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CONTAMINATION WITH MICROSCOPIC FUNGI AND THEIR METABOLITES IN CHICKEN FEEDS PRODUCED IN WESTERN POLAND IN THE YEARS 2009–2010

Kinga Stuper, Renata Cegielska-Radziejewska, Tomasz Szablewski, Anna Ostrowska, Maciej Buśko, Juliusz Perkowski

Poznań University of Life Sciences, Poland

Abstract. The aim of this study was to assess the level of contamination in feed mixes for broiler chickens produced in four commercial-scale feed mills located in western Poland in the years 2009–2010. Quality appraisal and quantitative analyses of feed contamination with microscopic fungi and trichothecenes were conducted in this study. It was found that starter type feeds were significantly less contaminated with microscopic fungi and mycotoxins than feed mixes for older chickens. In 2009 higher levels of contamination with the determined microorganisms and their toxic metabolites were found in feeds than it was in 2010. In terms of quality in the analysed feed samples the most frequently identified microscopic fungi were moulds from the genera *Aspergillus*, *Rhizopus* and *Mucor*. Among the detected mycotoxins DON, DAS, 3-AcDON and Fus-X were detected most frequently. The admissible DON contamination level imposed by the EU regulations was not exceeded in any of the analysed feed samples.

Key words: feeds, ergosterol, trichothecenes, microscopic fungi, CFU

INTRODUCTION

Diets for poultry are based on plant feeds, with cereal grain constituting their main components. Development, health, fertility and productivity of farm animals

Corresponding author: Kinga Stuper, Poznań University of Life Sciences, Department of Chemistry, Wojska Polskiego 75, 60-637 Poznań, Poland, e-mail: kstuper@up.poznan.pl

depend on appropriate feeding regimes. As a result of inadequate nutrition fertilizing capacity decreases, the number of healthy young offspring is reduced similarly to the number of laid eggs, growth is inhibited and at the same time animal conformation is changed.

Thus it is crucial to monitor the quality and safety of both the feed raw material and the final product, i.e. feed mixes. The nutritive value and health safety of cereal grain, being the primary components of feeds, depend to a considerable degree on the conditions under which crops were grown, harvested and stored until milling. Poultry is highly sensitive to microbiological and chemical contamination. For chickens, which are exceptionally sensitive, grain used in feed mixes needs to be of good quality. One of the hygienic criteria indicating the microbiological status of a feed is the level of contamination with microscopic fungi expressed in the number of colony forming units of microscopic fungi per 1 gram of feed (CFU $\cdot$ g^{-1}) as well as the concentration of their toxic metabolites, i.e. mycotoxins. The microscopic fungi most commonly found in feeds include genera *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor* and *Rhizopus* [Binder et al. 2007, Oxley-Goody 2009]. The primary sources of their occurrence in feeds are connected with plant-origin feed raw materials, mainly cereals. In wheat grain immediately after harvest typically a maximum level of 103 CFU $\cdot$ g^{-1} is detected [Kwiatek and Kukier 2009]. Fungi contaminating feed raw materials may be field and/or storage fungi and their counts to a considerable extent depend on the environmental conditions. Moulds developing on the surface of kernels contribute to losses of nutrients, organoleptic changes, the formation of mycotoxin and as a consequence a deterioration of feed quality and diseases in poultry [Wieliczko and Woźniak 2006, Mazanowski 2009]. In order to ensure feed safety the European Union imposed an obligation to control contents of microscopic fungi using analytical and microbiological methods (Ordinance of the Commission (EC) no. 1126/2007; Ordinance of the Commission (EC) 1881/2006). Next to classical microbiological methods in the assessment of the level of contamination with microscopic fungi with increasing frequency chemical methods are being applied for the analysis of fungal markers such as chitin, ATP and ergosterol (ERG). Literature sources on the subject indicate a high level of correlations between ERG concentration and CFU and with the total mycotoxin concentration, thus it may be seen as a good indicator of the level of contamination with microscopic fungi in the analysed material. Methods used to determine mycotoxin concentration, next to immunological methods, include instrumental chemical methods such as analyses with the application of HPLC, LC/MS and GC/MS [Perkowski et al. 2008].

Among the many genera of microscopic fungi found in the climatic zone of western Poland fungi from the genus *Fusarium* occupy a special position. The

species occurring most frequently on plant origin raw materials include *Fusarium oxysporum*, *Fusarium poae*, *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium sporotrichioides* [Meister 2009]. Their toxin-forming strains under advantageous conditions produce secondary metabolites, i.e. mycotoxins.

In Poland in produced feeds the most typically detected mycotoxins are those from the group of trichothecenes, mainly deoxynivalenol (DON), nivalenol (NIV) as well as T-2 and HT-2 [Grajewski et al. 2009]. DON is recorded most commonly and its established concentrations range from 0.033 to 6 mg $\cdot$ kg^{-1} in poultry feeds [Wiśniewska-Dmytrow et al. 2004].

Poultry exhibits moderate sensitivity to the toxic action of trichothecenes among all farm animals [Eriksen and Pettersson 2004], as birds are more susceptible to the disadvantageous effect of trichothecenes than ruminants, such as cattle, while they are less sensitive than pigs [Grajewski et al. 2009].

Generally two mechanisms have been assumed for the toxic action of the discussed mycotoxins. One of them concerns the role of the T-2 toxin and nivalenol indicating that they block the initiation of the synthesis of proteins. In turn, the other refers to e.g. deoxynivalenol and the HT-2 toxin, which disturb elongation of proteins [Pestka 2007]. It may be assumed that the generally predominant action of this type of mycotoxins is the inhibition of protein synthesis at the cellular level, combined with immunodeficiency. As frequently high contents of deoxynivalenol are found in feeds it is excreted in considerable amounts in poultry droppings, and the organism burdened with it is partly detoxified as a result of glucoronidation of that mycotoxin [Grajewski et al. 2009].

