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ORIGINAL ARTICLE

Microflora and mycotoxin contamination in poultry feed mixtures from western Poland

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Abstract

Objective: Contamination of feeds with pathogenic microflora and mycotoxins constitutes a serious threat both for animals and humans. The aim of the study was to determine the degree of risk of the occurrence of microscopic fungi, selected bacteria and mycotoxins from the trichothecene group in poultry feeds in western Poland.

Results: In feed mixtures, the concentration of ergosterol (ERG), being a specific quantitative biomarker for the content of microscopic fungi, was determined. Grower and finisher feeds were characterized by a higher count of bacteria and fungi in comparison to starter feeds. A considerable variation was found in the amount of ergosterol in analyzed feeds. Mean ergosterol content in feeds amounted to 19.34 mg/kg. The most common genera of fungi detected in the tested feeds included *Aspergillus, Rhizopus* and *Mucor*. Irrespective of the type of feed, the proportion of trichothecenes group B was five times higher than that of trichothecenes group A in relation to the total content of these mycotoxins in samples. In terms of the analyzed mycotoxins, feeds contained the highest concentration of deoxynivalenol (DON). A statistically significant correlation was shown between DON and ERG and between total trichothecenes and ERG.

Conclusion: Recorded results indicate that the level of microbiological contamination in feeds for broiler chickens produced in western Poland is within the requirements of the binding standards.

Key words

trichothecenes, ergosterol, bacteria, fungi, poultry feed

INTRODUCTION

Poultry feeds account for 70% of total commercial feed production in Poland. The scale of feed production, as well as the possible occurrence of pathogenic bacteria, microscopic fungi and mycotoxins in the general chain of nutrition, represent the most important potential risk to animal and human health. The main sources of fungal microflora in feeds originate from feed materials of plant origin, primarily cereals [1, 2]. Moulds developing on the surface of kernels under field and storage conditions may cause nutrient losses, organoleptic changes, potential formation of mycotoxins and, as a consequence, result in deteriorated feed quality and the incidence of diseases in poultry [3]. In relation to humans and animals, mycotoxins exhibit toxic action and are characterized by carcinogenic, mutagenic, teratogenic and estrogenic properties [4-8]. Due to the diversity of toxic effects, as well as their resistance to the action of high temperature, the presence of mycotoxins in feeds constitutes a potential threat to human and animal health [9, 10]. In Poland, because of the temperate climate, the most commonly found mycotoxins are those from the trichothecene group, mainly deoxynivalenol, zearalenone and toxins T-2 and HT-2, produced by fungi from the genus *Fusarium* [11, 12, 13]. There is limited information concerning microbiological contamination and the occurrence of fungi in feed mixtures for poultry produced in Poland. For the above-mentioned reasons, the aim of the presented study was to assess the degree of threat for the occurrence of selected groups of bacteria and fungi, as well as mycotoxins from the group of trichothecenes, in feed mixtures for broiler chickens produced in western Poland in 2010.

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MATERIAL AND METHODS

Material for analyses consisted of 45 samples of feed mixtures for broiler chickens collected from four different feed mills in western Poland in September and October 2010. Samples comprised feeds for different age groups of broiler chickens (starting chicken broilers, growing chicken broilers, finishing chicken broilers). The samples were analyzed in terms of counts of microscopic fungi, mesophilic aerobic bacteria, bacteria from the family *Enterobacteriaceae*, while the presence of anaerobic sporulating rods from the genus *Clostridium* was also assessed. Moreover, the concentration of ergosterol (ERG) was also determined in feed mixtures, as a specific quantitative biomarker of contents of microscopic fungi in the tested material [14], as well as that of mycotoxins from the trichothecene group.

