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Short communication

Levels of biogenic amines in maize silages

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Abstract

Seven biogenic amines (BAs) and polyamines were determined in 113 maize silages sampled from farm large-capacity silos 6–8 weeks after filling and sealing the silos. Amines were quantified as *N*-benzamides by micellar electrokinetic capillary chromatography. Mean dry matter (DM) contents of silages were 449±62 and 378±56 g/kg in 1999 and 2000, respectively. Tyramine (TY), putrescine (PUT), cadaverine (CAD) and spermidine were present in all samples, while tryptamine (TR), spermine (SPM) and histamine (HI) were detected only in a part of silages at very low levels. Mean tyramine, putrescine, cadaverine and spermidine contents were 482, 98, 48 and 17 mg/kg and 145, 136, 96 and 38 mg/kg in silages produced in 1999 and 2000, respectively. However, wide variations occurred. Possible effects on silage palatability and ruminants health are discussed.

Keywords: Biogenic amines; Polyamines; Maize silage

1. Introduction

Maize silage has been the main preserved forage fed to ruminants in many countries during winter period. High levels of lactic and acetic acids, mainly in combination with low dry matter (DM) content, limit intake in well preserved silages. Combined effects of volatile fatty acids (VFAs) and products of protein decomposition, such are biogenic amines (BAs), are considered to limit palatability of poorly preserved silages (Dulphy and Van Os, 1996).

Increased contents of dietary amines have been considered undesirable in ruminant nutrition. Ruminants potentially receive amines from both dietary and ruminal microbial sources and, thus, have the potential to absorb greater amounts than other species (Phuntsok et al.,

Abbreviations: BAs, biogenic amines; CAD, cadaverine; DL, detection limit; DM, dry matter; HI, histamine; ND, not detectable; PUT, putrescine; SPD, spermidine; SPM, spermine; TR, tryptamine; TY, tyramine; VFAs, volatile fatty acids

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1998). Biogenic amines, mainly putrescine (PUT), were implicated as a causative factor in ketonemia (Tveit et al., 1992; Lingaas and Tveit, 1992). Putrescine infusion treatment significantly reduced nitrogen degradability in rumen of steers (Dawson and Mayne, 1997) and tyramine (TY) infusion increased pH value and isovalerate proportion in rumen fluid (Dawson and Mayne, 1996). Aschenbach and Gabel (2000) concluded that absorption of ruminal histamine (HI) should be considered as an important cause of systemic histaminosis in acidotic ruminants. Histamine absorption is linked to luminal epithelial damage, which is primarily induced by luminal acidity and not by histamine. Moreover, histamine has been thought to worsen blood circulation in limbs. Roles of polyamines spermidine (SPD) and spermine (SPM) in ruminants are not yet clear.

High levels of biogenic amines are commonly observed in silages prepared from forages with high protein content (e.g. alfalfa, clovers, some grasses). However, as amines are produced by decarboxylation of amino acids not only by enzymes of putrefactive bacteria but also of many species and strains of lactic acid bacteria, their high levels were reported even in maize silages (Křížek et al., 1993). Cultivation of silage maize has changed considerably in the Czech Republic during the last decade. New hybrids (e.g. stay-green and two-line ones) have been grown. Decrease in plant density and delayed harvest have resulted in dry matter contents of ensiled maize 350–400 g/kg and even more. The increase of usual DM of ensiled maize has been about 120–150 g/kg as compared to the situation 10 years ago.

The objective of this work is a survey of biogenic amines levels in current farm-scale maize silages produced in the Czech Republic. Seven amines were determined: histamine, tyramine, putrescine, cadaverine (CAD), tryptamine (TR) and polyamines spermidine and spermine.

2. Materials and methods

2.1. Sampling

In total, 113 silages were sampled from large-scale clamp and bunker silos using a vertical corer, 6–8 weeks after filling and sealing of silos during 1999 and 2000. Samples originated from 86 farms and 113 ensilings. Most of silages were preserved by spontaneous fermentation, different biological additives (lactic acid bacteria inoculants and/or hydrolytic enzymes) were applied in 18 silages. The samples were transported in polyethylene bags with minimum air level and analysed on the day of sampling.

