Effect of Biological and Biochemical Silage Additives on Final Nutritive, Hygienic and Fermentation Characteristics of Ensiled High Moisture Crimped Corn

Daniel Bíro, Branislav Gálik, Miroslav Juráček, Milan Šimko, Eva Straková, Jaroslava Michálková, Erika Gyöngyová

Department of Animal Nutrition, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Slovak Republic
1Department of Nutrition, Animal Husbandry and Animal Hygiene, Faculty of Veterinary Hygiene and Ecology, University of Veterinary Sciences Brno, Czech Republic

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Abstract

The aim of this study was to determine the effect of different biological and biochemical additives on the final nutritive quality, fermentation process and concentration of mycotoxins of ensiled high moisture crimped corn. We created four variants for the experiment: control (UC), A1, and A2 (biological stimulators with the active principle of lactic acid bacteria) and variant B (combined additives with the active principle of lactic acid bacteria, benzoate sodium and active enzymatic complex of cellulases). After 6 months of storage in laboratory conditions, we determined in experimental silages the content of dry matter ranging from 608.9 to 613 g kg⁻¹. The significantly lower content of crude fibre was detected in silages with additives. In silages ensiled with additives we detected the highest content of nitrogen-free extract in variant B (834.3 g kg⁻¹ of DM, P < 0.05). A similar effect was determined also in the content of starch; significant differences were detected in variants A1 and B (P < 0.05) compared to the control variant. We detected a significantly (P < 0.05) higher content of total sugars in trial silages; the highest content was in variant A2 (6.1 g kg⁻¹ of DM).

In the trial variants we determined significantly the lowest content of acetic acid in variant B (2.82 g kg⁻¹ of DM). In case of butyric acid, whose content in the control variant was 0.22 g kg⁻¹ of DM, we detected the lowest content in variant A1 conserved with homo- and heterofermentative species of lactic acid bacteria. The lowest content of ammonia was determined in silages of variant B (0.074 g kg⁻¹ of DM).

We found lower concentrations of DON and FUM (P > 0.05) after the application of biological and biochemical silage additives. In concentration of T-2 toxin we detected a significantly (P < 0.05) lower value in variant A1. In concentration of AFL we found significant differences between variants A1 and B, as well as in concentration of OT between untreated control variant (UC) and variants conserved by additives. Application of silage additives influenced the nutritive and hygienic quality of the conserved fodders.

Feeds, maize, conservation, silage additives, mycotoxins

Maize (Zea mays L.) is a major source of energy in feeding rations for ruminants in the Slovak Republic. Generally, it produces feed with different nutritional and hygienic indicators. The differences lie mainly in the energy content (Bíro 2001). Energy limits the nutritive value of feeds, except for fresh and green forages (Hoffman 1998). During the harvest period, the technology of high moisture corn is economically more efficient due to lower losses and processing costs (Volkov et al. 1999; Bíro and Juráček 2003). High moisture corn has a higher nutritive value and higher digestibility of organic matter compared to dry corn (Woodarce 2004). Starch supplied from corn and corn silage is an important source of dietary energy for lactating dairy cows and other ruminants. However, various sources of corn starch have highly variable ruminal and total tract digestibility (Ørskov 1986; Threuer 1986). Factors such as particle size (Remond et al. 2004), conservation method (Oba and Allen 2003) and type of corn endosperm (Correa et al. 2002; Šimko et al. 2008) can influence ruminal and total tract digestion of starch in lactating dairy cows.
Lactobacillus plantarum, lactic acid bacteria (High moisture corn in variant B was ensiled by combined biochemical additive, where the biological part were 

Enterococcus highest content of crude protein was in silages of variant A1 (95.4 g·kg

deficient in corn, we did not detect significant differences influenced by additives. The 

bacteria species (Enterococcus 50 dm3. The additive used in variant A1 consisted of homo- and heterofermentative species of lactic acid 

bacteria (Lactobacillus rhamnosus, Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus buchneri and 

Pedioecoccus pentosaceus: 2.5 × 1011 CFU·g-1). In variant A2 we applied the additive compound of 5 lactic acid 

crude protein content, which is typically 

Pediococcus pentosaceus

Pedioecoccus pentosaceus: 150 × 10^9 CFU·g^-1). Inoculants used in variants A1 and A2 were in powder form. 

