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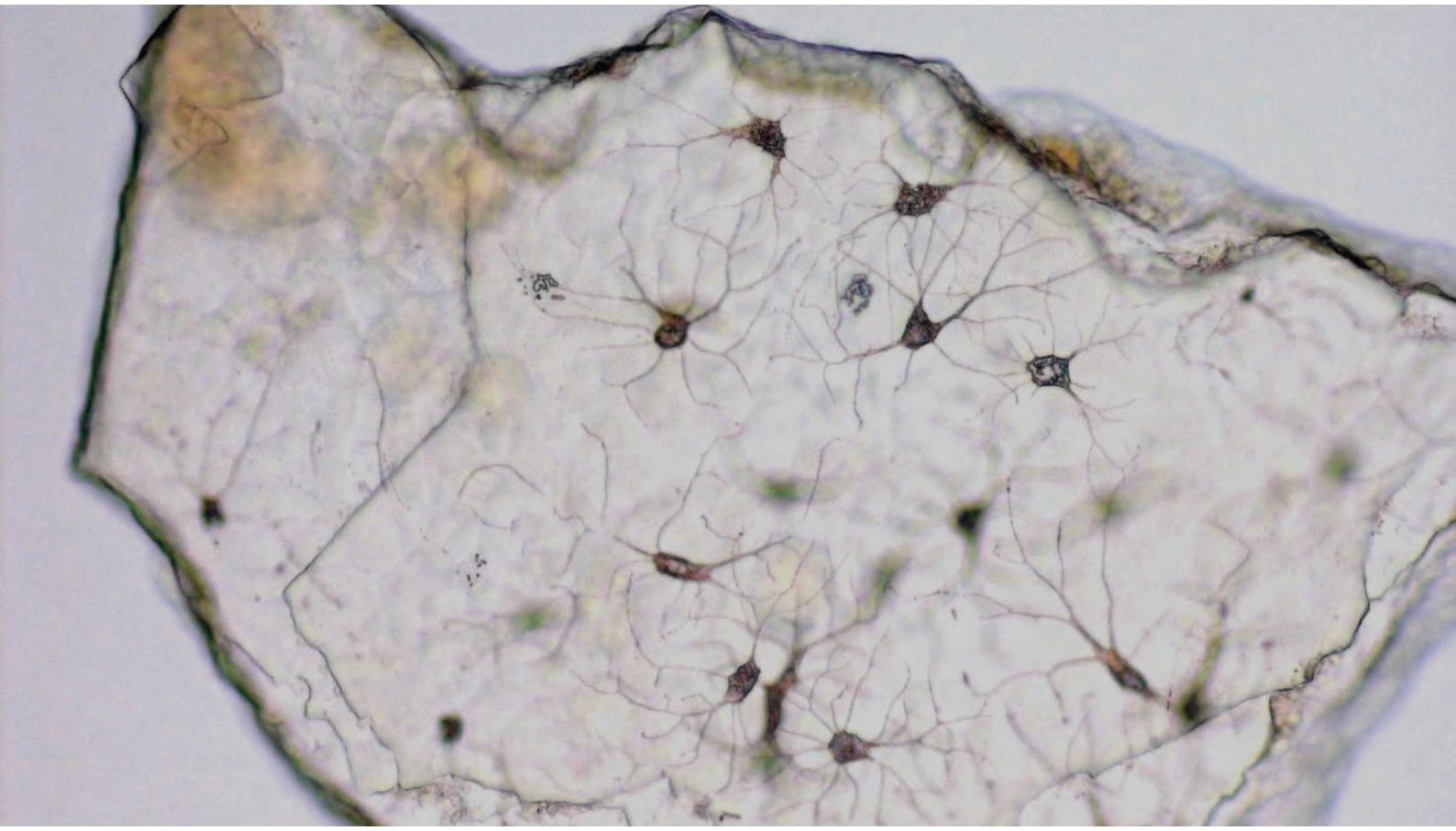

Animal proteins in feed

IAG ring test 2011

RIKILT Report 2011.015

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Summary

A ring test was organized for the detection of animal proteins in animal feed by microscopy in the framework of the annual ring tests of the IAG - International Association for Feeding stuff Analysis, Section Feeding stuff Microscopy. The organizer of the ring test was RIKILT - Institute of food safety, Wageningen University and Research Centre, The Netherlands. The aim of the ring study was to provide the participants information on the local implementation of the detection method for their individual quality systems. A further aim was to gather information about the application of the microscopic method.

Of the four samples prepared three were based on a ruminant feed as matrix: one containing no animal proteins (blank), one with 0.05% of terrestrial animal material, and one with 1% of feather meal. The fourth sample consisted of a fish meal contaminated with 1% of terrestrial animal material. All participants were requested to determine the presence or absence of land animal and/or fish protein material and to indicate the type of material found. The participants were also asked to report the amount of sediment found (the fraction containing minerals and bones, if present) and to answer questions on a series of parameters of the microscopic method. Reporting the estimated amount of land animal or fish protein was optional for all participants. 56 Participants returned results using the microscopic method, making this the largest ring test ever organized for animal proteins in feed.

Incorrect positive results (positive deviations) were expressed in a specificity score and incorrect negative results (negative deviations) were expressed in a sensitivity score. An optimal score is 1.0. Specificity scores for both the absence of fish meal and the absence of land animal material were 0.98 and 1.0, respectively in the blank sample. The detection of the absence of fish material (specificity) was suboptimal in both the samples with 0.05% of MBM (0.91) and in the sample with feather meal (0.88). The detection of the materials of land animals was good in all cases. The feather meal was primarily detected positively because of the presence of bone fragments (0.98), but the feather meal was only recognised by a minority of participants (sensitivity 0.33).