2.2. Analytical methods

Dry matter content was determined according to the Czech Standard ČSN 46 7092-42. DM determined by drying in an oven at 105 °C for 5 h was corrected to losses of volatile constituents using equation $DM_{corr} = DM_{oven} + 0.92 \times \text{formic acid} + 0.89(\text{acetic} + \text{propionic} + \text{butyric acids}) + 0.31 \times \text{lacticacid} + 1.0 \times \text{ethanol}$ (all constituents in g/kg). A water extract for the determination of quality criteria was prepared by homogenisation of 50 g of silage in 450 ml of water using a kitchen mixer. Mixture was filtered and filtrate was used for determination of pH value, total acidity, lactic acid, volatile fatty acids (VFAs), lower alcohols, alpha-amino groups and ammonia. Lactic acid was determined using an isotachophoretic method, VFAs and alcohols by gas chromatography, ammonia by Conway's microdiffusion method. All analytical procedures, their detection limits and repeatabilities were described in our previous work (Kalač et al., 1999).

An acidic extract for BAs determination was prepared from 20 g of silage shaken with about 75 ml of 0.6 M perchloric acid in a closed Erlenmeyer flask for 1 h. The mixture was filtered through a filter paper, washed with perchloric acid and filtrate volume was adjusted with the acid to 100 ml. Seven BAs were determined as *N*-benzamides by a method of micellar electrokinetic capillary chromatography, described in detail by Křížek and Pelikánová (1998), using Spectraphoresis 2000 (Thermo Separation Products, Fremont, CA). The detection limits were 1.0, 1.3, 1.4, 1.4, 2.1, 2.1 and 3.5 mg/kg for SPD, TR, CAD, SPM, PUT, HI and TY, respectively. The linearity of the method was maintained up to at least 960 mg of the individual amines/kg of sample. Correlation coefficients ranged from 0.9670 for TY to 0.9981 for TR. The relative standard deviations of five parallel determinations ranged from 1.51% for CAD to 8.82% for TY.

2.3. Statistical methods

Correlations between quality parameters and the individual amine contents were tested using multiple regression test at significance level $P < 0.05$ separately for silages produced in 1999 and 2000. The differences between the individual amine contents in silages produced in the both years were tested using analysis of variance at significance level $P < 0.05$. Values below the detection limits were used for the calculations as halves of the limits.

3. Results and discussion

Data on silage quality criteria and amine contents are given in Tables 1 and 2 separately for silages produced in 1999 and 2000. Methanol, releasing from pectin, was present in all samples at usual level about 0.2 g/kg. No C_3 and C_4 alcohols were detected at the detection limits ranging between 0.1 and 0.2 g/kg. Propionic acid was present in nearly all samples at mean content 0.6 g/kg, volatile fatty acids C_4–C_6 were detected only rarely at the detection limit 0.1 g/kg. Values for HI, TR and SPM are given only for samples with levels above the detection limits.

Harvest of silage maize was delayed due to relatively warm and dry weather in the both years and DM contents of silages were, thus, high. There were not found any significant differences in values of quality criteria between the harvest years. However, significantly higher levels of PUT, CAD, SPD and SPM and lower TY content were observed in 2000 than in 1999. Moreover, different distribution of the amine contents was found between years as can be seen from histograms given in Fig. 1. Only histograms of four amines occurring in all samples are presented.

Such differences cannot be explained by a simple way. Commonly, amine contents decrease with increasing DM contents of ensiled forage and resulting silage. Current amine levels are considerably lower than those observed by Křížek et al. (1993) in 54 maize silages

Table 1
Statistical values of silage quality criteria and amine contents in 1999 ($n = 62$)