High moisture corn in variant B was ensiled by combined biochemical additive, where the biological part were 

lactic acid bacteria (Lactobacillus plantarum, Enterococcus faecium, Pedioecoccus pentosaceus and Lactococcus lactis: 166 × 10^6 CFU·g^-1) and the chemical part were enzymatic complex of cellulases (Trichoderma viridae; activity 50 610 CU, 6478 IU) and preservative benzoate sodium. All variants were ensiled in 3 repetitions. After 

filling the matter into silos (density 1,100 kg·m^-3), we sealed them and stored in the laboratory of feeds, conserved 

by the temperature of 18-20 °C. The nutritive characteristic of fresh high moisture corn is presented in Table 1. 

After termination of the fermentation process (6 months of storage) we opened the silos and in average laboratory 
samples we determined the indicators of nutritive value and fermentation process. For analysing organic and 
inorganic nutrients, we used standard methods according to the Regulation of Ministry of Agriculture of the 
Slovak Republic no. 2145/2004-100 about sampling feeds and about laboratory testing and evaluating of feeds. 

The content of fermentative carboxyl organic acids was determined on analyser EA 100 (Villa Labeco, SR) using 

the method of ionic electrophoresis. Contents of alcohols and ammonia were detected by Conway microdiffusion 

method, titration acidity by alkalimetric titration and active acidity by the electrometric method. Energy (NEL and 

 NEG) and protein (PDI) values were calculated by regression scheme (Petrikovič and Sommer 2002). After 

opening the laboratory silos, we sensorically observed the occurrence of fungi. Concentration of mycotoxins 
(FUM: fumonisins, AFL: aflatoxins, ZON: zearalenone, DON: deoxynivalenol, T-2 toxin and OT: ochratoxin) 
was detected using the immunoenzymatic method in screening quantitative test of ELISA Reader (NEOGEN, 
U.S.A.). Before spectrophotometric measuring of concentration, the samples were processed by extraction in 
distilled water (DON), 70% methanol (FUM, ZON, AFL), respectively in 50% methanol (OT and T-2 toxin). 

Significance of determined differences was tested by single-factor analysis of variance (ANOVA). The evidence 
of differences of the mean values was assessed by t-test. 

Results 

The nutritive characteristic of high moisture corn is presented in Table 1. The content of 

nutrients in silages from high moisture corn is given in Table 2. After 6 months of storage we detected the content of dry matter in high moisture corn silages ranging from 596.3 (variant A1) to 607.4 g·kg^-1 (variant A2). In the crude protein content, which is typically 
deficient in corn, we did not detect significant differences influenced by additives. The 

highest content of crude protein was in silages of variant A1 (95.4 g·kg^-1 of DM). For corn grain low content of crude fibre is also typical; this is in negative correlation with
Table 1. Nutrient contents of high moisture crimped corn before ensiling

<table>
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<tr>
<th>n = 2</th>
<th>DM</th>
<th>CP</th>
<th>F</th>
<th>CF</th>
<th>A</th>
<th>NFE</th>
<th>OM</th>
<th>S</th>
<th>TS</th>
<th>NEL</th>
<th>NEG</th>
<th>PDIE</th>
<th>PDIN</th>
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<tbody>
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<td>UC</td>
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<td>91.7</td>
<td>42.4</td>
<td>31.8</td>
<td>16.6</td>
<td>817.5</td>
<td>983.4</td>
<td>666.8</td>
<td>28.65</td>
<td>8.68</td>
<td>9.33</td>
<td>98.3</td>
<td>60.3</td>
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<td>609.6</td>
<td>91.4</td>
<td>37.6</td>
<td>31.0</td>
<td>16.4</td>
<td>823.6</td>
<td>983.6</td>
<td>680.8</td>
<td>32.86</td>
<td>8.64</td>
<td>9.29</td>
<td>98.7</td>
<td>60.1</td>
</tr>
<tr>
<td>A2</td>
<td>610.7</td>
<td>88.9</td>
<td>35.9</td>
<td>31.2</td>
<td>14.1</td>
<td>829.9</td>
<td>985.9</td>
<td>686.0</td>
<td>30.85</td>
<td>8.66</td>
<td>9.31</td>
<td>98.4</td>
<td>58.5</td>
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<tr>
<td>B</td>
<td>608.9</td>
<td>89.9</td>
<td>37.8</td>
<td>29.4</td>
<td>15.2</td>
<td>827.6</td>
<td>984.8</td>
<td>643.8</td>
<td>30.84</td>
<td>8.67</td>
<td>9.32</td>
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<td>59.2</td>
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</table>


