

EFFECTS OF DIFFERENT MAIZE CULTIVATION SYSTEMS AND MICROBIAL INOCULATION ON THE AEROBIC STABILITY OF MAIZE SILAGE

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ABSTRACT

Freshly chopped whole-plant maize cultivated under narrow-row spacing (0.375 m) and conventional row spacing (0.75 m) was ensiled in laboratory-scale mini silos with or without a microbial inoculant containing *Lactococcus lactis* and *Lentilactobacillus buchneri*. The aim of the study was to compare the fermentation characteristics and quality parameters of silages produced under the two cultivation systems after six days of aerobic exposure. In addition, the effect of microbial inoculation on the aerobic stability of maize silage was evaluated.

Silages produced from maize grown under narrow-row cultivation had a significantly lower ($p < 0.05$) dry matter content than silages produced under the conventional cultivation system. The untreated narrow-row silage also contained significantly less ($p < 0.05$), crude fibre (205.48 ± 0.59 g/kg DM) than the untreated conventional-row silage (218.46 ± 1.08 g/kg DM). In both inoculated silages, the proportion of lactic acid in total fermentation acids was significantly lower (51.59 % and 52.04 %; $p < 0.05$), whereas the proportion of acetic acid was significantly higher (45.90 % and 45.08 %; $p < 0.05$) than in the untreated controls.

After six days of aerobic exposure, the untreated narrow-row silage exhibited the highest pH (5.82; $p < 0.05$). In contrast, silages treated with the microbial inoculant maintained a stable pH of approximately 4.0 throughout the aerobic exposure period. The untreated narrow-row silage also reached the highest temperature (38 °C), indicating intensive aerobic deterioration. Inoculation with a microbial inoculant containing *L. lactis* and *L. buchneri* significantly improved the aerobic stability of maize silage by reducing both silage heating and pH increase during aerobic exposure.

Keywords: maize silage; microbial inoculants; aerobic stability; row spacing; fermentation



INTRODUCTION

Maize cultivated under narrow-row spacing has attracted increasing attention because of its potential to improve crop performance under changing climatic conditions. Narrow-row cultivation promotes earlier canopy closure, thereby reducing soil water evaporation compared with conventional row spacing (Barbieri et al., 2012). In addition, denser plant stands help suppress weed growth and reduce the risk of soil erosion (Barbieri et al., 2008). Previous studies have shown that row spacing and plant density can influence both the yield and morphological characteristics of maize intended for silage production (Baron et al., 2006). These changes may subsequently affect the chemical composition, fermentation characteristics, and feeding value of maize silage.

Maize silage is one of the most important forage sources for ruminants worldwide owing to its high energy concentration, good palatability, and high dry matter yield (Pasa & Pasa, 2015). Whole-plant maize is generally harvested at a dry matter content of 30–35%, which provides favourable conditions for lactic acid fermentation and ensures efficient preservation of the forage (Kung et al., 2000). Successful ensiling depends on the rapid establishment of anaerobic conditions and the predominance of desirable lactic acid bacteria, resulting in a rapid decline in pH and inhibition of undesirable microorganisms.

Once the silo is opened, however, silage is exposed to oxygen, initiating a series of microbial and biochemical processes collectively referred to as aerobic deterioration. Yeasts are typically the first microorganisms to metabolise residual water-soluble carbohydrates and lactic acid, leading to heat production, carbon dioxide release, alcohol (especially ethanol), nutrient losses, and a gradual increase in silage pH (Muck, 2010). As aerobic deterioration progresses, the elevated pH creates favourable conditions for the growth of moulds and other spoilage microorganisms, resulting in further deterioration of silage quality (Woolford, 1990). Aerobic stability is therefore considered one of the key indicators of silage quality and is commonly defined as the time required for silage temperature to increase by 2 °C above ambient temperature after exposure to air (Kung et al., 2000).

