SUITABILITY OF THE FEEDCHECK TEST FOR THE DETECTION OF PROCESSED ANIMAL PROTEIN IN FEEDINGSTUFFS

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Abstract

The aim of the study was to determine the suitability of the FeedCheck test for the detection of processed animal protein (PAP) in comparison to the microscopic reference method. Among 72 examined samples of feedingstuffs there were raw plant materials, meat-and-bone meal (MBM), samples found as positive and negative after the microscopic method and samples with known levels of MBM’s. In all feed and feed material samples which contained mammalian and fish origin PAP positive results by microscopic and FeedCheck methods were obtained. It should be pointed out that although fish meal is classified as PAP, the producer of the test did not declare this material as detectable by the FeedCheck test.

Key words: feedingstuffs, animal meal, processed animal protein detection, FeedCheck test.

In the past, processed animal protein was widely used in animal feeding and it is suspected to be the main factor of bovine spongiform encephalopathy (BSE) epidemic in Europe and other countries around the world (7, 10, 14-16). At present, a ban on feeding farm animals on animal-derived proteins is in force in all EU countries. Any feed material or compound feedingstuffs should not contain processed animal protein of terrestrial animals and fish origin in the case of feeding ruminants, so it has to be controlled by competent authorities (1, 3, 8). There is still possibility of cross-contamination of feedingstuffs by processed animal protein (PAP) during production and after production stages. Currently, the microscopic method is a reference method for the detection of PAP. This method relies on the detection of the presence of meat particles, cartilage, bones, horn, hair, bristles, blood, feathers, eggs, shells, fish bones, and scales (1, 8, 9, 12). The microscopic method is time-consuming and requires high experience of laboratory staff. It may be useless when it is necessary to obtain results quickly in preliminary test of feed. Considering the legislative and practical aspects, there is a need for a new rapid and reliable method for routine use (2, 6, 13).

Recently a new FeedCheck test was elaborated and recommended for practical use (4). According to producers’s statement the FeedCheck is a simple and rapid test for the detection of the meat and bone meal (MBM) in feedingstuffs. The test was validated by producer using 17 varieties of final feed products. The test is highly sensitive because it detects 0.1% (w/w) of MBM and 1% (w/w) of mammalian MBM in animal feedingstuffs (5). Such kind of test parameters means that the test should be appropriate for monitoring and routine use in official laboratories.

Taking these into account, the study was undertaken to determine the suitability of FeedCheck test for the detection of PAP in feedingstuff samples and compare with the microscopic method.

Material and Methods

Feed samples. Seventy two samples were examined. The samples were divided into five groups: (1) meat and bone meals (pure ruminant MBM, mixed species MBM, poultry meal, feather meal, fish meal, horn meal, dried pig blood cells), (2) raw plant materials (sunflower, soya, maize, wheat-barley mixture, rape), (3) compound feedingstuffs with known content of mammalian MBM (2%, 1%, 0.5%, 0.2%, 0.1%), (4) feedingstuffs found as positive (positive control samples), and (5) feedingstuffs found as negative (negative control samples) in the microscopic examination in our laboratory.

Microscopic detection method. For the detection of PAP in the examined samples of feedingstuffs the microscopic method described in the Directive 2003/126/EC of 23 December 2003 was applied with modifications introduced by the laboratory.
instruction elaborated by the National Veterinary Research Institute for Regional Veterinary Laboratories (1, 8, 9). The Directive and laboratory instruction provide guidelines for the microscopic identification and estimation of constituents of animal origin. The constituents 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 sieved fractions and concentrated sediment of the sample.

