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CONTAMINATION OF FEED MIXTURES WITH MYCOFLORA IN SOUTH-WESTERN POLAND

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ABSTRACT

The analyses of feed contamination with mycoflora were carried on 1299 samples of feed mixtures for farm animals and pets. From those only in 12.2% the contamination was not detected. In Opole the presence of mycoflora was stated in 95.5% of samples, however in Wrocław the percentage of contaminated samples amounted to 80.13%. On the basis of these data it could be concluded that the evaluation of total number of fungi is not sufficient to evaluate the mycological quality of feed mixtures. For complete evaluation of mycological quality the simultaneous evaluation of mycotoxins concentration is indispensable.

Key words: micoflora, feed mixtures, contamination

INTRODUCTION

The hygienic quality of feed mixtures and raw plant materials is one of the most important factors decisive about the results obtained in animal production and influencing the health state of both animals and men [2,22]. Traditionally, the quality of feeds was described as function of nutritional value of both feed components and complete mixtures. It could be changed as an effect of many external factors, in them also microorganisms' activity, particularly bacteria or fungi. The moisture is the most important factor in decreasing of feeds quality because of significance for the microorganisms proliferation. However, among many features decisive about the nutritive value of raw plant materials, the contamination of them with bacteria and moulds is recognized as the most important one [14,16].

Numerous species of fungi create the normal mycoflora living on the surface of plants and raw materials, and feed mixtures [6,8,29,17]. The fungi always provide the potential threat for nutritive quality of produced feeds becoming in some sense the farm animals' competitors in the uptake and utilization of nutrients. Intensive growth of mycoflora triggered mainly by the incorrect conditions of storage leads to the decrease of quality and worsening of nutritive and technological value of cereal grains and produced feed mixtures [25,26,31] as well as to the worsening of the animal health and efficiency of animal production [12,13,23].

The purpose of carried out research was to evaluate the degree of fungal contamination of feed mixtures produced in the Opole and Lower Silesia provinces.

MATERIAL AND METHODS

The investigations were carried out on the basis of data collected during the period of 2003–2007. All results of fungal (yeasts and moulds) contamination analysis were received from the Regional Laboratories for Veterinary Hygiene (ZHW) in Opole and Wrocław, where the samples were evaluated according to the Polish Standard [28], which is the adaptation of international norm ISO 7954:1987 *"Microbiology – General guidance for enumeration of yeasts and moulds – Colony count technique at 25 °C"*. Fungi (cfu g⁻¹) were accounted to the log₁₀ value. Detected amounts below the determination limits were treated as traces.

The majority of collected samples came from manufacturers of the final products (mixtures, feed components). Sometimes the producers were not identified, therefore the results should be related to the concrete feeds only, and not to hygienic state in concrete farm or in the manufacture. The feeds and materials turnover is very large, therefore, the feeds and raw materials were analysed according to the principle "here and now" assuming that the samples concern the materials which were produced or imported in definite year and that they be used on the same area.

RESULTS AND DISCUSSION

In Poland production of commercial feeds for farm animals mixtures for poultry predominates. The mixtures (protein concentrates) for swine are produced in considerably smaller quantities. These are used mainly in the intensive pig fattening farms. The very low quantities of feeds are produced for ruminants (cattle and sheep) (Table 1).

Itam	Years						
Item	2003	2004	2005	2006	2007		
Total production of commercial feeds	5596	5464	5276	6341	7053		
– for poultry (hens, chickens)	3450	3667	3474	3815	4064		
– for swine	1352	1167	1082	1653	1874		
– for cattle	327	365	421	551	678		
- feeds for other animals (horses, sheep, fish, fur animals etc.)	259	186	195	253	280		
– other feeds	208	79	104	69	157		
Total foods for pets	181	208	225	254	265		
– for dogs	98	112	126	146	170		
– for cats	78	89	97	106	94		

 Table 1. Production of commercial feed mixtures (thousand tons) (Statistical data)

Source: Bull. PZPP, 10/2008 [4]

Fungal flora in feed mixtures for poultry

The results of investigations of fungi concentration in mixtures for poultry (chickens, laying hens in different phases of growth and production) are given in Table 2 and 3. In total, during 5 years in ZHW Opole 648 samples of feed mixtures designed for these animals were analyzed, in ZHW Wrocław 382 samples were analyzed. In ZHW Opole only in 5.25% of samples the presence of mycoflora was not detected during 5 years. In ZHW Wrocław this percentage amounted to 11.2%.

In majority of cases the range of mycoflora concentration did not exceeded the admissible levels $(2x10^5 \text{ cfu g}^{-1}; \log_{10} 5.30)$ [28]. The observed concentrations in both ZHW varied in range from 10^1 to 10^5 , but most the results were comprised in the range 10^2-10^4 cfu per g of mixture.

The highest concentrations of mycoflora exceeding admissible levels were observed in Opole in the year 2005 and 2006 (Table 2). These concentrations were stated in 0.9% of total analyzed samples only. In Wrocław, the highest concentrations were observed in the same years. In the year 2005 in 4 samples the average concentration was 5.70 log10 cfu g⁻¹, and in year 2006 in 2 samples (5.67 and 7.08 log10 cfu g⁻¹) were noted (Table 3). These samples make up only 1.6% of the total analyzed samples in this ZHW. The number of samples exceeding allowable levels stated in both ZHW were similar to the results obtained by other authors. Similar results were obtained in investigations carried out by Wojdat et al. (2005a,b), in which it was affirmed that only in 1.9% of examined samples of mixtures for poultry the concentration of mycoflora exceeding the admissible limits was noted.

