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Aerobic Stability of High-Moisture Corn Ensiled with *Lactiplantibacillus plantarum* During Prolonged Air Exposure

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Abstract: The evaluation of the aerobic stability of silages primarily involves monitoring temperature, while changes in composition are often neglected. In the present study, the effect of aeration on high-moisture corn ensiled with *Lactiplantibacillus plantarum* was investigated and compared with that of the control without inoculant. The corn used in this study was grown in five plots in a split-plot experimental design, and each plot represented the repetition of silage preparation, both with and without inoculant. In the silages, the temperature changes and the content of acids and alcohols were monitored during the 6 days of aeration, while the content of the main nutrients was compared before and after 6 days of aeration. The temperature difference between the silages and the environment was below 3 °C during the entire aeration period, regardless of the ensiling method. The content of lactic acid was higher in *L. plantarum* silages, while the contents of acetic and propionic acid, methanol and ethanol were higher in the control ($p < 0.001$). The content of all compounds changed during the aeration period ($p < 0.05$), regardless of the ensiling method and the stable silage temperature. Aeration affected the content of soluble crude protein and starch ($p < 0.05$), suggesting starch degradation in high-moisture corn during aeration. Therefore, in addition to monitoring silage temperature, monitoring lactic and acetic acid and the main nutrients should be considered, as the nutritional value of high-moisture corn could decrease during aeration.

Keywords: aeration of silages; lactic acid; acetic acid; methanol; ethanol; starch; soluble crude protein

Citation: Duvnjak, M.; Bogunović, I.; Kljak, K. Aerobic Stability of High-Moisture Corn Ensiled with *Lactiplantibacillus plantarum* During Prolonged Air Exposure. *Fermentation* **2024**, *10*, 580. https://doi.org/10.3390/fermentation10110580

Academic Editor: Fuyu Yang

Received: 1 October 2024
Revised: 4 November 2024
Accepted: 9 November 2024
Published: 12 November 2024

1. Introduction

Whole-plant corn and high-moisture corn silage are some of the most widely used energy sources for dairy and beef cattle production [1]. This type of feed is produced by the activity of lactic acid bacteria (LAB) under anaerobic conditions. LAB convert water-soluble carbohydrates (WSC) into lactic acid, which lowers the pH and preserves the nutrients in the feed for later use throughout the year [2,3].

The key parameter for silage stability is the anaerobic environment [2]. During the feedout phase, when silage is opened and aerated, this key factor for stability is no longer present. However, low pH in silage and the presence of some antimicrobial components, such as acetic and propionic acid, inhibit the activity of spoilage microorganisms [3,4]. The content of acids in the silage, lactic acid and acetic acid, is crucial for its stability [2–4]. The first and most important characteristic of silage is its low pH, which is a result of the acids contained in the silage, mainly lactic acid [2]. When lactic acid is broken down, the pH rises, and the silage becomes unstable [2–4]. Secondly, the acetic acid in the silage inhibits the activity of yeasts, the first microorganisms of aerobic degradation. In a semiaerobic and aerobic environment, the yeasts break down lactic acid, resulting in an increased pH and unstable silage [3,4].

The aerobic stability of silage is one of the most important parameters when it comes to silage quality [3,4]. It is defined as a period of aeration of the silage during which the

silage temperature does not rise by more than 2 °C [5] or 3 °C [6] compared to the ambient temperature. If silage has longer aerobic stability, its use is extended during the feedout period [7]. Detrimental activity is assessed by the increase in silage temperature during aeration as the microorganisms metabolize the nutrients and produce heat, water, and CO_2 [4].

The use of inoculants is often favored in silage production, as LAB control the active phase of silage or fermentation [2,3,8]. Inoculants promote the synthesis of lactic acid and volatile fatty acids (VFA) by controlling fermentation and influencing the quality of the silage [3,8]. Homofermentative and facultative heterofermentative LAB, such as *Lactiplantibacillus plantarum,* mainly produce lactic acid, while the obligate heterofermentative LAB, such as *Lentilactobacillus buchneri* and *Lentilactobacillus hilgardii* produce more VFA [8]. If more lactic acid is produced, as in silages ensiled with *L. plantarum*, the pH value in the silage drops faster and more prominently, which leads to better preservation of nutrients [2,3].

Corn is the most important cereal in animal nutrition, and its energy value depends on the amount of starch and its availability [1]. During aeration, detrimental microorganisms degrade nutrients, resulting in a lower nutritional quality of the silage [4]. We hypothesize that HMC ensiled with *L. plantarum* show better nutrient preservation during aeration than control silages and that, although ensiling with *L. plantarum* is often associated with lower aerobic stability (associated with lower content of acetic acid), the different changes in acids will affect aerobic stability, i.e., changes in acids during aeration will affect aerobic stability. Therefore, the aim of this study was to investigate the effects of the application of inoculant on aerobic stability and the change in acids in high-moisture corn during aeration over a period of 6 days and to determine how aeration affects the nutritional properties of silage.

