Occurrence of animal-origin constituents in feeds

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Abstract

The aim of the study was to gather and analyse available data concerning the presence of terrestrial animal constituents in feeds in Poland. A microscopic method of identification of the contaminants was used. Between 2012 and 2013, overall 16,139 samples of feeds were analysed, from which 282 (1.75%) contained elements from terrestrial animals. The percentage of feeds contaminated with such elements was lower in 2013. In 2012, among 8,499 samples analysed, 203 (2.39%) contained ingredients of animal origin, and in 2013, the elements were found in 79 (1.03%) out of 7,640 samples. The percentage of feed samples positive for processed animal protein was relatively low and steadily decreasing. Furthermore, the microscopic method demonstrated to be a very sensitive technique for the detection of constituents of animal origin.

Keywords: feeds, animal protein, contamination, microscopic method.

Introduction

Processed animal proteins (PAP) as well as meat and bone meal (MBM) of terrestrial animals are banned for use in animal feed in European Union (6, 9). The ban was implemented to eliminate the risk of TSE (transmissible spongiform encephalopathy) transmission in livestock and subsequent transmission to humans (2, 3, 22). The detection of animal proteins is based on the microscopic method (7). The current official description of the method is published in Commission Regulation (EU) No 51/2013 (8). The strength of the microscopic method is the combination of the relatively fast detection of animal proteins, sufficient detection of contamination levels as low as 0.02% (11, 24), indication of the type of the detected materials, insensibility to sterilisation temperature, and well established distinction between prohibited (e.g. meat and bone meal) and legally allowed (e.g. blood products) products of animal origin. Microscopy is a good choice for fast screening of samples and might provide the initial indication of the nature of the animal proteins (e.g. fish vs. terrestrial animals) if found (27).

The application of classical microscopy as a screening method was published for the first time in Directive EC/88/1998 (4). As a result of on-going research, a new protocol was published in Directive 2003/126/EC (5). In 2009, the European Commission decided to publish all methods for feed analysis in one Regulation. Annex VI of the Regulation 152/2009/EC (7) contains the protocol which is based on Directive 2003/126/EC. This regulation was replaced in January 2013 by Regulation Commission (EU) No 51/2013 (8). In Poland such laboratory analyses have been in use since 2003.

The aim of the study was the analysis of PAP and MBM in feeds produced in Poland between 2012 and 2013.

Material and Methods

Feed samples. In total, 16,139 samples of different feeds were analysed. They included 1,868 samples of feed materials (e.g. fish meal, blood meal, feather meal), 873 samples of fish meal, and 13,398 samples of compound feeds comprising 3,809 samples of feeds for ruminants and 9,589 samples for non-ruminants. As part of the official control programme, the samples of feeds and feed materials were taken from manufactures, storehouses, means of transport, feed mill, and farms. The samples were collected by official veterinary inspectors and, additionally, by manufactures and farmers. All samples were examined in 16 Regional Veterinary Laboratories and in the National Veterinary Research Institute. Feed sampling rules are

**Detection method.** In 2012 and in January and February of 2013, the microscopic method was used for the detection of PAP in accordance with the guidelines laid down in Commission Regulation (EC) No 152/2009. Next, the analyses were performed according to the method described in the Regulation Commission (EU) No 51/2013.

**The principles of detection.** The microscopy technique allowed detecting components of animal origin, such as bones, fish bones, muscle fibres, cartilages, scales, gills, hairs, and feathers.

**Preparation of the samples.** During the initial preparation of granular and particulate feed for analysis, sieving through meshes of 1 mm was recommended. The obtained fractions were examined as two separate samples. Fifty grams of the sample were taken for analysis and then ground.

**Preparation of sediment and the flotate of analysed suspensions.** According to the Commission Regulation (EC) No 152/2009, 5 g of the sample and 50 mL of tetrachloroethylene were weighed into a beaker. After thorough mixing it was allowed to stand for 5 min to separate the sediment from the liquid. Next, the fractions were transferred onto Petri dish and dried in digesters.

