EFFECT OF PHYSICAL STRUCTURE AND ADDITIVE APPLICATION ON MYCOTOXIN CONTAMINATION OF MAIZE SILAGE

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ABSTRACT

The aim of this study was to assess whether chop length and silage additives affect mycotoxin contamination in maize silage. Maize harvested at the milk wax stage was ensiled with urea, a lactic acid bacteria inoculant, or their combination, particle size was evaluated using the Penn State Particle Separator, and six mycotoxins were quantified by HPLC-MS/MS (88% dry matter basis). Deoxynivalenol and fumonisins were the predominant mycotoxins, HT-2 toxin occurred only in the untreated control, and urea alone or with inoculant reduced deoxynivalenol and prevented HT-2 toxin while chop length had no statistically significant effect on mycotoxin contamination.

Keywords: silage, additeves, urea, biological additive, corn silage

INTRODUCTION

Mycotoxins represent a group of toxic secondary metabolites produced by fungi, primarily belonging to the genera *Fusarium*, *Aspergillus*, and *Penicillium*. Major representatives of these toxins include deoxynivalenol (DON), fumonisins (FUM), HT-2 toxin (HT-2), zearalenone (ZEA), aflatoxins (AFL), and ochratoxins (OTA), which are frequently detected in both concentrate and forage feeds used in ruminant nutrition (Chhaya *et al.*, 2024). The aim of this study was to evaluate the occurrence and concentration of selected mycotoxins (DON, FUM, HT-2 toxin, ZEA, OTA, and AFL) in maize silages and to determine the effect of different silage additives (urea, biological additive, and their combination) and chop length on mycotoxin formation.

MATERIALS AND METHODS

In cooperation with PD Kozárovce (conventional farm), maize (FAO 480, dent grain type) was ensiled at the milk–wax stage of grain maturity. The biomass was harvested manually (several plants) and chopped using a shredder (H: 6284 XL). Variant C (control, without additives) was ensiled first, followed by variant U with the addition of urea (at a dose of 0.3%). + The physical structure of variants C and U was determined according to Heinrichs (2025) using the Penn State Particle Separator (PSPS), where the proportion of particles retained on the first sieve with 19 mm openings was 18.0% (longer chop length). Biomass from the same plot was subsequently harvested using a self-propelled harvester (KRONE BIG X), and a biological additive was applied simultaneously (containing *L. plantarum*, *L. rhamnosus*, and *L. buchneri*, min. 3.0×10^{11} CFU/g; applied at a dose of 1 g per 1 t of fresh matter). This biomass was ensiled as variant B (biological additive), after which urea was added (0.3%) and variant B+U was ensiled. The physical structure of variants B and B+U was assessed using the Penn State Particle Separator (PSPS), where the proportion of particles retained on the first sieve with 19 mm openings was 4.0% (shorter chop length). The biomass was compacted using a vacuum sealer (MSW, Motor Technics) and stored in plastic bags (n = 3). After six weeks, samples were collected, dried, and mechanically processed. In cooperation with Romer Labs (Tulln, Austria), the content of six mycotoxins (DON, FUM, HT-2 toxin, ZEA, OTA, AFL) was analysed using multi-mycotoxin analysis (HPLC–MS/MS). Mycotoxin concentrations are reported on an 88% dry matter basis.

