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THE EFFECTS OF LACTIC ACID BACTERIA INOCULANTS AND FORMIC ACID ON THE FORMATION OF BIOGENIC AMINES IN GRASS SILAGES

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Silages were prepared in six laboratory experiments from four direct-cut grassland swards and pure swards of perennial ryegrass and false oat with dry matter contents ranging between 180 and 325 g/kg. Grass was fermented at 22°C and silages were stored at the same temperature for 4 months. Untreated silages (negative control) and silages preserved with 3 g/kg of formic acid (positive control) were compared with silages inoculated with commercial strains of *Lactobacillus plantarum*, *Lactobacillus buchneri* and a mixed preparation Microsil. The inoculants were applied at a dose of 5.10^6 CFU/g of grass. Seven biogenic amines were extracted from silages with perchloric acid and determined as *N*-benzamides by micellar electrokinetic capillary chromatography. Common chemical quality parameters of silages were also determined. Tyramine, cadaverine and putrescine were the amines occurring at the highest concentration. As compared to untreated silages, formic acid was most effective to suppress formation of the main amines. Also the inoculants often decreased amine contents significantly ($P < 0.05$). The inoculants decreased levels of polyamine spermidine more efficiently than formic acid. Contents of histamine, tryptamine and polyamine spermine were very low, commonly below the detection limits.

Keywords: Biogenic amines; Polyamines; Grass silage; Formic acid; Inoculants; *Lactobacillus plantarum*; *Lactobacillus buchneri*

1. INTRODUCTION

At temperate climates grass silages are produced in large quantities for feeding ruminants during winter period. However, their palatability can be decreased by some components. Several biogenic amines, mainly histamine, tyramine and putrescine, fall into such silage constituents (Dulphy and Van Os, 1996). Nevertheless, the effects of amines on silage dry matter intake have been inconsistent in the literature. Ruminants potentially receive amines from both dietary and ruminal microbial sources and therefore they have a potential to absorb greater amounts than other species (Phuntsok *et al*., 1998). Van Os *et al*. (1995a) observed no direct impact of amines on the regulation

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of feed intake in dairy cows, which received good-quality grass silage with an addition of tyramine, putrescine, cadaverine and histamine at 1.0, 0.7, 0.6 and 0.5 g/kg silage dry matter, respectively. Nevertheless, a tendency to reduce dry matter intake due to the amines at the oro-pharyngal level of intake control was established in sheep at the same dose and feeding conditions (Van Os *et al*., 1995b). Moreover, amines, mainly putrescine, were implicated as the causal factors in ketonemia (Lingaas and Tveit, 1992). Infusion of putrescine significantly reduced nitrogen degradability in the rumen of steers (Dawson and Mayne, 1997), and a tyramine infusion treatment increased pH values and isovalerate proportion in rumen fluid (Dawson and Mayne, 1996). Aschenbach and Gäbel (2000) reported that absorption of ruminal histamine should be considered as an important cause of systematic histaminosis in acidotic ruminants. Furthermore, histamine has been thought to impair blood circulation in limbs. The role of polyamines, spermidine and spermine in ruminant nutrition and physiology are not yet clear.

Most of biogenic amines have been formed in silage by amino acid-decarboxylating activity of putrefactive bacteria and also by some species or strains of lactic acid bacteria. Free amino acids released from plant proteins become available and their decarboxylation takes place by metabolic activity of bacteria (Silla Santos, 1996). The most common amines, histamine, tyramine, cadaverine and putrescine are produced from histidine, tyrosine, lysine and arginine/ornithine, respectively. Polyamines, spermidine and spermine, originating from putrescine, seem to be natural components of forage.

In a survey of 30 farm silages prepared from wilted grass and red clover-grass swards with a DM content of about 450 – 500 g/kg, for tyramine and cadaverine a mean content of approximately 100 mg/kg were observed. About half of this level was analysed for putrescine (Steidlová and Kalač, 2002b). In a survey of 53 farm grass silages with low dry matter content considerably higher levels were reported by Křížek *et al*. (1993). Commonly, amine content increased with decreasing dry matter level and considerable histamine levels were observed. In both studies the maximum content of amines was several times higher than mean values.

Efficient grass wilting is difficult under poor weather conditions. Therefore, forage with low dry matter is often ensiled using either chemical or biological additives. Formic acid as a widespread silage preservative was proved to decrease efficiently biogenic amine contents in grass silages (Křížek, 1993; Van Os *et al*., 1996; Gasior and Brzóska, 1999a,b) and lucerne silages (Jambor, 2000).