Microbial inoculants are widely used to improve silage fermentation and enhance aerobic stability. Homofermentative bacteria, such as *Lactococcus lactis*, rapidly convert water-soluble carbohydrates into lactic acid, promoting a rapid decline in pH during the initial stage of fermentation (Hindrichsen et al., 2012). In contrast, heterofermentative species, particularly *Lentilactobacillus buchneri*, convert part of the lactic acid into acetic acid, which possesses strong antifungal activity and effectively inhibits the growth of yeasts and moulds during feed-out (Comino et al., 2014). Numerous studies have demonstrated that inoculants containing *L. buchneri*, either alone or in combination with other bacterial species, substantially improve the aerobic stability of maize silage by increasing acetic acid production (Ranjit & Kung, 2000; Tabacco et al., 2011; Silva et al., 2014; Kok et al., 2024).

The present study was conducted within the framework of the NAZV research project QL24010237 entitled *Cultivation of Maize Using Narrow-Row Technology in a Precision Agriculture System (Agriculture 4.0) under Climate Change Conditions – Stabilisation of Forage Production and Sustainable Intensification of Agricultural Production in the Czech Republic*. The objective of this study was to evaluate the effects of two maize cultivation systems and microbial inoculation with *L. lactis* and *L. buchneri* on the fermentation characteristics and aerobic stability of maize silage following six days of aerobic exposure.

MATERIAL AND METHODS

Plant material and silage preparation

Maize (*Zea mays* L.) hybrid KWS Monumento was cultivated at the experimental field station in Žabčice (Czech Republic) using two row-spacing systems: narrow-row cultivation (0.375 m) and conventional cultivation (0.75 m). The crop was harvested at a stubble height of 20–25 cm and chopped to a theoretical length of cut of 10 mm.

Freshly chopped forage from each cultivation system was ensiled in laboratory-scale silos (mini silos) in six replicates. Half of the silos were treated with a microbial inoculant containing *Lactococcus lactis* and *Lentilactobacillus buchneri*, while the remaining silos served as untreated controls. All silos were stored at an ambient temperature of 20–25 °C for five months before being opened for laboratory analyses.

Chemical analyses

After opening, the silages were analysed for dry matter (DM), pH, starch, acidity of the aqueous extract (KVV), lactic acid, volatile fatty acids (VFA), crude protein (CP), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), and ash content. Chemical analyses were performed according to Commission Regulation (EC) No. 152/2009, as amended by Commission Implementing Regulation (EU) 2024/771, which specifies official methods for feed sampling and laboratory analysis. All analyses were carried out at the Institute of Animal Nutrition and Forage Science, Mendel University in Brno.

Lactic acid and volatile fatty acids were determined by isotachopheresis (INOSEP 2017-2).

Determination of Ammonia Content

Ammonia concentration was determined using the Conway microdiffusion method. Approximately 5 mL of boric acid solution was placed in the central compartment of each Conway dish, while 10 mL of the aqueous silage extract and 1 mL of potassium carbonate solution were added to the outer compartment. The rim of each dish was sealed with glycerol, and the dishes were immediately closed to prevent ammonia losses.

After gentle mixing of the outer compartment, the dishes were incubated at room temperature for 20 h. Subsequently, Tashiro indicator was added to the boric acid solution, and the absorbed ammonia was quantified by titration with 0.05 N sulphuric acid until a stable pale-pink endpoint was reached.

Ammonia concentration was calculated using the following equation:

$$NH_3(\%) = V_{H_2SO_4} \times 0.0918 \times f_{H_2SO_4}$$

where

- $V_{H_2SO_4}$ = volume of 0.05 N sulphuric acid consumed during titration
- $f_{H_2SO_4}$ = correction factor of the sulphuric acid solution.

Preparation of Extracts

For each silage sample, two subsamples (50 g each) were homogenised with 450 mL of distilled water for 3 min using a blender. The homogenate was subsequently filtered through a 119- μ m (UHELON 59 S, SILK & PROGRESS, spol., s.r.o., Czech Republic) laboratory sieve and then through qualitative filter paper (FILTRAK 388) to obtain a clear aqueous extract for further analyses.

Aerobic stability

Aerobic stability was evaluated by continuously monitoring silage temperature during aerobic exposure. After the initial analyses, 350 g of silage from each treatment was transferred into individual polyethylene containers and exposed to air under laboratory conditions. Temperature probes were inserted into the centre of each sample, and silage temperature was recorded automatically at 1-h intervals.

