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The influence of the *lactobacillus buchneri*, *lactobacillus plantarum*, and *enterococcus faecium* on the nutritional value and health safety of the different maize hybrid silages

Aleksandra Ivetić [1*], Bojan Stojanović[2], Branko Petrujkić[3], Radmila Beskorovajni[1], Marijana Maslovarić[1], Milivoje Ćosić[4] and Stamen Radulović[3]

[1]*Institute for Science Application in Agriculture, Blvd. Belgrade, Serbia*
[2]*Department of Animal Science, Faculty of Agriculture, University of Belgrade, Belgrade, Serbia*
[3]*Department of Nutrition and Botany, Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia*
[4]*Institute of Forestry, Belgrade, Belgrade, Serbia*

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ABSTRACT

This research aimed to determine the impact of the identical inoculant mixture of lactic acid bacteria (LAB) on the silage quality of five different corn hybrids (*Zea mays*), which were ensiled separately. The hybrids (Pioneer Hi-Bred DuPont) differed in terms of FAO maturity group (from early H1, mid-early H2, medium H3, mid-late H4 to late H5). The inoculant consisted of a mixture homofermentative and heterofermentative LAB: *Lactobacillus buchneri*, *Lactobacillus plantarum*, and *Enterococcus faecium*. Ensiling was done in laboratory conditions, where the green mass of corn hybrids was ensiled with and without LAB. The process of ensiling lasted 60 days. The addition of the inoculant had a significant positive effect on the content of crude protein (CP, $P<0.05$) and non-fiber carbohydrates (NFC, $P<0.05$) in the silages obtained. Application of the inoculant also reduced acid detergent lignin, $P<0.05$) in the experimental silages. The chemical characteristics of the hybrids and inoculant composition affected the energy content. However, a positive influence of LAB inoculation on net energy for lactation (NE_L) content was only determined in early-maturity hybrid H1 ($P<0.05$). This silage had the highest value for NE_L concentration, with the lowest Neutral Detergent Fiber (aNDF) and acid detergent lignin (ADL) content. The addition of LAB inoculates influenced the health safety of the silages by increasing the content of acetic acid, with the potential of extending the duration of their aerobic stability and resistance to microbial degradation.

Key words: corn hybrids; inoculants; lactic acid bacteria; silage quality; animal health safety

*Corresponding author:
Aleksandra Ivetić, research associate, Institute for Science Application in Agriculture, Blvd. Despota Stefana 68b, 11000 Belgrade, Serbia, phone: +381 63 517 711, fax:+381 11 275 2959; e-mail: aivetic@ipn.bg.ac.rs

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Introduction

Ensiling is a microbial process used to preserve fresh feed (primary plants) in animal production for feeding, as well as bio-refinery raw material (OKOYE et al., 2023). In these processes, lactic acid bacteria have a key position among all the silage microorganisms, and the effects of exogenous LAB on silage quality have been widely studied (WANG et al., 2021). The addition of LAB inoculants is frequently used to speed up the process of ensiling, prevent the growth of harmful microorganisms, and improve the silage quality of different crops. Furthermore, LAB is one of the microorganisms that have the GRAS (generally recognized as safe) status (FABISZEWSKA et al., 2019).

Choosing the right corn hybrid, is very important for the nutritional value of silage and milk production, considering their different chemical compositions. Inconsistency in silage quality may also be due to a lack of information on the gene expression and molecular mechanisms of the microbiota involved in silage production (OKOYE et al., 2023). Nowadays, many researchers are focusing on obtaining high-quality animal feeds with improved LAB inoculants. Having in mind that LAB adaptability, establishment, and development in forages during ensiling are still partially unknown, additives with LAB inoculants do not always regulate silage fermentation successfully. One of the main reasons for the lack of sufficient domination by LAB inoculants is the influence of epiphytic LAB microflora in the ensiling plants.

Biotech companies have developed different commercial microbial inoculants to preserve the green mass of plants and reduce the loss of nutrients during the aerobic degradation of silage. The number of LAB colonies in such products is usually about 1×10^{11} CFU and only 10 g of such additives need to be added for ensiling 10 tons of green plant mass. Preservation depends on the ability of the LAB to produce sufficient acids to stop the growth and other activities of undesirable microorganisms (MO) under anaerobic conditions (CAI et al., 1999). Inoculants may consist of only homofermentative or heterofermentative LAB, or they may consist of a combination of both, with or without the addition of enzymes. The main differences between them are the production of lactic acid (LA) and acetic acid (AA), which greatly affect the fermentation process and aerobic stability of silage. Lactic acid rapidly lowers the pH of the ensiled mass, but unlike AA and propionic acid (PA), it has weak fungicidal properties (Mc DONALD et al., 1991).

