FOOD COMPOSITION AND ADDITIVES

Determination of Processed Animal Proteins, Including Meat and Bone Meal, in Animal Feed

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An intercomparison study was conducted to determine the presence of processed animal proteins (PAPs), including meat and bone meal (MBM) from various species, in animal feed. The performances of different methods, such as microscopy, polymerase chain reaction (PCR), immunoassays, and a protocol based on iquid chromatography (LC), were compared. Laboratories were asked to analyze for PAPs from all terrestrial animals and fish (total PAPs); mammalian PAPs; ruminant PAPs; and porcine PAPs. They were free to use their method of choice. In addition, laboratories using microscopy were asked to determine the presence of PAPs from terrestrial animals, which is applicable only to microscopy. For total PAPs microscopy, LC and some immunoassays showed sufficient results at a concentration as low as 0.1% MBM in the feed. In contrast, PCR was not fit for purpose. In differentiating between MBM from terrestrial animals and fishmeal, microscopy detected 0.5% of terrestrial MBM in feed in the presence of 5% fishmeal, but was less successful when the concentration of MBM from terrestrial animals was 0.1%. The animal-specific determination of MBM from mammals or, more specifically from either ruminants or pigs, by PCR showed poor results, as indicated by a high number of false-positive and false-negative results. The only PCR method that scored quite well was applied by a member of the organizer team of the study. Immunoassays scored much better than PCR, showing sufficient sensitivity but some deficiency in terms of specificity. The results also demonstrated that the reliable determination of MBM from ruminants has not been resolved, especially for low concentrations of MBM (0.1%) in feed. Comparison of the results for mammalian MBM from all methods indicated that, for control purposes, the immunoassay method, especially when applied as dipsticks, could be used as a rapid screening method combined with microscopy to confirm the positive samples. However, implementation of such a system would require that the immunoassays were previously validated to demonstrate that this approach is fit for purpose. The determination of ruminant or porcine PAPs by immunoassays was more difficult, partly because the MBM in this study contained about 50% bovine and porcine material, thereby reducing the target concentration level to 0.05%.

The ban on using processed animal proteins (PAPs), including meat and bone meal (MBM) as feed ingredient for all farmed animals is an important measure to prevent the spread of transmissible spongiform encephalopathies (TSE). Within the European Union (EU), the use of PAPs is regulated in principle by 2 regulations. The animal byproduct (ABP) Regulation (European Commision; EC) 1774/2002 (1) prohibits feeding animals with proteins from the same species and establishes 3 categories of ABPs which reflect different levels of food safety. Category 1, for instance, contains specified risk material such as the spinal cord; category 2 contains, among other materials, animal byproducts from fallen stock; and category 3 comprises material that is fit for human consumption. Therefore, only material from category 3 can be used for feeding farmed animals, because this class poses the lowest BSE risk. In addition, Regulation (EC) 999/2001 (2) explicitly prohibits the feeding of mammalian PAPs to ruminants. However, various problems such as the lack of appropriate methods to detect mammalian PAPs in the presence of PAPs from other animals led to the introduction of a temporary MBM ban for all farmed animals (total MBM ban) in 2001. The temporary ban was recently changed into a permanent MBM ban by

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amending Regulation (EC) 999/2001 (3). The revised regulation clearly indicated the need for a reappraisal of the total MBM ban once new and more specific control methods became available. For instance, reliable methods allowing for detecting mammalian MBM (MMBM) in the presence of fishmeal could be considered as justification for lifting the ban on the use of fishmeal in feed for ruminants. In the United States, feeding of various mammalian proteins to ruminants is prohibited by regulation 21 CFR 589.2000 (4).

In 1998, the microscopic method was validated by an intercomparison study, thereby becoming the only official method (5) in the EU suitable for the determination of PAPs in feed. Based on the results of the study reported in this paper the microscopic protocol was recently revised to improve the overall performance of European enforcement laboratories (6). In the meantime, alternative analytical methods, such as polymerase chain reaction (PCR), have been developed and validated. In 1998, Tartaglia et al. (7) proposed a method for the detection of bovine mitochondrial DNA in feed that was subsequently assessed by conducting an interlaboratory study (8). However, the results of that study indicated a lack of sensitivity of this protocol, which was most likely due to the severe temperature and pressure treatment of the MBM used to prepare the test material. Within the EU, MBM has to be sterilized at 133°C for 20 min and 3 bar caused by steam pressure, which can lead to decay of the target DNA (9). In 2000, an in-house validation of an immunoassay was reported (10), taking into account the impact of the heat treatment of the MBM on the response of the assay. This immunoassay is characterized by a very laborious sample preparation, whereas recently available alternative types of immunoassay have been developed as dipsticks not requiring any cleanup of the sample extract. The presence of MBM in feed is identified by a completely different concept based on measuring the IR spectrum from individual feed particles using near-infrared microscopy (NIRM) with subsequent statistical analysis of the obtained spectra (11, 12). More details of the different analytical approaches are given in a recent review (13).

