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OCCURRENCE AND CONCENTRATION OF MYCOTOXINS IN GRASS SILAGE FROM SELECTED FARMS

Summary

The aim of the study was to assess the frequency of occurrence and concentrations of mycotoxins in grass silages depending on meadow location, term of harvest and type of fertilizer used. The research material consisted of 24 grass silage samples collected in 2013 from three farms located in: Falenty, Kodeń and Kąty. The following mycotoxins were evaluated: deoxynivalenol (DON), nivalenol (NIV), T2 and HT2 toxin, zearalenone (ZEA) and roquefortine C (ROQ C). 22 samples were positive for at least one mycotoxin. The most frequently detected toxins were NIV (14 samples contaminated), DON and ZEA (10 samples). The concentration of mycotoxins in silage samples coming from individual farms varied and significant differences between farms were obtained only in the case of T2 toxin and ZEA. Silage made out of sward from the third cut was more likely to be contaminated, than silage made out of sward from the first cut. The type of fertilizer had no significant impact on mycotoxin concentrations except for NIV and HT2 toxin.

***Keywords**: cut, deoxynivalenol, NPK, manure, nivalenol, T2 and HT2 toxins, roquefortine C, zearalenone*

CZĘSTOTLIWOŚĆ WYSTĘPOWANIA I ZAWARTOŚĆ MYKOTOKSYN W KISZONCE Z RUNI ŁĄKOWEJ W WYBRANYCH GOSPODARSTWACH

Streszczenie

Celem pracy była ocena częstotliwości występowania i zawartości mykotoksyn w kiszonce z runi łąkowej w zależności od lokalizacji gospodarstwa, terminu zbioru runi łąkowej oraz rodzaju nawożenia stosowanego do nawożenia łąki. Materiał badawczy stanowiły 24 próbki sianokiszonki pobrane w 2013 r. w trzech gospodarstwach: w Falentach, Kodniu i Kątach. Oceniono zawartość następujących mykotoksyn i toksyn: deoksyniwalenolu (DON), niwalenolu (NIV), toksyn T2 i HT2, zearalenonu (ZEA) i roquefortyny C (ROQ C). 22 próbki były skażone co najmniej jedną mykotoksyną. Najczęściej wykrywanymi toksynami były NIV (14 próbek) DON i ZEA (po 10 próbek). Zawartość poszczególnych mykotoksyn w próbkach kiszonki pochodzących z poszczególnych gospodarstw była bardzo zróżnicowana, ale istotne różnice między gospodarstwami stwierdzono tylko w przypadku dwóch mykotoksyn: toksyny T-2 i ZEA. Kiszonki sporządzone z runi łąkowej zebranej w trzecim pokosie zwykle były bardziej zanieczyszczone niż kiszonki z pierwszego pokosu. Rodzaj nawożenia nie miał istotnego wpływu na poziom skażenia mykotoksynami, z wyjątkiem toksyn NIV i HT2.

***Słowa kluczowe**: pokos, deoksyniwalenol, NPK, obornik, niwalenol, toksyny T2 i HT2, roquefortyna C, zearalenon*

1. Introduction

Mycotoxins are a group of highly toxic secondary metabolites secreted by fungal organisms mostly belonging to the genera *Aspergillus*, *Fusarium*, *Alternaria,* and *Penicillium*. Mycotoxin synthesis depends on environmental and physiological conditions [1, 2].

Animal mycotoxin metabolism is a complex net of biactivation and detoxification pathways. Ruminants are less sensitive to some mycotoxins since rumen microbiota can effectively degrade them over time [3]. Some mycotoxins and the by-products of their detoxification may become fixed in animal or human tissues, but are mostly expelled in faeces, urine or milk. The presence of toxic residues in edible animal products (milk, meat, offal), may have some detrimental effects on human health [4]. In animals, mycotoxin toxicity can cause many different minor chronic illnesses, in rare situations high mycotoxin concentration may cause death. Symptoms of mycotoxicosis depend mainly on the type of mycotoxin, the age, overall health condition and dietary status of the affected organisms. Mycotoxins ingested by livestock can cause vomiting, reduced fertility, lameness, impaired resistance to infections, reduced feed intake and feed refusal [5]. There's approximately 400 types of mycotoxins that can cause undesirable effects in cattle and be harmful to human health [6]. Some of the mycotoxins harmful to ruminant health include: aflatoxins, ochratoxin A, zearalenone, fumonisins (B1 and B2), trichothecenes, ergot alkaloids, and gliotoxin and others [7]. There are methods that decrease the risk of mycotoxin occurrences: screening plant material for fungal contamination, improved cultivation, harvest and storage methods, eliminating mycotoxins from contaminated food [16].