Typical symptoms of trichothecene poisoning in poultry include swellings and pathological changes in the oral cavity. Moreover, growth inhibition is observed along with sluggishness, dysenteric diarrhoea and neurotoxic changes (the non-physiological position of wings, inadequate response to environmental stimuli). Moreover, slow feathering and symptoms of rickets are observed [Mello et al. 1999]. Enhanced susceptibility to other diseases results from the immunosuppressive action of trichothecenes. They cause deficient activity of T and B lymphocytes, inhibition of immunoglobulin and antibody production, as well as weakened interferon activity [Shlosberg and Hanji 2000].

The aim of this study was to determine the level of contamination with microscopic fungi and mycotoxins from the group of trichothecenes in feeds for broiler chickens of different ages produced at four commercial scale feed mills in western Poland in 2009 and 2010.

MATERIAL AND METHODS

Tested material

Material for analyses comprised a total of 120 samples of feed mixes for broiler chickens, collected from four different commercial scale feed mixes located in western Poland in September 2009 and 2010. Samples included feeds for broiler chickens from different age groups (chicken broilers – starting, chicken broilers – growing, chicken broilers – finishing). In August and September 2009 and 2010 from each of the four feed mixes a total of 5 samples were collected from each type of feed mixes, i.e. starter, grower and finisher feeds, of 1000 g each, which gave in each year a total of 60 feed samples for chemical and microbiological analyses.

Microbiological method to determine the number of colony forming units of microscopic fungi

Counts of moulds and yeasts per 1 g feed were assessed in accordance with the analytical procedure using the standard plate method from decimal dilutions [PN-ISO 21527-2: 2009. Microbiology of food and feeds. Horizontal method to determine counts of yeasts and moulds. Part 2: The method to determine the number of colonies in products with water activity of max. 0.95]. First 10 g of comminuted material for analyses from each sample were suspended in 90 ml of 0.1% peptone water. After 30 min samples were shaken for 2.5 min. Next from the prepared suspension decimal dilutions were prepared in 0.1% peptone water solution. The amount of 1 ml suspension was transferred with a sterile pipette from three prepared dilutions onto sterile Petri dishes (two for each dilution). In the next stage plates were flooded with 15 ml agar medium (DRBC with BTL chloramphenicol) at a temperature of 45°C. Plates were incubated under aerobic conditions, placed flat in a heater at a temperature of 25 $\pm$1°C for 5–7 days. After incubation colonies were counted on selected plates (making it possible to obtain from 15 to 150 colonies per plate) and based on the count of colonies the number of colony forming units of moulds and yeasts was determined in 1 g analysed material (CFU $\cdot$ g^{-1}). The final result was a mean and was expressed in log CFU $\cdot$ g^{-1}. The quality assessment of moulds was conducted using samples prepared previously to determine the count of colony forming units of fungi and yeasts per 1 g feed. For this purpose after the period of incubation grown fungal colonies were observed and the affiliation of microscopic fungi to a given genus was determined on the basis of morphological characteristics of growth the structure of the reproductive organs. Observations of microscopic fungi were made using in vivo

specimens prepared in the wet mount technique. Before the specimen preparation microscopic slides were defatted by passing them several times over the flame of a gas burner. Next a drop of water was placed on the microscopic slide and a small amount of material collected with a needle from the analysed colony was transferred onto the slide. After the preparation was covered with a cover glass mycelium was observed under an optic microscope at a x400 magnification in order to classify fungi to respective genera on the basis of their reproductive organs and spore shape [Trojanowska 2009a].

Analysis of trichothecenes [according to Perkowski et al. 2007]

Sub-samples (10 g) were extracted with acetonitrile/water (82:18) and purified on a charcoal column (Celite 545/charcoal Draco G/60/activated alumina neutral 4:3:4 (w/w/w). The trichothecenes of group A (H-2 toxin, T-2 toxin, T-2 tetraol) were analysed as TFAA derivatives. The amount of 100 µl of trifluoroacetic acid anhydride was added to the dried sample. After 20 min. the reacting substance was evaporated to dryness under nitrogen. The residue was dissolved in 500 µl of isooctane and 1 µl was injected onto a gas chromatograph-mass spectrometer. The trichothecenes of group B (DON, NIV, 3-AcDON, 15-AcDON) were analysed as TMS (trimethylsilylsilyl ethers) derivatives. The amount of 100 µl of TMSI/TMCS (trimethylsilyl imidazole/trimethylchlorosilane; v/v 100/1) mixture was added to the dried extract. After 10 min. 500 µl of isooctane were added and the reaction was quenched with 1 ml of water. The isooctane layer was used for the analysis and 1 µl of the sample was injected on a GC/MS system. The analyses were run on a gas chromatograph (Hewlett Packard GC 6890) hyphenated to a mass spectrometer (Hewlett Packard 5972 A, Waldbronn, Germany), using an HP-5MS, 0.25 mm $\times$ 30 m capillary column. The injection port temperature was 280°C, the transfer line temperature was 280°C and the analyses were performed with programmed temperature, separately for group A and B trichothecenes. The group A trichotheceneses were analysed using the following programmed temperatures: initial 80°C held for 1 min., from 80°C to 280°C at 10°C $\cdot$ min^{-1}, the final temperature being maintained for 4 min. For the group B trichothecenes initial temperature of 80°C was held for 1 min., from 80°C to 200°C at 15°C $\cdot$ min^{-1}. held for 6 min and from 200°C to 280°C at 10°C $\cdot$ min^{-1}, with the final temperature being maintained for 3 min. The helium flow rate was held constant at 0.7 ml $\cdot$ min^{-1}. Quantitative analysis was performed in the single ion monitored mode (SIM) using the following ions for the detection of STO: 456 and 555; T-2 tetraol 455 and 568; T-2 triol 455 and 569 and 374; HT-2 455 and 327; T-2 327 and 401. DON: 103 and 512; 3-AcDON: 117 and 482; 15-AcDON: 193 and 482; NIV: 191 and 600. Qualitative analysis was performed in the SCAN mode (100–700

