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A CONTAMINATION OF SWARD FROM THE GRASSLANDS OF ORGANIC AND CONVENTIONAL FARMS WITH AFLATOXINS AND OCHRATOXIN A

Summary

The presence of mycotoxins in feed for ruminants has been an ongoing problem, but still there is no regular programs monitoring mycotoxin contamination for green fodder, hay or silage. The aim of the study was to assess the amount of aflatoxins and ochratoxin A in dried green fodder (sward), which is a raw material for silages. The three-year study (2008-2010) was carried out in ten organic farms and six conventional farms, focused on breeding dairy cattle. The content of mycotoxins in dried sward and its contamination with moulds depended both on the production system as well as on the year of the study. The highest contamination with moulds was noticed in the first year of the study in sward from organic farms, which averaged 4.54 log CFU / g DM. At the same time the mycotoxin content in the sward was 4.46 ppb in the case of ochratoxin A, 6.20 ppb for aflatoxin B_1 and 15.18 ppb in regard to the sum of aflatoxins (B_1, B_2, G_1 and G_2). Between 2009 and 2010 the average amounts of ochratoxin A and aflatoxin B_1, both from organic and conventional farms, were significantly lower than those in 2008, but no significant difference was observed regarding the amount of total aflatoxins. The average content of aflatoxins and ochratoxin A taken from all years of the study was significantly higher for sward from organic farms than their content in sward in conventional farms. It was found also a positive correlation between the degree of moulds contamination and the contents of examined mycotoxins in forage.
***Key words**: moulds, mycotoxin, organic farming, sward, contamination*

POZIOM SKAŻENIA AFLATOKSYNAMI I OCHRATOKSYNĄ A RUNI ŁĄKOWEJ POCHODZĄCEJ Z GOSPODARSTW EKOLOGICZNYCH I KONWENCJONALNYCH

Streszczenie

Obecność mikotoksyn w paszach objętościowych dla przeżuwaczy jest wciąż aktualnym problemem. Brak również monitoringu dotyczącego poziomu skażenia tej grupy pasz (zielonki, siano, kiszonki). Celem pracy było określenie poziomu zawartości aflatoksyn i ochratoksyny A w podsuszonej zielonce, stanowiącej surowiec kiszonkarski. Trzyletnie badania (2008-2010) przeprowadzono w 10 gospodarstwach ekologicznych i 6 gospodarstwach konwencjonalnych, nastawionych na hodowlę bydła mlecznego. Zawartości badanych mikotoksyn w podsuszonej runi łąkowej oraz porażenie jej grzybami pleśniowymi zależały zarówno od sytemu produkcji, jak i roku badań. Największe porażenie runi łąkowej grzybami pleśniowymi stwierdzono w pierwszym roku badań w gospodarstwach ekologicznych, które wynosiło średnio 4,54 log. j.t.k. pleśni/g s.m. zielonki. Zawartości mikotoksyn w tej runi wynosiły w przypadku ochratoksyny A - 4,46 ppb, aflatoksyny B_1 - 6,20 ppb i sumy aflatoksyn (B_1, B_2, G_1 i G_2) - 15,18 ppb. W latach 2009 i 2010 średnie zawartości ochratoksyny A i aflatoksyny B_1, zarówno z gospodarstw ekologicznych, jak i konwencjonalnych, były istotnie niższe, natomiast w przypadku sumy aflatoksyn były na zbliżonym poziomie do roku 2008. We wszystkich latach badań średnia zawartość aflatoksyn i ochratoksyny A w runi łąkowej w gospodarstwach ekologicznych była istotnie wyższa od ich zawartości w runi łąkowej w gospodarstwach konwencjonalnych. Stwierdzono również dodatnią korelację między stopniem porażenia grzybami pleśniowymi a zawartością badanych mikotoksyn w zielonce.
***Keywords**: grzyby pleśniowe, mikotoksyny, rolnictwo ekologiczne, ruń łąkowa, skażenie*

1. Introduction

The permanent grasslands in Poland constitute about 22% of agricultural lands [1]. When properly used, grasslands are a source of valuable feed for ruminants, especially in organic farming [2], including pasture grass in summer and hay or silage during winter. Their share in the feed ration for dairy cattle can range from 50 to 100% depending on the level of intensity. Hence, their nutritional value and hygienic quality, resulting e.g. from the presence of potentially pathogenic microorganisms and toxic substances, are very important [3-4].

