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Microbiological quality of feed materials used between 2009 and 2012 in Poland

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Abstract

The study was conducted at all regional veterinary diagnostic laboratories. Feed materials were examined for *Salmonella* prevalence and contamination by *Enterobacteriaceae*, aerobic mesophilic bacteria, total plate count, fungi, *Clostridium* sp., and *Bacillus cereus*. Assays were done following international and Polish standards used in food and feed microbiology. *Salmonella* sp. were most often detected in oil seeds. In most of the examined feed ingredients, the number of *Enterobacteriaceae* did not exceed 10 cfu/g. The contamination by aerobic bacteria ranged most often from $10^1$to 10^7 cfu/g, and the highest mycological contamination was noted in cereal grains (10^8 cfu/g). The results showed that microbial contamination of feed materials in regard to *Enterobacteriaceae*, fungi, and total plate counts declined over the past years.

Key words: feed material, microbiological contamination, *Salmonella*, *Enterobacteriaceae*, fungi.

Introduction

The "farm-to-fork" concept, confirming that each link in the food production chain has a share in the state of public health, proves that feed quality is a part of food quality (7, 8, 29). Feed quality is defined as nutritional quality (value), technical quality (size of the pellets, fineness of the crumbs, flavour), safety for animals and environment, and emotional quality connected with ethics and ethology (*e.g.* feed for organic farming) (9). However, it seems that the animal health status is mainly determined by factors, such as nutritional value of the feed and its microbiological quality.

The past years have shown that a variant of Creutzfeldt-Jakob disease, chemical (dioxins, antibiotics, mycotoxins, pesticides) and microbiological (*Salmonella*, *Escherichia coli*, *Clostridium botulinum*) contamination of food of animal origin are the consequences of human errors at the primary production (12, 13, 18, 21). Therefore, if the feed is considered as a potential source of hazards to slaughter animals, products of animal origin, and thus to humans, it is important to control the quality of the feed materials and compound feed. The sources of microorganisms in the plant materials are soil, dust, water, animals (insects, vertebrata), or secondary contamination during processing, storage, and dispersal of the product (19).

Salmonella sp., one of the most important bacterial zoonotic agents, is an essential bacterium in the assessment of microbiological quality of feed (23). In the second place, ubiquitous sporeforming microorganisms, resistant to adverse conditions of processing (high temperature, pressure), should be mentioned. These include anaerobic bacilli of *Clostridium* sp., aerobic bacilli of *Bacillus* sp., and abundantly sporulating moulds (22, 20). A quality assessment of feed also comprises hygiene indicators as *Enterobacteriaceae* family, total aerobic bacteria, fungi, and total plate counts. The total plate count (TPC) defines how many aerobic, mesophilic microorganism colonies, such as bacteria, yeast, and mould fungi will grow for 72 h on an agar plate that was normalised for microbiological testing at 30°C. Microbiological requirements, currently being in force in the EU, exclude the presence of *Salmonella* sp. in 25 g in all feed materials. Besides, in accordance with EU Regulation (EC) No. 142/2011, *Enterobacteriaceae* count cannot exceed 300 colony forming units (cfu)/g in five batch samples of feed material derived from animal by-products.

The assessment of the microbiological quality of feed materials and compound feed used in Poland has been conducted since 2003 in the Department of Hygiene of Animal Feedingstuffs of the National Veterinary Research Institute (NVRI) in Pulawy (14–17). This paper describes microbiological quality of feed materials used between 2009 and 2012 in Poland.

Material and Methods

All feed material samples (17 512 meat meals; 3964 oil seeds; 1615 cereals; 791 feed materials of terrestrial animal origin; 336 water samples, and 8452 unidentified feed materials) were collected from the farmer granaries, feed factories, and imported batches. The samples were analysed at all veterinary diagnostic laboratories (national and regional) operating in frame of official laboratory system, and results of the analyses were collected at the Central Data Base CELAB System in the NVRI. The number of samples analysed in particular years and directions are presented in Tables 1 and 2.

Salmonella sp. were isolated and identified using the method described in ISO 6579:2002. *Enterobacteriaceae* were enumerated in accordance with ISO 21528-2:2004, *B. cereus* with ISO 7932:2004, and TPC with ISO 4833:2003. Aerobic bacteria and fungi count were enumerated following PN-R-64791:1994 "Animal feeding stuffs. Requirements and microbiological examination" with nutrient agar and dichloran-rose Bengal chloramphenicol (DRBC) agar (2), respectively. *Clostridium* sp. was isolated from 10 g of the sample under anaerobic conditions according to PN-R-64791:1994 on Wrzosek broth, and Willis-Hobbs and Wilson-Blair solid media, and presumed strains were confirmed by phase-contrast microscopy, mouse lethality assay, as well as PCR-based methods (1, 26).

