THE INFLUENCE OF FERTILIZATION AND PRESERVATION ON THE CONTENT OF MYCOTOXINS IN SILAGE OF COCKSFOOT (*DACTYLIS GLOMERATA* L.)

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Abstract: The aim of this work was to assess the content of mycotoxins with regard to habitat and fertilizationof cocksfoot crops (*Dactylis glomerata* L.) varieties Niva. Experimental forests were based on two sites with different altitudes. The harvest took place at the beginning of the earing (half of May) when the first cutsof the biomass were analyzed. The samples were analyzed for nutrients (ash, crude protein and crude fiber), the quality of the water extracts (ammonia, ethanol, pH, lactic acid, acetic acid) were evaluated, and the contents of mycotoxins (deoxynivalenol – DON, zearalenone – ZEA) were determined in green mass of silage. The data was assessed by analysis of variance ANOVA $P < 0.05$ on the surface. Fertilization did not influence the content of the organic nutrients in the silages. The difference ($P < 0.05$) in the quality of the silage extracts made from fertilized and unfertilized biomass, higher ($P < 0.05$) acetic acid content and a lower content of ethanol were noticeable. There was also noticeable higher content of ZEA in unfertilized stands. Silage did not show increased content of mycotoxins. The evaluation of the content of organic nutrients and acids proved that the quality of silage is very good.

Key Words: mycotoxins, silage, fertilization, cocksfoot, crude protein, lactic acid

INTRODUCTION

Mycotoxins are secondary metabolites produced by microscopic fungi - molds. Mold growth is determined by a number of factors that affect the ultimate composition of the mycoflora silage (Doležal 2009). Causes of mycotoxines production by moulds have not been entirely clarified yet (Kalač 2012). Their production is affected by a number of factors, either biotic (presence of more toxic mold species leads to different reactions) or abiotic (humidity, temperature, water activity, pH, O_2 content), (Skládanka et al. 2011).

Mycotoxins can cause fatal diseases at animals and they may have acute and chronic course (Barug 2004). Among the acute manifestations, degeneration of parenchym organs, circulatory system and CNS damage are included. Chronic course is manifested by teratogenic, mutagenic and carcinogenic effects (Doležal 2012). Effects of long-term exposure to the animal organism are diverse and depend on many factors (health, age, duration of exposure to the toxin). They can be e.g. disorders of reproduction, decrease in immunity, and allergic reactions. Research on mycotoxins is increasingly important as contaminated products directly present a threat to the health of the people (Nedělník et al. 2006). They affect the proper function of the ruminant rumen, may pass into the milk or meat, and further harm the sucking young animals (Alonso et al. 2013). The transition of mycotoxins to the animal products (milk, meat, eggs) is causing economic loss (Opitz von Boberfeld 1996).

Nowadays, there are known approximately 400 types of mycotoxins. The most commonly occurring mycotoxins in silage are deoxynivalenol (DON), zearalenone (ZON), fumonisin B1 (FB1), ochratoxin a (OTA), citrinin and patulin (Yiannikouris, Jouany 2002).

Monitoring of the contents of the DON in feed revealed i.e. there were 48 samples of dried forage positive for the contents of this mycotoxin in the year 2000 (Nedělník, Moravcová 2005). DON is probably the best known and the most common contaminant of grains and their products. Its positive occurrence in food and feed has been demonstrated in more than 90% of the total number of samples and it represents an indicator of potential occurrence of other mycotoxins (Sobrova et al. 2010). According to Skládanka et al. (2011) the highest contamination of mycotoxins arises already in the field or in the grasslands. Zearalenone (ZEA) and deoxynivalenon (DON) were mycotoxins mostly found at monitored grasses. This means that high amount of mycotoxins enter the food chain of animals from the green mass.

The importance is exemplified by the results of Zachariasova et al. (2014) indicating the expected intake of mycotoxins in corn silage per kg of living weight of the animals observed on the basis of the analyses. In cattle, there were determined amounts 2.3–5.4 $\mu g.kg^{-1}$ FW for nivalenol, 9.2–10.8 $\mu g.kg^{-1}$ FW for deoxynivalenol, 1–1.4 $ug.kg^{-1}$ FW for fusarenon X, 0.2–0.4 $ug.kg^{-1}$ FW for ochratoxin A, 0.03–1.8 $\mu g.kg^{-1}$ FW for enniatin and 0.5–5.4 $\mu g.kg^{-1}$ FW for mycophenolic acid.

The aim of the work was to determine whether fertilization of permanent crop of cocksfoot affects nutritional and quality parameters of silage, and whether manure is affected by the mycotoxin content in the harvested biomass and silage.