Epiphyte numbers, including moulds, are strongly affected by anthropogenic factors such as farming [8, 9]. Microorganisms present in the phyllosphere of grasses are influenced by changes in grassland management, particularly by change from intensive management to extensification due to reduced cutting frequencies and lower fertilizer applications [10].

Seasonal diversity in mould development is a common occurrence. In late autumn the vegetation of pastures gradually decreases and weather conditions stimulate the development of microscopic fungi, which, in consequence, may lead to the formation of mycotoxins [11].

There are considerable differences in mould-resistance amongst plant species. An example of mould-resistant grass species is *Festuca arundinacea* and its hybrids [12]. Most research studies have focused primarily on mycotoxin occurrence in cereal grains. Relatively few studies have surveyed mycotoxins in silages [10, 13]. Silage can become contaminated with fungi, either pre-harvest or post-harvest. The amount of mycotoxins in silage depends on many factors such as the quality of the plant material from which they are prepared, conditions of ensilaging and fodder storage [2, 14, 15].

2. Materials and methods
2.1. Grass silage samples collection

The research material consisted of 24 grass silage samples collected in 2013 from three farms located in: Falenty (52°08′ N 20°55′E) - Masovian Voivodoship, Kodeń (51°54′ N 23°36′ E) - Lublin Voivodeship and Kąty (53°22′ N 22°59′ E) – Podlasie Voivodeship.

Meadow sward intended for silage production was harvested on May 2013 (1st cut) and September 2013 (3rd cut). Before harvest the sward was wilted on the surface of the meadow to a dry matter content of 32 to 80%. Big-bale technology was used in all farms. Silages produced in Falenty came from meadow swards fertilized with mineral fertilizers NPK and organic fertilizer (Tab. 1). The time of forage ensilaging ranged from 9 to 12 weeks.

2.2. Mycotoxin analysis

The content of the following mycotoxins was evaluated: deoxynivalenol (DON), nivalenol (NIV), T2 and HT2 toxins, zearalenone (ZEA) and roquefortine C (ROQ C). Mycotoxin determinations were performed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) in Kazimierz Wielki University, Bydgoszcz, Poland.

Table 1. Experimental design
Tab. 1. Schemat doświadczenia

Experimental factor			Number of samples
Farm	Cut	Fertilisation	
Falenty	I	NPK	3
	III	NPK	3
	I	manure	3
	III	manure	3
Kodeń	I	NPK	3
	III	NPK	3
Kąty	I	NPK	3
	III	NPK	3

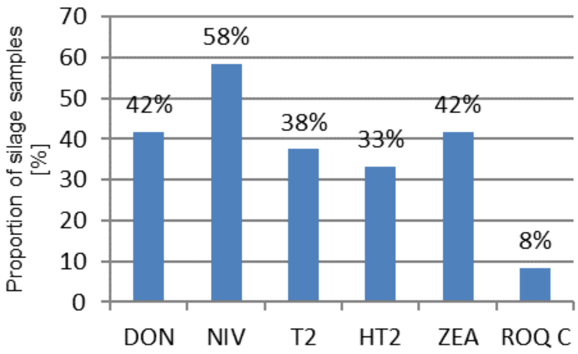
Source: own study / Źródło: opracowanie własne

2.3. Statistical analysis

Obtained data was compared using two-way analysis of variance (ANOVA) with place of sampling (farm) and time of harvest (cut) as factors. In samples collected from Falenty farm, the type of fertilisation and time of harvest was also analysed. Significance of differences was checked with the Tuckey HSD test at α=0.05. Correlations were calculated with a Spearman correlation test and were considered significant at a level of $p = 0.05$. All tests were made using Statistica ver. 6 (Statsoft, Poland).

3. Results
3.1. Frequency of mycotoxin occurrence

Out of 24 evaluated silage samples 22 were positive for at least one mycotoxin, and 12 samples contained three or more mycotoxins. NIV, ZEA and DON were amongst the most frequently encountered mycotoxins in grass silage and were found in 58% and 42% of the samples, with average concentrations at levels of 5.89 ppb (ZEA), 4.80 ppb (NIV) and 3.41 ppb (DON). Toxins T2 and HT2 were detected in grass silage samples at a frequency of 37% and 33%, respectively (Fig. 1).



Source: own study / Źródło: opracowanie własne

Fig. 1. Frequency of different mycotoxin occurrence in grass silage
Rys. 1. Procentowy udział prób sianokiszonki zanieczyszczonych poszczególnymi mykotoksynami

3.2. Mycotoxin concentration

The highest noticed concentration of mycotoxin occurred in grass silage samples from the farm located in Kąty (52.3 ppb), made of meadow sward harvested in the third cut where mineral fertilization had been used. The dominant mycotoxin being ZEA produced by *Fusarium* sp. fungi. In the first cut similar amounts of mycotoxins were noticed (44.3 ppb) with Roquefortin C being the dominant type of mycotoxin.