Microbiological analyses. For the determination of total bacterial counts (TBC), samples of 20 g were collected. They were ground using a WŻ-1 laboratory mill of Polish manufacture. A single sample for analyses was 10 g in weight. Microbiological analyses included total counts of aerobic bacteria, bacteria from the family *Enterobacteriacea*, titres of *Clostridium* and counts of fungi. The microbiological

analyses were carried by ISO reference methods (Polish Standard: PN-ISO). The total bacterial count was determined on Standard Plate Count Agar (CM 463, Oxoid, England). Incubation was run at 30 ± 1 °C for 72 h. For members of the family *Enterobacteriaceae,* 1 mL sample was inoculated into 15 mL of molten selective VRBG medium (P-0256, BTL, Poland). After setting, a 10 mL overlay of molten medium was added and incubation carried out at 37 °C for 24-48 h. In the determination of anaerobic sporulating rods, the Wrzosek medium with liquid paraffin was used (P-0192, D-037, supplemented with D-086, BTL, Poland). Anaerobic conditions were kept during incubation. Samples were incubated at 37 °C ± 1 °C for 48 h. Amounts of total mesophilic fungal microflora in feed were determined by the plate flooding method, according to Koch, using RBC Agar medium with chloramphenicol- (P-0117, BTL, Poland). Samples were incubated at 25 ± 1° for 5-7 days. Results were expressed in CFU/g feed. Qualitative identification of fungal genus was determined according to the manuals by Arx [15], Domsch *et al.* [16] and Nelson *et al.* [17].

Analysis of trichothecenes [14]. Briefly, determination of trichothecene amounts consisted in their extraction from the tested material using a acetonitryle–water mixture at 82:18 (v/v). Extracts were purified by extraction to the solid phase using columns filled with (5 mL) mixture of active carbon (Draco G 60, 100 mesh), celite (Celite 545) and neutral aluminum oxide (70-230 mesh), mixed at a weight ration of 1:1:1. Trichothecenes B (deoxynivalenol – DON, 3-acetyldeoxynivalenol – 3-AcDON, 15-acetyldeoxynivalenol – 15-AcDON, nivalenol –b NIV, fusarenon X – FUS-X) were analyzed as trimethylsilol derivatives using an external standard. Chromatographic separation and analyses of trichothecenes A and B were conducted using a gas chromatograph (Hewlett Packard 6890) coupled with a mass detector (Hewlett Packard 5972 A). For determinations of trichothecenes B, analyses were performed on selected ions (SIM): for DON ions 103 and 512; 3-AcDON 117 and 482; 15-AcDON 193 and 482; FUS 103 and 570; NIV 191 and 600. To confirm the presence of determined toxins in the samples, analyses were performed over an entire range of masses (100-700 amu) providing a mass spectrum, which was compared with an analogously obtained spectrum for the standard. Apart from quality analysis, concentrations of examined toxins were also determined. Results were processed by the ChemStation programme. In the applied methodology, the recovery of analyzed toxins was as follows: T-2 86 ± 3.8%, T-2 tetraol 88 ± 4.0%, HT-2 91 ± 3.3%, DAS 84 ± 4.6%, DON 84 ± 3.8%, 3AcDON 78 ± 4.8%, 15 AcDON 74 ± 2.2% and NIV 81 ± 3.8%, at a detection limit of 0.001 mg/kg.

Analysis of ergosterol [14, 18]. Briefly, samples were analyzed for the presence of ERG according to [14]. 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. The culture tubes were then 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: 3 × 4 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 by a nitrogen stream and dissolved in 1 mL of methanol. Prepared samples were analyzed 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. 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.

Statistical analysis. Statistical analyses were performed using Statistica 9.0 by StatSoft. One-way analysis of variance (ANOVA) was applied to evaluate the effect of the producer and feed type on mean total concentration of trichothecenes, counts of bacteria and fungi. The significance of Pearson's linear correlation coefficient was verified at the significance level $\alpha \leq 0.05$ for dependencies found between analyzed indexes.

