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The effect of additives on mycotoxic contamination of maize silages
Vplyv aditív na mykotoxickú kontamináciu kukuričných siláží

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ABSTRACT

Addition of silage additives is commonly concentrated on the improvement of nutritive and fermentative features of silages, but in recent years, mycotoxic contamination is also a feature monitored after the treatment. This study aimed to evaluate the effect of silage additives, in the concrete inoculant consisting of a mixture of *Lactobacillus buchneri* LN40177, *Lactobacillus casei* LC329090 and urea, on the concentration of major mycotoxins in maize silages. The ensilage mass was made in three variants, which consisted of maize silage (control variant C), maize silage with the addition of a commercial inoculant (variant A) and maize silage with the addition of urea (variant U). The commercial inoculant was added to the cut mass at a dose of 1 g/t of mass and urea at a dose of 5 kg/t of mass. After application of silage additives, the mass was stored and sealed in silage units. Mycotoxin analysis was performed using Veratox tests with ELISA reader, whereas average samples were prepared according to established protocols. The immuno-enzymatic method revealed that all samples of maize silage showed 100% contamination. The maize silages were characterized by the highest concentration of deoxynivalenol regardless of the treatment. The results confirmed the effect of silage additives on mycotoxic contamination of maize silages. Compared to the control variant, the commercial inoculant had negative increasing effect ($P<0.05$) on deoxynivalenol, ochratoxins and zearalenone, and a positive decreasing effect ($P<0.05$) on T-2 toxin and fumonisins. The urea addition resulted in significant reduction of T-2 toxin and increase of zearalenone.

Keywords: maize silage, inoculant, urea, mycotoxins

ABSTRAKT

Prídavkom silážnych aditív sa bežne sleduje zlepšenie nutričných a fermentačných vlastností siláží, ale v posledných rokoch je mykotoxická kontaminácia tiež sledovaným znakom po ošetrení. Táto štúdia bola zameraná na vyhodnotenie účinku silážnych aditív, konkrétne inokulantu pozostávajúceho zo zmesi *Lactobacillus buchneri* LN40177, *Lactobacillus casei* LC329090 a močoviny, na koncentráciu hlavných mykotoxínov v kukuričnej siláži. Silážovaná hmota kukurice bola vyhotovená v troch variantoch, ktoré pozostávali z kukuričnej siláže (kontrolný variant C), kukuričnej siláže s prídavkom komerčného inokulantu (variant A) a kukuričnej siláže s prídavkom močoviny (variant U). Komerčný inokulant bol do narezanej hmoty pridaný v dávke 1g/t silážnej hmoty a močovina v dávke 5 kg/t silážnej hmoty. Po aplikácií silážnych aditív sa hmota uskladnila a zasilážovala do silážnych jednotiek. Analýza mykotoxínov bola vykonaná pomocou Veratox testov na ELISA reader, pričom priemerné vzorky boli upravené podľa stanovených protokolov. Výsledky potvrdili efekt silážnych aditív na mykotoxickú kontamináciu kukuričných siláží. V porovnaní s kontrolným variantom mal komerčný inokulant negatívny stúpajúci efekt ($P<0,05$) na deoxynivalenol, ochratoxíny a zearalenon, a pozitívny klesajúci efekt ($P<0,05$) na T-2 toxín a fumonizíny. Výsledkom pridania močoviny bolo preukazné zníženie T-2 toxínu a zvýšenie zearalenonu.