The amount of land animal proteins in the feed was overestimated. The estimations of MBM in the fish meal and of the feather meal were lower than the actual amounts. All estimations appeared to be significantly deviating from the actual amount at or (far) below a level of p = 1.0%.

There were no clear correlations between the application of certain method parameters, e.g. the type of glassware, the embedding agent or the use of a binocular, and the results, nor in terms of specificity and sensitivity nor in quantification of the results. Only the application of Alizarin staining might have some effect on the method performance. There was still a large variety in the application of the method. A further harmonization is still possible.

The results for the PCR (two sets of results) and the immunoassay tests (two set of results) indicate that a proper detection of MBM in feed can be achieved at relatively low levels of contamination. However, in some cases false positive results were also reported, such a positive pig sample for avian contamination and an avian signal in a feed with mammalian material.

The results give a good overview of the performance of the labs performing the microscopic method, although further improvement is still possible.

Contents

1 Introduction

Member states of the European Union are requested by EU legislation to maintain an active monitoring program for the safety of feed. The monitoring of the presence of animal proteins in the framework of eradication of mad cow disease is an important part of it. A range of official control methods were in 2009 combined in one Regulation (152/2009/EC). With respect to animal proteins, the microscopic detection method is the only official control method until now. The description of the microscopic method was copied from the former Directive 2003/126/EC to Annex VI of the new Regulation without any modification. Although much efforts has been put in the development of improved methods for a range of techniques (Gizzi et al., 2003; van Raamsdonk et al., 2007; Woodgate et al., 2009; Liu et al., 2010; van Raamsdonk et al., 2011), these methods did not enter legislation at that stage.

The level of contamination of 0.1%, as stated as performance parameter for official control methods in Annex VI of Regulation 152/2009/EC, forms the basis of most proficiency tests and collaborative studies to establish lab performance and to validate new methods. It is nevertheless obvious that several methods, microscopy a.o., can detect contaminations at lower concentration levels (e.g. Veys et al., 2010).

The IAG - International Association for Feeding stuff Analysis, Section Feeding stuff Microscopy organises annually a ring test for animal proteins in feeds for all their members. In this report the ring test for animal proteins is presented, which was organised by RIKILT in 2011 on behalf of the IAG Section Feeding stuff Microscopy. A contamination level below 0.1 %, i.e. 0.05% of animal proteins from terrestrial animals is also part of the design of this ring test. The derogation to use fish meal for weaning ruminants, and the desire for further relaxation of the extended feed ban gave rise to inclusion of a contaminated fish meal, and a feed adulterated with feather meal.

The indication “ring test” fits in the history of annual proficiency tests for animal proteins carried out under the responsibility of the IAG. The main purpose of the ring test is to monitor the performance of the participating laboratories (internal quality assurance). The main part of this report presents and discusses the results in terms of sensitivity and specificity scores. For a further documentation of laboratory results each participant answered questions on details of the application of the method. These results can be used to interpret the effectiveness of some method parameters.

2 Methods

2.1 Materials

Four samples were produced, based on a cattle feed that was commercially produced, called Prima Biks Ambitie Z25-55873 (samples A, B, D), and a fish meal (sample C).

The feed material contained the following major ingredients in order of decreasing share: wheat gluten feed, sugar beet pulp, palm kernel flakes, rape seed meal, beet vinasse, beet molasses, maize, wheat, semolina, citrus pulp, vegetal fatty acids, calcium carbonate, and magnesium oxyde. The ingredients were glued together with molasse to form larger particles. Therefore, the feed material was sieved at 2 mm in order to exclude these particles from the samples, avoiding the possibility of animal proteins adhering to them. The composition might be modified as a result of this procedure. Five samples of this feed have been tested microscopically and by means of PCR at RIKILT for the presence of animal proteins. No material of animal origin was found.

The fish material consisted of a mixture of five different samples obtained from the RIKILT regular monitoring program. Five different portions of this mixture were tested by microscopy for other animal proteins than fish. No material of animal origin other than from fish was found.

The ring trial consisted of four samples with a composition as listed in Table 1.

Table 1: Composition of the samples in the NRL-IAG ring trial 2011.

Label	Content
2011-A	Feed with 0.05% MBM
2011-B	Blank feed
2011-C	Fish meal with 1% MBM
2011-D	Feed with 1% of feather meal

The meat and bone meal (MBM) used was obtained from a targeted monitoring program. It was proved to contain a high level of bovine material by PCR. Five different portions have been tested by microscopy for other animal proteins than land animals. No material of animal origin other than from land animals was found. The feather meal is a sample from practice. This feather meal sample was tested to assure the absence of animal proteins from fish.

2.2 Procedure for production

In order to avoid any cross contamination, the samples were produced in a strict order. Jars for sample 2011-B were filled with 40-45 grams of the pure feed, closed and set aside.

Samples 2011-A, 2011-C and 2011-D were produced according to the method of stepwise dilution. For sample 2011-A 1.5 g of MBM was used to prepare (finally) 3 kg of contaminated feed as follows. The initial 1.5 g of MBM was mixed in 1.5 g of feed and stirred for one minute. In nine subsequent steps the remaining amount of feed was added stepwise by mixing according to a fixed scheme.

For the preparation of sample 2011-D, 30 g of feather meal was mixed in 30 g of feed and stirred for one minute. In six additional steps the final amount of 3 kg mixture was obtained with a concentration of 1.0 % of feather meal. The final jars for sample 2011-D were filled with 40 – 45 grams of material.