Lactic acid bacteria cultures are widely used in farming practice as silage inoculants (starter cultures). The homofermentative bacterium *Lactobacillus plantarum* has been commonly used, while in recent years the heterofermentative *Lactobacillus buchneri* has been tested and recommended because of improving aerobic stability of silages due to the formation of acetic acid (Holzer *et al*., 2003). In our knowledge, the effect of *L. buchneri* on the formation of biogenic amines in silages was not yet tested. Mixtures of lactic acid bacteria with successive activities during ensiling process have been also widely applied for silage production.

The objective of the present work was to reduce the amine content of silages in laboratory experiments. For this purpose silages from direct-cut grasses with addition of lactic acid bacteria inoculants were compared with untreated silages prepared by spontaneous fermentation (negative control) and silages fermented with formic acid addition (positive control).

2. MATERIALS AND METHODS

2.1. Silage preparation

Four grassland swards and two grasses from pure swards were used. All ensiling materials were taken from the experimental plots of the Department of Forage Crops, University of South Bohemia. Characteristics of the used forages are given in Table I. The fresh materials were chopped 2 h after harvest by a semi-scale forage cutter to pieces of 1 – 2 cm length and ensiled immediately.

Five treatments were ensiled in experiments 1, 2, 4, 5 and 6 and 4 treatments in experiment 3 due to limited quantity of grass (Table I). The same volume of additives (25 ml) were applied per kg of grass: 3 g/kg of formic acid to positive control, suspensions of three inoculants or distilled water to negative control (untreated). Jars of 720 ml volume were completely filled with 315 – 425 g of a mixture (Table I). 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 replicates were prepared for each treatment.

The jars used as the laboratory silos allow fermentation gases to escape during the initial days of extensive fermentation. Later, during silage storage, the jars are hermetically sealed. Sporadically occurring jars with air access, showing mould growth and colour changes, were excluded.

2.2. Inoculants and sampling

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) (not used in Experiment 3), *Lactobacillus buchneri* (CCM 1819) and the mixed preparation Microsil containing *L. plantarum* (CCM 3769), *Lactobacillus casei* (CCM 3775), *Enterococcus faecium* (CCM 6226) and *Pediococcus pentosaceus* (CCM 3770) were applied at a $5 \cdot 10^6$ CFU/g of ensiled grass. The preparations were suspended in distilled water immediately prior to application.

The used dose has been usually applied in farming practice and it was found to be sufficient to decrease content of biogenic amines in sauerkraut (Špička *et al*., 2002).

Silages were sampled after 4 months of storage and four replicates were analysed for each treatment.

2.3. Analytical methods

Dry matter and nitrogen contents of ensiled grasses and chemical quality parameters of the silages were determined as described in our previous papers (Kalač *et al*., 1999, 2000; Steidlová and Kalač, 2002a). Analytical procedure for determination of seven observed biogenic amines 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 determination of tyramine, putrescine and cadaverine, respectively.

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

TABLE I Characteristics of grasses ensiled in laboratory experiments

Exp. no.	*Date*	*Botanical composition [% of wet weight] and prevailing species*	*Cut*	*Stage of vegetation of prevailing grass*	*DM [g/kg]*	*Nitrogen [g/kg DM]*	*Density of ensiled forage [kg/m^3]*
1	20th Sept. 2000	64% grasses (*Dactylis glomerata, Trisetum flavescens, Festuca pratensis*), 8% clovers (mainly *Trifolium pratense)*, 28% forbs (mainly *Taraxacum officinale* and *Achillea millefolium*)	3rd	Inflorescence emergence	235	21.5	590
2	4th Oct. 2000	77% grasses *(Dactylis glomerata, Agrostis vulgaris, Trisetum flavescens*), 10% clovers, 13% forbs (mainly *T. officinale*)	3rd	Inflorescence latent in sheath	180	20.3	590
3	28th May 2001	100% perennial ryegrass (*Lolium perenne*) var. Mustang	1st	Visible second node	212	19.7	520
4	28th May 2001	100% false oat (*Arrhenatherum elatius*) var. Medián	1st	Inflorescence latent in sheath	230	18.8	450
5	31st May 2001	69% grasses (*Poa pratensis, Lolium perenne, Alopecurus pratensis*) , 6% clovers, 25% forbs (mainly *Achillea millefolium, T. officinale* and *Galium verum*)	1st	Stigma fully developed	325	17.6	440
6	20th Sept. 2001	68% perennial ryegrass (*Lolium perenne*), 8% clovers, 24% forbs (mainly *T. officinale*)	3rd	Visible second node	255	19.1	440