Parameter	x	s_x	x_{min}	x_{max}	$n >$ DL[a]
Dry matter (g/kg)	449	62	295	584	62
pH	3.56	0.18	3.20	3.99	62
Total acidity (mg NaOH/100 g)	1050	255	683	1770	62
Lactic acid (g/kg)	21.3	4.6	13.6	30.4	62
Acetic acid (g/kg)	5.6	2.6	0.4	16.0	62
Ethanol (g/kg)	2.3	1.2	0.4	7.3	62
Alpha-amino groups (mg/100 g)	100.4	17.7	62.5	152	62
Ammonia (mg/100 g)	35.6	9.1	18.8	55.0	62
Amines (mg/kg)					
Histamine	2.6	8.0	ND[b]	48.5	10
Tyramine	482	197	3.5	924	62
Putrescine	97.8	72.0	9.7	459	62
Cadaverine	48.3	56.0	6.9	282	62
Tryptamine	4.2	12.0	ND[b]	92.4	44
Spermidine	16.6	6.0	1.6	43.0	62
Spermine	0.6	2.0	ND[b]	8.0	9

[a] $n >$ DL means values above the detection limit.
[b] ND value below the detection limit.

with DM contents between 166 and 326 g/kg. Mean contents 435, 388, 341, 72, 25 and 5 mg/kg were reported for TY, PUT, CAD, HI, SPD and SPM, respectively. Similar trends were observed for grass silages with different DM levels in the same work and by Van Os et al. (1996b). However, we did not observe any significant differences ($P < 0.05$) in BAs

Table 2
Statistical values of silage quality criteria and amine contents in 2000 ($n = 51$)

Parameter	x	s_x	x_{min}	x_{max}	$n >$ DL[a]
Dry matter (g/kg)	378	55.8	260	494	51
pH	3.64	0.16	3.20	4.10	51
Total acidity (mg NaOH/100 g)	1010	253	488	1700	51
Lactic acid (g/kg)	18.5	4.1	11.4	29.3	51
Acetic acid (g/kg)	6.1	3.4	1.0	19.6	51
Ethanol (g/kg)	3.1	2.0	0.6	9.4	51
Alpha-amino groups (mg/100 g)	64.5	21.6	26.8	174	51
Ammonia (mg/100 g)	39.6	18.3	12.3	93.3	51
Amines (mg/kg)					
Histamine	3.0	6.7	ND[b]	41.1	18
Tyramine	145	46.4	9.5	238	51
Putrescine	136	53.6	3.2	290	51
Cadaverine	96.2	80.8	4.1	349	51
Tryptamine	2.5	2.8	ND[b]	13.7	33
Spermidine	37.9	9.3	22.5	60.0	51
Spermine	2.8	2.5	ND[b]	9.6	37

[a] $n >$ DL means values above the detection limit.
[b] ND value below the detection limit.

