VERSITA

Bull Vet Inst Pulawy 56, 349-354, 2012
DOI: 10.2478/v10213-012-0061-x

MICROBIOLOGICAL QUALITY OF COMPOUND FEED USED IN POLAND

ELŻBIETA KUKIER, MAGDALENA GOLDSZTEJN, TOMASZ GRENDA, KRZYSZTOF KWIATEK, DARIUSZ WASYL[1], AND ANDRZEJ HOSZOWSKI[1]

Department of Hygiene of Feedingstuffs, [1]Department of Microbiology,
National Veterinary Research Institute, 24-100 Pulawy, Poland
elawoj@piwet.pulawy.pl

Received: July 5, 2012 *Accepted: September 7, 2012*

Abstract

The aim of the study was the assessment of microbiological quality of compound feed used in Poland in 2007-2010. The examinations were done at all veterinary diagnostic laboratories operating in the frame of official laboratory system. The occurrence of *Salmonella* sp. and counts of *Enterobacteriaceae* family, mesophilic aerobic bacteria, total microorganisms, and fungi were assessed. Assays were done following Polish, European, and international standards. Percentage of contamination of compound feed for poultry, pigs, and cattle by *Salmonella* sp. ranged from 0% to 3.5%. The highest contamination level by *Enterobacteriaceae* bacteria were detected in wet petfood. No more than 10^6 cfu/g of aerobic bacteria and no more than 10^5 cfu/g of fungi were detected in the feed. The results of the study revealed that the microbiological quality of compound feed used in Poland in 2007-2010 was better than the quality of the feed used in 2003-2006.

Key words: compound feed, microbiological quality, bacteria, fungi.

Permanent growth of human population and civilisation development require increasing production of food. Quality of animal food is directly depended on usage of nutritionally balanced and safe feed. In 2011, about 13 million tons of compound feed were produced in Poland (22). Industrial feed production amounted to 8 million tons and additional 5 million tons were produced by farmers. There are many evidences that pathogens in feed are transmitted through animals and food of animal origin to humans (7, 8). Besides, 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 (4). 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, what results in a decrease in animal production and its profitability. Feed contamination by fungi is responsible for animal mycotoxicoses and, through contaminated animal food, human intoxications. It is especially dangerous taking into account micotoxin properties (mutagenic, carcinogenic, teratogenic, oestrogenic, neurotoxic, immunosuppressive). Due to the short lifetime of slaughter animals, the problem of carcinoma is relatively rarely observed; however, economic losses result from reproduction disorders and immunosuppression. The immunotoxicity effect is usually observed in a number of animal infections caused by opportunistic pathogens (*Clostridium perfringens*, *Escherichia coli*).

In order to recognise microbiological quality of particular feed materials and compound feed used in Poland, the comparison of microbiological status of feed used in Poland during last eight years (9, 10, 11) was done. This paper describes microbiological quality of compound feed used in our country during 2007–2010.

Material and Methods

Compound feed samples were analysed at all veterinary diagnostic laboratories operating in the frame of official laboratory system. Results of analysis were collected at Central Data Base CELAB System leaded by the Department of IT Systems in the National Veterinary Research Institute. All the samples derived from the farmer granaries, feed factories, and imported batches. The number of analysed samples is presented in Tables 1 and 2. Procedures of sample examination were based on PN-EN ISO 6579:2003 (12), PN-EN ISO 4833:2004 (13), PN-ISO 7698:2004 (14), PN-ISO 21528-2:2005 (15), PN-ISO 21527-2:2009 (16), PN-R-64791:1994 (17).

Table 1

Number of compound feed samples analysed in 2007–2010

Kind of feed	*Enterobacteriaceae*				Aerobic mesophilic bacteria				Microorganisms				Fungi			
	2007	2008	2009	2010	2007	2008	2009	2010	2007	2008	2009	2010	2007	2008	2009	2010
Poultry feed			-		9	36	103	90	65	28	75	30	41	76	111	120
Pig feed			-		37	25	23	145	9	10	23	55	38	32	42	30
Cattle feed			-		27	36	32	89			-		192	83	291	214
Dry petfood	161	352	907	1902							-					
Wet petfood	30	56	123	23							-					
Total	1,484	814	2,195	3,159	45	79	158	429	1,139	933	1,391	1,292	271	191	444	364

Table 2

Compound feed contamination by *Salmonella* sp.