According to the guidelines of the Commission Regulation (EU) No 51/2013 for the extraction and preparation of the sediment and the flotate, a portion of 10 g of the ground sample was transferred into the separation funnel or conical beaker and 50 mL of tetrachloroethylene was added. In the case of fish meal or other pure animal products, or mineral ingredients or premixes constituting more than 10% of the sediment, the portion transferred into the separation funnel was limited to 3 g. The mixture was vigorously mixed for 30 s, next 50 mL of tetrachloroethylene was added to wash down the inner surface of separation funnel and remove all adherent particles. Next, the sediment was separated by opening the tap after standing for 5 min. If a conical beaker was used the mixture was mixed for 15 s. Next, 10 mL of tetrachloroethylene was added to wash down adherent particles on the inner surface. The mixture was left to stand for 3 min. Then, the mixture was vigorously mixed again for 15 s and all particles adhering to the inner surface of the conical beaker were washed out with 10 mL tetrachloroethylene. The resulting mixture was left to stand for 5 min. Next the flotate was removed by careful decanting to prevent the loss of the sediment.

The sediment was dried. If more than 5% of particles of the sediment were 0.50 mm in size, the sediment was sieved through the meshes of 0.25 mm and two obtained fractions were examined. The dried sediment, and the flotate were analysed for typical animal elements using stereo and biological microscopes.

**Detection and identification of fragments of animal origin using staining and embedding reagents.** The microscopic identification of elements of animal origin was facilitated by the use of the cystine reagent and paraffin oil. After a small amount of flotate and cystine reagent were transferred onto Petri dish, they were mixed and heated carefully on hot plate. During the heating process, the elements containing cystine, e.g. hair, or feathers, become black-brown. Paraffin oil was helpful in the identification of bone elements. The bone constituents were identified based on the presence and appearance of lacunae filled with air and visible under biological microscope as black holes about 5 to 15 µm of diameter.

The detection limit of the method was determined at the level of 0.1% (2, 10, 12-18, 26, 29-30). The presence of one typical component qualified the examined sample as positive.

**Results**

The microscopy technique allowed detecting components of animal origin, such as muscle fibres, cartilages, bones, fish bones, scales, gills, hair, and feathers (Figs 1-5).

**Fig. 1.** Mammal bone fragment with oval or round holes (lacunae), 100×

**Fig. 2.** Mammal bone fragment with oval or oblong holes (lacunae), 400×
Fig. 3. Fish bone particle with typical elongated, fusiform holes (lacunae), and numerous dispersed radiant lines (caniculae), 100×

Fig. 4. A fragment of fish gills with characteristic appearance, 200×

Fig. 5. Feather fragment after staining with cystine reagent, 20×

The results of analysis of animal constituents in animal feeds are shown in Tables 1 and 2. In 2012-2013, 16 139 samples of feeds were analysed (Table 1). The constituents of animal origin were detected in 282 (1.75%) samples. The percentage of samples contaminated with ingredients of terrestrial animals was higher in 2012; among 8499 samples of feeds, 203 (2.39%) samples were contaminated with elements from terrestrial animals. In 2013, among 7640 samples, 79 (1.03%) contained animal particles.

A total of 873 samples of fish meal, 1868 samples of feed materials, 3809 samples of feeds for ruminants, and 9589 samples of feeds for non-ruminants were examined for detection of processed animal protein (Table 2). Among 873 samples of fish meal, 13 (1.5%) samples contained elements from terrestrial animals. In 2012, 9 (2.5%) samples of fish meal were contaminated, while in 2013, only 4 (0.8%) samples were contaminated with elements from terrestrial animals.

Of 1868 samples of feed materials, 51 (2.7%) samples contained the constituents of terrestrial animals. In 2013, there was a decrease in the number of samples contaminated with meat and bone meals from terrestrial animals, and the level of contamination was 2.1%.

Additionally, out of 3809 samples of compound feeds for ruminants only 15 (0.4%) samples contained terrestrial animal constituents. The level of contamination was higher in 2012 (0.7%) than in 2013 (0.1%).

Moreover, as shown in Table 2, among 9589 samples of feeds for non-ruminants, the constituents of terrestrial animals were detected in 203 (2.12%) samples. The level of contamination was thus higher in 2012 (2.80%) than in 2013 (1.24%).

Discussion

The results obtained in our study show relatively low percentage of positive samples. Positive results, as indicated by literature data, ranged from 2.9% to 93.75%. In the study performed in Germany, the elements of animal origin were detected in 30 (93.75%) out of 32 samples of feeds for ruminants (16). As reported by Hahn (16), the reason for this was probably due to the possible contamination of the feeds during production, or contamination of containers used for transportation or storage.