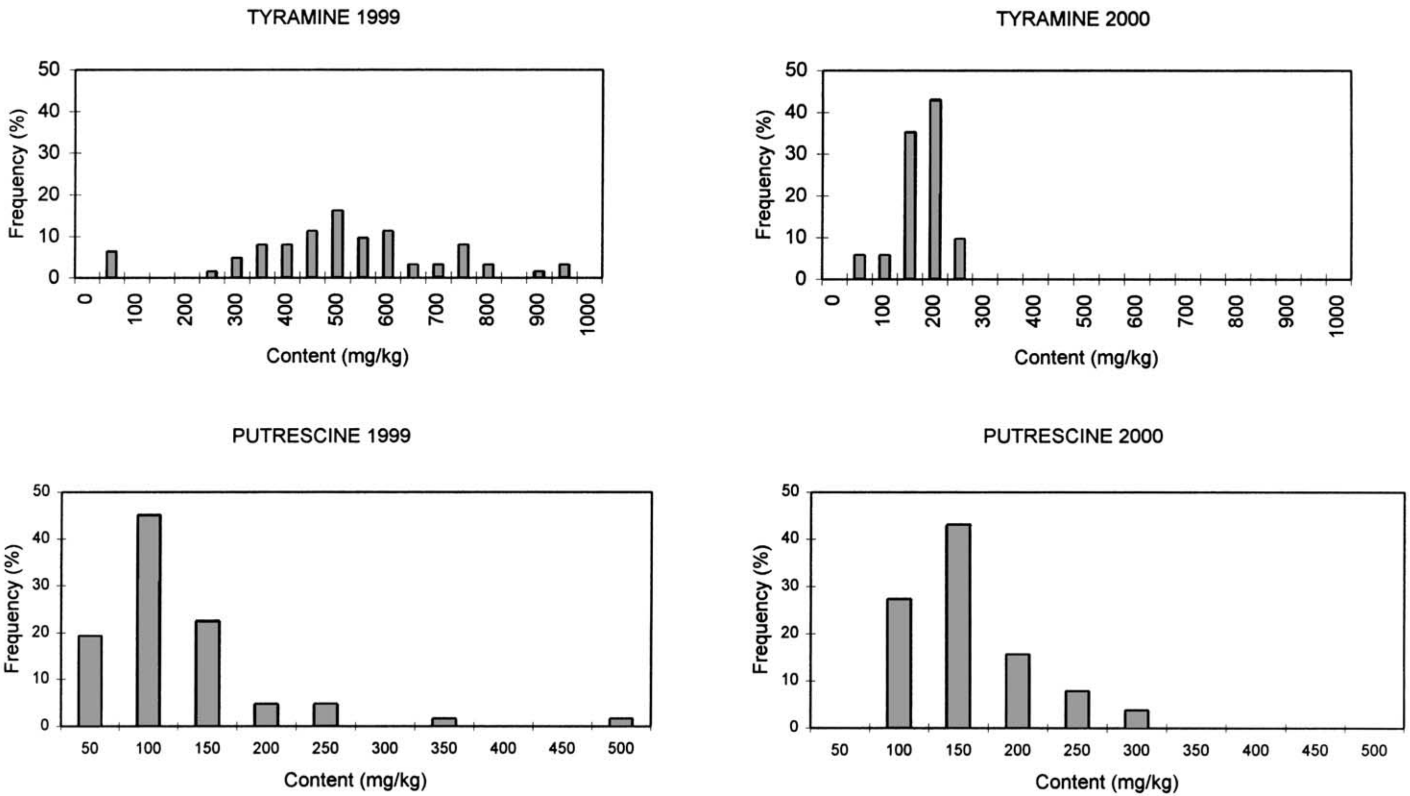

Fig. 1. Histograms of amine contents.