Kind of feed	2007			2008			2009			2010		
	Number of analysed samples	Number of positive samples	Positive samples (%)	Number of analysed samples	Number of positive samples	Positive samples (%)	Number of analysed samples	Number of positive samples	Positive samples (%)	Number of analysed samples	Number of positive samples	Positive samples (%)
Feed for poultry	2,559	56	2.2	1,151	13	1.1	1,169	16	1.4	1,643	20	1.2
Feed for pigs	1,853	30	1.6	851	10	1.2	577	6	1.0	876	8	0.9
Feed for cattle	1,011	10	1.0	465	3	0.6	260	9	3.5	406	6	1.5
Feed for fishes	3,281	36	1.1	2,371	33	1.39	4,643	41	0.88	4,947	108	2.18
Feed for rodents	15	0	0.0	6	0	0.0	128	2	1.56	14	1	7.14
Unidentified feed	18	0	0.0	12	1	8.33	42	0	0.0	36	0	0.0
Feed for slaughter animals – total	3,892	38	0.98	3,091	44	1.42	7,145	57	0.80	7,280	132	1.81
Dry petfood	655	1	0.15	844	9	1.07	1,695	22	1.30	3,049	53	1.74
Wet petfood	68	2	2.94	91	0	0.0	195	2	1.03	236	0	0.0
Petfood – total	723	3	0.41	935	9	0.96	1,890	24	1.27	3,285	53	1.61

Table 3

Total annual rainfall and average temperature in August in Poland in 2007–2010

Year	Total annual rainfall (mm)	Average temperature in August (°C)
2007	705.6	17.6
2008	648.6	17.8
2009	683.0	18
2010	832.0	20

Results

The obtained results for quantitative methods were analysed according to distributive series. Figures 1–10 present different microorganisms count in logarithm 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 9,999 cfu/g; $\log_{10}$=4 means range from 10^4 to 99,999 cfu/g, *etc.)*. Level of contamination of compound feed in particular years were compared applying the analysis of regression.

The highest contamination with *Salmonella* sp. in 2007–2010 were noted in feed for rodents with average 2.2% (Table 2). This pathogen were detected also in 1.4% of all analysed samples of fish feed. Percentage of compound feed samples in which *Salmonella* sp. were detected in the analysed period averaged 1.47 (poultry feed), 1.17 (pig feed), and 1.65 (cattle feed). There are unknown details about 2.08% of positive samples detected in this period in ready-to-feed products. Petfood analyses revealed that *Salmonella* sp. occurred in 1.06% of dry petfood and 0.99% of wet petfood.

Nearly 90% of the samples contained less than 10^2 cfu/g of bacteria of *Enterobacteriaceae* family (Fig. 1). Nevertheless, several unidentified feed samples collected in 2009 and 2010 were found to be seriously contaminated (10^{10}-10^{11} cfu/g). Besides, the high level of contamination with *Enterobacteriaceae* (10^5–10^6 cfu/g) was observed also in a few samples of feed for poultry, fish, and rodents. Between the analysed petfood samples, wet petfood revealed significantly higher level of contamination with *Enterobacteriaceae* than the dry products (Figs 2–3).

Only a few samples of compound feed were found to be contaminated with mesophilic bacteria in the level higher than 10^6 cfu/g (Figs 4-6). In most analysed samples, mesophilic bacteria in the number from 10^3 to 10^5 cfu/g were observed. It was similar to the level of feed contamination with microorganisms, where the majority of samples contained also from 10^3 to 10^5 cfu/g. However, a little more samples in which the number of microorganisms exceeded 10^6 cfu/g were noted (Figs 7–9).

Mycological contamination of the feeds (n=1,270) revealed that about 80% of the analysed samples did not exceed 10^4 cfu/g of fungi (Figs 10-12). Less than 10^5 cfu/g of fungi were noted in 96.5% of feed samples in 2007, 97.4% of samples in 2008, 97.3% of samples in 2009, and 94.8% of samples in 2010.

