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STUDY ON MONITORING THE APPLICATION OF EUROPEAN LAW REGARDING THE BAN OF USING PROCESSED ANIMAL PROTEIN FOR FARM ANIMALS IN ROMANIA

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Abstract

Entry into force of the ban on the use of the processed animal proteins (PAP) in feed for farmed animals and especially in ruminants is considered an important measure of prophylaxis to prevent BSE so the identification and the microscopic estimation of the constituents of animal origin became the official method and mandatory in all Member States. The method allows the identification of bone fragments, muscle tissue, hair, feathers, shell fragments and plant and mineral components. The microscopic analysis depends upon the identification of the hsitological characters macro-and microscopic structural of the processed animal tissue added in feed mixtures. To identify the microscopic animal constituents, some technical conditions are essential: optical microscope, stereo microscope, high-density solvent clarifying agents, microscope with digital visual images support as decision support. Between 2009-2012 were analysed to verify compliance 480 feed samples. Of the total samples analysed , 426 were compound feed (for ruminants, poultry, pigs) and 54 were raw materials (fish meal, premixes, animal fats). The incidence of processed animal proteins in this matrix was on average 0.46%, compared to 99.54% average of samples compliant. Nowadays four different approaches are applied to control the compliance on the prohibition of feeding with PAP: microscopic analysis, immunological analysis, infrared spectroscopy and microscopy (NIR), polymerization chain reaction (PCR). In this stage, the microscopic method is the only method validated and able to identify the nature of the animal in feed components with detection limit of <0.1%, but it cannot accurately detect the species of origin.

Key words: *feed, Bovine Spongiform Encephalopathy, microscopic identification, processed animal proteins*, *transmissible spongiform encephalopathies.*

INTRODUCTION

Zoonoses are defined by the World Health Organization as "diseases and infections which are naturally transmitted between vertebrate animals and man".

Although largely studied as infectious diseases are zoonoses, fungal or parasitic on animals, in this paper was aimed at increasing knowledge of control measures for surveillance and monitoring of transmissible spongiform encephalopathy's (TSEs) in terms of transmissibility of zoonotic diseases from animals to humans caused by prions, whose main source of infection by feeding ruminant processed animal proteins from the processing of animal by-products. Today it is accepted that the most likely route of infection with bovine spongiform encephalopathy (BSE) occurred as a result of consumption of feed containing protein derived prion infected.

The prion hypothesis, protein origin infectious particle, has been widely accepted, as defined by Prusiner et al (1982), as some non-conventional agents that are much lower than the viruses consist of a single type of protein noted acronyms PrP^S, free of nucleic acids. Prion diseases or transmissible spongiform encephalopathy's (TSEs) are considered neurodegenerative diseases that may occur in both humans and animals and can be transmitted by natural or experimental infection. Contamination of feed carnivorous encephalopathy BSE led to spongiform in domestic cats (ESF) and if the big cats bred in captivity and spongiform encephalopathy of exotic ungulates (EVE) that affected the species of hoofed animals in zoos (Wells et al. 2004). Problems derived from the the spread of prion diseases is not only to BSE, prions from other sources, especially in cervids susceptible to CWD (Chronic Wasting Disease) can manifest risks to humans (Belay at him, 2004).

The impact and consequences improper use of certain animal by-products, on public and animal health, the safety of food and feed chain, consumer confidence, has led to the ban of feeding processed animal proteins. This paper describes the analytical method for the detection and identification of processed animal proteins in feed and tissues.

MATERIALS AND METHODS

Microscopic identification of animal derivatives is based on knowledge of basic histology. Histological structure of components that have undergone heat treatment as is the case PAP is different from the normal structures. The differences can be accentuated, such as soft structures (muscle, epithelial tissue, connective tissue, etc.) or less accentuated, some unaltered, such as hard structures (bone tissue, teeth, scales, feathers, etc.). Drying and grinding are changed not only the initial histological structure but also macrostructure, so generally available for identification only small fragments. Because these fragments are then included in complex matrices (cereals, legumes, seeds ground and their derivatives, minerals, vitamins, feed additives etc) the extraction and separation of these fragments becomes all too important and time consuming to be identified. Bone fragments present in the mixture should be differentiated based on typical lacunae between the bones from fish and terrestrial animals (mammals and birds).