Rapid immunoassay FeedCheck test. In order to satisfy user-specific requirements, the FeedCheck Test for MBM combines two tests in one test strip (4). The first test indicates the presence of avian and mammal MBM, while the second one indicates the presence of mammalian-derived MBM in the sample. The assay uses a double antibody sandwich format. Antibodies specific to mammalian and avian MBM or exclusively mammalian MBM are coupled to a colour reagent and incorporated into the lateral flow test strip. When the test strip is placed in a feed extract containing mammalian and/or avian MBM, the antibody-colour reagent will bind to the MBM component. This complex then penetrates through the porous membrane. The membrane contains a control line and two test lines. The presence of one red-colour line (the control line) indicates the sample is negative for mammalian and avian MBM. The presence of 2 coloured lines indicates that the sample is positive for mammalian and avian MBM at or above 0.1% and the presence of 3 coloured lines indicates that the sample is positive for mammalian MBM at or above 1%. The examination was carried out inside the test tubes. The extraction buffer was poured into the test tube to the one-ounce mark and then the portion of the feed sample was added. After that the tube was covered and shaken for 15 s to extract the target protein from the sample. Then, one test strip was placed into the tube. The results were read between 10 and 15 min after placing the test strip into the tube.

Results

The number and sort of the examined samples and detailed results obtained are shown in Table 1 and Figs 1 to 6.

As shown in Table 1 and Fig. 1 all meat and bone meal samples were found positive both by microscopic and FeedCheck methods. In the case of fish meal samples examined by FeedCheck test (Fig. 1 strip f) two coloured lines were present on the test strip what means the positive result. It should be pointed out that although fish meal is classified as PAP, the producer of the test did not declare this material as detectable by FeedCheck.

Among 5 samples of feed material of plant origin and 5 control negative samples none of them were positive in both methods used (Table 1 and Figs 2 and 3). On all test strips there were present the control lines only. In the case of 5 naturally contaminated by PAP feed control samples found as positive in microscopic methods, all these results were confirmed by FeedCheck test (Table 1 and Fig. 4).

As it appears from the data presented in Table 1 and Figs 5 and 6, all samples with MBM at 0.1 to 2% level were positive in both methods used.
Fig. 1. Samples of animal meals: a) mixed species MBM, b) poultry meal, c) pure ruminant MBM, d) horn meal, e) feather meal, f) fish meal, g) dried pig blood cells.

Fig. 2. Samples of plant materials: a) sunflower, b) soya, c) maize, d) wheat-barley mixture, e) rape.

Fig. 3. Negative control samples.

Fig. 4. Positive control samples.

Fig. 5. Sample with 2% of mammalian MBM.

Fig. 6. Sample with 0.1% of mammalian MBM.
Discussion

Enforcing legislation on feed ban (3, 11) requires the availability of reliable analytical methods and sufficient proficiency of control laboratories to apply the methods in the proper way. Taking into account the practical aspects of PAP detection, there is a need for a simple, rapid and reliable test (2, 5-7, 9). Moreover, the new methods should show specific performance characteristics depending on which aspect of PAP ban is addressed (2, 5, 6). For instance, enforcing the PAP ban on feed for ruminants requires a method that detects all PAP’s, irrespective of the species composition, at the possible lowest level. In the case of the method of the analysis of feed for animals other than ruminants, there is a need to differentiate between total PAP’s and fish meal. In this study the main goal was to determine, whether FeedCheck Test allows detecting the presence of mammalian MBM and at what the lowest level.

The results obtained in this study showed that by the usage of FeedCheck it was possible to detect mammalian MBM at 0.1% level in feed and feed materials. Comparison of the results obtained in this study shows that FeedChek test yields results comparable with those received using the microscopic method which is recognised and used as the reference one (1). The results of a comparison study were reported also by Gizzi et al. (5). Microscopy, polymerase chain reaction (PCR), and immunoassay FeedCheck tests were compared in the study. The FeedCheck test was the only one that achieved 100% specificity, sensitivity, and accuracy when testing for total PAP. Our results showed that the FeedCheck test proved to be highly specific and sensitive, but it can give false positive result when the fish meal is present in the sample. It means that in the case of fish meal the FeedCheck lacks sufficient specificity.

In conclusion, provided that the assays can be further improved regarding specificity aspect, immunoassays should be considered as important tools for the detection of PAP in feed. But even right now, without such a kind of improvement, the FeedChek seems to be useful for screening feed and feed material production in feed meal companies.

References


11. 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, feedingstuffs and feed additives. (Law Gazette No. 22, item 254).

12. 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 (Law Gazette No. 66, items 613 and 614).


