Effect of various additives on the fermentation quality of corn silage

Vplyv rôznych aditív na fermentačnú kvalitu kukuričných siláží

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ABSTRACT

This study evaluated the effects of biological silage additives with and without chemical salts on the fermentation parameters of whole plant corn silage. The experiment consisted of five treatments: two with lactic acid bacteria (LAB without chemical salts), two with combined additives (LAB + chemical salts), and a control. Firstly, control (^C) and subsequently facultatively heterofermentative (^A) and obligately heterofermentative (^B) LAB (LAB1: *L. plantarum*^A, *L. buchneri*^B, *P. pentosaceus*^A) were examined. After that, LABCHSE inclusive of obligately homofermentative (^C) and facultatively heterofermentative (^A) LAB (*L. plantarum*^A, *L. salivarius*^C, *E. faecium*^A, *P. acidilactici*^C) + chemical salts (sodium benzoate, potassium sorbate) + enzymes (cellulase, hemicellulase, pentosanase, amylase) were evaluated. The last variant LABCHS with LAB (*L. plantarum*^A, *E. faecium*^A, *P. acidilactici*^C) + chemical salt (potassium sorbate) was consisted. After six weeks of storage, the results confirmed that the application of additives can affect the quality of the fermentation process by decreasing lactic acid as a result of heterofermentative bacteria fermentation, the most strongly marked additive based on *L. buchneri*. The addition of all additives increased the titratable acidity and acetic content, which manifested in a narrowed lactic/acetic acid ratio and an increased pH value because of heterofermentative bacteria fermentation. The addition of additives containing chemical salts affected the fermentation process more favourably compared to the addition of additives without chemical salts, particularly by inhibiting alcohol content.

Keywords: Zea mays L., whole plant, conserved feed, inoculants

ABSTRAKT

salivarius^c, E. faecium^A, P. acidilactici^c), chemické soli (benzoan sodný, sorban draselný), enzýmy (celuláza, hemiceluláza, pentosanáza, amyláza) a aditívum LABCHS s LAB (*L. plantarum^A*, *E. faecium^A*, *P. acidilactici^C*) a chemickou soľou (sorban draselný). Prídavok silážnych aditív na báze rôznych kmeňov LAB ovplyvnil fermentačnú kvalitu kukuričnej siláže. Po šiestich týždňoch skladovania výsledky potvrdili, že aplikácia aditív môže ovplyvniť kvalitu fermentačného procesu znížením množstva kyseliny mliečnej, čo bolo najvýraznejšie pri aditívach na báze *L. buchneri*. Pridaním všetkých aditív sa zvýšila kyslosť vodného výluhu a obsah kyseliny octovej, čo sa prejavilo zúžením pomeru kyseliny mliečnej a kyseliny octovej a zvýšením hodnoty pH. Prídavok aditív obsahujúcich chemické soli ovplyvnil proces fermentácie priaznivejšie v porovnaní s prídavkom aditív bez chemických solí, najmä inhibíciou obsahu alkoholu.

Kľúčové slová: Zea mays L., celá rastlina, konzervované krmivo, inokulanty

INTRODUCTION

The conservation of corn by ensiling is important in the production of silage used as a source of nutrients and energy, especially for the nutrition of ruminants. Corn silage is a stable component of ruminant feed rations throughout the year on high-producing farms (Khan et al., 2014). One way to influence silage quality is the application of silage additives (Alba-Mejía et al., 2016), which successfully improve the fermentation process (Kalúzová et al., 2022). Different groups of silage additives are known, biological on the base LAB (lactic acid bacteria), biological other inoculants (Bacillus species, P. acidipropionici, S. bovis, yeasts), chemicals and enzymes. Biological additives based on LAB are the most used stimulators for fermentation guidance and contain different combinations of the LAB groups (Muck et al., 2018). Chemicals as additives are mainly based on organic acids (formic, benzoic, propionic, sorbic, and acetic acid) and their salts (Weiß et al., 2019). Formic acid produces direct acidification and the suppression of undesired spoilage bacteria, thereby improving the preservation of silage. Benzoic, propionic, sorbic, and acetic acid improve the aerobic stability of silage by directly inhibiting yeasts and moulds to help improve the hygienic quality of silages (Muck et al., 2018). Additives based on chemical salts (benzoate, sorbate, propionate, and formate) are known to be efficient against the Clostridium population (König et al., 2017) and to eliminate yeast activity, thus decreasing ethanol content and enhancing the aerobic stability of the silage. In general, potassium sorbate is more effective than sodium benzoate against yeast (Bernardes et al., 2014). Additives featuring a combination of LAB and chemical salts, with or without enzymes, combine the positive effects of chemical inhibition (inhibition of undesirable microorganisms) and biological stimulation (increase of the LAB in the fermented forage matter) (Zhang et al., 2019). Moreover, the fermentation process can be directed by the silage additives addition on the base of the LAB strains with and without chemical salts, which stimulate fermentation and inhibit the undesirable microorganisms that affect the nutritional and fermentation parameters of silage.