		0	T		Fungal flora in	examined samples	$(\log 10 \text{ cfu g}^{-1})$	
	n	0	Traces	10^{1}	10^{2}	10^{3}	10^{4}	10^{5}
2003	212	15	74	3	46	34	37	3
Average				1.26	2.37	3.49	4.45	5.22
$\pm SD$				0.37	0.26	0.24	0.21	0.06
Range				1.00 - 1.78	2.00 - 2.86	3.04-3.90	4.08-4.95	5.15-5.30
2004	114	1	32	3	39	12	21	6
Average				1.86	2.65	3.37	4.38	5.25
$\pm SD$				0.04	0.25	0.18	0.17	0.07
Range				1.81 - 1.90	2.00 - 2.84	3.17-3.72	4.11-4.73	5.15-5.41
2005	117	2	38	-	18	23	31	5
Average					2.37	3.58	4.52	5.37
$\pm SD$					0.21	0.22	0.24	0.11
Range					2.04 - 2.75	3.15-3.96	4.00-4.95	5.25-5.53
2006	123	12	37	-	20	29	24	1
Average					2.43	3.58	4.46	5.49
$\pm SD$					0.25	0.22	0.17	0
Range					2.00-2.86	3.15-3.96	4.17-4.76	5.49
2007	82	4	28	-	13	25	12	
Average					2.28	3.64	4.42	
±SD					0.22	0.17	0.24	
Range					2.00-2.76	3.26-3.87	4.20-4.97	
Total	648	34	209	6	136	123	125	15

Table 2. Concentration of mycoflora in samples of feed mixtures for poultry (Opole)

 10^{1} – means the range from 1.00×10^{1} to 9.99×10^{1} 10^{2} – means the range from 1.00×10^{2} to 9.99×10^{2}

n - total number of analyzed samples

0 - number of negative (uncontaminated) samples

		0	т		Fungal flora in examined samples (log10 cfu g ⁻¹)					
	n	0	Traces	10^{1}	10^{2}	10^{3}	10^{4}	10 ⁵	10 ⁷	
2003	95	36	6	_	10	14	22	7	_	
Average					2.66	3.64	4.45	5.16		
$\pm SD$					0.29	0.26	0.33	0.06		
Range					2-2.95	3-3.95	4-4.95	5.04-5.23		
2004	91	7	13	9	16	26	16	4	_	
Average				1.56	2.37	3.47	4.31	5.16		
$\pm SD$				0.30	0.28	0.38	0.24	0.15		
Range				1-1.95	2-2.89	3-3.96	4-4.72	5-5.34		
2005	65		2	15	18	15	11	4	_	
Average				1.53	2.492	3.45	4.49	5.70		
$\pm SD$				1.54	2.434	3.45	4.49	5.73		
Range				1-1.93	2-2.98	3-3.86	4.15-4.83	5.48-5.86		
2006	67	0	3	7	24	20	11	1	1	
Average				1.61	2.47	3.36	4.32	5.67	7.08	
$\pm SD$				0.448	0.250	0.274	0.282	0	0	
Range				1.0.98	2.04-2.95	3.04-3.93	4.04-4.9	5.67	7.08	
2007	64	0	0	4	9	24	18	9	_	
Average				1.50	2.56	3.54	4.35	5.26		
±SD				0.33	0.23	0.33	0.26	0.30		
Range				1.18-1.90	2.11-2.86	3.0-3.96	4.0-4.72	5.0-5.94		
Total	382	43	24	35	77	99	78	25	1	

Table 3. Concentration of mycoflora in samples of feed mixtures for poultry (Wrocław)

 10^1 – means the range from $1.00x10^1$ to $9.99x10^1$ 10^2 – means the range from $1.00x10^2$ to $9.99x10^2$

n – total number of analyzed samples

0 - number of negative (uncontaminated) samples

The similar concentrations of mycoflora were detected in investigations carried out by Labuda et al. [20], who evaluated the contamination of 33 samples of feed mixtures for poultry with *Fusarium* fungi. In 48% of samples the *Fusarium proliferatum, moniliforme* and *oxysporum* were detected and their concentration varied at the range from 0.2×10^2 to 2.4×10^4 cfu g⁻¹. Slightly higher concentrations of mycoflora were detected by Labuda and Tancinova [21], who analysed 108 samples of feed mixtures for chickens. Average concentration observed in this research was defined on the level 1.8 $\times 10^3$ cfu g⁻¹ (log₁₀ 3.26) (range 1×10^2 –8.2 $\times 10^4$). The predominant fungi were *Fusarium*, *Aspergillus, Mucor* and *Rhizopus* and these, in opinion of the authors, were the typical fungi contaminating mixtures for poultry. Moreover the authors affirmed that the mixtures for poultry are the rich source of various mycotoxins, synthesized particularly by *Penicillium, Fusarium* and *Aspergillus*.

Krnjaja et al. [15] have analysed the moulds presence in 230 samples of feeds for all age-categories of poultry. The number of colonies varied at the range from 0 to $6x10^5$ cfu g⁻¹. Microbiological analysis executed in these investigations proved, that the predominant fungi were *Fusarium* (56.1%), *Aspergillus* (54%), *Rhizopus* and *Penicillium* (both above 30%) as well as *Alternaria* (34.5%). The contaminations exceeding admissible limits were affirmed in 2.6% of examined feeds for older birds and in 14.8% of samples of feeds for chickens. Similar research was executed earlier in Argentine, where in 130 samples of poultry feeds the predominant fungi were *Aspergillus* (85%) and *Fusarium* (70% of samples). The stated concentration of mycoflora varied from $6.6x10^3$ to $6.3x10^5$ cfu g⁻¹ (log10 3,82–5,80) [7].