Discussion

In the presented study, the microbiological quality of compound feed used in Poland in 2007-2010 were assessed. The occurrence of *Salmonella* sp. in samples of the feed destined for the most important farm animal species (poultry, pigs, cattle) in Poland in the last four years ranged from 0.6% to 3.5%.

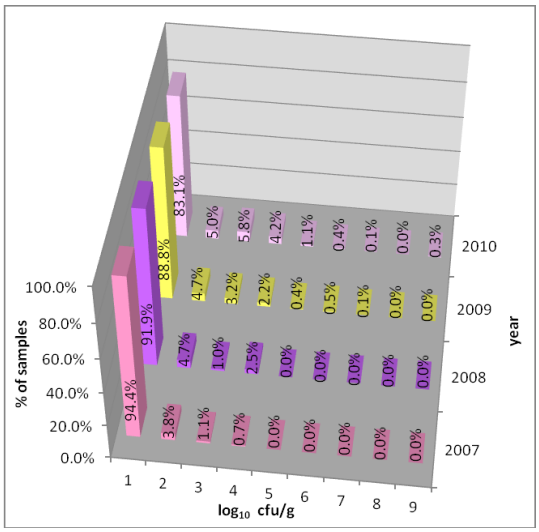

Fig. 1. *Enterobacteriaceae* count in all feed samples.

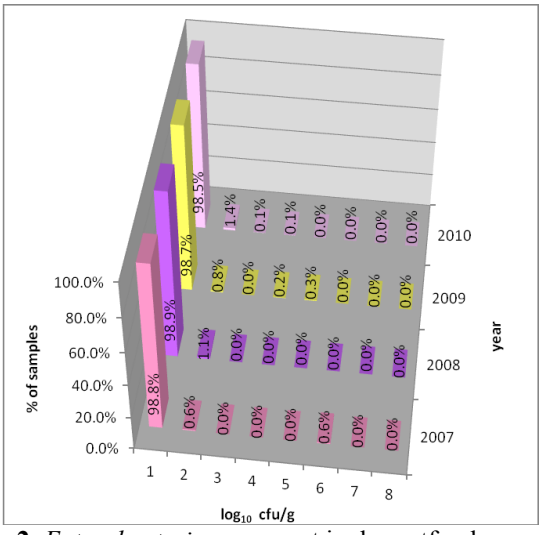

Fig. 2. *Enterobacteriaceae* count in dry petfood samples.

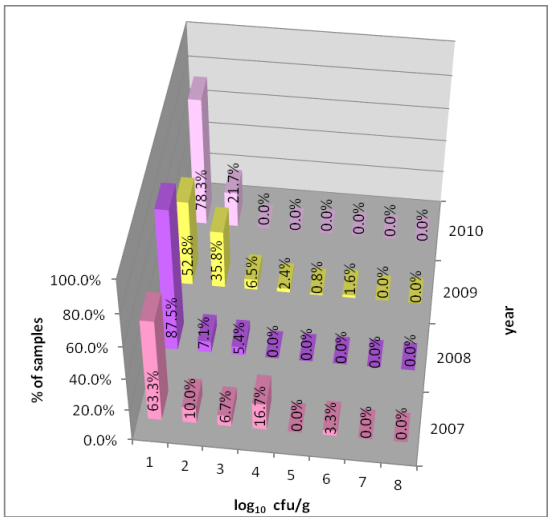

Fig. 3. *Enterobacteriaceae* count in wet petfood samples.

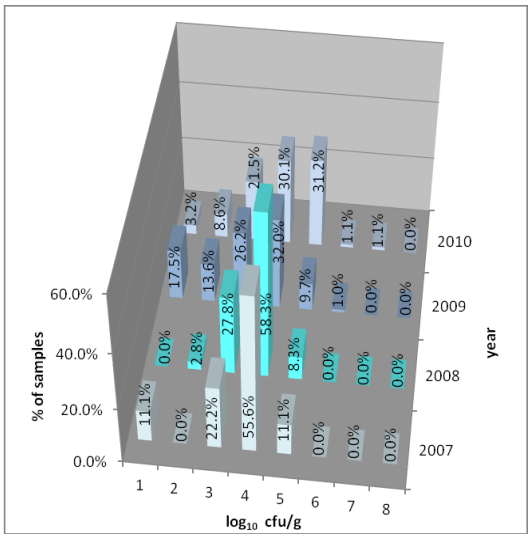

Fig. 4. Aerobic mesophilic bacteria count in poultry feed samples.