Following six days of aerobic exposure, the silage samples were analysed again for DM, pH, KVV, ammonia concentration, lactic acid, volatile fatty acids, and basic chemical composition.

Statistical analysis

Data were analysed using a two-way analysis of variance (ANOVA), with cultivation system and microbial inoculation as the main experimental factors. Treatment means were compared using Tukey's honestly significant difference (HSD) test. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

As shown in Table 1, silages produced from maize cultivated under the narrow-row system exhibited lower dry matter (DM) contents (350.0 and 352.6 g/kg) than silages produced under the conventional cultivation system, in which DM contents reached 397.7 and 389.96 g/kg.

The highest crude protein (CP) concentration was observed in the inoculated narrow-row silage (77.31 ± 0.09 g/kg DM; $p < 0.05$), whereas the untreated silage

produced from conventionally grown maize had the lowest CP content (67.55 g/kg DM). Similar increases in crude protein following inoculation with *L. buchneri* have previously been reported by SILVA et al. (2014) and BUMBIERIS Jr. et al. (2017). A comparable trend was observed in the present study, where microbial inoculation increased the crude protein content in both cultivation systems.

The untreated narrow-row silage also contained significantly less crude fibre (205.48 ± 0.59 g/kg DM) than the untreated conventional-row silage (218.46 ± 1.08 g/kg DM; $p < 0.05$).

Results obtained from the experiments, including their statistical evaluation and commentary, should be presented graphically or in table-form and the author should comment on the results and confront them with data published elsewhere.

The fermentation characteristics of the silages after opening are presented in Table 2. The lowest pH value (3.83; $p < 0.05$) was recorded in the untreated narrow-row silage, whereas the highest pH (3.96) was observed in the inoculated silage produced under the conventional cultivation system.

Barry et al. (1980) reported that lactic acid accumulation during fermentation lowers silage pH, thereby suppressing undesirable microbial activity and improving nutrient preservation. In the present study, the acidity of the aqueous extract was significantly higher in inoculated silages (1952.0 ± 193.82 and 2045.7 ± 116.16 mg KOH/100 g) than in the untreated controls ($p < 0.05$), indicating more intensive fermentation.

The concentrations and proportions of fermentation products are summarised in Table 3. According to SHAVER (2003), lactic acid should account for approximately 65–70% of total fermentation acids in well-preserved maize silage. This criterion was fulfilled by both untreated silages, in which lactic acid represented approximately 80% of total fermentation acids. In contrast, the inoculated silages contained significantly lower proportions of lactic acid (51.59% and 52.04%; $p < 0.05$). The reduced proportion of lactic acid resulted from the activity of the heterofermentative bacterium *L. buchneri*, which promoted the conversion of lactic acid into acetic acid. Consequently, the proportion of acetic acid increased significantly, reaching 45.90% and 45.08% in the inoculated silages.

The lactic acid concentration in well-fermented maize silage generally ranges from 2 to 4% of DM. In the present study, lactic acid concentrations ranged from 3.46 to 4.45% DM, which is consistent with values reported for high-quality maize silages

Krooneman et al. (2002) reported that acetic acid concentrations typically range between 1 and 3% DM. In the present experiment, untreated silages contained less than 1% acetic acid, whereas inoculated silages contained significantly higher concentrations (3.19% and 2.99% DM; $p < 0.05$).

These findings agree with those of Kung and Ranjit (2000), who demonstrated that inoculation with *L. buchneri* significantly increased acetic acid production. Similar results were reported by Kok et al. (2024), who observed enhanced acetic