The samples 2011-A, 2011-B and 2011-D were set aside in order to avoid any contamination with fish meal.

Finally sample 2011-C was prepared by initially mixing 30 g of MBM in 30 g of fish meal and stirred for one minute. A fixed scheme was followed to prepare the final 3 kg of mixture in six additional steps of stepwise dilution. The final jars for sample 2011-C were filled with 40 – 45 grams of material.

2.3 Homogeneity study

Two RIKILT microscopists examined independently three jars of sample 2011-A, of 2011-C, and of 2011-D. In all cases a correct result was obtained, as is shown in Table 2. Based on these results it was justified to send the sets of four samples around to all participants. The microscopy research group of RIKILT did not participate in the further laboratory analysis of this ring trial.

Table 2: Results of the homogeneity study. Sediment amounts are based on 10 grams. The number of portions is indicated for two microscopists independently.

Sample		Sediment amount	Fish	MBM
2011-A 0.05% MBM	(n= 3)	4.6 - 5.1 %	3 x negative	3 x positive
2011-B blank	(n= 5)	4.2 - 4.9 %	5 x negative	5 x negative
2011-C 99% fish meal, 1% MBM	(n= 3)	12.4 - 13.5 %	3 x positive	3 x positive
2011-D 1% feather meal	(n= 3)	4.4 - 5.5 %	3 x negative	3 x positive

2.4 Organization of the ring trial

All IAG members, all NRLs, and a series of putative interesting laboratories were informed about the ring test for 2011. In all cases an invitation letter, a participation form and an invoice were distributed. Until the beginning of March a total of 57 participants were listed. The sets of four samples with an accompanying letter (see Annex I) were sent to all participants on the 3rd of March 2011. On Friday March 4 an E-mail message was sent around to all participants, together with an electronic report form (see Annex II and III) and the request to confirm the receipt of the package. The report form also contained a sheet with instructions (see Annex IV).

The closing date for reporting results was fixed at April 6. In one occasion a participant received the package at a later date. Fifty-three sets of results were received not later than April 8. Three sets of results were submitted later, but were received before any communication about the contents of the samples was started. Therefore, a total of 56 sets of microscopic results could reliably be considered in the final evaluation. One participant did not submit its results. The report was finalised at May 5.

2.5 Participants

The 57 participants originated from 25 countries: 20 member states of the European Union, and five other countries (Canada, China, Norway, Peru and Switzerland). The list of participants is presented in Annex V. Five member states have been involved with three or more participating laboratories: Germany (16 labs), Italy (6), Belgium (5), Netherlands (3), and France (3). With the indicated number of participants and the coverage, this ring test is the largest one ever reported for microscopic detection of animal proteins in feed.

2.6 Analysis of 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. The sensitivity is the ability of the method used, to detect the contaminant when it is present, whereas the specificity is the ability to not detect the contaminant when it is absent. The following equations have been used to calculate the statistics:

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

$$\text{Sensitivity } SE = \frac{PA}{PA + ND}$$

$$\text{Specificity } SP = \frac{NA}{PD + NA}$$

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 are presented as fractions. Accuracy (specificity or sensitivity) has been calculated for each sample type.

As criterion for a good or excellent score a threshold of 0.95 for either sensitivity or specificity was applied.

Significancy of quantitative results were tested by using Student's t-test statistics; see, for example, Hand (2009).

3 Results

Fifty-seven packages with four samples were sent to all participants. Fifty-six participants returned results for the microscopic method, two sets of results were received for PCR analysis, and two sets from one participant for protein detection. Eight participants submitted only an e-mail message, and one participant send only a FAX message. Three participants submitted report sheets with the wrong participants number. The link with the original E-mail message and sender could be established beyond doubt; otherwise these reports would have been omitted. All reports were included.

The full results are presented in the tables of Annex VI, VII and VIII. Results indicated as "Blank" by the participants were considered to indicate the absence of the indicated type of animal protein.

3.1 Microscopic detection

Most of the specificity and sensitivity scores were at good to excellent levels (Table 3; Annex VII). There is a remarkable number of participants that reported the presence of fish in sample D with feather meal. Also in the exclusive presence of material of terrestrial animals (sample A) some participants reported the presence of fish material. The absence of animal proteins of any kind was remarkably well detected in the blank samples (B). The absence of fish material in the pure fish meal sample (C) in one report was not commented further by the reporting participant.

Table 3: Sensitivity and specificity scores for the detection of animal proteins in four samples. Abbreviations: n: number of participants per group. Capitals A to C: sample indication.

		Fish				MBM			
		A	B	C	D	A	B	C	D
N		0	0	99%	0	0.05%	0	1.0%	1.0%*
56	specificity	0.91	0.98		0.88		1.0		
	sensitivity			0.98		0.98		0.95	0.98

**: feather meal*

The presence of animal proteins in sample D was predominantly reported as the presence of bone particles. A minority of the participants (13 out of 40) reported the presence of feathers or feather meal. For these figures exclusively those participants were included which commented on the type of animal proteins found. Fifteen participants reported exclusively "present" or "absent" without further indication.

As far as commented by the participants the false positives were caused by only traces or low amounts of animal proteins (Table 4).

Factors such as laboratory skills, glassware used, and lab procedures on e.g. cleaning to avoid sample pollution (in the case of false positives) might influence laboratory performance. In some cases misidentifications might be caused by some confusing plant ingredients or by peculiar hydrolysed feather particles.

Table 4: Participants' comments on the background of the false positives reported for the calculations in Table 3a.