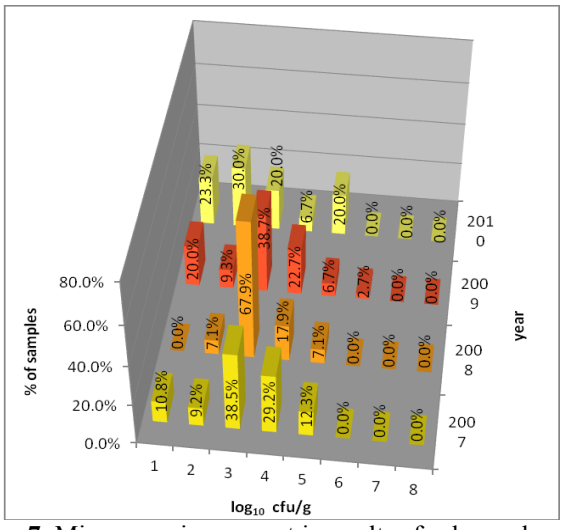

Fig. 7. Microorganisms count in poultry feed samples.

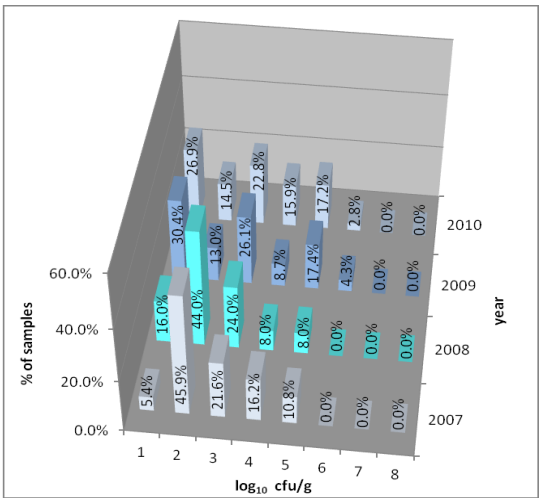

Fig. 5. Aerobic mesophilic bacteria count in pig feed samples.

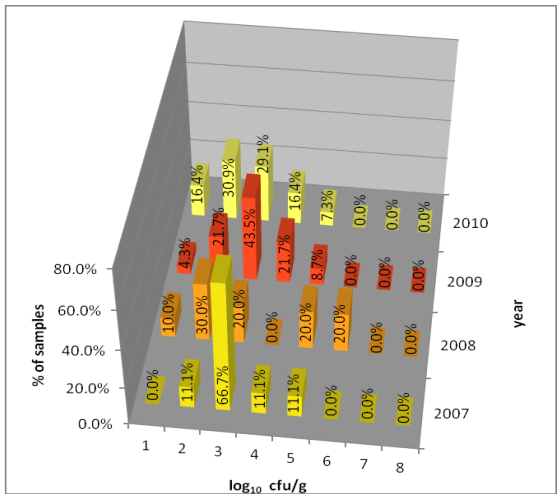

Fig. 8. Microorganisms count in pig feed samples.

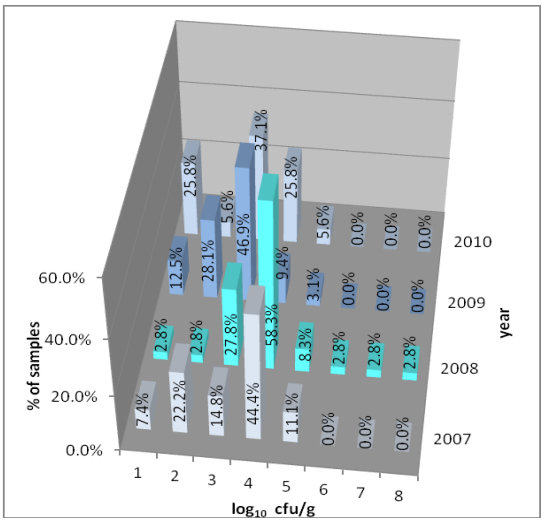

Fig. 6. Aerobic mesophilic bacteria count in cattle feed samples.