Moulds are a threat to animal and human health because of their ability to synthesize toxic metabolites called mycotoxins. Especially dangerous are aflatoxins and ochratoxin A. They affect the immune system and have a carcinogenic effect. Therefore, the presence of toxinogenic moulds pose a danger to the health of animals fed with contaminated feed and as a consequence imply a significant risk for people who consume animal products [5-10].

Among mycotoxins aflatoxins B_1 are the most commonly occurring and in the largest quantities in plants. In 2012, as a part of a European program for the evaluation of feed contamination with mycotoxins, including aflatoxin B_1, 359 samples of austrian feed were tested by Biomin Research Center. It was found that 41% of them were contaminated with aflatoxins. Amounts of measured aflatoxins ranged from 6 to 87 μg/kg (ppb) [11].

Initially it was thought that aflatoxin contamination of feed occurs after harvest and results from improper drying and storage of crops, what leads to mould growth [12]. It turned out, however, that contamination of these toxins appears already in the field, during plant vegetation [13-14]. Aflatoxins are synthesized primarily by some strains of *Aspergillus flavus*, most, if not all strains of *A. parasiticus*, and related to them species of *A. nomius*. It is believed that *A. parasiticus* is well adapted to living in soil and *A. flavus* is able to active development in the ground parts of the plant. *A. parasiticus* can produce all four aflatoxins (B_1, B_2, G_1 and G_2) and *A. flavus* usually synthesizes only aflatoxin B_1 and B_2 [13].

Aflatoxin B_1 is considered as a particularly dangerous. After the ingestion of contaminated feed it is metabolized in animals and excreted into the cow milk in the form of aflatoxin M_1, which is a significant threat to people consuming milk and milk products [15]. International Agency for Research on Cancer classified aflatoxin B_1 and aflatoxin M_1 as a human carcinogens. According to the European Commission Regulation no 466/2001 the maximum permissible level of aflatoxin M_1 in raw milk is 50 ppt, while in the milk intended for infant nutrition - 25 ppt [16]. In study by Pittet et al. who was monitoring the occurrence of aflatoxin M_1 in milk it was found that 12% of milk samples were contaminated with the toxin [17]. Based on the results of research conducted in Poland, it was found that aflatoxin M_1 was present in 17.3% of the 238 samples of human breast milk. Therefore it was proved that the problem of aflatoxin applies to infants too [14].

Ochratoxin A is produced by *Penicillium verrucosum* and some species of the genus *Aspergillus* e.g. *A. alataceus, A. sulphureus, A. mellus* and *A. sclerotium*. Studies conducted at the Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology showed that the presence of ochratoxin A is common in cereals and feed compounds. Moreover, ochratoxin A is accumulated in animals due to the carry over effect. Ochratoxin A bounds with blood proteins and enters to the muscles, what makes it dangerous for people consuming animal products [18-19].

The presence of mycotoxins in feed for ruminants has been an ongoing problem, but still there is no regular monitoring programs of contamination for green fodder, hay or silage. Under conditions of Southeastern Europe climate aflatoxins and ochratoxin A can be produced in "nests", what means that toxins can be produced only in certain layers of the silo heated by the sun or in some parts of cut forage subjected to the drying process [14].

In accordance with legal regulations presence of mycotoxins eliminates contaminated food from national and international trade [20]. European Food Safety Authority through scientific panels assesses the risk of mycotoxin contamination in food and feed. Both of them can be contaminated at any stage of the production process, from the growth of the plants in the field, through harvest, as well as during processing, storage and transport of the final product [21]. Therefore, it is essential to determine which mycotoxins, how often and in what frequency are present in feed.

2. Aim of the study

The aim of the study was to assess the amount of aflatoxins and ochratoxin A in dried green fodder (sward) collected in the years 2008 – 2010 from the grasslands of organic and conventional farms of Mazovia Region.

3. Materials and methods

The survey was carried out over a period of three years (in 2008, 2009 and 2010) in ten organic farms and six conventional farms specializing in the production of dairy cattle and located in the Masovia Region of Poland.
Animal nutrition in these farms was based on coarse fodder: pasture grass in summer and silages in winter. This feed made up around 65% of the daily feed ration for dairy cattle.