Table 2. Nutrient contents of high moisture corn silage

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<th>A</th>
<th>NFE</th>
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<th>TS</th>
<th>NEL</th>
<th>NEG</th>
<th>PDIE</th>
<th>PDIN</th>
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<tbody>
<tr>
<td>UC</td>
<td>603.8</td>
<td>94.9</td>
<td>36.2</td>
<td>27.6</td>
<td>14.1</td>
<td>827.2</td>
<td>985.9</td>
<td>679.8</td>
<td>1.0</td>
<td>8.66</td>
<td>9.32</td>
<td>96.97</td>
<td>62.5</td>
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<tr>
<td>A1</td>
<td>607.4</td>
<td>93.0</td>
<td>37.3</td>
<td>25.8</td>
<td>13.5</td>
<td>830.0</td>
<td>986.5</td>
<td>690.0</td>
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<td>8.69</td>
<td>9.35</td>
<td>96.60</td>
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<tr>
<td>A2</td>
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<td>92.5</td>
<td>36.2</td>
<td>23.5</td>
<td>13.5</td>
<td>834.3</td>
<td>986.5</td>
<td>698.6</td>
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<td>8.70</td>
<td>9.37</td>
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<td>60.8</td>
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<tr>
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<td>35.1</td>
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<td>0.010</td>
<td>0.012</td>
<td>0.321</td>
<td>0.306</td>
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</table>


Values with identical superscripts within one column are significant at $P < 0.05$
digestibility of organic matter. In our experiment, we determined a positive decrease of crude fibre content in silages with applied additives; the differences found were significant \((P < 0.05)\). The lowest content of crude fibre (23.5 g·kg\(^{-1}\) of DM) was detected in silages from high moisture corn conserved by combined biochemical inoculant in variant B. In the content of ash (13.5-14.1 g·kg\(^{-1}\) of DM) we did not detect significant differences influenced by additives. Nutritionally, corn grain is valued also for its high content of easily digestible carbohydrates as nitrogen-free extract (NFE). Additives positively influenced the content of NFE in silages. Significantly the highest content of NFE \((P < 0.05)\) was in silages conserved by combined biochemical additive (variant B). Likewise, in the content of starch as source of energy in corn grain, we determined a positive influence of additives. Compared to the variant UC, in which we ensiled high moisture corn without additives, we detected a significantly higher content of starch in silages of variant A1 and B \((P < 0.05)\). The content of total sugars analysed according to Luff-Schoorl ranged from 1.0 to 6.1 g·kg\(^{-1}\) of DM. Detected differences between variant UC and variants with applied additives were significant \((P < 0.05)\). We did not detect significant differences in the energetic and protein value of conserved silages from high moisture corn.

Detected values of fermentation process indicators are presented in Table 3. In silages with additives we determined a lower content of lactic acid compared to the variant without additives, mainly in variant A1 \((10.55 \text{ g·kg}^{-1}\) of DM). The content of acetic acid was low in all variants and it did not exceed 5.0 g·kg\(^{-1}\) of DM. In variant A1 we found a lower content of dry matter with a lower content of total sugars, and higher value of pH compared to variant A2. These factors affected lower production of lactic acid and a higher content of acetic acid in variant A1. The content of undesirable butyric acid was low in silages, non-significantly the highest concentration was in silages of variant A2 \((0.38 \text{ g·kg}^{-1}\) of DM), in this variant we applied a biological additive for stimulation of the fermentation process. Active acidity (pH) of water extracts ranged from 3.70 (variant B) to 3.85 (variant A1). The highest pH together with the lowest titration acidity (TA) was found in silages in which we detected the highest content of acetic acid (variant A1). Positive non-significant influence of applied additives was detected in the content of ammonia (\(\text{NH}_3\)), which compared to the control variant \((0.416 \text{ g·kg}^{-1}\) of DM) ranged in trial variants from 0.074 (variant A2) to 0.101 g·kg\(^{-1}\) of DM (variant A1).