2. Materials and Methods

2.1. High-Moisture Corn Preparation and Aeration

The corn used in this study (Bc 462, FAO 420; BC Institute d.o.o., Zagreb, Croatia) was grown in five plots under intensive conditions in the split-plot experimental fields of the University of Zagreb Faculty of Agriculture, Zagreb, Croatia. According to Köppen's climate classification [9], the climate is Cfb, with warm summers. The soil was silty clay loam Fluvisols [10]. Each plot was 14 m^2 in size, and corn was planted under the same agroclimatic conditions, following the recommendations of seed companies for seeding density (75,000 plants/ha) and using an intensive production system [fertilized with 400 kg/ha of NPK 7-20-30 (N-P_2O_5-K_2O); basic fertilizer was urea (100 kg/ha) and KAN N (MgO) was applied twice during vegetation, 175 kg/ha]. The production system was rainfed without irrigation. At the time of physiological maturity (70% DM), the corn was harvested, and each of the replicates was ground on a hammer mill with an 8 mm sieve (Ino Brežice d.o.o., Krška vas, Slovenia). The milled material from each of the five replicates was divided into two parts for ensiling.

The ensiling was carried out in airtight nylon bags of 280 × 360 mm (Status d.o.o., Metlika, Slovenia), each containing about 1000 g of ground corn ensiled with or without inoculant. The inoculant Bio-Sil LAB (Dr Pieper Technologie und Produktentwicklung GmbH, Wuthenow, Germany) contained two strains of *Lactiplantibacillus plantarum* and was used at a concentration of 300,000 CFU/g. After application, the bags were vacuumed and sealed using a SmartVac vacuum sealer (Status d.o.o., Metlika, Slovenia). The silages were stored at 25 ± 2 °C and sampled after 15 days, which corresponds to the early opening of the silages when the silage is ready for use [2]. After sampling, the first part of each silage was stored at −20 °C until the chemical properties and VFA were determined, whereas the other part was used for the determination of aerobic stability.

Approximately 500 g of each silage was weighed and analyzed for aerobic stability. Temperature probes were placed in the center of each silage to monitor the temperature. In addition, a temperature probe was placed near the silages to monitor the ambient temperature. The probes recorded temperature changes every 15 min via the mobile

application Fleettracer (Telematrix d.o.o. Zagreb, Croatia). The readings were recorded over a period of 6 days. The silages were covered with medical gauze. Samples were taken on each day of aeration for the analysis of acids and alcohols, while they were taken at the beginning and end of the aeration period for pH and chemical analyses. The samples were stored at −20 °C until analyses.

2.2. Chemical Analysis

Frozen samples of 15-day silage and aerated silage were tempered to 4 °C before chemical analysis. The silage samples taken at the beginning and end of the aeration period were divided into two parts, one for the determination of dry matter (DM) and crude protein (CP) and the preparation of a water extract for the determination of pH, WSC, acids, and alcohols. The other part was used to prepare a laboratory sample for the determination of DM, starch, and soluble crude protein (SCP). Samples from the remaining days of the aeration period were used to prepare a water extract for the determination of acids and alcohols.

The DM content of the fresh silage samples was determined by drying the samples in a UFE 400 oven (Memmert, Schwabach, Germany) at 103 °C for 24 h in accordance with the HRN ISO 6496:2001 standard [11]. The analytically determined DM values were corrected for volatile components lost during drying using a regression equation by Porter and Murray [12]. The Kjeldahl method [13] was used to determine the total N for CP evaluation in fresh high-moisture corn. The total N content was multiplied by 6.25 to obtain the CP content. Water-soluble carbohydrates were determined using the anthrone method [14]. The other half of the thawed sample was dried (60 °C, 24 h), ground to pass through the 1 mm sieve of a cyclone mill (Cyclotec 1093, FOSS Analytical, Hillerød, Denmark), and analyzed for DM (4 g of the sample, 103 °C, 4 h; [11]), starch (K-TSTA assay kit; Megazyme International, Bray, Ireland), and SCP [15].

A modification of the Nishino and Uchida method [16] was used to prepare the water extract. In brief, 20 g of fresh high-moisture corn samples were homogenized in 200 mL of distilled water and refrigerated at +4 °C for 24 h. The prepared water extracts were first used for pH determination using a digital benchtop pH meter (Inolab pH 720; WTW, Weilheim, Germany). The samples were then filtered to remove interfering substances that could affect detection by high-performance liquid chromatography (HPLC). The filtrate was stored in 2 mL Eppendorf tubes (Eppendorf, Hamburg, Germany) and placed in a freezer at −20 °C until HPLC analysis for lactic acid, VFA, and alcohols.

A modified HPLC method by Canale et al. [17] was used to analyze lactic acid, acetic acid, propionic acid, isobutyric acid, butyric acid, and the alcohols methanol, ethanol, propanol, and 1,2-propanediol. The separation was carried out using an Aminex HPX-87H column with different detection for the different compounds on a SpectraSystem HPLC instrument (Thermo Separation Products, Inc., Waltham, MA, USA). The alcohols were quantified using an RI detector, whereas the acids were quantified using UV-VIS (210 nm) and RI detection. All analytes were separated isocratically at 41 °C using diluted sulfuric acid (0.0025 M) as the mobile phase, and the injected sample volume was 10 µL. Standard solutions (Merck, Darmstadt, Germany) were used for identification and quantification. Calibration curves were generated for each analyte based on the standard measured values of 7 concentrations.

