



THE VALIDATION OF THE MICROSCOPIC METHOD SELECTED IN THE STRATFEED PROJECT FOR DETECTION OF PROCESSED ANIMAL PROTEINS IN FEED

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1 Introduction

The current ban on processed animal proteins (PAPs) including meat and bone meal (MBM), as ingredient for feedingstuffs requires the availability of appropriate control methods. In 2001 the European project STRATFEED was initiated to improve and to develop different methods covering polymerase chain reaction (PCR), spectroscopy and microscopy to address the requirements from current and upcoming European legislation. In overview of the various methods suitable for the detection of PAPs in feed is given by Gizzi et al [7]. The present report describes the results of the validation study of the microscopic method, which has been improved and finally selected within the STRATFEED project for being validated by an intercomparison study.

The validation of an analytical method is the final step before the protocol can be used for routine analysis. In a validation study experiments are conducted to estimate performance characteristics of the method. Based on the results of these trials it can be decided whether the method is suitable for the intended use or whether the method needs to be further refined.

2 Intercomparison studies

Recently various intercomparison studies for the detection of MBM in feed have been conducted, aiming at different objectives. For instance, the primary objective of the DG SANCO study [6] was to assess the proficiency of European control laboratories to classify correctly feed samples with and without MBM, whereas the present study aims at validating the microscopic method selected in the STRATFEED project. Since it is important to be aware of the differences between a validation study and a proficiency test, this report also elaborates on this aspect.

2.1 The objective of the method validation

Before conducting method validation studies it is important to address some basic aspects as outlined in *Figure 1*. Since the objective of method validation is to establish whether a method is fit for the purpose, the purpose of the method need to be defined. The primary aim of the analytical methods in the present case is characterised by the title of the Stratfeed project, aiming at the development of methods for the detection of mammalian tissue. However, it is also important to take into account the requirements from current European

legislation, introducing in 2000¹ a total ban on feeding ruminants with processed animal proteins (PAPs) whatever the origin of PAPs is. The legal situation is different for non-ruminants. Moreover, future legislation depends on the capacity of analytical methods to carry out an animal specific or even species determination of PAPs. This aspect will be discussed in section 3.

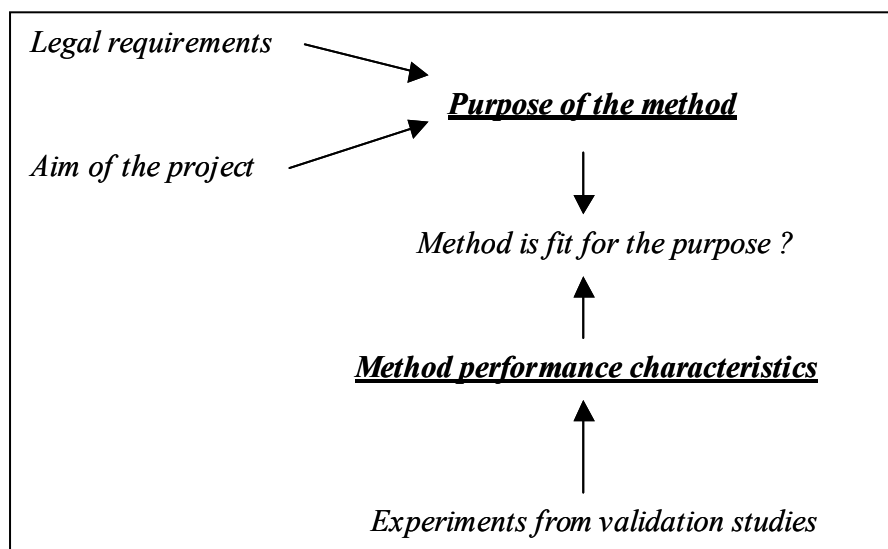


Figure 1: Structure of a validation study

Irrespective of whether the method is used to detect PAPs from terrestrial animals or exclusively from mammals, the corresponding ban as specified in European legislation does not foresee a legal limit. That means that the presence of bone particles indicates the infringement of EU legislation, whatever the actual concentration of MBM in the feed is. The purpose of the method is therefore to establish the presence of PAPs, whereas a quantification of PAPs is not required. When validating qualitative methods, the most important method performance characteristics are sensitivity indicating the achievable detection limit and specificity giving information on false positive results. These statistics will be discussed in section 5.3. In this study, 0.1 % of MBM in feed as target concentration was selected as required in European legislation establishing microscopy as official control method [2]. In addition the concentration level of 0.5 % was included in the Stratfeed validation study considering a recommendation of the DG SANCO's Scientific Steering Committee that cross-contamination with mammalian MBM should be condemned at a level above 0.5 % [11].

¹ The total MBM ban has been introduced after the approval of the Stratfeed project and could therefore not be taken into account in the design of the project.

In order to establish whether the microscopic method [2] is fit for this purpose, laboratories were asked to analyse samples and reporting the presence and absence of MBM to the organiser of the intercomparison study. The fraction of positive results at the target concentration was calculated as well as the fraction of negative results in the case of the blank samples. In both cases the fraction should be preferably above 95 %. Based on the calculated method performance characteristics it can be decided whether the method is fit for the purpose.

In particular, the aim of the validation study was to establish the performance characteristics of the microscopic method as modified within the Stratfeed project when used for the determination of MBM for the following purposes:

- Determination of terrestrial and more specifically mammalian MBM (MMBM) at a concentration level of 0.1 % in compound feed, and in compound feed with fishmeal, respectively
- Determination of MMBM in compound feed containing poultry meal and compound feed containing fishmeal and poultry meal

An additional objective of the study was to test whether the expert system ARIES developed within the Stratfeed project and which contains explanations on the microscopic method and many figures of particles specific for PAPs would help laboratories in identifying MBM in feed.

2.2 Validation study versus proficiency test

In an intercomparison study laboratories analyse the same batch of blind samples and report the results to the organiser of the study. Then the results are subjected to statistical analysis. In this field intercomparison studies have been applied to two purposes. The aim of the STRATFEED study is to assess *method* performance characteristics, whereas the major aim of the DG SANCO study was to look at the *proficiency* of European control laboratories. In the former case the laboratories have to apply a specific protocol and in the latter case the laboratories can utilise whatever method protocol they want. The major differences of both approaches are summarised in Table 1.

Table 1: Comparison of validation study and proficiency test.

| | Validation study | Proficiency test |
|--|--|--|
| Purpose | Estimating method performance characteristics | Estimating the proficiency of the laboratories |
| Analytical method | All laboratories apply the same method | The laboratories are free to use a method protocol of their choice |
| Extreme values | Extreme values need to be eliminated if justified | No elimination of extreme values |
| Principle of statistical analysis | Pooling results from all laboratories for each sample type | Pooling results from all sample types for each laboratory |

Since the objective of the current study was to estimate method performance characteristics it was necessary to be sure that the participating laboratories are experienced enough in the field of microscopic analysis and that they know the modified microscopic method. Therefore a training period was included in the study. During this stage the laboratories had to analyse samples and to report the results to the organiser of the study. Based on the results it was established whether each laboratory was fulfilling basic criteria in order to include them in the validation study. More details of the training period are given in chapter 5.5. Following the training phase the samples for the validation study were sent out.

3 The meat and bone meal ban according to European legislation

Within the European Union (EU) the use of PAPs is regulated in principle by two regulations. The animal by-product (ABP) Regulation (EC) 1774/2002 [3] prohibits feeding of animals with proteins from the same species and establishes three categories of ABPs according to their TSE risk. Only material from category 3 can be used for feeding farmed animal, since this class poses the lowest BSE risk. In addition, Regulation (EC) 999/2001 [4] prohibits explicitly 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 introducing a temporary MBM ban for all farmed animals (total MBM ban) in 2001. Therefore, the temporary ban was recently changed into a permanent MBM ban by amending [5] Regulation (EC) 999/2001. It is important to note that the revised regulation

clearly indicated the need for a reappraisal of the total MBM ban once new and more specific control methods are 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 of fishmeal as feed ingredient for ruminants.

4 Characterisation of the test material

When organising an intercomparison study a very important aspect is the preparation of suitable test material. The test material has to fulfil specific criteria in order to assure that the right conclusions can be drawn from the results of the study. In particular it was necessary to select appropriate feed to which the MBM is added and to establish the type of MBM used for the test material. Given the low target concentration level of the MBM in the feed it is also very important to apply a suitable preparation procedure that allows for a homogeneous distribution of the MBM particles in the test material.

4.1 The feed

The test material prepared for the various intercomparison studies in this field was typical compound feed without and with traces of MBM. Compound feed is a mixture of different ingredients such as grain, by-products from the food industry, minerals and feed additives. Since these compounds can interfere with the analysis of the sample it is important to select a typical material that is produced under “real-world” conditions. For instance, the microscopic analysis focuses among other target fractions on the analysis of the sediment of the test material, which contains particles of the sample of higher density such as bones. Minerals and other constituents of the sediment that do not indicate the presence of PAPs can interfere with the identification of bones and are therefore a challenge for the microscopic method. If they were not present in the compound feed, the analysis could be to “easy” and therefore not mirror a typical situation in a routine laboratory. In the STRATFEED study the test material was compound feed contained sediments at about 1.6 % and was prepared in a dedicated pilot plant of the feed company “Nutreco”, which was also a partner of the Stratfeed project. The composition of the material is shown in *Table 2*.

Table 2: Composition of the compound feed

| Raw Material | Mass fraction (%) |
|--------------------------|-------------------|
| Wheat | 8.0 |
| Corn | 2.0 |
| Corn gluten feed | 22.0 |
| Soyabean meal | 1.2 |
| Rapeseed meal | 8.0 |
| Palm kernel meal | 16.0 |
| Coconut meal | 9.0 |
| Beet pulp | 13.0 |
| Citrus pulp | 10.0 |
| Molasses | 10.0 |
| Vegetable fat | 0.2 |
| Magnesium oxide | 0.4 |
| Vitamins/mineral mixture | 0.2 |

4.2 The meat and bone meal (MBM)

The two MMBM samples (MMBM 134°C and MMBM 127°C) used in the DG-SANCO study were produced in the same commercial rendering plant, employing a batch type system as described elsewhere [12]. The MMBMs contained equal portions of porcine and bovine material and the portion of bones was respectively about 12.7 % and 10.5 %. The MMBM used in the STRATFEED validation study was also a mix of bovine and porcine processed material with a bone content about 12 %. The MMBM feed ingredients were analysed by PCR in order to check the species present in the sample, confirming that these samples mainly contained 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 %. The poultry meal was obtained from a pilot plant and produced from poultry by-products and its portion of bones was about 3.3 %. The fishmeal and the poultry meal were analysed 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.

4.3 Composition of the test material

In this study two different batches of test material were prepared. Batch A as shown in Table 3 contained samples for the training period, in which the participating laboratories also had the possibility to analyse known samples. Thereby they got familiar with the modified method protocol and learned how to use the decision support system ARIES.

Table 3: Composition of the samples for the training period (Batch A). MMBM: mammalian MBM.

| Code | Label | Description | Samples per laboratory |
|------|-------|-------------------------|------------------------|
| A | Known | MMBM 0.5% | 1 |
| B | Known | MMBM 0.5% + Fishmeal 5% | 1 |
| C | Blind | Fishmeal 5% | 1 |
| D | Blind | Blank | 1 |
| E | Blind | MMBM 0.5% | 1 |
| F | Blind | MMBM 0.5% + Fishmeal 5% | 1 |
| G | Blind | MMBM 0.5% + Poultry 5% | 1 |

For the validation a different sample design was selected in order to validate the method according to the objectives described in paragraph 2.1. The composition of the samples in this batch B is shown in Table 4. All samples were sent out as blind replicates.

Table 4: Composition of the samples analysed by participants in the validation study (Batch B).

| Code | Description | Samples per laboratory |
|-------|---|------------------------|
| MAT 1 | Blank compound feed | 4 |
| MAT 2 | Mat 1 with Poultry meal 5% | 3 |
| MAT 3 | Mat 1 with Fishmeal 5% | 4 |
| MAT 4 | Mat 1 with MMBM 0.1% + 5% Fishmeal | 4 |
| MAT 5 | Mat 1 with MMBM 0.5% + 5% Poultry meal | 3 |
| MAT 6 | Mat 1 with MMBM 0.1% | 3 |
| MAT 7 | Mat 1 with MMBM 0.5% + 2.5%Poultry meal + 2.5% Fishmeal | 3 |

5 The Stratfeed validation study

5.1 The organiser team

The study was jointly conducted by the European Commission Joint Research Centre Institute for Reference Materials and Measurements (IRMM) in Belgium, Walloon Agricultural Research Centre (CRA-W) in Belgium and Institute for Food Safety Wageningen (RIKILT) in The Netherlands. The study was coordinated by IRMM.