Domination of *Fusarium* was not observed in the investigations performed by Okoli et al., [27], who analysed the mycoflora in mixtures for poultry at the period of rainfall of year 2005 in Nigeria. Five genera of fungi were detected. The most was the *Mucor* spp. (77,3%), less *Rhizopus* (9.0%) and *Aspergillus* (6% of analysed samples). Results of these investigations confirm the opinion that the tropics are very good environment for fungi development, and can prove, that the *Fusarium* are identified mostly in the cooler regions of the world [5].

The trend of changes of fungi concentration was very stable with slightly increasing tendency, however trend observed in Wrocław shows the fall of mycoflora concentration in analysed mixtures in years 2003–2006. In 2007 the growth of mycoflora concentration was observed.

In routine analyses of the presence of mycoflora carried out in ZHW Opole and Wrocław the pathogenic fungi were not identified, however basing on cited investigations performed in similar climatical and geographical conditions (Slovakia) [21] it is possible to conclude that *Fusarium*, *Penicilium* and *Aspergillus* are the typical and predominant fungi that contaminate the feeds for poultry in Polish climatic conditions.

Fungal flora in feed mixtures for waterfowl

The results of the analysis of microflora presence in mixtures for waterfowl are given in Table 4. In total, in years 2003–2006 in ZHW Opole and Wrocław a small number of samples of mixtures for these birds was analysed, 8 and 21, respectively. Among the samples analysed in Opole in one case the exceeding of admissible level was stated in the year 2003 (5.36 vs. $5.30 \log 10 \text{ cfu g}^{-1}$). In ZHW Wrocław the samples in which the concentration of mycoflora would exceed the admissible level were not stated. The most samples were analysed in the year 2003 in Wrocław (12). In subsequent years the fall of number of submitted samples was observed, and in the year 2006 and 2007 the presence of mycoflora was not analysed..

The waterfowl, particularly geese, often fed the feeds containing a great amount of water and are susceptible to the fungi invasions, therefore can be exposed to the negative effects of their presence in feed. In contrast to older birds the young ducklings and goslings are particularly sensitive to the presence of fungi secondary metabolites – mycotoxins in feeds.

Fungal flora in feed mixtures for turkeys

The results relating the concentration of mycoflora in feed mixtures for turkeys are presented in Table 5. In total in the period 2003–2006 in Opole only 13 samples were studied, of them in 7.7% the fungi presence was not confirmed, and in 23.0% the trace quantities were detected. The average concentrations stated in remaining samples were comprised at the range of 10^{1} – 10^{4} .

In ZHW Wrocław, in the period of 2003–2007 only 15 samples of feeds for turkeys were studied. In 26.6% of samples the fungi presence was not detected (Table 5). Estimated concentrations, similarly as in Opole province were comprised at the range of 10^{1} – 10^{4} , and the most positive samples (6) were stated in year 2004 (averages varied at the range of 1.65–3.25 log10 cfu g⁻¹). In both ZHW the detected values did not exceed the levels appointed by Polish standard (5.30 log10 cfu g⁻¹).

		0			Fungal flora	in examined sample	es ($\log 10 \text{ cfu g}^{-1}$)	
	n	0	Traces	10^{1}	10^{2}	10 ³	104	10^{5}
				•	Opole			
2003	3	2	0				-	1
Average			-			-		5.36
$\pm SD$			-			-		0
Range			_			-		5.36
2004	1	0	1			—	—	—
2005	2	0	0			1	_	1
Average						3.85		5.20
$\pm SD$						0		0
Range						3.85		5.20
2006	2	0	0			1	1	_
Average						3.42	4.08	
\pm SD						0	0	
Range						3.42	4.08	
Total	8	2	1			2	1	2
				•	Wrocław			
2003	12	5	0	_	1	2	4	
Average					2.90	3.45	4.35	
\pm SD					0	0.45	0.23	
Range					2.90	3.00-3.90	4.00-4.60	
2004	5	1	1	_	1	2	_	
Average					2.15	3.32		
\pm SD					0	0.14		
Range					2.15	3.18-3.46		
2005	4	0	0	1	2	-	1	
Average				1.95	2.53		4.15	
±SD				0	0.10		0	
Range				1.95	2.43-2.62		4.15	
Total	21	6	1	1	4	4	5	

Table 4. Concentration of mycoflora	in samples of feed	mixtures for waterfowl
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 10^1 – means the range from 1.00×10^1 to 9.99×10^1

 10^2 – means the range from 1.00×10^2 to 9.99×10^2

n - Total number of analyzed samples

0 – number of negative (uncontaminated) samples

The considerable threat, especially for turkeys and pigeons may result from the presence of the fungi *Aspergillus fumigatus*. The spores of these fungi, present in aerial sacs may be the cause of dangerous disease, particularly of the respiratory tract – *aspergillosis* [32]. In young turkeys this disease can lead to the decrease of productivity, to the changes in carcass as well as to death in acute cases. Richard et al. [30] stated, that the clear interspecies differences exist between *Aspergillus* fungi in ability to infection. In majority of examined cases the concentration of individual species reached the high values, when the time of exposition was long enough. This assumption was confirmed by the investigations carried by Femenia et al. [9], who triggered the typical symptoms of acute aspergillosis in turkeys after application of isolated spores of *A. fumigatus* (CBS 144.89) in concentration 10^7 given via aerial bag.