2.3. Data Analysis

Microsoft Office Excel (Microsoft Corporation, Redmond, WA, USA) was used to compare the temperatures recorded by the temperature probes in the silage and the room temperature probe for aerobic stability evaluation.

The data were analyzed as a split-plot in time design using the SAS 9.4 software package (SAS Institute, Cary, NC, USA). The differences in the temperature, chemical properties (DM, WSC, starch, CP, and SCP), acids (lactic, acetic, propionic), and alcohols (methanol, ethanol) of the aerated silages were analyzed using the PROC MIXED procedure,

where the ensiling method and aeration time were the main factors and their interaction. The values were compared with the PROC PLM [18] and grouped. Statistical significance was set at $p \leq 0.05$.

3. Results

3.1. Aerobic Stability

After the silages were opened, temperature probes were placed in the center of the silages and the surrounding environment, and the temperature was recorded every 15 min. The differences between the temperature of the silages and the surrounding environment are shown in Figure 1. The differences were above 2 °C only in the first hours of the aeration period, whereas they were below 1 °C after 3 days of the aeration period for silages ensiled without the addition of inoculant, and below 1 °C after 4 days of the aeration period for silages ensiled with an inoculant containing *L. plantarum*. Regardless of the ensiling method, the temperature differences in both silages were below 2 °C during almost the entire aeration period, although silages ensiled with *L. plantarum* showed slightly higher temperature differences.

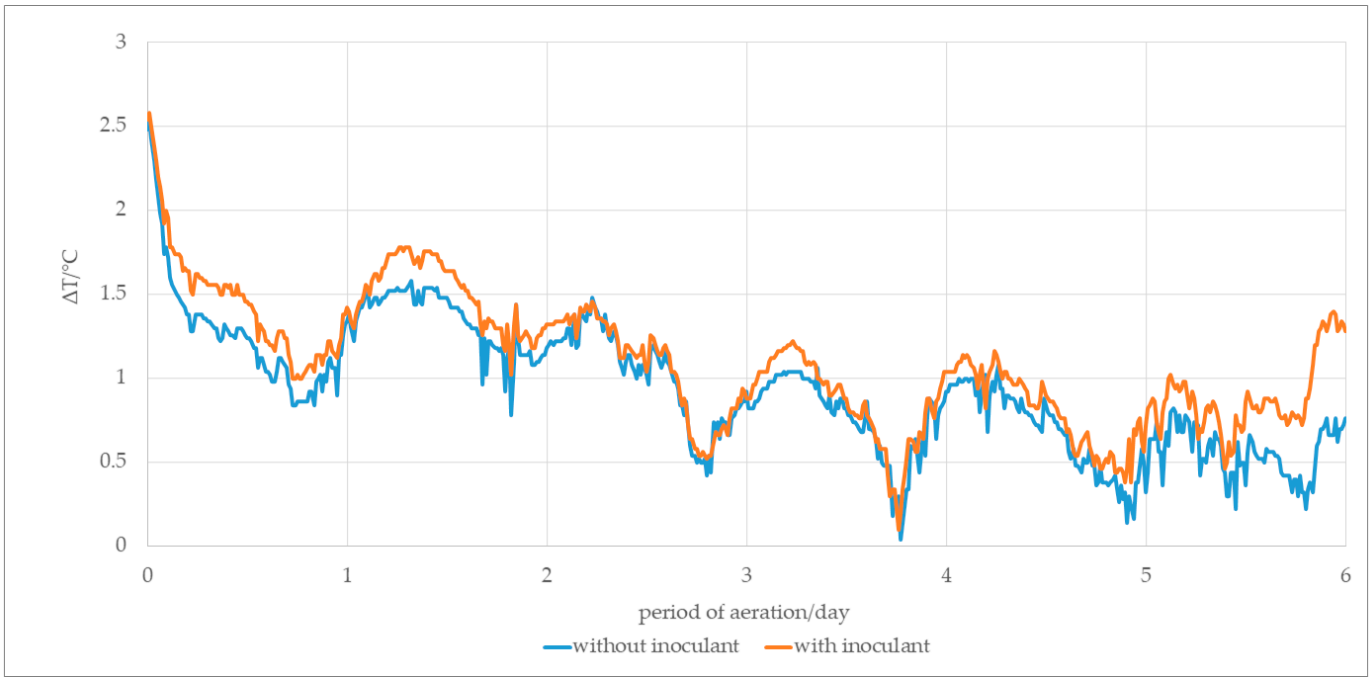

Figure 1. Temperature differences between silages ensiled with and without inoculant containing *Lactiplantibacillus plantarum* and the environment during the aeration period.