RESULTS

The level of microbiological contamination in feeds for broiler chickens is presented in Table 1. A significant diversification was found in the analyzed feeds in terms of their contents of microscopic fungi between individual groups of tested feeds. Within one type, the results did not differ statistically significantly. The mean amount of moulds and yeasts in analyzed feeds was 7.0×10^2 CFU/g. In the one-way analysis of variance, no effect of the producer or of the type of the analyzed feed was found for the amount of microscopic fungi. A statistically significant difference was recorded between the mean number of microscopic fungi in starter and finisher feeds. The mean count of microscopic fungi in starter and finisher feeds was 1.8×10^2 CFU/g and 1.6×10^3 CFU/g, respectively.

Apart from the determination of contents of microscopic fungi, also the content of ergosterol, as a specific biomarker for the presence of dead and live mycoflora, was determined. Concentration of ergosterol provides a more comprehensive picture of the level of contamination with microscopic fungi and it may supplement traditional determinations of fungi [14, 18]. Mean ergosterol content in feeds amounted to 19.35 mg/kg. Recorded results indicate a considerable diversification of the amount of ergosterol in analyzed feeds. An upward trend was observed for ergosterol content in feeds with a richer composition for older poultry. There is no information indicating what concentration of ERG found in feed pose a hazard for humans and animals. Maupetit *et al.* [19] proposed for healthy grain a range of ergosterol concentration from 1-9 mg/kg. Müller and Schwardorf [20] assumed the limit of 9 mg/kg as safe for grain for human consumption. Fungi from the genera *Aspergillus, Fusarium, Mucor, Penicillium* and *Rhizopus* were identified in feeds. The most frequently occurring genera were *Aspergillus* and *Rhizopus*, i.e. fungi developing intensively during grain

Table 1. Counts of bacteria and fungi as well as ergosterol content in feed mixtures for broiler chickens

Poultry feed	No of sample	Counts of bacteria and fungi CFU/g							
		Aerobic bacteria		*Enterobacteriaceae*		Fungal microflora		Ergosterol mg/kg	
		Range	Average	Range	Average	Range	Average	Range	Average
Starter	15	1.1×10^3-3.4×10^3	1.9×10^3	0-1.5×10^2	4.9×10^1	5.5×10^1-3.0×10^2	1.8×10^2	9.36-35.94	17.96±10.41
Grower	15	1.3×10^3-7.2×10^3	3.7×10^3	0-9.0×10^2	2.4×10^2	6.0×10^1-4.7×10^2	3.2×10^2	8.39-33.09	19.39±8.86
Finisher	15	2.4×10^3-3.8×10^3	3.2×10^3	0-6.8×10^2	2.9×10^2	8.5×10^1-7.0×10^3	1.6×10^3	1.19-53.69	20.69±21.39
Total	45	1.1×10^3-7.2×10^3	3.0×10^3	0-9.0×10^2	1.9×10^2	5.5×10^1-7.0×10^3	7.0×10^2	1.19-53.69	19.35±3.62

storage. A particularly high diversity of moulds was found in starter feeds.

Recorded values of total counts of aerobic bacteria for individual feed samples were varied. Mean TBC was 3.0×10^3 CFU/g. The admissible value of the total bacterial count for feeds, amounting to 3.0×10^6 CFU/g, was not exceeded in any of the tested samples [21]. On the basis of one-way analysis of variance no statistically significant effect of the type of producer or the type of feed was found on the total count of mesophilic bacteria in feeds. It was found that the highest TBC was found for finisher and grower feeds, while lower values were recorded for starter feeds.

Bacteria from the genus *Enterobacteriaceae* were detected in 60% tested feed samples. Contamination with *Enterobacteriaceae* in feeds varied and their mean count in samples amounted to 1.9×10^2 CFU/g. No statistically significant dependence was shown between TBC and the count of *Enterobacteriaceae*. A statistically significant dependence was found for the effect of the type of producer on the count of *Enterobacteriaceae*. In turn, no effect of the analyzed feed on the count of bacteria from the genus *Enterobacteriaceae* was observed. No presence of anaerobic sporulating rods from the genus *Clostridium* in 0.0001g feeds was shown, which is consistent with the hygienic recommendations concerning feed mixtures [21].