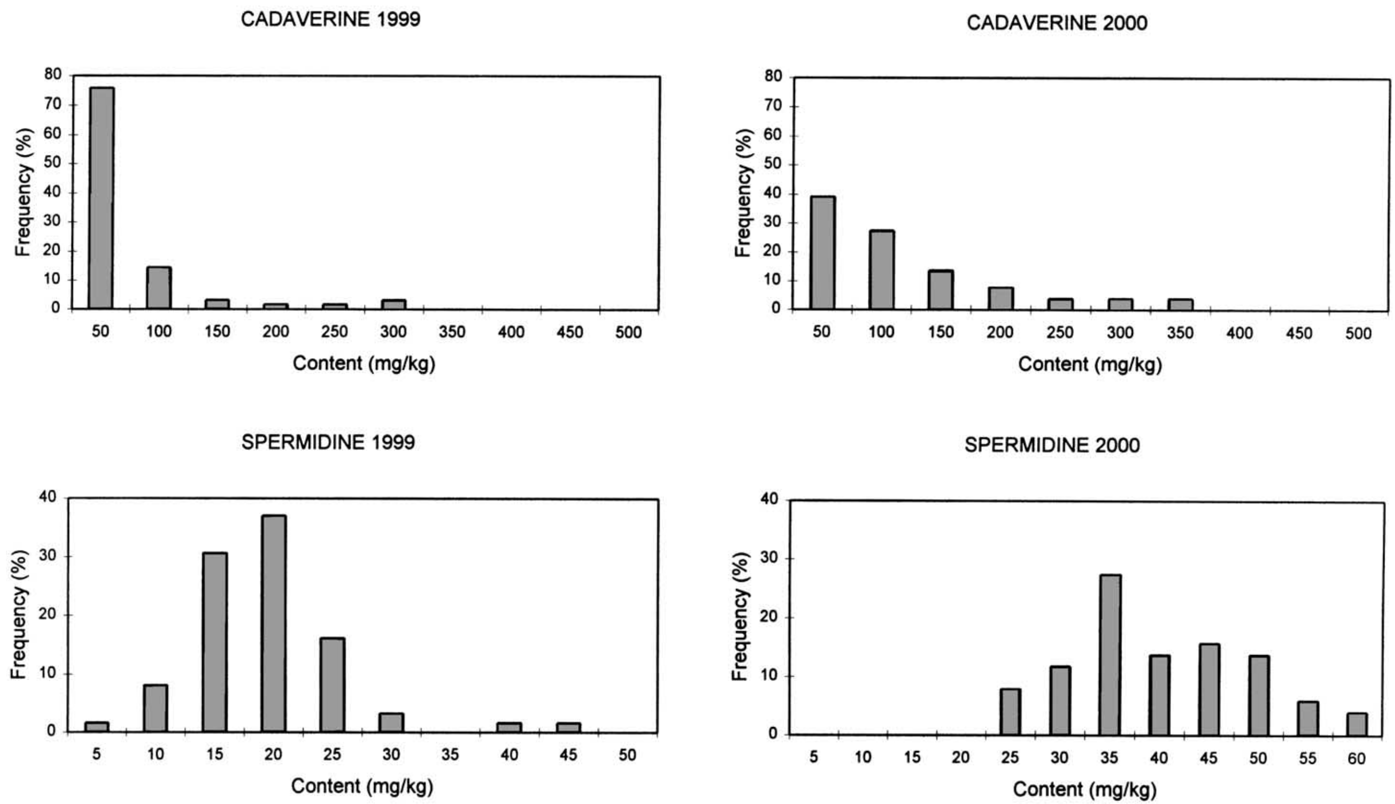

Fig. 1 (*Continued*).

level between silages with DM contents 350–400 and 450–500 g/kg produced in 1999 or between silages with DM contents 300–350 and 400–450 g/kg from 2000.

Moreover, formation of BAs has been affected by several other factors, such are temperature, quickness of pH decrease during the initial stage of fermentation and oxygen access (for reviews see Shalaby, 1996; Silla Santos, 1996). In ensiled maize with high levels of fermentable carbohydrates and quick decrease of pH values, these factors affect spontaneous lactic acid bacteria composition. Decarboxylating activity of these bacteria is determined not only by species composition and counts but also by very different activities of the individual strains within a species, which can be up to three orders of magnitude.

Polyamines SPD and SPM originate probably from maize and are not formed by bacterial activity.

BAs contents were determined in silages sampled 6–8 weeks after sealing silos. Investigating dynamics of BAs formation in laboratory silages of orchard grass, red clover and oats, Křížek (1993b) observed exponential curves for PUT and CAD levels during the initial 30–50 days, then a slight decrease, followed by a slow increase with maximum contents on days 200–230. Thus, levels of those amines during silage feeding in late winter and spring can be a little higher than those given in Tables 1 and 2. No explanation of the PUT and CAD decrease during silage storage was yet reported. However, HI and TY degrading lactic acid bacteria were found in meat products (Leuschner et al., 1998).

Correlation coefficients resulting from multiple regression test between amine contents and silage quality parameters are given in Table 3. No common conclusions can be done from such different between-year results. Van Os et al. (1996b) reported significant positive correlations between total and individual amine contents and ammonia or acetic acid contents in grass silages. No similar correlations were observed between sum of TY + PUT + CAD contents and these silage quality parameters either in silages from 1999 or 2000.

