PRESENCE OF PROCESSED ANIMAL PROTEIN IN FEEDINGSTUFFS FOR RUMINANTS

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The purpose of the study was to determine the presence of processed animal protein (PAP) in the selected feed materials and feedingstuffs for ruminants. For the detection of PAP in the examined samples the microscopic method was used. The constituents of animal origin were identified on the basis of typical, microscopically identifiable characteristics i.e. muscle fibres and other meat particles, cartilage, bones, horn, hair, bristles, blood, feathers, egg shells, fish bones, and scales. Overall the presence of PAP was detected in 4 (2.6%) out of 153 samples of compound feedingstuffs for ruminants. Among 21 examined samples of concentrates of feedingstuffs for ruminants none of them was positive. Out of 23 samples of feed mixture under cleaning 6 (26.1%) contained PAP. It should be also noticed that in the case of one examined sample of soya meal the result obtained was positive for presence of PAP. None of the examined samples of feed additives (lysine, phosphates) was positive.

Key words: feedingstuffs, processed animal protein, BSE, microscopy.

Transmissible spongiform encephalopathies (TSEs) are fatal neuro-degenerative diseases including Creutzfeld-Jacob disease in humans, bovine spongiform encephalopathy (BSE) in cattle and scrapie in sheep. The BSE epidemic is strongly suspected to have arisen from feeding of cattle with rendered protein supplements derived from scrapie-infected sheep tissues and its spreading was strictly correlated with the absence of stringent control of rendering processes (20, 23). Since 1988, a ban on enriching cattle feeds with ruminant-derived proteins has led to decline of BSE incidence in the UK. Nevertheless, almost 190 000 cases have been observed from that time in other EU countries (12, 22, 23) and a few in Poland in last four years (13, 14, 22, 23).

At present, a ban on feeding ruminants with animal-derived proteins is in force in EU and Poland (4, 15). There is still possibility of occurrence of cross-contamination of feedingstuffs for ruminants by processed animal protein (PAP) during production and needs to be controlled. Currently, the detection and identification of PAP in animal feedingstuffs in EU countries relies on microscopic evaluation of constituents of animal origin defined as products from processed carcasses and their parts such as muscle...
In 1998 the microscopic method was validated by an intercomparison study and became the only official method suitable for the determination of PAP in animal feedingstuffs in EU and the accession countries (3). Also in Poland, from 2002 animal feedingstuffs are examined by the recently introduced microscopic method, which is recognized by Polish competent authorities as the official method (11, 16). By using this method it was possible to detect PAP in animal feedingstuffs in some EU countries, which introduced ban on PAP in animal nutrition (1, 10). In Poland as yet no data are available on presence of PAP in feedingstuffs for ruminants.

Taking these facts into account, the study was undertaken to determine the presence of PAP in selected animal feedingstuffs produced in Poland for ruminants.

Material and Methods

Feed samples. A total of 221 samples of different feedingstuffs for ruminants were examined for the presence of PAP. Among them 153 samples were taken from compound feedingstuffs and 21 samples from concentrates. Moreover, 23 samples of feed additives for ruminants and 23 samples of feed mixtures taken at the end of cleaning process were checked for presence of PAP. One sample was classified as soya meal.

The sampling period lasted from May 2002 to June 2003. All the examined samples were taken from different Polish feed mills by official veterinary inspectors. It should also be added that in the total number of the examined samples 23 (10.41%) samples were taken from imported batches of feedingstuffs and comprised 1 sample of phosphate, 8 samples of lysine, 13 samples of wheat bran, and 1 sample of a concentrate of feedingstuffs for cattle. The sampling procedure was in accordance with the provisions laid down in the Directive 76/371/EEC (5) establishing Community methods of sampling for the official control of feedingstuffs and in the Regulation of the Minister of Agriculture and Rural Development on detailed principles for sampling for testing animal feedingstuffs (16). The samples were properly packed, sealed and sent to the laboratory by a special delivery service.

Detection method. For detection of PAP in the examined samples of feedingstuffs the microscopic method described in the Directive 98/88/EC of 13 November 1998 was applied with modifications introduced by the laboratory instruction elaborated by the National Veterinary Research Institute for Regional Veterinary Laboratories (3, 11). This Directive and laboratory instruction provide guidelines for the microscopic identification and estimation of constituents of animal origin. An outline of the method is shown in Fig. 1.