The obtained results for quantitative methods were analysed according to distributive series. Figs 1–16 present different microorganisms count in logarithmic scale (*e.g.* $\log_{10} = 1$ means range from 10^1 to 99 cfu/g; $\log_{10} = 2$ means range from 10^2 to 999 cfu/g; $\log_{10} = 3$ means range from 10^3 to 9999 cfu/g; $\log_{10} = 4$ means range from 10^4 to 99 999 cfu/g, *etc.*). The titre described the least amount of analysed sample (in grams), in which target microorganism was detected (Fig. 17). Microbial contaminations of feed ingredients in particular years were compared applying student's *t* test and regression analysis (Figs 18–21).

Results

The average prevalence of *Salmonella* sp. in feed materials analysed between 2009 and 2012 (Table 2) were as follows: 3.59% in unidentified feed materials, 2.15% in oil seeds, 1.39% in feed materials of terrestrial animal origin, 1.26% in cereals and derived, and 0.69% in animal meals. Average contamination by this pathogen of particular oil seeds was 3.8% in soya derived, 2.45% in rape seed derived, and 0.29% in sunflower seed derived. *Salmonella* sp. were less frequently detected in particular cereals, and ranged from 0.47% in wheat to 1.33% in barley. The permanent source of *Salmonella* sp. in food chain production was also confirmed in animal meals. An average prevalence of the bacteria in these feed materials was as follows: 2.59% in blood meals, 0.89% in fish meals, 0.74% in meat and bone meals, and 0.53% in poultry meals. The study revealed also positive samples of water for animals (2.97%). In the reported period, the percentage of feed materials contamination by *Salmonella* sp. ranged from 0.84% to 3.58% with an average value of 1.83%.

The lowest level of contamination by *Enterobacteriaceae* family was observed in meat meal samples (Fig. 1). Allowable limit of *Enterobacteriaceae* (300 cfu/g) for animal meals was exceeded in 0.53% of samples (0.95% in 2009; 0.2% in 2010; 0.17% in 2011; 0.74% in 2012). Nevertheless, a substantially high level of contamination (10^7 cfu/g) was noted in four samples of animal meals tested in 2009, 2010, and 2012. Relatively high faecal contamination was also observed in a few samples of oil seed (10^4 cfu/g), and in cereal samples (10^6 cfu/g) (Figs 2, 3). However, the analysis of all obtained results indicated few samples of feed ingredients, where these bacteria numbered 10^4, 10^5, 10^6, 10^7, or 10^8 cfu/g (Fig. 4).

Relatively high bacterial contamination was observed in some tested animal meals, where the number of aerobic bacteria reached up to 10^8 cfu/g (Fig. 5). Bacterial contamination of oil seeds was higher by one order of magnitude than cereals, and some samples in 2009 and 2011 contained nearly 10^8 cfu/g (Figs 6, 7). The highest bacterial contamination detected in all analysed feed ingredients amounted to 10^8 cfu/g (Fig. 8).

Almost all examined meat meal samples did not exceed 10^6 cfu/g of microorganisms (Fig. 9). Total plate count in oil seeds was about one order of magnitude higher than the bacterial level (Fig. 10). Contamination by microorganisms of cereals and derived was about two orders of magnitude higher than the level of fungi (Figs 11, 15). Four years' analysis indicates plant feed materials (oil seeds and cereals) as the most serious source of microorganisms in compound feed. Here, some samples with 10^9 cfu/g of microorganisms were noted, with an average value circa 10^6 cfu/g (Fig. 12).

Mycological contamination of feed materials revealed that 100% of meat meal samples did not exceed 10^4 cfu/g (Fig. 13). About two orders of magnitude higher was contamination level of oil seed samples (Fig. 14). The highest level of fungi was noted in cereals, where in some samples 10^8 cfu/g was observed (Fig. 15). The same value reached the highest mycological contamination of all tested feed materials in the reporting period (Fig. 16).

Assessment of meat meals contamination by the genus *Clostridium* revealed that the majority of samples contained less than 10 cfu/g of sample (titre of less than

0.1) (Fig. 17). The fact that some animal meals present on national marked in the examined period have shown permanent contamination with *Clostridium* sp. on the level of 10^3–10^4 cfu/g is alarming. Besides, two samples (4.08%) of the 49 analysed feed materials were contaminated with *B. cereus*. Detected levels of contamination were 1.2×10^3 cfu/g and 4.0×10^1 cfu/g in soya and rape derived, respectively. *B. cereus* was not detected in the rest of samples (contamination lower than 10 cfu/g).

Statistically significant improvement of microbiological quality of total feed materials was demonstrated ($P < 0.05$) with regard to *Enterobacteriaceae*, fungi, and total plate counts (Figs. 18, 20, 21). An increase in contamination with *Clostridium* sp. in tested meat meals was also statistically significant. No significant differences in contamination with aerobic bacteria were noted in the analysed years (Fig. 19).