### Table 3. Results of fermentation process of high moisture corn silages

<table>
<thead>
<tr>
<th></th>
<th>n = 3</th>
<th>DM g·kg(^{-1})</th>
<th>LA g·kg(^{-1}) of dry matter</th>
<th>AA g·kg(^{-1})</th>
<th>BA mg KOH</th>
<th>PA mg KOH</th>
<th>FA mg KOH</th>
<th>TA g·kg(^{-1}) of dry matter</th>
<th>pH</th>
<th>NH(_3) g·kg(^{-1})</th>
<th>Alc g·kg(^{-1}) of dry matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>UC</td>
<td>SD</td>
<td>603.8</td>
<td>24.27(^{a})</td>
<td>3.73(^{a})</td>
<td>0.22</td>
<td>0.16</td>
<td>0.19(^{a})</td>
<td>1134.3(^{a})</td>
<td>3.75(^{a})</td>
<td>0.416</td>
<td>2.61</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>6.087</td>
<td>0.678</td>
<td>0.267</td>
<td>0.012</td>
<td>0.013</td>
<td>0.012</td>
<td>19.707</td>
<td>0.006</td>
<td>0.035</td>
<td>0.166</td>
</tr>
<tr>
<td>A1</td>
<td>SD</td>
<td>596.3</td>
<td>10.55(^{a})</td>
<td>4.96</td>
<td>0.17</td>
<td>0.11</td>
<td>0.78(^{a})</td>
<td>691.09</td>
<td>3.85(^{a})</td>
<td>0.101</td>
<td>1.77</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>4.355</td>
<td>1.045</td>
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<td>0.014</td>
<td>0.008</td>
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<td>0.010</td>
<td>0.009</td>
<td>0.083</td>
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<tr>
<td>A2</td>
<td>SD</td>
<td>607.4</td>
<td>22.48</td>
<td>2.87</td>
<td>0.38</td>
<td>0.15</td>
<td>1.38</td>
<td>1078.25</td>
<td>3.73</td>
<td>0.074</td>
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<tr>
<td></td>
<td>V</td>
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<td>1.428</td>
<td>0.156</td>
<td>0.008</td>
<td>0.011</td>
<td>0.068</td>
<td>15.007</td>
<td>0.021</td>
<td>0.004</td>
<td>0.179</td>
</tr>
<tr>
<td>B</td>
<td>SD</td>
<td>596.6</td>
<td>22.96(^{a})</td>
<td>2.82(^{a})</td>
<td>0.22</td>
<td>0.17</td>
<td>1.36(^{a})</td>
<td>1072.64</td>
<td>3.70(^{a})</td>
<td>0.094</td>
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<td>V</td>
<td>2.021</td>
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<td>15.302</td>
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<td>0.009</td>
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<td></td>
<td></td>
<td>0.34</td>
<td>2.54</td>
<td>2.77</td>
<td>9.55</td>
<td>8.82</td>
<td>3.38</td>
<td>1.43</td>
<td>0.32</td>
<td>9.57</td>
<td>5.05</td>
</tr>
</tbody>
</table>

* DM: dry matter, LA: lactic acid, AA: acetic acid, BA: butyric acid, PA: propionic acid, FA: formic acid, TA: titration acidity, pH: active acidity, NH\(_3\): ammonia, Alc: alcohols