RESULTS AND DISCUSSION

Of the six monitored mycotoxins, only DON and FUM were detected in maize silages, and HT-2 toxin was found only in the control. DON was the predominant mycotoxin detected in maize silage, followed by FUM as the mycotoxins with the second highest concentration. Guidance level for DON in cereals and cereal products is 8,000 μg/kg and for maize by-products is 12,000 μg/kg in the EU (Romer Labs, 2024), and according to FDA (2019) for ruminating dairy cattle older than 4 months is advisory level for total ratio 5,000 μg/kg. For FUM is guidance level for maize and maize products 60,000 μg/kg and for compound feed for adult ruminants, 50,000 μg/kg in the EU (Romer Labs, 2024). For lactating dairy cattle is guidance level for FUM 30,000 μg/kg (no more than 50% of diet- dry weight) according to FDA (2019). None of the analysed samples of maize silage exceeded the specified guidance levels for DON and FUM. There is no EFSA and FDA guidance concerning T-2/HT-2, but potentially harmful levels for cattle are 700-1,500 μg/kg in total ration dry matter (Dairyland, 2016). The HT-2 toxin was only present in the C silage without additives, at a concentration below potentially harmful level. The application of additives (urea, biological additive, and their combination) inhibited the formation of HT-2 toxin in maize silages. Kalúzová *et al.* (2022) confirmed the significant effect of the addition of urea and biological additive on reducing the content of T-2 toxin in maize silages. The addition of urea and the combination of biological additive with urea significantly ($P < 0.05$) reduced the DON concentration, unlike the application of the biological additive alone. This agrees with the results of Kalúzová *et al.* (2022). In maize silages treated with the biological additive, a significantly higher ($P < 0.05$) content of DON and FUM was observed. Gallo *et al.* (2021) tested several inoculants containing lactic acid bacteria and found out a positive effect ($P < 0.05$) on reducing AFB1 concentration, but a different effect on DON concentration in maize silage (control: 2215.0 μg/kg vs. with biological additives 2629.0–4373.0 μg/kg, $P > 0.05$). Skládanka *et al.* (2013) found out that grass silage treated with a biological–enzymatic inoculant had a comparable DON content as control ($P < 0.05$) and significantly higher concentration ($P < 0.05$) in silage with chemical additive. In the present study, the addition of urea and the combination of the biological additive with urea did not significantly affect the FUM concentration. Maize silage with biological additive had statistically significant ($P < 0.05$) higher concentration of FUM. Skládanka *et al.* (2013) detected the presence of FUM only in grass silages treated with a biological–enzymatic inoculant, in contrast to the control and silages treated with a chemical additive. Overall, the shorter length of cut showed lower total mycotoxin average content compared with the longer length of cut (446.2 vs. 522.5 μg/kg) however, the differences were not statistically significant. Similarly, Toledo *et al.* (2020) found out that particle size did not affect the presence of mycotoxins in maize silage.

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Tab. 1: Influence of physical structure (stratified with the Penn State Particle Separator) and additive use on mycotoxin contamination of maize silage (in μg/kg)

Chop length	Variant	Parameter	DON	FUM	ZEA	HT-2	AFB1	OTA
Longer	C	$\bar{x}$	2131.2^{abc}	138.2^{a}	ND	235.8	ND	ND
		SD	206.9	21.8	/	86.0	/	/
	U	$\bar{x}$	612.4^{a}	136.8	ND	ND	ND	ND
		SD	45.1	10.8	/	/	/	/
Shorter	B	$\bar{x}$	1198.6^{b}	185.9^{a}	ND	ND	ND	ND
		SD	23.6	56.2	/	/	/	/
	B+U	$\bar{x}$	754.6^{c}	176.0	ND	ND	ND	ND
		SD	56.7	55.6	/	/	/	/
All Variants		$\bar{x}$	1174.2	160.2	ND	235.8*	ND	ND
		SD	626.8	45.2	/	86.0*	/	/
Longer chop length		$\bar{x}$	522.5 (total mycotoxins)					
Shorter chop length		$\bar{x}$	446.2 (total mycotoxins)					

C: control, U: urea, B: biological additive, ND: not detected, *only in C variant, values with the same index in the column are statistically significant at P<0.05

CONCLUSIONS

This study evaluated the occurrence of selected mycotoxins in maize silages and assessed the effects of silage additives and chop length on their formation. Among the monitored mycotoxins, maize silages from a Slovak conventional farm were predominantly contaminated with DON, followed by FUM, whereas ZEA, AFLB1, and OTA were not detected. The HT-2 toxin was detected only in the untreated control silage. Importantly, none of the detected mycotoxin concentrations exceeded the current EU or FDA guidance levels for ruminants. The application of silage additives affected mycotoxin formation. The use of urea alone and in combination with a biological additive significantly reduced DON concentrations, while the biological additive applied alone was associated with higher DON and FUM concentrations. The formation of HT-2 toxin was inhibited by all additive treatments, confirming their protective effect against this mycotoxin. In contrast, FUM concentrations were not significantly affected by urea or the combined treatment. Chop length had no statistically significant effect on mycotoxin concentrations; however, maize silages with a shorter length of cut showed a lower average total mycotoxin content compared with those with a longer length of cut. These findings suggest that the concentrations of mycotoxins in silage are influenced more significantly by the addition of additives than by physical structure.

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