Area of the grasslands in these farms ranged from 2.5 to 10.0 ha, which accounted for 15 to 50% of agricultural lands. The grasslands in certified organic farms were fertilized with manure and conventional farms used mineral fertilization. In the summer months cows were allowed to graze on pasture and a meadow. Sward was from the second mow. After drying on the air to the level of 40-60% of dry matter the sward was used for ensiling and silages were used in winter feeding.

In order to evaluate mycotoxin contamination of green fodder annually from each farm samples of second mow of sward were collected. The sward was the raw material for the production of silages. Samples were collected by random squares method. Plant material was mixed and separated into three average samples. In green fodder the following measurements were done:

- dry matter content using the gravimetric method in line with the PN-ISO 6496:2002 standard,
- the number of moulds in line with PN-ISO 7954: 1999 standard,
- the amount of total aflatoxins, aflatoxin B_1 and ochratoxin A using an immunoenzymatic method – ELISA tests and STAT FAX plate reader.

The test results were subjected to statistical analysis using Microsoft Excel 2003 and Statistica 8.0 (Statsoft, Poland) softwares. Correlation coefficients were calculated between the content of mycotoxins and number of moulds in the sward. The relationships between two variables was determined by linear regression model using the average values for each farm in the subsequent years of the study. P-values of $p \leq 0.05$ were considered to be statistically significant.

4. Results and discussion

The number of moulds in the dried sward was investigated and ranged on average from 2.60 to 5.00 log CFU/g DM (mean for all farms during three years of the study was 3.83 log CFU/g DM) in organic farms (Table 1). Regarding to conventional farms the number of moulds was significantly lower and totalled 3.21 log CFU/g DM on average for three years of the study (Table 2). In particular years of study the intensity of green fodder contamination with moulds was variable. The highest number of moulds occurred in 2008, probably because of the high humidity and temperature during its drying and harvesting. It averaged 4.54 log CFU/g DM for organic farms and 3.62 log CFU/g DM for conventional ones. In the following years of the research a degree of contamination with fungi was significantly lower. The average number of moulds in sward from grasslands of organic farms was 3.63 log CFU/g DM in 2009 and 3.33 log CFU/g DM in 2010 and for conventional farms it amounted to 3.17 and 2.85 log CFU/g DM respectively (Table 1 and 2). The observed values were compara-

ble to those published by authors in other studies [22].

Values of the measured mycotoxin levels in dried sward was significantly differentiated, both among individual years of the research and between two forms of agriculture – organic and conventional. A higher mycotoxin contamination of forage was observed in organic farms (difference between two types of farms was as following: ochratoxin A - 1.7 ppb, aflatoxin B_1 - 0.22 ppb, sum of aflatoxins - 2.26 ppb) comparing the average content of mycotoxins in sward derived from organic farms (Table 1 and 2).

The highest levels of mycotoxins were determined in 2008 in forage originated from organic farming. The average concentration of ochratoxin A in this group of farms was 4.46 ppb and the average concentration of aflatoxins totaled to 6.20 ppb and 15.18 ppb respectively for aflatoxin B_1 and total amount of the toxin (Fig. 1). The average contents of mycotoxins in material from conventional farms were relatively smaller and totaled to 2.30 ppb for ochratoxin A, 5.68 ppb for aflatoxin B_1 and 11.97 ppb for total aflatoxins (Fig. 2). Between 2009 and 2010, the average contents of ochratoxin A and aflatoxin B_1 in the sward were lower by 20 % irrespective of form of agriculture. No significant difference was observed regarding to the amount of total aflatoxins in those years (Fig. 1 and 2).

Table 1. Moulds, aflatoxins and ochratoxin A contamination of sward from organic farms
Tab. 1. Skażenie pleśniami, aflatoksynami i ochratoksyną A podsuszonej runi łąkowej w gospodarstwach ekologicznych