The principles of detection. The constituents of animal origin were identified on the basis of typical, microscopically identifiable elements, i.e. muscle fibres and other meat particles, cartilage, bones, horn, hair, bristles, blood, feathers, egg shells, fish bones, and scales. The identification was done both on the sieve fractions and concentrated sediment of the sample (3, 11).
Sample (70 g)
Grind the material (if necessary)

Tetrachloroethylene sedimentation
(2g of the sample + 15ml C₂Cl₄
1-3 min)

Discard supernatant

Sediment dried

Stereomicroscopical (25x, 40x) and microscopical (up to 400x) analysis of the sediment with paraffin oil

Sieve sample (50g)
divide fractions

Fraction I
throw-out
sieve 1.0 mm

Fraction II
through sieve 1.0 mm

Fraction III
through sieve 0.5 mm

Microscopical (40x, 100x) analysis of the fraction III (<0.5 mm)

Fig. 1. Outline of the protocol for microscopic detection of processed animal proteins in animal feedingstuffs.

Introductory preparation of the sample. The seventy g of the sample, depending on the nature of the material depelleted or ground using the suitable grinding equipment, was divided into two representative parts, one of at least 50 g for the sieve fraction and one of the least 20 g for the concentrated sediment (11).

Microscope detection and identification of PAP constituents visually in sieve fractions. The fifty g of the sample was sieved through the sieves (Retsch, Germany) with square meshes 1.00 mm and 0.50 mm of size. In this way it was possible to obtain three fractions of each examined sample. The sieve fraction > 0.5 mm or representative part of the fraction was applied as a thin layer on an object stage and screened thoroughly under the stereomicroscope (Olympus SZ40, Japan; PZO MST-ZOOM, Poland) at 25 and 40 magnifications for constituents of animal origin. Slides made with the sieve fraction < 0.5 mm were screened thoroughly under the compound microscope (Olympus CX41, Japan) up to 400 magnifications for constituents of animal origin (11, 16).
Detection and identification of constituents of animal origin in the concentrated sediment. From 20 g sediment portion 2 g of the sample was weighed with use of suitable balance (AG 285 Mettler Toledo, Spain, accuracy to 0.001g) into a test tube, and in the next step this sample was treated with at least 15 ml of tetrachloroethylene (POCh S.A., Poland). After that the mixture was shaken repeatedly and left to stand for at least one minute and no more than three minutes to allow the sediment to be separated. The sediment was dried in a fume cupboard. The entire dried sediment or a part thereof was examined for bone constituents under stereomicroscope and compound microscope (11, 16).

Detection and identification of PAP constituents by usage of embedding and staining reagents. The microscopic identification of constituents of animal origin was supported by the use of the cystine reagent and paraffin oil. The cystine reagent was prepared in laboratory in accordance with the rule described in the Directive 98/88 (3). The components of cystine reagent were: 2 g of lead (II) acetate trihydrate pure p.a. (POCh S.A., Poland), 10 g of sodium hydroxide pure (POCh S.A., Poland), 100 ml of distilled water. After suspending of suitable amount of the examined sieve fraction and the cystine reagent into Petri dish, the suspension was heated carefully. During heating, processed cystine-containing constituents such as: hairs, feathers become black-brown. The paraffin oil (P.P.F. GEMI, Poland) served as an embedding agent for the identification of bones. The bone constituents were identified in this embedding agent on the basis of presence of bone lacunae filled with air and seen under the microscope as black holes about 5 to 15 µm of diameter (11, 16).

The sensitivity of the microscopic method used was determined on 0.1% level (3).

Results

Overall, PAP were detected in 4 (2.6%) out of 153 samples of feedingstuffs for ruminants (Table 1). As shown in this table, all 4 positive samples originated from compound feedingstuffs. Among 21 examined samples of concentrates of feedingstuffs none of them was positive.

<table>
<thead>
<tr>
<th>Kind of feedingstuffs</th>
<th>No. of samples examined</th>
<th>No. of negative samples (%)</th>
<th>No. of positive samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound feedingstuffs</td>
<td>153</td>
<td>149 (97.40)</td>
<td>4 (2.60)</td>
</tr>
<tr>
<td>Concentrate of feedingstuffs</td>
<td>21</td>
<td>21 (100.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Total</td>
<td>174</td>
<td>170 (97.70)</td>
<td>4 (2.30)</td>
</tr>
</tbody>
</table>
Beside the feedingstuffs for ruminants there have been examined other feed materials and feed additives for ruminants as shown in Table 2. As it appears from the data presented in this table, out of 23 samples of feed mixtures under cleaning 6 (26.1%) contained PAP. It should be also noticed that in the case of 1 examined sample of soya meal the result obtained was positive for the presence of PAP. In all positive samples were detected most often feathers, hairs and bones. Other constituents i.e. muscle fibres and other meat particles, cartilage, bristles, blood were detected only sporadically. None of the examined samples taken from imported batches of feedingstuffs or feed additives (lysine, phosphates) was positive.