Mycoflora in feed mixtures for pigeons

The results of analysis of the mycoflora presence in feeds for pigeons are presented in Table 6. The mixtures for pigeons were analysed only in Wrocław and there the mycoflora was detected in 74% of analysed samples. The most samples were examined in the year 2004, but highest values exceeding admissible limits were observed in the year 2006 in 1.3% of all analysed samples (5.59 log10 cfu⁻¹). The fungi species were not identified, however the attention is paid to the decreased number of executed analyses of mixtures for pigeons as well as the increased average concentration of mycoflora.

		0	-	Fu	ngal flora in examined	samples (log10 cfu g	1)
	n	0	Traces	10^{1}	10 ²	10 ³	104
				Op	oole		
2003	10	1	3	1	5	_	_
Average					2.35		
$\pm SD$					0.28		
Range				1.95	2.00-2.70		
2004	1	0	0	-	_	_	1
Average							4.72
±SD							0
Range							4.72
2006	2	0	0	-	1	-	1
Average					2.75		4.32
±SD					0		0
Range					2.75		4.32
Total	13	1	3	1	6		2
				Wro	ocław		
2003	4	3	0	_	1	-	-
Average					2.00		
±SD					0		
Range					2.00		
2004	8	1	1	1	3	2	_
Average				1.65	2.49	3.25	
$\pm SD$				0	0.16	0.05	
Range				1.65	2.30-2.68	3.20-3.30	
2005	2	0	0	-	-	-	2
Average							4.36
±SD							0.04
Range							4.32-4.40
2007	1	0	0	_	-	1	-
Average						3.32	
±SD						0	
Range						3.32	
Total	15	4	1	1	4	3	2

Table	5.	Concentration	of my	coflora	in sam	ples of	f feed	mixtures	for	turkevs	(Opole	e)
	•••	001100110101	· · · · · · · · · · · · · · · · · · ·								(C P C-	~,

 10^{1} – means the range from 1.00×10^{1} to 9.99×10^{1} 10^{2} – means the range from 1.00×10^{2} to 9.99×10^{2} n – Total number of analyzed samples 0 – number of negative (uncontaminated) samples

Table 6. Concentration of mycoflora in samples of feed mixtures for pigeons (Wrocław)

		0	т		Fungal flora in e	xamined samples	$(\log 10 \text{ cfu g}^{-1})$	
	n	0	Traces	10^{1}	10^{2}	10^{3}	104	10 ⁵
2003	16	3	12	-	1	-	-	-
Average					2.30			
±SD					0			
Range					2.30			
2004	39	17	16	3	2	1	_	_
Average				1.54	2.43	3.49		
±SD				0.39	0.29	0		
Range				1.0-1.83	2.15 - 2.72	3.49		
2005	4	0	4	-	-	-	-	-
2006	18	0	5	8	3	-	1	1
Average				1.45	2.38		4.11	5.59
±SD				0.36	0.11		0	0
Range				1-4.54	2.26 - 2.48		4.11	5.59
Total	77	20	37	11	6	1	1	1

 10^{1} – means the range from 1.00×10^{1} to 9.99×10^{1} 10^{2} – means the range from 1.00×10^{2} to 9.99×10^{2} n – Total number of analyzed samples 0 – number of negative (uncontaminated) Samales

Mycoflora in feed mixtures for swine

Data relating to the contamination of feed mixtures for swine with mycoflora are presented in Table 7. In analysed samples high degrees of contamination were proved. In ZHW Opole only in 10.5% of samples the presence of mycoflora was not detected. The highest concentrations, exceeding admissible levels, were detected in the years 2004 and 2005. In Wrocław in the years 2003–2007 just 236 samples were analysed and the presence of mycoflora was stated in 78.4% of them. The highest concentrations exceeding admissible limits, were stated in year 2005 and 2006 (6.16 and 6.50 log10 cfu g⁻¹, respectively).