Silages obtained using different corn hybrids mostly differ in net energy content. The primary differences are in dry matter (DM) and crude protein (CP) content, as well as fiber content, (CHERNEY et al., 2004; CHERNEY et al., 2007). Under laboratory conditions, JURAČEK et al. (2013) conserved four early maize hybrids without additives and determined that the highest starch, nitrogen-free extract, and organic matter content was found in silages with the highest DM content. DANNER et al. (2003) inoculated corn plants with different homofermentative and heterofermentative LAB, and a visible difference in DM and energy content was observed in the amount of AA and LA produced, followed by significant amounts of 1, 2-propanediol in silages inoculated with *L. buchneri*.

The differences in the type of hybrid selected, as well as the inoculant used for ensiling, have an impact on the animals' production results. For example, in a study of the effects of three different corn hybrid silages on the lactation performance of Holstein dairy cows, it was reported that a corn silage hybrid with higher starch and lower NDF content resulted in higher milk, protein, and lactose yields, (AKINS and SHAVER, 2014). Between the different LAB strains used as ensiling agents for the reduction of methane emissions in dairy cows HUYEN et al.(2020) concluded an *in vitro* study where lower methane production was observed with silages inoculated with homofermentative *L. plantarum*. It is well known that during milk production, the volume of emission of enteric methane is an indicator of gross energy lost during the digestion process. It was found that among the different LAB strains for silage preparation, *L. plantarum* was the most potent in the reduction of enteric methane emissions.

Contamination with undesirable microbes is one of the major problems in silage production. The presence of yeasts and molds can negatively affect the nutritional value (NV) of silage because they produce toxic compounds that are harmful to ruminants (ALONSO et al., 2013). These microbes can proliferate massively once the silo is opened due to the presence of oxygen (PARADHIPTA et al., 2020). As a result, increasing yeast and mold populations decrease aerobic stability and reduce silage shelf life (WILKINSON and DAVES, 2013). Mycotoxins are secondary fungal metabolites that have been detected in a variety of feed ingredients, and they can affect animal health and productivity. Nevertheless, LAB is capable of producing acetate, proteinaceous compounds, peptides, and hydrogen peroxide, which exert antifungal activity (KLEINSCHMIDT et al., 2005). The ability of LAB to release antifungal substances varies among strains (SCHNÜRER et al., 2005). In an ensiling experiment conducted by PARADIHPTA et al. (2020), *L. brevis* and *L. buchneri* showed high antifungal and carboxylesterase activities in silage. The mixture of both strains improved corn silage quality by increasing nutrient digestibility and reducing yeast contamination.

The objective of the present research was to determine the impact of the same inoculant of LAB on the silage quality of five different corn hybrids H1-H5 (*Zea mays*). The hybrids differed in terms of FAO maturity group as early, mid-early, medium, mid-late, and late. To the best of our knowledge, there have been no reports of the influence of the same inoculate mixture of LAB on these different corn hybrids. This study aimed to provide a detailed insight into the chemical, fermentative, and microbial composition of silage made from five different corn hybrids treated with an LAB inoculant (mixtures of homofermentative and heterofermentative strains). Also, the study investigated the effect of LAB inoculants on the health safety of these silages. We hypothesized that the single identical LAB inoculant mixture could improve the quality and health safety of silages made from different maize hybrids.

Materials and methods

Ensiling. Studies on the influence of LAB on the nutritional value of silages were conducted on five different corn hybrids (Pioneer Hi-Bred DuPont), in Vojvodina, Serbia. Hybrids were harvested at the early dough stage of maturity. Hybrids differed in terms of FAO maturity group (from early to late), and those used in the trial were:

1. Early maturity (H1) - P37M34, FAO 380;
2. Mid early maturity (H2) - P36B08, FAO 450;
3. Medium maturity (H3) - P35P12, FAO 510;
4. Mid-late maturity (H4) - P35K67, FAO 530;
5. Late maturity (H5) – P32D12, FAO 730;

According to the specifications of the LAB inoculants manufacturer, the average level of LAB in the dry inoculant was 1×10^9 CFU/g and the recommended rate for use was 1×10^5 CFU/g of green mass, (the dry inoculant dissolved in water, and 1 g inoculants/1 t of green mass is used, mixed in the water in the amount of 1×10^5 LAB CFU/g green mass). However, to allow the dominance of inoculants over the epiphytic microflora of the plant, the inoculants were added at a higher rate of 1×10^6 LAB CFU/g green corn mass, as recommended (MUCK et al., 2007). The composition of the inoculant LAB used in the trial was: *Lactobacillus buchneri* 1×10^{11} CFU/g, *Lactobacillus plantarum* -LP 28 - 4×10^9 CFU/g, *Lactobacillus plantarum*-LP 329 - 4×10^9 CFU/g and *Enterococcus faecium* 2×10^9 CFU/g.

