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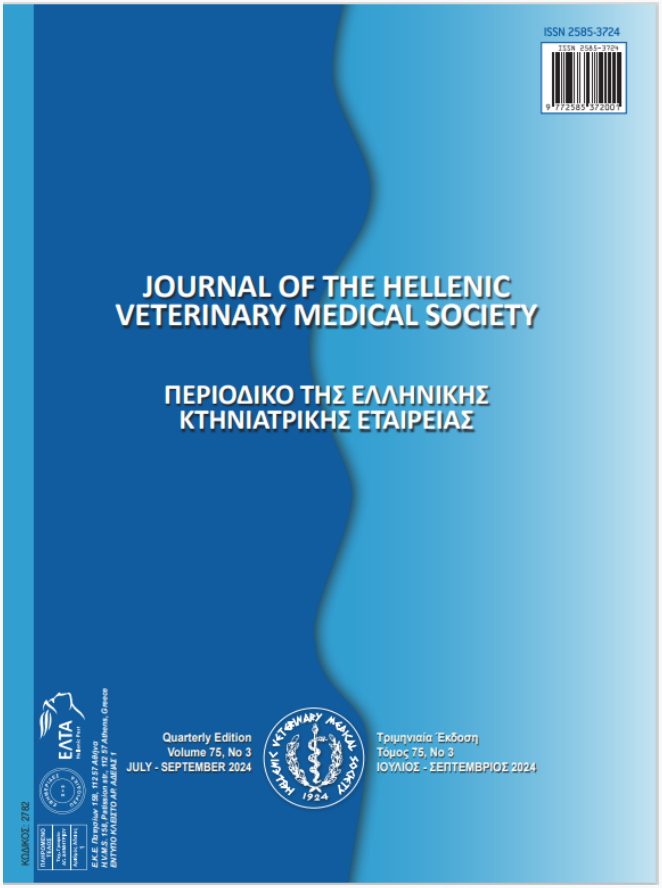

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Research article
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The influence of the homofermentative lactic acid bacteria and enzymes on the nutritional value, health safety, and aerobic deterioration of the different maize hybrid silages

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ABSTRACT: The research aimed to determine the impact of identicinoculants of lactic acid bacteria (LAB) with enzymes (ENZ)on the silage quality of 5 different corn hybrids (*Zea mays*). Hybrids (Pioneer Hi-Bred DuPont) differed by FAO maturity group (from early H1, mid-early H2, medium H3, mid-late H4 to late H5). Inoculant LAB consisted of a mixture of homofermentative bacteria:*Lactobacillus plantarum*, *Enterococcus faecium*,*Pediococcusacidilactici*, *Lactobacillus salivarius*, and ENZ: cellulase, α- amylase, hemicellulase, xylanase. Ensiling was doneat the laboratory conditions, where the green mass of corn hybrids has been inoculated with LAB and ENZ were added, before ensiling. The process of ensiling lasted 60 days. The addition of LAB inoculants with ENZ is frequently used to speed up the ensiling process, prevent the growth of harmful microorganisms, protect the aerobic stability of silage, and improve the silage quality of different crops. The phase of aerobic degradation of silage begins immediately after exposure to silage to air. This stage is inevitable during feeding with silage and takes place in all silages, regardless of quality.
Thesame pattern of reaction on identic LAB and EFEs addition was not found in silages ensiled from different hybrids. Among the evaluated corn hybrids, the additive to H1 early maturity hybrid had significantly improved the silage quality, and prolonged the time of aerobic stability, mainly due to the significantly highest CP, lowest ADL, and highest LA content. Thechemical composition, fermentation, and microbiology profiles of silage significantly influence CO_2 production in a test of aerobic stability (AS), and this is a causal relationship of aerobic stability duration. The results of this trial showed that the lower butyric acid, total microorganisms number, count of yeasts and mold, pH, and higher LAB, lactic acid, and acetic acidlevels ($P<0.05$), are accompanied by the production of lower CO_2 ($P<0.05$).The current research and previous studies suggest that nutritional value, health safety, and aerobic deterioration of the different maize hybrid silages are under the influence of epiphytic microflora of ensiled plants, used LAB inoculum, and EFEs addition in the appropriate amount, and the type of hybrid ensiled as well.

Keywords: Cornhybrids ensiling, aerobic stability,enzymes, andlactic acid bacteria inoculants,animal's health safety

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INTRODUCTION

Ensiling is a microbial process used to conserve fresh feed for animal nutritionand biorefinery (Okoye *et al.,* 2023). In those processes LABshave a key position between silage microorganisms, and the effects of exogenous lactic acid bacteria on silage quality have been widely studied, (Wang *et al.,* 2021). AddingLAB inoculantswith ENZ is frequently used to speed up the ensiling process, prevent the growth of harmful microorganisms, protect the aerobic stability of silage,and improve the silage quality of different crops. Furthermore, LAB belongs to the microorganisms that have the GRAS (generally recognized as safe) status, (Fabiszewska*et al*., 2019).

Corn hybrids differ in their nutritional value. Choosing the right corn hybrid has implications forsilage's nutritional value and milk production. Modern researchis focused on the improvement of ensiled forage quality with newly developed LAB inoculants due to inconsistency in silage quality which could belikely because of the lack of information on gene expression and molecular mechanisms of the microbiota involved in silage production, (Okoye *et al*., 2023). The additives of ENZ with LAB inoculants do not always result in successful regulation of silage fermentation because the adaptability, establishment, and development of LAB in forages during ensiling are partially unknown, (Cheng *et al*., 2022; Kobayashi *et al.,* 2010). One of the main reasons for not establishing sufficient domination of LAB inoculants is the influence of epiphytic microflora in the ensiling plants, (Ivetić, 2017).

To efficiently maintain the nutritional value ofgreen mass and reduce the loss of nutrients during aerobic degradation of silage, biotech companies have produced microbial inoculants. The number of LAB colonies in such products is usually about 1×10^{11}CFUg^{-1}and only 10 g of such additives need to be added for ensiling 10 tons of green plant mass. According to Cai *et al.* (1999), preservation depends on the ability of LAB to produce sufficient conservative acids to stop the growth and other activities of undesirable microorganisms (MO) under anaerobic conditions. The microbialcomposition ofthe inoculants is different. Inoculants maycontain only homofermentative or heterofermentative LAB, or they can consist of a combination of both, with or without the addition of an enzyme. The main differences among them arethe 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, (Woolford, 1984).

Silages obtained from different corn hybrids, mostly differ in net energy content. Primary, differences are in dry matter (DM) and crude protein (CP), (Cherney *et al*., 2004; Cherney *et al.,* 2007), as well as fiber content. Juráček*et al.* (2001) conserved four early maize hybrids without additives and found that the highest starch, nitrogen-free extract, and organic matter content in silages with the highest DM content. Danner *et al*. (2003) reported that inoculation of corn herbage using different homo-and heterofermentative LAB affectedthe amount of LA produced and significant amounts of 1, 2-propanediol were detected in silages inoculated with *L. buchneri*.

Huyen *et al*. (2020), have examined five different LAB strains as ensiling agents in the reduction of methane emissions in dairy cows. They concluded that in vitro CH_4 production was lower for silages inoculated with homofermentative *L. plantarum*.

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 Davies, 2012). Mycotoxins are secondary fungal metabolites that have been detected in a variety of feed ingredients and can affect animal health and productivity. Nevertheless, LAB is capable of producing acetate, proteinaceous compounds, peptides, and hydrogen peroxide which exert antifungal activity, (Kleinschmit *et al.,* 2005). This ability of LAB to release antifungal substances varies among strains, (Schnürer and Magnusson, 2005). Paradhipta*et al*. (2020) found that *L. brevis* and *L. buchneri*have high antifungal and carboxylesterase activities in silage. The mixture of both strains improves corn silage quality by increasing nutrient digestibility and reducing yeast contamination.

The addition of exogenous fibrolytic enzymes (EFEs) and length of storage can affect the quality of maize silage, (Salvo *et al*., 2022). The inclusion

of enzyme additives to forage aims to break down plant cell walls at ensiling, which can improve silage fermentation once provides sugars for homofermentative lactic acid bacteria, (Gandra *et al.,*2021). Zahiroddini*et al*. (2004) reported that a combination of LAB inoculants and enzymes improved the feed efficiency of cattle-fed barley silage.The addition of xylanase and cellulase, promoted dry matter degradation and increased energy availability, with increased *in vitro* gas production, (Martinez *et al.,* 2020). Applying cellulase and xylanase as the EFEs enhances the degradation of carbohydrates and cell walls in the phase of ruminal preincubation, which is consistent with the decrease in NDF (Martinez *et al.,* 2020).Enzymes may also increase the digestibility of cell walls, enhancing the nutritive value of silage (Muck *et al.,* 2018). Chauhan *et al.* (2023), reported that the combined effect of LAB (*L. plantarum* and *L.fermentum*) with enzymes(cellulase and xylanase) can reduce the ensiling period and improve silage quality. The fungus *Humicola grisea* is one of the enzyme producers (cellulase, β-glucosidase, and xylanase) that can be important in ruminant feed, (Cysneiros*et al.,* 2013).

As a main component of the rations, silages are characterized by the phase of aerobic degradation (deterioration) of silage that begins immediately after exposure to silage to air. Aerobic degradation of NV occurs in all silages that are open and exposed to air. Air (oxygen) is the main cause of deterioration in silage quality, because it allows unwanted chemical and microbiological activities to take place, which leads to aerobic silage degradation, (Woolford, 1990). During the feeding of animals, this stage is inevitable on the farms and takes place in all silages, regardless of quality. By Elferink*et al.* (1999) aerobic degradation consists of two stages. Thefirst represents the beginning of deterioration due to the degradation of protective organic acids. Withincreasing the pH value, the second stage of spoilage begins, in which the temperature and rate of development of microorganisms increase.The degree of degradation and duration of aerobic stability of the silage upon opening the silo depends on its quality.Visible signs of spoilage are heating of the silage surface and mold formation, (Kung *et al.,* 2003). According to Woolford (1984), if silage is of poor quality with a high pH value, high content of butyric acid and ammonia, and low content of lactic and acetic acid, it will be very stable when exposed to air, because BA and ammonia act as very effective preservatives.Aerobic degradation is more common in well-preserved silage, so improvements in ensiling technology are geared towards this problem since the role of aerobic microorganisms (MO) has been defined as harmful for maintaining the aerobic stability of silage after silo opening foranimal health.