2.4. Statistical methods

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

3. RESULTS AND DISCUSSION

Direct-cut grasses differing in botanical composition, cut order and DM content (Table I) were ensiled. Contents of biogenic amines and quality parameters of the experimental silages are given in Tables II and III. Evaluating chemical parameters of the untreated silages, together with sensorial assessment, good preservation efficiency was observed in Experiments 3 and 5, medium efficiency in Experiments 1, 2 and 6, while poor efficiency was observed in Experiment 4.

Both, the results of the individual experiments (Tables II and III) and the combined statistical test of all six experiments (Table IV) proved the high preservation efficiency of formic acid. The effects of the used inoculants were limited. The Microsil preparation seems to be of somewhat higher efficiency than both lactobacilli. This is different to our previous experiments after inoculation of maize silage, where *L. plantarum* showed a slightly better effect (Steidlová and Kalač, 2003). As reviewed by Holzer *et al.* (2003), under anaerobic conditions the common obligate heterofermentative bacterium *L. buchneri* is able to ferment hexoses and pentoses to lactic acid and acetic acid as well as to metabolize lactic acid to acetic acid. Since undissociated acetic acid was identified as the most important factor inhibiting yeast growth, *L. buchneri* increases the aerobic stability of silages against deterioration following aeration during silage handling (Danner *et al.*, 2003). However, the *L. buchneri* strain used in our experiments did not significantly increase the acetic acid contents as compared to other inoculants or untreated silages.

Proteolytic processes in forages and factors affecting their extent were described by Davies *et al.* (1998) and Winters *et al.* (2000, 2001). The released amino acids can be then decarboxylated. Putrescine and cadaverine seem to be the main amines formed by putrefactive bacteria, while some lactic acid bacteria produce mainly tyramine and histamine (Silla Santos, 1996). Great variations of decarboxylating abilities among both species and strains of lactic acid bacteria were reported (Bover-Cid and Holzapfel, 1999). Therefore, biogenic amine levels in untreated farm-scale grass silages have been relatively high and ranging widely. A high amine content has to be expected in silages with low dry matter (Křížek *et al.*, 1993; Steidlová and Kalač, 2002b).

The laboratory testing of amino acid decarboxylase activity of the used inoculants, which were cultivated in a defined microbiological medium revealed that *L. buchneri* and *E. faecium* from the Microsil preparation were able to produce biogenic amines. Particularly, both strains are able to decarboxylate tyrosine and to produce tyramine (Špička *et al.*, 2002) and are known to be strong tyramine producers (Bover-Cid and Holzapfel, 1999). However, such potential ability not necessarily leads to a tyramine production under the complex conditions of an ensiling process, which was confirmed by our experiments.

As mentioned above, histamine, tyramine, putrescine and cadaverine were reported as undesirable silage constituents. Histamine was detected at low level only in the

TABLE II Contents of biogenic amines and quality parameters of silages prepared from grassland sward with formic acid and starter cultures after 4 months storage (Experiment 1, 2 and 3) (Means, $n = 4$)