Among the analyzed mycotoxins, deoxynivalenol (DON), diacyloscirpentriol (DAS) and scirpentriol (STO) were the most frequently occurring metabolites (Tab. 2). DON and DAS were found in 100% tested samples. The presence of toxin T-2 was not detected in any of the samples. All feed samples were characterized by the highest contents of DON, the mean level of which was 33.58 µg/kg. Thist is a toxin produced mainly by *Fusarium graminearum* and *Fusarium culmorum*, being major field cereal pathogens characteristic of Europe [12, 22, 23]. The admissible level of deoxynivalenol contamination, recommended by the EU (5mg/kg), was not exceeded in any of the tested feed samples [24]. The highest concentration of this toxin recorded in the tested feeds was 99.36 µg/kg. In turn, high proportions of mycotoxins in feeds were found for DAS (4.25 µg/kg), 15-AcDON (3.94 µg/kg) and Fus-X (1.95 µg/kg). Contents of mycotoxins varied in the samples. For many toxins the threshold was found below the detection limit. Irrespective of the feed type the share of trichothecenes group B was five times higher than that of trichothecenes group A in relation to the total contents of mycotoxins of this group in samples. The highest contamination with mycotoxins was found in feed samples for finishing broiler chickens. No statistically significant effect of the producer was found in case of contents of trichothecene toxins in individual feed types. Moreover, no statistically significant dependence was observed between the type of analyzed feed and toxin content. A statistically significant correlation was shown between DON/ERG and total trichothecenes/ERG. Correlations between the contents of the other fungal toxins, as well as amounts of moulds and yeasts with ergosterol, were not statistically significant.

Table 2. Contens o trichotecenes in feed mixtures for broiler chickens

	Mycotoxins	No of samples positive/examined	Range µg/kg	Average ±SD µg/kg
Concentration of the trichotecenes [µg/kg]	Scirpentiol	36/45 (80%)	0.00-2.41	1.36±0.94
	T-2 Tetraol	15/45 (33%)	0.00-1.20	0.23±0.41
	T-2 Triol	12/45 (27%)	0.00-2.00	0.33±0.70
	DAS	45/45 (100%)	1.68-8.26	4.25±2.44
	HT-2	21/45 (47%)	0.00-2.46	0.35±0.67
	T-2	0/45 (0%)	0.00-0.00	0.00
Concentration of the B-trichotecenes [µg/kg]	DON	45/45 (100%)	3.05-99.36	33.58±26.97
	FUS-X	12/45 (27%)	0.00-12.30	1.95±3.92
	3-AcDON	18/45 (40%)	0.00-10.97	1.80±3.07
	15-AcDON	24/45 (53%)	0.00-17.10	3.94±5.54
	NIV	12/45 (27%)	0.00-7.24	0.94±2.0

DISCUSSION

The quality and safety of raw materials are important elements in the poultry feed production chain. Among the many biological contaminants, next to bacteria, a major role is played by microscopic fungi. These are commonly found microorganisms, with cereal grain being their main source in feed. The mean amount of microscopic fungi recorded in the presented study in feeds for broiler chickens was 7.0×10^2 CFU/g, which is a typical level of mycoflora contamination in feeds, as confirmed by literature sources [3].

Admissible limits for fungal contamination in animal feeds vary from country to country. The main sources of fungi in feeds include plant origin materials, while in fresh, good quality grain their count is typically max. 10^4 CFU/g [25, 26]. In studies conducted in the period of 2003-2006, concerning the evaluation of fungal counts in feed mixtures for poultry, values above 10^5 CFU/g were recorded for 0.7%-4% [27]. In a study by Kubizna *et al.* [28] it was stated that the count of fungi in samples of feed mixtures for poultry from South-Western Poland fell within the range of 10^2-10^4 CFU/g, and in most cases it did not exceed the admissible level of 2.0×10^5 CFU/g. Similar results were recorded in a study carried out by Labuda *et al.* [29], in which it was found that the count of fungi from the genus *Fusarium* in samples of feed mixtures for poultry ranged from 10^2-10^4 CFU/g.