Kľúčové slová: kukuričná siláž, inokulant, močovina, mykotoxíny

INTRODUCTION

Feed in the form of a silage is highly valued for several countries (Vaičiuliené et al., 2020). In many parts of the world, the most used forage is maize (*Zea mays* L.), thanks to its suitable properties for silage fermentation (Santos et al., 2019). Silva et al. (2018) attribute this mainly to its high nutritional value, suitable dry matter, high content of fermentable carbohydrates, low buffering capacity and high digestibility. The downside is, that silage poses a threat to animals because it can be a source of microscopic fungi capable of producing dangerous contaminants (mycotoxins) (Juan et al., 2020). As Glamočić et al. (2019) state, silage may be responsible for higher dry matter intake in dairy cows on the one hand but may be a source of mycotoxin intake on the other hand. Paradhipta et al. (2020) also report that the greatest concern in silage production is the activity of undesirable microflora (yeast and fungi), which can later produce toxic compounds harmful to ruminants. In addition, their concentration may accumulate due to the presence of oxygen after opening the silo. While dry matter, energy loss and nutritional value decrease, mycotoxin production potentially affects animal health (Ferrero et al., 2019; Nair et al., 2020). Except aerobic deterioration, the quality of silage and the concentration of mycotoxins are also affected by: unsuitable dry matter content, silo filling, imperfect hermetization and nutrient losses during storage (Gallo et al., 2015; Juan et al. 2020). Many mycotoxins have been identified (Udomkun et al., 2017; Kebede et al., 2020) but aflatoxins (AFL), deoxynivalenol (DON), ochratoxin A (OTA), T-2 toxin (T-2) and zearalenone (ZEA), are major feed contaminants (Pinotti et al., 2016). As cereals are found in the diet of dairy cattle as a potential source of mycotoxins, maize is regularly checked for aflatoxin B1 (AFB1) content (Glamočić et al., 2019). Due to the high incidence of mycotoxins in feed and food, the European Union has set regulations and recommendations for maximum permitted values for individual mycotoxins (Kalúzová et al., 2021). Therefore, Commission Directive 2003/100/EC regulates maximum concentrations for AFL, Commission Recommendation 2006/576/EC applies to DON, FUM, OTA and ZEA, and Commission Recommendation 2013/165/EU specifies limits for T-2 toxin. Various silage additives are used to prevent or reduce the development of toxigenic fungi in silage (Saylor et al., 2020). Additives in the form of lactic acid bacteria (LAB) can inhibit the growth of many genera of microscopic fungi due to their antibacterial character. The genus *Lactobacillus* produces metabolites with a marked antifungal effect (Fabiszewska and Zielińska, 2019). The most used silage inoculant is the heterofermentative species *Lactobacillus buchneri*, which improves the aerobic stability of silage. In manufacturing the microbiological additive, this species is predominantly combined with the homofermetative strain of LAB (Ellis et al., 2016; Saylor et al., 2020). An improvement in aerobic stability can also be achieved by the addition of nutrients such as urea and ammonia (Elferink et al., 2000; Bíro et al., 2020).

MATERIAL AND METHODS

In this experiment, the ensiling technology of late-season maize hybrid (KWS KATHEDRALIS) obtained from the University farm Oponice was applied. This two-line hybrid (FAO 480, dent grain type) was harvested using a self-propelled chopper and cut to a theoretical cut length of 20 mm. The mass was subsequently ensiled in the control (C) and in two experimental variants (A and U). Silage additives were applied homogeneously after cutting, in variant A using an applicator Pioneer Appli Pro SLV located on self-propelled chopper and in variant U by the hand on the untreated matter. Experimental variant A represented the application of a silage additive in a dose of 1 g/t of mass. The biological additive consisted of obligately heterofermentative (*Lactobacillus buchneri*) and facultatively heterofermentative (*Lactobacillus casei*) strains of lactic acid bacteria ($1.0x10^{10}$ CFU/g). In variant U, urea was used at an application dose of 5 kg/t of mass. All variants were stored in silage units (n=3; 1200 g of mass per one unit) using a sealing machine (Foodsaver FSGSSL0300) and stored at a constant temperature of 22 ± 2 °C in the laboratory of Feed preservation, Institute of Nutrition and Genomics, Slovak University of Agriculture. The average samples of maize silage for each variant (n=3) were taken after 82 days of storage. The concentration of mycotoxins (zearalenone, fumonisins, aflatoxins, ochratoxins, T-2 toxin and deoxynivalenol) was determined by immuno-enzymatic method using ELISA reader with the use of Veratox tests, thus samples were prepared according to Veratox protocols. The principle of this test is to capture the concentration of mycotoxin in the feed extract at a wavelength of 650 nm. Prior to analysis, silage extracts were prepared from average samples in 70% methanol (ZEA: zearalenone, FUM: fumonisins and AFL: aflatoxins), 50% methanol (OTA: ochratoxins and T-2 toxin) and distilled water (DON: deoxynivalenol). Significance of differences was tested by one-way ANOVA and the differences of mean values between groups were assessed by Tukey test at a level of $P<0.05$.

