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THE EFFECTS OF USING LACTIC ACID BACTERIA INOCULANTS IN MAIZE SILAGE ON THE FORMATION OF BIOGENIC AMINES

Š. STEIDLOVÁ and P. KALAČ*

Department of Chemistry, Faculty of Agriculture, University of South Bohemia, České Budějovice, Czech Republic

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Silages from five ripened varieties of silage maize with dry matter contents ranging between 275 and 410 g/kg were prepared in five laboratory experiments. Whole-plant maize was fermented at 22°C and silages were then stored at the same temperature for 4 months. Spontaneously fermented silages were prepared as control variants and compared with silages inoculated with commercial strains of *Lactobacillus plantarum*, *Lactobacillus buchneri* and a mixed preparation Microsil containing *L. plantarum*, *Lactobacillus casei*, *Enterococcus faecium* and *Pediococcus pentosaceus*. The starter cultures were applied at doses $5{\cdot}10^5$ and $5{\cdot}10^6$ CFU/g of chopped maize. Seven biogenic amines and polyamines were extracted from silages with perchloric acid and determined as *N*-benzamides by micellar electrokinetic capillary chromatography. Common chemical criteria of silage quality were also determined. All three inoculants, mainly at the higher dose, decreased significantly contents of tyramine, putrescine and cadaverine, three undesirable amines occurring at the highest levels. *L. plantarum* was the most effective. Contents of histamine and tryptamine were low in all experimental silages. Also relatively low were levels of polyamines spermidine and mainly of spermine.

Keywords: Biogenic amines; Polyamines; Maize silage; Inoculants; *Lactobacillus plantarum*; *Lactobacillus buchneri*

1. INTRODUCTION

Maize silage has been the main preserved forage for ruminants in many countries during the winter period. Some of its components can decrease its palatability and, moreover, show some detrimental effects on animal health. Several biogenic amines, namely histamine, tyramine and putrescine, fall into such silage constituents (Lingaas and Tveit, 1992; Dulphy and Van Os, 1996; Dawson and Mayne 1996, 1997; Phuntsok *et al*., 1998; Aschenbach and Gabel, 2000).

Mean tyramine, putrescine, cadaverine and spermidine contents at 482, 98, 48 and 17 mg/kg and 145, 136, 96 and 38 mg/kg were determined in a survey of 113 farm maize silages produced in 1999 and 2000, respectively. However, maximum values were several times higher. Histamine was detected only in a part of tested silages and its contents were only rarely above 10 mg/kg (Steidlová and Kalač, 2002). A risk both of

*Corresponding author: P. Kalač, Faculty of Agriculture, University of South Bohemia, 370 05 České Budějovice, Czech Republic. Tel. + 420-389032657; Fax: + 420-385310405; E-mail: kalac@zf.jcu.cz

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palatability decrease and of some deleterious effects mainly for cattle due to increased amine levels, cannot be excluded for a part of silages, mainly for those prepared under unfavourable weather conditions with relatively low dry matter content.

The objective of the present work was to test the possibility to decrease contents of undesirable amines during maize silage preparation with selected lactic acid bacteria inoculants (starter cultures) in laboratory experiments, as compared with silages prepared by spontaneous fermentation.

2. MATERIALS AND METHODS

2.1. Silage preparation

Five silage maize varieties of full ripeness were used. Whole-plant maize was chopped by a forage harvester under farm conditions to pieces of 1 – 2 cm length, which were transported in a polyethylene bag. Laboratory experiments were started within 2 h. Characteristics of the used maize are given in Table I.

The material was mixed with 25 ml/kg of an inoculant suspension. The same volume of distilled water was used instead of the suspension in the control variant for spontaneous fermentation. Jars (volume 720 ml) were fully filled with 375 g of the mixture. The jars were closed with Omnia caps 30 min after being filled and stored in the dark at 22°C for 4 months. At least four jars were prepared in each of the tested variants.

The jars used as the laboratory silos allowed the escape of gases during the initial 5 – 7 days of extensive fermentation and later, during silage storage, they were hermetically sealed. Sporadically occurring jars with air access, showing mould growth and silage deterioration, were excluded.