Sample, contaminant	Participant	Comment
A: fish material	5	none
	22	bone, muscle (0.01 %)
	33	bone
	42	bone (0.01 %)
	47	none
B: fish animal	17	7 bone fragments (< 0.01 %)
D: fish material	2	bone, scale
	12	bone (0.01 %)
	17	7 bone fragments (< 0.01 %)
	18	within LOD
	29	none
	37	5 bones
	42	bone, scale (0.0 1%)

3.2 Microscopic procedure

An inventory of ten different parameters was added to the report sheet of the actual results of the four samples. These results are shown in Annex VI and summarised in Table 5. The main purpose of this inventory was to provide information for the individual participants for comparison with the general application of the method. Although this has to be considered additional information only, a ring test with a random set of participants provides a good opportunity to collect meta-data on the application of the method. The current results provides the opportunity to discuss some parameters of the microscopic method. The frequencies of application of choices for several method parameters are presented in Table 5.

Table 5: Inventory of parameters for microscopic detection and their application.

Parameter	Parameter state	Number of participants	Amount
amount of material used for sedimentation	5 grams	3	
	10 grams	50	
	other	3	
type of glassware	chemical sedimentation funnel	33	
	beaker (flat bottom)	9	
	champagne glass	7	
	conical glass with cock	3	
	other	3	
sedimentation agent	TCE	54	
	TCE/Petroleumether	0	
	other	1	
use of staining of sediment	no	33	
	yes	22	
use of binocular for examination at lower magnifications	yes	44	
	no	12	
size of cover glass used	small (e.g. 20 x 20 mm)	36	
	medium	8	
	large (e.g. 26 x 50 mm)	12	
share of the total sediment used for examination	minimum		0.2%
	maximum		100%
embedding agent	paraffin oil	20	
	immersion oil	12	
	glycerine / glycerol	12	
	Norland Adhesive	6	
	other (water, glycerol:water mixture, mineral oil)	5	
Use of ARIES	yes	3	
	no	45	
f-factor for MBM	minimum		20%
	maximum		100%
	none estimated	23	

Fifty out of the 56 participants started the sedimentation procedure with an amount of 10 grams of material. A chemical sedimentation funnel was used primarily (33 out of 56 labs). Twenty-two participants used staining of the sediment (Alizarin Red) as primary treatment for evaluation of the materials. It was not stated if unstained examination (standard method) was applied as well. Examination of the sediment at lower magnifications by using a binocular is still requested in the official method, but 12 participants out of 56 reported to skip this part of the procedure. Only in one occasion a non-suited embedding agent was used for the examination of the sediment (water).

Correlations between specificity and method parameters are relevant only if some sort of causal relationship exists in order to avoid the analysis of random fluctuations of results. In the process of further harmonisation of the microscopic method, almost all participants made the same choice for the application of several parameters: amount of material used for sedimentation (10 grams), sedimentation agent (TCE), non-suited embedding agent: only one participant. As far as substantial numbers among the participants have applied different parameters of the method (see Table 5), there are no significant differences found between the results and whether or not staining was applied, a binocular was used, or with respect to using different types of glassware. As an example, the use of staining of the sediment will be documented further.

Only a very low share of the participants used the knowledge system ARIES (van Raamsdonk et al., 2004, 2010b). The information in this system could support the proper identification of hydrolised feather particles, or can help to discriminate between confusing particles of land animals and fish.

3.2.1 Staining of sediment

Staining of the sediment material with Alizarin Red is applied by 36% of the participants, with the goal to facilitate an initial recognition of bone particles. The results with respect to specificity and senstivity scores are presented in Table 6. The differences between the results after staining compared to the results without staining the sediment are not large, although the less optimal results for fish detection after staining are notable. It is nevertheless necessary for a final decision on the nature of individual particles to consider other features of the particle such as the structure, presence of lacunae and the visibility of canaliculae.

Table 6: Sensitivity/specificity scores for the detection of animal proteins in four samples, separate for analyses based on a stained or an unstained sediment. Abbreviations: n: number of participants per group. Capitals A to D: sample indication.

Staining of sediment	N		Fish				MBM			
			A	B	C	D	A	B	C	D
			0	0	100%	0	0.05%	0	1.0%	1.0%
Stained with alizarin	21	specificity	0.86	0.95		0.86		1.0		
		sensitivity			0.95		0.95		0.91	1.0
Unstained	33	specificity	0.94	1.0		0.88		1.0		
		sensitivity			1.0		1.0		0.97	0.97

3.2.2 Feather meal

The presence of 1.0 % of feather meal can be detected in three ways. The presence of bone fragments as part of feather meal can be noticed in most cases, since feather meal is usually contaminated with bone particles at a certain level. This was correctly reported except for one participant (Table 3). The second way is to detect (hydrolised) feather fragment by examining the flotation or the original sample. Fourty participants specified the type of fragments they encountered. Only one third of these participants reported positively the presence of feather material. The use of a binocular did not improve the results. Finally feathers and animal hairs can

be identified by using the Cystine reagent as indicated in the microscopic method section 6.3 of Regulation (EC) 152/2009.

3.3 Quantification

The starting amount of material for sedimentation will obviously influence the results of quantification. Presenting the results of the sedimentation procedure separated for the starting amount of material has no informatieve value, since only two participants using 5 gram submitted (partial) results. One of them (participant 31) obtained a very high amount of sediment for sample A (159.2 mg). In the following evaluation only those participants using 10 grams for sedimentation included.