Parameter	Experiment 1					Experiment 2					Experiment 3			
	Untreated	*Formic acid*	L. plantarum	L. buchneri	*Microsil*	*Untreated*	*Formic acid*	L. plantarum	L. buchneri	*Microsil*	*Untreated*	*Formic acid*	L. buchneri	*Microsil*
Amines [mg/kg]														
Histamine	ND[1]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tyramine	102^{b}	47.1^{a}	97.7^{b}	38.6^{a}	80.8^{ab}	75.8	59.6	46.1	40.1	49.1	82.1^{c}	18.8^{a}	53.8^{b}	68.7^{c}
Putrescine	111^{d}	9.8^{a}	40.8^{c}	13.6^{a}	49.7^{c}	88.0^{c}	14.4^{a}	27.9^{a}	18.8^{a}	55.3^{b}	11.7^{b}	7.7^{a}	7.5^{a}	6.5^{a}
Cadaverine	210^{d}	26.2^{a}	97.7^{c}	41.1^{b}	99.4^{c}	140^{c}	35.0^{a}	72.1^{ab}	51.6^{ab}	83.2^{b}	83.9^{c}	21.1^{a}	31.6^{ab}	41.1^{b}
Tryptamine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	4.4	5.9	2.0	ND
Spermidine	11.2^{c}	6.8^{a}	8.4^{ab}	10.3^{bc}	9.8^{bc}	7.7^{ab}	9.2^{b}	6.9^{ab}	6.2^{ab}	5.4^{a}	34.7^{c}	26.1^{b}	11.5^{a}	11.8^{a}
Spermine	ND	ND	ND	1.6	ND	3.7	ND	ND	ND	ND	ND	ND	ND	ND
Quality parameters														
pH	4.27^{a}	4.18^{ab}	4.85^{b}	4.48^{ab}	4.17^{ab}	4.21^{b}	3.89^{a}	4.07^{ab}	4.16^{b}	4.01^{ab}	3.83^{b}	4.02^{c}	3.71^{a}	3.81^{ab}
Total acidity [mg NaOH/100g]	775^{c}	630^{b}	480^{a}	555^{ab}	770^{c}	550	595	605	605	645	905	695	950	895
Lactic acid [g/kg]	26.3	18.5	22.6	21.8	16.7	17.1^{b}	15.5^{ab}	12.6^{ab}	10.0^{a}	12.6^{ab}	11.4^{a}	12.6^{ab}	13.8^{ab}	17.4^{b}
Acetic acid [g/kg]	6.1^{c}	2.8^{a}	4.1^{ab}	7.7^{c}	5.9^{bc}	3.9^{ab}	1.9^{a}	6.3^{b}	3.6^{ab}	4.1^{ab}	5.0^{c}	3.1^{a}	4.2^{bc}	3.2^{ab}
Propionic acid [g/kg]	0.2^{a}	0.6^{bc}	0.6^{c}	0.2^{ab}	0.4^{abc}	0.3	0.2	0.3	0.3	0.1	0.4	0.2	0.3	0.1
Butyric acid [g/kg]	2.9^{b}	0.2^{a}	7.6^{c}	1.1^{ab}	0.4^{a}	2.9	0.3	0.3	1.8	0.3	0.3	0.4	0.2	0.1
Isobutyric acid [g/kg]	0.2^{a}	0.1^{a}	0.5^{b}	1.2^{c}	0.1^{a}	0.3^{ab}	0.2^{a}	0.4^{ab}	0.6^{b}	0.4^{ab}	ND	0.1	0.1	0.1
Methanol [g/kg]	0.2	0.2	0.2	0.3	0.3	0.2	0.2	0.1	0.1	0.2	0.3	0.4	0.4	0.3
Ethanol [g/kg]	2.0^{b}	1.1^{a}	1.2^{a}	1.3^{a}	1.1^{a}	1.0^{d}	0.3^{a}	0.8^{c}	0.6^{b}	0.9^{cd}	2.6^{b}	2.0^{ab}	1.6^{a}	2.4^{ab}
α-amino groups [mg/100g]	105^{c}	80^{ab}	97^{bc}	75^{a}	90^{abc}	58^{c}	43^{ab}	51^{bc}	52^{a}	54^{abc}	58	54	50	47
Ammonia [mg/100g]	60^{b}	42^{a}	64^{b}	54^{b}	53^{b}	35^{b}	18^{a}	31^{b}	29^{b}	27^{b}	27	29	20	22

[1]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 false oat (Experiment 4) or grassland sward (Experiment 5 and 6) with formic acid and starter cultures after 4 months storage (Means, $n = 4$)