Thus, this study aimed to confirm the effects of biological silage additives with and without chemical salts on the quality of whole-plant corn silage, with a focus on fermentation parameters.

MATERIAL AND METHODS

Silage production

The experiment was carried out in cooperation with the University farm of SUA Kolíňany at the Oponice dairy farm. The corn (*Zea mays* L.) of a late hybrid FAO 450, a type of grain dent, was harvested at a cutting height of 30 cm when the grain was at milky-wax ripeness (1/2 milk line) and then ensilaged. Whole corn plants were processed on a stationary cutter (HR Agrostroj Jičín, Czech Republic) to a length of 15 mm via grain processing. The laboratory experiment consisted of 5 treatments (Table 1), with three replicates per treatment. Before ensiling for the C treatment, the average corn forage samples were taken (n = 3). The following groups were tested: control treatment C (without additives), treatments LAB1 and LAB2 treated with lactic acid bacteria (LAB without chemical salts), and combined treatments LABCHSE and LABCHS with the addition of LAB and chemical salts. All additives were applied at the doses recommended by the manufacturer. We applied the liquid application form (LAB1, LABCHSE, and LABCHS) using spray technology and the granular form (LAB2) uniformly on spread matter 10 kg in weight before ensiling. After application, the forage was homogenized separately for each treatment manually. The examined variants C, LAB1, LAB2, LABCHSE, and LABCHS are described in detail in Table 1. After the inclusion of silage additives (except for control treatment C), followed by homogenization and compaction, the corn matter (density 260 kg/m³ DM) was hermetically stored in silage units (glass jars) with volumes of 3.5 L⁻¹ at a temperature of 22±1 °C in an air-conditioned Feed Conservation Laboratory. Six weeks after storage, the silage units were opened, and the fermentation process indicators were determined in average silage samples - 5 treatments, each in 3 experimental units (glass jars) all in duplicate.

Silage nutritive value and fermentation parameters

According to the standard laboratory methods (AOAC, 2005), the nutritive composition and fermentation parameters of the laboratory samples were determined at the Laboratory of Quality and Nutritive Value of Feeds (Slovak University of Agriculture in Nitra, Slovakia; Department of Animal Nutrition). The average sample (1 kg) of forage matter before ensiling and corn silage (taken by hand from glass jars and mixed in the bin) was pre-dried (HS402PA Chirana Slovakia, for 27 hours at 60 °C). The samples for nutritive value were ground in a laboratory mill (Fritsch, Germany) to the particle size of 1 mm. Then the dry-matter content (method No. 934.01), ether extract (method No. 991.36), crude protein (method No. 976.05), crude fiber, neutral detergent fiber (method No. 2002.04), acid detergent fiber, acid detergent lignin (method No. 973.18), and ash (method No. 942.05) were determined. The contents of organic matter, hemicellulose, and cellulose were then calculated according to the following formulas:

Table 1. Composition and applica	tion doses of silage additives
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Treatments	Composition of Additive	Application form	Dose/t fresh forage
С	without additive (control)	/	/
LAB1	Lactiplantibacillus plantarum ^A DSM 19457, Levilactobacillus brevis ^B DSM 19456, Enterococcus faecium ^A DSM 3530, /2.5.00x10 ¹⁰ CFU/g*, Austria/	LAB1	4 g in l liter H ₂ O
LAB2	Lactiplantibacillus plantarum ^A DSM 12837, Lentilactobacillus buchneri ^B DSM 12856, Pediococcus pentosaceus ^A DSM 12834 (4.0x10 ⁸ CFU/g*, Austria)	LAB2	250 g
LABCHSE	Lactiplantibacillus plantarum ^A CNCM I-3235, Ligilactobacillus salivarius ^C CNCM I-3238, Enterococcus faecium ^A CNCM I-3236, Pediococcus acidilactici ^C CNCM I-3237 (6.67x108 CFU/g [*] , United Kingdom), cellulase, hemicellulase, amylase, pentosanase, sodium benzoate, potassium sorbate	LABCHSE	50 g in 2 liters H ₂ O
LABCHS	Lactiplantibacillus plantarum ^A NCIB 30085, Enterococcus faecium ^A NCIMB 11181, Pediococcus acidilactici ^c NCIB 30083, Pediococcus acidilactici ^c NCIB 30084 (2.00x10 ¹¹ CFU/g [*] , Austria), potassium sorbate	LABCHS	100 g in 1 liter H ₂ O

LAB1 and LAB2 = additives on the base of lactic acid bacteria without chemical salt, LABCHSE = additive on the base of lactic acid bacteria + chemical salts + enzymes, LABCHS = additive on the base of lactic acid bacteria + chemical salt, A = facultatively heterofermentative, B = obligately heterofermentative, C = obligately homofermentative.

*total counts of colony fermentation units (CFU/g of fresh forage).

Organic matter = Dry matter - Ash (% DM);

Hemicellulose = Neutral detergent fiber - Acid detergent fiber (% DM);

Cellulose = Acid detergent fiber – Acid detergent lignin (% DM).

Starch content was analyzed by the polarimetric method (% DM). The nutrient content of the forage matter before ensiling is presented in Table 2.

 Table 2. Nutritive value of forage matter in the control before ensiling (n = 3)

Parameter	Average ± SD
Dry Matter (%)	32.22 ± 0.40
Crude Protein *	9.07 ± 0.29
Ether Extract *	2.89 ± 0.18
Crude Fiber *	17.20 ± 1.18
Starch *	31.08 ± 1.90
Organic Matter *	94.93 ± 0.11
Ash *	5.07 ± 0.11
ADF *	19.68 ± 0.88
NDF *	39.35 ± 2.13
ADL*	1.97 ± 0.48
Cellulose *	17.99 ± 0.94
Hemicellulose *	19.63 ± 1.76

*(% dry matter); SD = standard deviation; ADF = acid-detergent fiber; NDF = neutral-detergent fiber; ADL = acid-detergent lignin

For the fermentation parameters determination, laboratory samples were extracted in distilled water. In the silage extracts, the fermentation acid content (lactic, butyric, acetic, formic) using the ionic electrophoresis method (analyzer EA 100, Villa Labeco, SK) was determined. Contents of ammonia (NH₃) and alcohols (Conway microdiffusion method), as well as active acidity (electrometric method), were also determined. The degree of proteolysis and fermentation products were calculated according to the following formulas:

Degree of proteolysis = $(NH_3 - N / \text{total N}) \times 100$;

Fermentation products = Lactic acid + Acetic acid + Butyric acid + Formic acid + Alcohols.

The acidity of the water extract was then analysed (alkalimetric titration of the silage extract to pH 8.5; expressed as milligrams of KOH per 100 grams of silage).

Statistical processing of the results

The results were statistically processed and evaluated in IBM SPSS 26.0. Descriptive statistics and the effects of each additive application were examined using a oneway Analysis of Variance. Statistical significance between the silage treatments was examined using Tukey's Post-Hoc test at a level of 0.05. Direct differences between the control and experimental treatments were examined by Independent-samples t-tests.