RESULTS AND DISCUSSION

Testing maize silage samples with the use of ELISA reader revealed and confirmed the presence of all 6 mycotoxins in all variants (Table 1). The highest concentration was reached by DON, while the highest value was recorded in variant A. The addition of urea (variant U) was able to reduce DON content in the silage, but it was nonsignificant ($P>0.05$) compared to the control variant. In variant A, the content of DON significantly ($P<0.05$) increased compared to both variants. Selwet (2011) treated maize silage with an inoculant consisting of both homofermentative and heterofermentative LAB strains, whereas a statistically significant ($P<0.05$) reduction in DON content towards untreated silage was observed. In present experiment, the concentration of ochratoxin was also ($P<0.05$) higher in the sample with applied inoculant (A) compared to the control (C) and silage with the addition of urea (U). Zielińska and Fabiszewska (2018) also observed a statistically significant ($P<0.05$) decrease in OTA for the *Lactobacillus buchneri* treated variant. The silage inoculant in the combination of *Lactobacillus buchneri* and *Lactobacillus reuteri* had even ($P<0.05$) better results than the application of *L. buchneri* alone in silage of maize kernel (Zielińska and Fabiszewska, 2018). Since the growth of toxigenic fungi is affected by many factors, researchers are monitoring more features that can reduce the concentration of mycotoxins (Kalúzová et al., 2021). Wang et al. (2018) found an interaction between additive supplementation and storage temperature. Lower ($P<0.05$) concentration of OTA was noticed at a storage temperature of 20 °C than at 28 °C or 37 °C. For aflatoxin, a statistically significant differences between the variants were not registered, although its concentration decreased in variant A (6.60 µg/kg) and U (3.79 µg/kg). The maximum permitted level for AFL in complete feedstuffs for adult ruminants is 20 µg/kg (Commission Directive 2003/100/EC), which was not exceeded. Strains of LAB exists, that have a wide range of mycotoxin adsorption abilities (Hsu et al., 2018). Chiocchetti et al. (2019) mentioned several authors who confirmed the ability of some LAB strains to bind contaminants such as AFB1, OTA, FUM, DON and ZEA. Gallo et al. (2021) used many types of inoculants in the experiment of maize silage, among which were inoculants similar in composition of variant A. The combination of *Lactobacillus buchneri* and *Lactobacillus plantarum* decreased the AFB1 content compared to the control, with a concomitant increase in DON. Another inoculant consisting of *Lactobacillus buchneri* and *Lactococcus lactis* did not affect AFB1 concentration but also negatively affected DON content. Apart from AFB1, AFB2 (aflatoxin B2) and DON, the presence of OTA, T-2, FUM or ZEA has not been reported (Gallo et al., 2021). Gallo et al. (2021) further state that the concentration of AFL can be also affected by ensiling time. A higher content of AFB1 and AFB2 was registered at 120 days versus 30 days of ensiling. A lower ($P<0.05$) content of T-2 toxin was registered in variants A and U. Although the T-2 toxin content decreased after inoculant and urea application, it still exceeded the maximum permitted limit for adult ruminants (250 µg/kg) set out in Commission Recommendation 2013/165/EU. After the application of *L. plantarum* in maize silage, Wang et al. (2018) didn´t noticed a remarkable effect on T-2, but its content was significantly ($P<0.05$) higher when treated by *Pediococcus pentosaceus*. The difference in concentration of FUM was ($P<0.05$) lower in variant A versus variant C. The same results were obtained by Gallo et al. (2018) when the content of FUM (fumonisin B1 and B2) was reduced after the addition of a microbiological additive (*Lactobacillus buchneri* and *Lactococcus lactis*) to maize silage, but only in FB2 statistically significant ($P<0.05$). A positive decreasing ($P<0.05$) effect on the content of T-2 was also performed by Latorre et al. (2015) after application of *L. buchneri*. ZEA was the only mycotoxin that responded to the addition of inoculant and urea by increasing its concentration in the silage, while significancy ($P<0.05$) was demonstrated in all variants among themselves. Drouin et al. (2021) reported an increased concentration of ZEA after the application of *L. buchneri* and *L. hilgardii* in maize silage, but not statistically significant ($P>0.05$).