The main objective of the present study was to evaluate the proficiency of control laboratories to enforce the current total MBM ban. Therefore, the laboratories were free to select a method that they considered suitable for the detection of MBM. In addition, we intended to compare the various techniques that are proposed to conduct a more specific analysis such as differentiation of MMBM from fishmeal or MMBM from poultry meal. In this paper, we focus on the performance characteristics of the different methods applied on the same type of samples.

Because there is no legal limit for the target PAPs in feed, the applied methods were qualitative. The target MBM concentrations in feed were set at 0.1 and 0.5%, because the achievable sensitivity of the microscopic method as described in Commission Directive 88/98 (5) was 0.1% of constituents of animal origin, and the EC's Scientific Steering Committee stated that cross-contamination with MBM should be condemned at a level >0.5% (14). In order to compare the performance of the various methods, the numbers of false-positive, false-negative, and inconclusive results were calculated.

Experimental

Organization of the Study

For the intercomparison study, each participant received: (*1*) a protocol of the study containing the required information for the participants; (*2*) a set of 24 coded feed samples consisting of 8 different materials, each in blind triplicate; (*3*) a report template in Excel format in which the participants had to record the results of their analysis for each parameter. In order to facilitate the evaluation of the results, the laboratory could only indicate "present," "not present," or "no result." In addition, the laboratories were also requested to provide the organizer of the study with information about the method applied using an electronic form.

Test Materials

The final composition of the 8 different materials (MAT I – MAT VIII) as shown in Table 1 was established, taking into account the following aspects: target concentration of MMBM in feed set at 0.1 and 0.5%; presence of fishmeal at 5% that could interfere with the detection of constituents from terrestrial animals when microscopy was used; presence of poultry meal at 5% to study the influence of this ingredient on the detection of MMBM; presence of MMBM treated at 2 different temperatures; inclusion of vegetable compound feed

Table 1. Description of samples included in the study

	MAT I	MAT II	MAT III	MAT IV	MAT V	MAT VI	MAT VII	MAT VIII
	Concentration, %[a]							
MMBM 134°C	—	—	0.10	0.50	—	—	—	0.5
MMBM 127°C	—	—	—	—	0.10	0.50	—	—
Poultry meal	—	—	—	—	—	—	5	5
Fishmeal	—	5	—	5	5	—	—	—

[a] The concentration is expressed in terms of % mass fraction of animal meal in compound feed.

without PAPs to assess the rate of false-positive results of the methods.

The base of all test materials (MAT I) was a compound feed for bovines containing typical feed ingredients such as maize, soybean, palm kernel/coconut, beet, molasses, sunflower, and minerals using a realistic formula. The sediment content of MAT I was about 1.16% (standard deviation (SD) = 0.27). The compound feed (MAT I) was tested by PCR, immunoassay kits, and NIRM for any contamination with processed animal proteins. The results of the analyses were negative for all methods.

The 2 MMBM samples (MMBM 134°C and MMBM 127°C) used in this study were produced in the same commercial rendering plant, using a batch type system as described elsewhere (15). The MMBMs contained equal portions of porcine and bovine material, and the portion of bones was, respectively, about 12.7% (SD = 0.11) and 10.5% (SD = 0.24). When differentiating between porcine MBM and ruminant MBM, the target level for these parameters was therefore 0.05%. The MMBM feed ingredients were analyzed by PCR in order to check the species present in the sample, confirming that these samples contained mainly bovine and porcine materials with low amounts of poultry material.

The fishmeal was obtained directly from a fishmeal producer and its portion of bones was about 12.4% (SD = 0.32). The poultry meal was obtained from a pilot plant and produced from poultry byproducts; its portion of bones was about 3.3% (SD = 0.09). The fishmeal and the poultry meal were analyzed by PCR in order to check the species composition in the sample. The results indicated that the fishmeal did not contain bovine, porcine, or poultry materials and that the poultry meal did not contain bovine and porcine materials.