The objective of this research was to determine the impact of the addition of the same LAB inoculant and EFEs on the silage quality of five different corn hybrids H1-H5, (*Zea mays*). To the best of our knowledge, no reports have shown the influence of the identic inoculate mixture of LAB with ENZ (enzymes) on the corn hybrids. The research aimed to determine the impact of the same inoculant LAB with ENZ (enzymes)on the silage quality of 5 different corn hybrids. Hybrids differed by FAO maturity group fromearly, mid-early, medium, mid-late, and late.We hypothesized that the identic LAB inoculant and EFEs mixture may improve the quality and health safety of silages made from different maize hybrids. Also, we hypothesize that the duration of aerobic stability of the nutritive value of silages isprolonged under the influence of LAB and EFEs addition.

MATERIAL AND METHODS

Ensiling

The investigation of the effect of using the mixture of LAB inoculant andENZon the nutritional value of silages was conducted on 5 different corn hybrids (Pioneer Hi-Bred DuPont). Hybrids differed by FAO maturity group (from early to late). Hybrids 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 specification of the LABinoculant'smanufacturer, the average level of LAB in dried inoculant was 1×10^{11}CFUg^{-1} and the recommended rate for use was 1×10^{5} CFUg^{-1} of green mass, with the dried inoculant dissolved in water. However, to allow the dominance of the inoculants over the epiphytic microflora of the plant, the inoculant was added at a higher rate of 1×10^{6}LAB CFUg^{-1}green corn mass, as recommended by Muck et al. (2007). The composition of the inoculant LAB (1×10^{11}CFUg^{-1}) used in the trial was: *Lactobacillus plantarum*, *Enterococcus*

faecium,*Pediococcusacidilactici*, *Lactobacillus salivarius*, with added enzymes: cellulase(EC 3.2.1.4), α-amylase, hemicellulase, xylanase(EC 3.2.1.8).

Before ensiling, in the green mass of each corn hybridwasadd inoculant of LAB with ENZ while the 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 released are used for ensiling. Freshly cut corn green mass is 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 room temperature (~22ºC). For each corn hybrid, five laboratory silos with inoculated (mixture of LAB and ENZ) ensiled plant material were prepared, while there was the same number of silos prepared without using inoculation.The process of ensiling lasted 60 days.

Chemical analyses

Silage's chemical and microbiology composition was analyzed in the Laboratory of Animal Nutrition at the Faculty of Agriculture, University of Belgrade. Immediately after the silo mass opening for the analyses, samples of 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 Official Analytical Chemists (AOAC, 2023).

The dry matter (DM) content was determined by drying at 80°C in an oven for 20h, (method 967.03). The ash content was determined after combusting samples at 600°C for 3h, (method 942.05). The organic matter content was calculated as OM, % = DM, % - Ash, %. The CP content was determined by the Kjeldahl method (method 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 the Soxhlet apparatus (method 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 ash content. 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 acid detergent lignin (ADL) content. Determining the contents of volatile fatty acids (VFA): lactic, acetic, butyric, and propionic acid, was done using liquid chromatography, HPLC with IR detection, using column 100×7.7mm 8ºm HyperREZ XP Organic, Thermo Fisher Scientific Inc., USA.

Microbiological analyses

Microbiological analyses of experimental silages were performed immediately after opening the silo, including parameters: total microorganisms' number (TMN), LAB colony count, as well as molds and yeasts count. The specific plates were used for microorganisms' determination: for microorganism's determination were used plates for TMN - Nutrient agar, LAB- MRS agar, and Molds and Yeasts -SDA (Sabour and Dextrose agar). For the CFU numeration, it was applied the Miles and Misra (1938) method. An anaerobic container (Becton Dickinson) was used to provide anaerobic conditions for the growth and development of LAB, and a BD GasPak EZ system for anaerobic conditions (manufacturerBecton Dickinson).

Aerobic stability test

After opening the experimental silos, a laboratory analysis of the aerobic stability of the silages of different hybrids of corn was performed method byAshbell*et al*. (1991)for the 48hduration. The method is based on the determination of the producedCO_2gas during aerobic silage exposure. This gas is produced as a result of aerobic activity that takes place in silages exposed to air.

For the AS test, the bottles are made from polyethylene terephthalate, photograph 1 and Figure 1. The pooled silage sample was loosely packed (200-250 gr on a wet basis), in the upper part of the bottles, and 100 ml of KOH (25%) solution was placed in the lower part of the unit to absorb the produced as a result of aerobic activity that may take place in silage samples exposed to the air (Ergin and Gumus 2020;Weinberg *et al*., 2011; Zhang *et al.,* 2009;Khanal, 2009).

$CO_2$is 1.5 times denser than air and falls to the bottom of the upper silage-patterned bottle, then absorbed in the lower unit with KOH. To determine the amount of CO_2 produced as a result of aerobic exposure, 10 ml of KOH is taken from the lower aerobic stability unit and diluted with 90 ml of *a. dest*.

This sample is titrated with 1N HCl. The volume of 1N HCl required to lower the pH value from 8.1

to 3.6 represents the value used in a given formula to calculate the amount of CO_2 produced per calculation ($CO_2 gkg^{-1}$ DM).

The chemical reaction during the aerobic exposure during the aerobic stability test is:

$KOH^* + CO_2^{**} \rightarrow K_2CO_3 + H_2O$

*In the lower bottle of the AS testing unit

** Produced as a result of aerobic exposure

Reactions during titration with HCL are:

$K_2CO_3 + HCl \rightarrow KHCO_3 + KCl$ (until pH 8.1)

$KHCO_3 + HCl \rightarrow CO_2 + H_2O + KCl$ (until pH 3.6)

The amount of HCL (1N) consumed during titration to displace CO_2 was used in the CO_2 determination formula (gr CO_2 /kg DM):

$CO_2 = 0.044\ T * V / (A * W * DM)$

0.044 - The weight of one CO_2 equivalent (kg)

T - Volume of HCl (1N) used in titration (ml)

V - Total volume 25% KOH (usually 100 ml)

A - Volume of KOH sampled for analysis (usually 10 ml)

W -Mass of fresh silage filled in the bottle (kg)

DM - dry matter

Energy value

The energy values of silages were calculated according to Tylutki *etal*. (2008). All obtained data were statistically processed using Statistics 6.0 Software, (Stat Soft Inc. 2003). As part of statistical processing of the obtained data, was performed Student`s t-test using a standard procedure.

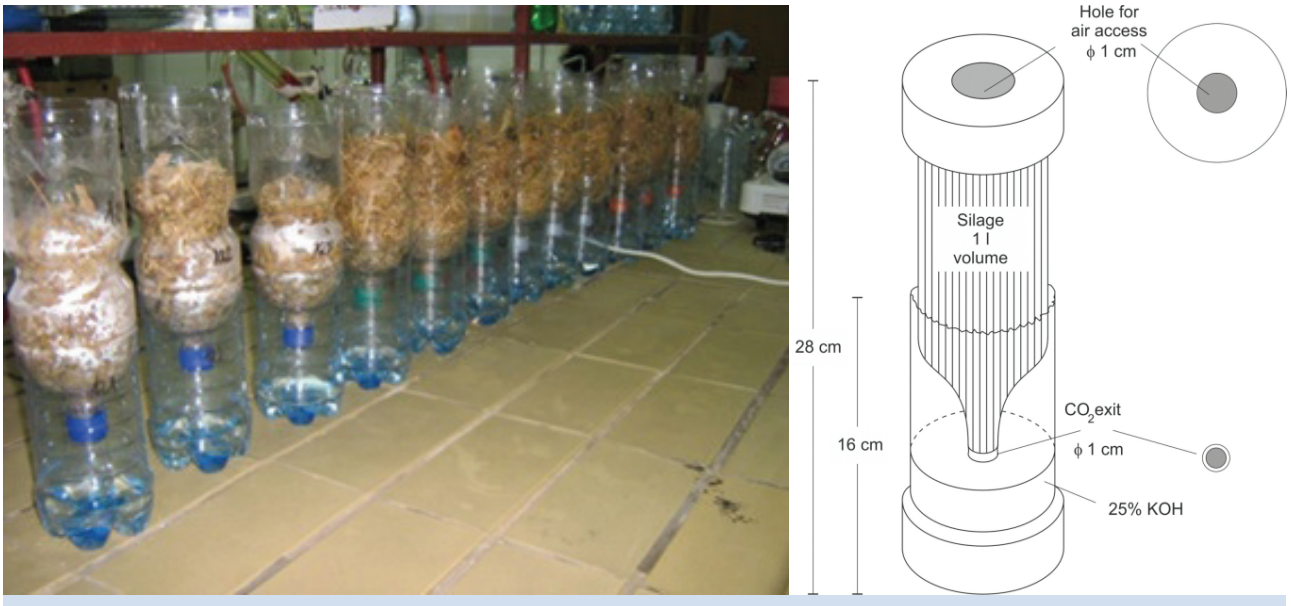
RESULTS

The influence of LAB inoculation and EFEs addition on nutritive parameters of corn silage

The effects of LAB inoculants and enzymes on the chemical composition and nutritive value of five different hybrid silages are presented in Table 1.

The statistically highest DM content at early corn maize H1 hybrid had an inoculated silage at 0 and 48h after silo opening, compared with the control treatments. The same trend was determined for OM content. The mid-early $H_2$and medium-maturity H3 corn hybrids had the same trend of statistical difference between DM comparing 0 with 48h in the control treatment. On the contrary, the inoculated silages had a significantly higher DM content in 0 compared with 48h aerobic exposure.