The application of staining with alizarin has some effect on the final amount of sediment. The presentation in Table 7 is based on the results of 45 out of 52 participants which submitted quantitative results based on 10 grams of material. As expected, the amount of sediment achieved after applying staining is generally lower than achieved without staining. Only for sample D a little higher amount of sediment was obtained after staining. This cannot be explained by the presence of feather meal in this sample. It has to be noted that the differences are very small in the view of the large standard deviations, and appeared to be not significant in any case (t-test). Participant 50 reported an amount of sediment for sample D of 1500 mg. This figure was considered a typing error and omitted for the calculations.

Table 7: Resulting amounts of sediment (in g) separate for the application of staining of the sediment. For every result the average (in normal) and standard deviation (in italics) is given. Ten participants did not report results for quantification.

	N	Amount of sediment (g)			
		A	B	C	D
total	45	0.674 (*0.321*)	0.657 (*0.422*)	1.264 (*0.313*)	0.711 (*0.458*)
10 gr, stained with alizarin	18	0.615 (*0.226*)	0.564 (*0.187*)	1.231 (*0.342*)	0.747 (*0.574*)
10 gr, unstained	27	0.724 (*0.370*)	0.727 (*0.515*)	1.291 (*0.301*)	0.699 (*0.374*)

The estimated amounts of MBM in three samples showed a very large variance (Table 8). The average estimate of MBM in sample A is considerably higher than the actual amount. For sample C and D the estimated amounts are too low. For sample D this can be due to the situation that most participants did report only the presence of bones instead of feather meal.

Table 8: Estimations (in %) for the amount of MBM in three samples. For every result the average (in normal) and standard deviation (in italics) is given. Twenty-two participants did not report these results.

	N	Estimated amount MBM		
		A 0.05%	C 1.0% in fish meal	D 1.0 % Feather meal
total	33	0.21% (*0.25*%)	0.70% (*0.67*%)	0.14% (*0.21*%)
t statistic		3.732 **	2.611 *	23.879 ***

**: 1% > p > 0.5%*
***: p = 0.05%*
****: p << 0.005%*

The estimated amount of feather meal is very significantly below the actual value.

3.4 Detection by other methods

Two participants made four PCR runs in total, all with primer sets for different target animals (Annex IX). Participant 19 indicated exclusively their positive signals; it is assumed that no indication should be interpreted as a negative result. With respect to the results as far as reported, some false positives are listed. The blank was reported to contain cattle, the fish meal with 1% MBM should contain avian material, and the feed with 1% of feather meal is reported to contain pig material. On the other hand, the presence of 0.05 % in feed was detected succesfully; avian material was not treported for this sample. No quantitative results were submitted.

One participant (nr 33) reported two sets of results for immunoassay analysis(Annex X). The Melisa-Tek kit detected three of the four samples correctly, whereas the Reveal kit encountered a flase negative in the fish meal sample. Both kits dit not detect the 0.05 % in the feed.

Participant 33 send in results for the microscopic detection, which were all correct. The microscopic results of participant 19 contained a false positive for fish in sample A (0.05 % MBM).

4 Discussion en conclusions

4.1 Method performance

In general the results of the participants in this study were very good.

For the detection of fish some remarks can be made. Fish material was usually not reported for blank materials (Table 9: specificity: 0.96-0.98). In the presence of land animal material a higher number of false positives was found. Also in the presence of feather meal a notable number of false positives was reported (Table 3: specificity: 0.87). These results might indicate that certain fragments of land animals were misinterpreted as fish material. Examination at lower magnification of the entire sediment should give a first impression of the presence of fish material, which could help to improve the specificity score. The recognition of fish (sensitivity), whether or not in the presence of land animal material is usually good (van Raamsdonk et al., 2009, 2010). On the other hand, the recognition of the absence of fish (specificity) needs improvement (Table 9).

With respect to the detection of animal proteins of terrestrial animals, the specificity in the blank sample is optimal in the current study (Table 9: 1.0). Land animal material at a reasonable low level (sample A: 0.05%), as contamination in fish meal (sample C: 1.0%) or present in the form of feather meal (sample D) did not cause serious problems, as the sensitivity score was at or above 0.95 in all these cases. Feather meal as such was only found by a minority of participants (0.33). In the IAG ring test 2007 (unpublished results of Danish Plant Direktorate) a feed sample with 0.8% of feather meal was included. Almost half of the participants (22 out of 45) did report feather meal, whereas 20 of them reported the presence of bone fragments (i.e. MBM). Two laboratories reported fish meal (specificity: 0.95; current study: 0.88). It is manifest that the detection of feather meal as such still is a major concern, and the presence of hydrolised feather particles is a confusing element for the proper detection of the absence of fish meal.

Table 9: Results for detection of material of terrestrial animals amd of fish of previous ring tests organised by J.S. Jørgensen (Danish Plant Directorate, Lyngby; 2003-2007) and RIKILT (2008-2011) on behalf of the IAG section Microscopy. Results have been communicated in the framework of this Section. Results indicate specificity in the case of the blank, and sensitivity in the case of the other sample types.

Detection of :	Land animals						Fish		
Content: fish	0	4-5%	2%	0	2%	0	0	0	0
year land animal	0	0	0.1%	0.1%	0.05%	0.05%	0	0.1%	0.05%
2003 (n=29)	0.86			1.0					
2004 (n=30)	0.93					0.97	0.97		0.93
2005 (n=42)			0.95	0.95				0.76	
2006 (n=43)	0.98		1.0				0.93		
2007 (n=45)		0.89	0.933						
2008 (n=45)	0.93			0.98		0.96	0.98	0.91	0.84
2009 (n=49)	0.96	0.98		1.0			0.96	0.88	
2010 (n=53)	0.96		0.98		0.91		0.98		
2011 (n=56) current study	1.0					0.98	0.98		0.91

The results for the PCR and immunoassay methods indicate that a proper detection can be achieved at relatively low levels of contamination (0.05% of MBM in feed). However, in some cases false positive results were also reported. Further ring tests are recommended to confirm these results.