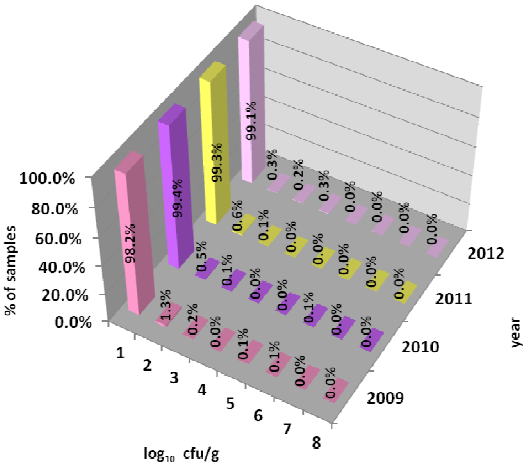

Fig. 1. *Enterobacteriaceae* count in meat meal samples

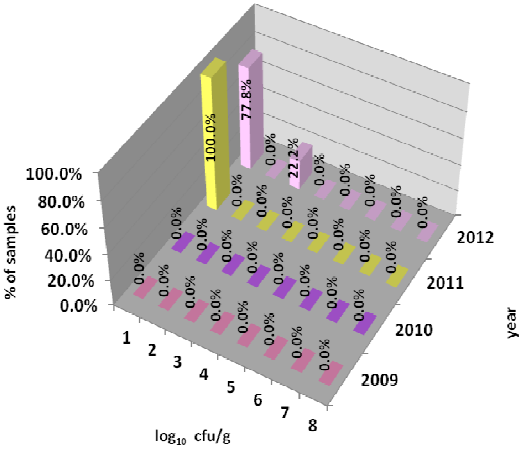

Fig. 2. *Enterobacteriaceae* count in oil seed samples

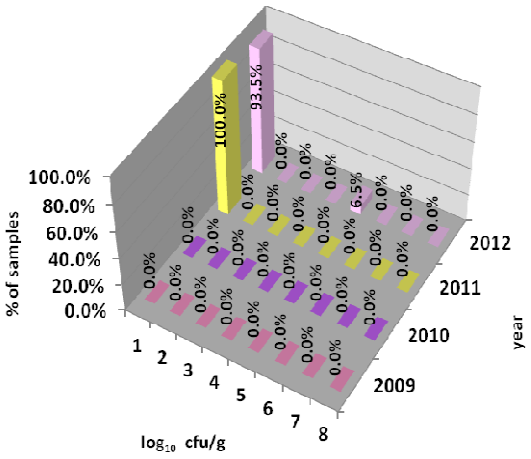

Fig. 3. *Enterobacteriaceae* count in cereal samples

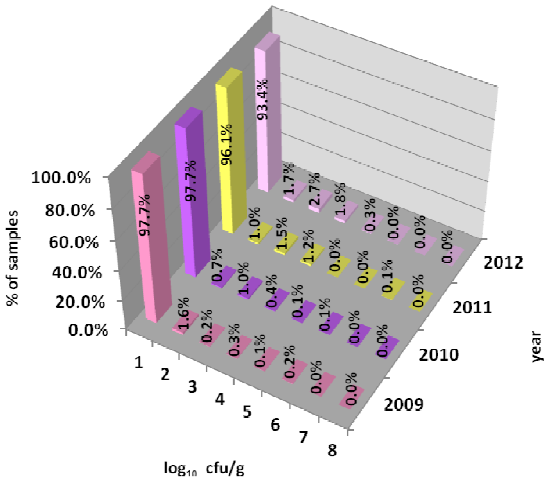

Fig. 4. *Enterobacteriaceae* count in all feed material samples

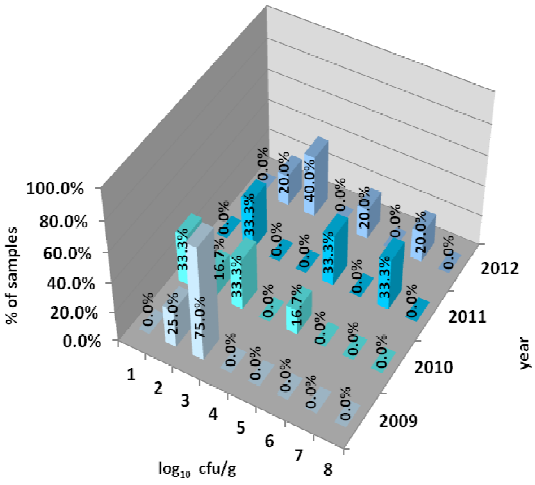

