Effect of different forms of bacterial inoculants on the fermentation process of ensiled crushed maize moisture grains

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ABSTRACT: Crushed high-moisture maize grains were ensiled in laboratory silos with different (water-soluble and granulated) forms of biological inoculants. The characteristics of the quality of silage fermentation process were analysed. The water-soluble inoculant product (WSI) Bonsilage and a microgranulated product (MGI) were compared with untreated control (UC). The water-soluble inoculant increased ($P < 0.01$) acetic acid (AA) production and the ratio LA/AA, decreased pH value, and inhibited production of ammonia ($P < 0.05$). The best results and the highest quality of preserved silage were obtained when the WSI preparation was used. In samples of WSI silage, the values of pH, AA content, LA/AA ratio, and ammonia content were much better; these differences were statistically highly significant ($P < 0.01$). In WSI silage, other parameters of the fermentation process were better as well; the contents of lactic acid (LA) and of all fermentation acids were higher than in the other variants while to content of alcohol was the lowest. However, these differences were statistically insignificant.

Keywords: lactic acid bacteria; fermentation process; inoculants; silage quality; high-moisture maize grain silage

When making silage, the actual preservation effect consists in a quick anaerobic fermentation of plant sugars, simultaneous rapid decrease in pH value and production of fermentation substances (Merry *et al*., 1997). To reach a good preservative effect and a high quality of fermentation processes it is necessary to stimulate the quick propagation of native lactic acid bacteria that occur in harvested plant material only in limited numbers because they suppress activities of competitive and harmful microbes. Regarding the fact that the epiphytic microflora of fodder crops is very different and that numbers of lactic acid bacteria (LAB) are usually relatively low, it is very important to know their composition and structure because such knowledge enables a successful application of preservative additives (Pahlow, 1984). It is well-known that the propagation of LAB is influenced not only by the type of forage and by the composition of epiphytic microflora but also by conditions of ensiling, e.g. dry matter content, temperature, rate of anaerobiosis, biological additives (inoculants), etc. (Beck, 1989). Biological additives with lactic acid bacteria were used for preservation of forage in many experiments (Chamberlain *et al*., 1987; Rooke *et al*., 1990; Flores *et al*., 1999; Kung *et al*., 1999; Rajnit and Kung, 2000 and others). Bolsen *et al*. (1999) used inoculants for an ensiling of maize. It was found out that within the first three days of fermentation the acidifying effect of some inoculants was similar to that of formic acid (Patterson, 1993). During the several first days of fermentation the rate of acidification is important not only with regard to the inhibition of undesirable aerobic enterobacters, yeasts and some lower fungi but also due to the fact that it helps to increase the production of lactic acid and to reduce the degradation of crude protein to ammonia. Rapid acidification influences the subse-

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. MSM 4321 00001).

quent aerobic decomposition of silages, especially of those with a higher content of dry matter and/or increased content of starch. Rammer and Lingvall (1999) studied different methods of application of bacterial inoculants and their influence on silage fermentation and found out that the LAB inoculation showed a positive effect on silage quality.

Results of many tests performed in Germany by the DLG proved also the efficiency of liquid and microgranulated biological additives. However, the form of application can influence the course of pH decrease, and thus the rate of silage acidification. Recent experiments (Pahlow and Weissbach, 1998) showed that liquid biological additives inhibited more efficiently harmful and undesirable enterobacteria that produce toxins and cause mastitis and other cattle diseases. In silage treated with liquid biological additives the decrease of pH was faster (3 days) than in that treated with the microgranulated additive and in untreated control (8 days and 10 days, respectively). Pflaum (1998) and Pflaum *et al.* (1999) also reported that the liquid form of biological additives showed a higher preserving effect, accelerated acidification and reduced degradation of proteins compared to microgranulated additives. Pieper (1999) considered the application of liquid additives to be more efficient and simpler as compared with granulates. Wooford (2000) stated that both forms of additives were comparable as far as their acidifying effects on the ensiled materials were concerned. According to Bolsen and Siefers (1998) there are only very small differences between the liquid and the granulated form of biological additives. However, it is important to apply both forms uniformly within the whole volume of ensiled material. In the case of bacterial inoculants it is necessary to preserve the biological activity of LAB for at least 72 hours. For the final evaluation of activity the temperature of the solution should not exceed 24°C. According to Woolford (2000) the durability of inoculant solutions should be 3 days.

The aim of this study was to evaluate the effects of different forms of additives on the quality of fermentation process of ensiled crushed maize grains with a higher content of moisture.

