude fiber	22,5	20,9	21,5	23,2	23,5	22,3	0,99
NDF <sup>1</sup>	95,1	89,4	91,1	97,1	98,9	95,6	2,90
ADF <sup>2</sup>	51,8	50,8	51,4	54,7	53,4	52,4	1,43
ADL <sup>3</sup>	16,1	16,3	16,7	17,0	16,5	16,5	0,31
WSC <sup>4</sup>	10,3 c	4,1 d	5,2 d	39,3 b	48,3 a	21,4	18,59
Starch	688,4	687,0	686,5	695,8	696,0	690,7	4,26

<sup>1</sup> NDF — neutral detergent fiber; <sup>2</sup> ADF — acid detergent fiber;

<sup>3</sup> ADL — acid detergent lignin; <sup>4</sup> WSC — water soluble carbohydrates;

<sup>5</sup> SD — standard deviation; a, b, c, d — (in rows) —  $P < 0,05$

The parameters of fermentation quality and aerobic stability of high-moisture maize grain are shown in Table 2. M0 silages were characterized by the highest pH value. All the additives used, particularly the bacterial inoculants, reduced ( $P < 0,05$ ) this indicator of fermentation quality.

The highest amount of NH<sub>3</sub>-N (6,9 g·kg<sup>-1</sup> of total-N) occurred in M0 silages. The level of this undesirable fermentation product was 2-fold lower in the MLF and MLM silages and 4-fold lower in the MK and ME silages on average.

Ensiling high-moisture grain with bacterial inoculants did not limit the alcohol fermentation in the silages. This effect was obtained by ensiling maize grain with chemical preservatives. The lactic acid content of the M0 silages did not exceed 12 g·kg<sup>-1</sup> DM. An increase ( $P < 0,05$ ) in the level of this acid to 20 g·kg<sup>-1</sup> DM was found in the MLF and MLM silages, and a decrease ( $P < 0,05$ ) to 7 g·kg<sup>-1</sup> DM in the ME and MK silages.

The level of acetic acid in the M0, MK and ME silages was low and did not exceed 6 g·kg<sup>-1</sup> DM. This acid was over twice higher ( $P < 0,05$ ) in the MLF and MLM silages.

M0 silages contained no propionic acid. It was found in all the other variants, being the highest ( $P < 0,05$ ) in the MLF and ME silages. No silages analysed were found to contain butyric or formic acids.

The shortest period of aerobic stability (45 h) was characteristic of the M0 silages. Resistance to aerobic deterioration was much longer ( $P < 0,05$ ) in the MLF and MLM silages (115 and 108 h) and in the MK and ME silages (153 and 168 h, respectively).

Table 2

**Fermentation parameters and aerobic stability  
of high-moisture maize grain silages**

Item	Type of silage					Mean (n=25)	SD
	M0	MLF	MLM	MK	ME		
pH	4,75 a	4,24 c	4,26 c	4,37 b	4,35 b	4,40	0,19
NH <sub>3</sub> -N (g·kg <sup>-1</sup> total-N)	6,9 a	3,4 b	3,5 b	1,6 c	1,2 c	3,3	2,01
	(g·kg <sup>-1</sup> DM)						
Ethanol	9,8 a	8,1 a	8,0 a	3,0 b	3,2 b	6,4	2,78
Lactic acid	11,5 b	17,8 a	19,5 a	6,4 c	6,0 c	12,2	5,60
Acetic acid	5,7 b	12,8 a	10,9 a	5,1 b	5,0 b	7,9	3,28
Butyric acid	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Propionic	0,0 d	1,9 b	1,0 c	0,9 c	2,5 a	1,3	0,86
Total acids	17,2 b	32,5 a	31,4 a	12,4 c	13,8 bc	21,5	8,71
LA:AA ratio <sup>1</sup>	2,0 a	1,4 bc	1,8 ab	1,1 c	1,2 c	1,5	0,35
LA in TA <sup>2</sup> [%]	66,9 a	54,8 b	62,1 a	51,6 c	43,5 d	55,8	8,16
Aerobic stability (h)	55 d	115 c	108 c	151 b	163 a	118,4	37,92

<sup>1</sup> Lactic acid: acetic acid ratio; <sup>2</sup> Lactic acid in total organic acids;

<sup>3</sup> SD — standard deviation; a, b, c, d — (in rows) —  $P < 0,05$

The higher level of crude protein and true protein in silages with addition of bacterial inoculants or chemical preservatives was due to the limited degradation of these components during fermentation process. During fermentation, *L. buchneri* bacteria produce large amounts of acetic acid by degrading lactic acid. By degrading lactic acid and glucose, *P. acidipropionici* bacteria produce acetic and propionic acids. By strongly acidifying the environment, these acids limit or inhibit the growth of protein-degrading bacteria [Ranjit et al., 2000; Holzer et al., 2003].