		٥	Tracas		Fungal fl	ora in examined	l samples (log1	0 cfu g ⁻¹)	
	п	0	Traces	10^{1}	10^{2}	10^{3}	10^{4}	10^{5}	10^{6}
					Opole				
2003	115	0	5	3	15	43	40	9	_
Average				6.50	2.51	3.50	4.31	5.18	
±SD				2.86	0.22	0.25	0.20	0.07	
Range				1.40-1.95	2.00-2.81	3.15-3.99	4.04-4.77	5.08-5.32	
2004	52	0	6	_	10	17	15	4	
Average					2.46	3.41	4.42	5.33	
±SD					0.17	0.23	0.21	0.17	
Range					2.20-2.73	3.04-3.90	4.04-4.77	5.20-5.62	
2005	52	0	9	-	10	12	16	5	
Average					2.60	3.51	4.55	5.48	
±SD					0.16	0.25	0.22	0.17	
Range					2.36-2.83	3.04-3.89	4.23-4.97	5.26-5.69	
2006	23	4	3	_	5	6	5	_	
Average					2.33	3.46	4.41		
±SD					0.16	0.19	0.19		
Range					2.15-2.56	3.23-3.72	4.28-4.78		
2007	15	0	5	_	1	2	7	_	
Average		-			2.00	3.46	4.45		
±SD					0	0.26	0.17		
Range					2.00	3.20-3.72	4.23-4.83		
			•••	-				10	
Total	257	4	28	3	41	80	83	18	
Total	257	4	28	3	41 Wrocław	80 N	83	18	
Total 2003	257	4	28	2	41 Wrocław 21	80 w 24	83	<u> 18 </u>	
Total 2003 Average	257 111	4	28 7	3 2 1.15	41 Wrocław 21 2.63	80 w 24 3.54	83 16 3.95	18 3 5.12	_
Total 2003 Average ±SD	111	4 38	<u>28</u> 7	2 1.15 0.15	41 Wrocław 21 2.63 0.25	80 w 24 3.54 0.26	83 16 3.95 1.47	3 5.12 0.06	
Total 2003 Average ±SD Range	111	4	7	2 1.15 0.15 1.30	41 Wrocław 21 2.63 0.25 2.30–2.95	80 x 24 3.54 0.26 3.00–3.90	16 3.95 1.47 0.08–4.91	3 5.12 0.06 0.06–5.08	
Total 2003 Average ±SD Range 2004	257 111 76	4 38 13	28 7 16	2 1.15 0.15 1.30 9	41 Wrocław 21 2.63 0.25 2.30–2.95 16	80 x 24 3.54 0.26 3.00–3.90 11	16 3.95 1.47 0.08–4.91 5	3 5.12 0.06 0.06–5.08 6	
Total 2003 Average ±SD Range 2004	257 111 76	4 38 13	28 7 16	2 1.15 0.15 1.30 9 1.58	41 Wrocław 21 2.63 0.25 2.30–2.95 16 2.51	80 x 24 3.54 0.26 3.00–3.90 11 3.45	16 3.95 1.47 0.08–4.91 5 4.46	3 5.12 0.06 0.06–5.08 6 5.35	
Total 2003 Average ±SD Range 2004 Average ±SD	257 111 76	4 38 13	7 16	2 1.15 0.15 1.30 9 1.58 0.23	41 Wrocław 21 2.63 0.25 2.30–2.95 16 2.51 0.31	80 x 24 3.54 0.26 3.00–3.90 11 3.45 0.24	16 3.95 1.47 0.08–4.91 5 4.46 0.25	3 5.12 0.06 0.06–5.08 6 5.35 0.25	
Total 2003 Average ±SD Range 2004 Average ±SD Range	257 111 76	4 38 13	7 16	3 2 1.15 0.15 1.30 9 1.58 0.23 1.11–1.95	41 Wrocław 21 2.63 0.25 2.30–2.95 16 2.51 0.31 2.00–2.95	80 x 24 3.54 0.26 3.00–3.90 11 3.45 0.24 3.00–3.91	83 16 3.95 1.47 0.08–4.91 5 4.46 0.25 4.11–4.82	3 5.12 0.06 0.06–5.08 6 5.35 0.25 5.08–5.79	
Total 2003 Average ±SD Range 2004 Average ±SD Range 2004 2005	257 111 76 33	4 38 13	<u>28</u> 7 <u>16</u> 2	3 2 1.15 0.15 1.30 9 1.58 0.23 1.11–1.95 5	41 Wrocław 21 2.63 0.25 2.30–2.95 16 2.51 0.31 2.00–2.95 15	80 x 24 3.54 0.26 3.00–3.90 11 3.45 0.24 3.00–3.91 6	83 16 3.95 1.47 0.08–4.91 5 4.46 0.25 4.11–4.82 3	3 5.12 0.06 0.06–5.08 6 5.35 0.25 5.08–5.79 –	
Total 2003 Average ±SD Range 2004 Average ±SD Range 2005 Average	257 111 76 33	4 38 13 0	28 7 16 2	3 2 1.15 0.15 1.30 9 1.58 0.23 1.11–1.95 5 1.28	41 Wrocław 21 2.63 0.25 2.30–2.95 16 2.51 0.31 2.00–2.95 15 2.43	80 x 24 3.54 0.26 3.00–3.90 11 3.45 0.24 3.00–3.91 6 3.42	83 16 3.95 1.47 0.08–4.91 5 4.46 0.25 4.11–4.82 3 4.75	3 5.12 0.06 0.06-5.08 6 5.35 0.25 5.08-5.79	
Total 2003 Average ±SD Range 2004 Average ±SD Range 2005 Average ±SD	257 111 76 33	4 38 13 0	<u>28</u> 7 <u>16</u> 2	3 2 1.15 0.15 1.30 9 1.58 0.23 1.11–1.95 5 1.28 0.29	41 Wrocław 21 2.63 0.25 2.30–2.95 16 2.51 0.31 2.00–2.95 15 2.43 0.22	80 v 3.54 0.26 3.00–3.90 11 3.45 0.24 3.00–3.91 6 3.42 0.26	83 16 3.95 1.47 0.08–4.91 5 4.46 0.25 4.11–4.82 3 4.75 0.17	3 5.12 0.06 0.06-5.08 6 5.35 0.25 5.08-5.79	 6.16 0.02
Total2003Average±SDRange2004Average±SDRange2005Average±SDRange2005Average±SDRange	257 111 76 33	4 38 13 0	<u> 28 </u>	3 2 1.15 0.15 1.30 9 1.58 0.23 1.11–1.95 5 1.28 0.29 1–1.78	41 Wrocław 21 2.63 0.25 2.30–2.95 16 2.51 0.31 2.00–2.95 15 2.43 0.22 2.04–2.81	80 x 24 3.54 0.26 3.00–3.90 11 3.45 0.24 3.00–3.91 6 3.42 0.26 3.04–3.88	83 16 3.95 1.47 0.08–4.91 5 4.46 0.25 4.11–4.82 3 4.75 0.17 4.51–4.90	3 5.12 0.06 0.06-5.08 6 5.35 0.25 5.08-5.79	
