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THE EFFECT OF BIOLOGICAL SILAGE ADDITIVE ON MYCOTOXIN CONCENTRATIONS IN MIXTURE SILAGES OF FABA BEAN, OAT AND ALFALFA

DJELOVANJE BIOLOŠKOG DODATKA SILAŽI NA KONCENTRACIJE MIKOTOKSINA U SMJESAMA SILAŽE FABA GRAHA, ZOBI I LUCERNE

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ABSTRACT

The influence of bacterial additive on concentration of mycotoxins in mixture silages of faba bean, oat and alfalfa with high dry matter content was studied. The fresh stuff was harvested in pods formation stage of faba bean and after considerable wilting it was cut in to 20 mm particles and stuffed into silage bags. We ensiled the mixture in the control variant without additives and in the trial variant with liquid additive with following biological constituents: *Lactobacillus paracasei, Lactobacillus lactis, Pedioccus acidilacti* at a dose of 0.5 liter per ton. The samples were examined for the nutritive value and mycotoxins content (deoxynivalenol, T-2 toxin, zearalenone and total fumonisins, ochratoxins) by direct competitive enzyme-linked immunosorbent assay. Zearalenone was the secondary metabolite of microscopic fungi with the highest concentration. This study suggests that application of bacterial additive can significantly reduce the concentration of zearalenone and total fumonisins in mixture silages of faba bean, oat and alfalfa.

Keywords: mixture silage, biological additive, mycotoxins

INTRODUCTION

Molds are filamentous fungi that commonly occur in feedstuffs, including roughages and concentrates (Kačániová and Tančinová, 2001; Whitlow and Hagler, 2010). The *Aspergillus, Fusarium* and *Penicillium* molds are among the most important in producing mycotoxins detrimental to cattle (Whitlow and Hagler, 2005). Molds grow over a temperature range of 10-40°C, a pH range of 4 to 8 and can grow on feeds containing more than 12 % to 13 % moisture. In wet feeds such as silage, molds will grow if oxygen is available and pH is suitable (Whitlow and Hagler, 2001). The mycotoxins of greatest concern include: aflatoxin, which is generally produced by *Aspergillus* mold; deoxynivalenol, zearalenone, T-2 toxin and fumonisin, which are produced by *Fusarium* molds and ochratoxin produced by *Penicillium* molds (Whitlow and Hagler, 2005; Skládanka et al., 2011). Contamination is usually caused by consumption of contaminated feeds but may also be by contact or inhalation (Whitlow and Hagler, 2010). Good management of the growing crop, the ensiling process and the unloading phase must be employed to minimize mold and mycotoxin contamination, and the use of silage additives should be implemented as a strategic tool in silage making technology. Adhering to those principles will certainly reduce the risk of nutrient losses by fungal development and the impact of their toxic metabolites on animal health and performance (Auerbach, 2006).

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MATERIALS AND METHODS

In farm experiments we ensiled mixture, which was sowed in two steps. In the first step was sowed 15 kg.ha^{-1}common oat (*Avena sativa*) variety Flämingsstern , along with faba bean (*Faba vulgaris*) variety Inovec, 250 kg.ha^{-1}, and in the second step alfalfa (*Medicago sativa*) variety Palava, 20 kg.ha^{-1}. In ensiling mixture the ratio was: oat 20%, faba bean 70% and alfalfa 10%. The mixture was harvested when bean was forming hulls. The experiment was realized in co-operation with the University Farm VPP SPU Kolíňany. The fresh stuff with average dry matter content of 156.53 g.kg^{-1} was wilted to dry content of 528.43 (before ensiling control variant). Wilted ensiling matter was cut the stuff into 20 mm particles and pressed into silage bags of length 60 cm, diameter 2.44 m and thickness 0.224 mm. We ensiled two variants: the control variant (C) without additives and the trial variant (A) with addition of biological additive, containing lactic acid bacteria: *Lactobacillus paracasei, Lactobacillus lactis, Pediococcus acidilactici* (the bacteria concentration 1.25x10^{11} cfu.g^{-1}) applied in liquid state in the dose of 0.5 l per ton (after dissolving 2 g of powder in 0.5 l of water). After 3 months of fermentation we took the average samples of silages in which the content nutrients was determined of nutrients according to Regulation no. 2145/2004-100 and concentration of mycotoxins. Mycotoxins content of the silages was determined by direct competitive enzyme-linked immunosorbent assays (ELISA). The samples of faba bean, oat and alfalfa mixture silages were analyzed for five mycotoxins including total fumonisins (FUM), total ochratoxins (OTA), zearalenone (ZON) deoxynivalenol (DON) and T-2 toxin (T-2). Samples of silages were dried at 50°C (20 hours) and grounded to a fine powder. Extraction of samples was carried out in destilled water (DON), in methanol: water (70:30 v/v) for FUM, ZON and 50:50 (v/v) for T-2 and OTA. The Veratox quantitative test kits (Neogen, USA) were used. Absorbance was determined using the microwell strip reader (Neogen, USA) at 650 nm. A calibration curve for the standards for each toxin dilution was plotted using standards concentration against the percentage inhibition of the standards. By using the microwell reader, the tests provided sample results in μg.kg^{-1} for all mycotoxins. Concentrations of mycotoxins were found in two repetitions of each silage sample. The results were statistically processed using one-factorial variance analysis (ANOVA) of SAS. Means were separated using Fischer LSD multiple range test.

The aim of this study was to determine the influence of bacterial additive on occurence of mycotoxins in mixture silages of faba bean, oat and alfalfa with high dry matter content.