The late hybrid H5 expresses significantly higher the content of DM and OM in 0 days whichon the contrary, decreases after 48h of air exposure from opening in the inoculated silages compared with the control one. Also, the content of DM was significantly decreased after 48h ($p<0.05$) in inoculated treat-



Photograph 1 and Figure 1. System for silage aerobic stability testing

ments. The prevention of OM losses was statistically influenced by inoculated treatments of early maturity hybrid H1 in 0 and 48h compared with control treatment, as well as comparing with inoculated treatment in the same duration itself.

In all experimental silages, it was not determined statistically important differencesin CP content, between the treatments (inoculated versus control) and the duration of air exposure (0 days at silage opening versus 48h of air exposure). The CP content was aerobic stable in allsilages.

The medium H2 and mid-lateH3 hybrids had statistically the biggest oscillations between EE content, from 48.2 gkg^{-1} in the control treatment to 17.3 gkg^{-1} after 48h of silo opening. In all experimental silages, it was observed a significantly similar trend of EE decreasing in control and inoculated treatments expressed after 48h.

Early H1, medium H3, and mid-lateH4 hybrid had the same changes in NDF content during aerobic exposure. These changes are expressed in the significant decreases of NDF contentcompared with 0 days of silo opening negative effects of aerobic exposure. The positive effects of inoculation on aerobic stability areexpressed at H2 mid-early and late H5 maturity hybrids in 48h with increasing the NDF content, compared to the content after silo opening.

Inoculated treated silages of H2, and H5 had significantly higher ADF content compared with control treatment in 0 days. The content of ADF statistically increased at all inoculated silages after 48h aerobic exposure. The control-treated silages H2, H3, and H5 silages had statistically increased content of ADF in 48h after aerobic exposure, compared with 0 days.

Table 1. Influence of LAB inoculation and enzymes on the chemical composition and nutritive value of corn hybrid silages, (gkg^{-1} DM)

		H1		H2		H3		H4		H5	
		0	48	0	48	0	48	0	48	0	48
DM	C	324.1^{bB}	366.7^{bA}	361.4^{B}	369.7^{aA}	357.1^{aB}	367.2^{aA}	345.9^{A}	328.9^{bB}	337.2^{bB}	340.1^{aA}
	I	371.1^{aB}	377.7^{aA}	362.1^{A}	357.8^{bB}	351.4^{bA}	333.4^{bB}	346.2^{A}	338.5^{aB}	343.8^{aA}	336.0^{bB}
OM	C	295.0^{bB}	338.5^{bA}	335.9^{aB}	340.9^{aA}	330.4^{aB}	339.1^{aA}	313.5^{aA}	295.3^{bB}	304.9^{b}	305.5
	I	345.5^{aB}	350.1^{aA}	332.5^{bA}	327.4^{bB}	321.4^{bB}	302.4^{bA}	312.8^{b}	305.5^{a}	310.9^{aA}	301.0^{B}
CP	C	84.0	86.6	46.8	47.5	46.8	48.9	47.6	52.4	44.6	45.2
	I	88.7	88.0	51.0	42.5	50.9	46.4	51.3	54.5	43.6	45.2
EE	C	25.1^{A}	18.2^{bB}	34.4^{a}	31.4^{a}	48.2^{aA}	17.3^{bB}	31.3^{bA}	21.3^{B}	23.4^{b}	20.3^{b}
	I	26.5^{A}	20.1^{aB}	22.3^{bA}	17.9^{bB}	31.9^{bA}	23.8^{aB}	35.0^{bA}	20.0^{B}	30.8^{aA}	21.4^{aB}
NDF	C	485.5^{aA}	451.3^{aB}	451.0^{bA}	448.6^{aB}	430.8^{a}	434.0^{a}	452.7^{bA}	449.2^{bB}	509.7^{bB}	512.4^{bA}
	I	447.4^{bA}	401.5^{bB}	533.0^{aB}	558.3^{bA}	444.0^{bB}	392.5^{bA}	479.4^{aA}	468.1^{aB}	522.5^{aB}	595.0^{aA}
ADF	C	204.9^{aA}	184.9^{aB}	224.0^{bB}	248.9^{aA}	188.1^{bB}	197.7^{bA}	205.7^{A}	198.6^{bB}	240.8^{bB}	264.9^{bA}
	I	195.2^{bB}	219.4^{bA}	234.3^{aB}	269.8^{bA}	198.9^{aB}	224.1^{aA}	207.7^{B}	240.6^{aA}	254.6^{BB}	309.2^{aA}
ADL	C	28.9^{a}	29.1^{a}	35.3^{b}	40.6^{a}	28.9	32.4	32.5^{bB}	33.7^{bA}	44.3	44.7^{b}
	I	20.8^{b}	22.3^{b}	37.5^{a}	38.3^{b}	30.6	34.3	34.3^{aB}	51.3^{aA}	41.4	52.3^{a}
NFC	C	389.0^{bB}	429.0^{bA}	434.0^{aB}	457.0^{aA}	482.0^{aB}	502.0^{aA}	449.0^{aB}	457.0^{aA}	403.0^{a}	401.0^{a}
	I	425.0^{aB}	477.0^{aA}	377.0^{bA}	364.0^{bB}	473.0^{bB}	536.0^{bA}	414.0^{bA}	437.0^{bB}	383.0^{bA}	316.0^{bB}
HC	C	280.6^{aA}	266.4^{aB}	227.0^{bA}	199.7^{bB}	242.7^{B}	236.3^{aA}	247.0^{bB}	250.6^{aA}	268.9	247.5^{b}
	I	252.2^{bA}	182.1^{bB}	377.0^{aA}	288.5^{aB}	245.1^{B}	168.4^{bA}	271.7^{aA}	227.5^{bB}	267.9	285.8^{a}
CEL	C	176.0^{A}	155.8^{B}	188.7^{B}	208.3^{bA}	159.5^{bB}	165.3^{bA}	173.2^{A}	164.9^{bB}	196.5^{bB}	220.2^{bA}
	I	174.4^{A}	154.3^{B}	198.6^{B}	231.5^{aA}	168.3^{aB}	189.8^{aA}	173.4^{B}	189.3^{aA}	213.2^{aB}	259.9^{aA}
Ash	C	29.1	28.2	25.5	28.8	26.7	28.1	32.4	33.6	32.3	34.6
	I	25.6	27.6	29.6	30.4	30.0	31.0	33.4	33.0	33.0	35.0
NEL	C	5.84	5.97	6.06^{a}	5.92^{a}	6.49	5.96	5.96	5.87	5.35	5.30^{a}
	I	6.25	6.31	5.33^{b}	5.22^{b}	6.05	6.18	5.95	5.52	5.43^{A}	4.79^{bB}

[a, b,] Between the values with different letters **in the same column, for each parameter, and each hybrid**, statistically significant differences were found, ($p < 0.05$); [A,B] Between the values with different letters **in the same line, for each parameter and each hybrid**, statistically significant differences were found, ($p < 0.05$); H1: early maturity hybrid. H2: mid-earlymaturity hybrid. H3: medium maturity hybrid. H4: mid-late hybrid. H5: late hybrid. DM: dry matter. OM: organic matter. CP: crude protein. EE: ether extract. NDF: neutral detergent fiber. ADF: acid detergent fiber. ADL: acid detergent lignin. NFC: nonfiber carbohydrates. NE_L: net energy for lactation

Table 2. Influence of LAB inoculation on the fermentation quality of corn hybrid silages, (g kg^{-1} DM)

		H1		H2		H3		H4		H5	
		0	48	0	48	0	48	0	48	0	48
LA	C	23.02^{bB}	32.72^{bA}	44.64^{aA}	29.70^{aB}	44.27^{aA}	19.33^{bB}	43.77^{bA}	14.87^{bB}	14.83^{aA}	10.32^{bB}
	I	25.33^{aB}	34.15^{aA}	25.19^{bA}	11.93^{B}	25.95^{bA}	17.49^{aB}	5.29^{aB}	27.06^{aA}	13.58^{b}	13.42^{a}
AA	C	17.83^{aA}	9.82^{B}	9.46^{bB}	11.39^{aA}	7.28^{bB}	9.99^{bA}	8.33^{bB}	11.19^{bA}	15.30^{aB}	25.37^{aA}
	I	14.28^{bA}	9.53^{B}	10.16^{aA}	3.52^{bB}	10.47^{aB}	22.08^{aA}	17.76^{aB}	20.21^{aA}	6.89^{bB}	17.49^{bA}
BA	C	0	0	7.06^{a}	6.82^{b}	0.84^{Bb}	3.05^{A}	0.64^{B}	2.22^{bA}	1.69^{B}	4.53^{bA}
	I	0	1.32^{aA}	4.56^{bB}	10.11^{aA}	4.69^{aA}	3.03^{B}	1.15^{B}	5.23^{aA}	1.42^{B}	5.27^{aA}
PA	C	0	0	0.77	1.05^{b}	5.24^{bA}	0.33^{bB}	3.87^{bA}	2.25^{aB}	1.07^{a}	1.73^{b}
	I	2.42^{aB}	6.35^{aA}	1.10^{B}	4.56^{aA}	1.14^{aB}	17.40^{aA}	0.84^{a}	1.09^{b}	0.41^{bB}	13.42^{bA}
pH	C	3.80	3.80	3.96	4.04^{b}	3.94^{A}	6.88^{B}	3.91^{B}	6.81^{aA}	3.70	3.82
	I	4.15	3.89	4.07^{B}	5.07^{aA}	3.99^{A}	6.80^{B}	3.80	4.13^{b}	3.72	3.37

$^{a, b,}$ Between the values with different letters **in the same column, for each parameter and each hybrid**, statistically significant differences were found, ($p < 0.05$); ^{A,B} Between the values with different letters **in the same line, for each parameter and each 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 hybrid. H5: late hybrid. LA: lactic acid. BA: butyric acid. PA: propionic acid.