Before ensiling, the green mass of each corn hybrid was sprayed with LAB inoculant dissolved in sterilized water, while a control group of each hybrid was treated with the same amount of sterilized water without LAB. Laboratory mini-silos with hermetic closure, 1.5 l volume, and one-way gas release were used for ensiling. Freshly cut corn green mass was ensiled at an average density of 500 g/l and each silo was loaded with approximately 750g of pressed fresh corn mass. The experimental silos were stored at a constant room temperature (~22°C ±1°C). The number of treatments was 10 (two treatments per hybrid, controls without inoculation, and ensiling with LAB, 5×2) and all treatments were performed in five replicates (quintuplicate). The process of ensiling lasted 60 days.

Chemical analyses. The chemical and microbiology composition of silages was analyzed at the Laboratory of Animal Nutrition at the Faculty of Agriculture, University of Belgrade, Serbia. Immediately after the silo mass was opened for the analyses, samples of the silages were taken. Dried samples of silages were ground to pass a 1 mm screen on a small-sample mill (Kinematica PX-MFC 90D). Ground samples of silages were analyzed according to the Official Methods of the Association of Analytical Chemists (AOAC, 2023).

The dry matter (DM) content was determined by drying at 80°C in an oven for 20 h (method 967.03). The ash content was determined after combusting samples at 600°C for 3 h (method 942.05). The organic matter content was calculated as OM, % = DM, % - Ash, %. The CP content was determined by the Kjeldahl method (AOAC 2001.11) using K_2SO_4/Cu catalyst-Kjeltabs S 3.5 using a Kjeltec Auto 1030 Analyzer-Tecator System. Ether extract (EE) content was determined by extraction using diethyl-ether in Soxhlet apparatus (method AOAC 920.39). The content of fibers insoluble in neutral detergent: aNDF content was determined using heat-stable α-amylase (A3306 Sigma Chemical Co., St Louis, MO, USA) according to (Method 2002.04), without using sodium sulfite and without correcting the ash content and the acid detergent fiber (ADF) was determined without correcting ash content (Method 973.18). The residues of ADF were incubated for 3 h in 72% sulfuric acid, to determine the content of acid detergent lignin (ADL). Determination of the contents of volatile fatty acids (VFA), lactic, acetic, butyric, and propionic acid, was done using liquid chromatography, HPLC with IR detection, using a column (100×7.7mm 8 m HyperREZ XP Organic, Thermo Fisher Scientific Inc., USA).

The energy values of the silages were calculated according to Tylutki *et al* (TYLUTKI et al., 2008).

Microbiological analyses. Microbiological analyses of the experimental silages were performed immediately after opening the silo, including the following parameters: total microorganism number (TMN), LAB colony count, and yeasts and molds (Y&M) count. The specific plates were used for determination of microorganisms: for TMN plates of Nutrient agar were used, for LAB plates of MRS were used (De Man–Rogosa–Sharpe) agar, and for Molds and Yeasts plates of SDA were used (Sabour and Dextrose agar). For numeration of the colony forming units (CFU), a surface viable count was used (MILES et al., 1938). An anaerobic container (Becton Dickinson, USA) was used to provide anaerobic conditions for the growth and development of LAB, and a BD GasPak EZ system (Becton Dickinson, USA) for anaerobic conditions, from the same manufacturer.

Statistical analyses. All obtained data were statistically processed using Statistics 6.0 Software, (Stat Soft Inc. 2003). As part of the statistical processing of the obtained data, the Student`s t-test was performed using the standard procedure.

Results

The influence of LAB inoculation on the nutritive parameters of corn silage. The effects of LAB inoculants on the chemical composition and nutritive value of five different hybrid silages are presented in Table 1.

The influence of LAB inoculation on the nutritive parameters differed significantly in the experimental and control groups ($P<0.05$). In the experimental silages, the content of DM differed among the treatments. The content of organic matter (OM) ranged from 304.9-335.9 g/kg DM in the experimental silages ensiled without LAB inoculation, and a similar range of 306.2-336.0 g/kg DM was found for inoculated silages. It should be emphasized that the influence of LAB addition was followed by a statistically significantly higher content of OM in the inoculated silages of H1 and H3, compared with the values in the control silages of the same hybrids. In contrary to the ash content in the H3 silage, in the early maturating hybrid H1 the ash content was significantly lower in the inoculated silage than in the silage without LAB addition.

The addition of LAB significantly improved the CP content in all silages, except for the mid-early maturing hybrid, where no difference was found between treatments.