Total 2003 Average ±SD Range 2004 Average ±SD Range 2005 Average ±SD Range 2005 Average ±SD Range 2005	257 111 76 33 7	4 38 13 0	28 7 16 2 0	2 1.15 0.15 1.30 9 1.58 0.23 1.11–1.95 5 1.28 0.29 1–1.78 –	41 Wrocław 21 2.63 0.25 2.30–2.95 16 2.51 0.31 2.00–2.95 15 2.43 0.22 2.04–2.81 –	80 x 24 3.54 0.26 3.00–3.90 11 3.45 0.24 3.00–3.91 6 3.42 0.26 3.04–3.88 3	83 16 3.95 1.47 0.08–4.91 5 4.46 0.25 4.11–4.82 3 4.75 0.17 4.51–4.90 1	18 3 5.12 0.06 0.06–5.08 6 5.35 0.25 5.08–5.79 -	- - 6.16 0.02 6.15-6.18 2
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Total 2003 Average ±SD Range 2004 Average ±SD Range 2005 Average ±SD Range 2005 Average ±SD Range 2006 Average ±SD Range 2006 Average ±SD Range	257 111 76 33 7	4 38 13 0 0	28 7 16 2 0	3 2 1.15 0.15 1.30 9 1.58 0.23 1.11–1.95 5 1.28 0.29 1–1.78 –	41 Wrocław 21 2.63 0.25 2.30–2.95 16 2.51 0.31 2.00–2.95 15 2.43 0.22 2.04–2.81 -	80 x 24 3.54 0.26 3.00–3.90 11 3.45 0.24 3.00–3.91 6 3.42 0.26 3.04–3.88 3 3.72 0.22 3.54–3.96	83 16 3.95 1.47 0.08–4.91 5 4.46 0.25 4.11–4.82 3 4.75 0.17 4.51–4.90 1 4.93 0 4.93	18 3 5.12 0.06 0.06-5.08 6 5.35 0.25 5.08-5.79 - 1 5.42 0 5.42	 6.16 0.02 6.15–6.18 2 6.50 0.06 6.46–6.54
Total 2003 Average ±SD Range 2004 Average ±SD Range 2005 Average ±SD Range 2005 Average ±SD Range 2006 Average ±SD Range 2006 Average ±SD Range 2007	257 111 76 33 7 9	4 38 13 0 0	28 7 16 2 0	3 2 1.15 0.15 1.30 9 1.58 0.23 1.11–1.95 5 1.28 0.29 1–1.78 –	41 Wrocław 21 2.63 0.25 2.30–2.95 16 2.51 0.31 2.00–2.95 15 2.43 0.22 2.04–2.81 –	80 x 24 3.54 0.26 3.00–3.90 11 3.45 0.24 3.00–3.91 6 3.42 0.26 3.04–3.88 3 3.72 0.22 3.54–3.96 -	83 16 3.95 1.47 0.08–4.91 5 4.46 0.25 4.11–4.82 3 4.75 0.17 4.51–4.90 1 4.93 0 4.93	18 3 5.12 0.06 0.06-5.08 6 5.35 0.25 5.08-5.79 - 1 5.42 0 5.42 5	 6.16 0.02 6.15-6.18 2 6.50 0.06 6.46-6.54
Total 2003 Average ±SD Range 2004 Average ±SD Range 2005 Average ±SD Range 2005 Average ±SD Range 2006 Average ±SD Range 2006 Average ±SD Range 2007 Average	257 111 76 33 7 9	4 38 13 0 0	28 7 16 2 0 4	3 2 1.15 0.15 1.30 9 1.58 0.23 1.11–1.95 5 1.28 0.29 1–1.78 –	41 Wrocław 21 2.63 0.25 2.30–2.95 16 2.51 0.31 2.00–2.95 15 2.43 0.22 2.04–2.81 	80 v 24 3.54 0.26 3.00–3.90 11 3.45 0.24 3.00–3.91 6 3.42 0.26 3.04–3.88 3 3.72 0.22 3.54–3.96 –	83 16 3.95 1.47 0.08–4.91 5 4.46 0.25 4.11–4.82 3 4.75 0.17 4.51–4.90 1 4.93 0 4.93 -	18 3 5.12 0.06 0.06-5.08 6 5.35 0.25 5.08-5.79 - 1 5.42 0 5.42 5 5.15	 6.16 0.02 6.15-6.18 2 6.50 0.06 6.46-6.54
Total 2003 Average ±SD Range 2004 Average ±SD Range 2005 Average ±SD Range 2005 Average ±SD Range 2006 Average ±SD Range 2007 Average ±SD	257 111 76 33 7 9	4 38 13 0 0	28 7 16 2 0 4	3 2 1.15 0.15 1.30 9 1.58 0.23 1.11–1.95 5 1.28 0.29 1–1.78 –	41 Wrocław 21 2.63 0.25 2.30−2.95 16 2.51 0.31 2.00−2.95 15 2.43 0.22 2.04−2.81 -	80 x 3.54 0.26 3.00–3.90 11 3.45 0.24 3.00–3.91 6 3.42 0.26 3.04–3.88 3 3.72 0.22 3.54–3.96	83 16 3.95 1.47 0.08–4.91 5 4.46 0.25 4.11–4.82 3 4.75 0.17 4.51–4.90 1 4.93 0 4.93 -	18 3 5.12 0.06 0.06-5.08 6 5.35 0.25 5.08-5.79 - 1 5.42 0 5.42 5 5.15 0.23	 6.16 0.02 6.15-6.18 2 6.50 0.06 6.46-6.54
Total 2003 Average ±SD Range 2004 Average ±SD Range 2005 Average ±SD Range 2006 Average ±SD Range 2006 Average ±SD Range 2007 Average ±SD Range 2007	257 111 76 33 7 9	4 38 13 0 0	28 7 16 2 0 4	3 2 1.15 0.15 1.30 9 1.58 0.23 1.11–1.95 5 1.28 0.29 1–1.78 –	41 Wrocław 21 2.63 0.25 2.30–2.95 16 2.51 0.31 2.00–2.95 15 2.43 0.22 2.04–2.81 -	80 24 3.54 0.26 3.00–3.90 11 3.45 0.24 3.00–3.91 6 3.42 0.26 3.04–3.88 3 3.72 0.22 3.54–3.96	83 16 3.95 1.47 0.08–4.91 5 4.46 0.25 4.11–4.82 3 4.75 0.17 4.51–4.90 1 4.93 0 4.93 -	18 3 5.12 0.06 0.06-5.08 6 5.35 0.25 5.08-5.79 - 1 5.42 0 5.42 5 5.15 0.23 5.00-5.70	 6.16 0.02 6.15-6.18 2 6.50 0.06 6.46-6.54