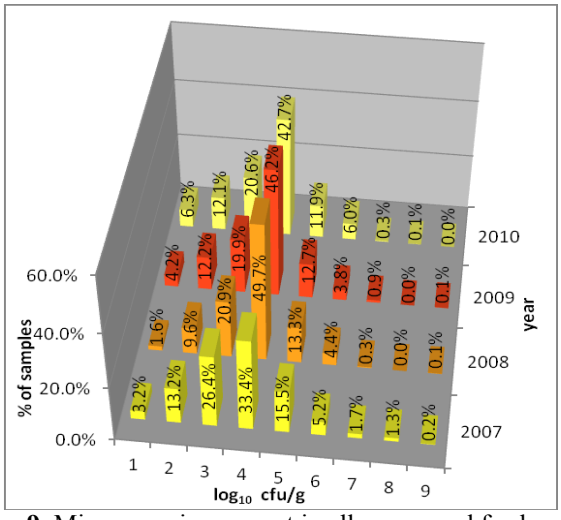

Fig. 9. Microorganisms count in all compound feed samples.

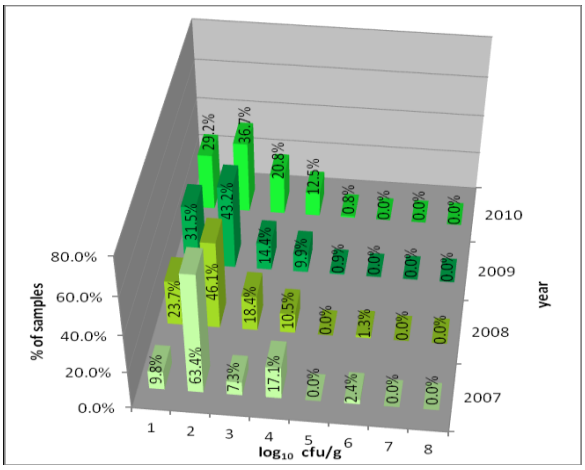

Fig. 10. Fungi count in poultry feed samples.

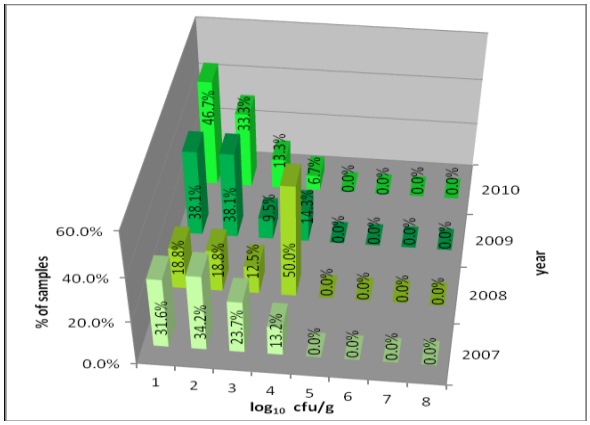

Fig. 11. Fungi count in pig feed samples.

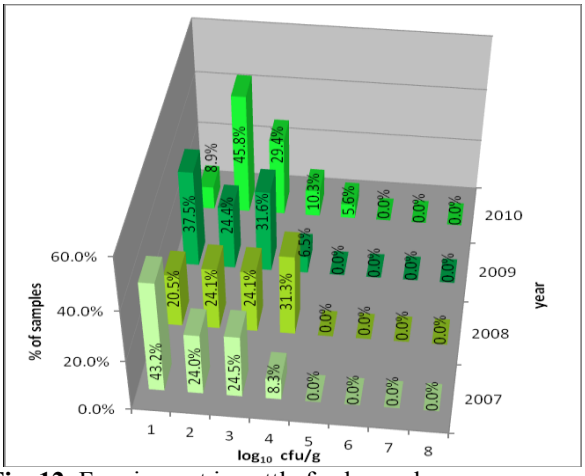

Fig. 12. Fungi count in cattle feed samples.