The average daily temperature difference between the silages and the environment was calculated and subjected to statistical analysis. An effect of the ensiling method, i.e., the addition of an inoculant containing *L. plantarum* and days of aeration, was detected ($p < 0.001$; Figure 2), while their interaction was not significant. Silages ensiled with an inoculant showed an average 0.16 °C higher temperature difference than those ensiled without inoculant. On the other hand, the temperature difference in the first two days of the aeration period averaged 1.40 °C, which then decreased and averaged 0.83 °C for the remainder of the aeration period.

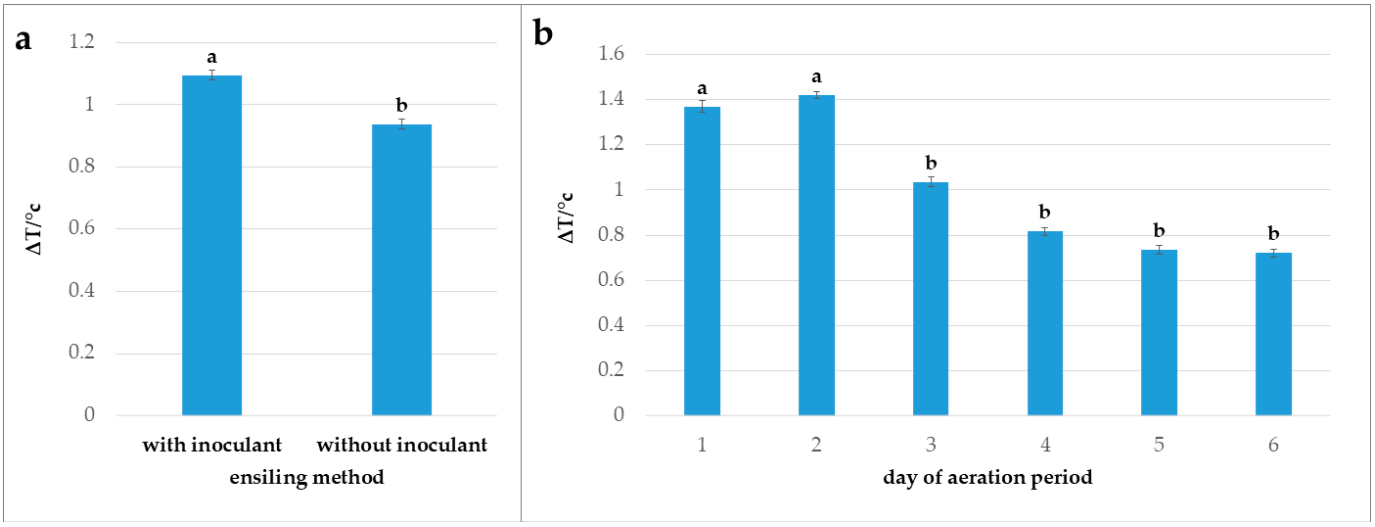

Figure 2. Difference in the average daily temperature difference between silages and environment for the ensiling methods (**a**) and days of the aeration period (**b**). [a,b] Columns with different letters indicate the differences between the ensiling methods or days of aeration ($p < 0.05$). Error bars represent standard errors.

3.2. Content of Acids and Alchocols During the Aeration Period

The content of acids and alcohols was monitored during each day of the aeration period. Neither isobutyric acid and butyric acid nor propanol and 1,2-propanediol were detected in the silages during aeration. In contrast, the ensiling method, i.e., the addition of an inoculant containing *L. plantarum*, affected the content of lactic acid, acetic acid, propionic acid, methanol, and ethanol ($p < 0.001$; Figure 3). Silages ensiled with the inoculant contained more lactic acid (14.64 vs. 7.69 g/kg DM), while a higher content of acetic acid and propionic acid was detected in silages ensiled without the inoculant (1.87 vs. 0.79 g/kg DM and 2.86 vs. 0.21 g/kg DM, respectively).

The day of the aeration period affected the content of lactic acid, acetic acid, methanol, and ethanol ($p < 0.001$). After the beginning of aeration, the content of these compounds decreased in the first two days of the aeration period, resulting in the lowest contents detected in the silages (lactic acid—5.22 g/kg DM, acetic acid—0.58 g/kg DM, methanol—4.25 g/kg DM, and ethanol—1.58 g/kg DM). Thereafter, the content of these compounds increased, but not to the content at the beginning of the aeration period [methanol (6.70 vs. 5.19 g/kg DM) and ethanol (6.43 vs. 2.84 g/kg DM)] or to the similar content as at the beginning of the aeration period [lactic acid (12.56 vs. 14.35 g/kg DM) and acetic acid (1.55 vs. 1.60 g/kg DM)]. In addition to the effects of the ensiling method and the day of the aeration period, an interaction between these two factors was found for contents of lactic acid, acetic acid, and methanol ($p < 0.05$), which is consistent with the greater changes during the aeration period for different ensiling methods. These changes were most pronounced for lactic acid in silages ensiled with an inoculant and for acetic acid and methanol in silages without an inoculant (Figure 3).