2.2. Inoculants

Three commercial lactic acid bacteria preparations were applied. The inoculants were produced by Medipharm CZ Ltd., Hustopeče near Brno, Czech Republic. The used strains have been registered in the Czech Collection of Microorganisms (CCM) in Brno. Pure freeze-dried cultures of *Lactobacillus plantarum* (CCM 3769), *Lactobacillus*

TABLE I Characteristics of the ensiled maize

Experiment No.	*Date of harvesting*	*Variety*	*Dry matter [g/kg]*	*Note*
1	Sept. 14, 2000	Felicia (FAO 260)	410	48% of plants were infested with common smut (*Ustilago maydis*, syn. *U. zeae*)
2	Sept. 14, 2000	Calimera (FAO 250)	373	
3	Oct. 10, 2000	80% CE-210S (FAO 210) and 20% Cester (FAO 220)	300	
4	Sept. 24, 2001	Tereza (FAO 240)	275	
5	Sept. 24, 2001	Markíza (FAO 290)	285	

buchneri (CCM 1819) and a mixed preparation Microsil containing *L. plantarum* (CCM 3769), *Lactobacillus casei* (CCM 3775), *Enterococcus faecium* (CCM 6226) and *Pediococcus pentosaceus* (CCM 3770) were applied in doses $5{\cdot}10^5$ and $5{\cdot}10^6$ CFU/g of ensiled maize. The preparations were suspended in distilled water immediately prior to application.

2.3. Sampling

Silages from four jars in each of seven variants (including control) were sampled after 4 months of storage. For each four replicates were analysed.

2.4. Analytical methods

Dry matter content of ensiled maize and chemical quality criteria of silage were determined as described in our previous papers (Kalač *et al*., 1999, 2000; Steidlová and Kalač, 2002). Analytical procedure for determination of seven observed biogenic amines (BA) as *N*-benzamides, using micellar electrokinetic capillary chromatography, was described in detail by Křížek and Pelikánová (1998).

The detection limits were 1.0, 1.3, 1.4, 1.4, 2.1, 2.1 and 3.5 mg/kg of silage for spermidine, tryptamine, cadaverine, spermine, putrescine, histamine and tyramine, respectively. Relative standard deviations were 11.2, 7.8 and 7.1% for tyramine, putrescine and cadaverine, respectively.

Similar information for the quality criteria was given in our above-cited papers.

2.5. Statistical methods

Results were tested by Duncan's test at a significance level of $P < 0.05$ for the individual experiments and also together for all five experiments. Correlations between silage quality parameters and the individual amine contents were also determined.

3. RESULTS AND DISCUSSION

Dry matter of ensiled maize ranged widely between 275 and 410 g/kg (Table I). Because whole-plant maize was chopped by a farm harvester it was inoculated with microflora occurring naturally under farm conditions. In the five experiments the quality parameters of the control differed mainly in contents of lactic acid and ethanol (Tables II – VI). However, such differences in spontaneously fermented maize silages have been observed commonly.

Maize used in experiment 1 (Table II) was massively infested with smut (*Ustilago maydis*). In spite of the highest dry matter content of ensiled maize, the silage had the highest levels of lactic acid, α-amino groups and ammonia. No other differences, either between the quality parameters nor biogenic amine contents, were observed as compared with the further experiments. Burgstaller *et al*. (1977) reported a decrease in net energy and digestible crude protein levels for maize silage fully infested with smut, but no negative effects on health status of dairy cows were observed.

The overall statistical testing of all experiments (Table VII) indicated that the application of the inoculants caused a decrease of active acidity in silages (increase of

TABLE II Contents of biogenic amines and quality parameters of silages prepared from maize var. Felicia in experiment 1 with different starter culture doses after 4 months storage[1]