There is a visible reduction in the number of positive samples of feed for pigs and poultry. According to the research carried out in the European Union countries, poultry and pig feeds are the most contaminated with *Salmonella* sp., which is in agreement with Polish national data only in respect to the poultry feed (2, 3). Divergence of the obtained results may be caused by the relatively low number of tested samples of feed for cattle. However, despite the apparently low number of positive samples, the average number of samples contaminated with *Salmonella* sp. in Poland in the analysed period is, unfortunately, two times higher than the average in the EU. This microorganism was also found in the dry and wet petfood, which identifies and indicates another source of the pathogen in the human environment (20). The likely source of *Salmonella* sp. in food chain can also be feed samples strongly contaminated with *Enterobacteriaceae*, according to the rule that the higher number of *Enterobacteriaceae* increases the probability of *Salmonella* sp. occurrence (5). The analysis of the contamination level of compound feed for farm animals with *Enterobacteriaceae* pointed at a significant improvement in this hygienic indicator. In 2003-2006 up to 22% of the samples contained 10^5 cfu/g of *Enterobacteriaceae* and this value did not exceed 4.2% in 2007–2010 (11).

Enumeration of fungi and identification of micotoxinogenic fungi may be helpful in identification of risky feed during epidemiological investigations (6). The level of compound feed contamination with fungi, as well as bacterial contamination was also lower in 2007-2010 than in 2003–2006, which undoubtedly reflects the improvement of hygienic quality of feed materials studied at the same time (9-11). This is confirmed by the percentage of feed samples with levels of contamination of 10^5 cfu/g in 2003-2006, which was significantly higher than in 2007-2010. In case of a few samples tested in 2007 and 2010, the obtained values were higher than the average, which undoubtedly was due to the weather conditions in these years in Poland (Table 3) (21). It is worth noting that the annual rainfall in Poland calculated on the basis of rainfall recorded in 1971-2000 amounted to 600 mm. A positive correlation, slightly less pronounced than in case of cereal grains, was also visible between the annual amount of rainfall in Poland and the level of compound feed contamination by fungi (9). When rainfall in 2008 exceeded annual rainfall by 8.1%, only 2.6% of compound feed samples contained more than 10^5 fungi per gram. When rainfall in 2009 exceeded average rainfall by 13.8%, 2.7% of the samples contained more than 10^5 cfu/g of fungi. But when rainfall in 2007 exceeded average rainfall by 17.5%, already 3.5% of feed samples contained more than 10^5 cfu/g. The percentage of samples containing more than 10^5 cfu/g of fungi was 5.2%, when rainfall in 2010 was higher by 38.7%. However, according to the Institute of Meteorology and Water Management in Warsaw, in some regions rainfall reached up to 180% of annual rainfall, which additionally deteriorates the feed materials harvested from these areas (21). Similar observations concerning the levels of compound feed contamination with fungi in Poland were reported by Twarużek *et al*. (19) in 2009-2011.

Despite the fact that a gradual improvement of the hygienic status of feed is observed, it appears that the incidence of harmful agents in feed is still underestimated. This is confirmed by numerous cases of so-called feed poisonings, clearly identifying the feed as a source of aetiological agent of the animal disease.

Most feed poisonings in Poland are observed during the time before the harvest (scarcity), when the feed is prepared from last year, long-stored feed materials. Based on these data, it can be assumed that the primary cause of feed poisonings are micotoxins, which by decreasing immunity of animals facilitate opportunistic infections caused by *C. perfringens* or *E. coli*, which was widely observed by the authors. However, taking into account the variety of micotoxins that occur in the nature and low levels of them in the compound feed, proving these relationships is extremely difficult.

According to the authors, there are several reasons why the occurrence of harmful agents in feed is underestimated. The first one concerns difficulties in obtaining representative samples when ingredients or compound feed are monitored (1). An additional source of uncertainty of the results presented above is an inadequate detection by used methodologies, resulting from the presence of various feed additives, for example antimicrobial substance, such as copper sulfate pentahydrate. Our not published studies revealed that when the analysed sample is contaminated by 2.0 x 10^2 cfu of *Salmonella* Enteritidis, and feed contains 16 mg/kg of $CuSO_4$ the sensitivity of the standard method for *Salmonella* sp. detection (12) is lower by 17.5%, compared to feed containing only ground cereal grains.

