

MICROBIOLOGICAL QUALITY OF ANIMAL FEEDINGSTUFFS IN POLAND

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Received for publication July 20, 2005.

Abstract

The study was undertaken to assess microbiological status of the samples of feedingstuffs taken during an official control. *Salmonella* was isolated from 6.7% of compound feedingstuffs and 4.6% of raw materials. The most often contaminated animal feedingstuffs were cereals, powdered milk and oil meals. The number of *Enterobacteriaceae* in feeds for fattening animals were as follows: lower than 10 cfu/g – 85% of the examined samples, from 10 to 300 cfu/g – 4.6%, higher than 300 cfu/g – 10.4%. The total plate count of bacteria in feedingstuff samples ranged from 10^3 to 10^5 cfu/g. The average level of yeast and moulds in animal feedingstuff samples ranged between 10^1 - 10^4 cfu/g.

Key words: feeds, microbiological quality, microbiological analysis.

Assessment of microbiological status is an important element in quality assurance system during animal feedingstuffs production, trade and feeding (1, 4, 5, 9). In agreement with the recently issued Regulation of the European Parliament No. 183/2005, since January 2006 it will be required from feed business operators to comply with specific microbiological criteria and take measures or adopt procedures necessary to meet specific target (8). This statement means that it will be needed to establish microbiological requirements for animal feedingstuffs on the country level. In Poland it should be relatively easy because in the past such kind of parameters existed and were obligatory in use till the end of 2002 (6). Nowadays, although there are not obligatory microbiological criteria, some zoonotic agents in animal feedingstuffs have to be under control i.e. *Salmonella* or other agents (2, 7). Besides, taking into account animal health and quality of raw materials of animal origin there is a need to establish recommended parameters for other microorganisms. It applies especially to total count of aerobic bacteria, total count of yeast and moulds, *Enterobacteriaceae* number and anaerobic bacteria. In this new situation it is

necessary to have more data concerning microbiological status of animal feedingstuffs produced in Poland.

Taking these aspects into consideration, the study was undertaken to assess microbiological quality of animal feedingstuffs produced and used in Poland.

Material and Methods

One hundred and sixty-nine samples of animal feedingstuffs were examined for the presence of *Salmonella* sp., 51 samples for the number of *Enterobacteriaceae*, 79 samples for the total plate count (TPC), and 93 samples for the number of yeast and moulds. The samples were taken from animal feed processing plants, imported batches, by Veterinary Inspectors during official controls and from farms. In the studies performed in 2004 there was involved Department of Hygiene of Animal Feeding Stuffs, National Veterinary Research Institute in Pulawy (Poland).

Procedures of samples examination. Procedures of sample examination were based on International Standard ISO 6579:2003 dealing with microbiology of food and animal feedingstuffs; horizontal method for the detection of *Salmonella* sp., Polish Standards: PN-R-64791:1994 regarding animal feedingstuffs, requirements and microbiological examination, PN-R-64792:1997 on animal feedingstuffs – enumeration of *Enterobacteriaceae* – most probable number (MPN) and colony count techniques. More detailed procedures for laboratory examination were as follows:

***Salmonella* detection:** for primary enrichment of 25 g of each of the supplied animal feedingstuffs sample, the samples were transferred to 225 ml of buffered peptone water. After 18 h incubation, 0.1 ml of the obtained culture was transferred to the tube containing 10 ml of RVS broth, and 1 ml to the tube containing MKTTn broth. Inoculated RVS broth was incubated at $42^\circ\text{C} \pm 1^\circ\text{C}$ and MKTTn broth at $37^\circ\text{C} \pm 1^\circ\text{C}$ for 24 h. From these broths, portions of the cultures were taken by

means of a loop for inoculating on the surface of plating media i.e. XLD and BGA agars. After incubation at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24 h, colonies suspected to be *Salmonella* sp. were selected for further confirmatory tests.

Enumeration of *Enterobacteriaceae*: from each sample (20g) serial dilutions 10^{-1} , 10^{-2} , 10^{-3} and 10^{-4} were prepared. One ml of each dilution was transferred to a plate and about 15 ml of VRBG agar at $43 - 47^{\circ}\text{C}$ was added. After incubation for 24 h at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$, the plates were examined for the number of colonies presumed to be *Enterobacteriaceae*.

Enumeration of total plate count and yeast and moulds: 1 ml of the following dilutions 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} of each sample was transferred to two plates and about 15 ml of nutrient agar (number of total plate count) and DRBC agar (number of yeast and moulds) at $43 - 47^{\circ}\text{C}$ was added. After incubation for 24 - 48 h at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ (number of total plate count) and for 5 - 7 d at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ (number of yeast and moulds), the plates were examined for the number of colonies.