5.2 Outline of the study

Since the laboratories had to apply a specific microscopy protocol a training period in order to familiarise the participating laboratories with the new protocol was included. To assure coherence with the official European [2] the following modifications of the Stratfeed protocol were accepted ²:

- Additional to an electric mill with specific requirements, a simple mortar might be used, especially for high fat containing samples.
- Sedimentation in an open beaker is allowed additional to a sedimentation funnel.
- Immersion oil is accepted as well as embedding agent, which results in range of media with more diverse viscosity

An invitation letter (Annex 9.1) was sent on the 1st July 2003 to 37 laboratories from 17 different countries asking whether they were interested to participate in the validation study of a modified microscopic method (Annex 1.1) for the determination of meat and bone meal (MBM) in feed. As a result, the Walloon Agricultural Research Centre (CRA-W) sent on 21 October 2003 batch A (a set of seven training samples) to 31 laboratories. Samples were prepared containing typical compound feed fortified with processed animal proteins (PAPs) from various species at different concentration level as shown in Table 3.

The laboratories were asked to report on two main parameters, which were used for validating the method, and to determine the method characteristics (1) PAPs derived from mammals and/or poultry (terrestrial animals), (2) PAPs derived from mammals. Two additional parameters were considered to assist in evaluating the participant's results: (3) PAPs derived from poultry (avian) and (4) PAPs derived from fish.

As the microscopic method gives only a qualitative response, the laboratories had three options to report their result of analysis: (1) present (2) not present and (3) no results. The latter response corresponded to inconsistent results or was indicated by laboratories when the method did not allow determining a specific parameter. For instance, laboratories that could not confirm the presence of PAPs derived from mammals when they confirmed the presence of bones from mammals or poultry.

In order to be able to look more carefully at the applied protocol, the laboratories were asked to indicate also the sediment percentages of each sample.

² The recent modification of the EU protocol for the microscopic identification was mainly based on the results of the STRATFEED project. However, after the consultation of all national representatives, deviations to the STRATFEED protocol have been introduced as they are applied in different European countries.

Part of the study was a test of the decision support system ARIES. This computer program supports the process of sample treatment and evaluation, and the identification of collected particles. The system helps to discriminate between animal particles and confusing plant material, terrestrial animal material and fish material and provides also information on the differences between mammalian and poultry material. A special web connection with the server in Wageningen was established for the participants applying the decision support system.

In order to handle the high number of information (more than 3100 data: 33 laboratories x 24 samples x 4 parameters), a special excel worksheet was designed to process the results submitted by the participants. Moreover, the excel sheet allowed for an automatic identification of the sample codes and an automatic calculation of the performance characteristics. When compiling the excel sheet for reporting results, a table summarising the results was automatically established. This last table from each laboratory was used for the analysis of results. To obtain some information about how strictly the laboratories followed the protocol, a sheet containing some important questions mostly about the sample preparation and the use or not of ARIES was included in the excel file for reporting results.

The laboratories participating in the study received together with the samples a detailed protocol on classical microscopy method developed within the Stratfeed project, a copy of the manual for the use of ARIES and the excel file for reporting results.

All results were reported by 10 December 2003 by using the designed excel file and the report facilities of ARIES. In addition, the laboratories gave specific information about their experiences when conducting the analysis using the Stratfeed protocol.

Based on the results the final set of laboratories participating in the study was established. More detailed information about the results of the training is given in paragraph 5.5.

On 7 January 2004, CRA-W sent the 24 samples of batch B which composition is shown in Table 4 to the 33 laboratories taking part in the validation study. After receiving the samples the laboratories had three weeks to analyse the samples and to report the results to the JRC in the same way and using the same excel file as in the training period.

The laboratories received the samples before 15 January and the organising team got the results back between 22 January and 16 February 2004.

5.3 Statistical analysis of the results

For binary results (yes/no, positive/negative, etc.) standard statistics are accuracy, sensitivity and specificity. The accuracy is the fraction of correct results, either positive or negative, calculated as

$$\text{Accuracy } AC = \frac{PA + NA}{PA + ND + PD + NA}$$

where PA is the number of correct positive identifications (positive agreements), NA the number of correct negative identifications (negative agreements), PD the number of false positives (positive deviations) and ND the number of false negatives (negative deviations). The statistics can be presented as fractions or as percentages after multiplication by 100.

The sensitivity is the ability of the method to detect PAP when it is present in the samples, whereas the specificity is the ability to indicate correctly the absence of PAP in the blank samples. The following equations have been used to calculate the statistics:

$$\text{Sensitivity } SE = \frac{PA}{PA + ND} \qquad \text{Specificity } SP = \frac{NA}{PD + NA}$$

Accuracy is numerically identical to either specificity or sensitivity depending on the test material and the specific PAP sought, since either the amount of false negatives (ND) when the feed is not spiked with the PAP, or the number of false positives (PD) when PAP is deliberately added, is equal to zero. For the evaluation of different treatments the statistics can generally be referred to as accuracy.

After collecting the raw results it appeared that at the level of distinction of terrestrial animal classes (mammals and birds) a lot of “No results” have been reported. For the calculation of accuracy this will greatly influence the calculated statistics. In order to get comparable results a modified equation was used, which is the *adjusted* accuracy:

$$\text{adjusted Accuracy } AC' = \frac{PA + NA}{PA + ND + PD + NA + mv}$$

where mv is the number of “No results”. The denominator is equal to the total number of data points for a specific treatment.

The 95 % percent confidence limits will be calculated, based on a Bernoulli distribution (equals a Binomial distribution with $n=1$). The values for PA or NA , and for n will be used to

calculate using the cumulative sampling distribution the limits for $p_i = 0.025$ and $p_i = 0.975$, which are limits of the 95 % confidence interval.

The validation study included seven different materials Table 4 and four parameters (PAPs at the various levels) resulting in 28 test material/parameters combinations. For each individual combination the accuracy was calculated.

5.4 Overview of the results

In this chapter a coarse overview of the results is presented without taking into account the individual capability of the participating of the laboratories. It is important to note that the assessment of the method performance characteristics require that results from laboratories, which are not experienced enough for conducting the microscopic analysis are excluded from the statistical evaluation. A detailed description of the valid results produced by the competent laboratories and the final calculation of the method performance characteristics will be given in chapter 5.5.

The participants were asked in the sheet for reporting about the method whether they followed strictly the protocol and whether they used the support system ARIES during the identification of the samples. Concerning the protocol, twenty-six of the thirty-three laboratories confirmed to have followed it. On the other hand, five laboratories reported different reasons for not having applied the protocol and two laboratories did not report any information about it.

Fourteen laboratories confirmed the use of ARIES during the identification process and seventeen stated not to have used it for different reasons. As before, two laboratories did not provide any information about the use of ARIES. We will elaborate on the method applied by the participants and on the use of ARIES in chapter 5.5 of this report.

In Table 5 and Table 6 the results submitted by the 33 laboratories for the 7 materials and for the parameters terrestrial animals and mammals are summarised.

Table 5. Total results for parameter terrestrial animals. Total observations (n) distributed in present (P), not present (NP) and no result (NR). Correct result in bold.

| PAPs from terrestrial animals | | | | | |
|--------------------------------------|---|-----------|------------|-----------|----------|
| MAT | Composition | P | NP | NR | n |
| 1 | Blank | 20 | 109 | 3 | 132 |
| 2 | Poultry meal 5% | 94 | 5 | 0 | 99 |
| 3 | Fishmeal 5% | 24 | 106 | 2 | 132 |
| 4 | MMBM 0.10% + 5% fish meal | 91 | 40 | 1 | 132 |
| 5 | MMBM 0.5% + 5% poultry meal | 97 | 2 | 0 | 99 |
| 6 | MMBM 0.10% | 94 | 5 | 0 | 99 |
| 7 | MMBM 0.5% + 2.5% poultry + 2.5% fish meal | 97 | 2 | 0 | 99 |

PAP detection, of different origin and at different concentrations, is the necessary and sufficient requirement to enforce the total ban of PAP in ruminant feed. The results for the parameter terrestrial animals show that the performance of the microscopic method to detect the presence of PAP in animal feed is in general good. For some materials (MAT #4, MAT #5) there is a notable number of false negatives or false positives, respectively, which is partly due to the fact that some participants did not follow the requested protocol. The number of reported “No results” is very low. The participants were apparently confident to draw a conclusion in almost all cases.

For the parameter PAPs from mammals as shown in Table 6 the high number of false positive when reporting MAT2 (poultry) and the high number of “No Results” demonstrated the limitations of the actual microscopic method to discriminate mammals and poultry materials.

Table 6. Total results for parameter mammals. Total observations (n) distributed in present (P), not present (NP) and no result (NR). Correct result in bold.

| PAPs from mammals | | | | | |
|-------------------|--|-----------|------------|----|-----|
| MAT | Composition | P | NP | NR | n |
| 1 | Blank | 11 | 113 | 8 | 132 |
| 2 | Poultry meal 5% | 34 | 33 | 29 | 96 |
| 3 | Fishmeal 5% | 13 | 109 | 10 | 132 |
| 4 | MMBM 0.10 % + 5% fish meal | 55 | 49 | 27 | 131 |
| 5 | MMBM 0.5% + 5% poultry meal | 61 | 9 | 26 | 96 |
| 6 | MMBM 0.10% | 57 | 12 | 29 | 98 |
| 7 | MMBM 0.5% + 2.5% poultry + 2.5%fish meal | 58 | 11 | 27 | 96 |

As already indicated a second objective of the study was a test on the decision support system ARIES. The participants had to indicate whether they used or not the program as a support on the identification process. Especially the distinction between mammalian and avian material is expected to be facilitated by the system, and therefore the results for PAPs from mammalian source is further presented in Table 7, separated for “users” (14 laboratories) and “non users” of ARIES (17laboratories).

When comparing the results reported by the group of laboratories using ARIES as a decision support system on the identification process with those that indicated to have not used it, no significant differences are found in the performance of the two groups for the detection of PAPs. It seems that the use of ARIES does not improve the performance of the method when detecting the presence of *terrestrial animals*. However when discriminating between mammals and poultry, ARIES demonstrated to be a very useful tool detecting MMBM in the presence of poultry and fishmeal as shown the substantial decrease in the number “No results” reported by “ARIES” users compared with those reported by the “non users” (Table 7).

Table 7. Compared results regarding the use or not of ARIES for the parameter mammals. Total results distributed in present (P), not present (NP) and no result (NR).

| PAPs from mammals | | | | | | | |
|-------------------|--------------------------------------|-------|----|----|----------|----|----|
| Mat | Composition | ARIES | | | NO ARIES | | |
| | | P | NP | NR | P | NP | NR |
| 1 | Blank | 5 | 60 | 3 | 6 | 45 | 5 |
| 2 | Poultry meal 5% | 22 | 21 | 5 | 9 | 12 | 21 |
| 3 | Fishmeal 5% | 9 | 57 | 2 | 4 | 44 | 8 |
| 4 | MBM 0.10%+ 5% fish meal | 32 | 31 | 4 | 23 | 12 | 21 |
| 5 | MBM 0.5%+ 5% poultry meal | 36 | 7 | 5 | 22 | 2 | 18 |
| 6 | MBM 0.10% | 36 | 10 | 4 | 19 | 1 | 22 |
| 7 | MBM 0.5% +2.5% Poultry+2.5%fish meal | 36 | 8 | 4 | 19 | 3 | 20 |

The combined results from *Table 5* and *Table 6* showed that for the parameter PAP from terrestrial animals all laboratories reported results on all materials (*Table 5*: MAT #1: 33 participants times 4 replicates is 132; etc.). However, for the parameter PAP from mammals a few data points were missing (*Table 6*: MAT #2: 33 participants times 3 replicates is 99, 96 reported, 3 void; MAT #4: 33 participants times 4 replicates is 132, 131 reported, 1 void; etc.)