amu). Recovery rates for the analyzed toxins were as follows: STO 82 ±5.3%; T-2 triol 79 ±5.1%;T-2 86 ±3.8%; T-2 tetraol 88 ±4.0%; HT-2 91 ±3.3%; DON 84 ±3.8%; 3AcDON 78 ±4.8%; 15 AcDON 74 ±2.2%; and NIV 81 ±3.8%. The limit of detection was 0.01 mg · kg^{-1}.

Analysis of ergosterol [according to Perkowski et al. 2008, Stuper et al. 2010]

Samples of 100 mg were placed into 17-ml culture tubes, suspended in 1 mL of methanol, treated with 0.1 mL of 2 M aqueous NaOH and sealed tightly. Then, the culture tubes were placed in 250-ml plastic bottles, sealed tightly and placed inside a microwave oven (model AVM 401/WH, Whirpool Warsaw Poland) operating at 2.450 MHz and 900 W maximum output. Samples were irradiated (370 W) for 20 s, after approximately 5 min for an additional 20 s and extracted with pentane (HPLC grade, Sigma-Aldrich, Steinheim, Germany: 3x4 ml) within the culture tubes. The combined pentane extracts were evaporated to dryness in a gentle stream of high-purity nitrogen. Before analysis samples were dissolved in 4 ml of methanol, filtered through 1-mm syringe filters with 0.5 μm pore diameter (Fluoropore membrane filters, Whatman POCH, Gliwice, Poland), evaporated to dryness with a nitrogen stream and dissolved in 1 ml of methanol. Prepared samples were analysed by HPLC. Separation was run on a 150 × 3.9 mm (length × diameter) Nova Pak C-18 4-μm particle size column (Waters, Milford, MA) and eluted with methanol-acetonitrile (90:10) at a flow rate of 0.6 ml · min^{-1}. Ergosterol was detected with a Waters 486 Tunable Absorbance Detector (Waters) set at 282 nm. Estimation of ERG was performed by a comparison of peak areas with those of an external standard (>95%, Sigma-Aldrich, Milwaukee, WI) or by co-injection with a standard. Detection level was 0.01 mg · kg^{-1}.

Statistical analysis

Statistical analyses were performed using Statistica 9.0 by StatSoft. The effect of the producer and the type of feed on the mean total concentration of trichothecenes and the count of fungi was assessed using a one-way analysis of variance ANOVA. The significance of Pearson's linear correlation coefficients was evaluated at the significance level $P \leq 0.05$ for dependencies found between the analysed parameters.

RESULTS

Microscopic fungi are microbiological hazards, which on the power of the Ordinance of the European Union should be controlled both in feed raw materials and final feed mixes. In this study the level of contamination with fungal microflora was determined in feed mixes for groups of broiler chickens differing in age (chicken broilers – starting, chicken broilers – growing, chicken broilers – finishing) produced in western Poland in the years 2009–2010. Quantitative evaluations of moulds were conducted using two methods: the classical microbiological method providing the level of contamination in feed mixes expressed in terms of CFU (colony forming units of microscopic fungi, Fig. 1), and the chemical method determining the concentration of a specific fungal marker such as ergosterol (ERG, Fig. 2). Recorded results are characterized by a wide range from a sample and a considerable standard deviation, which indicates a significant variation in terms of the contents of microscopic fungi in the analysed samples of feeds de-

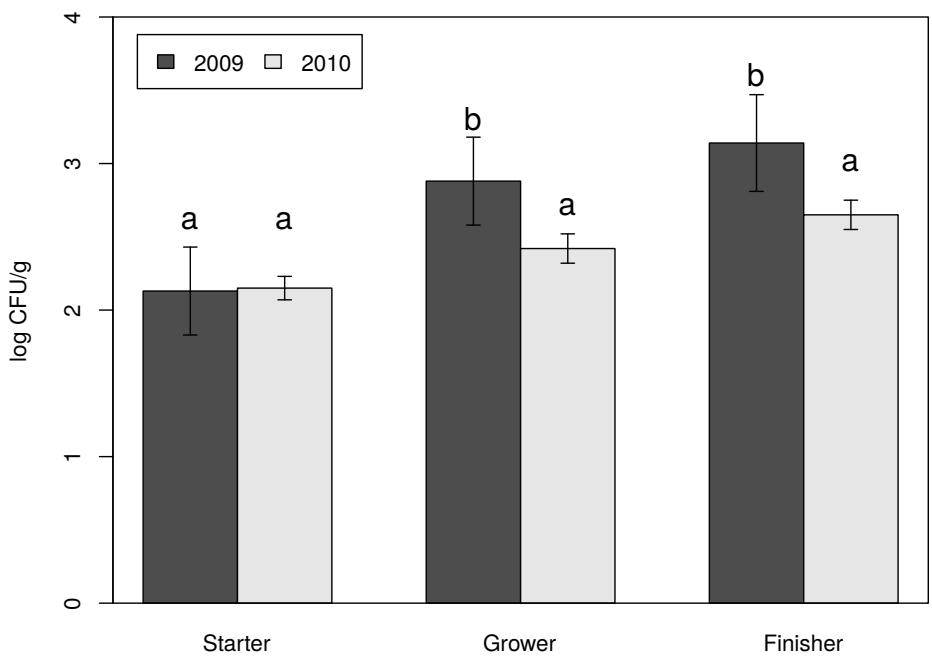

Fig. 1. Counts of microscopic fungi in samples of feeds collected in the years 2009–2010 in western Poland; a, b, c – identical letters denote a lack of significant differences at significance level P = 0.01