Particular emphasis was placed on preparation of the test material in order to ensure that the samples were homogenous enough for the purpose of this study. As basic requirements, the test material needed to reflect typical characteristics of real world material. Therefore, the PAPs (MMBM, poultry meal, and fishmeal) and the compound feed were used as raw product without grinding to a fine powder before the mixing procedure. Using fine powder would definitely facilitate the sample preparation, but the product from that process would be different from that of a routine feed sample. In addition, a sample that consists of a fine powder is difficult to analyze by microscopy. Another important criterion of the preparation procedure was the amount of each sample (12 g) sent to the laboratory. In order to obtain sufficient homogeneity, we prepared the various materials by stepwise, diluting the MBM with the blank feed so that , in each dilution step, the ratio of the 2 different materials to be mixed did not exceed a factor of 3. Depending on the target level of the PAP concentration, the procedure included between 4 and 10 steps. For instance, MAT III (0.1% MMBM) was prepared by mixing, in the first step, 1 g pure MMBM with 3 g compound feed (MAT I) to obtain 4 g MBM/feed mixture. In the second step, 4 g MAT I was added, followed by extensive mixing. Because the amount from the second dilution step was 8 g, the same amount of MAT I feed was added to obtain 16 g mixture. This procedure was repeated until 480 g material was prepared, which was later mixed with 520 g MAT I to provide 1000 g MAT III containing exactly 0.1% MBM. At each dilution level, the materials were mixed for 60 min. After preparation of the mixtures, aliquots of 12 g test material were filled directly into small plastic-aluminium bags.

Sufficient homogeneity of each material was established by analyzing 10 randomly selected bags using NIRM (12). This method is based on the analysis of several hundreds of particles of the sediment fraction using an N/R spectrometer coupled to an adapted microscope. A sedimentation step was performed to remove most of the unspecific feed matrix, thereby allowing the NIRM analysis to focus on particles that are more likely to indicate the presence of MBM. MBM particles are then unequivocally identified by their specific IR spectrum. By analyzing 10 g test material from several sachets, each containing 12 g test material, only between-sample homogeneity but not within-sample homogeneity was evaluated. The laboratories participating in the study were therefore advised to use the whole amount of sample for analysis or apply appropriate subsampling when less test material was needed for conducting the experiment. The results from the homogeneity study confirmed that, in all samples, a sufficient number of MBM particles were present. This also applied to MAT III, which contained only 0.1% MBM.

In addition to NIRM analysis, the homogeneity of part of the set of materials was measured by classical microscopy and PCR. Because of the low content of MMBM (0.1%) in MAT III, this material was also subjected to classical microscopy, with >1000 particles analyzed in the sediment of each sample. Between 20 and 40 particles, depending on the overall number of particles analyzed, were identified in each sample as particles of animal origin, demonstrating a very low relative standard deviation (RSD) of 22%. The homogeneity of MAT IV and MAT VIII containing 0.5% MMBM were also evaluated by analyzing 10 bags for the presence of the target bovine DNA using a PCR method described elsewhere (16). Ten bags were randomly selected and analyzed in triplicate. In this analysis, the bovine DNA target was used. The quantitative results were expressed as the mean threshold cycle (CT) value, revealing almost the same CT values of 35 for MAT IV and MAT VIII. All the samples were detected as positive for the presence of bovine DNA. These figures and all the results from the analyses by the other methods demonstrated the good homogeneity of the materials prepared.

Results and Discussion

Of 51 laboratories that participated in this study, 26 applied microscopy, 18 applied PCR, 6 applied immunoassay, and one applied LC. The results of the various methods revealed that the laboratories applied quite different protocols within the methods of microscopy, PCR, and immunoassays. Nevertheless, the results from each of the 4 methods were

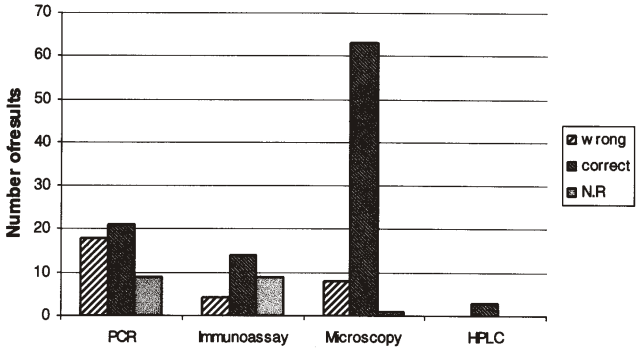

Figure 1. Results from analysis of blank material (MAT I). The number of correct results represents all negative results, whereas wrong results are the number of false-positive results for the parameter total PAPs. NR = No result.