Tab. 1: Chemical composition of maize silage immediately after silo opening

Row spacing (metre)		0.375		0.75		0.375		0.75	
Treatment		Control		Control		Lc. Lactis + Len buchneri		Lc. Lactis + Len buchneri	
n		6		6		6		6	
Variables	units	Mean $\pm$ SD		Mean $\pm$ SD		Mean $\pm$ SD		Mean $\pm$ SD	
DM	g/kg	350.00 ^a $\pm$ 0.6390		397.70 ^b $\pm$ 0.5623		352.60 ^a $\pm$ 0.9997		389.36 ^b $\pm$ 0.8084	
Ash	g/kg	35.48 ^a $\pm$ 0.0934		38.70 ^b $\pm$ 0.0688		38.53 ^b $\pm$ 0.0752		40.06 ^c $\pm$ 0.0611	
Crude protein	g/kg	71.61 ^b $\pm$ 0.1729		67.55 ^a $\pm$ 0.1967		77.31 ^c $\pm$ 0.0941		70.26 ^b $\pm$ 0.1324	
Crude fat	g/kg	26.57 ^a $\pm$ 0.1396		26.37 ^a $\pm$ 0.1306		29.84 ^b $\pm$ 0.0842		29.45 ^b $\pm$ 0.0826	
Starch	g/kg	304.60 ^a $\pm$ 1.4140		325.42 ^{ab} $\pm$ 1.0578		322.02 ^{ab} $\pm$ 2.1329		332.50 ^b $\pm$ 1.4700	
Crude fiber	g/kg	205.48 ^a $\pm$ 0.5851		218.46 ^b $\pm$ 1.0843		213.68 ^{ab} $\pm$ 0.6007		214.94 ^{ab} $\pm$ 0.7174	
ADF	g/kg	247.98 ^a $\pm$ 0.8371		273.64 ^d $\pm$ 0.5510		260.30 ^{ab} $\pm$ 0.7660		288.92 ^c $\pm$ 1.0415	
aNDF	g/kg	416.36 ^c $\pm$ 1.7677		457.23 ^{ab} $\pm$ 0.8125		444.50 ^a $\pm$ 1.2553		468.38 ^b $\pm$ 1.3003	

Means of value in a row marked with different letters are significantly different between groups ($p < 0.05$), Tukey's HSD test, n – number of cases, SD, standard deviation; DM, dry matter; aNDF, neutral detergent fiber determined with α -amylase; ADF, acid detergent fib

Tab. 2: Fermentation characteristics of maize silage immediately after silo opening

Row spacing (metre)		0.375		0.75		0.375		0.75	
Treatment		Control		Control		Lc. Lactis + Len buchneri		Lc. Lactis + Len buchneri	
n		6		6		6		6	
Variables	units	Mean $\pm$ SD		Mean $\pm$ SD		Mean $\pm$ SD		Mean $\pm$ SD	
pH	g	3.83 ^a $\pm$ 0.03688		3.89 ^b $\pm$ 0.02137		3.89 ^{ab} $\pm$ 0.01789		3.96 ^c $\pm$ 0.05820	
WEA mg KOH/100	g	1383.9 ^a $\pm$ 65.93		1382.1 ^a $\pm$ 78.16		1952.0 ^b $\pm$ 193.82		2045.7 ^b $\pm$ 116.16	
NH ₃	%	0.0372 $\pm$ 0.0142		0.0372 $\pm$ 0.0144		0.0389 $\pm$ 0.0131		0.0601 $\pm$ 0.0408	
NH ₃ – N	%	0.0307 $\pm$ 0.0117		0.0307 $\pm$ 0.0119		0.0321 $\pm$ 0.0108		0.0495 $\pm$ 0.0336	

Means of values in a row marked with different letters are significantly different between groups ($p < 0.05$), Tukey's HSD test, n – number of cases, SD, standard deviation; WEA, water extract acidity; NH₃, ammonium; NH₃-N, ammonia nitrogen.