Rys. 1. Ilość grzybów mikroskopowych w próbach pasz zebranych w 2009–2010 roku na terenie zachodniej Polski; a, b, c – takie same litery oznaczają brak istotnych różnic na poziomie ufności P = 0,01

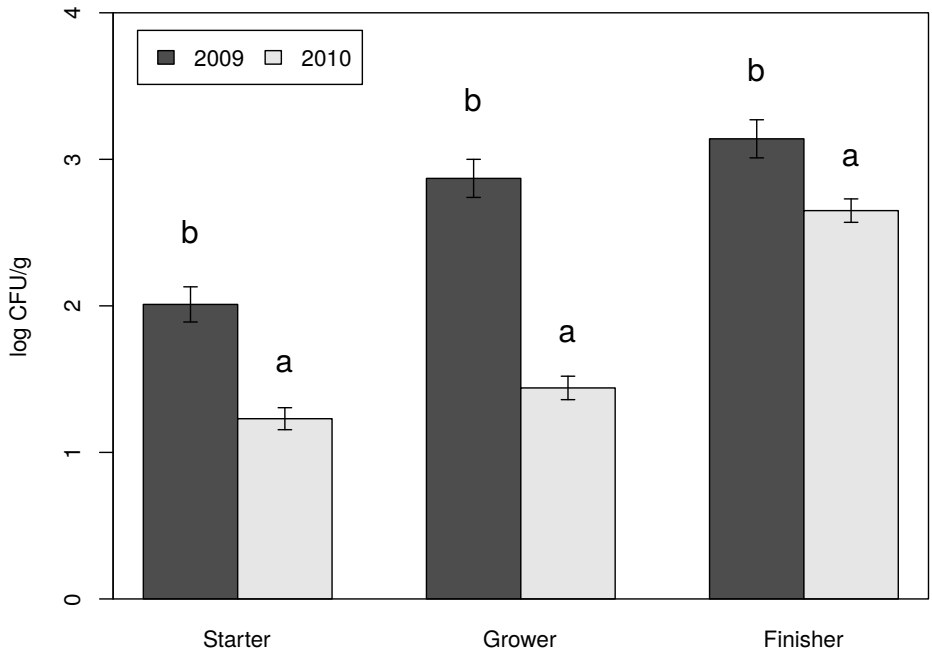

Fig. 2. Mean concentrations of ergosterol in feeds collected in the years 2009–2010 in western Poland; a, b, c – identical letters denote a lack of significant differences at significance level P = 0.01

Rys. 2. Średnie stężenie ergosterolu w próbach pasz zebranych w 2009–2010 roku na terenie zachodniej Polski; a, b, c – takie same litery oznaczają brak istotnych różnic na poziomie ufności P = 0,01

pending on the type of feed. A higher mean CFU count was recorded in samples of feeds for older grower and finisher chickens (2.7 log CFU $\cdot$ g^{-1} and 2.9 log CFU $\cdot$ g^{-1}, respectively) than in samples of starter mixes (2.1 log CFU $\cdot$ g^{-1}). A marked increase in the counts of moulds in the feed for older poultry probably results from its richer composition in comparison to feed mixes for younger chickens. When considering the total contamination with microscopic fungi in feed mixes in individual years it was found that in 2009 it was higher than in 2010, while significant differences between years were observed in samples of grower and finisher feeds (Fig. 1). Moreover, the significance of the effect of feed type and the producer on the counts of moulds and yeasts was also determined. The one-way analysis of variance ANOVA was applied to assess the power of these dependencies.

In the analysis of variance between the type of the producer and CFU no significant dependence was found, which was confirmed by Tukey's HSD test,

showing no differences between individual groups of mean log CFU · g^{-1} for each of the producers.

Apart from the quantitative determination of the number of moulds in analysed feed samples the concentration of ergosterol was also determined. Presented results show its mean concentration in tested feeds for broiler chickens to be 19.34 mg · kg^{-1}. Results are characterised by a large standard deviation and a considerable range from a sample (Fig. 2). Similarly as in the case of counts of moulds and yeasts, a trend was observed for an increase in ergosterol content in feeds with a richer composition, i.e. for older poultry. When comparing ERG concentration within the investigated years in all the tested samples, similarly as in the case of CFU, a higher ERG content was observed in 2009 in comparison to 2010. The conducted statistical analysis showed significant differences in ERG concentration between samples collected in 2009 and those collected in 2010. This pertained to individual groups of samples depending on the type of the feed mix.

The composition of mycoflora in the years of the study was identical and the percentages of individual genera of microscopic fungi in the years 2009–2010 did not differ significantly (Fig. 3). In contrast, considerable differences were found in quality and in terms of the percentage proportions of individual genera of fungi depending on the type of the feed mix (Figs. 3 A, B, C). Figure 3 D presents a joint share of individual filamentous fungi in all analysed samples. Storage fungi such as *Aspergillus*, *Rhizopus* and *Mucor* were detected most often.