RESULTS AND DISCUSSION

The analytical results for mycotoxins indicate that samples of mixture silages were contaminated with all the determined mycotoxins. *Fusarium* species are potential producers of a larger number of mycotoxins (diacetoxyscirpenol, T-2 toxin, deoxynivalenol, nivalenol, zearalenone and other) (Bočarov-Stančic et al., 2005). After 3 months of storage the most prevalent mycotoxin was ZON, followed by T-2 toxin and DON (control) and conversely in treated silage. These results are in accordance with Kalač (2011), that deoxynivalenol and zearalenone are the main mycotoxins formed in silage. Their content is reduced by the activity of both lactic acid bacteria in silage and rumen microflora (Kalač, 2011). Zearalenone is able to directly affect reproductive performance of dairy cows (Rossi et al., 2009). ZON was detected in concentrations of 435.5 (A) and 992.7 μg.kg^{-1} (C). Differences in ZON concentrations were statistically significant ($P<0.05$). Nedělník and Moravcová (2006) in the Czech Republic analyzed the average concentration of ZON in alfalfa silages (577 μg.kg^{-1}) and lower value of 500 μg.kg^{-1} in barley silage. Kegl and Vanyi (1991) observed bloody diarrhea, low feed consumption, decreased milk production, and absence of estrous cycles in cows exposed to T-2. The lowest T-2 toxin content was determined in the trial variant of mixture silages treated with bacterial additive (186.1 μg.kg^{-1}) and the highest T-2 toxin concentration was identified in the control variant of mixture silages (211.9 μg.kg^{-1}). Our results confirm previous findings that selected strains of *Lactobacillus* are able to inhibit T-2 toxin formation (El-Nezami et al., 2002). Deoxynivalenol is associated with reproductive disorders (Kolesárová et al., 2011/12). In the present study, the samples of the control mixture silages had lower mean level of DON (183.1 μg.kg^{-1}), while the samples of A variant contained the mean level of 361.1 μg.kg^{-1}. Higher DON values were observed by Bíro et al. (2007) in

Table 1 Concentrations of mycotoxins in mixture silages of faba bean+alfalfa+oat

Tablica 1. Koncentracije mikotoksina u smjesama silaže grah + lucerna + zob

Variant - Varijanta		ZON	T-2	FUM	OTA	DON
		μg.kg^{-1}				
C	$\overline{x}$	992.7^{a}	211.9	142.8^{a}	7.0	183.1^{a}
	S.D.	9.687	3.026	1.174	0.057	1.683
A	$\overline{x}$	435.5^{a}	186.1	31.9^{a}	7.45	361.1^{a}
	S.D.	1.485	18.385	1.047	0.354	9.758

$\overline{x}$: average, S.D.: standard deviation, C: control, A: biological additive, ZON: zearalenone, T-2: T-2 toxin, FUM: total fumonisins, OTA: total ochratoxins, DON: deoxynivalenol, the values with identical superscripts in column are significantly different at $P<0.05$ – vrijednosti s istim slovima u kolonama se značajno razlikuju ($p<0.05$)

alfalfa silages (200-300 μg.kg^{-1}). Concentrations of DON in treated silages were significantly different in comparison with the control. The lowest contamination of mixture silage samples was by OTA mycotoxin. The primary toxic effect of OTA is inhibition of protein synthesis (Creppy et al., 1984). Nonsignificantly higher OTA content ($P>0.05$) was found in silages treated with bacterial additive than in untreated silages. Cabo et al. (2002) reported apparent antifungal activity of several lactic acid bacteria against genera *Penicillium*, which is a potential producer of ochratoxins. Our results do not confirm previous finding but suggest that concentrations of FUM were affected ($P<0.05$) by treatment with a commercial preservative based on a lactic acid bacteria. Dairy cattle (Holsteins and Jerseys) fed diets containing 100 mg fumonisin for approximately seven days prior to freshening and for 70 days thereafter demonstrated lower milk production (6 kg/cow/day), explained primarily by reduced feed consumption (Diaz et al., 2000).

CONCLUSION

Occurrence of observed mycotoxins was detected in all faba bean, oat and alfalfa mixture silages. The results showed that zearalenone was the secondary metabolite with the highest concentration. These data confirmed the application of bacterial additive (*Lactobacillus paracasei, Lactobacillus lactis, Pediococcus acidilactici*) were not sufficient to inhibit the concentration of deoxynivalenol and total ochratoxins, whereas significantly reduced the concentration of zearalenone, total fumonisins and non-significantly T-2 toxin content.

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SAŽETAK

Proučavan je utjecaj bakterijskog dodatka na koncentraciju mikotoksina u smjesama silaže faba graha, zobi i lucerne visokog sadržaja suhe tvari. Svježa tvar je brana u stadiju stvaranja mahuna faba graha te nakon znatnog uvenuća narezana na male dijelove od 20 mm i utrpana u vreće za silažu. Kontrolnu smjesu smo silirali bez dodatka a pokusnu varijantu s tekućim dodatkom sljedećih bioloških sastojaka: Lactobacillus paracasei, Lactobacillus lactis i Pedioccus acidilacti u dozi od 0.5 l po toni. Uzorci su ispitani na hranjivu vrijednost i sadržaj mikotoksina (deoksinivalenol,T-2 toksin, zearalenon i ukupni fuminozini, ohratoksini) direktnim kompetitivnim imunosorbentnim enzimskim testom. Zearalenon je bio sekundarni metabolit mikroskopskih gljivica najviše koncentracije. Ovaj rad navodi na zaključak da primjena bakterijskog dodatka može značajno smanjiti koncentraciju zearalenona i ukupnih fumonizina u smjesama silaže faba graha, zobi i lucerne.

Ključne riječi: smjesa silaže, biološki dodatak, mikotoksini