The trend of a decreasing content of aNDF and ADL, which was significant ($P<0.05$), was established in the early H1 and late H5 maturity hybrids. A statistically higher ($P<0.05$) level of ADF was found in all inoculated silages, compared with the control treatments. The ADL content in the silage of the inoculated late-maturity hybrid H5 was higher by almost 63% compared with the silage from the early maturing hybrid H1. The second trend we recognized was the increased ash and ADL content from the early to late maturity hybrid. The amount of ADL ranged from 25.3-41.2 g/kg DM, and ash content was in the range of 28.1-35.3 g/kg DM. Contrary to the trend of an increase in the content of ADF and ADL from early to late maturity hybrids, the content of NFC showed a trend of decreasing in general. For the inoculated H1 early maturity hybrid silage the NFC value was 470.0 g/kg DM, whereas in the inoculated H5 late hybrid it was 411.0 g/kg DM. It is worth mentioning that in the inoculated silages of both hybrids, these contents were statistically higher than in the control silages of the same hybrid.

The positive influence of LAB inoculation on NEL content was determined in the early maturity hybrid H1, where the energy content was statistically higher ($P<0.05$) than in the control treatment of the same hybrid silage. Also, these were the highest values among all the experimental silages in this trial.

The influence of LAB inoculation on the fermentation quality of corn silage. Table 2 presents the influence of LAB inoculation on the fermentation quality of corn hybrid silages.

Table 1. The influence of LAB inoculation on the chemical composition and nutritive value of corn hybrid silages, (g/kg DM)

Hybrid		DM	OM	CP	EE	aNDF	ADF	ADL	NFC	Ash	NE_L
H1	Control	342.1[b]	313.0[b]	84.0[b]	25.1[b]	485.5[a]	204.9[b]	28.9[a]	385.0[b]	29.1[a]	5.84[b]
	Inoculant	362.3[a]	334.2[a]	91.2[a]	28.7[a]	395.1[b]	212.0[a]	25.3[b]	470.0[a]	28.1[b]	6.40[a]
H2	Control	361.4[a]	335.9[a]	46.8	34.4[a]	451.0[b]	224.0[b]	35.3[a]	434.0[a]	25.5[b]	6.06
	Inoculant	329.2[b]	300.5[b]	46.8	33.8[b]	539.1[a]	232.3[a]	33.7[b]	364.0[b]	29.4[a]	5.65
H3	Control	357.1[b]	330.4[b]	46.8[b]	48.2[a]	430.8[b]	188.1[b]	28.9	482.0[a]	26.7[b]	6.49
	Inoculant	365.8[a]	336.0[a]	49.2[a]	43.3[b]	484.0[a]	198.0[a]	28.9	424.0[b]	29.8[a]	6.16
H4	Control	345.9[a]	313.5[a]	47.6[b]	31.3[a]	452.7	205.7[b]	32.5	449.0[b]	32.4[a]	5.96
	Inoculant	337.5[b]	306.2[b]	52.6[a]	13.2[b]	451.1	213.2[a]	33.4	464.0[a]	31.3[b]	5.72
H5	Control	337.2[b]	304.9[b]	44.6[b]	23.4[b]	509.7[a]	240.8[b]	44.3[a]	403.0[b]	32.3[b]	5.35
	Inoculant	345.6[a]	310.3[a]	48.0[a]	26.4[a]	492.4[b]	267.9[a]	41.2[b]	411.0[a]	35.3[a]	5.59

[a, b] Between the values with different letters in the same column, for each individual hybrid, statistically significant differences were found ($P<0.05$)

H1: early maturity hybrid; H2: mid early maturity hybrid; H3: medium maturity hybrid; H4: mid late maturity hybrid; H5: late maturity hybrid; DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; aNDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NFC: non-fiber carbohydrates; NE_L: net energy for lactation

Table 2. The influence of LAB inoculation on the fermentation quality of corn hybrid silages, (g/kg DM)

Hybrid		LA	AA	BA	PA	Total VFA	pH
H1	Control	83.02 [a]	17.83 [b]	0	0	100.85	3.80
	Inoculant	20.70 [b]	23.44 [a]	0	0	44.14	3.70
H2	Control	44.64 [a]	9.46 [b]	7.06 [b]	0.77 [b]	61.93	3.96
	Inoculant	40.63 [b]	13.38 [a]	7.89 [a]	2.57 [a]	64.47	4.01
H3	Control	44.27 [a]	7.28 [b]	5.24 [a]	0.84 [b]	57.63	3.94
	Inoculant	38.71 [b]	13.23 [a]	3.61 [b]	2.76 [a]	58.31	4.15
H4	Control	43.77 [a]	8.33 [b]	3.87	0.64 [b]	56.61	3.91
	Inoculant	41.60 [b]	18.70 [a]	3.50	1.96 [a]	65.76	4.00
H5	Control	14.83 [b]	15.30 [b]	1.07 [b]	1.69 [b]	32.89	3.70
	Inoculant	22.28 [a]	16.35 [a]	2.95 [a]	2.55 [a]	44.13	3.80