Smaller amounts of mycotoxins were noticed in Falenty, where mineral fertilization was applied, in grass silage samples from the first cut (18.6 ppb) and from the third cut (17.4 ppb). The most abundant mycotoxin types being DON and NIV, mycotoxins from the Trichotocene group which are produced by *Fusarium* sp.

No mycotoxins were detected in two silage samples from Falenty: one made out of meadow sward coming from the first cut that was fertilized with manure and one from the third cut fertilised with NPK.

The concentration of mycotoxins in silage samples coming from individual farms varied. Statistically significant differences between farms were obtained only in the case of T-2 mycotoxin and ZEA (Table 2). The highest concentration of both toxins was measured in Kąty and the lowest in Falenty.

The factor differentiating the amount of mycotoxins in silage samples was the cut in which the material for silage production was harvested. Silages made of meadow swards harvested in the third cut were more contaminated with mycotoxins than silages made from meadow swards coming from the first cut. Although significant differences were proven only in the case of ZEA (Table 2). The concentrations of other analysed mycotoxins showed no statistically significant differences.

Table 3 shows how the type of used fertilizer impacts the level of mycotoxin concentrations in samples of grass silage from Falenty. Mycotoxin amounts were very similar in samples from meadow swards fertilized with mineral fertilizers NPK and manure. Statistically significant differences were noted only between the levels of two mycotoxins: NIV and toxin HT2. On average the concentration of NIV was higher in silages from meadow sward fertilized with manure (10.47 ppb) than with mineral fertilizer (2.76 ppb). The opposite was observed in the case of HT2 toxin level (Table 3). Silages from meadow sward fertilized with manure had 0.0 ppb of HT2 toxin and those fertilized with mineral fertilizer - 1.89 ppb.

The term of cut had a significant impact only on HT2 toxin concentrations (Table 3).

3.3. Co-occurrences of mycotoxins

Table 4 shows that there was a statistically significant positive correlation between toxins T2 and HT2 (0.50) as well as between T2 toxin and ROQ C (0.50). Statistically significant negative correlation was obtained between toxin T2 and NIV mycotoxins (-0.48).

Table 2. Mycotoxin concentrations in grass silage depending on farm and term of harvest
Tab. 2. Stężenia mykotoksyn w sianokiszonce w zależności od gospodarstwa i terminu zbioru runi łąkowej

Farm	Cut	DON (ppb)	NIV (ppb)	Toxin T2 (ppb)	Toxin HT2 (ppb)	ZEA (ppb)	Roquefortine C (ppb)
Falenty	I	4.56	6.28	0.0a	0.0	0.32a	0.0a
	III	3.34	6.95	0.10ab	1.89	0.88a	0.0a
Kodeń	I	1.52	5.60	0.0a	0.0	0.0a	0.0a
	III	8.95	5.17	0.92bc	5.30	11.3ab	0.0a
Kąty	I	1.00	0.00	1.11c	1.33	0.19a	21.0b
	III	0.00	1.18	0.70abc	0.67	33.2b	0.0a
Mean for farms							
Falenty		3.95	6.62	0.05a	0.95	0.60a	0.0
Kodeń		5.23	5.38	0.46ab	2.65	5.65ab	0.0
Kąty		0.50	0.59	0.91b	1.0	16.7b	10.5
Mean for cuts							
I		2.91	4.54	0.28	0.33	0.21a	0.0
III		3.91	5.06	0.45	2.44	11.57b	5.25

Means in columns followed by the same letter are not significantly different at 5% level of probability (Tukey's test, p<0.05)

Source: own study / Źródło: opracowanie własne

Table 3. Mycotoxin concentrations in grass silage samples in Falenty depending on fertilization type and cut
Tab. 3. Stężenia mykotoksyn w próbkach kiszonki z trawy w Falentach w zależności od rodzaju nawożenia i pokosu

Fertiliser type	Cut	DON (ppb)	NIV (ppb)	Toxin T2 (ppb)	Toxin HT2 (ppb)	ZEA (ppb)	Roquefortine C (ppb)
Mineral	I	7.2	10.85	0	0.0a	0.53	0.0
	III	5.1	10.1	0	0.0a	1.38	0.0
Manure	I	1.92	1.72	0	0.0a	0.12	0.0
	III	1.59	3.80	0.2	3.79b	0.38	0.0
Mean from fertilisation							
Mineral		1.75	2.76a	0.0	1.89b	0.25	0.0
Manure		6.16	10.47b	0.1	0.0a	0.95	0.0
Mean from cuts							
I		4.56	6.28	0.0	0.0a	0.32	0.0
III		3.34	6.95	0.1	1.89b	0.88	0.0

Means in columns followed by the same letter are not significantly different at 5% level of probability (Tukey's test, p<0.05)