		L. plantarum		*L. buchneri*		*Microsil*	
Parameter	*Control*	$5 \cdot 10^5$ *CFU/g*	$5 \cdot 10^6$ *CFU/g*	$5 \cdot 10^5$ *CFU/g*	$5 \cdot 10^6$ *CFU/g*	$5 \cdot 10^5$ *CFU/g*	$5 \cdot 10^6$ *CFU/g*
Amines [mg/kg]							
Histamine	ND[2]	ND	ND	ND	ND	ND	ND
Tyramine	134^{bc}	110^{ab}	176^{c}	157^{bc}	141^{bc}	123^{ab}	86.7^{a}
Putrescine	154	181	151	141	155	152	143
Cadaverine	27.3^{a}	40.2^{b}	38.9^{b}	36.7^{b}	33.9^{ab}	27.8^{a}	29.7^{a}
Tryptamine	3.5	2.0	5.3	1.5	5.4	2.3	1.8
Spermidine	37.9	33.3	34.6	37.9	33.7	31.5	32.8
Spermine	8.3	4.4	7.4	5.8	7.5	6.5	7.6
Quality parameters							
pH	3.80^{a}	3.85^{c}	3.87^{d}	3.82^{b}	3.86^{c}	3.82^{b}	3.80^{a}
Total acidity [mg NaOH/100 g]	1090	980	995	1020	1030	1060	965
Lactic acid [g/kg]	21.2	20.4	21.0	19.8	21.0	18.5	22.0
Acetic acid [g/kg]	8.6	7.9	8.6	8.1	7.7	7.4	7.5
Propionic acid [g/kg]	0.5^{ab}	0.4^{a}	0.6^{b}	0.4^{ab}	0.4^{ab}	0.4^{ab}	0.3^{a}
Butyric acid [g/kg]	2.4^{b}	0.6^{a}	0.6^{a}	0.6^{a}	0.8^{a}	1.1^{a}	0.9^{a}
Ethanol [g/kg]	1.7^{ab}	2.8^{c}	3.1^{bc}	3.1^{bc}	3.0^{c}	1.0^{a}	2.0^{abc}
α-amino groups [mg/100 g]	82^{ab}	86^{ab}	94^{b}	94^{b}	88^{ab}	74^{a}	71^{ab}
Ammonia [mg/100 g]	36^{a}	50^{b}	52^{b}	47^{ab}	56^{bc}	66^{c}	47^{ab}

[1]Data are mean values from four replicates.
[2]ND: values were below detection limit.
Means with different superscript letters in a line indicate significant differences at $P < 0.05$.

TABLE III Contents of biogenic amines and quality parameters of silages prepared from maize var. Calimera in experiment 2 with different starter culture doses after 4 months storage[1]

		L. plantarum		*L. buchneri*		*Microsil*	
Parameter	*Control*	$5 \cdot 10^5$ *CFU/g*	$5 \cdot 10^6$ *CFU/g*	$5 \cdot 10^5$ *CFU/g*	$5 \cdot 10^6$ *CFU/g*	$5 \cdot 10^5$ *CFU/g*	$5 \cdot 10^6$ *CFU/g*
Amines [mg/kg]							
Histamine	ND[2]	ND	ND	ND	ND	ND	ND
Tyramine	122^{ab}	166^{c}	153^{c}	112^{ab}	156^{c}	137^{bc}	104^{a}
Putrescine	121^{ab}	131^{b}	128^{ab}	125^{ab}	136^{b}	107^{a}	120^{ab}
Cadaverine	21.1^{a}	32.6^{b}	30.0^{ab}	28.2^{ab}	30.3^{ab}	19.5^{a}	36.9^{b}
Tryptamine	ND	ND	ND	ND	1.4^{a}	4.5^{b}	5.4^{b}
Spermidine	29.3	22.4	29.9	25.5	26.3	28.8	32.8
Spermine	1.7^{a}	1.5^{a}	3.3^{a}	ND	ND	ND	ND
Quality parameters							
pH	3.75^{a}	3.81^{c}	3.79^{b}	3.79^{bc}	3.80^{bc}	3.76^{a}	3.76^{a}
Total acidity [mg NaOH/100 g]	850^{a}	855^{a}	895^{ab}	835^{a}	925^{ab}	895^{ab}	995^{b}
Lactic acid [g/kg]	13.8^{a}	21.8^{b}	15.3^{ab}	19.1^{ab}	17.3^{ab}	14.9^{a}	19.1^{ab}
Acetic acid [g/kg]	6.2^{bc}	4.4^{a}	4.9^{ab}	6.9^{c}	6.4^{bc}	5.7^{abc}	7.0^{c}
Propionic acid [g/kg]	0.4^{b}	0.2^{a}	0.1^{a}	0.3^{ab}	0.3^{ab}	0.2^{ab}	0.4^{ab}
Butyric acid [g/kg]	0.7^{ab}	0.2^{a}	0.2^{a}	0.6^{ab}	0.4^{a}	1.6^{b}	0.8^{ab}
Ethanol [g/kg]	2.0^{a}	4.7^{d}	4.0^{d}	2.8^{b}	3.0^{c}	1.9^{a}	3.0^{bc}
α-amino groups [mg/100 g]	54^{a}	62^{ab}	66^{ab}	56^{ab}	69^{b}	57^{ab}	58^{ab}
Ammonia [mg/100 g]	29^{a}	42^{d}	38^{c}	37^{c}	43^{d}	39^{c}	37^{bc}

[1]Data are mean values from four replicates.
[2]ND: values were below detection limit.
Means with different superscript letters in a line indicate significant differences at $P < 0.05$.