MATERIAL AND METHODS

Effects of liquid and microgranulated forms of inoculants on the quality of fermentation process. The effect of liquid and microgranulated forms of inoculants on the quality of fermentation process of ensiled crushed maize grains with the average humidity 39.01% was studied under experimental model conditions. The additive contained as an effective agent two homofermentative strains of LAB (*Lactobacillus rhamnosus* and *Enterococcus faecium*). When preparing an aqueous solution, it is necessary to ensure the required concentration of lyophilised LAB cells. Dilution was carried out using drinking tapwater (it is possible to use also chlorinated water with the maximum concentration of chlorides equal to 1 mg/l); this does not reduce the viability LAB. Maize grains (cv. Romario) were harvested and mechanically treated in the stage of pastry ripeness with the average water content of 609.9 g/kg.

A model experiment with maize grain containing on average 609.9 g of dry matter per kg was carried out in laboratories of the Mendel University of Agriculture and Forestry in Brno. Maize grains were ensiled into aluminium experimental vessels of the volume 50 litres. Three separate batches of 40 kg of fresh maize grains were collected. Untreated control maize grains and grains supplemented with either 1.0 g of Bonsilage liquid per ton or 0.5 kg of Bonsilage microgranulate per ton were pressed into experimental vessels. In each treatment altogether 40 kg of maize grains were mixed with inoculants and pressed. Untreated ensiled material was used as a negative control. The vessels were closed with lids covered with a diluted solution of molasses and stored at 20–25°C. In each treatment three vessels were incubated for a period of 180 days. The silos were then opened and representative sub-samples (6) were analysed for DM, starch content and fermentation parameters.

Analytical methods

Dry matter was determined at 103 ± 2°C (drying to the constant weight) according to Czech National Standard CSN 467092-42. All analytical procedures including the preparation of aqueous extracts were described earlier (Doležal, 2002). The samples of silage were analysed for the pH value and contents of volatile fatty acids and lactic acid. Contents of ethanol, volatile fatty acids and lactic acid were determined as described by Hartman (1974, 1980). The contents of organic nutrients in ensiled material were also analysed using the methodology published in Public Notice No. 222/1996. The content of metabolisable energy (ME) was calculated using regression equations published by Sommer *et al.* (1994). Results were evaluated by the analysis of variance using the software Statgraphic (ver. 5.0).

RESULTS AND DISCUSSION

At the moment of ensiling, the content of DM in ensiled crushed maize grains was 609.9 g/kg. Contents of crude protein, crude fat and crude fibre in 1 kg of DM were 109.13 g, 48.89 g and 28.77 g, respectively. The content of organic matter was 982.70 g/kg and that of metabolisable energy was 14.427 MJ/kg dry matter. The average content of starch in DM was 675 g/kg. The rumen degradability of maize starch was 41.63%. In comparison with starch of other cereals maize starch shows lower rumen degradability (Loose, 2000; Dvořáček *et al.*, 2001). The degradability of starch was similar to previous results (Dvořáček *et al.*, 2001). Characteristics of the fermentation process taking place in model silages made of crushed wet maize grains are presented in Tables 1–3. Statistical evaluation of differences between average values of inoculated silages and those of control is presented in Table 4.

The results of the model experiment indicate that although there were not any statistically significant differences between untreated control (UC) and experimental silages treated with bacterial inoculants in the content of lactic acid and total content of acids in 1 kg of DM, silage with WSI showed more favourable and higher values. No statistically significant differences were found in the content of ethanol but the lowest concentration of this component was found in the WSI-treated silage. In the above parameters differences between the average values of both experimental silages under study were not significant either. The MGI silage showed statistically insignificantly lower parameters. These results corresponded with data published by some other authors (Bolsen and Siefers, 1998; Woollford, 2000), who obtained similar results. On the other hand, they did not coincide with data published by Pieper (1999) and Pflaum (1998). If the bacterial inoculant is applied uniformly into the ensiled material, it seems possible to obtain comparable results at least in some parameters.

However, there were statistically significant ($P < 0.01$) differences in pH values. As compared with control (UC), a statistically significantly lower ($P < 0.01$) pH value was found in WSI-treated maize grains. In treated silage, the pH value decreased from 4.38 ± 0.02 to 4.18 ± 0.02. A statistically highly significant difference ($P < 0.01$) was also found between pH values measured in both experimental variants. As compared with UC silage, the microgranulated inoculant did not show a significant effect on a decrease in the pH value.