Source: own study / Źródło: opracowanie własne

Table 4. Spearman's Rank Correlation Coefficient
Tab. 4. Korelacja porządku rang Spearmana

	DON	NIV	Toxin T2	Toxin HT2	ZEA	ROQ C
DON	1.00	0.18	-0.04	0.01	0.18	-0.06
NIV	0.18	1.00	-0.48*	-0.17	-0.13	-0.32
T2	-0.04	-0.48*	1,00	0.50*	0.39	0.50*
HT2	0.01	-0.17	0.50*	1.00	0.04	0.05
ZEA	0.18	-0.13	0.39	0.04	1.00	-0.07
ROQ C	-0.06	-0.32	0.50*	0.05	-0.07	1.00

* correlations significant at p <0.05

Source: own study/ Źródło: opracowanie własne

4. Discussion

So far only a few studies on the occurrence of mycotoxins in grass silage have been published [10, 12, 17; 18]. Among the few studies in Poland focused on mycotoxins contaminating silages there's a paper by Panasiuk et al. [19] in which 87 maize silage and 33 grass silage samples were collected in 2015 from farms in Poland. All silage samples were positive for at least one mycotoxin, and 61% of the samples contained five or more mycotoxins simultaneously.

The most frequently detected toxins were deoxynivalenol, nivalenol, zearalenone, enniatins and beauvericin. In grass silage DON and ZEN were detected at a frequency of 37% and 3%, respectively, but the concentration levels of these toxins were relatively low. In their study deoxynivalenol mean concentration was 40.6 and zearalenon 80.6 ppb, respectively, and the two toxins were positive-correlated. In the study conducted by Skladanka et al. [10], the maximum content of DON was 167 μg/kg and ZEN at 66.9 μg/kg.

In our study the most frequently detected toxins were NIV (14 samples contaminated) DON and ZEA (10 samples) but their concentrations were relatively low and considerably lower than the current EU directive or guidance thresholds [20, 21]. Similar results, where mycotoxin concentrations in grass silages were relatively low, were obtained by McElhinney et al. [17, 18].

The simultaneous presence of different mycotoxins can be more toxic than the toxicity predicted for one mycotoxin only. In our study 22 samples were positive for at least one mycotoxin. But 50% of silage samples were contaminated by 3 or 4 toxins. A positive correlation between toxins T2 and HT2 was noted. T2 toxin is rapidly transformed into HT-2 toxin during the fermentation process [19]. The stated co-occurrence of toxins in examined grass silage samples, could pose chronic problems for exposed cattle, with possible synergistic and/or additive effects. The presence of multiple mycotoxins in animal feed should be considered as a potential threat to livestock wellbeing.

Obtained results confirm the observation that the occurrence of mycotoxins in grass silage is lower than in maize silage or wheat silage [22]. This is probably a result of the fact that fungi and other pathogens can easily survive on maize crops, which contain more necessary proteins and polysaccharides than grasses [23].

The results obtained in our study show that the concentrations of some mycotoxins (T2 and ZEA) in grass silage depends on the place and term of meadow sward harvest intended for silage production (the cut). The differences in occurrence of the main mycotoxins (DON, NIV and ZEN) could be caused by climate differences (temperature and precipitations). Kang'ethe et al. [24] study from 2017 showed differences in the number of grain samples contaminated with aflatoxins and levels of contamination depending on sampling site. The authors explain this finding by differences in weather conditions: hot and dry weather could possibly promote *Aspergillus* growth and consequently the production of aflatoxins.

It's hypothesized that the amount of mycotoxins is affected by the plant's condition, time of harvest and utilization. Our results, as well as Baholet et al. study [25] show that the driving factor behind mycotoxin contamination is the term of harvest. Silage made of sward coming from the third cut tend to be more contaminated than silage made of sward from the first cut. The reasons for this phenomenon are different weather condition during the growing season that promote mould development and synthesis of mycotoxins. Skládanka et al. [10] examined how forage grasses can become contaminated with deoxynivalenol (DON) and zearalenone (ZEA) during the growth season. July and October were presented as months where mycotoxin contamination was most common.

In our study the type of fertilizer had no significant impact on mycotoxin concentrations in silage except for NIV and HT2 toxin, similar results were observed in Baholet et al. study [25]. Results of our research can be important for organic farming systems as they show that natural fertilizers do not affect mycotoxin content in grass silages.

5. Conclusions

The concentrations of measured mycotoxins were generally low and considerably lower than current EU directive or guidance thresholds.

The content of individual mycotoxins in silage samples coming from individual farms varied but significant differences between farms were obtained only in case of T-2 toxin and ZEA.

Silage made of sward coming from the third cut tend to be more contaminated than silage made of sward from the first cut.

The type of fertilizer used had no significant impact on the mycotoxins concentrations except for NIV and HT2 toxin.

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