Among the analysed mycotoxins the highest frequency was recorded for deoxynivalenol (DON), diacetoxyscripenol diacyloscirpentriolu (DAS) and scirpentriol (STO) (Table 1). In contrast, the T-2 toxin was not detected in the samples. DON was found in all analysed feed samples, with its mean content of 33.28 µg/kg. In the case of DAS, 3-AcDON and Fus-X their mean concentrations were significantly lower and amounted to 4.09 µg · kg^{-1}, 3.94 µg · kg^{-1} and 1.96 µg · kg^{-1}. All the recorded results were characterized by a large range from sample and the admissible DON contamination of 5 mg · kg^{-1} was not exceeded in any of the analysed feed samples (Ordinance of the Commission (EC) no. 1126/2007; Ordinance of the Commission (EC) 1881/2006). In each feed type the proportion of group B trichothecenes was almost five times higher than that of group A trichothecenes in their total content (Fig. 4). The highest mean mycotoxin contamination was found in finisher feeds. The level of contamination in starter and grower feed mixes was similar in most cases. As far as group A trichothecenes are concerned, in the years 2009–2010 their level was similar and mean concentrations did not differ significantly. However, when analysing the concentrations of these toxins depending on the type of feed mix in 2009 we may state that starter feeds contained significantly less group A trichothecenes than the other types of analysed feeds. In contrast, in 2010 no significant differences were observed. In

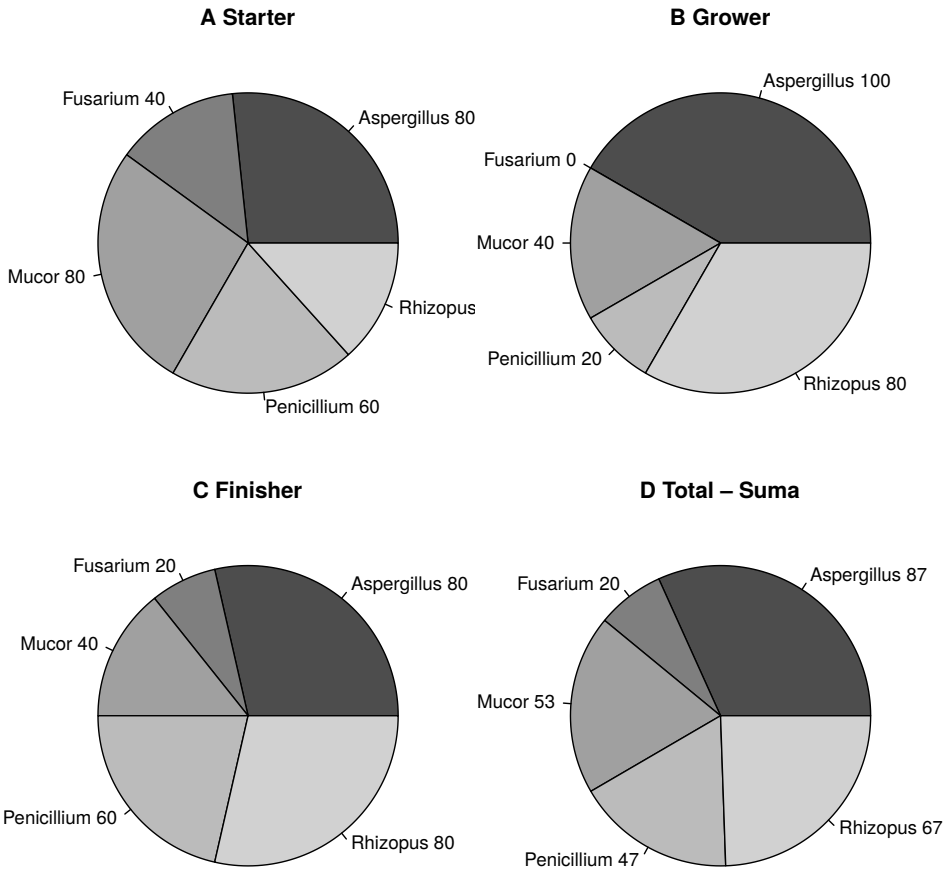

Fig. 3. Percentages of identified genera of microscopic fungi in individual types of feed mixes (A, B, C) and total percentages of genera of moulds in all feed samples (D)

Rys. 3. Procentowy udział zidentyfikowanych rodzajów grzybów mikroskopowych w ogólnej populacji grzybów mikroskopowych w próbach poszczególnych mieszanek paszowych (A, B, C) oraz we wszystkich sumarycznie rozpatrywanych próbach pasz (D)

the case of group B trichothecenes no significant differences were found in mean concentrations between years. In turn, in 2009 significant variation was recorded in terms of the total content of these toxins depending on the feed type. In 2010 significantly higher concentrations of total group B trichothecenes were found in the finisher feed mix. When analysing the sum of all mycotoxins in the group of trichothecenes an identical trend was observed as in the case of group B trichothe-

cenes. No significant effect of the type of producer or the type of feed was found on the trichothecene content (ANOVA).

DISCUSSION

Recorded counts of microscopic fungi were referred to the data supplied by Grajewski et al. [2009] in the years 2006–2008 in Poland. They gave values of log CFU per 1 g for all feeds jointly. Counts of microscopic fungi for feeds from 2010 were much lower in relation to the studies conducted in previous years, in which – starting from 2006 – they were 4.08 log CFU $\cdot$ g^{-1}, 3.82 log CFU $\cdot$ g^{-1} and 4.00 log CFU $\cdot$ g^{-1} [Dalcero et al. 1998, Basalan et al. 2004]. In the investigations conducted in the period 2003–2006, concerning the assessment of fungal counts in feed mixes for poultry, values of more than 105 cfu $\cdot$ g^{-1} were recorded in 0.7 to 4% samples [Kwiatek et al. 2008]. In a study by Kubizna et al. [2011] it was stated that the count of fungi in samples of feed mixes for poultry originating from south-western Poland fell within the range of 102–104 cfu $\cdot$ g^{-1}. Similar results concerning feed mixes for poultry were reported by Labuda et al. [2003], who stated that the count of fungi from the genus *Fusarium* fell within the range of 102–104 cfu $\cdot$ g^{-1}.