Tab. 3: Fermentation products of maize silage immediately after silo opening

Row spacing (metre)		0.375	0.75	0.375	0.75
Treatment		Control		<i>Lc. Lactis</i> + <i>Len buchneri</i>	
n		11		12	
Variables	units	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
LA/VFA	% of DM	4.01 ^b $\pm$ 0.2238	4.16 ^b $\pm$ 0.5497	1.09 ^a $\pm$ 0.2243	1.10 ^a $\pm$ 0.1659
LA	% of DM	4.45 ^c $\pm$ 0.6413	4.27 ^{bc} $\pm$ 1.1702	3.58 ^{ab} $\pm$ 0.5107	3.46 ^{ab} $\pm$ 0.5852
AA	% of DM	0.91 ^a $\pm$ 0.1485	0.80 ^a $\pm$ 0.2578	3.19 ^b $\pm$ 0.5273	2.99 ^b $\pm$ 0.5336
PA	% of DM	0.12 ^c $\pm$ 0.0180	0.11 ^{ac} $\pm$ 0.0261	0.08 ^b $\pm$ 0.0168	0.09 ^{ab} $\pm$ 0.0276
BA	% of DM	0.08 $\pm$ 0.0232	0.14 $\pm$ 0.2054	0.09 $\pm$ 0.0097	0.10 $\pm$ 0.0116
VFA _s	% of DM	1.11 ^a $\pm$ 0.1594	1.05 ^a $\pm$ 0.3538	3.37 ^b $\pm$ 0.5483	3.19 ^b $\pm$ 0.5604
Total organic acids	% of DM	55.56 ^{ac} $\pm$ 7.9338	53.21 ^c $\pm$ 14.6952	69.51 ^b $\pm$ 8.4874	66.42 ^{ab} $\pm$ 9.9396
Proportion of LA	%	80.03 ^b $\pm$ 0.9271	80.36 ^b $\pm$ 2.7786	51.59 ^a $\pm$ 4.6586	52.04 ^a $\pm$ 4.0002
Proportion of AA	%	16.36 ^a $\pm$ 0.5149	14.72 ^a $\pm$ 1.3765	45.90 ^b $\pm$ 4.5690	45.08 ^b $\pm$ 4.0262
Proportion of PA	%	2.14 ^b $\pm$ 0.2972	2.20 ^b $\pm$ 0.4314	1.20 ^a $\pm$ 0.1495	1.32 ^a $\pm$ 0.3134
Proportion of BA	%	1.47 ^{bc} $\pm$ 0.6509	2.73 ^c $\pm$ 3.3040	1.31 ^a $\pm$ 0.1031	1.55 ^{ab} $\pm$ 0.1151

Means of values in a row marked with different letters are significantly different between groups ($p < 0.05$), Tukey's HSD test, n – number of cases, S – Standard deviation, DM, dry matter; LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, butyric acid; VFA_s, volatile fatty acids; LA/VFA ratio, ratio of lactic acid to volatile fatty acids. Total organic acids represent the sum of lactic, acetic, propionic and butyric acids

acid production and improved aerobic stability in silages treated with *L. lactis* and *L. buchneri*. As expected, propionic acid was absent or detected only in trace amounts, which is typical of well-fermented maize silages with relatively high dry matter contents.

As shown in Table 4, weight loss after fermentation was significantly higher ($p < 0.05$) in the untreated narrow-row silage (21.08 g). Following six days of aerobic exposure, both narrow-row silages contained significantly higher crude protein concentrations than the corresponding conventional-row silages. The crude protein content increased significantly in the untreated narrow-row silage, from 71.61 to 79.98 g/kg DM. Woolford (1990) reported that exposure of silage to air results in the depletion of readily oxidisable forage components. Consequently, structural carbohydrates, crude protein, and ash represent a greater proportion of the remaining dry matter. Similar findings were reported by Tabacco et al. (2011), who observed increases in crude protein and ADF concentrations with increasing duration of aerobic exposure.

According to Rezende et al. (2011), exposure of silage to aerobic conditions is associated with several characteristic changes, including increases in pH, temperature, the proliferation of aerobic microorganisms, and ammonia nitrogen concentration. Similar trends were observed in the present study.

The fermentation characteristics of the silages after opening are presented in Table 5. Following six days of aerobic exposure, ammonia and ammonia nitrogen concentrations increased in all silages except for the untreated narrow-row silage. Bumbieris Jr. et al. (2017) reported that exposure to air promotes the growth of aerobic microorganisms, particularly yeasts and moulds, which utilize nutrients present in the silage. During this process, protein degradation may also occur as a result of the activity of *Clostridium* spp. and other undesirable microorganisms. Deamination of amino acids releases ammonia, contributing to an increase in ammonia nitrogen concentration.