	Experiment 4					*Experiment 5*					*Experiment 6*				
Parameter	*Untreated*	*Formic acid*	L. plantarum	L. buchneri	*Microsil*	*Untreated*	*Formic acid*	L. plantarum	L. buchneri	*Microsil*	*Untreated*	*Formic acid*	L. plantarum	L. buchneri	*Microsil*
Amines [mg/kg]															
Histamine	7.4	ND[1]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tyramine	120	112	83.3	75.9	111	53.4	19.8	39.6	65.8	86.8	70.6^{ab}	42.9^{a}	99.6^{b}	102^{b}	76.2^{b}
Putrescine	182	94.4	151	73.7	103	46.2^{ab}	36.0^{a}	58.0^{ab}	72.2^{b}	59.2^{ab}	87.5	133	129	153	125
Cadaverine	218^{b}	102^{a}	87.2^{a}	55.2^{a}	102^{a}	115^{c}	27.2^{a}	46.2^{ab}	55.0^{ab}	82.6^{bc}	106^{ab}	87.0^{a}	167^{c}	156^{c}	107^{ab}
Tryptamine	ND	1.6	2.1	ND	ND	ND	9.1	ND	1.3	ND	ND	ND	ND	ND	ND
Spermidine	16.0^{ab}	23.8^{b}	13.0^{a}	19.0^{ab}	19.7^{ab}	53.0^{b}	49.0^{b}	17.6^{a}	23.2^{a}	23.0^{a}	12.6	5.7	3.6	8.7	7.1
Spermine	ND	ND	ND	ND	ND	1.8	1.5	ND	ND	ND	ND	ND	ND	ND	ND
Quality parameters															
pH	5.17^{b}	4.00^{a}	4.11^{a}	4.22^{a}	4.17^{a}	4.12^{b}	4.05^{b}	3.84^{a}	3.84^{a}	3.88^{a}	3.80	3.86	4.28	4.44	4.10
Total acidity [mg NaOH/100g]	410^{a}	705^{b}	800^{b}	805^{b}	735^{b}	645^{b}	550^{a}	850^{c}	710^{b}	830^{c}	780	665	575	370	640
Lactic acid [g/kg]	14.0^{b}	12.9^{b}	9.4^{a}	10.1^{ab}	10.6^{ab}	17.4^{b}	7.9^{a}	10.6^{a}	11.1^{a}	10.6^{a}	3.4	10.0	7.3	7.7	7.4
Acetic acid [g/kg]	4.5^{a}	3.7^{a}	5.6^{b}	7.7^{c}	5.1^{ab}	5.0	3.4	3.0	2.9	3.4	3.8	3.8	4.5	4.9	7.2
Propionic acid [g/kg]	1.4^{b}	0.3^{a}	0.1^{a}	0.2^{a}	0.2^{a}	0.1	0.2	0.2	0.1	0.3	0.1	0.1	0.2	0.1	0.2
Butyric acid [g/kg]	15.0^{b}	0.8^{a}	0.2^{a}	0.3^{a}	2.2^{a}	0.1	0.1	0.1	0.2	0.2	0.1^{a}	ND^{a}	2.6^{b}	0.5^{ab}	0.5^{ab}
Isobutyric acid [g/kg]	0.4^{b}	0.2^{a}	0.1^{a}	0.1^{a}	0.2^{a}	0.1	0.1	0.2	0.1	0.2	0.2^{ab}	0.1^{a}	0.3^{c}	0.2^{ab}	0.3^{bc}
Methanol [g/kg]	0.2	0.2	0.2	0.2	0.2	0.5	0.6	0.5	0.5	0.5	0.2	0.2	0.2	0.2	0.2
Ethanol [g/kg]	2.7^{b}	1.5^{a}	1.9^{ab}	1.8^{a}	1.5^{a}	2.1^{a}	2.4^{a}	10.4^{c}	9.5^{c}	6.6^{b}	1.5^{bc}	1.2^{ab}	1.3^{b}	2.1^{c}	0.6^{a}
α-amino groups [mg/100g]	137^{b}	74^{a}	85^{a}	100^{a}	90^{a}	117^{b}	78^{a}	76^{a}	70^{a}	80^{a}	137	111	137	102	127
Ammonia [mg/100g]	100^{c}	39^{a}	51^{b}	38^{b}	35^{a}	63^{b}	43^{a}	35^{a}	38^{a}	35^{a}	53	52	62	54	60

[1]ND: Values were below detection limit.
Means with different superscript letters in a line indicate significant differences at $P < 0.05$.

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

Parameter	*Untreated*	*Formic acid*	L. plantarum	L. buchneri	*Microsil*
Amines					
Tyramine	b	a	b	ab	b
Putrescine	b	a	ab	a	ab
Cadaverine	c	a	b	b	ab
Spermidine	c	bc	a	ab	ab
Quality parameters					
Lactic acid	b	a	a	a	a
Acetic acid	b	a	b	b	b
Butyric acid	c	a	bc	ab	ab
α-amino groups	b	a	a	a	a
Ammonia	c	a	bc	b	ab

Means with different superscript letters in a line indicate significant differences at $P < 0.05$.

untreated silage of Experiment 4 (Table III). Tyramine, putrescine and cadaverine were the amines occurring at the highest levels. Cadaverine contents in the untreated silages of all six experiments were higher than the content of putrescine. The same relation was observed in our survey of farm-scale grass silages (Steidlová and Kalač, 2002b). This is an inverse relation than commonly found in maize silages (Steidlová and Kalač, 2002a, 2003). Several factors should be taken under consideration. Contents of lysine as cadaverine precursor have been commonly lower in silage maize than in grasses. Putrescine can be produced either from ornithine or from arginine via agmatine. Contents of arginine have been comparable in maize and grasses, data on ornithine contents have been scarce. Moreover, different rates of proteolysis and different composition of microflora can also have some influence.