Table 1. Chemical characteristics of the fermentation process quality in untreated silage

Item	Dry matter (%)	pH	TA (mg KOH/100 g)	LA (g/kg DM)	AA (g/kg DM)	Σ acids (g/kg DM)	LA/AA	Alcohols (g/kg DM)	Ammonia (mg/kg DM)
1	60.46	4.22	1 190.80	26.30	6.62	32.92	3.97	3.64	400
2	60.41	4.26	1 197.10	25.66	6.29	31.95	4.08	3.31	390
3	60.27	4.23	1 178.30	23.06	5.64	28.70	4.09	3.15	400
4	60.24	4.26	1 165.70	22.74	4.81	27.55	4.73	2.82	380
5	60.42	4.21	1 190.80	21.18	4.63	25.81	4.57	3.48	360
6	60.23	4.24	1 059.30	21.58	4.48	26.06	4.82	3.82	380
Average	60.34	4.24	1 163.67	23.42	5.41	28.83	4.38	3.37	385.00
s.e.g.	0.0937	0.0189	47.7858	1.9282	0.8291	2.7359	0.3393	0.3271	13.8444
Variation coefficient (%)	0.1553	0.4451	4.1065	8.2330	15.3211	9.4892	7.7514	9.7065	3.5959

TA = titration acidity; LA = lactic acid; AA = acetic acid

Table 2. Chemical characteristics of the fermentation process quality in silage treated with the water-soluble form of inoculant

Item	Dry matter (%)	pH	TA (mg KOH/100 g)	LA (g/kg DM)	AA (g/kg DM)	Σ acids (g/kg DM)	LA/AA	Alcohol (g/kg DM)	Ammonia (mg/kg DM)
1	59.51	4.19	1 059.30	26.55	5.54	32.09	4.79	2.86	360
2	59.67	4.16	1 215.90	25.98	5.19	31.17	5.01	2.51	350
3	59.68	4.18	1 203.30	28.65	6.03	34.68	4.75	3.18	330
4	59.89	4.21	1 190.80	28.89	5.51	34.40	5.24	3.01	370
5	59.37	4.16	1 209.60	21.22	7.07	28.29	3.00	3.54	360
6	58.86	4.18	1 215.90	21.07	6.79	27.86	3.10	3.06	350
Average	59.50	4.18	1 182.47	25.39	6.02	31.42	4.32	3.03	353.33
s.e.g.	0.3266	0.0173	55.7454	3.1786	0.6922	2.6611	0.9084	0.3118	12.4722
Variation coefficient (%)	0.5489	0.4144	4.7143	12.5176	11.4952	8.4709	21.0464	10.3002	3.5299

Table 3. Chemical characteristics of the fermentation process quality in silage treated with the microgranulated form of inoculant

Item	Dry matter (%)	pH	TA (mg KOH/100 g)	LA (g/kg DM	AA (g/kg DM)	Σ acids (g/kg DM)	LA/AA	Alcohol (g/kg DM)	Ammonia (mg/kg DM)
1	60.20	4.21	1 303.60	24.75	8.64	33.39	2.86	5.48	400
2	59.80	4.23	1 316.20	24.41	8.19	32.60	2.98	5.85	400
3	60.19	4.22	1 052.90	15.12	6.48	21.60	2.33	2.66	350
4	60.60	4.24	1 015.30	14.69	6.78	21.47	2.17	2.81	340
5	59.41	4.21	1 059.30	32.82	8.25	41.07	3.98	3.20	370
6	59.76	4.28	1 197.10	31.96	8.53	40.49	3.75	3.68	380
Average	59.99	4.23	1 157.40	23.96	7.81	31.77	3.01	3.95	373.33
s.e.g.	0.3833	0.0241	121.7266	7.1578	0.8539	7.9095	0.6672	1.2616	22.8522
Variation coefficient (%)	0.6389	0.5694	10.5172	29.8760	10.9314	24.8961	22.1554	31.9664	6.1211

The above data indicated that in conditions of this model experiment the granulated form of inoculant showed a less marked effect on the decrease in silage acidity than its water-soluble form, even when it was perfectly distributed throughout the ensiled material. This also indicated that a different rate of acidification was obviously associated with differences in lactic acid production because, as compared with MGI silage, WSI silage showed not only an insignificantly higher value of volumetric acidity (expressed in mg of KOH per 100 g of silage) but also a higher content of lactic acid.

As compared with inoculated model silages, untreated control (UC) showed a lower content of acetic acid. In experimental MGI silage, the content of acetic acid was higher by 44.36% than in UC. This increase was statistically significant ($P < 0.01$). The difference between average contents of acetate in both inoculated silages was also statistically highly significant ($P < 0.01$); higher values were recorded in samples of WSI variant. As compared with both UC and WSI silages, the content of acetic acid in MGI silage was higher than expected; however, this difference was statistically insignificant.