TABLE IV Contents of biogenic amines and quality parameters of silages prepared from maize varieties CE- 210S and Cester in experiment 3 with different starter culture doses [CFU/g] after 4 months storage[1]

		L. plantarum		*L. buchneri*		*Microsil*	
Parameter	*Control*	*5·10⁵ CFU/g*	*5·10⁶ CFU/g*	*5·10⁵ CFU/g*	*5·10⁶ CFU/g*	*5·10⁵ CFU/g*	*5·10⁶ CFU/g*
Amines [mg/kg]							
Histamine	ND[2]	ND	ND	ND	ND	ND	ND
Tyramine	153^{e}	53.4^{b}	16.3^{a}	85.4^{c}	80.8^{c}	131^{d}	80.7^{c}
Putrescine	147^{d}	76.4^{bc}	38.7^{a}	60.7^{ab}	5U 49.1^{a}	158^{d}	93.9^{c}
Cadaverine	94.0^{c}	39.3^{a}	31.9^{a}	39.1^{a}	32.1^{a}	4.1^{b}	38.9^{a}
Tryptamine	1.8	ND	ND	ND	ND	1.3	ND
Spermidine	28.9	25.4	24.8	27.3	26.7	27.8	29.1
Spermine	4.5	2.4	2.9	3.5	1.6	ND	2.8
Quality parameters							
pH	3.60^{a}	3.70^{e}	3.69^{de}	3.66^{c}	3.68^{d}	3.65^{b}	3.65^{bc}
Total acidity [mg NaOH/100 g]	1110^{c}	935^{ab}	815^{a}	1020^{bc}	975^{bc}	1000^{bc}	1010^{bc}
Lactic acid [g/kg]	17.3	15.9	14.7	12.6	13.3	14.9	15.5
Acetic acid [g/kg]	6.8	3.9	3.6	7.6	5.6	5.5	5.5
Propionic acid [g/kg]	0.2	0.4	0.3	0.2	0.4	0.2	0.2
Butyric acid [g/kg]	0.3	0.2	0.3	0.1	0.1	0.2	0.2
Ethanol [g/kg]	0.8^{a}	2.1^{b}	3.2^{c}	1.9^{b}	3.0^{c}	0.8^{a}	2.0^{b}
α-amino groups [mg/100 g]	74^{bc}	62^{b}	48^{a}	64^{bc}	65^{bc}	74^{c}	59^{b}

[1]Data are mean values from four replicates.
[2]ND: values were below detection limit.
Means with different superscript letters in a line indicate significant differences at $P < 0.05$.

TABLE V Contents of biogenic amines and quality parameters of silages prepared from maize var. Tereza in experiment 4 with different starter culture doses after 4 months storage[1]