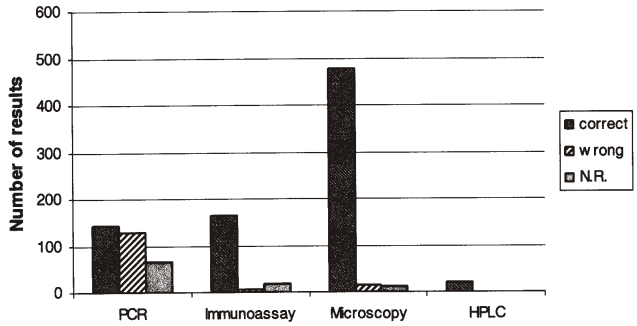

Figure 2. Results from analysis of materials (II–VIII). The number of correct results represents all positive results for the parameter total PAPs, whereas wrong results are the number of false-negative results. NR = No result.

pooled to get an overview of their performance, followed by a more detailed evaluation of the results.

Overview of the Performance of the Different Methods

The results for total PAPs from the analysis of the blank material (Figure 1) and the samples containing PAPs (Figure 2) indicating the number of false-positive and false-negative results showed that microscopy and PLC scored better than the other methods. Furthermore, the results from the PCR method are characterized by a high number of false-positive results for the blank material, hinting at a lack of selectivity. Similarly, many samples containing PAPs were erroneously classified as negative, demonstrating a lack of sensitivity.

The results from the analyses for the other parameters were evaluated separately when focusing on the individual methods, because most of these parameters are method-specific, e.g., PAPs from terrestrial animals (microscopy) and PAPs from mammals (PCR and immunoassays).

Microscopy

The participants were asked to apply their own routinely used laboratory protocol for microscopy. In general, these protocols comply with the EU Directive 88/98 (5), which provides guidelines allowing individual laboratories to include deviations provided that the amendments are comparable to the guideline.

As shown in Figure 2, almost all samples containing PAPs from mammals, poultry, and/or fish were correctly classified as positive for the total PAPs. Fourteen out of 493 (2.8%) results were wrongly classified as negative. This value held true, irrespective of the microscopic protocol that was applied.

Table 2. Possible protocol parameters of the microscopic method[a]

Protocol parameter	All laboratories	Best-performing laboratories (9 laboratories)[b]
Sample size, g	Various amounts	At least 5 g or more
Grinding of sample	Yes/no	Yes/no
Mixing of sample	Yes/no	Yes (–1)
Type of solvent	TCE, TCE/TBE, other, none	TCE (2)
Type of separation funnel	Open, closed, none	Open, closed
Type of action	Shaking, stirring, none	Shaking (-3)
Time for sedimentation, min		0.2–5
Observed fractions	Flotation, sediment, both, feed only	Sediment (3), both (6)
Sieved	Yes/no	Yes/no
No. of slides examined	1, 2, 3, >3	At least 2 or more
Use of stereo microscope	Yes/no	Yes/no

[a] TCE = Tetrachloroethylene; TBE = tetrabromoethylene applied by the laboratories.

[b] Of the 10 best-performing laboratories, one did not submit details on its specific protocol. Indication in parentheses in the third column = number of laboratories deviating from the indicated parameter.

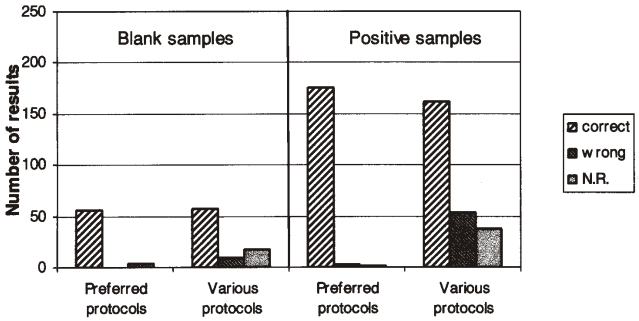

Figure 3. Performance comparison for the parameter PAPs from terrestrial animals of the laboratories applying preferred protocols for microscopy compared to those applying other protocols. Blank samples were MAT I and II; positive samples were MAT III–VIII. NR = No result.