Table 3. Influence of LAB inoculation on the microbiology composition of corn hybrid silages, (log CFU/g DM) g kg^{-1} DM)

		H1		H2		H3		H4		H5	
		0	48	0	48	0	48	0	48	0	48
TMN	C	8.37^{b}	8.64	6.92^{bB}	8.54^{aA}	8.62^{a}	8.57	7.76^{B}	8.33^{A}	7.96	8.17
	I	9.86^{aA}	8.69^{B}	7.44^{a}	7.45^{b}	6.93^{bB}	8.91^{A}	8.07^{B}	8.55^{A}	7.72^{B}	8.45^{A}
LAB	C	9.07^{Ab}	8.04^{B}	6.34	6.43^{b}	8.36^{aA}	7.48^{B}	7.57^{A}	6.96^{bB}	7.95^{b}	8.07^{b}
	I	9.58^{Aa}	8.33^{B}	6.74	7.29^{a}	7.31^{b}	7.62	7.76	7.47^{a}	9.46^{a}	9.45^{a}
Y&M	C	3.16^{B}	4.44^{bA}	4.34^{a}	4.39^{b}	3.15^{bB}	7.13^{A}	4.94^{aA}	7.18^{aB}	5.68^{aB}	6.77^{aA}
	I	3.43^{B}	5.72^{aA}	3.28^{bB}	5.29^{aA}	4.15^{aB}	7.95^{A}	3.64^{bB}	4.47^{bA}	3.46^{B}	3.65^{b}

$^{a, b,}$ Between the values with different letters **in the same column, for each parameter, and each hybrid**, statistically significant differences were found, ($p < 0.05$); ^{A,B} Between the values with different letters **in the same line, for each parameter and each 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 hybrid. H5: late hybrid. TMN: total microbiology number. LAB: lactic acid bacteria. Y&M: yeasts and molds

Determination of ADL showed that itsvalue did not significantly change at H3 medium maturity silages for both treatments and the time of aerobic exposure. The statistically highest content of ADL had medium H4and late maturity H5 hybrid silage with inoculated treatment after 48h of aerobic exposure with a range of 51.3-52.3 gkg^{-1}DM value.

Theearly H1maturity hybridsilage had statistically increased content of NFC at inoculated treatments, at silo opening, and after 48h of air exposure compared with control treatment. In all other hybrids silages, the content of NFC was significantly lower at inoculated treatments, at the silo opening, compared with the control treatments of the same hybrid.For the point of aerobic stability, the content of NFC was significantly increased after 48h air exposure in all silages with control treatment, as well as in the H1, H3, and H4inoculated hybrids silages.

In the early H1 maturity hybridsilages, it was determined significantly lowerthe content of HC atinoculated treated silages compared with control ones, at the time of silo opening and after 48h of air exposure, which was an opposite trend for the H2 mid-early hybrid silages. The content of HC was significantly decreased at medium H3 andH4 mid-late maturity hybrid after 48h of aerobic exposure to the inoculated treatment compared with the control silages and with the content at the moment of silo opening.

In the silages of early H1 maturity hybrid, was level of CELsignificantlydecreased after 48h of air exposure in the inoculated treatment. The opposite trend was present in the H2, H3, and H5 silages, where the CEL content increased after 48h in the AS test in both treatments. The positive influence of inoculation and enzyme addition was followed by statistically higher CEL content in H2 and H4 in 48h compared to the 0h and in the late H5 maturity hybrid for both du-

ration in AS test and both treatments. However, all hybridcontrolexperimental silages (without LAB and EFEs),hadstatistically lower CEL content compared with inoculated treatments.

The ash content did not significantly vary in all experimental silages in both treatments and during the AS test, and primary changes in OM matter were due to the differences in DM content.

Experimental silages H1, H3, and H4 were aerobic stable, without changes of NE_L during the aerobic exposure. In the medium H2 and late H5 maturity hybrid silages treated with LAB and EFEsthere was a determinate negative trend of significantly decreasing the NE_L content in the inoculated treatment compared with the control one. Those changes in treated silages werecorrelated with less OM and EE content, followed by the highest NDF and ADL content.

The influence of LAB inoculation and EFEs addition on the fermentation quality of corn silage

Table 2 presents the influence of LAB inoculation EFEs addition on the fermentation quality of corn hybrid silages.

The content of VFAs varied between hybrids and treatments, table 2. Only H1 and H4 control silages at 48h had significantly higher LA content than in 0 days. Positive influence on aerobic stability and LA content can be noticeable in H4 inoculated silage where the 5.39 gkg^{-1} DM was increased fivefold from 0days to 48h. But, the highest content of LA ranged from 43.77 - 44.64 g kg^{-1}DM was present in the H2, H3, and H4 control treatments at 0 days, immediately after silo opening. In the late hybrid,there was nostatistical difference between 0 and 48h after aerobic exposure of silages. On average, the content of LA after 48h of silo opening was double reduced.

The scope of AA was significantly different between control and inoculated treatments after silage opening. In the H1 silages, both treatments had a smaller content whereas inoculated had smaller AA participation after 48h of aerobic exposure and it was not recorded significant difference between these treatments. After the inoculated silages H2, H3, and H4 opened it was determineda significant increasein AA content compared with the control treatment. This positive influence of inoculum and enzyme addition was observed after 48h of air exposure in H3 and H4 silages, the content was 20.21gkg^{-1} DM which is double more than in control silages during the AS test. The highest content of AA of 25.37 g kg^{-1} DM was found in H5 control silages indicating the presence of other parameters influence such as hybrid type and epiphytic microflora on the corn plants.

Observing the changes of BA, PA, and pH values it was found the aerobic instability of those parameters in H2, H3, and H4 silages. After the H2 silage opening the control silage had statistically higher content of BA than the inoculated treatments. But, after 48h of aerobic exposure, silages with both treatments, had significantly increased content of BA. The presence of PA varied in the range of 0-4.56 g kg^{-1}DM in H1, H2, and H4 silages. At H3 medium maturity corn silage the inoculated treatment after 48h had a significantly higher PA content of 17.40 gkg^{-1} DM, followed by pH 6.88. The pH value in H1 and H5 silages did not change after air exposure and it was not detected differences between controls and inoculated treatments.

The influence of LAB inoculation and EFEs addition on the microbiology of corn silage

The influence of LAB inoculation and EFEs addition on the microbiology composition of corn hybrid silages is represented in Table 3.

The obtained results are heterogenic and differ by the hybrid type ensiled. The influence of the homofermentative inoculant used for the ensiling of five different hybrids tended to exert different trends in changes in microbiology profile in all experimental silages. The statistically higher levels of LAB ($p<0.005$) were present in early H1 and mature H5 silages, ensiled with LAB and EFEs addition over the control silages (for each hybrid *per se*) at the moment of silo openings, as well as after 48 hours of aerobic exposure (H5). However, the positive effects of inoculation are expressed in the lower amount of TMN in silages compared with the ensiling without LAB addition. In the H2 mid-late corn hybrid, the treated silages with LAB and EFEs had a content of TMN statistically lower ($p<0.005$) than in control ones (without additive) and the moment of silage opening, and after 48 hours of aerobic exposure, indicating on the additive positive influence. The LAB and EFEs addition had an impact on yeasts and molds (Y&M) statistically lower count of 3.65 gkg^{-1} DM (that value was the lowest for all experimental silages) in the late H5 maturity silages compared with control treatment after 48hof aerobic exposure.

Aerobic stability tests

Table 4 presents the effects of treatment on change in temperature, CO_2 content, and pH of different hybrid silages (H1-H5) during the 48h aerobic stability test.

The silages of the early hybridwere aerobic stabile during 48h of aerobic exposure, without the significant production of CO_2, changes of pH, and temperature values. The mid-early hybrid, after 48h had higher CO_2 production (12.27-20.26 gkg^{-1} DM) indicating the early onset of the aerobic deterioration. This production was not followed by changes in pH and T values. Thehighest level of CO_2 produced was in the medium corn hybrid H3 experimental silages (29.03-48.94 gkg^{-1} DM) and the highest pH value of 6.80 compared with experimental silages of other hybrids.Insilages of H1, H2, and H4, the level of produced CO_2 was doubled in inoculated and enzymatic silages, compared with the control ones.

In this trial, we suggest according to the obtained results, thatthe maximum acceptable level of CO_2 production be 15g kg^{-1} DMfor 48h of AS of corn silages in the applied test of Ashbell*et al*. (1991).The suggested maximum acceptable level of CO_2 production in 48hof aerobic exposure during the test of AS is separate in this trial on aerobic stable H1 and H5 from unstable H2, H3, and H4 hybrids.

The corn silages of H1 and H5 are below this limit for control and treated silages. At the H1 early maturity hybrid, the approximate level of LA produced was about 33 g kg^{-1} DM (which was the highest level among all silages after the 48h AS test), and AA levels were about 10 g kg^{-1} DM. The inverse relation of LA: AA after 48h of aerobic exposure, was found in the H5 late maturity silages, with the level of AA 17.49-25.37g kg^{-1} DM. Under the level of 10 g kg^{-1} DM for AS, was also H4 silage treated with LAB and EFEs. The characteristics of these silage were a ratio LA: AA in the range 1:1, followed by twice less content of Y&M than in the control treatment after 48h aerobic exposure.

During 48h in the AS test, it was not recorded the significant T changes in all experimental silages.

The inoculated H2 mid-early hybrid silages wereanaerobically unstable due to the significantly lowest content of AA (3.52 g kg^{-1}DM) and the highest BA content (10.11 g kg^{-1}DM), compared with the control treatment (without LAB and EFEs addition). The experimental silages of medium H3 maturity hybrids.