In the tested feed samples, fungi from the genera *Aspergillus, Fusarium, Mucor, Penicillium* and *Rhizopus* were identified, with *Aspergillus* and *Rhizopus* being the most commonly found genera. The recorded results confirm literature data indicating that in Poland, and under similar climatic and geographical conditions, the predominant mould fungi in poultry feeds include those from the genera *Aspergillus, Fusarium*, *Rhizopus* and *Penicillium* which, as typical phytopathogens, cause plant diseases and reduce yields [30]. Labuda and Tancinova [31] also indicated *Fusarium*, *Aspergillus*, *Mucor* and *Rhizopus* as typical fungi contaminating feed mixtures for poultry. *Fusarium*, *Aspergillus*, *Penicillium* and *Rhizopus* were also the most frequently found genera of fungi in cereal samples from Poland and eastern Slovakia, as well as those in feed mixtures for poultry in Serbia [32, 33].

It may be stated that the level of microscopic fungi in poultry feeds from western Poland in the year 2010 was much lower in relation to the count of fungi determined in feeds in Poland in the years 2006-2008. The counts of moulds and yeasts in feeds in the period 2006-2008 amounted to 10^4 -10^5 CFU/g [34].

Apart from the conventional microbiological method of determining the level of contamination with microscopic fungi in the tested material, a chemical method to analyze the concentration of ergosterol (ERG) was also applied. The compound is a cell wall component in microscopic fungi, and due to the fact that it is not found in the feed material, it could be applied as a specific marker indicating the level of feed contamination with microscopic fungi [18]. In the conducted studies, variation was found both in terms of the count of microscopic fungi and ergosterol content in feeds for all age categories of poultry.

In the tested samples of feeds for older poultry the counts of moulds and yeasts were higher in comparison to their counts recorded in starter feeds. A similar dependence was observed in the case of ergosterol content. The observed trend may result from the richer composition of feed mixtures in relation to mixtures for younger chickens. In the literature on the subject, there are no data on ERG concentration in feed mixtures. European researchers have provided only the content of this metabolite in the grain of feed cereals. Extensive studies on that subject were conducted by Maupetit *et al*. [19]. They stated that among legumes used in feed production the highest ERG concentration was found in soy, where it ranged from 0.6-7.1 mg/kg, while it was lowest in lupine and pea, at mean values of 3.4 and 3.7 mg/kg, respectively. Analyzed sunflower seed contained from 1.0-60.0 mg/kg, maize feed gluten contained 2.0-47.0 mg/kg, maize gluten flour contained 7.4-72.7 mg/kg, while in the dried seed of distillery maize it was 31.0-83.0 mg/kg. Müller and Schwardorf [20] tested similar plant materials in terms of the concentration of this fungal metabolite and recorded lower results than Maupetit *et al*. [19]. Concentration of ERG in maize grain was 0.3-2.4 mg/kg, in feed maize gluten it was 3.1-13.0 mg/kg, while ordinary maize gluten contained 2.9-19.6 mg/kg. Legumes (broad beans and peas) contained from 0.1-4.5 mg/kg of this compound. They also tested different types of ground grain, detecting the highest ERG concentration in ground sunflower seed at 1.4-9.9 mg/kg, a lower level being found in ground rapeseed at 1.5-3.6 mg/kg, whereas the lowest was recorded in ground soy beans at 0.4-2.8 mg/kg.