It can also be concluded that the structure of the excel reporting sheet, the type of questions asked, and the explanation to the sheet were in general clear to the users. In some cases, illogical results have been reported, e.g. absence of both mammalian and avian material and presence of terrestrial animals. The questions regarding details of the method performed were part of the report sheet. Most participants answered these questions; only two labs do not send in their answers. It can be concluded that currently a good structure for reporting results of a qualitative proficiency test has been developed.

The next chapter will elaborate on the analysis of results of both the training period and the STRATFEED validation study. The selection criteria used to define the final set of laboratories participating in the study after the training will be presented. A detailed evaluation of the comments reported by the laboratories regarding the methodology applied and the use of ARIES will be tackled. Finally, a comparison between the Stratfeed results and the results of two other studies, which are the DG-SANCO [6, 8] and IFFO [10] studies, in terms of accuracy will be presented.

5.5 Detailed evaluation

A training phase was organised prior to the validation study, in which 31 laboratories participated. Due to organisational reasons two Stratfeed partners were allowed to enter the final validation study without studying the training samples. The purpose of this training phase was twofold: allowing the participants to get experienced with the type of materials and to test the performance of the participants. Out of 31 laboratories 25 of them presented good results for the five blind training samples of batch A. The remaining six failed for at least two of the five samples. These labs were still allowed to enter the final validation study and it was decided to accept their results of the final set of 24 samples of batch B provided that a considerable improvement was achieved.

Table 8. Parameters in the protocol for the preparation of slides for microscopic evaluation. The requested and the deviating type of the indicated parameters are listed, with the number of participants applying the parameters in brackets. Two participants did not report details on their protocol.

| Parameter | Requested (# users) | | Deviating (# users) | |
|---------------------|---------------------|------|---------------------|------|
| sample size (grams) | 5 | (23) | 10 | (8) |
| grinding | mill | (13) | mortar | (16) |
| solvent | TCE | (30) | CCl ₄ | (1) |
| separation funnel | Closed | (21) | open | (10) |
| time (min) | => 4 | (28) | =< 3 | (3) |
| sieving | yes | (19) | no | (12) |
| binocular | yes | (27) | no | (4) |

A total of 33 laboratories submitted their full results for the set of 24 samples in the validation study. Two laboratories appeared to have not achieved any improvement compared to the training. Two laboratories produced an average amount of sediment that was considerable higher than the declared amount of sediment in the feed used (8,6 % and 7,8 %, respectively, compared to 0,6 – 0,9 % obtained by the other laboratories). This high amount indicates an improper application of the protocol. Four laboratories applied principally other parameter states than allowed, which can be expected to substantially influence the observations in a negative sense: embedding the sieve fraction in ether petroleum combined with sedimentation in CCl₄, embedding the sieve fraction in several agents not included in the required protocol combined with a sedimentation time of 2-3 minutes, embedding the fine sediment fraction in

water combined with a sedimentation time of only 1 minute, and embedding the sieve fraction in immersion oil combined with a high viscosity for the embedding agent of the fine sediment fraction. All these eight laboratories have been excluded from the final evaluation of the results of the validation study for the mentioned reasons. One laboratory showed a substantial improvement of their results for the final set of 24 samples compared to the training set and was accepted for the final evaluation.

As shown in Table 8, a substantial number of participants used a deviating state for several parameters. A major number of participants used the starting amount of 5 g of the sample that is required in the EC directive 126/2003 [2]; the original Stratfeed protocol stated the use of 10 grams of original material. More than half of the participants used the mortar because of the small size of the original samples. The use of the open sedimentation beaker was excluded in the original Stratfeed protocol, but allowed in the procedure as currently accepted by the EU [2] (see paragraph 3). The lack of sieving and of the use of the binocular for examination of the coarse fractions was allowed in the final evaluation, although requested in the protocol. After excluding the mentioned eight laboratories the results of 25 participants were used for the final evaluation. The basic results are presented in Table 9. The measure “accuracy” means either specificity in the case of false positives (e.g. terrestrial material in a blank (#1), mammalian in #3, etc.) or sensitivity in the case of false negatives (e.g. mammalian in #6, avian in #2, etc.).

The detection of terrestrial animal material and of fish material offers generally no considerable problems. Exceptions are the report of some false positives for terrestrial animal material in material #3 and for fish material in material #5. A special case is the detection of 0.1 % terrestrial material in the presence of 5 % fish meal (#4), for which 23 false negatives of a total of 99 observations were reported (and 1 No-results). The detection of specific mammalian or avian material appears to be more difficult for certain materials. This is not only illustrated by the lower values for accuracy, but also by the number of “No results” as already indicated in paragraph 5.4, which is in some materials more than half the number of data points for the presence of avian material. In order to have a more detailed and balanced view on the results, an equation for an adjusted accuracy has been developed (see paragraph 5.3).

Table 9. Basic results expressed in accuracy for the detection of different types of animal proteins in seven differently contaminated feeds. Number of “No results” in brackets. n: total number of observations.

| | Material | n | AC | | | |
|----|---|-----|-------------|------------|------------|------------|
| | | | terrestrial | mammalian | avian | fish |
| #1 | blank | 100 | 0.908 (2) | 0.933 (7) | 0.953 (11) | 0.880 (0) |
| #2 | poultry 5% | 75 | 0.960 (0) | 0.587 (29) | 0.769 (36) | 0.907 (0) |
| #3 | fish 5% | 100 | 0.857 (2) | 0.919 (10) | 0.963 (15) | 0.990 (1) |
| #4 | MBM 0.1% + fish 5% | 100 | 0.768 (1) | 0.639 (27) | 0.800 (39) | 0.970 (0) |
| #5 | MBM 0,5% + poultry 5% | 75 | 1.0 (0) | 0.959 (26) | 0.878 (34) | 0.865 (1) |
| #6 | MBM 0,1 % | 75 | 0.987 (0) | 0.896 (27) | 0.632 (37) | 0.920 (0) |
| #7 | MBM 0,5% + poultry 2,5% + fish 2,5% | 75 | 1.0 (0) | 0.898 (26) | 0.718 (36) | 0.947 (0) |

The adjusted accuracies per material and target contaminant are presented in Table 10. The detection of classes of terrestrial animals is expectedly more difficult than the detection of terrestrial animals in general. There appear to be generally more positive observations for the detection of mammalian material compared to avian material except for material #4, which results in higher values for adjusted accuracy. Only for material #2 the AC' value for mammalian material is lower, since a positive in this material is a false positive, whereas for materials #5, #6 and #7 positives are correct ones.

Table 10. Recalculated results expressed in adjusted accuracy for the detection of different types of animal proteins in seven differently contaminated feeds. n: total number of observations.

| | Material | n | AC' | | | |
|----|-------------------------------------|-----|-------------|-----------|-------|-------|
| | | | terrestrial | mammalian | avian | fish |
| #1 | blank | 100 | 0.890 | 0.865 | 0.844 | 0.880 |
| #2 | poultry 5% | 75 | 0.960 | 0.253 | 0.400 | 0.907 |
| #3 | fish 5% | 100 | 0.840 | 0.823 | 0.813 | 0.980 |
| #4 | MBM 0,1% + fish 5% | 100 | 0.760 | 0.465 | 0.485 | 0.970 |
| #5 | MBM 0,5% + poultry 5% | 75 | 1.0 | 0.627 | 0.480 | 0.853 |
| #6 | MBM 0,1 % | 75 | 0.987 | 0.573 | 0.320 | 0.920 |
| #7 | MBM 0,5% + poultry 2,5% + fish 2,5% | 75 | 1.0 | 0.587 | 0.373 | 0.947 |

Participants have been asked to indicate whether they have used the decision support system ARIES or not. No difference was made between the use for providing knowledge and/or for active support of their detection and identification process. Laboratories that used ARIES exclusively for providing background knowledge have been considered as ARIES users. On the other hand, three partners of Stratfeed that indicated to have not used ARIES have been classified in the group of non-users. The group of ARIES users consisted of 13 participants and the group of non-users of 12 participants. There is no significant difference in the performance of both groups for the detection of terrestrial animal material and of fish material.

Table 11. *Basic results expressed in accuracy for the detection of two types of animal proteins in seven differently contaminated feeds split for the use of the ARIES system. Number of “No results” in brackets. n: total number of observations.*

| material | | AC with ARIES | | | AC without ARIES | | |
|----------|---|---------------|------------|------------|------------------|------------|------------|
| | | n | mammalian | avian | n | mammalian | avian |
| #1 | blank | 52 | 0.935 (2) | 0.935 (2) | 48 | 0.930 (5) | 0.974 (9) |
| #2 | poultry 5% | 39 | 0.353 (5) | 0.800 (6) | 36 | 0.583 (24) | 0.667 (27) |
| #3 | fish 5% | 52 | 0.891 (2) | 0.932 (3) | 48 | 0.950 (8) | 1.0 (11) |
| #4 | MBM 0.1% + fish 5% | 52 | 0.702 (4) | 0.718 (9) | 48 | 0.520 (23) | 0.952 (27) |
| #5 | MBM 0.5% + poultry 5% | 39 | 0.941 (5) | 0.871 (5) | 36 | 1.0 (21) | 0.900 (26) |
| #6 | MBM 0.1 % | 39 | 0.857 (4) | 0.607 (8) | 36 | 1.0 (23) | 0.700 (26) |
| #7 | MBM 0.5% + poultry 2.5% + fish 2.5% | 39 | 0.917 (3) | 0.759 (7) | 36 | 0.846 (23) | 0.600 (26) |

This situation fits in the predominantly high level of the values for accuracy as presented in Table 9 and Table 10. Also for the critical detection of terrestrial land material in the presence of fishmeal (material #4) hardly any difference is found. The results for the detection of mammalian and avian material are presented in Table 11. Also at this level there seems to be mostly no seriously deviating results whether or not ARIES has been used, except for a few situations: e.g. detection of mammalian material in material #2 (0.353 vs. 0.583) and in material #4 (0.702 vs. 0.520) and detection of avian material in material #7 (0.759 vs. 0.6). However, the number of “No results” is significantly higher for the group of participants that did not use ARIES. Therefore, the adjusted accuracy has been calculated for both groups of participants. The results are shown in Table 12. In all cases the adjusted accuracy is higher when using ARIES compared to ignoring the information provided by ARIES. The report of correct negatives for mammalian material in feed that is exclusively adulterated with poultry material and vice versa the detection of avian material in pure mammalian contaminated feed are special cases for further attention. Without using ARIES there is no difference in adjusted accuracy (0.194; values in italics in Table 12). With using ARIES it seems easier to correctly

indicate the absence of avian material in material #6 (0.436) than for mammalian material in material #2 (0.308).

Table 12. Recalculated results expressed in adjusted accuracy for the detection of different types of animal proteins in seven differently contaminated feeds divided in two groups of users and non-users of the ARIES system. n: total number of observations. For values in italics see text.

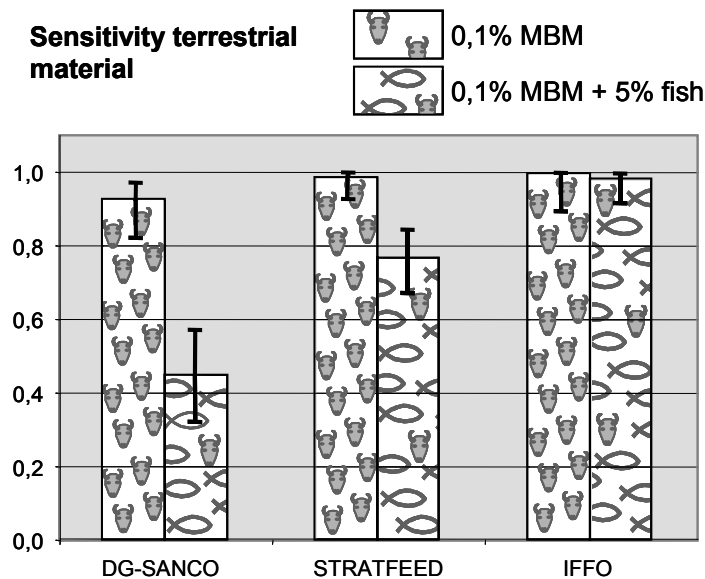
| | Material | n | AC' with ARIES | | n | AC' without ARIES | |
|----|---|----|----------------|-------|----|-------------------|-------|
| | | | mammalian | avian | | mammalian | avian |
| #1 | blank | 52 | 0.896 | 0.896 | 48 | 0.833 | 0.792 |
| #2 | poultry 5% | 39 | 0.308 | 0.615 | 36 | 0.194 | 0.167 |
| #3 | fish 5% | 52 | 0.854 | 0.854 | 48 | 0.792 | 0.771 |
| #4 | MBM 0.1% + fish 5% | 52 | 0.647 | 0.549 | 48 | 0.271 | 0.417 |
| #5 | MBM 0.5% + poultry 5% | 39 | 0.821 | 0.692 | 36 | 0.417 | 0.250 |
| #6 | MBM 0.1 % | 39 | 0.769 | 0.436 | 36 | 0.361 | 0.194 |
| #7 | MBM 0.5% + poultry 2.5% + fish 2.5% | 39 | 0.846 | 0.564 | 36 | 0.306 | 0.167 |

Reclassification of the three Stratfeed partners from the group of non-users to the group of ARIES users made a substantial difference for some materials. For the detection of mammalian material in material #4 most correct positives were accountable to the three Stratfeed partners; reclassification resulted in AC' values of 0.667 and 0.110 for the group of users (n=16) and non-users (n=9, respectively). All correct positives for avian material in material #2 were exclusively reported by the three Stratfeed partners in the group of non-users.