4.2 Method parameters

A proficiency test is meant to reveal information on the performance of individual labs. It is not possible to draw conclusions about the validity of the method(s) applied (von Holst et al., 2005). In certain occasions a questionnaire is send around with the samples, which can be used to evaluate the way in which the method is implemented. The current and previous ring tests of IAG are examples of those "extended proficiency tests". Although method validation is principally impossible, improvements of method implementation and relationships with the results can be discussed (van Raamsdonk et al., 2011b).

Table 10: Comparison between parameters distribution in the IAG 2008, 2009, 2010 and 2011 study.

Parameter	Parameter choice	2008	2009	2010	2011
amount of material used for sedimentation	5 grams	16	5	3	3
	10 grams	26	41	48	50
	other	3	3	2	3
type of glassware	chemical sedimentation funnel	22	28	31	33
	beaker (flat bottom)	11	13	10	9
	champagne glass	6	5	8	7
	conical glass with cock	3	1	2	3
	other	3	2	2	3
use of staining of sediment	no	31	35	34	33
	yes	14	14	19	22
use of binocular for examination at lower magnifications	yes	29	40	45	44
	no	16	9	8	12
number of slides used	minimum	1	1	n.d.	n.d.
	maximum	7	14	n.d.	n.d.
size of cover glass used	small (e.g. 20 x 20 mm)	34	27	27	36
	medium	1	9	10	8
	large (e.g. 26 x 50 mm)	9	13	16	12
share of the total sediment used for examination	minimum	4%	2%	2%	0.2%
	maximum	100%	100%	100%	100%
embedding agent for sediment	paraffin oil	18	20	23	20
	immersion oil	8	12	14	12
	glycerine / glycerol	8	10	12	12
	Norland Adhesive	0	2	2	6
	chloral hydrate	3	1	0	0
	other (e.g. Depar 3000, water)	8	4	2	5

As shown in Table 9, a status quo in the shift of method parameters can be found. Still some participants use only 5 grams of material for sedimentation, the use of glassware allowing the release of the sediment at the bottom is slightly increasing, as is the number of participants that apply staining of the sediment. Eleven participants did not use a binocular, although this is requested according to the official protocol. The range in the amount of sediment used for examination is even running from 0.2% to 100%. A further harmonisation of the application of the method is still recommended.

4.3 Quantification

The amounts of sediment can be used as a parameter for the application of the method. However, in specific cases of a deviating amount it is not obvious to reach a conclusion about correct or wrong application. Furthermore, in case of alizarin staining it is not clear if figures indicate the amounts obtained before or after staining.

The averages of the quantification results of the animal proteins of terrestrial animals show a diverse pattern. The estimation for MBM in the feed (sample A: 0.05%) show an overestimation. The usual situation is that ingredients with a low share in the total composition are overestimated (unpublished results of ring trials of IAG Section Feeding stuff Microscopy). The Student's t-test show significant differences between the actual and estimated amounts (below or far below p = 1.0%). This means that individual results should be expected to be no reliable indicator of the real amount of fish or MBM in a sample.

The amount of feather meal is underestimated, which is obvious considering the situation that generally feather meal was not reported, but only the presence of bone fragments.

5 General conclusions and recommendations

5.1 Conclusions

The response of the participants showed a larger number of reporting errors than in previous years. These problems mainly apply to inconsistent reporting (wrong or missing unique laboratory number: three occasions), incomplete reporting (no Fax or no E-mail: nine occasions), and too late reporting (three occasions). The latter problem was partly due to severe delays in the custom procedures of certain countries.

Specificity and sensitivity for the detection of fish and land animal material was generally good to excellent (higher than 0.95). The only two exceptions are the erroneous finding of fish material in the presence of 0.05% of land material (specificity = 0.91) and in the presence of 1.0% of feather meal (specificity = 0.88). The legislation states that a method for detection of animal proteins in feeds should be able to detect a level of contamination of 0.1 % at the least. The current study indicates that a level of 0.05% of MBM in a feed does not give any problems. Confusion of fish material and land animal material, and the proper detection of feather meal, still needs attention.

The reported amounts of sediment show a large variation. The estimation of the amounts of MBM in either feed or fish meal show a diverse pattern. In any case a significant difference exist between the eactual and estimated amount. Quantification based on microscopic observations still shows a disputable reliability.

A further harmonization of the application of the microscopic method was achieved in the past years. This is especially indicated in the predominant use of 10 grams of material for sedimentation, and the use of a stereo microscope for the examination of the entire sediment. In the current ring test no gain was achieved in a further optimisation. A further harmonization is still possible for some other parameters.

5.2 Recommendations

- The specificity of the microscopic method for proper detection of the lack of fish meal still needs attention. Training of microscopists remains important.
- The full application of the method (e.g. examination of sample or flotate, use of binocular) still needs attention.
- It is recommended to evaluate further the effect of several method parameters because of large variation of application.
- Further ring tests are recommended to confirm the results of the tests with PCR and immunoassays.

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Annex I
Invitation letter

Dear colleague, Dear IAG member,

The IAG section Feeding stuff Microscopy organizes annually a ring test for the detection of animal proteins in animal feeds. As in previous years, the presidium of the IAG section Feeding stuff Microscopy and RIKILT have agreed to organize together the 2011 ring test for animal proteins under certain conditions.