Maize is the cereal added most frequently to feed mixtures. In 2004, ERG concentration was analyzed in maize kernels by Mille-Lindblom *et al*. [35]. They reported a low level of this metabolite in analyzed samples, ranging from 0.32-0.97 mg/kg. Several years earlier, Seitz *et al*. [36] determined ERG concentration in the same type of plant material at 0.15-200 mg/kg. The most recent reports on that subject indicate that the level of contamination with microscopic fungi found in maize kernels has been changing rapidly over the years, and considerably more than in case of other cereals, is dependent on weather conditions due to the specific structure of maize ears and the overall morphology of the plant itself. High humidity and temperature contribute to an intensive development of diseases with fungal etiology, which is equivalent to an increased ERG level and the occurrence of mycotoxins. Macri *et al* [37] compared ERG concentrations in maize grain samples in the years 2001 and 2002 and recorded a significant difference in the concentration of this metabolite, amounting in 2001 to 0.2-72 mg/kg, on average 6.4 mg kg/kg, while in 2002 it was 0.2-9.7 mg/kg, on average 2.1 mg/kg.

The presence of microscopic fungi affects the quality of feeds, their organoleptic attributes and nutritional quality. However, secondary metabolites produced by certain strains, referred to as mycotoxins, pose a serious direct threat to poultry, and also indirectly to consumers. These metabolites, mostly thermoresistant, are capable of accumulating in the soft tissues of broiler chickens [9, 38]. In view of the above, it is crucial to control the level of mycotoxins, both in feeds and in the poultry house environment [39, 40]. Among mycotoxins identified in the climatic zone of Poland under field conditions the most common compounds are trichothecenes [41, 42, 43].

Literature data indicate contamination with microscopic fungi as well as mycotoxins from the group of trichothecenes in forage cereals and feeds in Europe [11, 41, 44]. In 2009 in Europe, seven cases were reported in which the admissible DON concentration was exceeded in forage cereals and feeds, while in 2010, 4 such cases were recorded [45, 46]. Conducted studies confirm literature data, indicating that the level of mycotoxin contamination in cereals as well as feeds in Poland in comparison to the data from the rest of Europe is low, and in most analyzed cases the concentration of DON and other mycotoxins from the group of trichothecenes is at the threshold of detectability using instrumental methods [42]. It may be stated that mean DON content, as well as that of HT-2 in feeds from the Wielkopolska region of western Poland, in 2010 was many times lower in comparison to data recorded in previous years. The content of DON in 2006, 2007 and 2008 was 167μg/kg, 230μg/kg and 160μg/kg, respectively [34]. In studies conducted by Burek *et al*. [47], DON content was detected in 32% of samples of feed mixtures at 130-1125 μg/kg. The presence of DON in feeds was also stated in many other European countries [31, 33, 48]. Mean DON content in samples of poultry feed mixtures from Slovakia was 303 μg/kg [29]. Meister [22] reported that in 2007 in Germany, the presence of DON was detected in 86% analyzed wheat samples, while in the case of 17% samples its content exceeded the limit value recommended by the EU. In feed samples analyzed in 2010, collected in the Wielkopolska region of western Poland, mean content of mycotoxin HT-2 was 0.35μg/kg, while in feeds produced in 2007 and 2008, it was 15.1μg/kg and 7.3μg/kg, respectively.

In feeds analyzed in the presented study in 2010, the presence of the T-2 toxin was not detected, while Grajewski *et al* [34] recorded its slight content, amounting on average to 5 μg/kg in 2007 and 7.5 μg/kg in 2008. Mycotoxin T-2 was not detected in samples of feed mixtures in Poland analyzed in the years 2004-2009 [47]. In turn, in Slovakia in the period of 2003-2004, mycotoxin T-2 was the mycotoxin found most commonly in chicken feeds, the presence of which was recorded in 90% of samples; however, it must be stressed that the content was relatively slight. Trichothecenes HT-2 and DON were detected in 76% and 56% of samples, respectively. Mean DON content was 303 μg/kg and in case of 4% of samples it exceeded 1,000 μg/kg [29].