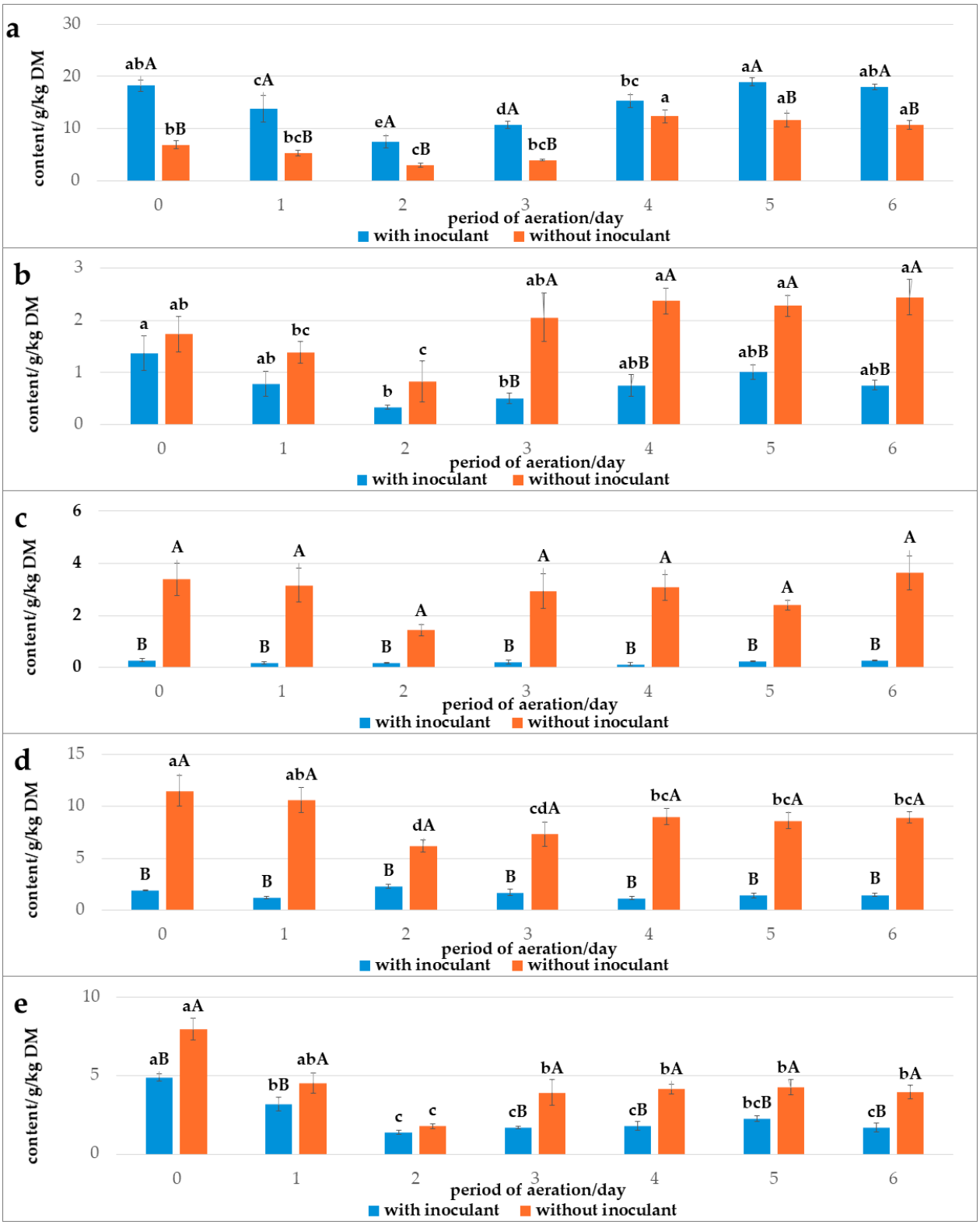

Figure 3. Content of lactic acid (**a**), acetic acid (**b**), propionic acid (**c**), methanol (**d**), and ethanol (**e**) in silages ensiled with and without inoculant containing *Lactiplantibacillus plantarum* during the aeration period. $^{a–e}$ Columns with different lowercase letters indicate differences between days of the aeration period within the same ensiling method ($p < 0.05$). ^{A,B} Columns with different capital letters indicate differences between ensiling methods on the same day of the aeration period ($p < 0.05$). Error bars represent standard errors.

3.3. pH and Contents of Main Nutrients at the Beginning and End of Aeration

The pH and contents of DM, CP, SCP, WSC, and starch were determined in silages at the beginning and end of the aeration period. The ensiling method, i.e., ensiling with an inoculant containing *L. plantarum*, only affected the pH of the silages. As expected, silages ensiled with an inoculant had a lower pH than silages ensiled without an inoculant (4.07 vs. 4.74; $p < 0.001$; Table 1). The differences between the beginning and the end of the aeration period, i.e., the effect of aeration, were detected in the contents of SCP and starch ($p < 0.05$). Silages at the end of aeration had a higher content of SCP (337.3 vs. 266.4 g/kg DM) and a lower content of starch (693 vs. 710 g/kg DM) compared to the beginning of aeration. In addition, there was a tendency for lower pH (4.30 vs. 4.52; $p = 0.096$) and higher CP content (114.2 vs. 107.8 g/kg DM; $p = 0.099$) in silages at the end of aeration compared to the beginning of aeration (Table 1).