Table 4. Effect of treatment on change in temperature, CO_2 content, and pH of different hybrid silages (H1-H5) during 48h aerobic stability test

	Treatment	Days inAS tests	CO_2 (gkg^{-1}DM)	pH	Temperature of silage (°C)	Δ T** (°C)
H1	Control	0*	0	3.80	20.80	-1.10
		2	1.34	3.80	23.30	-0.80
	LAB+Enz	0	0	4.15	20.70	-1.70
		2	3.26	3.89	23.80	0.10
H2	Control	0	0	3.96	20.20	-3.20
		2	12.27	4.04	21.50	-1.30
	LAB+Enz	0	0	4.07	20.20	-3.20
		2	20.26	5.07	22.20	-0.50
H3	Control	0	0	3.94	22.40	-0.80
		2	29.03	6.88	24.70	1.10
	LAB+Enz	0	0	3.99	19.90	-1.60
		2	48.94	6.80	24.10	0.50
H4	Control	0	0	3.91	21.20	-1.20
		2	23.65	6.81	22.00	1.30
	LAB+Enz	0	0	3.80	20.20	-3.00
		2	9.37	4.13	18.30	-0.50
H5	Control	0	0	3.70	19.80	-2.30
		2	6.60	3.82	19.40	-1.10
	LAB+Enz	0	0	3.72	20.60	-0.70
		2	5.96	3.37	19.00	-1.50

*0 day – opening the experimental silages and starting exposure to air in the AS test. ** Δ T- difference between the external temperature and the silage temperature of the maize

for both treatments (with and without additive) were aerobically unstable due to the highest content of Y&M (7.13-9.95 g kg^{-1}DM) among all hybrids included in the trial. The pH value in these silages was 6.80 followed by CO_2 production of 29.03 g kg^{-1}DM for control and 48.94g kg^{-1}DM treatment with LAB and EFEs. A similar level of CO_2 production had aerobic unstable mid-late H4 control silage (without LAB and EFEs) with a similar pH value of 6.81 and significantly higher content of Y&M 7.18 g kg^{-1}DM over treated H4 silage with additive.

DISCUSSION

The hybrid and additive types might affect the nutrient composition and digestibility of maize silages. In some cases, effects are missing. When discussing the obtained results, it should be noted that there is an intermediate space because according to our knowledge, there is no published scientific work on the effects of the same LAB inoculant and EFEs on the different maize varieties of hybridization.

In this trial, only in the H1 early and H5 late maturity treated LAB and EFEs silages, the contents of DM were statistically higher than in control silages at the moment of silo opening (0 days). However, at H1 inoculated and enzymatic treated silage, after 48h of aerobic exposure, the content of DM remained higher than the control treatment, indicating aerobic stability of this content. On the contrary, in the silages of other hybrids H2, H3, and H4 it was not affected by the additive addition for the DM content. Those findings are in agreement with the research of Salvo*et al.*(2022), who reported that the addition of EFEs in maize silages did not affect nutrient digestibility and DM losses during fermentation.

It is well known that increasing the ash content during ensiling is negatively correlated with OM content. The increase in the ash content could indicate in practice on existence of a few problems: loss of nutrients during ensiling, soil-born yeasts, clostridial microorganisms incorporated into silage, winds with rain, and organic manure contamination, and others. The quality of lactic acid fermentation affects the levels of DM and ash during ensiling. The higher levels of ash may indicate soil contamination and poor quality of fermentation with higher acetic and butyric acid content (low DM silages). Higher DM silages often indicate bacterial spoilage and restrict fermentation (Kung and Shaver, 2001), as well as ash levels. In all experimental hybrid silages from early to late maturity, the content of ash was not significantly different between the treatments and between content at the moment of silage opening and after 48 hours of aerobic exposure. The stability of ash content indicates the good quality of ensiling but, it points to a lack of LAB and EFE influence in treated silages because there were no statistically significant differences between treatments.

The addition of LAB and EFEs did not significantly improve the CP content in all silages,and a difference between treatments (inoculated with ENZ *versus* control) was not found as well as during the 48h AS test.

The impact of EFEs on the NDF content in silage is not consistent and the NDF content could be increased during the longer time of the ensiling (Salvo *et al.,* 2022; Sanderson *et al.,* 1993). On the contrary, the same pattern of NDF content increases over time was established for wheat silages at the milk stage but not for maize silages. (Weinberg and Chen 2013; Der Bedrosian *et al.,* 2012). Furthermore, a decrease in the NDF content by the application of EFEs at ensiling was observed in several studies (Higginbotham *et al.*, 1994; Spoelstra *et al.,* 1992; Sheperd *et al.,*1996; Li *et a*l., 2017; Lynch *et al.,* 2015) in which the NDF content reduction was attributed to the degradation of the cell wall carbohydrates. (Salvo *et al*., 2022). However, in the present study the effects of EFE treatment on the NDF content were not clear). Because of the complex fibrous structures, NDF and ADF can be hardly degraded in silage (Nair *et al.,* 2020). In this trial,early H1 and medium H3, hybridsshowed the same changes in NDF content expressed in the significant decreases in LAB and ENZ silages after 48h of aerobic exposure compared with content at the time of silageopening and with the content in control silages at the same time of the AS test. It is well known. that a higher level of NDF (as a measure of total fiber in the silage), could slow down digestion. restrict intake and reduce performance in animals. The trend of statistically significant increases in the NDF and ADF contents in the mid-early H2, H3 medium, and H5 late silages with LAB and EFEs over control silages of the same hybrids were determined at 0 days (silage opening) and after 48h of AS testing. The second recognized trend was statistically increasing the ADL levels in the treated H2 mid-early and mid-late H4 silages with LAB and EFEs over control silages of the same hybrids was determined at 0 days and after 48h of AS testing. The measure of the amount of undi-

gestible material in silage is used for the evaluation of ADL content and the measure of digestibility is often used for the estimation of the ADF level (high NDF and ADF values could indicate low DM digestibility, energy, and protein level). The content of ADL in H1 silage with LAB and ENZ was significantly lower at the moment of silage opening and after 48h of aerobic exposure, comparing the control silages of the same hybrid (without additive). These changes indicate the positive influence of LAB and ENZ addition in early maturity H1 hybrid. Opposite to the previous, in the H5 late-maturity hybrid silages, the amount of ADL at 48h in the treated silage was significantly higher than in the control one (without LAB and EFEs addition) of the same hybrids. Zahiroddini*et al*. (2004) reported that ensiling with the addition of LAB and enzymes attained pH 4.0 by day 3 and did increase ($P<0.01$) the soluble NDF fraction. Also, in the feedlot study of over mention authors, DM intake did not differ among treatments. but average daily gain (ADG) by steers fed diets with LAB and EFEs treated silage was higher ($P=0.1$). by 7.6%, respectively. and feed efficiency of steers fed was improved ($P=0.01$) relative to those fed with the silage ensiled without additive.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 to reduce the rumen fill and improve the DMI (Borreani*et al*., 2018; Kammes and Allen, 2012).

Corn forage is a fibrous feed with high concentrations of cellulose and hemicellulose, which can create a structural complex of carbohydrates and lignin to reduce the digestibility ofcarbohydrates and thereby decrease the efficient utilization of feed by ruminants (Elghandour*et al*., 2014). In some previous research. the carbohydrate content was unaffected by the enzyme treatment and length of storage (Salvo *et al.,* 2022). On the contrary the other observed that carbohydrate content differs between the ensiled hybrids with the addition of EFEs, where that content decreases slightly over time (Der Bedrosian *et al.,*2012).Settimi *et al.* (2013) mention that the direct application of enzymes to the substrate favors the formation of a stable enzyme-substrate complex which increases the effectiveness of the exogenous enzymes, improving fiber solubility or availability for microbial attack in the rumen. In this trial, the positive effects of LAB and EFEs addition on silage aerobic stability were recorded by the significant increase in the NFC content over control and over 0 days (silage opening) in the same treatment at the early H1 and H3 medium hybrid silages, after 48h of aerobic exposure. The NFC content in the control silages (ensiled green mass of hybrids without LAB and EFEs) for H1-H4 was higher (statistically significant) at 48h after aerobic exposure than at the moment of silage opening. Those changes were due to decreasing the HC content in the same periods. The positive influence of LAB and enzyme addition was detected in the mid-early H2 hybrid silages, where significantly increased the content of HC at inoculated silage, compared with the control treatment.Xylan is the main component of hemicellulose, and by the action of a complex enzymatic system, it is hydrolyzed and converted into its constituent sugars (Breccia *et al*., 1998). Determination of CEL for the treated silages (LAB and EFEs) showed statistically higher levels in H2-H5 hybrid silages compared with the control treatments, at 0 days and 48 hours of AS testing, indicating the positive influence of added EFEs. For the early H1 corn hybrid silages, it was not recorded the overmentioned trend. The possible explanation given by Martinez *et al*. (2020), suggests that in some hybrids the use of EFEs could increase cellulose and hemicellulose degradation. Cellulases are inducible enzymes synthesized by a wide range of microorganisms including fungi and bacteria (Campioni*et al.,*2020).

In the trial, it was found the inconsistency of the effects in experimental silages with LAB and EFEs addition on the fermentation profile. Those findings are in agreement with previous reports. The EFEs treatment did not increase the digestibility of nutrients but increased the AA concentration, (Salvo *et al.,* 2022). The authors reported that at 60 days (the same period of ensiling as in our trial), the same pattern of increased lactic acid content with higher doses was also observed, but the pH remained equally low for all the treatments, andusually changes in pH or the lactic acid are not observed (Salvo *et al.,* 2022). Because the pH of the silage is lower and its temperature at fermentation onset is relatively higher than that in the rumen, the application of EFEs at ensiling, rather than providing it directly to the animal, can optimize the enzyme's activity and its effects (Adesogan*et al.,* 2014). The higher concentration of AA in silages stored for long periods is a consequence of the conversion of lactic acid to equimolar parts of acetic acid and 1.2-propanediol (Oude Elferink*et al.,* 2001).The increases in the butyric acid (BA) content from 30 to 90 days of storage suggest a possible action of proteolytic microorganisms, such as clostridia, (Muck, 2010).Butyric acid can be present in poorlyfermented

silage from the fermentation of water-soluble carbohydrates and lactic acid, principally by clostridia that are present on the crop at harvest (Dreihuis*et al.,*2018).