The pivotal material is the adulteration of feed with 0.1 % of MBM in combination of 5 % fishmeal (#4). The AC value for all participants is 0.768. This means that a part of the laboratories is not capable of correctly distinguish between the two sources of animal proteins. It is, however, important to emphasize the required expertise and proficiency of the laboratories to correctly analyse the samples. The current study (to be referred to as

“Stratfeed”) can be compared to the proficiency study carried out by JRC (commissioned by DG-SANCO; [6; 8]) and a validation study organised by RIKILT (commissioned by IFFO; [10]).

Figure 2. Sensitivity values with indication of 95 % confidence limits for the detection of two types of animal meals in feeds (terrestrial animal meal versus fishmeal). Names in capitals refer to the stakeholder of the original studies



The DG-SANCO study aimed at providing information on the proficiency of the laboratories and therefore all participants were allowed to apply their routine procedures. The participants of the IFFO study strictly applied the Stratfeed procedure, which is stricter than the protocol currently accepted by the EU [2]. The DG-SANCO study did not report statistics on specificity and sensitivity for individual materials, since this is not an aspect of proficiency testing. However, the reports of 18 laboratories from the DG SANCO study that were classified in the groups A and B (see [6] for details) have been used to calculate the required statistics for the comparison shown in Figure 2. The results for the material with 5 % fish meal and 0.1 % MBM (MAT #4 here) of the DG-SANCO study indicate an accuracy of 0.444 (n=63 data points). The IFFO study reports an accuracy of 0.984 (n=64). The current study indicates a value of 0.768 (n=99), which is higher than the DG-SANCO value but lower than the IFFO value. A second observation of interest is the detection of terrestrial animal material in feed adulterated with exclusively 0.1 % MBM (#6). The values for accuracy were 0.921 (n=63), 1.0 (n=32) and 0.987 (n=75) for the DG-SANCO study, the IFFO report and the current Stratfeed study, respectively. For both materials the results for detection of terrestrial

animal material of the current study are intermediate between both other studies. A major improvement has been achieved compared to the normal situation as described in the proficiency test commissioned by DG-SANCO. Since the characteristics of the test materials amongst the three studies were not the same, the reason of the different outcome of these studies cannot be completely inferred from the results. However, differences of the applied methodology might have caused the superiority of the IFFO study compared to the other, but this requires further investigation. Presumed reasons for the lower AC values in the current study compared to those of the IFFO study are the additions to the original strict Stratfeed protocol that are allowed in the current legislation [2]. The use of an open sedimentation beaker is not expected to give a major influence, since previous proficiency studies indicate a good performance. The use of 5 grams instead of 10 grams for sedimentation, however, might decrease the chance of detecting the few particles that are present in the sediment. Furthermore, the small quantity of the samples as provided to the participants in the current study induced the use of a mortar. For future studies (either for validation or for proficiency testing) a higher amount of material per sample is necessary. Focusing on the results of the Stratfeed validation study might indicate that microscopy is not fit for the purpose when analysing compound with 0.1% MBM in the presence of fishmeal. However, the results from the well performing laboratories of the DG SANCO study and all laboratories from the IFFO studies clearly confirmed that microscopy is capable of detecting MBM at this level, provided that the laboratories have enough experience in applying the microscopic method in a proper way. It is clear that the use of ARIES enhance the possibilities for detection and identification of material of the classes of terrestrial vertebrate classes. The proper indication of absence of mammalian material in poultry contaminated feed appeared to be more difficult than vice versa indicating the absence of avian material in mammalian adulterated feed ($AC' = 0.31$ vs. $AC' = 0.44$, respectively). This might be due to the fact that some poultry long bones show an appearance that is pretty much similar to that of mammalian bones [9]. These bones can be indicated as confusing elements, whereas in the vice versa situation these confusing particles are almost absent. These results indicate that a further improvement of ARIES can be achieved. The first official release ARIES version 1.0 will include such improvements.

6 Conclusions

We can summarise the results of the study as follows:

- The overall performance of the microscopic detection of animal proteins in feeds is generally good.
- In the Stratfeed validation study the detection of terrestrial MBM 0.1 % in the presence of fishmeal turned out to be not sufficient. Additional investigations need to be conducted in order to establish whether a further harmonisation of the analytical protocol improves the performance of the microscopic method as indicated by the results of the IFFO study.
- Comparing the current results with those of the previous study commissioned by DG-SANCO [6; 8], it can be concluded that the improved protocol [2] is significantly better than the one established by former Directive 98/88/EC [1].
- When comparing the current Stratfeed study with the IFFO study [10], the laboratories trained in applying the optimised Stratfeed protocol are capable to obtain an acceptable low rate of false negative results for the detection of terrestrial MBM 0.1 % in presence of fish meal (64 data points in the IFFO Study). Sufficient experience of the control laboratories is extremely important.
- Use of ARIES doesn't improve the performance in the determination of terrestrial MBM, but ARIES helps in detecting mammalians MBM in the presence of poultry and fishmeal. Three of the four laboratories (from a total of twelve) that indicated not using ARIES and that classified correctly MAT 4 (mammalians MBM in the presence of fishmeal) were STRAFFEED partners. Since they all participated in the development of ARIES, they had the main features of ARIES available without being physically connected to the Aries Server. The other eight laboratories reported exclusively false negatives.

7 Recommendations

Based on the results of the studies we put forward the following recommendations:

- It is important to organise training courses in critical aspects, such as cleaning to avoid cross contamination, the correct application of the sedimentation procedure, use of a binocular and the proper identification of assumed animal particles.
- Proficiency Tests should be organised regularly. The amount of material send for each sample should be sufficient to use the right grinding equipment and to be able to perform all aspects of the protocol.

- The information of ARIES needs to be updated as a planned result of the validation study.
- Harmonisation of the interpretation of level of detection (i.e. is one bone particle already the prove of a positive sample, status of a duplicate analysis) should be approved by the Commission.

8 Bibliographic references

1. EC/88/1998 Commission Directive establishing guidelines for microscopic identification and estimation of constituents of animal origin for the official control of feedingstuffs. Official Journal of the European Communities 27.11.98. L318. 45-50.
2. EC/2003/126 Commission Directive of 23 December 2003 on the analytical method for the determination of constituents of animal origin for the official control of feedingstuffs. Official Journal of the European Communities L 339 . 24.12.2003. 78-84.
3. EC Regulation 1774/2002 of the European Parliament and of the Council laying down health rules concerning animal by-products not intended for human consumption. Official Journal of the European Communities. 10.10.2002. L 273. 1-95
4. EC Regulation 999/2001 of the European Parliament and of the Council laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. Official Journal of the European Communities. 31.5.2001. L 147. 1 – 40
5. EC Regulation 1234/2003 amending Annexes I, IV and XI to EC Regulation 999/2001. Official Journal of the European Communities. 11.07.2003. L 173. 6-13
6. Gizzi, G., von Holst C., Baeten V., Berben G., L. van Raamsdonk (2003a). Intercomparison study for the determination of processed animal proteins including meat and bone meal in animal feed. Joint Research Centre. Ispra. Italy.
7. Gizzi, G., Raamsdonk, L.W.D. van, Baeten, V., Murray, I., Berben, G., Brambilla, G. & Holst, C. (2003b). An overview of tests for animal tissues in animal feeds used in the public health response against BSE. In: Risk analysis of BSE and TSEs: update on BSE and use of alternatives to MBM as protein supplements. Rev. Sci.Tech.Off. Int. Epiz. 22(1): 311-331.
8. Gizzi, G., V. Baeten, G. Berben, L. van Raamsdonk and C. von Holst (in press). Intercomparison study for the determination of processed animal proteins including meat and bone meal in animal feed. J. A.O.A.C.

9. Mondini S., Calocchio E., Altissimi M.S., Haouet M.N. & Cenci T. (1999). - Validita' dell'esame microscopico per la individuazione dell'origine delle farine di carne. *La Selezione Veterinaria* **7**. 453-463.
10. Raamsdonk L.W.D. van and H. van der Voet (2003). A ring trial for the detection of animal tissues in feeds in the presence of fish meal. Report 2003.012. RIKILT. Wageningen. pp. 17 with 6 addenda.
11. Statement of the Scientific Steering Committee (SSC) on its Report and Scientific Opinion on mammalian derived meat and bone meal forming a cross-contaminant of animal feedstuffs. adopted on 24-25 September 1998. adopted at the SSC meeting of 26-27 October 2000 (http://europa.eu.int/comm/food/fs/sc/ssc/out144_en.html)
12. von Holst C., Unglaub W. & Anklam E. (2001) *J. AOAC International*. **84** (6). 1793-1799

9 Annexes

9.1 Annex I: Invitation letter to participate in the validation study



EUROPEAN COMMISSION
DIRECTORATE GENERAL JRC
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
Food Safety and Quality Unit

Call for participation in the validation study of a modified microscopic method for the determination of meat and bone meal (MBM) in feed, possibly containing processed animal proteins (PAP) from various animals

Introduction

The use of processed animal by-products as ingredient for animal feedingstuffs within the European Union is regulated by Council Decision 2000/766/EC imposing a ban of processed animal proteins (PAP) to farmed animals (extended feed ban). Enforcing this legislation requires the availability of reliable analytical methods. In 1998 the microscopical method has been validated by an intercomparison study thereby becoming the only official method (Commission Directive 88/1998) suitable for the determination of PAP in feed.

Within an international project (“Stratfeed”) funded by the European Commission we are developing and validating methods for the detection of mammalian tissue in feedingstuffs. One of these methods we are working on is the microscopic method. For more details about the project please look at the web site of the project (www.stratfeed.cra.wallonie.be).

This letter is to invite you to participate in a validation study of the microscopic method for the determination of processed animal protein (PAP) in feed that has been improved in the “Stratfeed” project.

The organiser team

The study will be jointly conducted by the European Commission Joint Research Centre Institute for Reference Materials and Measurements (IRMM), Centre de Recherches Agronomiques de Gembloux, CRAGx (Belgium) and RIKILT Institute for Food Safety Wageningen (The Netherlands). The study will be coordinated by the IRMM.

Objective

The objective of this validation study is to assess the performance characteristics of a modified microscopic method by organising an intercomparison study in which the participating laboratories analyse feed samples with unknown content of PAP from various



animals. In detail, we are looking at the capability of the method to detect the presence of (1) PAPs from all animals, (2) PAPs from land animals when there is also fishmeal present and (3) PAPs from mammals when there is also poultry meal present. In order to meet this target the participating laboratories analyse the samples by applying exactly the same protocol that will be provided by us. In this context we would like to place emphasis on the fact, that in this study we are looking at the performance of the method and not at the proficiency of the attending laboratories.

Short description of the modified method

The actual protocol to be used is a modification to the currently used method as indicated by EU Directive 98/88/EU. Improvements are the strict use of a closed sedimentation funnel allowing shaking instead of stirring, TCE as solvent, an enhanced time for sedimentation (5 minutes) and the use of up to three slides for evaluation when no bones can be found. An essential part of the protocol is the use of the Decision Support System “ARIES”. This computer program assists in training, and provides support for the identification of the different particles and for reporting the results. Access to ARIES will be provided by a secured internet connection to a specific IP address.