		L. plantarum		*L. buchneri*		*Microsil*	
Parameter	*Control*	*5·10⁵ CFU/g*	*5·10⁶ CFU/g*	*5·10⁵ CFU/g*	*5·10⁶ CFU/g*	*5·10⁵ CFU/g*	*5·10⁶ CFU/g*
Amines [mg/kg]							
Histamine	3.0	ND[2]	ND	ND	ND	ND	ND
Tyramine	145^{d}	70.7^{b}	36.8^{a}	58.5^{b}	65.4^{b}	100^{c}	52.4^{ab}
Putrescine	97.8^{d}	54.7^{b}	40.1^{ab}	41.6^{ab}	25.5^{a}	73.3^{c}	53.9^{b}
Cadaverine	113^{c}	87.2^{ab}	73.5^{a}	82.1^{a}	80.7^{a}	109^{bc}	104^{bc}
Tryptamine	14.4	2.7	4.9	4.4	11.3	7.1	5.6
Spermidine	36.4	28.7	31.2	31.5	40.5	33.7	34.4
Spermine	6.9^{ab}	6.3^{ab}	4.4^{a}	8.7^{ab}	4.6^{a}	10.9^{b}	8.9^{ab}
Quality parameters							
pH	3.39^{ab}	3.42^{b}	3.33^{a}	3.51^{c}	3.50^{c}	3.41^{ab}	3.36^{ab}
Total acidity [mg NaOH/100 g]	1160^{ab}	1090^{a}	1250^{b}	1120^{ab}	950^{ab}	1060^{ab}	1110^{ab}
Lactic acid [g/kg]	7.9^{c}	4.7^{a}	5.8^{ab}	6.2^{bc}	5.7^{bc}	6.9^{bc}	6.6^{bc}
Acetic acid [g/kg]	5.2^{abc}	4.0^{ab}	3.7^{abc}	5.9^{c}	5.7^{bc}	3.9^{abc}	2.4^{a}
Propionic acid [g/kg]	0.2	ND	ND	0.2	0.2	0.2	0.2
Butyric acid [g/kg]	0.3	0.1	ND	0.2	0.2	0.2	0.2
Ethanol [g/kg]	1.8	3.0	2.4	1.3	1.9	2.9	2.0
α-amino groups [mg/100 g]	65^{ab}	64^{a}	70^{b}	59^{ab}	56^{ab}	60^{ab}	49^{a}
Ammonia [mg/100 g]	24^{a}	32^{a}	34^{b}	34^{b}	26^{ab}	26^{ab}	27^{ab}

[1]Data are mean values from four replicates.
[2]ND, values were below detection limit.
Means with different superscript letters in a line indicate significant differences at $P < 0.05$.

TABLE VI Contents of biogenic amines and quality parameters of silages prepared from maize var. Markíza in experiment 5 with different starter culture doses after 4 months storage

		L. plantarum		*L. buchneri*		*Microsil*	
Parameter	*Control*	$5 \cdot 10^5$ *CFU/g*	$5 \cdot 10^6$ *CFU/g*	$5 \cdot 10^5$ *CFU/g*	$5 \cdot 10^6$ *CFU/g*	$5 \cdot 10^5$ *CFU/g*	$5 \cdot 10^6$ *CFU/g*
Amines [mg/kg]							
Histamine	ND	12.0	3.5	ND	ND	ND	ND
Tyramine	185^{d}	141^{c}	45.8^{a}	90.6^{b}	67.0^{ab}	121^{c}	53.2^{a}
Putrescine	112	117	46.6	57.1	127	105	44.8
Cadaverine	105^{bc}	156^{d}	72.8^{a}	91.9^{ab}	79.5^{a}	117^{c}	88.5^{ab}
Tryptamine	7.0^{b}	5.7^{ab}	5.3^{ab}	3.6^{ab}	1.8^{a}	6.9^{b}	2.6^{ab}
Spermidine	33.4^{ab}	40.2^{b}	25.1^{a}	32.7^{ab}	24.0^{a}	34.1^{ab}	34.2^{ab}
Spermine	ND	8.1^{c}	2.1^{ab}	4.4^{bc}	3.3^{ab}	5.0^{bc}	4.4^{bc}
Quality parameters							
pH	3.41^{a}	3.40^{a}	3.41^{a}	3.48^{c}	3.48^{c}	3.43^{b}	3.43^{b}
Total acidity [mg NaOH/100 g]	1240^{b}	1120^{b}	945^{a}	1130^{b}	955^{a}	1120^{b}	1140^{b}
Lactic acid [g/kg]	5.6	7.4	6.0	5.6	5.0	9.4	7.8
Acetic acid [g/kg]	4.7^{b}	4.2^{ab}	3.6^{ab}	5.7^{b}	2.2^{a}	4.4^{ab}	4.2^{ab}
Propionic acid [g/kg]	0.1	0.1	ND	0.2	0.1	0.2	0.2
Butyric acid [g/kg]	0.1	0.1	ND	0.2	ND	0.2	ND
Ethanol [g/kg]	2.7^{a}	3.6^{ab}	5.9^{c}	3.7^{ab}	4.2^{abc}	3.7^{a}	5.4^{bc}
α-amino groups [mg/100 g]	60	65	63	63	62	60	59
Ammonia [mg/100 g]	30^{ab}	23^{a}	28^{ab}	28^{ab}	28^{ab}	31^{b}	28^{ab}

[1]Data are mean values from four replicates.
[2]ND: values were below detection limit.
Means with different superscript letters in a line indicate significant differences at $P < 0.05$.

pH values), total acidity and contents of acetic acid and butyric acid as compared with the control variants. On the contrary, levels of ethanol and ammonia were largely increased. However, from the point of view of ruminant nutritional physiology such changes can be assessed as acceptable.