The next level of reporting was the detection of terrestrial animal and fish material separately. The outcome of the evaluation for PAPs for terrestrial animals revealed false-negative results of 13% and false-positive results of 6.2%, without distinction between the variants of the microscopic method used. Comparison of the results from MAT III (0.1% MBM) with the results from MAT V (0.1% MBM and 5% fishmeal) showed that the presence of fishmeal could make the detection of MBM more difficult; the rate of false-negative results was 8% for MAT III and 50% for MAT V. However, the effect of fishmeal was less pronounced, as shown by the results from the material containing 0.5% MBM without fishmeal (MAT VI) and that containing 0.5% MBM/5% fishmeal (MAT VIII). At this level of MBM, the rate of false-negative results was almost the same for both materials and varied only from 8 to 9%. Because the overall performance for this parameter was considered too low, we elaborated the methods used by the laboratories, indicating that quite different protocol parameters were applied, as shown in the second column of Table 2.

Focusing on the protocol parameters applied by the best-performing laboratories (n = 10), the results indicated that the use of the solvent (e.g., tetrachloroethylene versus tetrachloro ethylene/tetrabromoethylene and other solvents) had the most pronounced impact on the performance of the laboratories (Figure 3). The numbers of correct and wrong results for PAPs from terrestrial animals obtained with the preferred protocol and deviating protocols were compared, revealing that the number of wrong results for both the blank and the positive materials were much lower when the preferred protocol was used. Table 2 gives an overview of the preferred protocol parameters. The choice of these parameters is also supported by the results of a simultaneously conducted ring trial (17).

Similarly, results from the most difficult material, MAT V containing 0.1% MBM and 5% fishmeal, showed that laboratories applying the preferred protocol parameters reported 10% false-negative results, whereas the other laboratories reported >50% false-negative results. Based on these results, the EC's Directorate General for Health and Consumer Protection discussed with Member States' experts a revision of the former Directive about the microscopic method (5), introducing a more specified protocol for proper application of this method (6).

Immunoassay

This study included 9 immunoassays from 5 suppliers, and the performance characteristics of the tests as specified by the supplier are presented in Table 3. The methods applied were predominantly based on dipsticks (6 out of 9 laboratories) and microplates.

Mammalian MBM.—Figure 4 shows the performance of the immunoassays at 0.1 and 0.5% MBM. For this presentation, we pooled the results from all immunological tests. The results clearly demonstrated that the immunoassays used in this study failed at the 0.1% MBM concentration level, but obtained satisfying results when the samples contained 0.5% MBM. For instance, the results of MAT III and MAT V (0.1% MBM) contained a considerable number of false-negative results, whereas the analyses of MAT V and MAT VI (0.5% MBM) contained no wrong results. Careful examination of the results from the various immunoassays revealed that the performance differed considerably between the different types of immunoassays. However, none of the immunological tests used in this study performed consistently on all samples. For instance, the most sensitive test, such as

Table 3. Characteristics of the immunoassays specified by the supplier of the test

Immunoassay[a]	Target	LOD[b], %
A	Ruminant	1
	Pig	
B	Ruminant	1
C	Ruminant	Variable
	Pig	
D	Mammal and Avian	0.1
E1	Mammal and Avian	0.1
	Mammal	0.5
E2	Mammal and Avian	0.1
E3	Mammal and Avian	0.05
	Mammal	0.1
F	Ruminant	0.11
	Pig	0.028
	Poultry	0.021

[a] Letters indicate that the tests are from different suppliers. The immunoassays E1, E2, and E3 are from the same supplier but available in different formats.

[b] LOD = Limit of detection. Information was provided by the laboratories without indication whether the LOD refers to MBM or to the target analytes; in the table, "and" indicates that the species are identified together by a single response.