The protocol including the supporting computer program already received much interest from the European Commission for future legislation and the laboratories participating in this validation study will have an excellent opportunity to get acquainted with these new developments.

Test material

Samples have been prepared containing typical compound feed fortified with PAP from various species at different concentration level. Each laboratory will receive approx. 20 blind samples for analysis and will submit the results to the coordinator of the study. The exact number of samples will be set when the number of participating laboratories is known. In addition, a training period prior to the validation study is foreseen, in which the laboratories will analyse about 7 samples.



General outline of the exercise

Since the laboratories are supposed to apply the modified microscopy protocol developed within the “Stratfeed” project we will include a training period in order familiarise the participating laboratories with the new protocol. This training phase will take place in September 2003 and it is foreseen that the participating laboratories will analyse labelled as well as blind samples according to the protocol. All results in the final validation study will be reported by using a result sheet as well as by using the report facilities of ARIES. In addition, the laboratories will give specific information about their experiences when conducting the analysis using the “Stratfeed” protocol. Based on the results and the submitted form the final set of laboratories participating in the study will be established. The actual validation study will follow in October 2003 and it is planned that the laboratories analyse the set of validation samples in November 2003.

At the end of the validation study scheduled for January/February 2004 the coordinator will send out a report on the outcome of the exercise to the involved laboratories. In addition you will receive a certificate about the participation in this study. Those laboratories which will finally participate in the validation will receive a financial support of about 200 Euro to 400 Euro depending on the final number of laboratories.

Please send your indication of interest including the official contact person, **E-mail address** and IP adress(es) (for establishing the internet connection) to the coordinator.



EUROPEAN COMMISSION
DIRECTORATE GENERAL JRC
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
Food Safety and Quality Unit

Letter of agreement

Please indicate whether you are interested to participate in the validation study for the determination of meat and bone meal feed. The number of samples to be analysed in the training period will be about 10 samples and the number of samples in validation study will be between 20 to 30 samples. Based on the outcome of the analyses from the training period the final set of laboratories participating in the study will be established.

| | |
|---|--------------------------|
| We want to participate in the validation study of a modified microscopic method for the determination of meat and bone meal in feed | <input type="checkbox"/> |
|---|--------------------------|

Please send this information via fax or **e-mail** within 2 weeks from receiving this invitation to:

Dr. Stefan Stratmann
European Commission
Institute for Reference Materials and Measurement, Food Safety and Quality Unit
Retieseweg
2440 Geel, Belgium
phone: +32 14 571 826
e-mail: stefan.strathmann@jrc.it

Your address:

| | |
|------------------------|--|
| Organisation: | |
| Contact: | |
| Street: | |
| Postal Code | |
| Town: | |
| Country: | |
| Tel.No.: | |
| Fax No.: | |
| e-mail address: | |
| IP address: | |

Place

Date

Signature

9.2 Annex II: Protocol of the microscopic method

Annex III: List of participating laboratories

| Organization Name | City | Country |
|--|---------------------------|----------------|
| Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH (AGES) | Vienna | Austria |
| Federal feed and food laboratory | Tervuren | Belgium |
| Ministry of Food, Agriculture and Fisheries, Danish Plant Directorate | Lyngby | Denmark |
| Plant Production Inspection Centre (KTTK), Agricultural Chemistry Department | Vantaa | Finland |
| IDAC | Nantes | France |
| Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit | Oberschleißheim | Germany |
| Landesanstalt für Landwirtschaftliche Chemie, Universität Hohenheim | Stuttgart | Germany |
| Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer | Speyer | Germany |
| Sächsische Landesanstalt für Landwirtschaft Fachbereich Landwirtschaftliche Untersuchungen/LUFA | Leipzig | Germany |
| LUFA - Kassel | Kassel | Germany |
| Aristotle University of Thessaloniki | Thessaloniki | Greece |
| Official Seed testing Station, Department of Agriculture and Food | Castleknock, Dublin | Ireland |
| Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Laboratory for Diagnosing Transmissible Animal Diseases | Teramo | Italy |
| Dipartimento di Scienze e Tecnologie Vet. per la Sicurezza Alimentare | Milano | Italy |
| RIKILT - Institute for Food Safety | Wageningen | Netherlands |
| Maasweide Laboratory Services NUTRECO | Boxmeer | Netherlands |
| LabCo B.V. Laboratory Services | Rotterdam | Netherlands |
| CCL-Research | Veghel | Netherlands |
| Laboratorio Nacional De Investigacao Veterinaria | Lisboa | Portugal |
| National Sanitary Veterinary Agency, Inst.for Hygiene and Vet. Public Health | Bucharest | Romania |
| CNIEP Larex (National Centre for Products Testing and Appraisalment) | Bucurest | Romania |
| Central Control and Testing Institute of Agriculture | Bratislava | Slovakia |
| Navarra de Servicios S.A. (NASERSA) | Villava , Navarra | Spain |
| Laboratorio de Nutrición Animal Área de Nutrición, Pastos y Forrajes Regional (SERIDA) | Villaviciosa, Asturias | Spain |
| Laboratorio Agrario y de Medio Ambiente (LAYMA) | Murcia | Spain |
| Laboratorio Agroalimentario de Cordoba | Cordoba | Spain |
| OFFICE – Laboratori d'Investigació Cerealista | Castellgalí, Barcelona | Spain |
| Generalitat de Catalunya (GENCAT) | Cabrils, Barcelona | Spain |
| Centro de Sanidad Animal | Zaragoza | Spain |
| TROUW NUTRITION MasterLab Madrid (before TROUlab) | Tres Cantos, Madrid | Spain |
| SVA, Dept. of Animal Science | Uppsala | Sweden |
| Swiss Federal Research Station for Animal Production and Dairy Products | Posieux | Switzerland |
| Veterinary Laboratories Agency | Warwickshire | U.Kingdom |

9.3 Annex IV: Results of laboratories and identification of the samples.

Laboratory # 1

| Material | Sample | Mammalian & Poultry | Mamaliens | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 1387 | not present | not present | not present | not present |
| | 1835 | not present | not present | not present | not present |
| | 2395 | not present | not present | not present | not present |
| | 2752 | not present | not present | not present | not present |
| Poultry meal 5% | 1150 | present | no result | no result | not present |
| | 2081 | present | no result | no result | not present |
| | 2473 | present | no result | no result | not present |
| Fishmeal 5% | 745 | not present | not present | not present | present |
| | 1011 | not present | not present | not present | present |
| | 2075 | not present | not present | not present | present |
| | 2481 | not present | not present | not present | present |
| MMBM 0.1% + 5% Fishmeal | 767 | present | no result | no result | present |
| | 1201 | present | no result | no result | present |
| | 1978 | present | no result | no result | present |
| | 2412 | present | no result | no result | present |
| MMBM 0.5% + 5% Poultry meal | 845 | present | no result | no result | not present |
| | 1202 | present | no result | no result | not present |
| | 2245 | present | no result | no result | not present |
| MMBM 0.1% | 160 | present | no result | no result | not present |
| | 510 | present | no result | no result | not present |
| | 1497 | present | no result | no result | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 112 | present | no result | no result | present |
| | 637 | present | no result | no result | present |
| | 938 | present | no result | no result | present |

Laboratory # 2

| Material | Sample | Mammalian & Poultry | Mamaliens | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 1597 | not present | not present | not present | not present |
| | 1681 | not present | not present | not present | not present |
| | 2402 | not present | not present | not present | not present |
| | 2647 | not present | not present | not present | not present |
| Poultry meal 5% | 814 | present | present | no result | not present |
| | 1066 | present | present | no result | not present |
| | 1962 | present | present | no result | not present |
| Fishmeal 5% | 682 | not present | not present | not present | present |
| | 1165 | not present | not present | not present | present |
| | 1984 | not present | not present | not present | present |
| | 2432 | not present | not present | no result | present |
| MMBM 0.1% + 5% Fishmeal | 746 | present | present | no result | present |
| | 1166 | not present | not present | not present | present |
| | 2195 | present | present | no result | present |
| MMBM 0.5% + 5% Poultry meal | 2391 | not present | not present | no result | present |
| | 1006 | present | present | no result | not present |
| | 2042 | present | present | no result | present |
| MMBM 0.1% | 2476 | present | present | no result | not present |
| | 153 | present | present | no result | not present |
| | 440 | present | present | no result | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 860 | present | present | no result | not present |
| | 133 | present | present | no result | present |
| | 497 | present | present | no result | present |
| | 1141 | present | present | no result | present |

Laboratory # 3

| Material | Sample | Mammalian & Poultry | Mamalians | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 1765 | not present | not present | not present | not present |
| | 2514 | not present | not present | not present | not present |
| | 2773 | not present | not present | not present | not present |
| | 737 | present | not present | present | not present |
| Poultry meal 5% | 1227 | present | present | not present | not present |
| | 2417 | present | not present | present | not present |
| | 871 | not present | not present | not present | present |
| Fishmeal 5% | 1221 | not present | not present | not present | present |
| | 2054 | not present | not present | not present | present |
| | 2404 | not present | not present | not present | present |
| | 739 | not present | not present | not present | present |
| MMBM 0.1% + 5% Fishmeal | 1019 | not present | not present | not present | present |
| | 1999 | present | present | not present | present |
| | 2321 | present | present | not present | present |
| | 796 | present | present | present | not present |
| MMBM 0.5% + 5% Poultry meal | 1342 | not present | not present | not present | not present |
| | 2308 | present | present | not present | not present |
| | 2392 | present | present | present | not present |
| MMBM 0.1% | 363 | present | no result | no result | not present |
| | 475 | not present | not present | not present | not present |
| | 916 | present | no result | no result | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 525 | present | present | present | present |
| | 798 | present | present | not present | present |
| | 1134 | present | present | present | present |

Laboratory # 4

| Material | Sample | Mammalian & Poultry | Mamalians | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 1590 | no result | no result | no result | no result |
| | 1709 | not present | not present | not present | present |
| | 2360 | not present | not present | not present | not present |
| | 2682 | not present | not present | not present | present |
| Poultry meal 5% | 933 | present | present | present | present |
| | 1234 | present | not present | present | present |
| | 1969 | present | not present | present | present |
| Fishmeal 5% | 703 | present | present | not present | present |
| | 1039 | present | present | present | present |
| | 2068 | present | present | not present | present |
| | 2411 | present | present | not present | present |
| MMBM 0.1% + 5% Fishmeal | 725 | present | not present | present | present |
| | 1124 | present | present | present | present |
| | 2020 | present | present | not present | present |
| MMBM 0.5% + 5% Poultry meal | 2314 | present | not present | present | present |
| | 768 | present | present | present | present |
| | 1209 | present | present | not present | not present |
| MMBM 0.1% | 2252 | present | present | present | present |
| | 132 | present | present | present | present |
| | 1140 | present | not present | present | present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 1490 | present | present | present | present |
| | 308 | present | present | present | present |
| | 805 | present | present | not present | present |
| | 1169 | present | present | not present | present |

Laboratory # 5

| Material | Sample | Mammalian & Poultry | Mamalians | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 1331 | not present | not present | not present | not present |
| | 1793 | not present | not present | not present | not present |
| | 926 | present | no result | no result | not present |
| | 1038 | present | no result | no result | not present |
| Poultry meal 5% | 2186 | present | no result | no result | not present |
| | 892 | not present | not present | not present | present |
| | 1053 | not present | not present | not present | present |
| Fishmeal 5% | 1942 | not present | not present | not present | present |
| | 2320 | not present | not present | not present | present |
| | 858 | present | no result | no result | present |
| | 1131 | present | no result | no result | present |
| MMBM 0.1% + 5% Fishmeal | 1908 | not present | not present | not present | present |
| | 712 | present | no result | no result | not present |
| | 1111 | present | no result | no result | not present |
| | 2161 | present | no result | no result | present |
| MMBM 0.5% + 5% Poultry meal | 48 | present | no result | no result | not present |
| | 433 | present | no result | no result | not present |
| | 1203 | present | no result | no result | not present |
| MMBM 0.1% | 56 | present | present | no result | present |
| | 224 | present | no result | no result | present |
| | 441 | present | no result | no result | present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 665 | present | no result | no result | present |
| | 770 | present | present | no result | present |
| | 1225 | present | present | no result | present |