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of samples</th>
<th>Number of samples with constituents from terrestrial animals (%)</th>
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</thead>
<tbody>
<tr>
<td>2012</td>
<td>8499</td>
<td>203 (2.39)</td>
</tr>
<tr>
<td>2013</td>
<td>7640</td>
<td>79 (1.03)</td>
</tr>
<tr>
<td>Total</td>
<td>16 139</td>
<td>282 (1.75)</td>
</tr>
</tbody>
</table>
In Switzerland, the introduction of feed ban on MBM feeding resulted in a rapid decrease in the level of contamination. As reported by Boss (1), between 1990 and 1999, 38% of samples were positive; in 2000, 14% of samples were positive; however, in 2001, only 2.9% samples were positive. From 2002, about 1600 samples were analysed and almost all were negative. In 2004, no constituents from terrestrial animals were found in all feeds (15).

In Slovenia, between 1999 and 2008, only 95 (3.46%) samples of feeds, among 2748 samples examined, contained constituents of animal origin. Most of these samples (73.68%) contained fish meal, which was even declared in some cases, or feed for animals allowed to be fed fish meal (23).

In Latvia, between 2005 and 2011, out of 1574 samples examined, 12 (0.76%) contained constituents of animal origin. The highest levels of contamination were detected in 2006 (1.60%) and in 2011 (1.25%). Then, in 2007 and 2008, the lowest level (0.33%) was found (19).

In Poland, a current trend in the reduction of feed contamination with proteins from terrestrial animals is observed. In 2005, 3.79% of samples contained fragments from terrestrial animals and 1.04% of the samples of feeds for ruminants were positive. In 2006, a higher number of samples were contaminated by processed animal proteins. Out of 6149 samples, 429 (6.98%) were positive for the presence of constituents from terrestrial animals; however, only 8 samples of feeds for ruminants contained this kind of proteins (29).

The above data indicate that the presence of animal-origin constituents in feeds for ruminants and non-ruminants, which are declared as free of animal-origin components, varies depending on the region and time of examination. Based on the literature (1) and our studies, it can be claimed that the level of contamination of feeds of animal origin, defined as free of MBM, is becoming lower, reaching most often required zero tolerance standard.

The study describes the microscopic method of detection and identification of PAP in feeds, which is commonly used in EU and Poland, and presents the results obtained during a two-year examination of feeds. The results confirmed that using microscopy technique enables detection of animal-origin constituents. Such constituents as feathers, bones, fish bones, and scales were detected most frequently. The use of chemical reagents, especially cystine reagent and paraffin oil (embedding agent) improved the visualisation and identification of the constituents of animal origin in the samples examined.

Currently, the microscopic analysis is one of the accepted methods in the European Union for the detection and identification of constituents of animal origin in feeds. This method is very sensitive, because it allows the detection of animal-origin elements at the requested level of 1 g/kg (2, 20, 21, 25, 28).

In conclusion, feed contaminated with elements of terrestrial animal origin could play a role in the transmission and epidemiology of BSE. Currently, when using constituents of terrestrial origin in feeding food-producing animals is banned, the most important control measure is to avoid cross-contamination or illegal usage. Moreover, one should state that the microscopic feed examination is a universally useful method for hygiene and quality assessment, because it is quite simple, fast, and sensitive in the detection of animal-origin constituents in feeds.

Conflict of Interests Statement: The authors declare no conflict of interests regarding the publication of this article.

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References

**Table 2.** Results of feeds analysis for the presence of constituents of terrestrial animal origin in the Regional Veterinary Laboratories and National Veterinary Research Institute in 2012-2013

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of samples tested for PAP</th>
<th>Number of samples of non-compliant samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fish meal</td>
<td>Feed materials</td>
</tr>
<tr>
<td></td>
<td>For ruminants</td>
<td>For non-ruminants</td>
</tr>
<tr>
<td>2012</td>
<td>361</td>
<td>932</td>
</tr>
<tr>
<td>2013</td>
<td>512</td>
<td>936</td>
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<tr>
<td>Total</td>
<td>873</td>
<td>1868</td>
</tr>
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