Apart from T-2 toxin, none of the listed mycotoxins in table 1 exceeded the maximum permitted limit set by the European Union. Several authors paid attention to the effect of urea addition (Santos et al., 2021; Fiantok, 2020; Kaewpila et al., 2020; Santos et al., 2020; Zamir et al., 2020; Santos et al., 2018; Kang et al., 2018; Kebede et al., 2018), but monitored the changes in nutritional and fermentation parameters rather than mycotoxin concentrations. Most of the studies are focused on the aerobic stability of silages after the addition of urea, and research on the concentration of mycotoxins is not known. Generally, according to One-way Anova there was confirmed the effect of the addition of additives on mycotoxin contamination in maize silage, however only FUM and T-2 toxin positively.

Table 1. Concentration of major mycotoxins in maize silage variants expressed in 88% dry matter content (µg/kg of DM)

Mycotoxin	Silage variant	Mean	S.D.
DON	C	591.11[a]	40.24
	A	784.23[b]	22.88
	U	503.22[a]	103.99
OTA	C	13.77[a]	0.19
	A	16.40[b]	0.81
	U	13.57[a]	0.62
AFL	C	6.72	2.07
	A	6.60	1.71
	U	3.79	0.10
T-2	C	337.68[a]	36.51
	A	275.90[b]	2.98
	U	275.32[b]	17.39
FUM	C	324.36[a]	5.90
	A	270.19[b]	14.67
	U	375.61[a]	43.79
ZEA	C	260.78[a]	10.79
	A	423.91[b]	21.62
	U	356.71[c]	4.13

DON: deoxynivalenol, OTA: ochratoxins, AFL: aflatoxins, T-2: T-2 toxin, FUM: fumonisins, ZEA: zearalenone, S.D.: standard deviation, C: control, A: *Lactobacillus buchneri* + *Lactobacillus casei*, U: urea, [a,b,c]: different superscripts in columns for each mycotoxin, indicate significance at value $P<0.05$

CONCLUSIONS

Treating maize silage with microbiological silage additive consisting of *Lactobacillus buchneri* LN40177 and *Lactobacillus casei* LC329090 had both positive and negative impact on the concentration of mycotoxins. Statistically significant (P<0.05) higher concentration of DON, OTA and ZEA compared to untreated variant was observed. The positive effect was expressed by statistically significant (P<0.05) lower concentration of T-2 and FUM. The effect of urea addition on mycotoxic contamination was also demonstrated. In comparison with control variant, concentration of T-2 toxin was significantly (P<0.05) lower in variant treated by urea. On the other hand, statistically significant (P<0.05) increase of ZEA in this variant was noticed. Nonsignificant (P<0.05) decrease in AFL concentration was found in both treated variants against the control. The use of additives affect mycotoxic contamination of silages contradictory, but further research is needed.

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