Table 1. The properties of silages ensiled with and without inoculant containing *Lactiplantibacillus plantarum* at the beginning and end of the aeration period.

Ensiling Method	Aeration		SEM	*p*		
	Beginning	End		Ensiling Method	Aeration	Ensiling Method × Aeration
			pH			
With inoculant	4.14	4.01	0.121	<0.001	0.096	0.471
Without inoculant	4.89	4.59				
			DM/g/kg			
With inoculant	693	704	8.6	0.218	0.766	0.133
Without inoculant	695	679				
			CP/g/kg DM			
With inoculant	106.6	112.6	3.65	0.445	0.099	0.914
Without inoculant	109.0	115.8				
			SCP/g/kg CP			
With inoculant	275.5	321.3	13.6	0.619	<0.001	0.084
Without inoculant	257.3	353.3				
			WSC/g/kg DM			
With inoculant	23.47	10.69	11.3	0.293	0.221	0.877
Without inoculant	37.67	21.32				
			Starch/g/kg DM			
With inoculant	709	697	6.20	0.703	0.015	0.401
Without inoculant	712	689				

DM—dry matter, CP—crude protein, SCP—soluble crude protein, WSC—water-soluble carbohydrates.

4. Discussion

The breakdown of nutrients in silage during aeration can be clearly seen with an increase in silage temperature, as the microorganisms break down the nutrients and generate heat [4]. In the present study, the temperature differences between the silages and the environment were consistently below 3 °C for all silages, regardless of the ensiling method, during the entire duration of the experiment, which corresponds to the DLG guidelines and their definition of aerobically stable silage [6].

In the present study, however, the silages with the addition of facultative heterofermentative *L. plantarum* had significantly higher temperatures than the control silages (as shown for the average daily temperature difference between silages and the environment in Figure 2). Inoculation with facultative heterofermentative LAB is often associated with the rapid production of lactic acid, but some authors also associate it with lower aerobic

stability compared to control silages, as this type of inoculation lacks the production of acetic acid [8].

Indeed, the inoculated silages in the present trial had a 21% lower acetic acid content at opening than the control silages (Figure 3b) and were comparable to Filya [19], Wardynski et al. [20], and Da Silva et al. [21], who showed a lower acetic acid content in silages inoculated with *L. plantarum* compared to the control silages (76%, 17%, and 5%, respectively). However, similar to the present study, Da Silva et al. [21] found the same aerobic stability in control silages and silages inoculated with *L. plantarum*. In contrast, Filya reported a significantly lower stability of *L. plantarum* silages compared to that of control silages [19]. In a study with corn silages and an aerobic stability test over 5 days, Filya had a 6% higher pH in inoculated silages compared to control silages [19]. These results are not consistent with the results of the present study, as the pH of the inoculated silages at the end of aeration was significantly lower compared to that of the control (Table 1, 15% lower pH in the inoculated silages). Similar to the results in the present study, Ranjit and Kung [22] reported lower pH in corn silages inoculated with two different strains of *L. plantarum* (*L. plantarum* 30114 and *L. plantarum* 30115) on day 5 of aeration compared to the control silages: 3.88, 4.85, and 5.02, respectively. This shows that although temperature is a crucial parameter in the evaluation of aerobic stability, it is not the only determinant. As Liu et al. [23] stated, aerobic stability can be evaluated by chemical parameters, such as pH and the amount of lactic acid and VFA in aerated silages, as well as by temperature variations.

The acids contained in silage act as antimicrobial agents. They control what happens in silage at this delicate moment. Lactic acid, whose concentration corresponds to the pH value of silage, is the most important acid. The more lactic acid present, the lower the pH value, and a low pH value inhibits the activity of microorganisms [2,3]. The lactic acid content in high-moisture corn silages varies between 5 and 20 g/kg DM [3], and the lactic acid levels in both inoculated and control silages in the present study were within this range. It is generally believed that the activity of homofermentative/facultative heterofermentative LAB is associated with higher lactic acid production and acidity, which is reflected by a lower pH [2,3]. Consistent with this general view, the *L. plantarum* silages in the present study had significantly higher lactic acid content (Figure 3a) and lower pH (Table 1) compared to the control silages. Other authors, such as Wardynski et al. [20], Filya [19], Hu et al. [24], and Da Silva et al. [21] have also found higher lactic acid contents in inoculated corn silages when using *L. plantarum* compared to the control (63%, 53%, 22%, and 6% higher lactic acid content, respectively).