No. of farm	Year of the study	Dry matter %	Number of moulds, log CFU/ g DM	Content of mycotoxins in dry matter, µg/kg (ppb)		
				ochratoxin A	aflatoxin B_1	aflatoxins B_1, B_2, G_1, G_2
1	2008	47.8	5.00	5.76	3.50	15.26
	2009	46.8	4.30	4.06	4.85	13.42
	2010	56.9	3.30	4.43	3.18	10.78
	average:	**50.5**	**4.20**	**4.75**	**3.84**	**13.15**
2	2008	43.5	4.60	5.24	8.22	18.63
	2009	45.0	3.00	3.05	3.78	11.05
	2010	44.5	2.60	2.98	3.46	8.89
	average:	**44.3**	**3.40**	**3.76**	**5.15**	**12.86**
3	2008	43.4	4.60	5.24	7.73	18.90
	2009	58.2	3.30	4.20	4.86	14.94
	2010	47.9	3.00	2.98	5.60	12.52
	average:	**49.8**	**3.63**	**4.14**	**5.73**	**15.45**
4	2008	50.2	4.30	4.25	5.24	10.16
	2009	49.2	4.60	4.09	4.85	16.87
	2010	56.9	2.60	3.46	4.09	11.73
	average:	**52.1**	**3.83**	**3.93**	**4.73**	**12.92**
5	2008	58.7	5.00	5.54	8.63	19.62
	2009	56.8	4.30	5.28	8.16	12.94
	2010	60.0	2.60	4.86	5.28	9.98
	average:	**58.5**	**3.97**	**5.23**	**7.36**	**13.18**
6	2008	41.4	4.60	4.27	6.67	18.28
	2009	49.0	4.30	4.44	4.15	15.85
	2010	43.0	5.00	3.48	4.87	18.82
	average:	**44.5**	**4.63**	**4.06**	**5.23**	**17.65**
7	2008	47.2	5.00	3.00	5.72	17.81
	2009	49.7	3.90	1.30	4.46	11.27
	2010	43.6	3.60	1.99	4.78	10.27
	average:	**46.8**	**4.17**	**2.10**	**4.99**	**13.12**
8	2008	50.7	3.90	4.18	5.63	12.84
	2009	55.5	3.00	3.24	4.24	11.25
	2010	54.2	3.00	2.20	5.76	11.38
	average:	**53.5**	**3.30**	**3.21**	**5.21**	**11.82**
9	2008	56.7	3.78	3.88	4.26	9.62
	2009	56.2	3.00	3.18	3.49	9.90
	2010	45.8	3.30	2.38	4.62	10.24
	average:	**52.9**	**3.36**	**2.81**	**4.12**	**9.92**
10	2008	47.0	4.60	3.20	6.35	10.65
	2009	46.2	2.60	2.88	4.28	8.89
	2010	39.6	4.30	2.78	5.66	18.04
	average:	**44.3**	**3.83**	**2.95**	**5.43**	**12.53**
	Average for all farms:	**49.7 ± 5.9**	**3.83 ± 0.83**	**3. 73 ± 1.11**	**5.21 ± 1.46**	**13.36 ± 3.53**
	Average for 2008	48.7 ± 5.3	4.54 ± 0.41	4.46 ± 0.91	6.20 ± 1.58	15.18 ± 3.80
	Average for 2009	51.3 ± 4.7	3.63 ± 0.69	3.57 ± 1.04	4.71 ± 1.23	12.64 ± 2.49
	Average for 2010	49.2 ± 6.8	3.33 ± 0.75	3.15 ± 0.88	4.73 ± 0.86	12.27 ± 3.23

± *standard deviation / ± odchylenie standardowe*

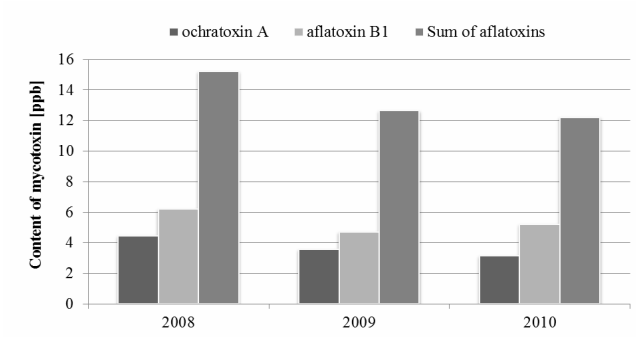
Source: own work / Źródło: opracowanie własne

Table 2. Moulds, aflatoxins and ochratoxin A contamination of sward from conventional farms
Tab. 2. Skażenie pleśniami, aflatoksynami i ochratoksyną A runi łąkowej w wybranych gospodarstwach konwencjonalnych