Microbial inoculation effectively prevented the increase in pH during aerobic exposure. In contrast, the pH of untreated silages increased to values exceeding 5.0. The untreated narrow-row silage exhibited the highest pH value (5.82; $p < 0.05$), indicating the greatest degree of aerobic deterioration. According to Kung and Ranjit (2001), an increase in pH following exposure to air is one of the most reliable indicators of aerobic spoilage. Lactate-assimilating yeasts, including species of *Saccharomyces*, *Candida*, *Cryptococcus*, and *Pichia*, are considered the primary initiators of aerobic deterioration because they metabolise lactic acid, thereby eliminating the principal factor responsible for maintaining a low silage pH (Kung & Ranjit, 2000).

The acidity of the aqueous extract was significantly lower ($p < 0.05$) in the untreated silages from both cultivation systems (402.7 ± 360.36 and 672.6 ± 699.84 mg KOH/100 g), reflecting the depletion of fermentation acids during aerobic deterioration.

Tab. 4: Chemical composition of maize silage after six days of aerobic exposure

Row spacing (metre)	0.375	0.75	0.375	0.75
Treatment	Control	Control	<i>Lc. Lactis + Len buchneri</i>	<i>Lc. Lactis + Len buchneri</i>
n	6	6	6	6
Variables	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Fermentation weight loss				
DM	21.08 ^b $\pm$ 14.038	10.68 ^{ab} $\pm$ 3.3075	6.38 ^a $\pm$ 0.9071	6.28 ^a $\pm$ 0.6847
Ash	351.71 ^a $\pm$ 1.1026	405.74 ^b $\pm$ 0.7255	361.61 ^a $\pm$ 0.6567	399.19 ^b $\pm$ 0.2690
Crude protein	39.76 $\pm$ 0.3723	40.95 $\pm$ 0.0758	38.50 $\pm$ 0.1066	40.668 $\pm$ 0.0682
Crude fat	79.98 ^b $\pm$ 0.6110	68.87 ^a $\pm$ 0.1420	76.22 ^b $\pm$ 0.1804	69.56 ^a $\pm$ 0.0735
Starch	27.72 ^a $\pm$ 0.1767	26.48 ^a $\pm$ 0.1610	31.82 ^b $\pm$ 0.0870	28.71 ^a $\pm$ 0.1527
Crude fiber	309.04 $\pm$ 1.6887	318.57 $\pm$ 1.3142	323.09 $\pm$ 1.1828	324.45 $\pm$ 1.1330
ADF	214.53 $\pm$ 1.5247	215.95 $\pm$ 0.6430	203.81 $\pm$ 0.6003	212.45 $\pm$ 0.8066
aNDF	285.28 $\pm$ 2.4305	289.80 $\pm$ 0.9642	271.15 $\pm$ 1.0824	278.98 $\pm$ 0.7347
	458.07 $\pm$ 2.6304	457.96 $\pm$ 1.3385	433.66 $\pm$ 1.2989	450.28 $\pm$ 1.6680

Means of values in a row marked with different letters are significantly different between groups ($p < 0.05$), Tukey's HSD test, n – number of cases, SD, standard deviation; DM, dry matter; aNDF, neutral detergent fiber determined with α -amylase; ADF, acid detergent fiber

Tab. 5: Fermentation characteristics of maize silage after six days of aerobic exposure

Row spacing (metre)	0.375	0.75	0.375	0.75
Treatment	Control	Control	<i>Lc. Lactis + Len buchneri</i>	<i>Lc. Lactis + Len buchneri</i>
n	6	6	6	6
Variables	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
pH	5.82 ^b $\pm$ 1.4017	5.07 ^{ab} $\pm$ 1.0821	3.92 ^a $\pm$ 0.0337	3.98 ^a $\pm$ 0.0316
WEA	402.7 ^a $\pm$ 360.36	672.6 ^a $\pm$ 699.84	2123.7 ^b $\pm$ 161.68	2134.3 ^b $\pm$ 113.29
NH₃	0.0371 $\pm$ 0.0129	0.0484 $\pm$ 0.0138	0.0479 $\pm$ 0.0163	0.0484 $\pm$ 0.0150
NH₃ – N	0.0306 $\pm$ 0.0106	0.0398 $\pm$ 0.0113	0.0395 $\pm$ 0.0134	0.0398 $\pm$ 0.0123

Means of values in a row marked with different letters are significantly different between groups ($p < 0.05$), Tukey's HSD test, n – number of cases, SD, standard deviation; WEA, water extract acidity; NH₃, ammonium; NH₃–N, ammonia nitrogen.