Assessing the observed levels of BAs from ruminants health point of view, HI, TR and SPM levels in the tested maize silages seem to be negligible. Roles of SPD, though present in low levels, are not clear. SPD participates in cells and tissues growth and in human

Table 3
Results (*r* values) of multiple regression test between amine and silage quality parameters in harvest years 1999 and 2000

Amine	Dry matter	pH	Total acidity	Lactic acid	Acetic acid	Ethanol	Alpha-amino groups	Ammonia
Year 1999								
Tyramine	−0.165	−0.256	0.006	0.212	0.118	0.117	0.056	0.201
Putrescine	−0.479*	−0.156	−0.495*	−0.156	0.300*	−0.084	0.543*	−0.192
Cadaverine	−0.480*	−0.373*	−0.444*	0.045	0.454*	−0.023	0.155	0.104
Spermidine	−0.069	0.068	0.073	0.357*	−0.071	0.139	−0.015	0.064
Year 2000								
Tyramine	−0.071	0.249	0.782*	−0.116	−0.608*	−0.141	−0.048	−0.223
Putrescine	−0.443*	−0.313	−0.380	0.215	−0.201	−0.145	−0.143	0.068
Cadaverine	−0.263	−0.429	−0.555	0.450	0.046	−0.117	−0.198	0.239
Spermidine	0.182	−0.156	0.382	−0.130	−0.291	−0.244	0.090	−0.177

* Values significant at $P < 0.05$.

nutrition may be deleterious by stimulating growth of tumours. Thus, TY, PUT and CAD have been amines present in maize silages in the highest levels, forming about 90% of total amine contents. Similar results with decreasing order TY, CAD and PUT reported Van Os et al. (1996b) for grass silages. However, occurrence of several other BAs must be supposed, mainly of agmatine and 2-phenylethylamine. Moreover, some synergistic effects among dietary amines increasing deleterious impacts are known in human nutrition. Such information has been yet lacking in nutrition of ruminants.

Mean daily intake of TY + CAD + PUT by an animal given 20 kg of maize silage (i.e. about 7.5–9 kg DM) was 12.5 and 7.5 g in 1999 and 2000, respectively. In an experiment of Van Os et al. (1995a), no direct role of amines on feed intake regulation was established in dairy cows fed with good-quality grass silage with the addition 2.8 g amines/kg DM (1.0, 0.6, 0.7 and 0.5 g of TY, CAD, PUT and HI, respectively). However, a tendency to reduce DM intake due to amines effect at the oro-pharyngal level of intake control was observed in sheep under the same dose and feeding conditions (Van Os et al., 1995b). Simulating poor-quality grass silages, addition of 4.9 g amines/kg DM was tested in sheep (Van Os et al., 1996a). There was not observed a direct effect on chemostatic regulation of grass silage intake, however, a slight negative effect on silage palatability could not be excluded. Feeding grass silage supplemented with very high level 7.2 g amines/kg DM (2.8, 1.4, 1.8 and 1.2 g of TY, CAD, PUT and HI, respectively), Van Os et al. (1997) observed that in sheep non-adapted to dietary amines acutely decreased DM intake due to reduced palatability and with high probability also due to stress of intermediary metabolism. Sheep preconditioned to amines showed a slight reduction of DM intake. The animals adapted within 2 weeks to high BAs intake in diet increased their daily DM intake. Thus, there was clear evidence that amines have a negative effect on palatability. From an in vitro study with wether sheep rumen content resulted that in animals adapted to silage with high BAs level, the accumulation of amines in the rumen is prevented by an increase of the amine-degrading capacity of rumen microbes (Van Os et al., 1995c).

In conclusion, mean daily TY + CAD + PUT exposures from the tested maize silages ranged between 1.0 and 1.4 g/kg DM. Such intakes should not be of negative effects on cattle. However, the maximum observed levels of BAs in maize silages were up to several times higher than the mean values. A risk both of palatability decrease and of some detrimental health effects for cattle, probably in combination with high silage acidity, cannot be thus, excluded for a part of maize silages.

Application of formic acid as an preservative, known in grass silages (Křížek, 1993a; Van Os et al., 1995a,b, 1996b; Gasior and Brzóska, 1999a,b) and alfalfa silages (Jambor, 2000) as an efficient treatment for BAs level decrease, cannot be used for maize silages. Some lactic acid bacteria inoculants (starter cultures) were found to decrease significantly BAs contents in sauerkraut (fermented white cabbage) (Kalač et al., 2000). Research work testing efficiency of some inoculants in maize and grass silages is, therefore, in progress.

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