RESULTS AND DISCUSSION

The main fermentation parameters of corn silage

The fermentation characteristics (acids and pH value) of corn silage after 6 weeks of ensiling are shown in Table 3. Significant effects of additives on lactic acid (P = 0.046), acetic acid (P < 0.001), the lactic/acetic acid ratio (P < 0.001), and pH value (P < 0.001) were observed. Lactic acid is an important product of silage fermentation that contributes to a decrease in pH (Loučka et al., 2018) but also has antifungal functions, as confirmed by recent studies (Xu et al., 2017; Wu et al., 2019). In comparison with C silage, in LAB2, significantly different (P < 0.05) lactic acid content was found in all treatments with additives. The addition of additives reduced the content of lactic acid in the LAB2 treatment. while in other treatments the addition of additives did not statistically significantly affect the content of lactic acid compared to the control treatment. Treatments with biological additives (based on L. brevis and L. buchneri) affected the lactic acid content compared to C. These results correspond with the findings reported by Santos et al. (2020) in corn silage treated with the activated inoculant L. buchneri. Decreases in lactic acid are related to the conversion of lactic to acetic acid by obligately heterofermentative LAB during anaerobic fermentation (Muck et al., 2018). Silages inoculated with L. buchneri are characterized by the moderate conversion of lactic acid to acetic acid; 1,2-propanediol, and ethanol (Oude Elferink et al., 2001). Thus, there is a tendency towards lower lactic acid content after treatment. The addition of biological additives with chemical salts did not affect lactic acid content in the corn silage. The silages of all treatments were characterized by superior preservation of lactic acid content (2.00% in kg of the fresh matter), except for the LAB2 silage, which preserved 1.75%.

The acetic acid content was significantly (P < 0.05) affected by the biological additive (LAB2) and both additives with chemical salts (LABCHSE, LABCHS) compared to the control treatment. Acetic acid content increased after the addition of additives, which is profitable for ruminants because acetic acid is a source of energy available for milk fat synthesis (Urrutia et al., 2019). Higher acetic acid concentrations in obligately heterofermentative strains are effective in inhibiting the growth of yeast, which is involved in the aerobic deterioration of silage as oxygen penetrates the silage (Borreani et al., 2018). Likewise, Paradhipta et al., (2020) observed a significant effect of L. brevis and L. buchneri on increases of acetate in corn silage. Conversely, Santos et al. (2020) observed a non-significant effect of activated L. buchneri on acetic acid content compared to the present study. The present study showed a significant effect of additives containing chemical salts on acetic acid. Similarly, Tyrolová et al. (2017) found that chemical additives (organic acids and ammonium formate) increased acetic acid by forming favourable conditions for the growth of lactic acid bacteria with the heterofermentative type of fermentation. Another study by Weiß et al. (2019) observed the variable influence of chemical additives containing potassium sorbate, ammonium propionate, and sodium benzoate on acetate content in corn silage. Potassium sorbate and sodium benzoate were also shown to improve the aerobic stability of silages and the production of antifungal compounds, such as acetic acid (Seppälä et al., 2016).

The widest lactic/acetic acid ratio (5.07/1.00) in silage without additive (C) and narrower ratios (from 1.78 to 4.35/1.00) in other treatments were observed. The differences in lactic/acetic acid ratio between C and treatments with additives were significant (P < 0.05).

These results were also in agreement with Paradhipta et al. (2020), who observed a lower lactate-to-acetate ratio vs. the control (5.22 vs. 6.22) after the addition of *L. brevis* and *L. buchneri*. These findings are in accordance with the results of Zhang et al. (2020), who investigated the significant effects of chemical additives (sodium benzoate and potassium sorbate) on the lactate-to-acetate ratio of corn silage (P = 0.009). Silages with a very high lactic/ acetic acid ratio may sometimes be more aerobically unstable than those with the recommended ratio (3:1) because low concentrations of acetic acid may not be sufficient to prevent lactate from assimilating yeast. A lactic/acetic acid ratio below 1 is usually an indication of abnormal fermentation (Kung et al., 2018a).