[a,b] Between the values with different letters in the same column, for each individual hybrid, statistically significant differences were found (P<0.05)

H1: early maturity hybrid; H2: mid early maturity hybrid; H3: medium maturity hybrid; H4: mid late maturity hybrid; H5: late maturity hybrid; LA: lactic acid; BA: butyric acid; PA: propionic acid

The fermentation profiles differed significantly between inoculated and control silages in all treated hybrids ($P < 0.05$). The participation of the specified acids in the total VFA content of the control treatments was in the range of: 45%-82% LA, 13%-47% AA, 0-11% BA, and 0-5% PA. In control silages of mid-early, medium, and mid-late hybrid silages, LA participated with 72-77% of the total VFAs, while in the inoculated silages the range was 63-66%. All the control treatments, ensiled without LAB addition, except the late maturity hybrid, had a statistically higher presence of LA than in the inoculated silages. In contrast, in all the inoculated silages the content of AA was significantly higher ($P<0.05$) than in the control treatments. The ratio between LA: AA in the H2 and H3 inoculated silages was 3:1, while in H4 it was 2:1. However, the LA: AA ratio was greater in the control treatments of the same hybrid, at 5:1. The presence of BA and PA was not detected in the H1 early maturity experimental silages in both treatments. The highest participation of VFAs, with 11%-12% BA of total, was recorded in the H2 mid-early maturity corn silages. The pH value was within optimal limits for high-quality silages.

The influence of LAB inoculation on the microbiology of corn silage. The influence of LAB inoculation on the epiphytic microflora composition of corn hybrid is presented in Table 3.

In the H3 mid-early and H4 medium maturity hybrid silages, significantly different content of TMN was determined, followed by different impacts of LAB addition. The positive effect of LAB inoculation was observed in H3 silage, with a significantly lower presence of TMN. All the other experimental silages had similar content for both treatments except for H2 where TMN was higher in the inoculated treatment.

The highest content of LAB was recorded in H1 silages, with both treatments, but without statistical differences (as well as in H3 silages) between the control and the inoculated silage. A positive trend of LAB addition can be seen for H2, H4, and H5 silages, due to the significantly higher LAB content in the inoculated silages compared with the controls. The range of yeast and molds present was 2.50 -5.68 log CFU/g DM with an inconsistent effect from the added inoculant.

Table 3. The influence of LAB inoculation on the epiphytic microflora composition of corn hybrid silages, (log CFU/g DM)

Hybrid		TMN	LAB	Y&M
H1	Control	8.37	9.07	3.16 [b]
	Inoculant	8.44	9.04	5.44 [a]
H2	Control	6.92 [b]	6.34 [b]	4.34 [a]
	Inoculant	8.27 [a]	8.34 [a]	2.50 [b]
H3	Control	8.62 [a]	8.36	3.15 [b]
	Inoculant	7.83 [b]	8.31	4.74 [a]
H4	Control	7.76	7.57 [b]	4.94 [b]
	Inoculant	7.57	8.61 [a]	5.50 [a]
H5	Control	7.96	7.95 [b]	5.68 [a]
	Inoculant	7.31	9.11 [a]	4.94 [b]

[a,b] Between the values with different letters in the same column, for each individual hybrid, statistically significant differences were found (P<0.05)

H1: early maturity hybrid; H2: mid early maturity hybrid; H3: medium maturity hybrid; H4: mid late maturity hybrid, H5: late maturity hybrid; TMN: total microorganism number, LAB: lactic acid bacteria; Y&M: yeasts and molds

Discussion

In all experimental silages, the content of DM differed between the treatments. Inoculants of LAB in silages H1, H3, and H5 had positive effects on DM and OM content, which were statistically higher (P<0.05) than in the control silages (without LAB addition) of the same hybrid. A high DM level in silages is usually followed by high starch, nitrogen-free extract, and organic matter content (JURÁČEK et al., 2013) . It is well known that increasing the ash content during ensiling is negatively correlated with OM content. In early maturity hybrid H1, inorganic content (ash) was significantly lower in the inoculated silage than in the silage without LAB addition.

The nutritional value of whole-crop corn silage largely depends on its DM and starch content, as well as the rate of starch degradation (FERRARETTO et al., 2015; BUENO et al., 2020; DONG et al., 2022). Silages provide energy, protein, and digestible fiber for ruminant diets; therefore, the ensiling process has a significant effect on the nutritional value of silage (GRANT and ADESOGAN, 2018). Since loss of DM is an important parameter of poor silage nutrition value, it should be minimized as much as possible (JOHNSON et al., 2002). Also, an important parameter is the maturity of corn during harvesting as this significantly influences the nutrient content (HRISTOV et al., 2020) and consequently influences dry matter intake (DMI), (ANDRAE et al., 2001). The maturity of the plants at harvest affects yield and has an impact on silage quality and nutritional value (NV). However, in practice, field conditions usually dictate harvest and ensiling timing, which influence silage quality (HRISTOV et al., 2020).