Since the feed may be a reservoir or vector of pathogens, including zoonotic agents, it is essential for feed producers and farmers to care for the best microbiological quality of the used products. Taking into account the European Union law by which "primary responsibility for feed safety rests with the feed business operator", providing the information about the maximum level of microbial contamination of feed by feed business operator can effectively advertise the product on the market and pose a challenge to competition (18).

The maximum levels of microbial contamination of compound feed proposed by the authors on the basis of the obtained results are 10^2 cfu/g of *Enterobacteriaceae*, 10^6 cfu/g of other bacteria, and 10^5 cfu/g of fungi.

References

1. Andersson, G., Häggblom, P.: Sampling for contaminants in feed. Feed Int 2009, **30**, 16–19.
2. Anon.: Scientific report of EFSA and ECDC. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2009. www.efsa.europa.eu/en/efsajournal/doc/2090.pdf
3. Anon.: Scientific report of EFSA and ECDC. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2010. www.efsa.europa.eu/en/efsajournal/doc/2597.pdf
4. Colditz I.G.: Effects of the immune system on metabolism: implications for production and disease resistance in livestock. Livest Prod Sci 2002, **3**, 257-268.
5. Cox N.A., Bailey J.S., Thomson J.E., Juven B.J.: *Salmonella* and other *Enterobacteriaceae* found in commercial poultry feed. Poult Sci 1983, **62**, 2169-2175.
6. Dalcero A., Magnoli C., Luna M., Ancasi G., Reynoso M.M., Chiacchiera S., Miazzo R., Palacio G.: Mycoflora and naturally occurring mycotoxins in poultry feeds in Argentina. Mycopathology 1998, **141**, 37-43.
7. Davis M.A., Hancock D.D., Rice D.H., Call D.R., DiGiacomo R., Samadpour M., Besser T.E.: Feedstuffs as a vehicle of cattle exposure to *Escherichia coli* O157:H7 and *Salmonella enterica*. Vet Microbiol 2003, **95**, 199-210.
8. Hinton M.H.: Infections and intoxications associated with animal feed and forage, which may present a hazard to human health. Vet J 2000, **159**, 124-138.
9. Kukier E., Kwiatek K.: Microbiological quality of feed materials used in Poland. Bull Vet Inst Pulawy 2011, **55**, 709-715.
10. Kwiatek K., Kukier E., Wasyl D., Hoszowski A.: Microbiological quality of feed materials in Poland. Medycyna Wet 2008, **64**, 183-188.
11. Kwiatek K., Kukier E., Wasyl D., Hoszowski A.: Microbiological quality of compound feedstuffs in Poland. Medycyna Wet 2008, **64**, 949-954.
12. PN-EN ISO 6579:2003 "Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* sp.".
13. PN-EN ISO 4833:2004 "Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of microorganisms - Colony-count technique at 30° C".
14. PN-ISO 7698:2004 "Cereals, pulses and derived products - Enumeration of bacteria, yeasts, and moulds",
15. PN-ISO 21528-2:2005 "Microbiology of food and feeding stuffs - Horizontal method for the detection and enumeration of *Enterobacteriaceae* – Part 2: colony-count method".
16. PN-ISO 21527-2:2009 "Microbiology of food and feeding stuffs - Horizontal method for the enumeration of yeasts and moulds - Part 2: Colony count technique in products with water activity less than or equal to 0.95".
17. PN-R-64791:1994 "Feeding stuffs - Requirements and microbiological examination".
18. Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene.
19. Twarużek M., Błajet-Kosicka A., Grajewski J.: Problem zanieczyszczeń mikologicznych surowców i pasz w świetle aktualnych badań. Pasze Przem 2012, **No. 2**, 86-93.
20. Wojdat E., Kwiatek K., Zasadny R.: Microbiological quality of petfood in Poland, Pol J Vet Sci 2004, **7**, 207-209.
21. www.imgw.pl/images/stories/biuletyn_monitoringu/2010/17_monitoring_rok_2010.pdf
22. www.ppr.pl/dzial-rynek-pasz-w-polsce-2363.