According to Velho et al. (2006), the increase in silage temperature during aerobic exposure is caused by the metabolic activity of spoilage microorganisms. As these microorganisms degrade nutrients, energy is released in the form of heat, resulting in progressive heating of the silage mass.

Table 6 shows the aerobic stability of corn silage after six days of aerobic exposure. In the present study, untreated silages reached higher maximum temperatures than inoculated silages (38.35 and 31.83 °C, respectively), indicating greater susceptibility to aerobic deterioration. Consequently, the aerobic stability of the untreated silages was reduced. In contrast, silages treated with *Lactococcus lactis* and *Lentilactobacillus buchneri* exhibited improved aerobic stability under both cultivation systems with aerobic stability increasing to 79.4 h and 63.4 h, respectively. These findings are consistent with those of KOK et al. (2024), who also reported enhanced aerobic stability in maize silage treated with the same bacterial combination.

According to Doležal et al. (2012), aerobic deterioration inevitably results in nutritional losses. Similarly, the untreated narrow-row silage reached a temperature of approximately 38 °C after six days of aerobic exposure. Muck and Pitt (1993) suggested that prolonged exposure to temperatures of this magnitude may lead to gradual protein denaturation, thereby reducing the nutritional value of the silage.

In the present study, inoculation with *L. lactis* and *L. buchneri* increased the aerobic stability of narrow-row silage by approximately 50 h, whereas the corresponding improvement in conventionally grown maize silage was only 11.8 h. Although these results confirm the positive effect of microbial inoculation on aerobic stability, the observed improvement was considerably lower than that reported by KOK et al. (2024), who achieved an extension of aerobic stability of approximately 150 h.

Tab. 6: Aerobic stability of maize silage after six days of aerobic exposure

Row spacing (metre)	0.375	0.375	0.75	0.75
Treatment	Control	<i>Lc. Lactis</i> + <i>Len buchneri</i>	Control	<i>Lc. Lactis</i> + <i>Len buchneri</i>
Aerobic stability (hours)	28.4	79.4	51.6	63.4
Average environmental temperature	24.20	24.20	24.20	24.20
Maximum temperature	38.35	26.27	31.83	27.66
Maximum pH	5.82	3.92	5.07	3.98

Maize silage is particularly susceptible to aerobic deterioration after silo opening because of its high concentration of water-soluble carbohydrates. The application of microbial inoculants is therefore an effective strategy for improving aerobic stability during the feed-out period. Inoculants containing a combination of homofermentative and heterofermentative lactic acid bacteria are especially suitable for maize silage.

In the present study, maize cultivated under two different row-spacing systems was ensiled with a microbial inoculant containing *Lactococcus lactis* and *Lentilactobacillus buchneri*. Silage produced from maize grown under narrow-row cultivation had a significantly lower dry matter content (350.0 and 352.6 g/kg) than silage produced under the conventional cultivation system (397.7 and 389.96 g/kg; $p < 0.05$). The untreated conventional-row silage contained significantly more crude fibre (218.46 ± 1.08 g/kg DM) but the lowest crude protein concentration (67.55 g/kg DM). The highest pH after fermentation was recorded in the inoculated conventional-row silage (pH 3.96; $p < 0.05$).

Inoculation with *L. buchneri* significantly reduced the proportion of lactic acid (51.59% and 52.04%) while increasing the proportion of acetic acid (45.90% and 45.08%), reflecting the metabolic activity of this heterofermentative species.

Following six days of aerobic exposure, the untreated narrow-row silage exhibited the greatest fermentation losses, the highest pH (5.82), and the lowest aerobic stability. In contrast, silages treated with the microbial inoculant maintained a stable pH of approximately 4.0 and reached lower maximum temperatures during aerobic exposure. Overall, the results demonstrate that microbial inoculation with *L. lactis* and *L. buchneri* effectively enhanced the aerobic stability of maize silage produced under both cultivation systems.

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