The analysis of correlations between counts of microscopic fungi and ergosterol did not show a statistically significant dependence at P = 0.05 (the correlation coefficient was 0.54). When analysing ERG contents in feed mixes it needs to be stated that the authors gave only the content of this metabolite in grain of fodder cereals [Perkowski et al. 2008].

Maize is the cereal most commonly added to feed mixes. The concentration of ERG was analysed in maize grain in 1998 by Müller and Lehn, who stated that it ranged from 0.48 to 0.81 mg $\cdot$ kg^{-1}. Earlier Seitz et al. [1979] determined ERG concentration at 0.15–200 mg $\cdot$ kg^{-1}. The latest reports on the contamination with microscopic fungi in maize grain indicate that the ERG level changes rapidly over the years, which is caused by sensitivity of maize to weather conditions due to the specific ear structure. In relation with the above, results reported by Macri et al. [2003] seem justified, as they compared ERG concentration in samples of maize grain in the years 2001 and 2002 and found a significant difference in the concentration of this metabolite, with the concentration of ERG in 2001 at 0.2–72 mg $\cdot$ kg^{-1}, with a mean of 6.4 mg $\cdot$ kg^{-1}, while in 2002 it was 0.2–9.7 mg $\cdot$ kg^{-1}, with a mean of 2.1 mg $\cdot$ kg^{-1}.

The analysis of correlations between selected characteristics showed a significant correlation between DON/ERG and the sum of mean concentrations of trichothecenes/ERG, as it was previously indicated by Wiśniewska and Buśko [2005] and Stuper et al. [2010]. In view of the above it may be inferred that the

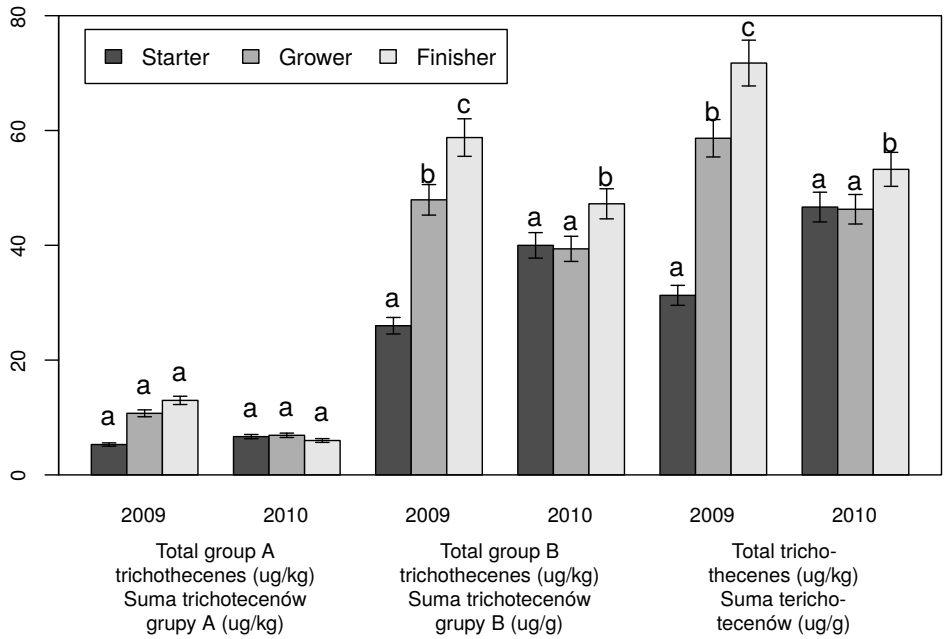

Fig. 4. Mean concentrations of group A and B trichothecenes and the sum of mean concentrations of trichothecenes in samples of feeds collected in the years 2009–2010 in western Poland; a, b, c – identical letters denote a lack of significant differences at significance level P = 0.01

Rys. 4. Średnie stężenie sumy trichotecenów grupy A, sumy trichotecenów grupy B oraz sumy trichotecenów w próbach pasz zebranych w 2009–2010 roku na terenie zachodniej Polski; a, b, c – takie same litery oznaczają brak istotnych różnic na poziomie ufności P = 0,01

concentration of mycotoxins is significantly correlated with the level of not only live, but also dead mycoflora, as indicated by ERG concentration. Recorded contents of mycotoxins from the group of trichothecenes were next referred to the results reported by Grajewski et al. [2009] in the years 2006–2008 in Poland for contents of DON, the T-2 and HT-2 toxins in µg/kg feed mixes. The mean DON content in feeds determined in 2010 was much lower in comparison to the studies conducted in 2006, amounting to 167 $\mu g \cdot kg^{-1}$; in 2007 to 230 $\mu g \cdot kg^{-1}$ and in 2008 to 160 $\mu g \cdot kg^{-1}$, respectively. In relation to HT-2 its lower content was also observed in feeds from 2010.