Quality criteria of silages prepared with homofermentative *L. plantarum* in both doses seem to be a little more favourable than parameters of silages inoculated with *L. buchneri* or Microsil preparation. *L. buchneri,* the obligate heterofermentative bacterium, was observed to metabolize lactic acid to acetic acid and 1,2-propanediol in anaerobic cultures (Driehuis *et al*., 1999). Undissociated acetic acid content was identified as the most important inhibitory factor of yeast growth in spontaneously fermented silages. Acid-tolerant yeasts oxidize residual fermentable carbohydrates and lactic acid to carbon dioxide in silage aerated during its handling. As the process proceeds, pH value rises and other aerobic microorganisms start to proliferate. Thus, the role of *L. buchneri* as compared to homofermentative inoculants has been mainly to increase aerobic stability of silage (Ranjit and Kung, 2000). However, *L. buchneri* strain used in our experiments did not significantly change the levels of acetic acid as compared to the other starter cultures used.

Maximum content of propionic acid was 0.6 g/kg. Activity of *Propionibacterium* spp., present within epiphytic microflora, finishes as soon as pH value decreases to about 5.0. Thus, propionic acid is much more likely the result of alanine deamination by limited clostridial activity. Propionic acid also participates in silage stability against aerobic deterioration (Merry and Davies, 1999), however, its contents in the prepared silages were too low.

Isobutyric acid was detected at low levels (0.1 – 0.3 g/kg) without any significant differences between the variants. It can be formed from valine by oxidative deamination followed by decarboxylation. Similarly as for propionic acid, its contents was too low to improve aerobic stability of silage.

Both the acids at the observed levels are readily metabolized by ruminants. No C_5 and C_6 volatile fatty acids were detected at detection limit of 0.1 g/kg. Methanol contents were very low, only up to 0.2 g/kg without any significant differences between the variants. It was released probably from pectin. Methanol is readily metabolized by rumen microflora to methan (Pol and Demeyer, 1988; Neumann *et al*., 1999). Ethanol contents were higher in the inoculated variants. Application of *L. plantarum* seems to be the most effective. However, absolute levels are low enough to be metabolized by ruminants (Jeanblain *et al*., 1992). No C_3 and C_4 alcohols were detected at a detection limit of 0.1 g/kg.

In conclusion, the effects of the inoculants on maize fermentation evaluated by traditional chemical quality criteria were relatively limited at both applied doses. Ensiling ability of silage maize by spontaneous fermentation has been due to the combination of high content of fermentable carbohydrates, low levels of buffering constituents and usually also to both appropriate dry matter content and composition of epiphytic microflora.

Most biogenic amines present in feeds and foods have been produced by decarboxylation of free amino acids, released during proteolysis, by activity of bacteria possessing relevant decarboxylases. Two bacterial groups are important. The role of putrefactive bacteria is limited in maize silage fermentation due to relatively quick decrease of pH value of the ensiled mass. A lot of lactic acid bacteria species and strains form the second group. Great variations of decarboxylating abilities occur even among strains within a species (Bover-Cid and Holzapfel, 1999). Thus, biogenic amine levels in spontaneously fermented farm-scale maize silages have been relatively high and ranging widely (Křížek *et al*., 1993; Steidlová and Kalač, 2002).

Very limited data were published on changes of free amino acid contents available for decarboxylating bacteria during maize ensiling. Fairbairn *et al*. (1992) reported relatively slow increase of free tyrosine, histidine and lysine contents during the initial 7 days, then considerable increase between days 7 and 42 followed by decrease until day 90 of ensiling. The most extensive changes were observed in lysine.