The addition of LAB inoculation had a significant influence on the content of aNDF, ADF, and ADL in the experimental silages of early H1 and late H5 maturity hybrids. For these hybrids, higher levels of ADF and lower levels of aNDF and ADL were determined in the inoculated silages, compared with the control treatments. It is not enough to produce a large amount of DM and protein on the farm, it is also necessary to produce forages with high NDF degradability and low ADL content, in order to reduce the rumen fill score and improve the DMI (KAMMES and ALLEN, 2012, BORREANI et al., 2018).

LAB addition significantly affected ADL content in the experimental silages of early, mid, and late maturity hybrids (H1, H2, and H5 respectively). Due to their complex fibrous structures, it is difficult for NDF and ADF to degrade in silage (NAIR et al., 2020). The positive effects of LAB inoculation were determined for the ADF content, which was significantly lower than in silages without its addition. SUN et al. (2022) reported that the levels of NDF and ADF slightly increased after ensiling. The same phenomenon was also described in research by NAIR et al. (2020), and this might be attributed to the reduction of DM content. Fiber plays a fundamentally important role in ruminant livestock production, health and welfare, being an important energy source. It stimulates chewing and salivation, rumination, gut motility and health, buffers ruminal acidosis, regulates feed intake, produces milk fat precursors, and is the structural basis that is vital for the digestion of solid feed particles in the rumen (VYAS and ADESOGAN, 2022). Milk fat and protein concentrations had a strong quadratic relationship with forage NDF intake (as a percentage of body weight), and consequently, when forage NDF intake (as a percentage of body weight), drops below 0.70%, a rapid decline in milk fat and protein concentrations can be expected (JOHNSON et al., 2002). As shown in Table 1, inoculant treatment in the experimental H1 silages caused a significantly lowest aNDF content compared to the control silages.

This trial showed the statistically significant effect of using LAB inoculant on silage NFC content. The determined content of NFC in the experimental silages of early H1 and late-maturity hybrids H5 (both treatments), were inversely linearly proportional to the content of ADL.

Cellulose and hemicellulose, as the main components of fiber, are intrinsically rumen digestible, but partially indigestible due to their close association with lignin and hydroxycinnamic acid in the plant cell wall (VYAS and ADESOGAN, 2022). The experimental H5 silage had the lowest NE_L, due to the significantly higher ADL content. Due to the high lignification of the lignocellulosic content, a large amount of cellulose could not be realized as an energy source.

The chemical characteristics of the hybrids and inoculant composition affect the energy content in the silages. The results of NAIR et al. (2020) showed that the energy content of NEm and NEg in silage inoculated with *Lactobacillus hilgardii* and *Lactobacillus buchneri* were higher compared with the control. In this trial, the same heterofermentative strain was used (*Lactobacillus buchneri*) in the inoculant mixture, which also resulted in significantly higher CP, NFC, and NE_L contents in inoculated H1 silage. This silage also had the lowest ADL value (25.3 g/kg DM) of all the experimental silages. In this trial, the positive effect of using the inoculant on energy content was determined in all H1-H5 silages, but there was a significant influence on NE_L content only in the early maturity hybrid.

The results for the fermentation profile are presented in Table 2. The contents of the VFAs were significantly different ($P<0.05$) between treatments in all the hybrids used. In all control treatments, except the late maturity hybrid, a statistically higher presence of LA was recorded than in the inoculated silages. In contrast, in all the inoculated silages the content of AA was significantly higher ($P<0.05$) than in the control silages. This is likely due to the content of heterofermentative *L. buchneri*. The higher content of AA in silages obtained using LAB can improve the aerobic stability duration of silage. In this trial, a combination of homofermentative strains of *L. plantarum* and *E. faecium* with heterofermentative strains of *L. buchneri* was used. MUCK et al. (2018) highlighted the positive effects of combination inoculants to provide the benefits of the aerobic stability of *L. buchneri* together with the silage fermentation efficiency and animal productivity benefits of homofermentative LAB. In the study by Der BEDROSIAN et al. (2012), the only significant effect of hybrids on LA concentration was determined for the interaction of DM and ensiling duration, but it did not affect AA concentration. The highest content of AA was determined in silage H1 with a value of 23.44 g/kg DM. Similarly, TABACCO et al., 2009 reported that corn maize sorghum silage inoculated with *L.*

buchneri had a higher AA content than the control treatment and the *L. plantarum* treatment. This is likely because *L. buchneri* degrades LA to AA, 1, 2 propanediol, and ethanol under anaerobic conditions (OUDE ELFERINK et al., 2001).