Laboratory # 6

| Material | Sample | Mammalian & Poultry | Mamalians | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 1268 | not present | not present | not present | not present |
| | 1786 | not present | not present | not present | not present |
| | 2388 | not present | not present | not present | not present |
| | 2654 | not present | not present | not present | not present |
| Poultry meal 5% | 660 | present | present | not present | not present |
| | 1101 | present | present | not present | not present |
| | 2298 | present | not present | present | not present |
| Fishmeal 5% | 787 | not present | not present | not present | present |
| | 1305 | not present | not present | not present | present |
| | 1928 | not present | not present | not present | present |
| | 2509 | not present | not present | not present | present |
| MMBM 0.1% + 5% Fishmeal | 634 | present | present | not present | present |
| | 1096 | present | present | not present | present |
| | 2090 | present | present | not present | present |
| | 2468 | present | present | not present | present |
| MMBM 0.5% + 5% Poultry meal | 873 | present | present | not present | not present |
| | 1153 | present | present | present | not present |
| | 2273 | present | present | present | not present |
| MMBM 0.1% | 293 | present | present | present | not present |
| | 881 | present | present | present | not present |
| | 1329 | present | present | not present | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 49 | present | present | present | present |
| | 721 | present | present | present | present |
| | 1281 | present | present | present | present |

Laboratory # 7

| Material | Sample | Mammalian & Poultry | Mamalians | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 1289 | not present | not present | not present | not present |
| | 1744 | not present | not present | not present | not present |
| | 2283 | present | present | not present | not present |
| | 2794 | not present | not present | not present | not present |
| Poultry meal 5% | 856 | present | present | not present | not present |
| | 2067 | present | not present | present | not present |
| | 2368 | present | present | not present | not present |
| Fishmeal 5% | 577 | present | present | not present | present |
| | 1088 | not present | not present | not present | present |
| | 2089 | present | present | not present | present |
| | 2467 | not present | not present | not present | present |
| MMBM 0.1% + 5% Fishmeal | 669 | present | present | not present | present |
| | 1117 | present | not present | present | present |
| | 1950 | present | present | not present | present |
| | 2349 | present | present | not present | present |
| MMBM 0.5% + 5% Poultry meal | 852 | present | not present | present | not present |
| | 1132 | present | present | not present | not present |
| | 1972 | present | present | not present | not present |
| MMBM 0.1% | 76 | present | present | not present | not present |
| | 706 | present | not present | present | present |
| | 1077 | present | present | not present | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 196 | present | not present | present | present |
| | 455 | present | not present | present | present |
| | 1071 | present | present | not present | present |

Laboratory # 8

| Material | Sample | Mammalian & Poultry | Mamalians | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 1408 | present | present | present | not present |
| | 1688 | present | present | present | not present |
| | 2381 | present | present | not present | not present |
| | 2661 | present | not present | present | not present |
| Poultry meal 5% | 800 | present | present | present | not present |
| | 1087 | present | not present | present | not present |
| | 2221 | present | not present | present | not present |
| Fishmeal 5% | 731 | present | present | present | present |
| | 850 | present | present | present | present |
| | 2166 | present | not present | present | present |
| | 2376 | present | not present | present | present |
| MMBM 0.1% + 5% Fishmeal | 718 | present | present | present | present |
| | 1082 | present | present | present | present |
| | 2048 | present | present | present | present |
| | 2293 | present | not present | present | present |
| MMBM 0.5% + 5% Poultry meal | 817 | present | present | present | not present |
| | 1174 | present | present | present | not present |
| | 2266 | present | not present | present | not present |
| MMBM 0.1% | 195 | present | present | present | not present |
| | 531 | present | present | present | not present |
| | 1070 | present | present | present | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 294 | present | present | present | not present |
| | 483 | present | present | present | not present |
| | 1106 | present | not present | present | present |

Laboratory # 9

| Material | Sample | Mammalian & Poultry | Mamaliens | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 1541 | not present | not present | not present | present |
| | 1828 | not present | not present | not present | not present |
| | 2451 | present | not present | present | not present |
| | 2738 | not present | not present | not present | not present |
| Poultry meal 5% | 961 | present | present | not present | not present |
| | 2165 | present | present | not present | present |
| | 2487 | present | present | present | not present |
| Fishmeal 5% | 696 | not present | not present | not present | present |
| | 794 | not present | not present | not present | present |
| | 1319 | not present | not present | not present | present |
| | 2152 | not present | not present | not present | present |
| MMBM 0.1% + 5% Fishmeal | 655 | not present | not present | not present | present |
| | 1040 | not present | not present | not present | present |
| | 1922 | not present | not present | not present | present |
| | 2265 | not present | not present | not present | present |
| MMBM 0.5% + 5% Poultry meal | 992 | present | present | present | not present |
| | 1300 | present | present | not present | not present |
| | 2063 | present | present | not present | not present |
| MMBM 0.1% | 83 | present | not present | present | not present |
| | 384 | present | present | present | not present |
| | 839 | present | present | not present | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 147 | present | present | not present | present |
| | 490 | present | present | not present | present |
| | 910 | present | present | not present | present |

Laboratory # 10

| Material | Sample | Mammalian & Poultry | Mamaliens | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 1401 | not present | no result | no result | not present |
| | 1758 | not present | no result | no result | not present |
| | 2346 | not present | no result | no result | not present |
| | 2745 | not present | no result | no result | not present |
| Poultry meal 5% | 765 | present | no result | no result | present |
| | 1157 | present | no result | no result | not present |
| | 2046 | present | no result | no result | present |
| Fishmeal 5% | 724 | not present | no result | no result | present |
| | 997 | not present | no result | no result | present |
| | 2040 | not present | no result | no result | present |
| | 2327 | not present | no result | no result | present |
| MMBM 0.1% + 5% Fishmeal | 662 | present | no result | no result | present |
| | 1194 | present | no result | no result | present |
| | 1936 | present | no result | no result | present |
| MMBM 0.5% + 5% Poultry meal | 2272 | present | no result | no result | present |
| | 775 | present | no result | no result | present |
| | 1328 | present | no result | no result | not present |
| MMBM 0.1% | 2280 | present | no result | no result | not present |
| | 139 | present | no result | no result | not present |
| | 489 | present | no result | no result | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 1084 | present | no result | no result | not present |
| | 119 | present | no result | no result | present |
| | 609 | present | no result | no result | present |
| | 763 | present | no result | no result | present |

Laboratory # 11

| Material | Sample | Mammalian & Poultry | Mamaliens | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 1359 | not present | not present | not present | not present |
| | 1632 | not present | not present | not present | not present |
| | 2311 | present | not present | present | not present |
| | 2675 | present | not present | present | not present |
| Poultry meal 5% | 898 | present | not present | present | not present |
| | 1206 | present | not present | present | not present |
| | 2172 | present | present | present | not present |
| Fishmeal 5% | 647 | not present | not present | not present | present |
| | 906 | not present | not present | not present | present |
| | 2250 | not present | not present | not present | present |
| | 2299 | present | not present | present | present |
| MMBM 0.1% + 5% Fishmeal | 690 | present | present | not present | present |
| | 1187 | present | not present | present | present |
| | 2174 | present | not present | present | present |
| | 2258 | not present | not present | not present | present |
| MMBM 0.5% + 5% Poultry meal | 789 | present | present | present | not present |
| | 1244 | present | not present | present | not present |
| | 2231 | present | present | present | not present |
| MMBM 0.1% | 146 | present | present | not present | not present |
| | 468 | present | present | not present | not present |
| | 1259 | not present | not present | not present | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 336 | present | present | not present | present |
| | 812 | present | present | present | present |
| | 1183 | present | not present | present | present |

Laboratory # 12

| Material | Sample | Mammalian & Poultry | Mamaliens | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 1394 | not present | not present | not present | not present |
| | 1499 | not present | not present | not present | not present |
| | 2563 | not present | not present | not present | not present |
| | 2619 | not present | not present | not present | not present |
| Poultry meal 5% | 779 | present | not present | present | not present |
| | 1073 | present | present | not present | present |
| | 2179 | present | not present | present | not present |
| Fishmeal 5% | 864 | not present | not present | not present | present |
| | 1207 | not present | not present | not present | present |
| | 2082 | not present | not present | not present | present |
| | 2502 | not present | not present | not present | present |
| MMBM 0.1% + 5% Fishmeal | 648 | present | not present | present | present |
| | 970 | present | present | present | present |
| | 2041 | present | present | present | present |
| | 2125 | not present | not present | not present | present |
| MMBM 0.5% + 5% Poultry meal | 824 | present | present | present | not present |
| | 1160 | present | present | present | not present |
| | 2028 | present | present | present | not present |
| MMBM 0.1% | 125 | present | not present | present | not present |
| | 349 | present | no result | no result | not present |
| | 867 | present | not present | present | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 126 | present | not present | present | present |
| | 539 | present | present | not present | present |
| | 952 | present | present | present | present |

Laboratory # 13

| Material | Sample | Mammalian & Poultry | Mamalians | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 1254 | not present | not present | not present | not present |
| | 1604 | not present | not present | not present | not present |
| | 2374 | not present | not present | not present | not present |
| | 2633 | present | no result | no result | not present |
| Poultry meal 5% | 786 | present | no result | no result | not present |
| | 884 | present | no result | no result | not present |
| | 2249 | present | no result | no result | not present |
| Fishmeal 5% | 654 | not present | not present | not present | present |
| | 1109 | not present | not present | not present | present |
| | 2145 | not present | not present | not present | present |
| | 2425 | not present | not present | not present | present |
| MMBM 0.1% + 5% Fishmeal | 781 | present | no result | no result | present |
| | 1054 | present | no result | no result | present |
| | 2153 | present | no result | no result | present |
| | 2433 | present | no result | no result | present |
| MMBM 0.5% + 5% Poultry meal | 866 | present | present | no result | not present |
| | 943 | present | present | present | not present |
| | 2210 | present | present | no result | not present |
| MMBM 0.1% | 258 | present | no result | present | not present |
| | 811 | present | present | no result | not present |
| | 1294 | present | no result | present | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 266 | present | no result | present | present |
| | 693 | present | present | no result | present |
| | 1274 | present | no result | present | present |

Laboratory # 14

| Material | Sample | Mammalian & Poultry | Mamalians | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 1534 | not present | not present | not present | not present |
| | 1639 | not present | not present | not present | not present |
| | 2521 | present | present | present | present |
| | 2766 | not present | not present | not present | not present |
| Poultry meal 5% | 751 | present | present | present | not present |
| | 1164 | present | present | present | not present |
| | 2039 | present | present | present | not present |
| Fishmeal 5% | 633 | present | not present | present | present |
| | 1095 | not present | not present | not present | present |
| | 2061 | not present | not present | not present | present |
| | 2334 | present | not present | present | present |
| MMBM 0.1% + 5% Fishmeal | 697 | present | present | present | present |
| | 1180 | present | present | present | present |
| | 1964 | not present | not present | not present | present |
| | 2363 | not present | not present | not present | not present |
| MMBM 0.5% + 5% Poultry meal | 691 | present | present | present | not present |
| | 1195 | present | present | present | not present |
| | 2007 | present | present | present | not present |
| MMBM 0.1% | 118 | present | present | present | not present |
| | 573 | present | present | not present | not present |
| | 1063 | present | present | not present | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 7 | present | present | present | present |
| | 504 | present | present | present | present |
| | 896 | present | present | present | present |

Laboratory # 15

| Material | Sample | Mammalian & Poultry | Mamalians | Poultry | Fish |
|---------------------|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 1373 | not present | not present | not present | not present |
| | 1485 | not present | not present | not present | not present |
| | 2339 | not present | not present | not present | not present |
| | 2689 | not present | not present | not present | not present |
| Poultry meal 5% | 674 | present | present | present | not present |
| | 947 | present | not present | present | not present |
| | 2431 | present | not present | present | not present |
| Fishmeal 5% | 857 | not present | not present | not present | present |
| | 983 | not present | not present | not present | present |
| | 2047 | not present | not present | not present | present |
| | 2257 | not present | not present | not present | present |
| MMBM 0.1% + 5% Fist | 788 | present | present | not present | present |
| | 1047 | present | present | not present | present |
| | 2006 | present | present | not present | present |
| | 2335 | present | not present | present | present |
| MMBM 0.5% + 5% Po | 964 | present | present | present | not present |
| | 1986 | present | present | present | not present |
| | 2287 | present | present | present | not present |
| MMBM 0.1% | 90 | present | present | present | not present |
| | 594 | present | present | not present | not present |
| | 902 | present | not present | present | not present |
| MMBM 0.5% + 2.5% F | 154 | present | present | present | present |
| | 574 | present | present | present | present |
| | 945 | present | present | present | present |