No. of farm	Year of the study	Dry matter, %	Number of moulds, log CFU/g DM	Content of mycotoxins in dry matter, µg/kg (ppb)		
				ochratoxin A	aflatoxin B_1	aflatoxins B_1, B_1, G_2, G_2
1	2008	45.8	3.90	3.26	4.54	10.27
	2009	48.6	4.30	1.38	3.82	11.42
	2010	46.9	3.30	1.96	4.15	8.78
	average:	**47.1**	**3.83**	**2.20**	**4.17**	**10.16**
2	2008	53.5	3.30	2.68	6.22	12.61
	2009	55.0	2.60	1.37	4.73	11.08
	2010	54.5	2.60	2.31	3.94	9.86
	average:	**54.3**	**2.83**	**2.12**	**4.96**	**11.18**
3	2008	43.4	3.00	1.37	5.74	12.92
	2009	50.2	3.60	1.95	4.88	13.96
	2010	47.2	2.60	2.38	5.67	10.58
	average:	**46.9**	**2.73**	**1.90**	**5.43**	**12.49**
4	2008	40.2	2.30	3.44	5.09	9.24
	2009	49.2	2.60	2.26	4.85	9.80
	2010	52.3	3.30	1.98	4.42	10.73
	average:	**47.2**	**2.73**	**2.56**	**4.79**	**9.92**
5	2008	48.1	4.60	1.72	6.64	14.60
	2009	56.8	2.60	2.35	5.16	10.98
	2010	57.4	2.30	2.28	5.42	9.96
	average:	**54.1**	**3.17**	**2.12**	**5.74**	**11.85**
6	2008	51.4	4.60	1.35	5.82	12.20
	2009	52.0	3.30	0.78	4.19	10.84
	2010	49.6	3.00	1.72	4.52	9.88
	average:	**51.0**	**3.63**	**1.28**	**4.84**	**10.97**
	Average for all farms:	**50.1 ± 4.5**	**3.21 ± 0.74**	**2.03 ± 0.68**	**4.99 ± 0.80**	**11.10 ± 1.60**
	Average for 2008	47.1 ± 4.5	3.62 ± 0.84	2.30 ± 0.86	5.68 ± 0.69	11.97 ± 1.76
	Average for 2009	52.0 ± 3.0	3.17 ± 0.64	1.68 ± 0.56	4.61 ± 0.47	11.35 ± 1.27
	Average for 2010	51.3 ± 3.8	2.85 ± 0.38	2.11 ± 0.24	4.69 ± 0.64	9.97 ± 0.63

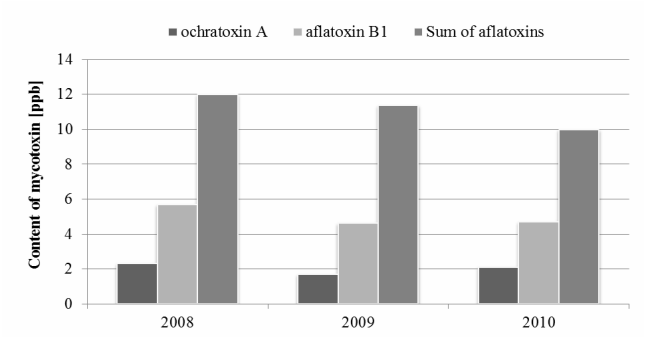
± *standard deviation* / ± *odchylenie standardowe*

Source: own work / Źródło: opracowanie własne



Source: own work / Źródło: opracowanie własne

Fig. 1. C ontent of mycotoxins in sward of grasslands from organic farms in 2008-2010
Rys. 1. Poziom zawartości mikotoksyn w runi łąkowej w gospodarstwach ekologicznych w latach 2008-2010