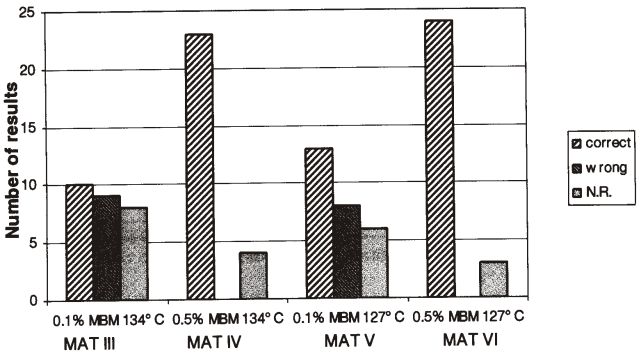

Figure 4. Influence of concentration and heat treatment of MBM on performance of immunoassay methods for the parameter mammalian PAPs. NR = No result.

type E, produced some false-positive results in analyzing the material containing fishmeal in the absence of MBM, thereby indicating a lack of selectivity. The analysis of the blank samples (MAT I) correctly identified 14 negative, 4 false-positive, and 6 inconclusive results. Three of the 4 false-positive results were from method A, thereby indicating that this particular test is not specific enough. In contrast, the specificity of the methods used by other laboratories in analyzing blank material was sufficient. Evaluating the results from the materials containing no MMBM but fishmeal (MAT II) or poultry meal (MAT VII) revealed 14 false-positive and 10 correct negative results for MAT II. For MAT VII, 10 results were false-positive and 9 results were correctly negative. In both cases, a considerable number of results were reported as "no result." Figure 4 also depicts the impact of the heat treatment of the MBM on the sensitivity of the tests. Comparing the results from MAT III (134°C, 0.1% MBM) with those from MAT V (127°C, 0.5% MBM) and the results from MAT IV (134°C, 0.5% MBM) with those from MAT VI (127°C, 0.5% MBM) clearly showed that the sensitivity of the tests did not depend on the temperature of the sterilization. This important outcome demonstrates a significant improvement of the available immunological methods compared to former tests that did not show a positive response when the temperature of sterilization was > 133°C (15).

Ruminant MBM.—Four of the applied assays also allowed the determination of PAPs from ruminants, but the outcome of the analyses indicated a serious problem because none of the reported results were positive when for materials containing 0.1% MMBM (MAT III and MAT V). The situation was slightly improved at the 0.5% concentration level (MAT IV, VI, and VII); 11 results were positive, 12 were negative, and 13 were inconclusive. On the other hand, the content of MBM from ruminants in the MMBM was only 50%, thereby leading to a final concentration of ruminant MBM of 0.05% in MAT III and MAT V, and 0.25% in MAT IV, VI, and VII. This means that the criteria for this parameter were more demanding than for the other parameters. Moreover, it could be expected that the results are improving when materials containing 0.5% pure MBM from ruminants are analyzed, but the answer to this question was beyond the scope of this study.

It can be concluded that the sensitivity of the currently available immunoassays has been significantly improved over that of immunoassays previously evaluated by the organizer of the study (results not published). It is also important that MMBM can be detected even if the material was rendered at the current European standard, 133°C, 3 bar, for 20 min. Provided that the assays can be further improved in terms of selectivity, immunoassays should be considered as important tools for the detection of PAPs in feed.

Polymerase Chain Reaction (PCR)

All PCR techniques were used by the participants of the study, including classical PCR with agarose gel electrophoresis, PCR-enzyme linked immunosorbent assay (ELISA) and real-time PCR with either SYBR Green or use of hybridization probes (exclusively Taqman probes).

The method descriptions given by the participants about sample preparation indicated that, although grinding the samples entirely before extraction was strongly recommended, some participants skipped this step. Others went through it but sometimes with questionable devices (e.g., pestle or mortar) for a feed matrix. The amount of test portion used varied from 50 mg to 8 g. Extraction–purification steps were mostly done with kits. Concerning the reaction itself, the involved amount of DNA per reaction vial varied largely from laboratory to laboratory, and the number of cycles for amplification was between 30 and 50. The targets to be amplified reached sizes between 60 and 359 base pairs (bp), the latter size already very large, given the severe heat treatment of the target PAPs (13).

The general assessment of the performance of the PCR method in this study is quite negative. It is as if there was no clear discriminative power for PCR, certainly when considering determination of PAPs from all animals and PAPs from mammals, ruminants, or pig separately. For the determination of PAPs from fish or poultry, results are much better (respectively 94 and 81% of correct positive assignments, no false negatives on fish, and only 4% for poultry). However, only few participants were able to give a response for these animal groups, though the content of PAPs from fish or poultry was rather high, thus not too difficult to detect.