The *in vitro* testing of amino acid decarboxylase activity of the used strains of starter cultures showed that *L. buchneri* and *E. faecium* from the Microsil preparation are able to produce biogenic amines. Particularly, they decarboxylated tyrosine and produced tyramine (Špička *et al*., 2002). Both the species have been described as strong tyramine producers (Bover-Cid and Holzapfel, 1999). However, such potential ability not necessarily have to occur under *in vivo* conditions of ensiling process. No such decarboxylation activity was confirmed in our experiments.

Four amines, histamine, tyramine, putrescine and cadaverine, originating mainly from histidine, tyrosine, arginine/ornithine and lysine, respectively, were reported as undesirable silage components. Histamine occurred in the experimental silages very rarely, commonly below the detection limit 2.1 mg/kg. Such contents are of no risk for ruminants. Levels of tyramine, putrescine and cadaverine were significantly decreased by the inoculants application, especially at the higher dose $5{\cdot}10^6$ CFU/g (Table VII). *L. plantarum* seems to be the most effective at that dose. Similar results were observed at

TABLE VII Overall statistical results of Duncan's test at a significance level of $P < 0.05$ for all five laboratory experiments tested together. Data are given only for parameters with significant differences between the variants

		L. plantarum		*L. buchneri*		*Microsil*	
Parameter	*Control*	$5 \cdot 10^5$ *CFU/g*	$5 \cdot 10^6$ *CFU/g*	$5 \cdot 10^5$ *CFU/g*	$5 \cdot 10^6$ *CFU/g*	$5 \cdot 10^5$ *CFU/g*	$5 \cdot 10^6$ *CFU/g*
Amines							
Tyramine	d	bc	ab	b	bc	c	a
Putrescine	d	bcd	a	ab	abc	dc	abc
Cadaverine	c	c	a	ab	a	bc	abc
Quality parameters							
pH	a	cd	bc	de	e	ab	a
Total acidity	c	a	ab	abc	ab	abc	bc
Acetic acid	bc	a	a	c	ab	ab	ab
Ethanol	a	bc	c	b	bc	a	b
Ammonia	a	bc	bcd	bcd	cd	d	ab

TABLE VIII Correlation coefficients between silage quality parameters and amine contents in all five experiments together ($n = 138$ except for ammonia where $n = 114$)

Amine	*pH*	*Total acidity*	*Lactic acid*	*Acetic acid*	*Ethanol*	*α-amino groups*	*Ammonia*
Tyramine	0.3942*	0.0026	0.3941*	0.3278*	− 0.0337	0.3395*	0.3051*
Putrescine	0.5052*	− 0.1275	0.5115*	0.3699*	− 0.1632	0.3839*	0.3161*
Cadaverine	− 0.7905*	0.4553*	− 0.6342*	− 0.3999*	0.0315	− 0.2040*	− 0.2607*

*Values significant at $P < 0.05$.

sauerkraut fermentation as white cabbage has been also well fermentable plant material (Kalač *et al*., 2000; Špička *et al*., 2002). However, current trends in silage starter cultures development prefer combination of several species of lactic acid bacteria affecting both primary fermentation and aerobic stability to application of a single species.

Tryptamine levels were also decreased by the inoculants application. Its contents in maize silage have been commonly low and its roles in ruminant physiology have not yet been explained.

The polyamines spermidine and mainly spermine were detected at relatively low levels. Unfortunately, their contents were not determined in the ensiled maize and therefore it is not possible to conclude, if they originate from maize or are produced during fermentation. Data on their levels in maize are very scarce. Nemeth *et al*. (2002) reported a maize content of about 17 and 4 mg spermidine and spermine per kg, respectively in young plants. These values are comparable with our findings in silages. The roles of the both polyamines in ruminant physiology have not been explained yet. They participate in cell and tissue growth, *e.g*. of ruminal epithelium (Eliassen and Sjaastad, 2000).

Correlation coefficients between silage quality parameters and contents of four main amines in all five experiments are given in Table VIII. Although many correlations were significant at $P < 0.05$, it is not easy to draw a common conclusion as these results differ from those in our survey of farm-scale silages (Steidlová and Kalač, 2002).

Much more scientific information will be necessary for elucidation of the processes of proteolysis, degradation of released amino acids both by decarboxylation and deamination in relation to fermentation and other processes. Moreover, histamine and tyramine degrading lactic acid bacteria were found in meat products (Leuschner *et al*., 1998) and their potential occurrence in plant products was not yet investigated.

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