Source: own work / Źródło: opracowanie własne

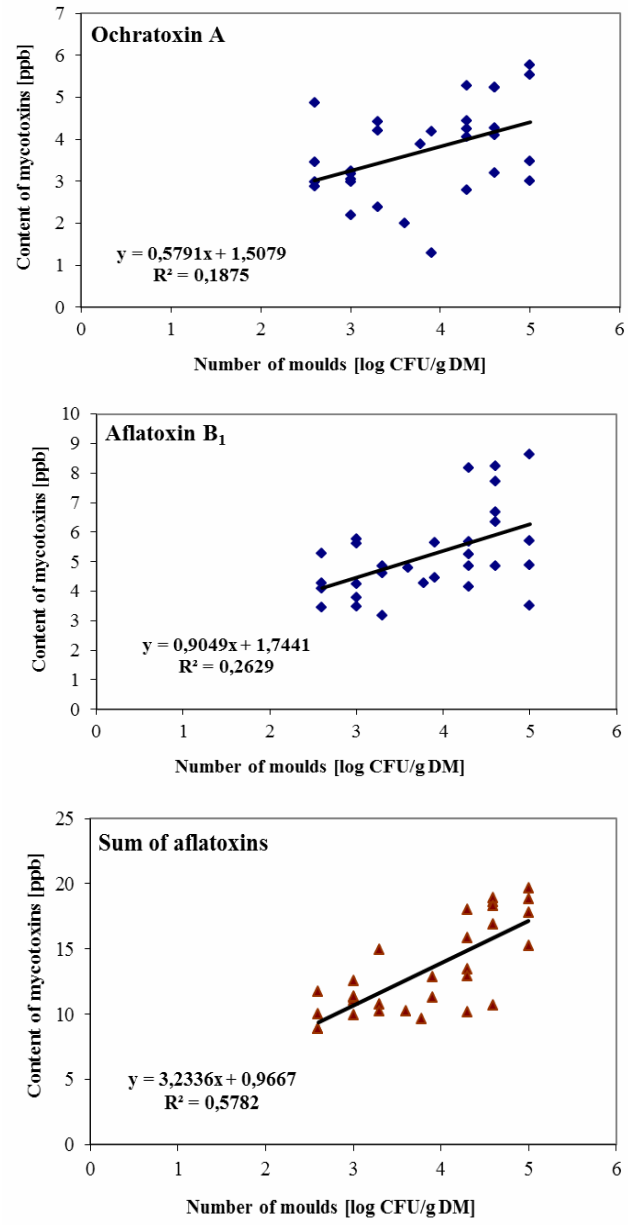
Fig. 2. Content of mycotoxins in sward of grasslands from conventional farms in 2008-2010
Rys. 2. Poziom zawartości mikotoksyn w runi łąkowej w gospodarstwach konwencjonalnych w latach 2008-2010

Conditions under which mycotoxins are produced by moulds in high concentrations are not fully described. In general, the synthesis of mycotoxins is genetically determined and related to the basic metabolic pathways. Additionally it is phenotypically determined and dependent on environmental factors. Fungi produce mycotoxins often under stress conditions when the temperature, humidity and the availability of oxygen change or in case of aggressive substances action against moulds. E.g. humidity which exceeds 70% and moisture of plant material by more than 15% favour synthesis of mycotoxins [23].

Statistical analysis allowed to find some weak linear relationships between the degree of mould contamination and the values of the measured amount of mycotoxins in forages (Fig. 3 and 4). The strongest relationship between the number of moulds and mycotoxins concentration was found for total aflatoxins content in the sward from organic farms (R2 = 0.578) and then for conventional farms (R2 = 0.339).

On the basis of calculated correlation coefficients for two types of farming it was proved that there was a positive correlation between the content of all mycotoxins in the sward and the number of moulds as well as between the content of total

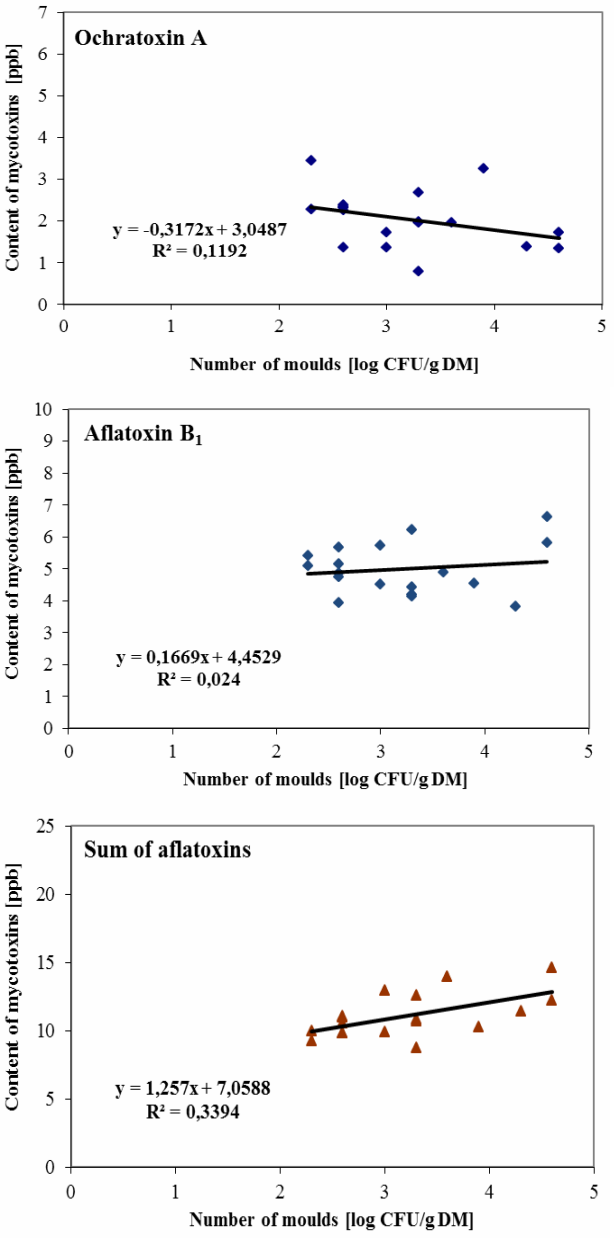
aflatoxins in the sward and the number of moulds (Table 3). The highest correlation was calculated for total aflatoxin content, both for organic farms (r = 0.76) and for conventional farms (r = 0.58). Lower values of the correlation coefficient were found regarding aflatoxin B_1 (r = 0.51) and ochratoxin A (r = 0.43) in sward from organic farms (Table 3).