MATERIAL AND METHODS

The experimental crops were based in the Research Forage station Vatín located in the Czech-Moravian highlands (560 m. a. s. l.) and the Research Institute of Forage Troubsko near Brno (270 m. a. s. l.). The monitored variety of cocksfoot (NIVA) was sown in the spring in 2013 on the experimental plot with an area of 1.25 x 8 m in three repetitions on the habitats Vatín and Troubsko. The assessed factor was fertilization (unfertilized and fertilized with the dose of 170 $kg.ha^{-1}$ N) and the treatment of ensiled biomass with chemical treatment (control and chemical treatment). The subject of the evaluation was silage biomass from the harvesting of the first cut in the year 2014 (the first utilitarian year) at the beginning of the earing (May). Experimental silages were prepared in containers with a diameter of 150 mm. Preparation of experimental silage is described in Vyskočil et al. (2011). Samples of silage were taken after 60 days of ensilage. Samples of forage and silage were dried at a temperature of 60 °C, milled to a particles of size <1 mm, and then the analysis of the content of the mycotoxin deoxynivalenol (DON) and zearalenone (ZEA) using the ELISA test according to a Skládanka et al. (2011) was carried out. The concentration of the toxins is expressed in parts per million (ppm) and a billion (ppb). The quality was further evaluated using the extracts (pH, lactic acid, acetic acid, the content of ammonia and ethanol) and nutrient parameters (ash, crude protein and crude fibre). Analytical procedures including the preparation of the water extract is described by Doležal (2002). The results were evaluated by analysis of variation (ANOVA) and subsequently by Tukey test. The evaluation was carried out at the level of significance of $P<0.05$.

RESULTS AND DISCUSSION

DON levels ranged in the levels from 0.9 to 2.23 $mg.kg^{-1}$ in green mass and from 0.39 to 0.63 $mg.kg^{-1}$ in the silage. In the monitored samples, DON levels reached the lowest limits. The maximum quantity of mycotoxins shall be 0.9 to 12 $mg.kg^{-1}$ at DON and 0.1–3 $mg.kg^{-1}$ at ZEA in the EU. *Fusarium* and *Alternaria* are the main genera of fungi which are already present in the green mass (Rasmussen et al. 2010) and are able to survive the acidic and anaerobic environment of the fermentation process (Samson et al. 2002). From the results obtained, it is evident that the content of DON was about the same on both sites. The amount was not affected by the fertilization at the reference crop. Ensilaging of contaminated mass did not cause any further increase of mycotoxins.

Table 1 Content of DON [ppm] in the silage and green mass of unfertilized and fertilized variants of cocksfoot.

Variation	Site	Green mass	Silage
Unfertilized	Vatín	2.23	0.43
	Troubsko	1.27	0.63
Fertilized	Vatín	1.33	0.39
	Troubsko	0.9	0.39

Table 2 Content of ZEA [ppb] in the silage and green mass of unfertilized and fertilized variants of cocksfoot.

Variation	Site	Green mass	Silage
Unfertilized	Vatín	74.1	28.64
	Troubsko	22.57	30.7
Fertilized	Vatín	67.49	41.31
	Troubsko	18.63	40.01

The green mass of silage contained the permissible amount of ZEA. Values ranged from 0.0009 mg.kg^{-1} to 0.074 mg.kg^{-1} in the green mass and from 0.029 to 0.040 mg.kg^{-1} in the monitored silage.

Driehuis et al. (2008) indicate the average content of ZEA 0.017 mg.kg^{-1} and the maximum content 0.308 mg.kg^{-1} in the green mass. Fertilized grassland of cocksfoot showed a lower amount of ZEA compared to the unfertilized one. According to the D'Mello (2006), the concentration 0.2–1 mg.kg^{-1} of zearalenone is toxic even for rodents. Forage containing more than 0.5 mg.kg^{-1} of zearalenone is not recommended for feeding (Marasas et al. 1979). According to the determined values, the contents of mycotoxins did not exceed the standard allowable its use for feeding animals.

Ensilage is the process where lactic acid bacteria cleave simple sugars and produce acids. This process decreases the pH and which reduces the growth of undesirable microorganisms. Due to the fact that the anaerobic environment reduces the growth of mold, from this point of view, silage should be an effective strategy in order to avoid further formation of mycotoxins (Cheli et al. 2013).

Table 3 Content of ash, crude protein and crude fiber in the unfertilized and fertilized variants after use of chemical additive in comparison with non-protective variant of cocksfoot [g.kg^{-1}]

Variation	Treatment	Ash	Crude protein	Crude fiber
Unfertilized	Control	45.05	85.33	243.63
	Kemisile	37.85	81.8	242.5
Fertilized	Control	53.6	99.1	283.25
	Kemisile	48.4	111.5	288.85

Legend: Different letters in the columns indicate statistically significant differences at a level of $P<0.05$

The amount of ash content was observed in the standard levels at both variations (see the Table 3). In the samples treated with organic acids, lower ash content was recorded. The latter is an indicator of the pollution of the biomass with the soil. During the harvest of fodder, the ash is able to get there especially in case when low height of the stubble is chosen and there are unsuitable weather conditions (Skládanka 2011). The content 139 g.kg^{-1} of ash in the grass silage shows slight pollution with soil. The ash content of 265 g.kg^{-1} indicates high degree of contamination of silage with soil (Jakobe 1987). This pollution can lead to the development of undesirable microflora and increase further content of natural harmful substances in silage. Zeman (1995) indicates contents 93 g.kg^{-1} of the CP in average at green mass of cocksfoot crop. This value is referred to silage made from unfertilized stands of cocksfoot.