Values with identical superscripts within one column are significant at \(P < 0.05\).
Concentration of mycotoxins in high moisture corn before ensiling is presented in Table 4. In fresh high moisture corn we did not detect the concentration of deoxynivalenol at the detection level of mg·kg\(^{-1}\). The most prevalent were *Fusarium* toxins: FUM, followed by ZON and T-2. The samples of high moisture corn before ensiling were the least contaminated by toxin producers of the genera *Penicillium* and *Aspergillus*. Concentration of mycotoxins in silages of high moisture corn is given in Table 5. The lowest concentration of zearalenone (29.83 µg·kg\(^{-1}\)) was determined in silages of the variant conserved by the combined biochemical additive (variant B). In concentration of deoxynivalenol, we detected lower values in all variants conserved by different silage additives (0.067 mg·kg\(^{-1}\)). Average concentration of deoxynivalenol in untreated control variant was 0.133 mg·kg\(^{-1}\). The same effect of silage additives was found in concentration of fumonisins. Significantly the lowest concentration of T-2 toxin was in variant A1 which we conserved by homo- and heterofermentative species of lactic acid bacteria. The positive effect of the biochemical additive (variant B) was detected also in the concentration of aflatoxins (2.13 µg·kg\(^{-1}\)). Significant differences in the concentration of aflatoxins were found between variants A1 and B. The concentrations of ochratoxin were lower in silages of variants conserved by biological and biochemical additives (the lowest in variant A2, 0.533 µg·kg\(^{-1}\)). Compared to untreated control variant, the differences were significant \((P < 0.05)\).

**Discussion**

In a similar experiment, Doležal and Zeman (2005) determined the average content of dry matter of 603.4 g·kg\(^{-1}\) which partially corresponds with our results. In the content of crude protein, we confirmed the results of Zebrowska et al. (1997) who determined the average content of crude protein in corn of 100 g·kg\(^{-1}\) of DM. In the content of crude protein we did not detect significant effects of additives. Similar results were also reported by Wardynski et al. (1993). The content of crude fibre ranged in silages from 23.5 to

<table>
<thead>
<tr>
<th>n = 2</th>
<th>ZON µg·kg(^{-1})</th>
<th>DON mg·kg(^{-1})</th>
<th>FUM µg·kg(^{-1})</th>
<th>T-2</th>
<th>AFL µg·kg(^{-1})</th>
<th>OT µg·kg(^{-1})</th>
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<tr>
<td>UC</td>
<td>43.5</td>
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<table>
<thead>
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<th>n = 3</th>
<th>ZON µg·kg(^{-1})</th>
<th>DON mg·kg(^{-1})</th>
<th>FUM µg·kg(^{-1})</th>
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Values with identical superscripts within one column are significant at \(P < 0.05\)
27.6 g·kg⁻¹ of DM. Petrikovič et al. (2000) report the average content of crude fibre in 72 samples as 24 g·kg⁻¹ of DM. Results comparable with Petrikovič et al. (2000) were detected also in the content of nitrogen-free extract, whose content limits the production of sufficient lactic acid. Silages from high moisture corn in our experiment had a higher content of starch as reported in a similar work of Doležal and Zeman (2005).

After termination of the fermentation process we detected a lower content of desirable lactic acid in trial silages, similar to Bíro et al. (2006). The opposite effect of bacterial inoculants was found by Doležal and Zeman (2005) who reported a positive increase of the lactic acid content influenced by the applied additives. Compared to the results of these authors we detected lower concentrations of acetic acid and undesirable butyric acid in our samples. Active acidity (pH) of silage extracts ranged from 3.70 to 3.85. Sebastian et al. (1996) who analysed silages from high moisture corn conserved by different biological and chemical additives, detected higher values of pH (4.0-4.3). The content of ammonia in control silages was comparable to the results of Doležal and Zeman (2005). The cited authors report the average content of ammonia in high moisture corn silages ranging from 0.353 to 0.373 g·kg⁻¹ of DM. In our experiment we determined markedly lower concentrations (0.074 to 0.101 g·kg⁻¹ of DM). The content of alcohols in silage extracts was identical with the results of Driehuis et al. (1999) according to whom microbial stimulation of silage fermentation is important in elimination of alcohol fermentation and activity of enterobacteria. Our results correspond only partially in acidity of aqueous extract with the results published by Pyrochta et al. (2005).

The concentration of zearalenone ranged in silages from 29.83 to 48.9 µg·kg⁻¹ and it was twice higher than that reported by Leibetseder (1995). The determined concentration of zearalenone corresponds with the results of Whitlow et al. (1998). We confirmed partially the results of Stryzewska and Pys (2006) according to whom applied silage additives can influence species composition of epiphytic microflora and thereby also the production of secondary metabolites. Ström et al. (2002) explains this effect as bacterial additives containing Lactobacillus plantarum and producing metabolites that inhibit growth of moulds and production of mycotoxins. The results of Mokoena et al. (2005) indicate that lactic acid bacteria fermentation can significantly reduce the concentration of fumonisins and zearalenone in maize. The positive effect of Lactobacillus on mould growth and aflatoxin production was reported by Gourama and Bullerman (1995).