In all silages, it was expressed extremely heterogenic effects of LAB inoculation influence the different hybrids. It was not found the similar pattern expressed between different corn hybrids for nutritional value changes. In other words, different hybrid reacts differently. The role of epiphytic microflora is visible at H2, H3, and H4 control silages (ensiled without additive), where the contents of LA were significantly higher than in silages of the same hybrids ensiled with LAB and EFEs. In the second case for this claim, the experimental silages of early maturity hybrid, with and without additive, showed a similar trend of raising the content of LA after 48h of aerobic exposure and, these trends also indicate on positive influence of epiphytic microflora. However, it is worth mentioning that levels of LA in H1-treated silages were statistically higher than in control ones, and we supposed that these changes were partly due to the LAB and EFEs addition. Liu *et al.* (2018) stated that lower VFA content and higher lactic acid content in silage might limit aerobic deterioration after exposure to air.Completely, under the positive influence of LAB and EFEs addition on AS, where homofermentative LAB mixture in inoculum overwhelms the epiphytic microflora is observed at H4 mid-late hybrid experimental silages, with statistically higher content of LA and AA than in control ones, after the 48 hours of aerobic exposure. In these silages, acetate and lactate levels were remarkably increased ($p<0.05$),while propionate levels were decreased ($p<0.01$) by inoculant treatmentsduring AS testing. High-level acetate production might be attributed to the fact thatthe lactic acid was converted into acetic acid during the ensiling (Ni *et al.,* 2017).The homofermentative LAB ferment hexoses mainly to lactic acid whileheterofermentative LAB produces acetic acid, CO_2, as well as lactic acid (Muck *et al.,* 2018). Usually, no or low levels of butyric acid (BA) are desired in the silage as it negatively affects thesilage quality by reducing the nutritional value of silages (Nkosi and Meeske, 2010).Similarly, Yang *et al.* (2018) found *Lactobacillus* species increased by *L. plantarum* due to its high activity in the low pH. A similar finding was also indicatedby Ni *et al*. (2017).whose silage inoculated with *L. plantarum* and*Pediococcuspentosaceus* tended to have a greater LAB number than the control silage. This positive LAB inoculum influence is detected at H1, H4, and H5 treated silages, where the range of pH values were approximately in optimum levels at the moment of silage opening and after 48h of aerobic exposure, as well. In the optimum fermentation. homofermentative LAB (as present in our trial in inoculum), produces only LA followed by a high recovery of DM and energy, (Pahlow *et al.,* 2003). However, the fermentation of forage crops is very complex and involves many types of MO (Kung *et al.,* 2018).

The effectiveness of preserving the quality and nutritional value of silage in a closed silo depends solely on the degree of anaerobicity of the environment. At the beginning of fermentation. residual air remains trapped between parts of the ensiled mass and allows respiratory processes to take place in which the ether necessary for the formation of LA and AA is used to release heat (Driehuis*et al.,* 2006). The prolonged exposure to the air of the silage mass in the first place prolongs the life of yeast and mold and thus delays the development of LAB needed to conserve silage. A rapid removal of air from silage during storage and shorter exposure during storage in the diet are two important factors that determine the quality of silage and the preservation of its nutritional value. During the feeding,by the process of aerobic degradation of the silage, there is a growth of aerobic acid-tolerant MO, which oxidizes the created fermentative products for their development (Filya, 2003). Several types of MO have been identified so far, which affect the length of the aerobic stability of silage, primarily in the silage of whole maize plants (Middlehoven*et al.,* 1990), but also new types of yeast (Lu *et al.,* 2004).Different varieties of maize ensiled, as native corn maize genotypes treated with xylanase and cellulase, showed the highest gas production and better fermentation profiles than the silage of other genotypes, (Martinez *et al.,* 2020).

The obtained results about microbiology composition were heterogenic, depending on hybrid ensiled (with identic LAB and EFEs). It was observed two trends. First, anaerobic unstable silages, with statistically higher amounts of Y&M were found in the control treatments of medium H3, mid-late H4, and late H5 hybrids, after 48 hours of aerobic exposure, compared with Y&M composition in control silages (without additive) at the moment of silos openings. The second trend was noticeable in all treated silages (with additive ensiled), with higher levels of LAB after 48h of aerobic exposure, indicating the positive influence of LAB and EFEs addition. High numbers

of Y&M, are most often connected with high concentrations of ethanol, and their numbers are often inversely related to the AS, primary corn-based silages (Kung *et al*., 2018). It was established the negative correlation y=315.4-45.7×; r=0.79. where x is the log $_{10}$total number of yeasts in corn silage and y is the predicted hours of AS, (defined as ≥ 2°C increase in the temperature of silage mass after air exposure), (Kung *et al.,* 1998). The author suggests that this equation predicts that aerobic stability is zero when there are ≥6 $\log_{10}$CFUyeasts g^{-1} of wet corn silages.

Proper selection of silos with reduced porosity, fast filling, good green mass compaction, and quick closure are just prerequisites for the aerobic stability of the silage. These prerequisites are insufficient to prevent the growth of undesirable MO in aerobic exposure to silage (Muck *et al*., 2005). During the gradual emptying of silos on farms in the removal of silage, according to Weinberg and Ashbell, (1994), air penetrates 1-2 m from the front side of the object into the depth. Depending on the rate of removal, the silos may be exposed to air for 3-5 days before feeding. During this period, the development of unwanted MO occurs in silage and losses of initial nutritional value. In silos where the silage is open, daily DM losses range from 1.5 to 4.5%, while the content of DM and NDF was significantly lower compared to silagethat is still covered (Weinberg *et al*.,2009). Because of that, up to 50% of the DM loss occurs primarily due to aerobic degradation atthe open surface of the silage, (Seglar, 2003). Under aerobic conditions, many yeast strains break down LA to CO_2 and H_2O, thus increasing the pH of silage, which allows the growth of other undesirable MO. Aerobic stability could be defined as the resistance of silage against spoilage after it has been exposed to air. There was another description of aerobic stability according to Liu *et al*. (2016) who stated that the silages showing a change of <0.5 units in pH over 5 days are deemed to be stable. The mentioned authors describe in detail the *in vitro* procedures for H_2, CO_2, and CH_4 determination in the rumen fluid treated with inoculated silages. The LAB inoculants improved the silage quality by reducing the content of butyric acid and by increasing the effective degradation of ADF upon ruminal degradation and produced more CO_2 during gas production compared with non-inoculated silage. (Liu *et al.,*2016). The results of this trial showed that the lower BA, TMN, Y&M, pH, and higher LAB, LA, and AA levels ($p<0.05$), are accompanied by the production of lower CO_2 ($p<0.05$). The CO_2 production depends on the inoculum used, and a mixture of *L. buchneri* and *L. plantarum*-inoculated silage was less than that only with homofermentative L. *plantarum*-inoculated silage ($p< 0.05$). (Zhang *et al.,* 2009; Zhao *et al.,* 2019). Arriola *et al.* (2015) reported that silage treated with LAB inoculant improved the fermentation strength resulting from the decline of silage pH that causes a low DM loss and high aerobic stability by inhibiting yeast fermentation. By our observation, we supposed that the maximum acceptable level of corn aerobic stability in CO_2 production in 48 hours is 15 $CO_2 gkg^{-1}$ DM. In the current study, the lower levels of CO_2 production from the suggested maximum AS acceptable level of CO_2 production in 48h of aerobic exposure were present at H1 and H5 silages followed by a statistically less Y&M present in the treated silages (with LAB and EFEs) compared with control ones of each hybrid. Also, it was detected as significantly higher LA content in H1 and AA content in H5 treated with LAB and EFEs silages, after 48 hours of aerobic exposure. Theincreased acetic acid concentration suppressed the yeast fermentation during the ensiling. resulting in lower CO_2 production. The chemical composition, fermentation, and microbiology profiles of silage significantly influence CO_2 production in a test of AS and this is a causal relationship of aerobic stability.

During silo emptying, silage is exposed to air and becomes a suitable environment for aerobic MO, affecting aerobic decomposition and increasing silage temperature. According to Ranjit and Kung (2000), aerobic stability is defined as the number of hours in which the temperature of the sink is not more than 2°C above the outside temperature. Therefore, Tabacco *etal*. (2009) defined the difference between outdoor temperature and slope temperature as ΔT. However, monitoring temperature changes alone is not enough to define the AS length of a given slope, Weinberg *et al*. (2001) hypothesize that when higher amounts of WSC residues for aerobic yeast are present in silage, the pH of the silage will not change during aerobic decomposition. The authors state that when LA is the only source of energy for yeast, the pH value of silage will increase; or in the case of silage, the content of WSC and LA residues is less than that of aerobic yeast substrates, then silage is more aerobically stable as there is no substrate for the growth and development of undesirable MO. In the AS silage test, Weinberg *et al*. (2009) indicate that the inoculant-treated silages did not have a statistically significantly different pH value compared to the control-treated silages. According to Woolford *et al.* (1977), degradation is

accompanied by an increase in temperature and is directly related to oxidative losses of DM in the form of CO_2. The rate of CO_2 production is also an indicator of the intensity of aerobic degradation of silage and loss of DM,and the correlation coefficient between CO_2 production and pH change during aerobic exposure is 0.99 for corn silage (Ashbell*et al*., 1991). The current research and previous studies suggest that nutritional value, health safety, and aerobic deterioration of the different maize hybrid silages are under the influence of epiphytic microflora of ensiled plants, used LAB inoculum, and EFEs addition in the appropriate amount, and the type of hybrid ensiled as well. The used method by Ashbell*et al.* (1991) in this trial, is easy to set up and standardize, allowing samples can be withdrawn at different intervals.

CONCLUSION

This study evaluated the effect of identical LAB inoculant and EFEs addition on the ensiling process of five hybrids that differed by FAO group of maturity. Hybrids (Pioneer Hi-Bred DuPont) differed by FAO maturity group (from early H1, mid-early H2, medium H3, mid-late H4 to late H5). Inoculant LAB consisted of a mixture of homofermentative bacteria: *Lactobacillus plantarum*.*Enterococcus faecium*.*Pediococcusacidilactici*.*Lactobacillus salivarius*, and exogenous fibrolytic enzymes: cellulase, α- amylase, hemicellulase, and xylanase.