When comparing recorded data with literature reports on the contamination of fodder cereals and feeds in Europe we may observe a varied level of contamination both with microscopic fungi and mycotoxins [Binder et al. 2007] depending on the year of analysis, while e.g. in feeds the mean content recorded in central Europe

Table 1. Mean concentrations of identified trichothecenes in analysed samples of feeds collected in the years 2009–2010 in western Poland

Tabela 1. Średnie stężenie zidentyfikowanych trichoitecenów w próbach pasz zebranych w 2009–2010 roku na terenie zachodniej Polski

Mycotoxin Mikotoksyna	Statistics* Parametry statystyczne*	Type of feed – Rodzaj paszy					
		Starter		Grower		Finisher	
		2009	2010	2009	2010	2009	2010
Group A Trichothecenes – Trichoteceny grupy A							
STO	Min – Max	0.00–2.47	0.00–2.79	0.00–6.42	0.00–3.28	0.00–2.79	0.00–4.75
	$\bar{x}$	2.01	1.95	2.77	1.62	1.20	0.94
	%	75	81	76	73	66	86
T-2 Tetraol	Min – Max	0.00–1.44	0.00–2.56	0.00–1.31	0.00–2.18	0.00–4.09	0.00–2.11
	$\bar{x}$	0.24	1.66	0.45	0.61	1.32	0.68
	%	30	34	27	29	29	20
T-2 Triol	Min – Max	0.00–2.10	0.00–1.23	0.00–2.76	0.00–1.42	0.00–1.94	0.00–2.00
	$\bar{x}$	0.71	1.19	0.59	0.79	0.98	0.15
	%	22	28	33	24	11	8
DAS	Min – Max	0.00–2.17	0.00–2.00	0.00–13.48	0.00–9.27	0.00–18.29	0.00–10.80
	$\bar{x}$	1.36	1.25	6.67	3.40	7.45	4.42
	%	86	95	79	89	93	81
HT-2	Min – Max	0.00–1.63	0.00–0.89	0.00–1.22	0.00–3.94	0.00–5.05	0.00–1.97
	$\bar{x}$	0.97	0.62	0.34	0.55	2.03	0.85
	%	36	41	46	55	40	0
Group B Trichothecenes – Trichoteceny grupy B							
DON	Min – Max	2.78–30.15	1.00–39.81	2.90–48.92	1.72–49.02	3.75–67.98	1.86–50.18
	$\bar{x}$	19.62	33.28	38.41	31.17	43.15	34.06
	%	100	100	100	100	100	100
Fus-X	Min – Max	0.00–3.18	0.00–5.09	0.00–5.49	0.00–7.38	0.00–14.37	0.00 –9.53
	$\bar{x}$	2.34	3.02	1.05	1.72	8.78	6.77
	%	22	37	35	46	20	31
3-AcDON	Min – Max	0.00–2.66	0.00–4.72	0.00–2.37	0.00–5.20	0.00–6.81	0.00–6.74
	$\bar{x}$	1.72	1.57	0.92	1.13	3.95	2.47
	%	35	42	52	40	43	31
15-AcDON	Min – Max	0.00–1.27	0.00–2.31	0.00–3.11	0.00–3.43	0.00–2.44	0.00–4.77
	$\bar{x}$	0.51	0.95	1.27	0.38	0.84	1.72
	%	47	34	50	26	10	19
NIV	Min – Max	0.00–3.49	0.00–2.10	0.00–12.08	0.00–14.21	0.00–5.12	0.00–7.03
	$\bar{x}$	1.62	1.24	6.23	5.29	2.05	2.21
	%	23	36	39	52	31	24

*Min–Max – range, $\mu g \cdot kg^{-1}$; $\bar{x}$ – mean, $\mu g \cdot kg^{-1}$; % – percentage of positive samples, %.
*Min–Max– zakres, $\mu g \cdot kg^{-1}$; $\bar{x}$ – średnia, $\mu g \cdot kg^{-1}$; % – procent pozytywnych prób, %.

in previous years for DON, HT-2 and T-2 amounted to 168 $\mu g \cdot kg^{-1}$, 20 $\mu g \cdot kg^{-1}$ and 51 $\mu g \cdot kg^{-1}$, respectively [Slikova et al. 2008].

Overall in Europe in 2009 seven cases of exceeded admissible DON concentration were detected in fodder cereals and feeds (Report RASSF 2009), while in 2010 four such cases were recorded (Report RASSF 2010). On this basis it may be stated that the level of contamination in cereals and their milling products, in-

cluding feeds, is low in Poland when compared to Europe and the reported levels worldwide.

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REFERENCES

Basalan, M., Hismiogullari, S.E., Hismiogullari, A.A., Filazi, A. (2004). Fungi and aflatoxin B1 in horse and dog feeds in Western Turkey. Rev. Méd. Vétér., 5, 248–252.

Binder, E., Tan, L., Chin, L., Handl, J., Richard, J. (2007). Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. Anim. Feed Sci. Tech., 137, 265–282.

Dalcero, A., Magnoli, C., Luna, M., Ancasi, G., Reynoso, M. M., Chiacchiera, S., Miazzo, R., Palacio, G. (1998). Mycoflora and naturally occurring mycotoxins in poultry feeds in Argentina. Mycopathologia, 141, 37–43.

Grajewski, J., Błajet-Kosicka, A., Twarużek, M., Kosicki, R., Miklaszewska, B. (2009). Wyniki wieloletnich badań mikotoksyn w produktach rolno-spożywczych z uwzględnieniem pasz [Results of long-term studies on mycotoxins in agricultural produce including feeds]. Pasze Przem., 7/8/9, 34–39 [in Polish].

Kubizna, J., Jamroz, D., Kubizna, J.K. (2011). Contamination of feed mixtures with mycoflora in South-Western Poland. Electron. J. Pol. Agric. Univ., Veterinary Med. 14, 2–8.

Kwiatek, K., Kukier, E. (2009). Criteria of microbiological quality of feeds. Pasze Przem., 7/8/9, 58–63.