Assessing the detection of total PAPs by PCR with the given result was difficult because it seemed that participants filled in that part of the reporting template, although probably their target was not properly designed for the purpose of detecting PAP of all animal species. It is nevertheless rather surprising that the overall rate of false positives is 46%. Indeed, a significant number of laboratories found positive PAP results for the 3 samples of blank material. A rather amazing result of the survey is that a large number of laboratories declared not to use extraction controls.

Results obtained for detection of mammals, ruminants and pig are slightly better than for total PAPs, with the rate of false

negatives of 41, 15, and 19%, respectively. Detection performance remains nevertheless very poor.

Results for mammals, ruminants, and pig were also evaluated by assuming that the 0.1% level was actually below the achievable detection level of PCR; the actual concentration of PAPs from these animals was about 0.05%, given the fact that the MBM used in this study consisted approximately of half cattle and half pig. Even when considering MAT III and MAT V (containing 0.1% MBM) as negative, the overall results of the participants are not that much improved (e.g., correct assignments in detection of pig increases from 31 to 36% and, for ruminants, from 31 to 37%).

Some reasons why PCR failed completely in this study could be put forward. There were probably several interacting causes to explain this:

(**a**) *Inappropriate methodology.*—Since the previous validation study of a specific PCR protocol (8) failed, no really standardized PCR method has been made available. Therefore, each participant used techniques that were supposed to be fit for purpose, but several of these techniques are not appropriate for the specific goal of the test. For instance, target sizes >175 bp should be avoided, as DNA in MBM is highly degraded during rendering and it is even better to be <100 bp (13).

(**b**) *Sampling problem.*—Some participants did not grind the sample but at the same time used very tiny test portions. It was then very likely that the resulting test portion was not representative of the sample, thereby leading to false-negative results.

(**c**) *Lack of expertise of some participants.*—Absence of the use of extraction controls in some laboratories already gives an idea of PCR practices that may not be appropriate.

The results of the study clearly showed that the present state of the art for the use of PCR to detect processed animal byproducts in feed is insufficient. No statement could be made according to the type of PCR method used, except that a commercially available test used by 2 laboratories and based on PCR coupled to ELISA was certainly inappropriate as it completely failed to detect PAPs in the positive samples. It should, however, absolutely be stressed that the bad results of the test did not reflect the real potential of PCR, which still deserves to be considered in the future as one of the methods for this kind of analysis, especially when considering the ban of feeding animals with PAPs from the same species as outlined in EC Regulation 1774/2002 (1).

Liquid Chromatography (LC)

One laboratory used an LC method for the detection of the dipeptides anserine (-alanyl-L-1-methylhistidine), carnosine (-alanyl-L-histidine) and balenine (-alanyl-L-3-methylhistidine). The identification of these compounds indicated the presence of products of animal origin in feed. In addition, the quantification of these compounds and calculation of appropriate ratios were expected to allow differentiation between animals, since anserine is mainly present in chicken, whereas carnosine prevails in pork and beef. However, a further differentiation between PAPs from ruminants and PAPs from pigs was not possible when this protocol was used.

The results for total PAPs obtained with this method revealed that all material containing PAPs were correctly classified as positive and all blank samples were correctly classified as negative. The parameter MMBM is also correctly classified in the feed samples irrespective of the temperature treatment of the MBM and the corresponding concentration. However, when the samples contained poultry meal or fishmeal in addition to MMBM, the laboratory reported no results. This was observed irrespective of whether the MMBM concentration was 0.1 or 0.5%. Though there is no further information available on why the laboratory reported no results, it is very likely that the presence of fishmeal or poultry meal interfered significantly with the detection of MMBM. The interference could result because the content of fishmeal and poultry meal in the samples is much higher than the content of MMBM.

Conclusions

The present study for the determination of PAPs in feed demonstrated that microscopy is the most reliable method for enforcing the current total MBM ban in the EU. Immunoassay demonstrated potential as an alternative test, especially in determining PAPs from mammals, but some improvements in terms of specificity are still required. With the exception of one protocol, all PCR methods failed to detect MBM with the required sensitivity. Another problem could be the low sample amount (frequently <1000 mg) that poses a problem when dealing with heterogeneous material such as compound feed containing MBM at low concentration. Improving sample preparation or extracting much more material (preferably >5 g) could be a solution. The homogeneity study of the material clearly showed that NIRM applied to the sediment fraction of the sample is a very reliable tool for the detection of meat and bone meal in feed. This technique can also be considered as an alternative method for the detection of MBM, especially when the required instrumentation becomes more affordable.

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