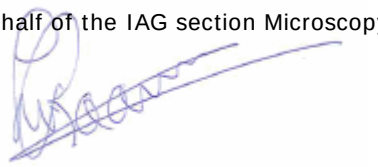
On behalf of the IAG section Feeding stuff Microscopy, RIKILT will invite you for participation in this next ring test. The share in the costs of the 2011 ring test as asked from every participant will be a fee of € 200, which is the same as in the previous years.

Three or four samples will be send around late February or early March 2011. Also a questionnaire will be sent by E-mail. A time slot of four weeks is planned for the analyses of the samples by every participants This means that late March or early April all results are expected to be returned to RIKILT. Pooling and evaluation of the results will take place during April and May, and a preliminary report will be presented during the annual IAG meeting in Tervuren (Belgium) in June. After that, a final report will be made depending on the outcome of the discussions during the meeting. All communications of the evaluation will be fully anonymous.

If you are interested to participate in the ring test 2011 for animal proteins, please return the application form and make a payment of € 200 to RIKILT. For smoothing the administrative procedure, an invoice is already included with this letter. In case of participation, please hand this invoice over to your financial department, and make sure that the reference number, your name and your institute's name is mentioned. This information is necessary to avoid loss of payments that can not be linked to participating institutes.

We are looking forward to have a nice cooperation for the next ring test and to have results which will support your laboratory quality system.

On behalf of the IAG section Microscopy and the RIKILT organizing team,



Dr. L. van Raamsdonk

Annex II
Report form for procedure details

IAG ring test 2011		
Please select your unique lab number		
Have you read the ring test instructions?		
What detection method do you use?	Microscopy	
Please skip this line		
Please continue here		
Please indicate your starting amount of material for sedimentation		
if other, please specify		
Indicate your glassware for sedimentation		
if other, please specify		
Describe your sedimentation agent		
if other, please specify		
Did you apply staining of the sediment (e.g. alizarin staining) as standard procedure?		
Did you examine at lower magnifications (using a binocular)?		
Indicate the size of cover glass		
Please estimate the amount of sediment you have used for preparing the slide(s) (in %)		
Please describe your embedding agent for the sediment material		
if other, please specify		
When estimating amounts:		
please indicate the f-factor used for fish meal		
please indicate the f-factor used for terrestrial animal meal		

Annex III
Report form

IAG ring test 2011

lab number 0

sample number	2011-A	2011-B	2011-C	2011-D
weight of sediment				
presence of fish material				
if present, estimated amount				
presence of material of land animals				
if present, estimated amount				
Comment, if necessary				

Signature:

Date:

Annex IV
Instructions as included in the report form

IAG ring test 2011

Instructions for the IAG ring trial

1 You have received a box with an introduction letter and three vials containing 50 grams of possibly contaminated animal feed. Please report the receipt of your package as soon as possible by E-mail to the address mentioned below.

2 The samples have to be analysed according to Regulation 152/2009/EC from the European Union. Identical procedures can be found in the module Methods of the computer program ARIES. It is recommended to start the sedimentation procedure with 10 grams of material. Take care to homogenise the content of each vial before taking the amount for analysis.

3 Reporting consists of the following steps:

3a Please fill in the questionnaire on the page "Procedure". Depending on your chosen method, different questions will show up.

Most of the cells contain a drop-down list. These lists can be used to select an answer as follows. When clicking on a cell, the cursor changes into a hand. A second click will open the drop-down list.

Your unique lab number is mentioned in the introduction letter.

All the fields with a drop-down list have to be completed.

3b Please enter your results in the fields at page "Results". Your unique lab number automatically shows up after your have entered it at the page Procedure. Select "yes" if fish or land animal material is detected, or "no" if the respective type of material is absent. You are free to give an estimation of the amount of material found.

All fields with a drop-down list have to be completed. Please add the exact sediment weight in 0.01 g.

4 After completing the two forms "Procedure" and "Results", they have to be sent to the organisers in two ways:

4a A print out of both forms have to be sent by Fax to RIKILT, Wageningen, the Netherlands. The FAX number will appear in the forms as soon as they are completed.

4b The forms have to be sent to by E-mail as well. Save the Excel file by using "Save as …", add your unique lab code to the end of name (just before ".xls") and send the file to leo.vanraamsdonk@wur.nl.

4c Results will be included in the final analyses and report only if both forms are send in by FAX as well as by electronic mail, and after the proper receipt of the requested fee.