The double reduction in the WSC content of silages treated with lactic and propionic bacteria compared to the control silages resulted from the more intensive fermentation that required degradation of a higher amount of carbohydrates by a larger population of bacterial, which resulted in the production of a considerably greater amount of organic acids. The decrease in the WSC content of silages made from high-moisture maize grain with the addition of propionic acid bacteria was reported by Dawson et al. [1998].

Formic and propionic acids show strong antibacterial properties, which cause them to inhibit the activity of desirable fermentative bacteria [Slottner and Bertilsson, 2006]. As a result, silages made with organic acids contain high amounts of WSC. This action of chemical preservatives containing propionic and formic acids was confirmed in the present experiment.

All the silages made from high-moisture maize grain with the additives were characterized by a significantly lower pH compared to untreated silages. In the case of the silages with bacterial inoculants, this resulted from a greater amount of metabolic products of *L. buchneri* and *P. acidipropionici*, which acidified the grain biomass environment more strongly. Meanwhile, the chemical preservatives enriched the ensiled grain with formic and propionic acids, which also increased biomass acidification. In other studies, ensiling high-moisture maize grain with propionic

bacteria [Dawson et al., 1998] or homofermentative lactic bacteria [Kung et al., 2004] also significantly reduced the pH of the silages obtained. A reduced value of this fermentation parameter was also found in a study by Kung et al. [2004], in which high-moisture grain was ensiled with buffered propionic acid.

The additive used in the ensilage of high-moisture maize grain considerably limited the degradation of protein to ammonia. In the silages with *L. buchneri* or *P. acidipropionici*, the large amounts of produced organic acids (lactic, acetic and propionic) caused a rapid and strong acidification of the environment, which limited the activity of bacteria degrading protein to ammonia. Better results in limiting protein degradation to ammonia are obtained by chemical preservatives containing organic acids, which strongly limit the growth of protein-degrading bacteria [Woolford, 1990; Purwin, 2005; Slottnér and Bertilsson, 2006]. This effect of formic and propionic acids on greatly reducing the degradation of proteins to ammonia was confirmed in the present study.

Ensiling high-moisture maize grain with bacterial inoculants did not limit alcohol fermentation, which is consistent with the findings of other authors [Kung et al., 2004]. Organic acids and their salts, added to ensiled materials can limit the growth of yeast responsible for this type of fermentation [Woolford, 1990; Kung et al., 2004]. This activity of organic acids was confirmed in the present experiment.

The small amounts of lactic acid in silages from high-moisture maize grain without additives indicated that lactic acid fermentation was a slow process. This could result from both the inadequate population of lactic acid bacteria living on maize grain and the inadequate amount of water necessary for intensive growth of these bacteria. The addition of bacterial inoculants in aqueous solutions increases the population of fermentative bacteria, whereas the increased moisture of the ensiled material creates better conditions for their growth. In the present study, ensiling high-moisture maize grain with the *L. buchneri* or *L. plantarum* and *P. acidipropionici* bacteria increased the lactic acid content in the silages. Other authors did not find increased amounts of lactic acid when ensiling high-moisture maize grain with *L. plantarum*, *L. bulgaricus* and *L. acidophilus* [Kung et al., 2004] or *P. acidipropionici* [Dawson et al., 1998].

By strongly restricting bacteria, organic acids also limit the growth of lactic and acetic acid bacteria during fermentation [Kung et al., 2004; Slottnér and Bertilsson, 2006]. This action of formic and propionic acids was confirmed in the present experiment. Silages from high-moisture maize grain with these acids contained the smallest amounts of lactic and acetic acids.

In the present study, the bacterial inoculants added to the ensiled high-moisture maize grain also stimulated acetic fermentation. The greatest amount of acetic acid occurred in the variants with *L. buchneri*. This resulted from the activity of these bacteria, the main product of which is acetic acid [Holzer et al., 2003]. The greater acetic acid content of silages with *L. plantarum* and *P. acidipropionici* bacteria than in the control variant was due to the activity of *P. acidipropionici*, of which acetic acid is one of metabolites [Dawson et al., 1998; Ohmomo et al., 2002]. In a study by Dawson et al. [1998], ensiling high-moisture maize grain with *P. acidipropionici* bacteria caused an over two-fold increase in the acetic acid content of the silages obtained. The decreased rate of acetic fermentation was found in a study by Kung et al. [2004], in which high-moisture grain was ensiled with *L. plantarum*, *L. acidophilus* and *L. bulgaricus* bacteria.

Silages from high-moisture maize grain without any additives were the least resistant to aerobic deterioration, which resulted from the unfavourable profile of organic acids — the high lactic acid to acetic and propionic acid ratio and the greatest ratio of lactic acid in total organic acids. In addition to WSC, lactic acid is a nutrient substrate for yeasts and moulds responsible for the aerobic deterioration of silages after exposure of the silage heap [Ohmomo et al., 2002].

In the present study, silages from high-moisture maize grain with *L. buchneri* or *P. acidipropionici* bacteria were characterized by longer period of aerobic stability, compared to untreated silages. These silages were richer in antifungal acetic and propionic acids [Ohmomo et al.,

2002] and had a considerably lower ratio of acetic acid in total organic acids. The increased resistance of high-moisture maize grain silages to secondary fermentation as a result of using *P. acidipropionici* was also reported by Dawson et al. [1998].