Table 7. Concentration of mycoflora in samples of feed mixtures for swine

 10^1 – means the range from 1.00×10^1 to 9.99×10^1

 10^2 – means the range from 1.00×10^2 to 9.99×10^2

n – Total number of analyzed samples

0 - number of negative (uncontaminated) samples

Considering the analysis of presence of mycoflora in subsequent years in ZHW Opole the decrease of number of the analysed samples could be seen. The similar tendency was observed in the number of samples analysed in ZHW Wrocław.

In the results a slight rise of mycoflora concentrations could be seen in the samples analysed in ZHW Opole. In ZHW Wrocław the clear tendency to an increase of mycoflora concentration in mixtures for swine was observed. Swine are particularly sensitive to the presence of *Fusarium* fungi and to the product of their metabolism – zearalenone (ZEA). The piglets from the sows fed the diets contaminated with ZEA are weaker, often are born dead. In next stages of life the piglets are characterized by low growth rate and increased mortality. The symptoms of the poisoning are often accompanied by diarrhoea, lack of appetite and weak body weight gain [10,11].

Mycoflora in feed mixtures for cattle

The results of the analysis of the samples of mixtures for cattle are given in Table 8. In ZHW Opole totally only 29 samples were analysed and only in one case the mycoflora was not detected as well as in one case, in the year 2007, the concentration exceeding admissible level (5.36 log10 cfu g⁻¹) was detected. These results can testify about substantial contamination of feeds for cattle, despite of relatively low concentrations of mycoflora. In ZHW Wrocław in total 194 samples were analysed in the period of three years and in 28.3% of these the presence of mycoflora was not detected. Estimated average values (1.52–5.26 log10 cfu g⁻¹) were lower than the averages stated in ZHW Opole.

	n	0	Traces		s (log10 cfu g^{-1})			
	11	0	Traces	10^{1}	10^{2}	10^{3}	10^{4}	10^{5}
					Opole			
2003	9	1	0	1	2	1	4	_
Average				1.85	2	3.36	4.52	
±SD				0	0	0	0.20	
Range				1.85	2.00	3.36	4.20-4.74	
2004	6	0	1	_	2	2	1	_
Average					2.43	3.49	4.72	
±SD					0.11	0.13	0	
Range					2.32-2.53	3.36-3.63	4.72	
2005	8	0	0	_	1	1	6	_
Average					2.49	3.53	4.54	
±SD					0	0	0.22	
Range					2.49	3.53	4.34-4.94	
2006	2	0	0	_	_	1	1	_
Average						3.69	4.53	
±SD						0	0	
Range						3.69	4.53	
2007	4	0	0	-	1	2	-	1
Average					2.20	3.57		5.36
±SD					0	0.15		0
Range					2.20	3.42-3.72		5.36
Total	29	1	1	1	6	7	12	1
					Wrocław			
2003	74	43	3	_	13	7	8	-
Average					2.64	3.19	4.44	
±SD					0.29	0.34	0.33	
Range					2.00-2.95	3.00-3.95	4.00-4.95	
2004	77	12	24	13	21	5	2	-
Average				1.55	2.41	3.48	4.21	
±SD				0.24	0.24	0.35	0.07	
Range				1.00 - 1.88	2.08 - 2.88	3.15-3.99	4.15-4.28	
2005	43	0	4	15	18	3	0	3
Average				1.52	2.38	3.57		5.26
\pm SD				0.26	0.22	0.22		0.13
Range				1.00-1.93	2.08-2.95	3.26-3.74		5.08-5.40
Total	194	55	31	28	52	15	10	3

Table 8. Concentration of mycoflora in samples of feed mixtures for cattle (Opole)

 10^1 – means the range from 1.00×10^1 to 9.99×10^1

 10^2 – means the range from 1.00×10^2 to 9.99×10^2

n – Total number of analyzed samples

0 - number of negative (uncontaminated) samples

The analysis of these results pointed out on the clear rise of mycoflora concentrations noted in ZHW Opole. In ZHW Wrocław the clear tendency of the decrease of mycoflora concentration in mixtures for cattle was observed. Moreover, it could be seen that all values were comprised at admissible range.

Mycoflora in feed mixtures for horses

In the period of five years only one sample of feed for horses was analysed in ZHW Wrocław. Estimated value $(2.85 \times 10^2 \text{ cfu g}^{-1})$ was below of the admissible limits valid in the European Union (10^5) . Higher values of concentrations were stated in investigations carried out by Basalan et al. [3]. Average concentration observed in these investigations amounted to 0.97×10^4 cfu g⁻¹. The species of fungi were not identified, however on the basis of presence of mycoflora the authors concluded, that so contaminated feeds can make up the threat for animals. In ZHW Opole the feeds for these animals were not studied.