Formic acid at the usually applied dose of 3 g/kg decreased biogenic amine contents very efficiently. Similar results were reported for forages wilted to different dry matter content, e.g. perennial ryegrass (Van Os *et al*., 1996), meadow and pasture swards (Gasior and Brzóska, 1999a,b), orchardgrass, oat and red clover (Křížek, 1993) and lucerne (Jambor, 2000). Rapid acidification of ensiled forage during the initial stage of fermentation seems to be an efficient factor due to limitation of proteolysis as a source of free amino acids (McKersie, 1985; Fairbairn *et al*., 1992).

None of the tested inoculants at $5 \cdot 10^6$ CFU/g reached efficiency of formic acid to decrease tyramine, putrescine and cadaverine contents. Similar results were observed by Van Os *et al*. (1996) who ensiled perennial ryegrass wilted to 250 g DM per kg with *L. plantarum,* a mixture of *L. plantarum* and *Enterococcus* (formerly *Streptococcus*) *faecium* or *Enterobacter sakazakii* at doses of $1 \cdot 10^7$, $1 \cdot 10^5$ or $6 \cdot 10^6$ CFU/g, respectively.

A small decrease of about 13% of total amines was reported from two laboratory experiments preparing grass silages with Microsil preparation at a low dose of $2 \cdot 10^5$ CFU/g as compared to untreated silages (Gasior and Brzóska, 1999a,b). Using the preparation Bactozym, which contains the same bacteria as the preparation Microsil and additionally cellulase, hemicellulase and glucose oxidase, even a lower decrease was observed in both experiments.

In many cases the tested starter cultures were able to decrease the main amine levels significantly, however, they were less effective as compared to maize silages (Steidlová

TABLE V Correlation coefficients between silage quality parameters and amine contents in all six experiments together ($v = 114$)

Amine	*pH*	*Total acidity*	*Lactic acid*	*Acetic acid*	*Ethanol*	*α-amino groups*	*Ammonia*
Tyramine	0.1813	0.0880	0.0484	0.2108*	0.1194	0.1978*	0.2125*
Putrescine	0.1230	− 0.2427*	− 0.2286*	0.1325	0.0117	0.5810*	0.5564*
Cadaverine	0.2367*	− 0.1713	0.1259	0.1984*	− 0.0982	0.6013*	0.5031*
Spermidine	0.0652	0.1615	0.0106	− 0.0214	0.2513*	0.0901	0.0875

*Values are significant at $P < 0.05$.

and Kalač, 2003). It probably results from a limited ability of the starter cultures to acidify the ensiled grass rapidly.

Histamine and tryptamine contents were commonly below the detection limits. The spermidine levels were decreased significantly by inoculation, but only to a limited extent by formic acid (mainly Experiments 3, 5 and 6). Spermine contents were mostly below the detection limit. It is not yet proved if the both polyamines originate from plant material or are produced during fermentation. Unfortunately, their contents were not determined in the ensiled grasses. Information on their roles in ruminant physiology has been scarce. They participate in cell and tissue growth, e.g. for ruminal epithelium (Eliassen and Sjaastad, 2000).

The statistical evaluation of the combined data of all experiments proved that many correlations between silage quality parameters and the contents of the four main amines were significant at $P < 0.05$ (Table V). Particularly, positive correlations between the contents of putrescine and cadaverine and the contents of α-amino group and ammonia seem to be plausible. Also Van Os *et al*. (1996) reported significant correlations between the contents of total and individual putrescine, cadaverine and tyramine and the ammonia or acetic acid contents.

In conclusion, formic acid at a usual dose of 3 g/kg of direct-cut grasses was more effective in depressing tyramine, cadaverine and putrescine formation than the tested inoculants. However, the inoculants also decreased the levels of the amines considerably.

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