The corn silage of all treatments did not contain undesirable butyric acid, which is consistent with the results of Paradhipta et al. (2020). Butyric acid, as the main product of Clostridium sp., should not be detectable in well-fermented silage (Kung et al., 2018a). In contrast to the present study, Santos et al. (2020) observed the presence of butyric acid in all samples of corn silage (0.03-0.04%) along with a positive effect of activated L. buchneri. The different content of formic acid was observed in silage LAB2 in comparison with C (P < 0.05). A high concentration of formic acid was accompanied by a slower decrease in pH within the silages. This phenomenon was confirmed in the LAB2 silage. The effect of the addition of biological additives, with and without chemical salts, on the pH value was also significant (P <0.001). The pH values of the silages in all treatments were below 4.00 in the present study; these pH values indicate well-ensiled silage, as described by Zhang et al. (2020). Compared to the C silage, significantly (P < 0.05) higher pH values were found in all treatments with additives, which relates to the lower lactic acid concentrations in the treated silage. Silage additives increased the pH silage values, which could be effective in lowering the risk of developing rumen acidosis (Khorrami et al., 2021).

In the previous experiment published by Santos et al. (2020) the value of pH increased (3.52 in the control vs. 3.66 in the treated silage) in corn silage inoculated with activated *L. buchneri*, albeit non-significantly.

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Treatments/ Effects	С	LAB1	LAB2	LABCHSE	LABCHS	Total	P additives
Lactic acid (% DM)	7.11±0.43ª	6.84±0.06 ^{ab}	5.56±0.18 ^b	6.88±0.14 ^{ab}	6.53±0.25ab	6.58±0.60	0.046
Acetic acid (% DM)	1.40±0.04ª	1.58±0.15ª	3.13±0.17 ^b	2.17±0.19°	2.20±0.24c	2.10±0.64	<0.001
Lactic acid/ Acetic acid	5.07±0.21ª	4.35±0.46 ^b	1.78±0.15°	3.19±0.34 ^d	3.00±0.36d	3.48±1.21	<0.001
Butyric acid (% DM)	ND	ND	ND	ND	ND	ND	ND
Formic acid (% DM)	0.25±0.02ª	0.25±0.01 ^{ab}	0.30±0.01 ^b	0.25±0.02 ^{ab}	0.23±0.01ab	0.26±0.03	0.373
рН	3.71±0.001°	3.74±0.001 ^b	3.84±0.001°	3.74±0.001 ^b	3.75±0.002b	3.76±0.048	<0.001

Table 3. Effect of silage additives on fermentative acids and the pH values of corn silage

C = control; LAB1 = L. plantarum, L. brevis, and E. faecium; LAB2 = L. plantarum, L. buchneri, and P. pentosaceus; LABCHSE = L. plantarum, L. salivarius, E. faecium, P. acidilactici, cellulase, hemicellulase, amylase, sodium benzoate, and potassium sorbate; LABCHS = L. plantarum, E. faecium, P. acidilactici, and potassium sorbate; ND = not detected; a-d = means with different superscripts in the columns indicate significant differences (P < 0.05) relative to the control (Independent-Samples T-test and Tukey HSD test).

In the research of Kung et al. (2018b) treatment with an inoculant (*L. plantarum*, *E. faecium*, and *L. buchneri*; pH 3.74) and chemical additive (potassium sorbate, sodium benzoate, and sodium nitrite, in dosage 3.0 L/t; pH 3.71) also resulted in corn silage with a significantly higher pH than the control (pH 3.60). Conversely, da Silva and Kung (2022) published a review showing that a chemical additive containing potassium sorbate, sodium benzoate, and sodium nitrite can decrease the pH value in corn silage.

The other fermentation parameters of corn silage

The fermentation characteristics (other parameters) of corn silage after 6 weeks of ensiling are shown in Table 4. The addition of silage additives affected the alcohol content (P = 0.045) and acidity of the water extract (P = 0.009) in the corn silage. However, additives did not affect the degree of proteolysis (P = 0.161) or the content of fermentation products (P = 0.384). The alcohol content in silages from whole-plant corn is usually low (0.5-1.5%) (Kung et al., 2018a), which was confirmed by the current experiment. Thus, this could be physiologically beneficial for ruminants because higher concentrations of alcohol are toxic to desirable microbiomes (Iruzubieta et al., 2020). Significant (P < 0.05) differences in alcohols