The content of PA was low in all experimental silages, and ranged from 0 to 1.69 g/kg DM. Since there were differences in PA concentrations between treatments, conversion of 1, 2-propanediol to PA was likely under inoculation with primary *L. buchneri*. According to KUNG et al. (2021), a possible explanation for these results is the activity of the heterofermentative metabolism that was affected by air stress, even though the inoculant dominated the fermentation. As for PA, the same explanation could be applied to the content of BA (detected only in traces) for good-quality silages. The experimental silage (H1) with the highest NE_L content, did not contain detectable levels of either BA or PA. Propionic acid is usually undetectable in good silages, or present at very low levels (<0.1%), (KUNG et al., 2018). However, the aerobic stability of silage could be prolonged by the antifungal activity of PA (SUN et al., 2022), but the use of PA as a silage additive has not been sufficiently demonstrated (FEEDAP, 2011), although ruminants have a high tolerance to PA.

The silage pH value did not statistically differ between inoculated and control silages in this experiment, and the obtained values were in the optimal range for excellent quality silage. These findings agree with other studies (WEINBERG et al., 2001; KANG et al., 2009) involving ensiling different hybrids inoculated with *L. buchneri* and *L. casei*, in different temperature regimes. These authors found that silage pH was not significantly affected by inoculation and in all cases low pH values were obtained. Silage can be well preserved only when the pH value, the most basic index evaluating the fermentation quality of silage, is kept approximately between 3.80 and 4.20 (KUNG and SHAVER, 2001).

The influence of the inoculant used for the ensiling of five different hybrids tended to exert the same trends in changes in microbiology profile in all experimental silages. This can be partially explained by the dominant role of epiphytic microflora over the added inoculant. Also, the inoculum concentration recommended by the manufacturer of the inoculant used in these trials was probably not sufficient to overcome epiphytic microflora efficiently. However, the positive effects of inoculation are expressed in the lower amount of TMN in silages compared with ensiling without LAB addition.

The microbial ecology of ensiling is very complex, with the dominant role of epiphytic populations on silage quality. Silage additives can generate microbiomes in a way more conducive to the production of high-quality silage (Mc ALLISTER et al., 2018). Research focusing on the effects of homofermentative *L. plantarum* (also a strain of the LAB inoculant used in our experiment), reported that this LAB increased the relative abundance of heterofermentative *L. buchneri* in silage, while the other dominant species from the epiphytic plant microflora decreased in the silage (XU et al., 2020). These authors highlight that compared with the control (silage fermentation under the epiphytic microflora), the inoculation of *L. plantarum* mainly affected the metabolic pathways related to carbohydrates and nitrogen. It also should be mentioned that when bacteria are involved in the ensiling process, carryover of antimicrobial resistance genes (ARGs) in the silage increases in parallel with the bacterial number (NAGY et al., 2022), and consequently silage as a bulk feed can continuously supply the gastrointestinal tract of animals with ARG-carrying bacteria. This problem can be overwhelmed by LAB inoculate addition due to their probiotic effects. Probiotics and prebiotics are used to enhance animal health and production (AZZAZ et al., 2022), and are considered safe and sustainable alternatives to antibiotics (REUBEN et al., 2022). Previously, AZZAZ et al. (2022) reported that daily supplementation of cows with *E. faecium* in their feed improved *in vitro* nutrient digestibility, milk production, and feed efficiency.

The most apparent positive effect on the count of Y&M in our study was observed in the inoculated mid-early maturity hybrid H2 (P<0.05) silage, where there was 2.50 log CFU/g DM silage. This result was similar to the findings of TABACCO et al. (2009) where corn green mass was inoculated with *L. buchneri*. These results are particularly important because of the secondary metabolites of Y&M and their presence in feed. A recent investigation (SCHWANDT et al., 2022) evaluated the presence and contamination levels of mycotoxins in 72 corn silage samples, and preliminary results showed detectable levels of aflatoxins and type B trichothecenes in 90% of the samples, with zearalenone and fumonisin detected less frequently, in 67 and 24%, respectively. It was thought that LAB has different effects on the fungal communities of different forage species (CHENG et al., 2022).

In the study by NAIR et al. (2020) evaluating only the LAB (*L. buchneri* and *L. hilgardii*) inoculation heterofermentative effect, it was found that yeast and mold counts were lower than in the control silage from ensiling a single hybrid. The data in these trials indicate the positive effects of LAB on silage quality and health safety preservation. However, the structure and abundance of fungal community members (such as *Saccharomyces*, *Cladosporium,* or *Issatchenkia*) during the ensiling process also affected the fermentation quality of silage (LI et al., 2021). In the process of ensiling, epiphytic homofermentative LABs ferment soluble carbohydrates in fresh forage into organic acids, mainly lactic acid, thereby reducing pH and inhibiting harmful microorganisms (DONG et al., 2023), including yeasts and molds.