A reduction in lactic acid content during aeration is expected [4]; however, distinctive changes in the content of acids during aeration are often neglected, and few studies have evaluated the changes in acids during aeration. Kljak et al. [25] showed that lactic acid content decreases in rehydrated corn silages on day 10 of aeration. In the same study, the silages showed a stable lactic acid concentration from day 0 to day 5 of aeration. Furthermore, Dawson et al. [26] showed an increase in lactic acid from 7.5 to 16.5 g/kg DM during the 5-day aeration of high-moisture corn ensiled with *Propionibacteriumacidi propionici* DH42 and a decrease in lactic acid from 7.2 to 5 g/kg DM in control silages. The authors explained this increase in lactic acid content with the higher aerobic stability of corn ensiled with *P. propionici* DH42 [26]. Canibe et al. [27] also showed that the patterns of lactic acid changes differed depending on the DM of the silages. The authors reported a constant reduction in lactic acid content during the one-week aeration in the control and independent of the additive used (acid additive, homofermentative LAB inoculant, heterofermentative LAB inoculant). However, in high-moisture corn with higher DM, the reduction was constant during all sampling time points, while in high-moisture corn with lower DM, a different pattern was observed. The latter observation was similar to the changes in lactic acid content in the present study, where the content decreased until day 3 of aeration and then increased in both *L. plantarum* silages and the control (Figure 3). Similarly, Canibe et al. [27] showed a reduction in lactic acid, followed by an increase in

silages with low DM content, regardless of the additive used. The authors related the observed differences in lactic acid degradation to the DM content of the silages, with a higher DM content leading to higher lactic acid degradation. It is worth mentioning that in the trial by Canibe et al. [27], high-moisture corn with a low DM content had a 75% higher content of acetic acid than high-moisture corn with a high DM content.

Acetic acid is the second most important acid in silage in terms of quantity; however, its content should be below 5 g/kg DM in high-moisture corn [3], as an excessive amount can be an indicator of high nutrient degradation and secondary fermentation [2,3]. In the present study, the acetic acid content was below 2.5 g/kg DM and was lower in the silages inoculated with *L. plantarum* than in the control silages (Figure 3b). The activity of facultative heterofermentative LAB *L. plantarum* is associated with a higher production of lactic acid and faster acidification, which also inhibits the synthesis of acetic acid [2]. However, acetic acid is important for aerobic stability, as it controls yeasts, the microorganisms that metabolize lactic acid at the onset of aeration [3,4]. In his trial with 15-day-old corn silage, Filya [19] showed that the amount of acetic acid in *L. plantarum* silages is 76% lower compared to control silages, while it is 205% higher in *L. buchneri* silages. In the same study, *L. buchneri* silages showed the highest aerobic stability, with the lowest pH and CO_2 production [19]. Ensiling with the obligate heterofermentative LAB inoculant *L. buchneri* is often associated with higher acetic acid production and longer aerobic stability [8].

In the study by Da Silva et al. [21], corn silages inoculated with *L. buchneri*/*L. plantarum* combination showed a 21–33% higher acetic acid content on opening and a 39–41% higher aerobic stability compared to control silages, depending on the *L. buchneri*/*L. plantarum* combination was used. Interestingly, however, Pang et al. [28] showed that acid degradation during aeration is more intense in corn silages ensiled with *L. buchneri* than in control silages, even if the acetic acid content is higher. In the same study, the contents of lactic and acetic acid in corn silages ensiled with *L. buchneri* were reduced from 71.7 to ND and from 18.56 to 4.21 g/kg DM, respectively, during the one-week aeration, while in the control silage, they were in ranges 54.83–4.33 and 16.16–10.65 g/kg DM, respectively [28]. On the other hand, the corn silages ensiled with two different *L. plantarum* strains in the study by Wardynski et al. [20] had different acetic acid contents: 2.04 g/kg DM for *L. plantarum* 30114 and 1.68 g/kg DM for *L. plantarum* 30115, while the control had 1.82 g/kg DM. The authors reported a longer aerobic stability for both inoculated silages than for the control, 32.8 and 33 vs. 26.5 h ($p < 0.05$), indicating that the amount of acetic acid is not the main factor for aerobic stability.

In the present study, the degradation of acetic acid in silages inoculated with *L. plantarum* showed a similar pattern to that of the control silages. This observation was similar to the degradation of high-moisture corn observed by Canibe et al. [27], where silages with lower DM content initially showed a decrease and then an increase in acetic acid content, regardless of the additive used (acid additive, homofermentative LAB inoculant, or heterofermentative LAB inoculant). At the same time, and in contrast to the present results, the low DM corn silages showed a reduction in acetic acid content during aeration [27] and are similar to the results of Dawson et al. [26], where acetic acid content was reduced from 2.9 to 1.7 g/kg DM in the control and from 6.3 to 4.8 g/kg DM in high-moisture corn ensiled with *P. propionici* DH42.