In the analyses, a statistically significant correlation was found between DON/ERG and total mean concentrations of trichothecenes/ERG. The correlation coefficient for DON/ERG, as well as trichothecenes/ERG, was 0.97 at $\alpha \leq 0.05$. Similar results for the analysis of correlation in this respect have been given by other researchers. Wiśniewska and Buśko [49] indicate that the DON/ERG dependence in the analyzed wheat grain is expressed by the correlation coefficient of 0.91 at $\alpha \leq 0.05$. In a study by Stuper *et al*. [50] on the basis of an analysis of cereal samples, it was stated that the total mean trichothecene concentration is significantly correlated with the mean ERG concentration, while the correlation coefficient for wheat amounts to 0.88, whereas for all cereals it is 0.92. In view of the above, it may be concluded that the amount of mycotoxins is inseparably connected with the amount of mycoflora.

Another significant criterion, informing both on the quality of applied feed materials and sanitary and hygienic conditions in course of harvesting, processing and turnover of feeds, is connected with the total count of aerobic bacteria [27]. Bacteria commonly found in feeds, including pathogenic strains, constitute a direct health hazard for animals and, as a consequence, raw materials and products of animal origin [39, 51]. Feeds containing considerable amounts of bacteria may not be the object of national or international trade. The count of aerobic bacteria in analyzed feed mixes varied, with their mean level being 3.0×10^3 CFU/g. The highest number of aerobic bacteria was detected in samples of feeds for older poultry. Kwiatek *et al*. [27] stated that the total count of aerobic bacteria in different types of feed mixtures for animals falls within the range of 10^3-10^6 CFU/g. The authors indicate that contamination with aerobic bacteria in poultry mixtures was higher in comparison to feed mixes for pigs or cattle. In case of feed mixes for poultry, contamination levels exceeding 10^6 CFU/g were found in 2.3% to 19.4% of analyzed samples. The count of aerobic bacteria in feed mixtures in Greece in the period of 1999-2003 was 10^5 CFU/g [52].

The count of *Enterobacteriaceae* constitutes an indicator of faecal contamination and indirectly indicates the presence of *Salmonella* rods in analyzed samples. Certain bacteria from this family may colonize plants and their count in the feed material is 10^2 CFU/g [27]. In the analyzed samples of feed mixes, contamination with bacteria from the family *Enterobacteriacea* varied; however, the mean number of bacteria was also 10^2 CFU/g. A higher bacterial count was found in finisher feed mixtures for older poultry. In analyses of microbiological quality of feed mixtures in Poland, conducted in the years 2003-2006, the count of *Enterobacteriacea* in most cases was found not to exceed 10^4 CFU/g. In the case of feed mixtures for poultry, this level was exceeded in 0 to 14.3% of samples. This indicates that the admissible contamination with *Enterobacteriaceae* in feed mixtures may amount to 10^5 CFU/g [27].

CONCLUSIONS

Studies on the microbiological status of feeds produced in western Poland, conducted in 2010, showed that immediately after production those feeds were characterized by low levels of contamination, both with bacteria and microscopic fungi. These levels did not exceed standards specified by EU Ordinances for such contaminants. In the case of the analyzed mycotoxins from the group of trichothecenes, the presence of DON and DAS was found in all samples. Concentrations of these mycotoxins were low and did not exceed standard limits specified by EU Ordinances.

Among the three investigated groups of feeds, significantly lower microbiological contamination and levels of analyzed fungal metabolites were found in starter feeds, in comparison to the two other groups.

On the basis of the conducted statistical analysis, a highly significant correlation was recorded between the concentration of DON/ERG and total toxin/ERG concentration, higher than the dependence of CFU/DON or total toxin/CFU concentration. This proves that ERG is a better indicator of the level of contamination with microscopic fungi in the analyzed material than CFU.

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