To identify the animal components are essential following technical conditions (equipments, reagents and standards):

Equipments: electronic analytical balance, laboratory electric mill, sieve with size of 250 μm, oven drying sediment compound microscope with binocular and phase contrast and polarized light, adapter for digital camera, stereomicroscope with side illumination device on optical fibber, used for microscopic examination and identification of animal components and visual digital image database for decision support

Reagents and standards: chlorhydrate, paraffin oil or glycerol for microscopic examination sediment, alcohol, acetone, tetrachloroethylene, alizarin Red, cystine reagent, iodine potassium iodide solution; commercial solution of sodium hypochlorite, CRM (Certified Reference Material).

Constituents of animal nature (bone fragments, scales, shells, feathers, egg shells fragments) are separated from the concentrated sediment through flotation technique in a high-density solvent and identify by macroscopic examination on stereomicroscope and by microscopic examination on compound microscope.

Separation of constituents is facilitated by sieving and concentrated sediment technique, which consists in suspending the sample in a solvent with high density (chloroform, tetrachloroethane) which makes the bones, minerals and other high-density fragments to settle and plant tissues and other low-density constituents, to be found in the floating. Alizarin red staining for identification is very important because only colored bone and cartilage making them easy to recognize separately. Observation of lacunae by clarifying preparation is a densely agent (paraffin, glycerol) that cannot penetrate the capillary so that they remain filled with air and appear black on a light background of the bone fragment. The main elements are microscopic diagnosis of bone fragments. The size of the fragments identified on compound microscope is about 30 μm. Details included are identifiable dimensions between 2.5-5 μm.

The sensitivity of the method depending on the type of constituents of animal nature can be detected very small amounts (<0.1%) of constituents in feed mixtures as assessed by microscopic identification detection capability demonstrated in the validation of the method. Method which formed the basis of this study is accredited to ISO/CEN 17025 by RENAR (Accreditation Association Romania).

RESULTS AND DISCUSSIONS

In our institute in the microscopy laboratory in the period 2009-2012 were analysed for verification of compliance about 480 feed samples. Of the total 426 samples were feed mixtures, 4 non-compliant and 54 samples were raw materials all compliant. Distribution of samples analysed per years was as follows: 117 samples in 2009, 120 samples in 2010, 161 samples in 2011 and 82 samples in 2012 (Figure 1).

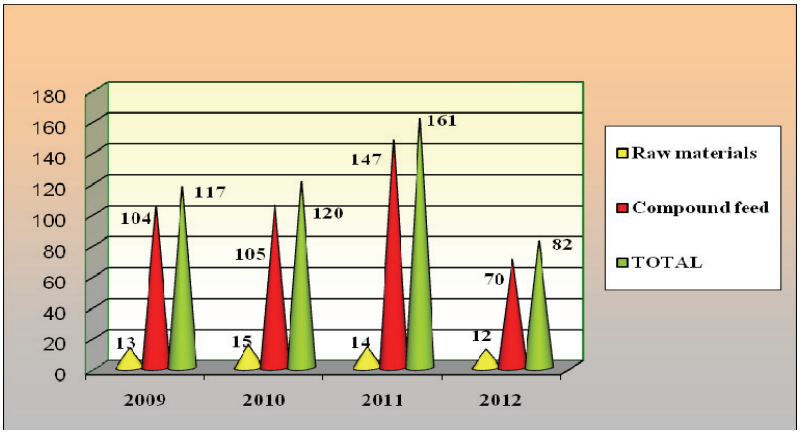

Figure 1. The distribution of analised samples for years

Distribution of samples analyzed by types of matrices was as follows: 155 samples for ruminant compound feed, compound feed for pigs 120 samples, 151 samples combined fodder for birds, 24 fish meal samples, 28 samples of premixes and 2 samples animal fat (Table 1).

Incidence of processed animal proteins in this matrix analysed ranged from an average 0.46% from 99.54% compliant media samples (Figure 2).

Table 1. Distribution of samples analyzed by types of matrices

Type of samples analyzed	No. of samples analised	No. of negative samples	% negative samples	No. of negative samples	% positive samples
Compound feed for ruminants	155	153	98.71	2	1.29
Compound feed for pigs	120	119	99.17	1	0.83
Compound feed for poultry	151	150	99.34	1	0.66
Fishmeal	24	24	100.00	0	0.00
Premixes (PVM)	28	28	100.00	0	0.00
Animal fats	2	2	100.00	0	0.00
TOTAL	480	476	M= 99.54	4	M= 0.46