The content of the crude fiber was comparable (243.63 and 283.25 $g.kg^{-1}$) in the silage made of fertilized and unfertilized crops. The content of the crude fiber was not affected by treatment or fertilization of the crop (Table 3). Mikyska (2013) presents the content of the crude fiber 256.9 $g.kg^{-1}$in the silage of permanent grassland. Its value is close to the silage made of unfertilized crop. According to Zeman (1995), the content of crude fiber should be 322. 9 $g.kg^{-1}$ in the grass. This value is higher compared to ensiled samples. Skládanka (2009) indicates 210 $g.kg^{-1}$ of the crude fiber content at the beginning of the earing in the green crop of cocksfoot.

Table 4 Content of ammonia, ethanol, pH and acids in the unfertilized and fertilized variants after use of chemical additive in comparison with non-protective variant of cocksfoot [$g.kg^{-1}$]

Variation	Treatment	Ammonia	pH	Lactic acid	Acetic acid	Ethanol
Unfertilized	Control	0.33^{a}	4.08^{a}	23.18	4.53^{a}	4.53^{a}
	Kemisile	0.28^{a}	3.96^{a}	21.15	2.2^{b}	8.96^{b}
Fertilized	Control	0.68^{b}	4.76^{b}	17.68	5.3^{a}	3.5^{a}
	Kemisile	0.35^{a}	4^{a}	22.58	3.48^{c}	2.43^{a}

Legend: Different letters in the columns indicate statistically significant differences at a level of P<0,05

From our results (Table 4), it is apparent that the amount of ammonia was higher in the fertilized compared to the unfertilized samples of silage. A very good result was achieved using treatment with organic acids at fertilized stand of cocksfoot. The amount of ammonia was reduced (P<0.05) from 0.68 $g.kg^{-1}$ to 0.35 $g.kg^{-1}$. The content of the ammonia did not exceed standard values indicated by Doležal (2010), (0.3–0.7 $g.kg^{-1}$). The use of chemical preservatives caused reduction (P<0.05) of the concentration of ammonia in both silages made of unfertilized and unfertilized stands. Jakobe et al. (1987) list the value of ammonia 0.68 $g.kg^{-1}$ in dry matter of silage made of grass after seventy days. Animals are able to tolerate ammonia in the range of 1.4–2 $g.kg^{-1}$ in dry matter (Kalač 2012).

According to the Wilkinson (2005), the ideal pH of the silage should be in the range of 4–4.2. Doležal (2012) reported 3.7–5. These values were achieved in the experimental silage of cocksfoot. At both variants after the addition of organic acids, reduction (P<0.05) of pH values (4.08 to 3.96 in unfertilized and 4.76 on 4 fertilized variants) was achieved.

Kotal (1962) reported that the content of lactic acid in quality silage should be 2/3 of the total quantity of acids. Zeman et al. (2006) also reports the tables of evaluation of the quality of the silage where the minimum content of the lactic acid is 70% from the total amounts of acids. Higher amount of lactic acid was detected at unfertilized variants - 23.18 $g.kg^{-1}$ and 21.15 $g.kg^{-1}$ at variant treated with Kemisile. Silage made of fertilized crop showed higher representation of lactic acid in the samples that had been treated with chemical treatment (22.58 $g.kg^{-1}$). The content of acetic acid was influenced by the fertilization and way of treatment. The addition of organic acids decreased (P<0.05) acetic acid at both monitored variations - unfertilized variants from 4.53 to 3.96 $g.kg^{-1}$ and fertilized from 5.3 to 3.48 $g.kg^{-1}$.

The optimum content of the acetic acid should be 20–30% in dry matter from the total representation of the acids in the silage (Wilkinson 2005). Drevjany et al. (2004) reported the proportion of acetic acid 4–9 $g.kg^{-1}$ in dry matter as 35–35 %.

CONCLUSION

It was found that the addition of nitrogen does not cause further increase of the content of DON and ZEA in the green mass of the crop. During the process of ensilage, the concentration of the monitored mycotoxins was not increased. The content of ash, crude fiber and crude protein was slightly higher in the silage made from fertilized crop. However, these differences were not significant. At the silage produced from fertilized stand, content of ammonia and acetic acid was a higher and a concentration of ethanol lower. Nevertheless, all of the monitored parameters did not exceed the standards. Silages were very good with sufficient content and fermentation acids. Fertilizing does not have significant impact on the quality and nutritive value of silage.

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