The concentrations of tested mycotoxins were below the limit levels. Analysed samples of silages were not visibly moulded. Driehuis et al. (2008) found higher concentrations of mycotoxins in silages.

In conclusion, after application of biological and biochemical additives we detected a significant influence on several nutritive characteristics of high moisture corn silage. Additives positively influenced mainly the content of carbohydrate complex, structural and non-structural carbohydrates (total sugars, starch, NFE and crude fibre). The highest content of lactic acid was determined in silages without additives. Contents of other volatile fatty acids (mainly acetic and butyric acid) were low, below the limit of 5 g·kg⁻¹ of DM, and 0.4 g·kg⁻¹ of DM, respectively. A positive effect of applied additives was detected on sensory evaluation of the hygienic quality of silages. In the upper layers of high moisture corn silages ensiled with additives we did not detect presence of moulds as potential biological contaminants.

In terms of nutritive value and fermentation process we found the most positive results in silages conserved with the biochemical additive that contained lactic acid bacteria, complex of enzymes and benzoate sodium.

These results confirmed that addition of biological and biochemical additives may significantly reduce the concentration of mycotoxins.
Vplyv rozdielnych biologických a biochemických aditív na výslednú výživnú, hygienickú a fermentačnú charakteristiku silážovaného vlhkého miaganého kukuričného zrna

Cieľom experimentu bolo zistenie vplyvu rôznych biologických a biochemických konzervačných aditív na výslednú nutričnú kvalitu, fermentáciu a koncentráciu mykotoxínov silážovaného vlhkého miaganého kukuričného zrna. V experimente sme sledovali 4 varianty: kontrolný (UC), A1, A2 (použité biologické stimulátory s účinnou zložkou baktérií mliečneho kvasenia) a variant B (použité kombinované aditívum s účinnými zložkami baktérií mliečneho kvasenia, benzoanu sodného a aktívneho enzymatického komplexu celuláz). Po 6 mesiacoch skladovania v laboratórnych podmienkach sme v silážach experimentu zistili obsah sušiny v rozpätí od 608,9 do 613 g·kg⁻¹. Preukazne najnižší obsah vlákniny sa zistil v silážach s prídavkom aditív. V silážach zakonzervovaných prostredníctvom aditív bol zistený najvyšší obsah bezdusíkatých látok výťažkových, preukazne vo variante B (834,3 g·kg⁻¹ sušiny, P < 0,05). Podobný efekt sa zistil aj v obsahu škrobu, zistené rozdiely boli v porovnaní s kontrolným variantom preukazné v silážach variantov A1 a B (P < 0,05). Preukazne (P < 0,05) vyšší obsah sa významne aplikácie konzervačných aditív zistil aj v obsahu celkových sacharidov, kde sa najvyšší obsah zaznamenal vo variante A2 (6,1 g·kg⁻¹ sušiny).

Vo variantoch experimentu sme preukazne (P < 0,05) najnižší obsah kyseliny ocitovej zistili vo variante B (2,82 g·kg⁻¹ sušiny). V obsahu kyseliny maslovej, ktoré priemerný obsah bol v kontrolnom variante 0,22 g·kg⁻¹ sušiny, sme najnižší obsah zistili v silážach variantu A1 zakonzervovaných prostredníctvom homono heterofermentatívnych kmeňov baktérií mliečneho kvasenia. Najnižší obsah amoniaku sme zaznamenali v silážach variantu B (0,074 g·kg⁻¹ sušiny).

Po aplikácii biologických a biochemických silážnych aditív sme zistili nižšiu koncentráciu DON a FUM (P > 0,05). V koncentrácií T-2 toxínu sme preukazne (P < 0,05) nižšiu hodnotu zaznamenali vo varianti A1. V koncentrácií AFL sme preukazne rozdiely zistili medzi variantmi A1 a B, rovnako aj v koncentrácií OT medzi kontrolným variantom (UC) a variantmi zakonzervovanými pomocou aditív. Aplikácia silážnych aditív ovplyvňuje nutričnú a hygienickú kvalitu konzervovaných krmív.

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