Low number of examinations executed in ZHW Wrocław could result from the fact that horses are very fastidious in choice of feeds, and that the fungal contaminations make a specific taste and scent of feeds and therefore they are avoided by these animals.

Mycoflora in fodders for rodents

The concentration of mycoflora in feed mixtures for rodents are given in Table 9. This category comprised of the feeds applied in feeding of rabbits, chinchillas, hamsters, guinea pigs and other rodents kept as pets.

In ZHW Opole at the period of 2003–2007 only 6 samples of mixtures for rabbits were analysed. One of them was negative, the traces of fungi were detected in two samples, and in another 3 (2003) the concentration was lower than 10^2 (2.20 log10 cfu g⁻¹; 2.17–2.20) (Table 9). In ZHW Wrocław just 36 samples were examined and there in 66.6% the presence of mycoflora was detected. All values were below the limits appointed by Polish standard. The majority of results was comprised at the range of 10^1-10^2 , and only in one sample the concentration amounted to 4.79 log10 cfu g⁻¹. The traces of mycoflora were detected in one sample of feed for chinchilla.

	n	0	Traces	Fun	gal flora in examined	l samples (log10 cfu g	g ⁻¹)
	11	0	Traces	10^{1}	10^{2}	10^{3}	10^{4}
				Opole			
2003	4	0	1		3		
Average					2.20		
±SD					0.01		
Range					2.17-2.20		
2006	2	1	1	_	_	_	_
Total	6	1	2		3	-	_
				Wrocław	v		
2003	13	8	2	_	3	-	_
Average					2.62		
±SD					0.27		
Range					2.30-2.95		
2004	10	4	0	4	2	-	_
Average				1.48	2.38		
±SD				0.30	0.38		
Range				1.18-1.98	2.00-2.76		
2005	8	0	1	5	2	-	_
Average				1.37	2.43		
±SD				0.32	0.35		
Range				1.00-1.70	2.08-2.78		
2006	4	0	0	1	_	2	1
Average				1.70		3.46	4.79
±SD				0		0.36	0
Range				1.70		3.20-3.72	4.79
2007	1	0	0	_	_	1	_
Average	1					3.56	
±SD						0	
Range						3.56	
Total	36	12	3	10	7	3	1

Table 9. Concentration of mycoflora in samples of feed mixtures for rodents (Opole)

 10^{1} - means the range from 1.00×10^{1} to 9.99×10^{1}

 10^2 – means the range from 1.00×10^2 to 9.99×10^2

n – Total number of analyzed samples

0-number of negative (uncontaminated) samples

The feeds strongly contaminated with fungi are characterized by low digestibility and usually are omitted by animals. In the cases of intake of contaminated feed by rabbits the water intake by these animals considerably grows up. In the died animals the changes in liver and spleen were observed [1].

Mycoflora in feeds for dogs and cats

Feeds for dogs and cats comprised of the vegetable materials which easily may be contaminated with fungi – maize, corn gluten, rice, chicken, beef or fish meat – as well as mineral and vitamin supplements. These feeds are subjected to the intensive technological procedures in which the majority of mycoflora is destroyed.

Only 3 samples of feeds for dogs and cats were analyzed in ZHW Wrocław. In all of them the traces of mycoflora were detected. Similar values of mycoflora concentration were obtained by Martins et al. [24]. They studied 20 samples of feeds for dogs and 20 samples of feeds for cats. In the positive samples presence of mycoflora in concentration $10^{1}-10^{2}$ cfu g⁻¹ was detected. The most often detected fungi were *Aspergillus* (58,3%) and *Penicillium* and *Mucor* (each 38.3% of studied samples).

The possibilities of increasing the production of industrial feeds for different animals in Poland are very large due to the centralization of production, enlarging of the herds as well as decreasing of number of individual farms dealing with the animal production. This puts the duty to keep the norms of proper quality of production on the manufacturers. Industrial feeds produced in correct conditions seldom show the low hygienic quality. However, the hygienic quality can become worse as a result of incorrect storage.

It total, during the period of 2003–2007 in the investigations relating to the presence of mycoflora 1922 samples of feed mixtures applied in animal feeding were analysed. In 12.2% of samples the presence of mycoflora was not shown. Considering the degree of samples contamination in both ZHW's it could be stated that in Opole just 961 samples were examined and only in 4.47% of them the presence of mycoflora was not detected, in 25,39% were the traces and in 70.14% of samples the presence of mycoflora was shown. In ZHW Wrocław also 961 samples were analysed and the estimated values amounted to 19.87, 13.11 and 67.02%, respectively.

On the basis of these results it can be stated, that the correct agricultural practices and proper storage of feed products allow to maintain their proper hygienic quality. However, in the available literature the opinions about the necessity of decrease of the admissible limits of presence of mycoflora from 10^5 to the 10^4 are met [18,19]. It can affect the interpretation of the threat posed by the mycoflora.

CONCLUSIONS

As a final thought it should be stated that the evaluation of total number of fungi is not sufficient criterion for the determination of feed mixtures quality. For the complete determination the simultaneous evaluation of mycotoxins concentration seems to be necessary.

For obtaining a general picture of threat of mycological contamination, the monitoring of feeds and raw materials carried by the veterinary services as well as gathering of such data from different sources (the mills, feed mills etc.) supported with information on the weather conditions as well as conditions of storage are indispensable.

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