Table 4. Statistical differences in silage fermentation characteristics

Item	Dry matter (%)	pH	TA (mg KOH/100 g)	LA (g/kg DM)	AA (g/kg DM)	Σ acids (g/kg DM)	LA/AA	Alcohol (g/kg DM)	Ammonia (mg/kg DM)
Average – untreated silage	60.34 ± 0.0937 NS	4.24 ± 0.0189 B	1163.67 ± 47.7858 NS	23.42 ± 1.9282 NS	5.41 ± 0.8291 A	28.83 ± 2.7359 NS	4.38 ± 0.3393 B	3.37 ± 0.3271 NS	385.00 ± 13.8444 b
Average – WSI treated silage	59.50 ± 0.3266 NS	4.18 ± 0.0173 A	1182.47 ± 55.7454 NS	25.39 ± 3.1786 NS	6.02 ± 0.6922 A	31.42 ± 2.6611 NS	4.32 ± 0.9084 B	3.03 ± 0.3118 NS	353.33 ± 12.4722 a
Average – MGI treated silage	59.99 ± 0.3833 NS	4.23 ± 0.0241 B	1157.40 ± 121.7266 NS	23.96 ± 7.1578 NS	7.81 ± 0.8539 B	31.77 ± 7.9095 NS	3.01 ± 0.6672 A	3.95 ± 1.2616 NS	373.33 ± 22.8522 ab

A, B = means of untreated and treated silage are significantly different ($P < 0.01$); a, b = differences between the values marked with different letters in the same row are significant ($P < 0.05$); NS = not significantly different

Not only total contents of individual acids and other compounds but also their ratios, especially that of lactic (LA) and acetic (AA) acid, belong to important parameters of the fermentation process quality. In this experiment, no statistically significant differences were found out between WSI-treated and untreated control (UC). In MGI-treated silage, however, a statistically significant ($P < 0.01$) narrowing of this ratio was recorded (from 4.38 ± 0.339 to 3.01 ± 0.667) due to an increased production of acetic acid. Statistically highly significant differences ($P < 0.01$) in LA/AA ratio existed also between both experimental variants; better values were recorded in samples of WSI silage. Statistically significant differences between all silage samples under study were also found out in the content of ammonia. As compared with UC, the application of WSI decreased significantly ($P < 0.05$) the content of this compound in silage from 385.00 ± 13.84 mg/kg DM to 353.33 ± 12.47 mg/kg DM. This corroborated data published by other authors who obtained similar results in inoculated silages (Cai *et al.*, 1999; Rammer and Lingvall, 1999; Ismail *et al.*, 1999 and others). A decrease in the content of ammonia in MGI silage was statistically insignificant. The difference in ammonia contents recorded in both experimental variants was insignificant as well ($P > 0.05$).

This experiment demonstrated that the liquid form of microbial inoculants showed a statistically significant positive effect on some parameters of fermentation process. The form of applied inoculants influenced not only changes in pH values but also the course of the process of silage acidification. This corresponds with conclusions published by Pahlow and Weissbach (1998), who observed a faster decrease in pH in WSI-treated silage than in samples of MGI and UC silages. These marked differences in the rate of acidification were caused above all by a different intensity of lactic acid production. Within the first 4 days of ensiling, these authors found out 6.8%, 2.2% and only 0.9% of lactic acid in samples of WSI, MGI and UC silages, respectively. Our results corresponded with data published by Pflaum (1998) and Pflaum *et al.* (1999), who also observed a higher preservation effect, especially the faster rate of acidification and lower losses of protein, in silages inoculated with the water soluble form of additives than in silages treated with microgranulated preparations.

Crushing and ensiling of maize grain increased starch degradation in the rumen. The level of starch degradability in UC silage was 48.21%, WSI 44.36% and MGI 44.51%. These results corresponded with data published by some other authors (Mills *et al*., 1999).

CONCLUSIONS

The present study showed that inoculation with different forms of inoculants improved the quality of the fermentation process of high-moisture maize grain. The precision crushed samples of all treatments were fermented in experimental containers (aluminium silos 50 litres in volume). The positive effects of individual inoculants were demonstrated under laboratory conditions. The LAB inoculation showed a positive effect on silage quality. Individual forms of additives showed different effects on quality parameters of silage fermentation. As compared with untreated (UC) and microgranulate-treated (MGI) variants, the application of water-soluble LAB inoculant (WSI) showed the best effect on the quality of silage fermentation.

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Received: 05–01–03
Accepted after corrections: 05–01–26

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