Results

The numbers of examined samples of different animal feedingstuffs and the results obtained are presented in Tables 1-3. As shown in Table 1, out of 169 feedingstuff samples examined, 10 (5.9%) contained *Salmonella* species. These bacteria were isolated from 6.7% of compound feeds and 4.6% of the examined raw materials. Among different feedingstuff samples examined the most often contaminated were cereals (12.5%), powdered milk (11.1%) and oil meals (5.5%). The number of *Enterobacteriaceae* in feedingstuffs for fattening animals were as follows: lower than 10 cfu/g – 85% of examined samples, from 10 to 300 cfu/g – 4.6%, higher than 300 cfu/g – 10.4% (Table 2). As shown in Table 3, the total plate count (TPC) of bacteria in the majority of the examined feedingstuff samples ranged from 10^3 to 10^5 cfu/g. It was observed sporadically that the highest values of TPC (10^6 to 10^7 cfu/g) were found in feedingstuffs destined for poultry, swine, and cattle. The most often stated levels of yeast and moulds in feedingstuff samples were ranging between $10^1 - 10^4$ cfu/g (Table 3). In 9% of the examined samples the numbers much higher than 10^5 cfu/g of yeast and moulds were found in compound feed.

Table 1
Prevalence of *Salmonella* sp. in examined animal feedingstuffs

| Kind of feedingstuffs | Total number of samples examined | Number of positive samples (%) |
|-----------------------|----------------------------------|--------------------------------|
| compound feed | 104 | 7 (6.7) |
| raw materials | 65 | 3 (4.6) |
| total | 169 | 10 (5.9) |

Table 2
Levels of contamination of animal feedingstuffs by *Enterobacteriaceae*

| Level of contamination | Number of samples at the examined level of contamination (%) | | Total number of samples at the examined level of contamination (%) |
|----------------------------|--|---------------|--|
| | compound feed | raw materials | |
| lower than 10 cfu/g* | 12 (30) | 6 (64.5) | 18 (35.3) |
| from 10 to 300 cfu/g | 5 (12.5) | 0 | 5 (9.8) |
| higher than 300 cfu/g | 23 (57.5) | 5 (45.4) | 28 (54.9) |
| number of samples examined | 40 | 11 | 51 |

Table 3
Levels of contamination of animal feedingstuffs by total plate count of bacteria and yeast and moulds

| Level of contamination | Total plate count of bacteria | | | Number of yeast and moulds | | |
|----------------------------|--|---------------|--|--|---------------|--|
| | number of samples at the examined level of contamination (%) | | total number of samples at the examined level of contamination (%) | number of samples at the examined level of contamination (%) | | total number of samples at the examined level of contamination (%) |
| | compound feed | raw materials | | compound feed | raw materials | |
| 10^{-7} ** | 1 (1.5) | 0 | 1 (1.3) | 0 | 0 | 0 |
| 10^{-6} | 4 (6.1) | 0 | 4 (5) | 0 | 0 | 0 |
| 10^{-5} | 20 (30.8) | 8 (57.1) | 28 (35.4) | 7 (9) | 0 | 7 (7.5) |
| 10^{-4} | 23 (35.4) | 2 (14.3) | 25 (31.6) | 14 (18.1) | 5 (31.3) | 19 (20.4) |
| 10^{-3} | 16 (24.6) | 0 | 16 (20.2) | 14 (18.1) | 5 (31.3) | 19 (20.4) |
| 10^{-2} | 0 | 1 (7.1) | 1 (1.3) | 28 (36.3) | 4 (25) | 32 (34.4) |
| 10^{-1} | 1 (1.5) | 3 (21.4) | 4 (5) | 14 (18.1) | 2 (12.5) | 16 (17.2) |
| number of samples examined | 65 | 14 | 79 | 77 | 16 | 93 |

* - colony forming unit/g.

** - 10^7 mean range from 1.0×10^7 to 9.99×10^7 , 10^6 mean range from 1.0×10^6 to 9.99×10^6 , etc.

Discussion

The results obtained indicate that most of animal feedingstuff samples examined had proper microbiological quality and fulfil criteria established in PN-R-64791:1994 (6), which were obligatory in use till the end of 2002. However, there was small percentage of samples which did not fulfil the parameters required, especially for the absence of *Salmonella*. In comparison to our earlier study, percentage of contaminated animal feeding stuffs by *Salmonella* sp. has increased (10, 11). Details given from several Member States demonstrate that *Salmonella* represents high risk prevalence in animal and vegetable derived proteins as well as in feedingstuffs. But as described already by EU reports from several past years, there is no common trend (3). In some countries, mostly contaminated are the same feed material categories as in the previous year. In EU the overall contamination rate with *Salmonella* varied in animal derived proteins between 0% and 12%, and vegetable derived proteins from 0% to 18% in 2002, but in 2003 these numbers amounted 0.7% and 2.6%, respectively.

Level of contamination by aerobic mesophilic bacteria in the examined animal feedingstuffs was diversified and sometimes relatively high. It depended on feed composition (protein, fat, and carbohydrate level), production technology, microbiological quality of feed materials, and storage conditions. It should be pointed that occurrence of pathogenic bacteria in feed is directly correlated with the level of contamination by microorganisms.

In conclusion it has to be emphasized that the results obtained will be useful for establishing microbiological criteria for animal feedingstuffs in Poland.

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