The highest aerobic stability was characteristic of silages from high-moisture maize grain with chemical preservatives, especially with a preparation containing propionic acid, formic acid and ammonium propionate. The silages with chemical preservatives were characterized by the lowest lactic to acetic acid ratio and the lowest ratio of lactic acid in total organic acids. Based on such a high resistance of these silages to aerobic deterioration, it is concluded that chemical compounds found in the preservatives used caused a large reduction of the population of yeasts and moulds during the fermentation of high-moisture maize grain. In the study of Kung et al. [2004], the addition of buffered propionic acid to the ensiled high-moisture maize grain reduced the yeast population by a factor of 2 and 7, compared to the population of these microorganisms in untreated silages.

### **Conclusions**

Ensiling high-moisture maize grain with chemical preservatives containing propionic acid, formic acid and ammonium formate or potassium sorbate made it possible to obtain silages with the lowest protein and WSC degradation during fermentation process and the highest resistance to aerobic deterioration after exposure of the silage heap. Bacterial inoculants containing *L. buchneri* or *P. acidipropionici* bacteria could be effective additives alternative to ensiling high-moisture maize grain when no chemical preservatives can be used.

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## **CHEMICAL COMPOSITION AND AEROBIC STABILITY OF HIGH MOISTURE MAIZE GRAIN SILAGE MADE WITH BACTERIAL OR CHEMICAL ADDITIVES**

### **S u m m a r y**

The experiment was carried out to determine the effect of bacterial inoculants or chemical preservatives on fermentation quality, nutritive value and aerobic stability of high-moisture maize grain silages. The lowest ( $P < 0.05$ ) degradation of crude and true protein, water-soluble carbohydrates and starch was characteristic of MK and ME silages. The highest lactic acid in total organic acids ratio was found in M0 silages, what corresponds with the lowest resistance to aerobic deterioration of these silages. The lowest ( $P > 0.05$ ) ethanol and ammonia content was obtained by ensiling high-moisture maize grain with formic and propionic acids and ammonium formate. There was no butyric acid in the silages obtained. The longest period of aerobic stability (163 h) was observed for ME silages.

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## **ХІМІЧНИЙ СКЛАД І АЕРОБНА СТАБІЛЬНІСТЬ СИЛОСУ ЗЕРНА КУКУРУДЗИ З ВИСОКИМ ВМІСТОМ ВОЛОГИ ВИГОТОВЛЕНОГО З БАКТЕРІАЛЬНИМИ АБО ХІМІЧНИМИ ДОБАВКАМИ**

### **Р е з ю м е**

Метою проведення дослідів було визначити вплив бактеріальних інокулянтів або хімічні способи збереження якості бродіння, поживну цінність, і аеробну стабільності силосів зерна кукурудзи з високим вмістом вологи. Найнижча ( $P < 0.05$ ) деградація загального і справжнього білка, розчинних у воді вуглецю і крохмалю була характерна для

силосів МК і МЕ. Найвищий вміст молочної кислоти в загальному органічному співвідношенні кислот виявлено в силосах М0, що відповідає найнижчій опірності до аеробного псування силосів.

Найнижчий ( $P > 0.05$ ) вміст етанолу і аміаку був отримано при силосуванні зерна кукурудзи з високим вмістом вологи з мурашиною, пропріоновою кислотами та форміатом амонію. У силажі не отримано масляної кислоти. Найдовший період аеробної стабільності (163 год) спостерігався в силосів МЕ.

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## ХИМИЧЕСКИЙ СОСТАВ И АЭРОБНАЯ СТАБИЛЬНОСТЬ СИЛОСА ЗЕРНА КУКУРУДЗЫ С ВЫСОКИМ СОДЕРЖАНИЕМ ВЛАГИ ИЗГОТОВЛЕННОГО С БАКТЕРИАЛЬНЫМИ ИЛИ ХИМИЧЕСКИМИ ДОБАВКАМИ

### А н н о т а ц и я

Целью проведения опыта было определить влияние бактериальных инокулянтов или химические способы сохранения качества брожения, питательную ценность, и аэробную стабильность силосов зерна кукурузы с высоким содержанием влаги. Самая низкая ( $P < 0.05$ ) деградация общего и настоящего белка, растворимых в воде углерода и крахмала была характерная для силосов МК и МЕ. Наивысшее содержание молочной кислоты в общем органическом соотношении кислот выявлено в силосах М0, что отвечает самой низкой сопротивляемости к аэробной порче силосов. Самое низкое ( $P > 0.05$ ) содержание этанола и аммиака было полученный при силосовании зерна кукурузы с высоким содержанием влаги с муравьиной, пропріоновою кислотами и формиатом аммония. В силосе не получена масляная кислота. Самый длинный период аэробной стабильности (163 год) наблюдался у силосов МЕ.

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