Similarly, our findings also indicated there is a need for more research about the influence of the same inoculum on ensiling different corn hybrids. The positive effect of LAB inoculation on NE_L concentration is visible when ensiling the early-maturity H1 hybrid. Experimental silages from late-maturing hybrids (H5) would have the weakest nutritive characteristics in animal nutrition. It is an encouraging fact that in practical conditions, the early maturing hybrids are commonly used for effectively ensiling and for mitigating negative effects due to climate change.

When discussing these results, it should be noted that there is a lack of comparative studies because, according to our knowledge, no scientific work has been published on the effects of the same LAB inoculant on different maize hybridization varieties.

Conclusions

This study evaluated the effect of identical LAB inoculants on the ensiling process of five hybrids that differed by FAO group of maturity. This inoculant consists of homofermentative and heterofermentative LAB: *L. plantarum*, *L. buchneri*, and *E. faecium*.

The chemical characteristics of hybrids and inoculant addition affected the energy content in the silages as well as several other factors. In this trial, the effect of the same inoculant on energy content differed among H1-H5 hybrid silages.

Silage pH value was not affected by hybrid type and the low pH values obtained indicated that all silages had adequately fermented under the inoculation of *L. buchneri*, *L. plantarum*, and *E. faecium*. The microbial ecology of ensiling is very complex, emphasizing the role of silage additives in the production of high-quality silage. Compared to the treatment with inoculants containing heterofermentative LAB, the contents of AA were statistically significantly different from the ensiled hybrids.

According to NV parameters in experimental hybrid silages, the effect of treatments and hybrids is statistically significant on the content of CP, NDF, and ADF. Among the evaluated corn hybrids, the H1 early maturity hybrid had significantly improved the silage quality, mainly in terms of high CP content. The H1 treatment had a statistically significant higher energy content of 6.40 MJ/kg than the other silages. The H5 silage had the lowest energy content at 5.59 MJ/kg and the highest ADF content (statistically significant) compared to the other corn hybrid silages. In practice at farms, the earlier maturing hybrids are commonly used for effectively ensiling and to mitigate negative effects due to climate change.

The addition of LAB inoculates influenced the health safety of silages by increasing the content of acetic acid, with the potential to extend the duration of aerobic stability and resistance to degradation.

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Declaration of Competing Interest

All authors declare that they have no conflicts of interest.

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SAŽETAK

Cilj istraživanja bio je utvrditi utjecaj identične inokulantne smjese bakterija mliječne kiseline (LAB) na kvalitetu silaže pet različitih hibrida kukuruza (*Zea mays*). Hibridi (Pioneer Hi-Bred DuPont) razlikovali su se prema FAO skupini zrelosti (od ranog H1, sredine ranog H2, srednjeg H3, srednjeg kraja H4 do kasnog H5). Inokulant se sastojao od mješavine homofermentativnog i heterofermentativnog LAB-a: *Lactobacillus buchneri*, *Lactobacillus plantarum* i *Enterococcus faecium*. Siliranje je obavljeno u laboratorijskim uvjetima, gdje je zelena masa hibrida kukuruza odvojeno silirana sa i bez LAB-a. Proces siliranja trajao je 60 dana. Dodavanje inokulanta imalo je znakovito pozitivan učinak na sadržaj sirovih proteina (CP, $P<0,05$) i ugljikohidrata bez vlakana (NFC, $P<0,05$) u dobivenim silažama. Primjena inokulanta također je smanjila sadržaj lignina (ADL- kiselinski deterdžent lignin, $P<0,05$) u eksperimentalnim silažama. Kemijska svojstva hibrida i sastav inokulanata utjecali su na energetski sadržaj. Međutim, pozitivan utjecaj LAB inokulacije na sadržaj NEL (neto energija za laktaciju) određen je samo u hibridu H1 rane zrelosti ($P<0,05$). Ova silaža imala je najveću vrijednost za koncentraciju NEL-a, s najnižim sadržajem aNDF-a (neutralna vlakna deterdženta) i ADL-om. Dodavanje LAB inokulanta utjecalo je na zdravstvenu sigurnost silaže povećanjem sadržaja octene kiseline, s potencijalom produljenja trajanja aerobne stabilnosti i otpornosti na razgradnju mikroba.

Ključne riječi: hibridi kukuruza; inokulanti; bakterije mliječne kiseline; kvaliteta silaže; sigurnost zdravlja životinja