Fig. 5. Aerobic bacteria count in meat meal samples

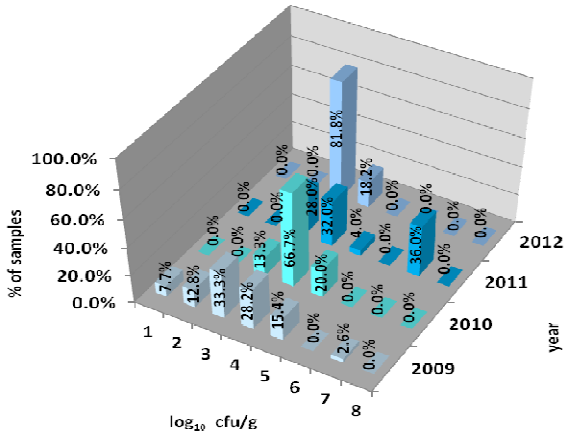

Fig. 6. Aerobic bacteria count in oil seed samples

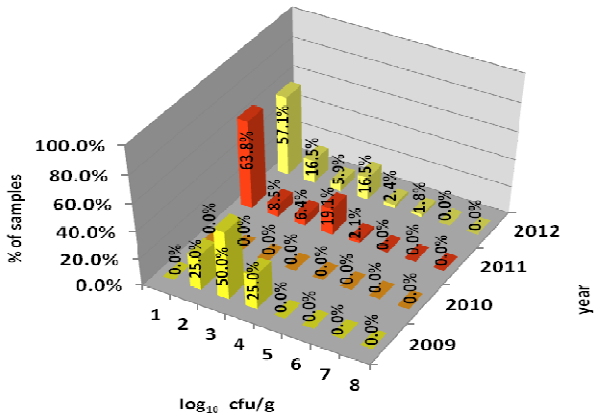

Fig. 9. TPC in meat meal samples

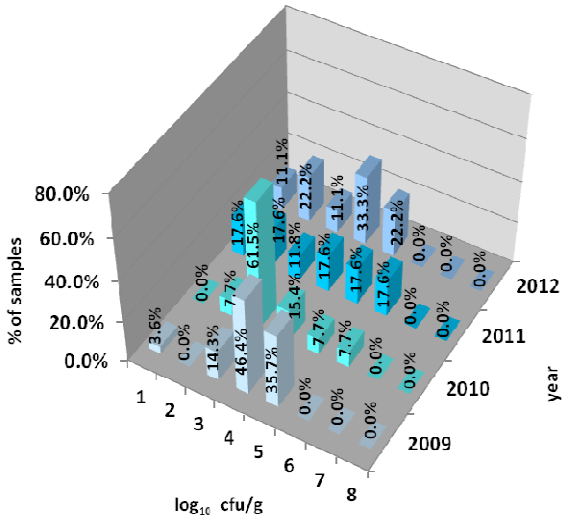

Fig. 7. Aerobic bacteria count in cereal samples

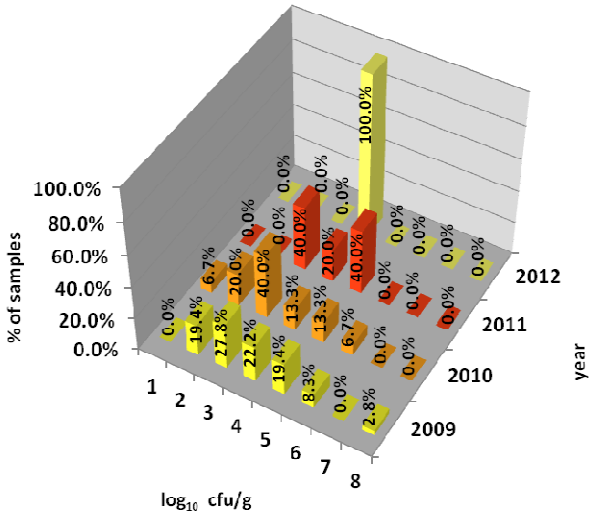

Fig. 10. TPC in oil seed samples

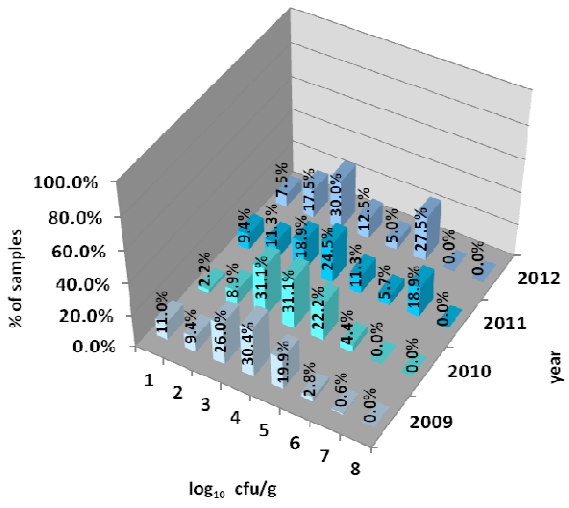