In this trial, the effect of the same LAB inoculant and EFEs on nutritive value, fermentation profile,microbial composition, and aerobic stability differed among H1-H5 hybrid silages. In all silages, it was expressed extremely heterogenic effects of LAB inoculation influence the different hybrids. It was not found the similar pattern expressed between different corn hybrids for nutritional value changes. In other words. different hybrid reacts differently to the addition of identic LAB and EFEs.

Among the evaluated corn hybrids, the additive to H1 early maturity hybrid had significantly improved the silage quality,and prolonged the time of aerobic stability, mainly due tothe significantly highest CP, lowest ADL,and highest LAcontent. Experimental silages from late-maturing H5hybrids had the weakest nutritive characteristics for animal nutrition, but good AS properties. On farms, nowadays the early maturing hybrids are more often used for effectively ensiling and for mitigating negative effects due to climate change.

In this trial, the used additive did not provide the same pattern of changes for five different hybrids. Is well known that microbiology of ensiling is a very complex process and these findings indicate that an identic additive will not ensure the quality of ensiling of different hybrids in some cases. Second, the importance of epiphytic microflora presents a barrier for overwhelming the lactic acid fermentation under the used LAB inoculum. Following EFEs, as an additive in inoculum used has the potential to provide more carbohydrates, but the obtained results indicate thatthe difference in the effects that are in some hybrids were absent.

Also, it should be emphasized that the chemical composition, fermentation, and microbiology profiles of silage significantly influence CO_2 production in a test of AS and this is a causal relationship of aerobic stability duration. The results of this trial showed that the lower BA, TMN, Y&M, pH, and higher LAB, LA, and AA levels ($P<0.05$), are accompanied by the production of lower CO_2 ($p<0.05$).

Under aerobic conditions, many yeast strains break down LA to CO_2 and H_2O, thus increasing the pH of silage, which allows the growth of other undesirable MO. Aerobic stability could be defined as the resistance of silage against spoilage after it has been exposed to air. The addition of LAB inoculates influenced the health safety of silages by the potential to extend the duration of aerobic stability and resistance to degradation.The current research and previous studies suggest that nutritional value, health safety, and aerobic deterioration of the different maize hybrid silages are under the influence of epiphytic microflora of ensiled plants, used LAB inoculum, and EFEs addition in the appropriate amount, and the type of hybrid ensiled as well.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

REFERENCES

Adesogan, A.T.; Ma, Z.X.; Romero. J.J.; Arriola. K.G. RUMINANT NUTRITION SYMPOSIUM: Improving cell wall digestion and animal performance with fibrolytic enzymes. *J. Anim. Sci.* 2014. *92*. 1317-1330.

Alonso, V.A.; Pereyra. C.M.; Keller. L.A.M.; Calcero. A.M.; Rosa. C.A.R.; Chiacchiera. S.M.; Cavaglieri, L.R. Fungi and mycotoxins in silage: an overview. J. Appl. Microbiol. 2013. 115. 637-643.

AOAC, 2023. Official Methods of Analysis. 17th ed. 1st revision. Association of Analytical Communities. Gaithersburg. MD. USA.

Ashbell G., Weinberg Z. G, Azrieli A., Hen Y.. Horev B.. 1991. A simple system to study the aerobic deterioration of silage. Can.Agric.Eng. 33. 391-394.

Borreani,G.. Tabacco.E.. Schmidt. R. J.. Holmes. B. J.. and Muck. R. E. (2018). Silage review: Factors affecting dry matter and quality losses in silages. Journal of Dairy Science 101. 3952-3979.

CaiYimin, Benno Y., Ogawa M..Kumai S.,1999. Effect of Applying Lactic Acid Bacteria Isolated from Forage Crops on Fermentation Characteristics and Aerobic Deterioration of Silage. Journal of Diary Science. 82:52-526

Campioni,T.S.,de Azevedo Carvalho.A.F.. de Figueiredo.F.C.. da Silva.D.F..& de Oliva Neto. P. 2020. Xylanases and cellulases biosynthesis by selected fungi in a simple and economic bio system using sugarcane straw. International Journal of Environmental Agricultural and Biotechnology. 5(1):217-230. doi:10.22161/ijeab.51.3

Chauhan, N., Kumari. N., Mishra. D.B. et al. Dynamic Changes in Microbial Succession and Fermentation Profiles of Sugarcane Tops Silage Treated With Exogenous Enzymes and Lactic Acid Bacteria Following Various Duration of Ensiling. Sugar Tech 25. 592-602 (2023).

Cheng Q, Li M., Fan X., Chen Y., Sun H.,Xie Y., Zheng Y., Chen C., Li P. , 2022. Effects of epiphytic and exogenous lactic acid bacteria on fermentation quality and microbial community compositions of paper mulberry silage. Front Microbiol. 2022 Aug 25;13:973500. doi: 10.3389/fmicb.2022.973500. PMID: 36090070; PMCID: PMC9453674.

CherneyD.J. ,R.. Cherney.,J. H. Cox. W. J., 2004. Fermentation characteristics of corn forage ensiled in mini-silos. Journal of Dairy Science 87.124238-4246.

Cherney, D. J. ,R.. J. H. Cherney, and W. J. Cox. 2007. Forage quality differences of corn hybrids as influenced by ensiling. Forage and Grazinglands. 10.1094/FG-2007-0918-01-RS

Cysneiros, Cristine & Ferreira, Reginaldo & Oliveira. Michelly &Favoretto. Adriano & Arnhold. Emmanuel & Ulhoa. Cirano. (2013). Production. characterization and evaluation of fibrolytic enzymes on digestibility of forage maize. Ciência Animal Brasileira. 14. 426-435.

Danner, H., M. Holzer, E. Mayrhuber. and R. Braun (2003): Acetic acid increases stability of silage under aerobic conditions. Appl. Environ. Microbiol. 69:562-567

Der Bedrosian, M.C.; Nestor.K.E.. Jr.; Kung.L.. Jr. The effects of hybrid. maturity. and length of storage on the composition and nutritive value of corn silage. J. Dairy Sci. 2012. 95. 5115-5126.

Driehuis F., J.M. Wilkinson. Y. Jiang. I. Ogunade. A.T. Adesogan.2018. Silage review: Animal and human health risks from silage. Journal of Dairy Science.Volume 101. Issue 5.2018.Pages 4093-4110.ISSN 0022-0302.https://doi.org/10.3168/jds.2017-13836.

ElferinkOude, S.J.W.H.; Janneke Krooneman. H.; Gottschal. J.C.; Spoelstra. S.F.; Faber. F.; Driehuis. F. Anaerobic Conversion of Lactic Acid to Acetic Acid and 1.2-Propanediol by *Lactobacillus buchneri*. *Appl. Environ. Microbiol.* 2001. *67*. 125-132

Elferink Oude, Stefanie &Driehuis. Frank & Gottschal, Jan & Spoelstra.S.F.. (1999). Paper 2.0: Silage fermentation processes and their manipulation. Proceedings of the FAO Electronic Conference on Tropical Silage. 1 September to 15 December 1999. FAO plant production and protection paper 161

Elghandour, M.M.Y., Vazquez Ch.J.C.. Salem.A.Z.M..Kholif.A.E.. Martinez.C.J.S.. Camacho.L.M..& Cerrillo-Soto. M.A. 2014. Effects of Saccharomyces cerevisiae at direct addition or pre-incubation on in vitro gas production kinetics and degradability of four fibrous feeds. Italian Journal of Animal Science. 13:295-301

Ergin,S. andGumus. H. 2020. Silagequality.fermentationdynamicsandchemicalcompositionofalfalfasilagepreparedwithsaltandlacticacidbacteriainoculants. AnimalNutritionandFeedTechnology. 20: 367-380

Fabiszewska AU, Zielińska KJ.Wróbel B. Trends in designing microbial silage quality by biotechnological methods using lactic acid bacteria inoculants: a minireview. World J MicrobiolBiotechnol. 2019 May 3;35(5):76. doi: 10.1007/s11274-019-2649-2. PMID: 31054017; PMCID: PMC6499736.

Gandra J.R., A. T. Nunesc. E. R. Oliveirab. M. S. J. Silvab. C.A. P. . F. S. Machadob2 .G.K..Silvab R. E. R. S. Gandrad. P.V. C. Mendesd. A.G. S. Pausea. 2021. Effect of amylolytic and cellulolytic enzymes on whole plant corn silage: characteristics of silage and animal digestion. Research Square.

Higginbotham, G.E.; Mueller. S.C.; Collar. C.A.; Shultz. T.A.; Brazle. F.K.; Bush. L.J. Effects of an Enzyme Addition on Corn Silage Fermentation. Prof. Anim. Sci. 1994. 10. 163-168

Huyen Nguyen Thi, . Ines Martinez. Wilbert Pellikaan. 2020. “Using Lactic Acid Bacteria as Silage Inoculants or Direct-Fed Microbials to Improve In Vitro Degradability and Reduce Methane Emissions in Dairy Cows” Agronomy 10. no. 10: 1482.

Ivetić Aleksandra, 2017. Uticaj mikrobioloških inokulanata na hranljivu vrednost i aerobnu stabilnost silaže kukuruza i senaže lucerke. Doktorska disertacija. Poljoprivredni fakultet - Univerzitet u Beogradu. The influence ofmicrobiologicalinoculants on thenutritionalvalueandaerobicstabilityofcornsilageandalfalfahaylage. Doctoraldissertation. FacultyofAgriculture - UniversityofBelgrade.

Juracek, Miroslav & Biro. Daniel &Šimko. Milan & Gálik. Branislav &Rolinec. Michal &Dorszewski.Piotr&Gajdošík. Peter &Majlat. Marian. (2013). Nutritive value and fermentation characteristics of maize silages from different hybrids. Acta fytotechnica et zootechnica. 16.

Kammes. K. L., and Allen. M. S. (2012). Rates of particle size reduction and passage are faster for legume compared with cool-season grass. resulting in lower rumen fill and less effective fiber. J Dairy Sci 95. 3288-97.