Kwiatek, K., Kukier, E., Wasyl, D., Hoszowski, A. (2008). Jakość mikrobiologiczna mieszanek paszowych w Polsce [The microbiological quality of compound feedstuffs in Poland]. Med. Weter., 64(7), 949–954 [in Polish].

Labuda, R., Tancinova, D., Hudec, K. (2003). Identification and enumeration of *Fusarium* species in poultry feed mixtures from Slovakia. Ann. Agric. Environ. Med., 10, 61–66.

Macri, A., Schollenberger, M., Dancea, Z., Drochner, W. (2003). Zearalenone and ergosterol contents in corn samples of Transsylvania. Mycotoxin Res., 19, 190–193.

Mazanowski, A. (2009). Mikotoksyny w paszach dla drobiu [Mycotoxins in poultry feeds]. Hod. Drobiu, 138 (2), 3-9

Meister, U. (2009). Fusarium toxins in cereals of integrated and organic cultivation from the Federal State of Brandenburg (Germany) harvested in the years 2000-2007. Mycotoxin Res., 25, 133-139.

Mello, J., Placinta, C., Macdonald, A. (1999). *Fusarium* mycotoxins: a review of global implications for animal health, welfare and productivity. Anim. Feed Sci. Tech., 80, 183–205.

Oxley-Goody, D. (2009). Kropidlak na fermie brojlerów [Aspergillus on the broiler farm]. Hod. Drobiu, 143(8), 26–27 [in Polish].

Perkowski, J., Buśko, M., Stuper, K., Kostecki, M., Matysiak, A., Szwajkowska-Michałek, L. (2008). Concentration of ergosterol in small-grained naturally contaminated and inoculated cereals. Biology, 63, 542–547.

Pestka, J. (2007). Deoxynivalenol: Toxicity, mechanism and animal health risk. Anim. Feed Sci. Tech., 137, 283–298.

Polish Standard PN-ISO 21527:2009. Microbiology of food and feed. Horizontal method to determine yeast and mould counts. Part 2: Colony counting method for products with water activity of max. 0.95.

Seitz, L.M., Mohr, H.E., Burroughs, R., Sauer, D.B. (1979). Ergosterol as an indicator of fungal invasion in grains. Cereal Chem., 54, 1207–1217.

Shlosberg, A., Hanji, V. (2000). Natural mycotoxicoses in broilers and broiler breeders in Izrael induced by T-2 toxin and diacyloscirpenol – a diagnostic challenge. Oryginal work presented at IUPAC Conference on Mycotoxins in Brazil. Proceedings, 76–79.

Slikova, S., Sudyova, V., Gregova, E. (2008). Deoxynivalenol in wheat from growing areas of Europe. Cereal Res. Comm., 36, 279–287.

Stuper, K., Buśko, M., Matysiak, A., Perkowski, J. (2010). Zanieczyszczenie zbóż grzybami mikroskopowymi oraz ich metabolitami zebranych na terenie Wielkopolski [Contamination with microscopic fungi and their metabolites in crereals harvested in the Wielkopolska region Poland]. ABiD, 4, 89–96 [in Polish].

Trojanowska, K. (2009). Moulds. [Pleśnie]. W: Mikrobiologia żywności. Red.: Trojanowska K., Giebel H., Gołębiewska B., Wyd. Uniwersytet Przyrodniczy Poznań, 74-92.

Wieliczko, A., Woźniak, A. (2006). Aspergiloza drobiu [Aspergillosis in poultry]. Hod. Drobiu, 6/7, 42–44 [in Polish].

Wiśniewska, H., Buśko, M. (2005). Evaluation of spring wheat resistance to *Fusarium* seedling blight and wheat blight. Biology, 60, 287–293.

Wiśniewska-Dmytrow, H., Kozak, A., Żmudzki, J. (2004). Occurrence of *Fusarium* mycotoxins in feedstuffs from farms with husbandry problems. Bull. Veter. Ins. Pulawy, 48, 117–122.

ZANIECZYSZCZENIE GRZYBAMI MIKROSKOPOWYMI ORAZ ICH METABOLITAMI PRZEZNACZONYCH DLA KURCZĄT PASZ PRODUKOWANYCH NA TERENIE ZACHODNIEJ POLSKI W LATACH 2009–2010

Streszczenie. Celem niniejszych badań była ocena poziomu zanieczyszczenia mieszanek paszowych przeznaczonych dla kurcząt brojlerów produkowanych w czterech przemysłowych mieszalniach pasz na terenie zachodniej Polski w latach 2009–2010. Przeprowadzono ocenę jakościową i ilościową poziomu zanieczyszczenia pasz grzybami mikroskopowymi oraz mikotoksynami z grupy trichothecenów. Stwierdzono, iż pasze typu starter były istotnie mniej zanieczyszczone grzybami mikroskopowymi oraz mikotoksynami niż mieszanki przeznaczone dla starszych kurcząt. W roku 2009 zaobserwowano wyższe skażenie pasz oznaczanymi mikroorganizmami oraz ich toksycznymi metabolitami, niż w roku 2010. Pod względem jakościowym w analizowanych próbach pasz najczęściej identyfikowanymi grzybami mikroskopowymi były pleśnie z rodzaju: *Aspergillus*, *Rhizopus* i *Mucor*. Wśród wykrytych mikotoksyn najczęściej występującymi były: DON, DAS, 3-AcDON oraz Fus-X. W żadnej z analizowanych prób pasz nie stwierdzono przekroczenia zalecanego dopuszczalnego przez UE poziomu skażenia DON.

Słowa kluczowe: pasza, ergosterol, trichoteceny, grzyby mikroskopowe, JTK

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