Fig. 8. Aerobic bacteria count in all feed material samples

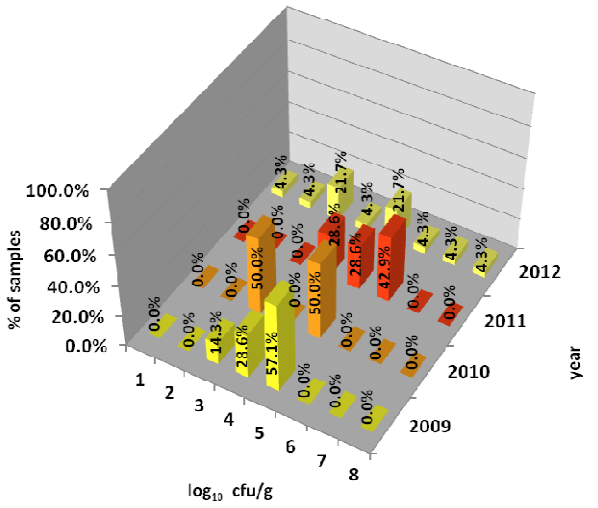

Fig. 11. TPC in cereal samples

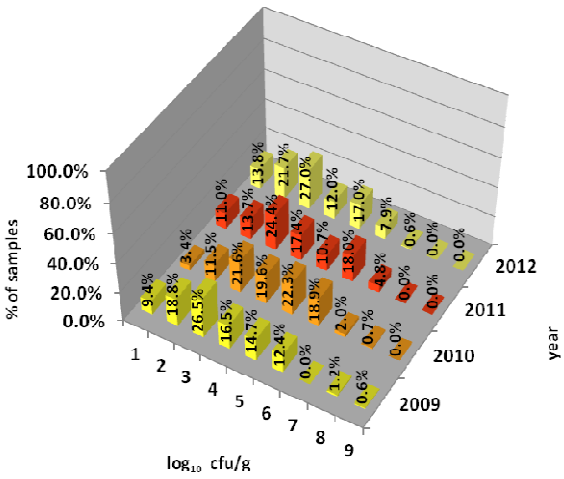

Fig. 12. TPC in all feed material samples

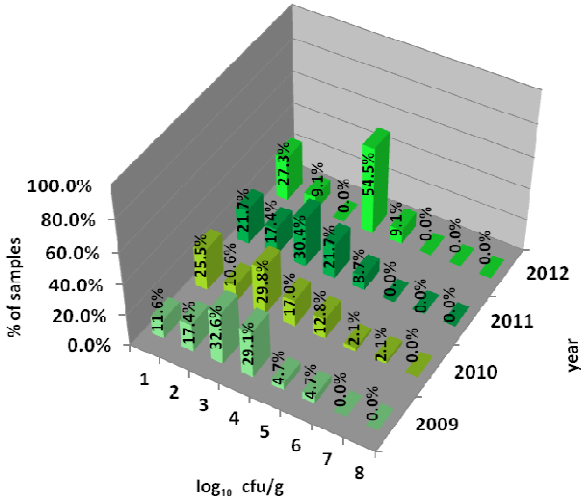

Fig. 15. Fungi count in cereal samples

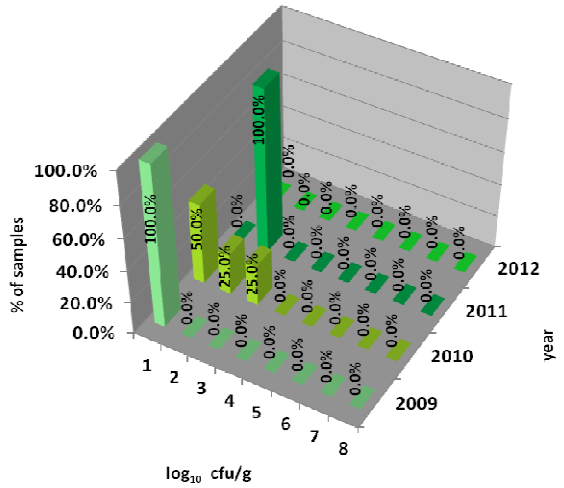

Fig. 13. Fungi count in meat meal samples

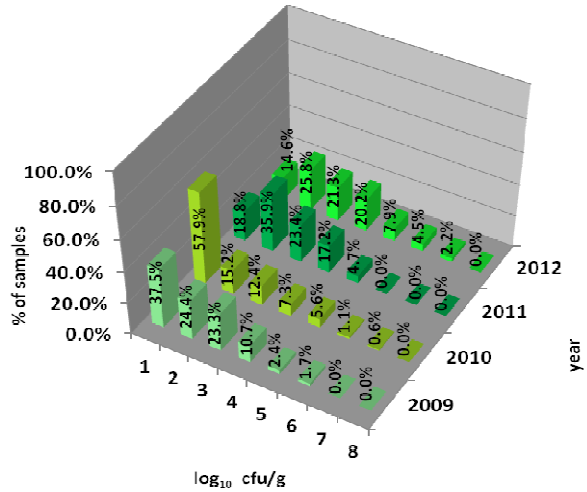

Fig. 16. Fungi count in all feed material samples