were observed in treatments with LAB + chemical salts (LABCHSE and LABCHS). These findings agree with those in studies by Kung et al. (2018b) and Weiß et al. (2019), which reported that silages with additives containing salts of acids (with antifungal properties) significantly decreased the alcohol concentrations in corn silages. The decrease in the concentration of alcohols after the addition of sodium benzoate, potassium sorbate, and salts of propionic acid was related to the inhibition of mould growth, especially the growth of yeasts, which can improve the aerobic stability of silage (Muck et al., 2018). The effect of biological additives on alcohol concentration was insignificant, similarly reported by Bernardi et al. (2019). Conversely, Santos et al. (2020) observed significantly lower alcohol content after the addition of activated L. buchneri. The acidity of the water extract is an important quality indicator of the fermentation process and depends on the concentration of fermentation acids, mainly acetic acid (Skládanka et al., 2014). All treatments with additives had significantly (P < 0.05) different waterextract acidity (except for treatment LAB1) in comparison to the untreated silage (C). Moreover, plant and microbial proteolytic activity can affect the degree of proteolysis in silage (Kung et al., 2018a). Further, the end products of proteolysis are usually free amino acids and peptides.

Table 4. Effect of silage additives on alcohol content, acidity of the water extract, proteolysis, and the fermentation product con	1-
tent in the corn silage	

Treatments/ Effects	С	LAB1	LAB2	LABCHSE	LABCHS	Total	P additives
Alcohols (% DM)	0.69±0.09ª	0.61±0.07 ^{ab}	0.64±0.02ª	0.47±0.04 ^b	0.54±0.04 ^b	0.59±0.09	0.045
Acidity of Water Extract (mg OH/100 g)	1621.33±43.13ª	1658.00±36.39ªb	1740.33±46.01 ^b	1752.33±80.09 ^b	1780.00±65.48 ^b	1710.40±78.62	0.009
Degree of Proteolysis (%)	5.83±0.36v	5.51±0.77 ^{ab}	6.77±0.45 ^b	5.93±0.36 ^{ab}	6.33±0.44 ^{ab}	6.08±0.62	0.161
Fermentation products (% DM)	9.20±0.40	9.04±0.10	9.32±0.03	9.51±0.32	9.26±0.31	9.27±0.28	0.384

C = control; LAB1 = L. plantarum, L. brevis, and E. faecium; LAB2 = L. plantarum, L. buchneri, and P. pentosaceus; LABCHSE = L. plantarum, L. salivarius, E. faecium, P. acidilactici, cellulase, hemicellulase, amylase, sodium benzoate, and potassium sorbate; LABCHS = L. plantarum, E. faecium, and P. acidilactici, potassium sorbate.

 a,b = means with different superscripts in the columns indicate significant differences (P < 0.05) relative to the control (Independent-Samples T-test and Tukey HSD test).

Ammonia and amines are commonly the end products of microbial activity rather than plant enzyme activity, and the degree of proteolysis can be affected by many factors, such as pH. Moreover, slow acidification increases the degree of proteolysis (Borreani et al., 2018). The differences were significant in the degree of proteolysis (P < 0.05) in LAB2 in comparison with the C variant. This result is consistent with those in (Rabelo et al., 2017), where an increase was found in the ammonia-N concentration in corn silage inoculated with L. buchneri. This result is related to the proteolytic activity of LAB such as L. buchneri (Kunji et al., 1996). An increase in the ammonia nitrogen concentration in corn silage with activated L. buchneri was confirmed in an experiment by Santos et al. (2020), but the differences were not significant. No differences (P > 0.05) were observed between the control and additive-treated silage in the content of their fermentation products.

CONCLUSION

The addition of silage additives considerably influenced the fermentation quality of silage, by decreasing lactic acid content, and increasing the concentration of acetic acid, which led to a narrowing of the lactate-to-acetate ratio, increased pH value, and the titratable acidity, and positively reduced alcohol content. The application of biological additives (with *L. brevis* - LAB1 and *L. buchneri* - LAB2) significantly increased the value of pH, as well LAB2 significantly increased the acetic acid content, the acidity of the water extract and degree of proteolysis, and significantly decreased concentration of lactic acid. Treatment with combined additives (LABCHS, LABCHSE) significantly increased the content of acetic acid, pH value, and titratable acidity and decreased the concentration of alcohols in the corn silage.

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