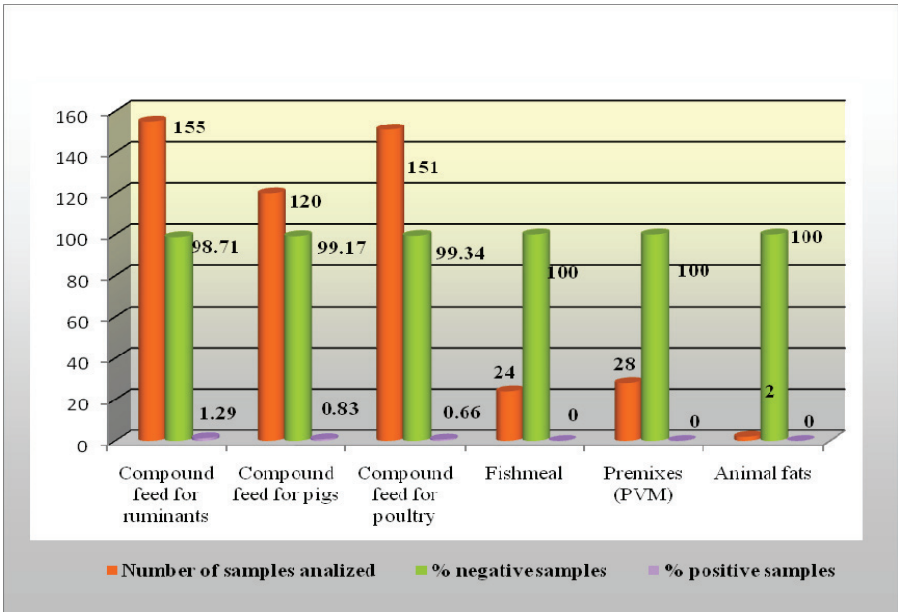

Figure 2. Incidence of processed animal proteins in the samples analised

In positive samples the epidemiological surveys are performed by veterinary inspectors for establishing traceability of raw materials, farms, animal feed, transport means for establishing the way of contamination (cross or illicit). In all situations apply their withdrawal procedure of consumption and distortion, and supplement control measures and surveillance of livestock. Following investigations have not revealed any cases of illicit use.

Laboratory records - for samples analysed are kept digital micrographs recorded in a folder with the number of the sample that is applied by editing (AxioVision LE 4.1) sample number and corresponding objective and micrometer scale that worked.

In the descriptive part (view info) denotes the number of the sample , the name of the analyst, microscope objective used (numerical aperture NA and specifies the device using phase contrast), opening value of the condenser, the filter used (neutral, colored or polarized light).

The rest of the descriptive data (date, room type, image resolution) are automatically retrieved from the camera. During description of constituents identified lacunae will specify appearance when bone fragments including the ratio between the diameter and length / width and the portions of muscle tissue will be mentioned and muscle rate calculated. Saved file will contain the image of the sample number and the descriptive part.

CONCLUSIONS

Testing laboratory has developed a laboratory microscopy applied to control feed with original contributions to the evaluation of digital photomicrographs, future work is focused on microscopic identification by using an expert system to correct recorded images.

Given the average non-compliant (%0.46) samples correlated with the number of samples analyzed (480) but also the diversity matrices, can conclude that operators comply with the requirements for processed animal proteins in feeds for farm animals.

Currently compliance on the prohibition of feeding with PAP, are based on four different approaches: microscopic analysis, immu-noassays, spectroscopy and infrared microscopy (NIR), the polymerization chain

reaction (PCR). According interlaboratory studies conducted by European Union Reference Laboratories in collaboration with Member States, all methods have advantages and disadvantages of technique used and performance parameters.

In Romania, microscopic method is the only method accredited and able to identify the nature of animal feed components with a detection limit of <0.1%, but cannot accurately detect the species of origin.

Based on studies and scientific opinions adopted, the quantitative assessment of BSE risk posed by processed animal proteins assuming a contamination of 0.1 % (detection limit), the European Union increasingly taking into account the perspective of eliminating the ban on the use of PAP in non-ruminant animal feed, subject to intraspecific recycling requirements.

Feed ban on the use of processed animal proteins interspecies constitutes a new challenge for analytical methodologies and identification methods will require better and more precise control.

Testing laboratory which formed the basis of this study, is accredited for microscopic method and validate alternative method based on polymerase chain reaction (PCR) for detection of ruminant constituents with the prospect of enlargement and to identify species from non-ruminant origin.

Using this new method in conjunction with or replace, as applicable, the microscopic method is very valuable for the official control in Romania, in order to meet the requirements of European legislation, implementing a feed ban on correct nutrition.

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