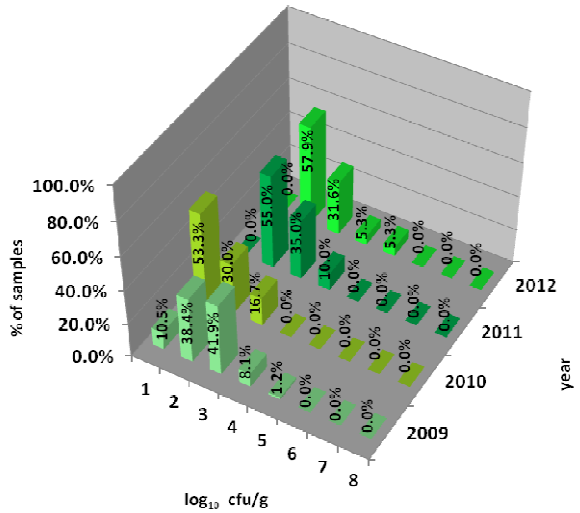

Fig. 14. Fungi count in oil seed samples

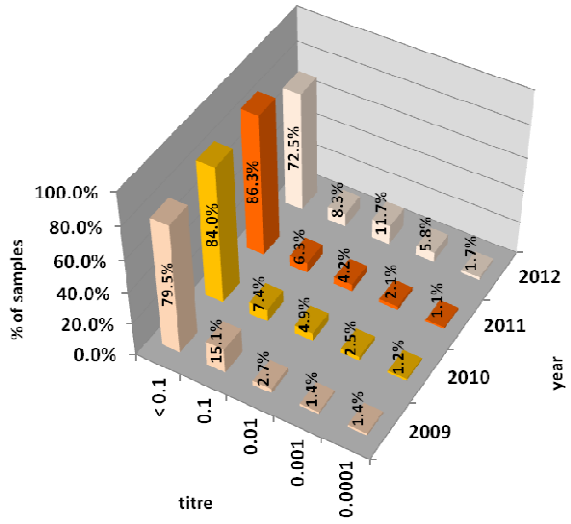

Fig. 17. *Clostridium* sp. titre in meat meal samples

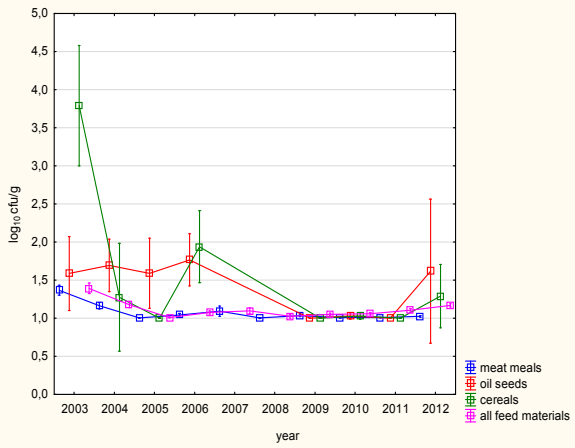

Fig. 18. Average *Enterobacteriaceae* count (±95% confidence interval) in all feed materials

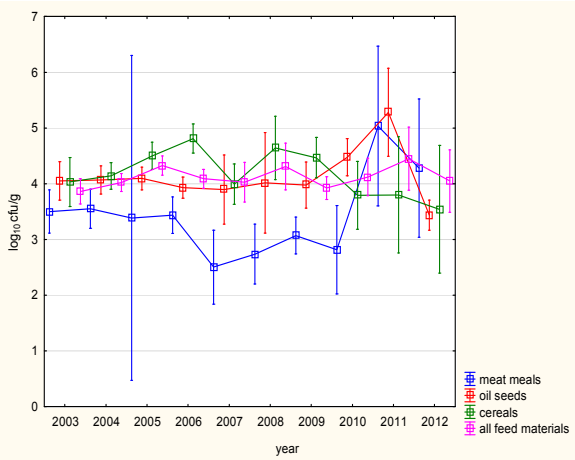

Fig. 19. Average aerobic bacteria count (±95% confidence interval) in all feed materials