Source: own work / Źródło: opracowanie własne

Fig. 3. Content of mycotoxins in dry matter of pasture grass collected from organic farms depending on the number of moulds

Rys. 3. Zawartość mikotoksyn w suchej masie zielonki w gospodarstwach ekologicznych w zależności od liczby grzybów pleśniowych



Source: own work / Źródło: opracowanie własne

Fig. 4. Content of mycotoxins in dry matter of pasture grass collected from conventional farms depending on the number of moulds

Rys. 4. Zawartość mikotoksyn w suchej masie zielonki w gospodarstwach konwencjonalnych w zależności od liczby grzybów pleśniowych

A positive correlation between the occurrence of moulds and ochratoxin A concentration was also found in previous studies on the reduction of microbial contamination in silages made of sward [24].

Table 3. Correlation coefficients between content of mycotoxins and number of moulds in sward from the examined farms

Tab. 3. Korelacje między zawartością mikotoksyn a liczebnością grzybów pleśniowych w runi łąkowej w badanych gospodarstwach

	ochratoxin A	aflatoxin B_1,	aflatoxins B_1, B_2 ,G_1, G_2
Number of moulds, log CFU/ g DM in sward from organic farms	0.43*	0.51*	0.76*
Number of moulds, log CFU/ g DM in sward from conventional farms	-0.35	0.15	0.58*

**significance of correlations at P≤0.05 / *wyznaczone współczynniki korelacji są istotne na poziomie P≤0,05*

Source: own work / Źródło: opracowanie własne

On the basis of a few research conducted in Poland it can be pointed that there exist a danger of mycotoxins presence in roughages made of permanent grassland harvest. It is indicated inter alia by the results of the assessment of raw cow milk in five organic farms, which showed the presence of aflatoxin M_1 at level of 8,45-8,60 ppt. Therefore, it could be thought that there occurred also aflatoxin B_1 contamination of the pasture sward as well as silages [3].
Still there have not yet been provided a large-scale monitoring studies in Poland on the levels of mycotoxin contamination of roughages, such as fodder or hay. Little is also known on the mycotoxin contamination in silages [25-26]. Hence, presented in the paper data cannot be compared with the results obtained by other authors.

5. Conclusions

1. The highest number of moulds in green forage occurred in 2008, it averaged 4.54 log CFU/g DM for organic farms and 3.62 log CFU/g DM for conventional ones and was probably caused by high humidity and temperature during sward drying and harvesting.
2. The highest contamination with mycotoxins was noticed in 2008 in sward from organic farms, which averaged 4.46 ppb in the case of ochratoxin A, 6.20 ppb for aflatoxin B_1 and 15.18 ppb regarding the sum of aflatoxins (B_1, B_2, G_1 and G_2).
3. In the 2009 and 2010 the level of aflatoxins and ochratoxin A contamination of the dried sward, originating both from organic and conventional grasslands, was lower by about 20%.
4. It was found a positive correlation between the number of mould contamination and the content of examined mycotoxins in forage.

6. References

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