The other two VFAs, propionic acid and butyric acid, also have antifungal properties [29,30]; however, their presence in silage is an indicator of negative secondary fermentation [3]. Butyric acid is an indicator of Clostridia activity in silage, and this acid should not be present in high-moisture corn silage [3]. No butyric acid was detected in the silages in the present trial, regardless of the ensiling method. Propionic acid should not exceed 1 g/kg DM in high-quality high-moisture corn silage, and silages with high propionic acid content (more than 3–5 g/kg DM) can be assumed to have negative Clostridia activity [3]. Clostridia activity in silages leads to high DM losses and poor energy utilization as well as health problems in this type of silage [31]. Sometimes, both propionic acid and acetic acid can be products of propionic acid bacteria activity [26]; however, this type of bacteria

is present in low numbers in most silages [3]. Higher levels of propionic acid are found when propionic acid bacteria are used as inoculants [26], or when propionic acid is added as an acid additive during ensiling [2,3]. In the present study, the ensiling method had a significant effect on propionic acid content, which was almost 10 times higher in the control silages (Figure 3c), as another indicator of heterofermentative activity in the control silages [3].

Alcohols are normally found in silages [3]. Ethanol is a product of different types of microorganisms, such as heterofermentative LAB, and various yeasts can produce ethanol [2]. Methanol is mainly the result of the activity of plant enzymes before ensiling as a product of pectin demethylation during leaf expansion and cell wall synthesis [32]. The ethanol content in high-moisture corn should be between 2 and 20 g/kg DM, while a higher content could be an indicator of high yeast activity in the silages and possibly lower aerobic stability [3].

Both the silages inoculated with *L. plantarum* and the control silages in the present study had ethanol contents in the range of the optimal high-moisture corn silage, although the ethanol content of the control silages was significantly higher (Figure 3e). A lower ethanol content in *L. plantarum* silages is expected, as fermentation with facultative heterofermentative LAB inoculants mainly leads to lactic acid production [8,20,26]. However, in some silages inoculated with *L. plantarum*, the ethanol content corresponds to that of the control silages [19,21,24] or is even higher [33]. This provides further evidence that ethanol is a product of several microorganisms. The reduction in ethanol during aeration of the silages, which was observed in the present study regardless of the ensiling method (Figure 3e), corresponds to the changes in ethanol in Dawson et al. [26], where ethanol was reduced from 16.7 to 0.5 g/kg DM in the control silages and from 11.3 to 3 g/kg DM in the inoculated silages. This also corresponds to Kljak et al. [25], where ethanol was reduced 10-fold in various rehydrated corn silages that were aerated for 10 days. In the same study, the methanol content stagnated during the 10-day aeration, which corresponds to the changes in methanol content during aeration in the present study (Figure 3d). In the present study, the methanol content was higher in the control silages than in the silages inoculated with *L. plantarum*, which is a further indication of homolactic fermentation with facultative heterofermentative LAB inoculants [2].

In general, it is considered that silages exposed to air lead to the degradation of the main nutrients [4], but changes in their contents have not been commonly determined in studies investigating the aerobic stability of high-moisture corn. Therefore, the changes in DM, CP, WCP, WSC, and starch contents in the silages were determined at the beginning and end of the 6 days aeration period. Although the effect of the ensiling method was not found, aeration had an effect on the SCP and starch content. Ensiling has a major influence on the nutritional quality of corn [34], as it leads to higher degradability of starch in the rumen [3,35]. During ensiling, starch becomes more available to enzymes and microorganisms due to the degradation of the proteins surrounding the starch granules [36,37]. The more available starch could also lead to higher starch degradation during aeration, as starch is more available for aerobic microorganisms. The results of the present study show that starch degradation during aeration occurs in all silages, although the silages were aerobically stable. This observation could also have a major impact on the nutritional quality of corn silage. Another possible indicator of the higher availability of starch is the soluble protein. When proteins are degraded, the soluble protein fraction (SCP) increases [38]. In the present trial, the SCP actually increased during aeration, which is similar to the results obtained by other authors in a trial with aerated, rehydrated corn silage [25].

5. Conclusions

Even if silages are classified as aerobically stable on the basis of temperature rise, there are significant changes in the characteristics of silages; therefore, changes in lactic and acetic acid should be added as a standardized method to classify the aerobic stability of silages, which is often neglected.

The results of this trial show that the general rule that facultative heterofermentative LAB are associated with lower aerobic stability in silages should not be taken as a tenet. If the acidity of silage is maintained during aeration, it will remain stable. This raises questions and makes future studies on the aerobic stability of silages ensiled with facultative heterofermentative LAB necessary to obtain an accurate overview of this type of silage.

Author Contributions: Conceptualization, M.D.; methodology, M.D. and K.K.; validation, M.D. and K.K.; formal analysis, M.D.; investigation, M.D.; resources, M.D. and I.B.; data curation, K.K. and I.B.; writing—original draft preparation, M.D.; writing—review and editing, K.K. and I.B.; visualization, K.K.; supervision, M.D.; project administration, M.D.; funding acquisition, M.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Science, Education, and Sports of the Republic of Croatia (research grant 178–1780496–0368, 'Nutritional, antioxidant, and prebiotic attributes of corn for domestic animals').

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors upon request.

Conflicts of Interest: The authors declare no conflicts 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.

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