KhanalPrabhat, 2009. Changesandlosses in silageduringaerobicexposure. The Robert H. SmithFacultyofAgriculture.FoodandEnvironment. TheHebrewUniversityofJerusalem.

Kleinschmit D.H., Kung L., 2006.. A meta-analysis of the effects of Lactobacillus buchneri on the fermentation and aerobic stability of corn and grass and small-grain silages. Journal of Diary Science.89:4005-4013.

Kleinschmit, D.H.; Schmidt. R.J.; Kung.L.. Jr. The effects of various antifungal additives on the fermentation and aerobic stability of corn silage. J. Dairy Sci. 2005. 88. 2130-2139.

Kobayashi, H., Cai.Y..Uegaki.R.. Shimizu.M.. Nakajima.M.. Kanaya.C.. et al. (2010). Microorganism composition of high moisture italian ryegrass (Lolium multiflorum lam.) and its fermentation characteristics of silage inoculated with lactic acid bacteria. JPN J. Grassl. Sci. 56. 39-46. doi: 10.14941/grass.56.39

Kung,Limin&Shaver. R., 2001. Interpretationanduseofsilagefermentationanalysisreports. Focus on Forage. Vol.3. No13. 20-28.

Kung L Jr., Shaver RD. Grant RJ.Schmidt RJ. Silagereview: Interpretationofchemical.microbial.andorganolepticcomponentsofsilages. J Dairy Sci. 2018 May;101(5):4020-4033. doi: 10.3168/jds.2017-13909. PMID: 29685275.

LiYing, H.; Borjigin. N.; Yu. Z. Effect of inoculants and fibrolytic enzymes on the fermentation characteristics. in vitro digestibility and aflatoxins accumulation of whole-crop corn silage. Grassl. Sci. 2017. 63. 69-78

Liu, C., Lai. Y..Lu. X..Guo. P. andLuo. H. 2016. Effectoflacticacidbacteriainoculants on alfalfa (Medicagosativa L.) silagequality: assessmentofdegradation (in situ) and gas production (in vitro). JournalofIntegrativeAgriculture. 15: 2834-2841.

Liu. Q.H.,Dong. Z.H. andShao. T. ,2018. Effectofadditives on fattyacid

profile ofhighmoisturealfalfasilageduringensilingandafterexposure to air. AnimalFeedScienceandTechnology. 236:29-38

Lynch, J.P.; Baah. J.; Beauchemin. K.A. Conservation. fiber digestibility. and nutritive value of corn harvested at 2 cutting heights and ensiled with fibrolytic enzymes. either alone or with a ferulic acid esterase-producing inoculant. J. Dairy Sci. 2015. 98. 1214-1224.

Martínez, José & López. Delfina & Serrato. Rodolfo &A.Z.M.. Salem.& Robles Jimenez. Lizbeth & Ronquillo. Manuel. (2020). Effect of xylanase. cellulase and natural maguey extract on the chemical composition of corn silage and in vitro rumen gas production. International Journal of Agriculture and Natural Resources. 47. 23-34. 10.7764/ijanr.v47i1.2128.

Muck R.E., Filya I., Contreras- Govea F.E., 2007. Inoculant Effects on Alfalfa Silage: In Vitro Gas and Volatile Fatty Acid Production. Journal of Dairy Science. 90:5115-5125.

Muck. R. E.; Nadeau. E. M. G.; Mcallister. T. A.; Contreras-Govea. F. E.; Santos. M. C.; and Kung Jr. L.,2018. Silage review: Recent advances and future uses of silage aditives. Journal of Dairy Science. 101(5). 3980-4000.

Muck, R.E. Silage microbiology and its control through additives. *Rev. Bras. Zootec.* 2010. *39*. 183-191

Okoye C. O., Wang Y., Gao L., Wu Y.. Xia Li, Sun J., Jiang J., 2023. The performance of lactic acid bacteria in silage production: A review of modern biotechnology for silage improvement. Microbiological Research. Volume 266. 127212. ISSN 0944-5013

Nair, J.,Huaxin, N., Andrada, E., Yang, H. E.,Chevaux, E., Drouin. P., McAllister, T. A., and Wang. Y, 2020. Effects of inoculation of corn silage with Lactobacillus hilgardii and Lactobacillus buchneri on silage quality. aerobic stability. nutrient digestibility. and growth performance of growing beef cattle. J Anim Sci 98.

Pahlow, G., Muck. R. E.,Driehuis.F..Elferink. S. J. O..& Spoelstra. S. F. (2003). Microbiology of ensiling. Silage science and technology. 42. 31-93.

Paradhipta, D.H.V.; Lee. S.S.; Kang. B.; Joo. Y.H.; Lee. H.J.; Lee. Y.; Kim. J.; Kim. S.C. 2020. Dual-Purpose Inoculants and Their Effects on Corn Silage. Microorganisms 2020. 8.

Proceedings. 146-152.

Ranjit N.K, Kung L..2000. The effect of Lactobacillus buchneri. Lactobacillus plantarum. or a chemical preservative on the fermentation and aerobic stability of corn silage. Journal of Diary Science.83:526-535

Salvo, Pedro. Viviane Gritti, Érica da Silva, Elisabet Nadeau,João Daniel, Maria Spindola. and Luiz Nussio,2022. "Exogenous Fibrolytic Enzymes and Length of Storage Affect the Nutritive Value and Fermentation Profile of Maize Silage" *Agriculture* 12. no. 9: 1358.

Sanderson, M.A. Aerobic stability and in vitro fiber digestibility of microbially inoculated corn and sorghum silages. J. Anim. Sci. 1993

Schnürer Johan, Jesper Magnusson,2005. Antifungal lactic acid bacteria as biopreservatives. Trends in Food Science & Technology. Volume 16. Issues 1-3.Pages 70-78.ISSN 0924-2244.

Settimi,C.C.S.. Nassar.F.R.. Ayres.O.M.. Oliveira.F.A.. Arnhold.E.. and Ulhoa. C.J. 2013. Production. characterization and evaluation of fibrolytic enzymes on digestibility of forage maize. Ciencia Animal Brasileira. 14(4):426-435

Sheperd. A.C.; Kung. L. Effects of an Enzyme Additive on Composition of Corn Silage Ensiled at Various Stages of Maturity. J. Dairy Sci. 1996. 79. 1767-1773

Spoelstra. S.F.; Van Wikselaar. P.G.; Harder. B., 1992. The effects of ensiling whole crop maize with a multi-enzyme preparation on the chemical composition of the resulting silages. J. Sci. Food Agric. 1992. 60. 223-228.

Tabacco E., Piano S., Cavallarin L, Bernandes T.F., Borreani G., 2009. Clostridia spore formation during aerobic deterioration of maize and sorghium silages as influenced by Lactobacillus buchneri and Lactobacillus plantarum inoculants. Journal of Applied Microbiology 107:1632-1641

Tylutki, T.P., Fox. D.G.,Durbal.V.M.. Tedeschi.L.O.. Russell J.B.. Van Amburgh.M.E.. Overton.T.R.. Chase. L.E., Pell. A.N., 2008. Cornell Net Carbohydrate and Protein System: A model for precision feeding of dairy cattle. Animal Feed Science and Technology. 143: 174-Kung L..StokesM.R..Lin C.J..2003. Silage additives. Silage science and Technology. D.R. Buxton.R.E.Muckand Harrison J.H.,ed.Am.Soc. Agron.,CropSci.Soc.Am.,SoilSci.Soc. Am.,Madison.WI. p.305-360.

WangX, Cao X, Liu H, Guo L, Lin Y, Liu X, Xiong Y, Ni K, Yang F, 2021. Effects of Lactic Acid Bacteria on Microbial Metabolic Functions of Paper Mulberry Silage: A BIOLOG ECO Microplates Approach. Front Microbiol. 2021 Jun 25;12:689174. doi: 10.3389/fmicb.2021.689174. PMID: 34248912; PMCID: PMC8267872.

Weinberg Z.G, Hen Y.., Solomon R., 2009. The quality of commercial wheat silages in Israel. Journal of Dairy Science. 92:638-644.

WeinbergZ.G.,Szakacs G.,Ashbell G., Hen Y., 2001. The effects of temperature on the ensiling process of corn and wheat. Journal for Applied Microbiology.90.561-566

Weinberg, Z.G.,Khanal. P.,Yildiz. C.,Chen. Y. andArieli. A. ,2011.Ensilingfermentationproductsandaerobicstabilityofcornandsorghumsilages. GrasslandScience. 57: 46-50.

Weinberg, Z.G.; Chen.Y.. 2013. Effects of storage period on the composition of whole crop wheat and corn silages. Anim. Feed Sci. Technol.. 185. 196-200

Wilkinson, J.M. and Davies. D.R. (2013). The aerobic stability of silage: key findings and recent developments. Grass Forage Sci. 68: 1-19.

Wilkinson, J.M.; Davies. D.R. The aerobic stability of silage: Key findings and recent developments. Grass Forage Sci. 2012. 68. 1-19.

Woolford M.K., 1984. The Silage Fermentation.Microbiological Series. 14. Marcel Dekker.Inc..New York and Basel.

Woolford, M. K., H. Honig. J. S. Fenlon, 1977. Untersuchungenueber aerobe Umsetzungen in Silage mitHilfeeinerLabortechnik. Das wirtschaftseigene Futter 23:10-22.

Woolford, M.K.,1990. The detrimental effects of air on silage. Journal of Applied Bacteriology. 68.101-116.

Zahiroddini H., J. Baah. W, Absalom. T.A, McAllister,2004. Effect of an inoculant and hydrolytic enzymes on fermentation and nutritive value of whole crop barley silage. Animal Feed Science and Technology. Volume 117. Issues 3-4. 2004. Pages 317-330. ISSN 0377-8401.

Zhang, T,. Li. L..Wang.Xf. *et al*. 2009, EffectsofLactobacillusbuchneriandLactobacillusplantarum on fermentation.aerobicstability.bacteriadiversityandruminaldegradabilityofalfalfasilage. World J Microbiol-Biotechnol 25. 965-971.