Laboratory # 16

| Material | Sample | Mammalian & Poultry | Mamalians | Poultry | Fish |
|-----------------------------------|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 1366 | not present | not present | not present | not present |
| | 1772 | not present | not present | not present | not present |
| | 2332 | no result | no result | not present | not present |
| | 2668 | not present | not present | not present | not present |
| Poultry meal 5% | 975 | present | not present | present | not present |
| | 1059 | present | present | present | not present |
| | 2361 | present | present | present | not present |
| Fishmeal 5% | 626 | not present | not present | not present | present |
| | 1074 | no result | no result | no result | present |
| | 2033 | not present | not present | not present | present |
| | 2439 | not present | not present | not present | present |
| MMBM 0.1% + 5% Fishmeal | 795 | no result | no result | not present | present |
| | 1152 | present | present | no result | present |
| | 1985 | present | present | not present | present |
| MMBM 0.5% + 5% Poultry meal | 2342 | not present | not present | not present | present |
| | 978 | present | present | present | not present |
| | 2315 | present | present | present | not present |
| MMBM 0.1% | 2385 | present | present | not present | not present |
| | 104 | present | present | not present | not present |
| | 601 | present | present | not present | not present |
| MMBM 0.5% + 2.5% Poultry meal+ | 1322 | present | present | not present | not present |
| | 105 | present | present | not present | present |
| | 567 | present | present | present | present |
| 2.5 % Fishmeal | 1155 | present | present | present | present |

Laboratory # 17

| Material | Sample | Mammalian & Poultry | Mamaliains | Poultry | Fish |
|---------------------|---------------|------------------------------------|-------------------|----------------|-------------|
| Blank material | 1352 | not present | not present | not present | not present |
| | 1674 | not present | not present | not present | not present |
| | 2318 | not present | not present | not present | not present |
| | 2731 | not present | not present | not present | not present |
| Poultry meal 5% | 695 | present | no result | no result | not present |
| | 1178 | present | no result | no result | not present |
| | 2193 | present | no result | no result | not present |
| Fishmeal 5% | 885 | present | no result | no result | present |
| | 1158 | present | no result | no result | present |
| | 2019 | present | no result | no result | present |
| | 2306 | not present | not present | not present | present |
| MMBM 0.1% + 5% Fisl | 760 | present | no result | no result | present |
| | 1061 | present | no result | no result | present |
| | 1957 | present | no result | no result | present |
| | 2356 | present | no result | no result | present |
| MMBM 0.5% + 5% Po | 985 | present | no result | no result | not present |
| | 2098 | present | no result | no result | not present |
| | 2371 | present | no result | no result | not present |
| MMBM 0.1% | 69 | present | no result | no result | not present |
| | 1161 | present | no result | no result | not present |
| | 1427 | present | no result | no result | not present |
| MMBM 0.5% + 2.5% F | 14 | present | no result | no result | present |
| | 511 | present | no result | no result | present |
| | 1113 | present | no result | no result | not present |

Laboratory # 19

| Material | Sample | Mammalian & Poultry | Mamaliains | Poultry | Fish |
|---|---------------|------------------------------------|-------------------|----------------|-------------|
| Blank material | 1576 | not present | not present | not present | not present |
| | 1667 | not present | not present | not present | not present |
| | 2017 | not present | not present | not present | not present |
| | 2304 | not present | not present | not present | not present |
| Poultry meal 5% | 940 | present | not present | present | not present |
| | 1213 | present | not present | present | not present |
| | 2032 | present | not present | present | not present |
| Fishmeal 5% | 843 | not present | not present | not present | present |
| | 1144 | not present | not present | not present | present |
| | 2243 | not present | not present | not present | present |
| | 2460 | not present | not present | not present | present |
| MMBM 0.1% + 5% Fishmeal | 704 | present | present | not present | present |
| | 1145 | present | present | not present | present |
| | 2139 | present | not present | present | present |
| | 2475 | present | present | not present | present |
| MMBM 0.5% + 5% Poultry meal | 971 | present | present | present | not present |
| | 1293 | present | present | present | not present |
| | 2224 | present | present | present | not present |
| MMBM 0.1% | 55 | present | present | not present | not present |
| | 559 | present | present | not present | not present |
| | 1112 | present | present | not present | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 98 | present | not present | present | present |
| | 581 | present | not present | present | present |
| | 980 | present | present | present | present |

Laboratory # 20

| Material | Sample | Mammalian & Poultry | Mamaliains | Poultry | Fish |
|---|---------------|------------------------------------|-------------------|----------------|-------------|
| Blank material | 603 | present | present | present | present |
| | 1303 | not present | not present | not present | not present |
| | 1849 | not present | not present | not present | not present |
| | 2241 | not present | not present | not present | not present |
| Poultry meal 5% | 2787 | not present | not present | not present | not present |
| | 772 | present | not present | present | not present |
| | 1171 | present | not present | present | not present |
| Fishmeal 5% | 2340 | present | not present | present | not present |
| | 1032 | not present | not present | not present | present |
| | 1277 | not present | not present | not present | present |
| | 2229 | not present | not present | not present | present |
| MMBM 0.1% + 5% Fishmeal | 2355 | not present | not present | not present | present |
| | 942 | not present | not present | not present | present |
| | 1222 | not present | not present | not present | present |
| | 2027 | not present | not present | not present | present |
| MMBM 0.5% + 5% Poultry meal | 2307 | not present | not present | not present | present |
| | 1027 | present | present | present | not present |
| | 1314 | present | present | present | not present |
| MMBM 0.1% | 2455 | present | present | present | not present |
| | 41 | present | present | not present | present |
| | 496 | present | present | no result | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 1154 | present | present | not present | not present |
| | 756 | present | not present | present | present |
| | 1176 | present | no result | present | present |

Laboratory # 21

| Material | Sample | Mammalian & Poultry | Mamaliains | Poultry | Fish |
|---|---------------|------------------------------------|-------------------|----------------|-------------|
| Blank material | 1569 | not present | not present | not present | not present |
| | 1842 | not present | not present | not present | not present |
| | 1982 | not present | not present | not present | not present |
| | 2570 | not present | not present | not present | not present |
| Poultry meal 5% | 702 | not present | not present | present | not present |
| | 1003 | present | present | present | not present |
| | 2410 | present | present | present | not present |
| Fishmeal 5% | 710 | not present | not present | not present | present |
| | 1235 | not present | not present | not present | present |
| | 2173 | not present | not present | not present | present |
| | 2488 | present | present | not present | present |
| MMBM 0.1% + 5% Fishmeal | 753 | present | present | present | present |
| | 1075 | present | present | not present | present |
| | 1992 | present | present | not present | present |
| MMBM 0.5% + 5% Poultry meal | 2286 | present | present | not present | present |
| | 803 | present | present | present | not present |
| | 1286 | present | present | present | not present |
| MMBM 0.1% | 2238 | present | present | present | not present |
| | 34 | present | present | not present | not present |
| | 615 | present | present | not present | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 1455 | present | present | not present | not present |
| | 140 | present | present | not present | present |
| | 616 | present | present | present | present |
| | 1127 | present | present | present | present |

Laboratory # 22

| Material | Sample | Mammalian & Poultry | Mamalians | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 1506 | not present | not present | not present | present |
| | 1856 | not present | not present | not present | not present |
| | 1954 | present | present | present | present |
| | 2556 | not present | not present | not present | not present |
| Poultry meal 5% | 807 | not present | not present | not present | present |
| | 1262 | present | present | not present | not present |
| | 2403 | present | present | not present | not present |
| Fishmeal 5% | 619 | not present | not present | not present | present |
| | 1200 | not present | not present | not present | present |
| | 2026 | not present | not present | not present | present |
| | 2474 | not present | not present | not present | present |
| MMBM 0.1% + 5% Fishmeal | 620 | present | present | not present | present |
| | 1229 | present | present | not present | present |
| | 1929 | not present | not present | not present | present |
| | 2419 | not present | not present | not present | present |
| MMBM 0.5% + 5% Poultry meal | 810 | present | present | not present | not present |
| | 1237 | present | present | present | not present |
| | 2084 | present | present | present | not present |
| MMBM 0.1% | 27 | present | present | no result | not present |
| | 622 | present | present | present | not present |
| | 832 | present | present | not present | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 91 | present | present | no result | present |
| | 560 | present | present | not present | present |
| | 917 | present | present | not present | not present |

Laboratory # 23

| Material | Sample | Mammalian & Poultry | Mamalians | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 1380 | not present | not present | not present | not present |
| | 1695 | not present | not present | not present | present |
| | 1961 | not present | not present | not present | not present |
| | 2724 | not present | not present | not present | not present |
| Poultry meal 5% | 905 | present | not present | present | not present |
| | 1276 | present | present | not present | not present |
| | 2382 | present | not present | present | present |
| Fishmeal 5% | 675 | not present | not present | not present | present |
| | 1102 | not present | not present | not present | present |
| | 2096 | present | present | not present | present |
| | 2236 | present | no result | no result | present |
| MMBM 0.1% + 5% Fishmeal | 774 | present | present | no result | present |
| | 844 | present | present | no result | present |
| | 2034 | not present | not present | not present | present |
| | 2461 | present | not present | present | present |
| MMBM 0.5% + 5% Poultry meal | 1020 | present | present | present | not present |
| | 1279 | present | not present | present | not present |
| | 2035 | present | present | present | not present |
| MMBM 0.1% | 20 | present | present | no result | not present |
| | 111 | present | present | no result | not present |
| | 1049 | present | present | not present | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 322 | present | present | no result | present |
| | 749 | present | present | no result | present |
| | 1246 | present | present | present | present |

Laboratory # 24

| Material | Sample | Mammalian & Poultry | Mamalians | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 1338 | not present | not present | not present | not present |
| | 1821 | not present | not present | not present | not present |
| | 2031 | not present | not present | not present | not present |
| | 2549 | not present | not present | not present | not present |
| Poultry meal 5% | 688 | present | no result | no result | not present |
| | 1311 | present | no result | no result | not present |
| | 2347 | present | no result | no result | not present |
| Fishmeal 5% | 752 | not present | not present | not present | present |
| | 1179 | not present | not present | not present | present |
| | 1956 | not present | not present | not present | present |
| | 2159 | not present | not present | not present | present |
| MMBM 0.1% + 5% Fishmeal | 921 | not present | not present | not present | present |
| | 1348 | not present | not present | not present | present |
| | 1817 | not present | not present | not present | present |
| | 2377 | present | no result | no result | present |
| MMBM 0.5% + 5% Poultry meal | 831 | present | no result | no result | not present |
| | 1321 | present | no result | no result | not present |
| | 2441 | present | no result | no result | not present |
| MMBM 0.1% | 174 | present | no result | no result | not present |
| | 580 | present | no result | no result | not present |
| | 1091 | present | no result | no result | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 175 | present | no result | no result | present |
| | 728 | present | no result | no result | present |
| | 1260 | present | no result | no result | present |

Laboratory # 25

| Material | Sample | Mammalian & Poultry | Mamalians | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 1345 | not present | not present | not present | not present |
| | 1779 | not present | not present | not present | not present |
| | 2024 | not present | not present | not present | not present |
| | 2780 | not present | not present | not present | not present |
| Poultry meal 5% | 758 | present | no result | no result | not present |
| | 1325 | present | no result | no result | not present |
| | 2375 | present | no result | no result | not present |
| Fishmeal 5% | 738 | not present | not present | not present | present |
| | 1081 | not present | not present | not present | present |
| | 2012 | not present | not present | not present | present |
| | 2446 | not present | not present | not present | present |
| MMBM 0.1% + 5% Fishmeal | 928 | not present | not present | not present | present |
| | 1362 | present | no result | no result | present |
| | 1719 | present | no result | no result | present |
| MMBM 0.5% + 5% Poultry meal | 2405 | not present | not present | not present | present |
| | 880 | present | no result | no result | not present |
| | 1188 | present | no result | no result | present |
| MMBM 0.1% | 2126 | present | no result | no result | no result |
| | 314 | present | no result | no result | not present |
| | 720 | present | no result | no result | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 1343 | present | no result | no result | not present |
| | 385 | present | no result | no result | present |
| | 595 | present | no result | no result | present |
| | 1148 | present | no result | no result | present |