Fig. 20. Average TPC (±95% confidence interval) in all feed materials

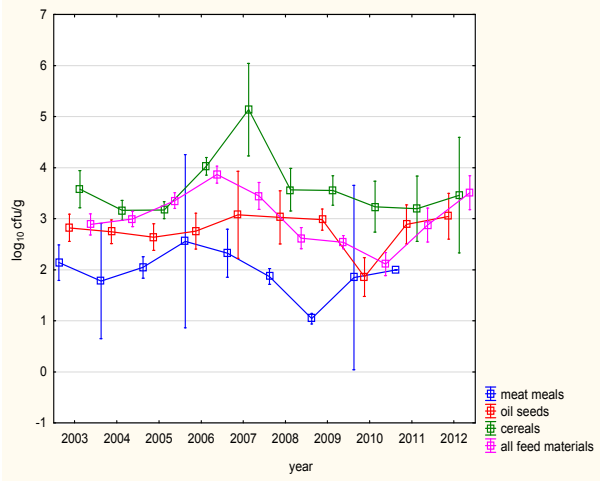

Fig. 21. Average fungi count (±95% confidence interval) in all feed materials

Discussion

Microbial contamination of feed materials used in Poland between 2009 and 2012 was assessed. The results of the study showed that oil seeds and derived are still "critical feed material" among other feed ingredients (24). However, comparing the results from the years 2009–2012 to the years 2003–2010, a slight decline in the number of samples contaminated with *Salmonella* sp. is permanently noticeable (14, 16). The same trend can be observed by comparing the contamination of feed materials of terrestrial animal origin, water, or unidentified feed materials. Barley was indicated as the main source of *Salmonella* sp. among cereals, and blood with fish meals among animal meals (16), similarly like in the previous study. It should be noted that the use of animal meals in EU feed industry is now banned, with the exception of fish meal and blood products (30). Positive findings in poultry meals, and meat and bone meals are relevant to organic fertilisers, in which these kinds of feed materials are used nowadays in the EU. According to the research conducted between 2008 and 2010 by the European Union countries, fish meals are the most contaminated with *Salmonella* sp. (27), which was only partially confirmed by Polish national survey. The discrepancy of the obtained results may be caused by the relatively low number of tested samples of blood meals in Poland. The EU data also revealed decreasing *Salmonella* sp. prevalence in meat and bone meals, which has not been confirmed in this type of meals from the Polish market. In turn, *Salmonella* sp. were nearly twice more often isolated from soya derived from Denmark and rape seed derived from the Czech Republic than from Poland (27). However, despite the apparently low number of positive samples, the average number of cereal and oil seed samples contaminated with *Salmonella* sp. in Poland in the analysed period is, unfortunately, almost two times higher than the average in the EU.

Table 1. Number of feed samples analysed between 2009 and 2012

Kind of feed material	*Enterobacteriaceae*				Aerobic mesophilic bacteria				TPC				Fungi				*Clostridium* sp.				*Bacillus cereus*
	2009	2010	2011	2012	2009	2010	2011	2012	2009	2010	2011	2012	2009	2010	2011	2012	2009	2010	2011	2012	2012
Meat meals	1471	1972	1756	2974	12	18	9	10	4	0	47	170	7	4	2	0	73	81	95	120	35
Oil seeds	0	0	8	9	39	15	25	11	36	15	5	1	86	30	20	19	0	0	0	0	14
Cereals	0	0	29	31	28	13	17	9	14	10	7	23	86	47	23	11	0	0	0	0	0
Unidentified feed materials	375	324	461	998	102	0	0	10	116	123	314	834	280	97	19	56	0	0	0	0	0
Total analysed samples	1846	2296	2254	4012	181	46	51	40	170	148	373	1028	459	178	64	86	73	81	95	120	49

Table 2. Feed contamination by *Salmonella* sp.

Kind of feed material	2009			2010			2011			2012		
	Number of analysed samples	Number of positive samples	Percentage of positive samples	Number of analysed samples	Number of positive samples	Percentage of positive samples	Number of analysed samples	Number of positive samples	Percentage of positive samples	Number of analysed samples	Number of positive samples	Percentage of positive samples
Oil seeds or fruit origin – total	976	14	1.43	1001	25	2.5	1024	29	2.83	630	10	1.59
Rape seed derived	208	7	3.37	202	4	1.98	196	6	3.06	126	1	0.79
Sunflower seed derived	370	0	0	252	0	0	203	0	0	197	3	1.52
Soya derived	299	7	2.34	312	17	5.45	365	16	4.38	180	4	2.22
Linseed derived	0	0	0	0	0	0	13	0	0	17	0	0
Animal meals – total	1578	7	0.44	1861	8	0.43	1950	20	1.03	3263	25	0.77
Poultry meal	245	0	0	465	0	0	352	7	1.99	631	2	0.32
Feather meal	0	0	0	0	0	0	277	0	0	409	0	0
Meat and bone meal	801	7	0.87	994	0	0	818	10	1.22	1578	14	0.89
Blood meal	0	0	0	0	0	0	29	1	3.45	48	1	2.08
Fish meal	532	0	0	402	8	1.99	474	2	0.42	597	8	1.34
Cereals and derived – total	217	2	0.92	247	0	0	526	14	2.66	277	0	0
Maize	31	0	0	28	0	0	30	0	0	27	0	0
Barley	16	1	6.25	15	0	0	24	0	0	20	0	0
Oats	5	0	0	7	0	0	15	0	0	7	0	0
Wheat	87	0	0	106	0	0	132	2	1.52	94	0	0
Rye	0	0	0	0	0	0	21	0	0	21	0	0
Triticale	0	0	0	0	0	0	18	0	0	9	0	0
Millet	0	0	0	0	0	0	1	0	0	1	0	0
Sorghum	0	0	0	0	0	0	3	0	0	5	0	0
Feed material of land animal origin	22	7	31.82	201	2	1.0	395	1	0.25	173	1	0.58
Unidentified feed material	1203	10	0.83	998	115	11.52	1200	24	2.0	942	7	0.74
Water for animals	102	1	0.98	102	8	7.84	106	1	0.94	26	0	0
Feed materials – total	4098	41	1.0	4410	158	3.58	3500	63	1.8	4170	35	0.84