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<th>Kind of feedingstuffs</th>
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<th>No. of negative samples (%)</th>
<th>No. of positive samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed mixtures under cleaning</td>
<td>23</td>
<td>17 (73.90)</td>
<td>6 (26.10)</td>
</tr>
<tr>
<td>Feed additives:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- lysine</td>
<td>8</td>
<td>8 (100.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>- phosphates</td>
<td>15</td>
<td>15 (100.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Feed materials-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>soya meal</td>
<td>1</td>
<td>0 (0.00)</td>
<td>1 (100.00)</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>40 (85.10)</td>
<td>7 (14.90)</td>
</tr>
</tbody>
</table>

Discussion

This is a first study in Poland which describes the detailed methodology of the microscopic detection of PAP in feedingstuffs and presents the results obtained during examination of selected animal feedingstuffs for ruminants for the presence of PAP. The results showed that by the usage of the microscopy technique it was possible to detect constituents of animal origin such as: muscle fibres and other meat particles, cartilage, bones, horn, hair, bristles, blood, feathers, egg shells, fish bones and scales. The constituents most often possible to detect were feathers and bones. The other mentioned constituents were detected only sporadically. By usage of embedding and staining reagents it was possible to make constituents of animal origin more apparent. Cystine reagent and paraffin oil were found to be especially useful as agents.

The percentage of positive samples from compound feedingstuffs found in the presented study is generally lower than that reported by other authors (1, 10) where positive results ranged from 2.9 to 93.75%. For example, Hahn (10) in a study performed in northern Germany showed that 30 (93.75%) of 32 feed samples for ruminants contained traces of constituents of animal origin at concentration level equal or lower than 1%. In author’s opinion the reason for this was probably the possibility of switching the production of compound feedingstuff containing animal meal to producing compound feedstuff for ruminants or contamination from containers used to transport or store. Boss (1) reported that in Switzerland after introduction of general
ban on feeding of meat and bone meal (MBM) the number of cases of contamination in feed samples decreased sharply i.e. of 707 samples examined for MBM in 2001 only 2.9% were positive in comparison to 14% in 2000, while in the period from 1990 - 1999 an average 38% of all samples tested were positive by using the microscopy technique. On the other hand, the studies done in Northern Ireland (6) and Great Britain (21) by using ELISA test or microscopy method showed lower values of contamination, where positive results i.e. presence of PAP in batches of feeds declared as for ruminants or free of MBM for other animals were ranging from 0 to 1.13%.

These data indicate that the degree of PAP contamination of animal feedingstuffs for ruminants or declared as free of MBM varies dependently on an area and time of examination. It appears from the literature data that the presence of MBM in feedingstuffs for ruminants and feeds labeled as free of MBM is becoming lower and presently reaching most often zero-tolerance standard which is required in EU countries and will be obligatory in Poland very soon. Moreover, the results of the examination of feed mixture taken at final stage of the cleaning process show that such a process does not guarantee complete removing of PAP from processing line. Taking this into account, a credible structure, based on dedicated feed processing and handling plants, authorized and supervised by the competent authority for the production and handling of feeds for specific animal species, is vital to avoid risks of cross-contamination and the likelihood of feeding banned feed for ruminants. In the absence of such dedicated plants, and where banned and permitted materials are processed and handled in the premises, there must be complete separation of lines and facilities throughout the feed chain, also covering transport, storage and packaging, to guarantee prevention of the possibilities of cross-contamination. Good cleaning and disinfection are also necessary.

In conclusion, since PAP may be present in feedingstuffs for ruminants the consumption of feed for ruminant contaminated by PAP could be an important factor in the transmission and epidemiology of BSE infection. Presently, when PAP is allowed to use in our country in feeding of the other food animals, the most important control measure is avoiding of on-line cross-contamination. For this reason, separated lines for the production of feeds for ruminants or declared as free of PAP are recommended.

References

6. Final report of mission carried out in the United Kingdom (Northern Ireland) from 24 to 28 June 2002 in order to evaluate the implementation of certain EC measures aimed at the eradication, control and prevention of Transmissible Spongiform Encephalopathies (TSE) and amendments proposed by the UK as regards the date based export scheme (DBES).


15. Regulation by the Minister of Agriculture and Rural Development of 19 March 2001 amending regulation concerning veterinary conditions required during production, processing, marketing or storage of unedible raw materials of animal origin, feedstuffs and feed additives. (O. J. No 22, item. 254).

16. Regulation by the Minister of Agriculture and Rural Development of 23 January 2003 on methodology of analysis procedures within determination of contents of food components and feed additives in feed material, premixes and feed mixes (O.J. No 66, item 613, 614).

17. Regulation by the Minister of Agriculture and Rural Development of 13 March 2001 on detailed rules of animal feedingstuffs sampling for tests (O. J. No 49, item 418).


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