Laboratory # 26

| Material | Sample | Mammalian & Poultry | Mamalians | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 785 | not present | not present | not present | not present |
| | 1037 | not present | not present | not present | not present |
| | 2059 | not present | not present | not present | not present |
| | 2542 | not present | not present | not present | not present |
| Poultry meal 5% | 744 | present | no result | no result | not present |
| | 1283 | present | no result | no result | not present |
| | 2158 | present | no result | no result | not present |
| Fishmeal 5% | 717 | not present | not present | not present | present |
| | 1249 | not present | not present | not present | present |
| | 2005 | not present | not present | not present | present |
| | 2418 | not present | not present | not present | present |
| MMBM 0.1% + 5% Fishmeal | 977 | not present | not present | not present | present |
| | 1397 | not present | not present | not present | present |
| | 1754 | present | no result | no result | present |
| | 2440 | present | no result | no result | present |
| MMBM 0.5% + 5% Poultry meal | 726 | present | no result | no result | not present |
| | 1265 | present | no result | no result | not present |
| | 2434 | present | no result | no result | not present |
| MMBM 0.1% | 377 | present | no result | no result | not present |
| | 461 | present | no result | no result | not present |
| | 1007 | present | no result | no result | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 476 | present | no result | no result | present |
| | 735 | present | no result | no result | present |
| | 1295 | present | no result | no result | present |

Laboratory # 28

| Material | Sample | Mammalian & Poultry | Mamalians | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 764 | not present | - | - | not present |
| | 1107 | not present | - | - | not present |
| | 1947 | not present | - | - | not present |
| | 2472 | not present | - | - | not present |
| Poultry meal 5% | 730 | present | - | - | not present |
| | 1185 | present | - | - | not present |
| | 2214 | present | - | - | not present |
| Fishmeal 5% | 941 | not present | - | - | present |
| | 1270 | not present | - | - | present |
| | 2222 | not present | - | - | present |
| | 2453 | not present | - | - | present |
| MMBM 0.1% + 5% Fishmeal | 956 | not present | - | - | present |
| | 1383 | not present | - | - | present |
| | 1838 | not present | - | - | present |
| MMBM 0.5% + 5% Poultry meal | 2426 | present | - | - | present |
| | 1076 | present | - | - | present |
| | 2021 | present | - | - | not present |
| MMBM 0.1% | 2490 | present | - | - | not present |
| | 538 | not present | - | - | not present |
| | 937 | not present | - | - | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 1406 | present | - | - | present |
| | 182 | present | - | - | present |
| | 448 | present | - | - | present |
| | 1099 | present | - | - | present |

Laboratory # 29

| Material | Sample | Mammalian & Poultry | Mamalians | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 722 | not present | not present | not present | not present |
| | 1128 | not present | not present | not present | not present |
| | 2073 | not present | not present | not present | not present |
| | 2465 | not present | not present | not present | not present |
| Poultry meal 5% | 681 | present | present | no result | not present |
| | 1255 | present | present | no result | not present |
| | 2494 | present | present | no result | not present |
| Fishmeal 5% | 913 | not present | not present | not present | present |
| | 1312 | not present | not present | not present | present |
| | 1921 | not present | not present | not present | present |
| | 2292 | not present | not present | not present | present |
| MMBM 0.1% + 5% Fishmeal | 830 | present | present | no result | present |
| | 1376 | present | present | no result | present |
| | 1698 | present | present | no result | present |
| | 2300 | not present | not present | not present | present |
| MMBM 0.5% + 5% Poultry meal | 1069 | present | present | present | not present |
| | 1146 | present | present | present | not present |
| | 2056 | present | present | no result | not present |
| MMBM 0.1% | 188 | present | present | present | not present |
| | 699 | present | present | no result | present |
| | 930 | present | present | no result | present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 427 | present | present | no result | present |
| | 819 | present | present | no result | present |
| | 1239 | present | present | no result | present |

Laboratory # 30

| Material | Sample | Mammalians & Poultry | Mamalians | Poultry | Fish |
|---|---------------|-------------------------------------|------------------|----------------|-------------|
| Blank material | 743 | not present | not present | not present | not present |
| | 939 | present | present | not present | not present |
| | 1940 | present | not present | present | not present |
| | 2297 | not present | not present | not present | not present |
| Poultry meal 5% | 989 | present | not present | present | not present |
| | 1269 | present | not present | present | present |
| | 1990 | present | not present | present | not present |
| Fishmeal 5% | 1018 | not present | not present | not present | present |
| | 1263 | not present | not present | not present | present |
| | 1998 | not present | not present | not present | present |
| | 2271 | not present | not present | not present | present |
| MMBM 0.1% + 5% Fishmeal | 879 | not present | not present | not present | present |
| | 1516 | not present | not present | not present | not present |
| | 1803 | not present | not present | not present | present |
| MMBM 0.5% + 5% Poultry meal | 2447 | not present | not present | not present | present |
| | 859 | present | not present | present | present |
| | 1251 | present | not present | present | present |
| MMBM 0.1% | 2294 | present | not present | present | not present |
| | 335 | present | present | not present | not present |
| | 741 | present | present | not present | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 1252 | present | not present | present | not present |
| | 252 | not present | not present | not present | present |
| | 630 | not present | not present | not present | present |
| | 994 | present | not present | present | present |

Laboratory # 31

| Material | Sample | Mammalians | | | |
|---|---------------|----------------------|-------------------|----------------|-------------|
| | | & Poultry | Mamaliains | Poultry | Fish |
| Blank material | 694 | not present | not present | no result | not present |
| | 1100 | present | present | no result | not present |
| | 2045 | not present | not present | no result | not present |
| | 2409 | present | present | no result | present |
| Poultry meal 5% | 723 | present | present | no result | not present |
| | 1318 | present | present | no result | not present |
| | 2235 | present | present | no result | not present |
| Fishmeal 5% | 668 | present | present | no result | present |
| | 1228 | present | present | no result | present |
| | 1914 | not present | not present | no result | present |
| | 2495 | no result | no result | no result | no result |
| MMBM 0.1% + 5% Fishmeal | 851 | present | present | no result | present |
| | 1544 | present | present | no result | present |
| | 1768 | present | present | no result | present |
| | 2454 | present | present | no result | present |
| MMBM 0.5% + 5% Poultry meal | 915 | present | present | no result | not present |
| | 2182 | present | present | no result | not present |
| | 2525 | present | present | no result | not present |
| MMBM 0.1% | 202 | present | present | no result | not present |
| | 678 | present | present | no result | not present |
| | 1357 | present | present | no result | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 56 | present | present | no result | present |
| | 770 | present | present | no result | present |
| | 1225 | present | present | no result | present |

Laboratory # 32

| Material | Sample | Mammalian | | | |
|---|---------------|----------------------|-------------------|----------------|-------------|
| | | & Poultry | Mamaliains | Poultry | Fish |
| Blank material | 687 | not present | not present | not present | present |
| | 1205 | not present | not present | not present | not present |
| | 2066 | no result | no result | no result | present |
| | 2486 | not present | not present | not present | present |
| Poultry meal 5% | 996 | present | no result | present | present |
| | 1220 | present | present | present | present |
| | 2018 | present | no result | present | present |
| Fishmeal 5% | 829 | present | present | no result | present |
| | 1291 | not present | not present | not present | present |
| | 1970 | not present | not present | not present | present |
| | 2516 | not present | not present | not present | present |
| MMBM 0.1% + 5% Fishmeal | 802 | present | present | present | present |
| | 1390 | present | present | no result | present |
| | 1747 | present | present | not present | present |
| MMBM 0.5% + 5% Poultry meal | 2398 | present | present | not present | present |
| | 838 | present | present | present | present |
| | 1223 | present | no result | present | present |
| MMBM 0.1% | 2203 | present | no result | present | present |
| | 6 | present | present | present | present |
| | 713 | present | present | present | present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 1350 | present | present | no result | not present |
| | 301 | present | present | present | present |
| | 833 | present | no result | present | present |
| | 1092 | present | present | present | present |

Laboratory # 33

| Material | Sample | Mammalian & Poultry | Mamalians | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 757 | not present | not present | not present | not present |
| | 1198 | not present | not present | not present | not present |
| | 2052 | not present | not present | not present | not present |
| | 2479 | not present | not present | not present | not present |
| Poultry meal 5% | 954 | present | not present | present | not present |
| | 1290 | present | not present | present | not present |
| | 2319 | present | not present | present | not present |
| Fishmeal 5% | 689 | present | not present | not present | present |
| | 1214 | present | not present | not present | present |
| | 1963 | present | not present | not present | present |
| | 2390 | present | not present | not present | present |
| MMBM 0.1% + 5% Fishmeal | 865 | present | present | not present | present |
| | 1299 | present | present | not present | present |
| | 1831 | present | present | not present | present |
| | 2384 | present | present | not present | present |
| MMBM 0.5% + 5% Poultry meal | 740 | present | present | present | not present |
| | 1230 | present | present | present | not present |
| | 2462 | present | present | present | not present |
| MMBM 0.1% | 13 | present | present | not present | not present |
| | 643 | present | present | not present | not present |
| | 958 | present | present | not present | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 378 | present | present | not present | present |
| | 784 | present | present | present | present |
| | 1197 | present | present | not present | present |

Laboratory # 34

| Material | Sample | Mammalian & Poultry | Mamalians | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 673 | not present | - | - | not present |
| | 1135 | not present | - | - | not present |
| | 1975 | not present | - | - | present |
| | 2500 | not present | - | - | not present |
| Poultry meal 5% | 653 | present | not present | present | not present |
| | 1136 | present | not present | present | not present |
| | 2137 | present | not present | present | not present |
| Fishmeal 5% | 934 | not present | - | - | present |
| | 1298 | not present | - | - | present |
| | 2131 | not present | - | - | present |
| | 2362 | not present | - | - | present |
| MMBM 0.1% + 5% Fishmeal | 809 | present | present | present | present |
| | 1285 | present | present | not present | present |
| | 1733 | not present | - | - | present |
| | 2223 | present | present | present | present |
| MMBM 0.5% + 5% Poultry meal | 936 | present | present | present | not present |
| | 2119 | present | present | present | not present |
| | 2378 | present | present | present | not present |
| MMBM 0.1% | 272 | present | present | not present | not present |
| | 923 | present | present | not present | not present |
| | 1462 | present | present | not present | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 84 | present | present | not present | present |
| | 623 | present | present | not present | present |
| | 1085 | present | present | present | present |

Laboratory # 50

| Material | Sample | Mammalian & Poultry | Mamaliens | Poultry | Fish |
|---|---------------|------------------------------------|------------------|----------------|-------------|
| Blank material | 211 | not present | not present | not present | not present |
| | 610 | not present | not present | not present | not present |
| | 1877 | not present | not present | not present | not present |
| | 2290 | not present | not present | not present | not present |
| Poultry meal 5% | 191 | present | no result | no result | not present |
| | 527 | present | no result | no result | not present |
| | 1948 | present | no result | no result | not present |
| Fishmeal 5% | 129 | not present | not present | not present | present |
| | 549 | not present | not present | not present | present |
| | 1445 | not present | not present | not present | present |
| | 2194 | not present | not present | not present | present |
| MMBM 0.1% + 5% Fishmeal | 298 | present | no result | no result | present |
| | 592 | present | no result | no result | present |
| | 1523 | not present | not present | not present | present |
| | 2104 | present | no result | no result | present |
| MMBM 0.5% + 5% Poultry meal | 383 | present | no result | no result | not present |
| | 1062 | present | no result | no result | not present |
| | 2469 | present | no result | no result | not present |
| MMBM 0.1% | 244 | present | no result | no result | not present |
| | 685 | present | no result | no result | not present |
| | 1280 | present | no result | no result | not present |
| MMBM 0.5% + 2.5% Poultry meal+ 2.5 % Fishmeal | 70 | present | present | present | present |
| | 672 | present | no result | no result | present |
| | 1022 | present | no result | no result | present |