Comparison of faecal contamination of feed ingredients tested between 2003 and 2008 with the data from 2009–2012 also showed a slight improvement (14, 16). Compared to previous years, the percentage of animal meals with exceeding a current allowable *Enterobacteriaceae* limit (7.4% in 2003; 4.9% in 2004; 1.47% in 2006; 1.75% in 2007) decreased. On the other hand, the presence of bacteria from *Enterobacteriaceae* family in some animal meals in the level of 10^7 cfu/g proved that some feed business operators are still ignoring the rules of good practices of producing, manufacturing, and hygiene at the primary production stage. It was proven, that *Enterobacteriaceae* count is a useful indicator of *Salmonella* contamination, and faecal contamination is higher in *Salmonella*-positive samples than in the negative feed samples (10). This principle has been confirmed by results of our study, where similar percentage of animal meal samples does not fulfil the EU limit of *Enterobacteriaceae* count and *Salmonella* contamination (0.53 and 0.69, respectively).

The studies conducted in the last decade showed that microbial contamination of animal meals decreased substantially, and the main source of bacterial and fungal contamination of compound feed are plant ingredients (cereals, oil seeds and derived) (14, 16). Analysis of feed material contamination by mesophilic aerobic bacteria between 2009 and 2012, confirms this tendency. The contamination of plant feed materials (cereals, oil seeds, and derived) by fungi remains constant or increases in rainy years, when the total annual rainfall exceeds the national average (16). It is worth noting that an increase in mycological contamination of cereals in rainy years was correlated with a decrease in *Salmonella* sp. prevalence in cereal grains. The average values of contamination of cereals and cottonseed meals in Greece were 5.50 $\log_{10}$ cfu/g and 4.60 $\log_{10}$ cfu/g for bacteria, and 4.32 $\log_{10}$ cfu/g and 3.19 $\log_{10}$ cfu/g for fungi, respectively (29). Lower average values of bacterial and mycological contamination were obtained in our study in Polish feed ingredients, and they were as follows: 4.04 $\log_{10}$ cfu/g and 4.36 $\log_{10}$ cfu/g for bacteria, and 3.41 $\log_{10}$ cfu/g and 2.76 $\log_{10}$ cfu/g for fungi, respectively. In this comparison, climate differences in Poland and Greece should be taken into account, as they have a significant impact on contamination levels by microorganisms.

B. cereus is an ubiquitous microbe in the environment (soil, vegetation) and it can easily contaminate the feed production or processing systems. The bacterium is of a hygienic importance for processes involving heat treatment, which may activate the germination of *Bacillus* spores and kill the competing, non-sporeforming microflora (11, 22). Besides, *B. cereus* present in feed may pose a health hazard for animals (25) and humans through contaminated food of animal origin (4, 20). Low water activity of feed materials theoretically excludes *B. cereus* growth in these types of products. However, contamination of soya and rape derived reveals that feed material of 0.6 water activity, can also be a source of this pathogen in food production chain. As far as it is known, there is no published data on feed materials contaminated by aerobic sporeformers. Australian authors recorded an outbreak of foodborne disease in humans caused by consumption of raw, sprouted soya seeds contaminated by *B. cereus* (28). *B. cereus* was also detected in dog food sausages of 0.9 water activity, which was associated with food-poisoning in these pets (25), although our unpublished data indicates the presence of the bacteria also in dry pet foods of 0.6 water activity. In our study, only a low percentage of feed materials was highly contaminated in regard to this parameter. However, a great variability of contamination by *B. cereus*, observed by the authors, revealed this parameter as an important hygiene indicator of feed materials and compound feeds.

Feed materials may serve as carriers for a wide variety of microorganisms. It is believed that lower number of microorganisms decreases the probability of pathogens occurrence (3). When pathogens, either to animal or human hosts, contaminate feed, it becomes a potential route of transmission of disease to both populations, and consequently is of a great concern to producers and consumers (6). Besides, the exposure of the organism to a high microbial burden in an unhygienic environment and in feed stimulates the immune system and affects homeostatic pathways that regulate metabolism, nutrient partitioning, behaviour, thermoregulation, and hypothalamic-pituitary-adrenocortical activity (5). Excessive immune activation causes production of the pro-inflammatory cytokines and interferon, activation of the acute phase response, fever, inappetance, amino acid resorption from muscles, redirection of nutrients from accretion in meat, milk, and wool towards liver anabolism of acute phase proteins, and stimulation of leptin production. Consequently, the catabolism predominates over anabolism, resulting in a decrease in animal production and its profitability. Feed contamination by fungi brings animal mycotoxicoses, and through contaminated food of animal origin, human intoxications. It is especially dangerous due to mycotoxin properties (mutagenic, carcinogenic, teratogenic, oestrogenic, neurotoxic, and immunosuppressive). Besides, proteolytic and lipolytic bacteria lead to disintegration of proteins and lipids, which decreases feed nutritive value.

The presence of microorganisms is unavoidable on account of the permanent contact of plant feed materials with soil and dust during the growing season and harvesting. However, the efforts to reduce the number of microorganisms in feed as much as possible show that the ultimate goal is not sterile feed but feed with "safe contamination level".

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