5 Direct any questions to leo.vanraamsdonk@wur.nl

6 Closing date is April 2nd, 2011.

Annex V
List of participants

Institute	City	Country
Austrian Agency for Health and Food Safety-AGES	A-1226 Vienna	Austria
Oleotest N.V.	B-2660 Antwerpen	Belgium
FLVVT	B-3080 Tervuren	Belgium
AFSCA/FAVV	B-4000 Liege	Belgium
CRA-W	B-5030 Gembloux	Belgium
Laboratorium ECCA nv	B-9820 Merelbeke	Belgium
Ottawa Laboratory (Carling), Science Branch, Canadian Food Inspection Agency	Ottawa, Ontario, K1A 0C6	Canada
China Agricultural University	100083 Bejing	China
Central Institute for Supervising and Testing in Agriculture	Prague 5-Motol	Czech Republic
Danish Plant Directorate	DK-2800 Lyngby	Denmark
IDAC	F-44327-Nantes cedex	France
IPL Atlantique	F-33000 Bordeaux	France
S.C.L. Laboratoire de Rennes	F-35000 Rennes	France
Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft, GB6-Labore Landwirtschaft / LUFA, FB62	D-04159 Leipzig	Germany
LLFG Landesanstalt für Landwirtschaft	D-06120 Halle	Germany
Thüringer Landesanstalt für Landwirtschaft	D-07743 Jena	Germany
Landeslabor Berlin-Brandenburg	D-14473 Potsdam	Germany
Inst. Fur Veterinar-Pharmakologie und Toxicologie	D-16321 Bernau Bei Berlin	Germany
LUFA Rostock	D-18057 Rostock	Germany
SGS Germany GmbH	D-21035 Hamburg	Germany
Futtermittelinstitut Stade (LAVES)	D-21680 Stade	Germany
LUFA Nord-West	D-26121Oldenburg	Germany
Q-vis GmbH	D-38112 Braunschweig	Germany
CVUA-RRW	D-47798 Krefeld	Germany
Agri Q-service GmbH	D-48155 Münster	Germany
LUFA-Speyer	D-67346 Speyer	Germany
Universität Hohenheim, LA Chemie (710)	D-70599 Stuttgart	Germany
LTZ Augustenberg	D-76227 Karlsruhe	Germany
Bayerisches Landesamt fur Gesundheit und Lebensmittelsicherheit	D-85764 Oberschleissheim	Germany
Feedstuffs Control Laboratory, Min. of Rural Development & Food	GR-14123 Likovrissi Attikis, Athens	Greece
MGSZH ÉTBI TAKARMÁNYVIZSGÁLÓ NEMZETI LABORATÓRIUM	H-1144 Budapest	Hungary
Department of Agriculture, Fisheries and Food, Backweston Agri Laboratories	Celbridge, Co. Kildare	Ireland
Equine Centre	Naas, County Kildare	Ireland

Institute	City	Country
Istituto Zooprofilattico Sperimentale Abruzzo & Molise "G. Caporale"	I-64100 Teramo	Italy
Istituto Zooprofilattico Sperimentale della Sardegna	I-07100 Sassari	Italy
IZS PLV Torino - CReAA	I-10154 Torino	Italy
Ist. Zooprofilattico Sperimentale delle Lombardia e dell'Emilia Romagna	I-25121 Brescia	Italy
Inst. Zooprofilattico Sperimentale delle Venezie	I-35020 Legnaro	Italy
IZSLT Sezione Firenze	I-50010 San Martino alla Palma (FI)	Italy
Natl. Food and Veterinary Risk Assessment Institute	LT-08409 Vilnius	Lithuania
Labco	NL-3198 LC Europoort-Rotterdam	Netherlands
CCL - Nutricontrol	NL-5462 GE Veghel	Netherlands
MasterlabBV	NL-5831 JN Boxmeer	Netherlands
Nofima Ingredients	N-5141 Fyllingsdalen	Norway
Inspectorate Services Perú S.A.C., Agricultura & Pesquería, Jefa de Laboratorio de Microbiología	Callao 1	Peru
National Veterinary Research Insitute	P-24-100 Pulawy	Poland
Laboratório Nacional de Investigação Veterinária INRB, IP	PT 1549-011 Lisboa	Portugal
Lab. Regional de Veterinária	PT 9700-236 Angra do Heroismo	Portugal
Institute of Veterinary medicine of Serbia	11070 Belgrade	Serbia
Scientific Veterinary Institute "Novi Sad"	21000 Novi Sad	Serbia
State Veterinary and Food Institute	04001 Kosice	Slovakia
University of Ljubljana, Veterinary Faculty, Natl. Veterinary Institute, Unit for Pathology of Animal Nutrition and Environmental Hygiene	SLO-1000 Ljubljana	Slovenia
Dirección General de Produccion Agropecuaria, Laboratorio Agrario Regional	E-09071 Burgos	Spain
Trouw nutrition Espana	E-28760 Tres Cantos (Madrid)	Spain
SVA	SE-75189 Uppsala	Sweden
Agroscope (ALP), Swiss Research Station	CH-1725 Posieux	Switzerland
LGC	Middlesex TW11 0LY	UK

Annex VI
Details of procedures applied, microscopic method

	Amount	Glassware *)	Agent	Staining	Binocular	Size	Sed. used	Embedding	ARIES	F-factor
1	10	chem.sed.funnel	TCE	yes	yes	large	10%	immersion oil	no	40%
2	5	chem.sed.funnel	TCE	no	yes	small		water	no	40%
3	10	chem.sed.funnel	TCE	no	no	small	25%	paraffin oil	no	
4	10	chem.sed.funnel	TCE	no	yes	large	100%	paraffin oil	yes	40%
5	10	beaker (flat bottom)	TCE	yes	yes	small	5%	paraffin oil	no	
6	10	chem.sed.funnel	TCE	yes	yes	medium	30%	glycerol	no	40%
7	10	mensur	TCE	no	yes	large	20%	mineral oil		
8	10	chem.sed.funnel	TCE	no	yes	medium	100%	paraffin oil	no	60%
9	10	conical champagne glass	TCE	no	yes	small	100%	immersion oil	no	
10	10	chem.sed.funnel	TCE	no	yes	small	25%	immersion oil	no	
11	10	beaker (flat bottom)	TCE	no	yes	small	100%	immersion oil	no	
12	10	beaker (flat bottom)	TCE	no	no	small	100%	immersion oil	no	50%
13	10	conical champagne glass	TCE	no	yes	small	100%	paraffin oil	no	50
14	10	chem.sed.funnel	TCE	no	yes	small	2%	paraffin oil	no	60%
15	10	chem.sed.funnel	TCE	no	yes	large	100%	glycerol		
16	10	chem.sed.funnel	TCE	yes	no	small	20%	glycerol	no	
17	10	chem.sed.funnel	TCE	yes	yes	medium	100%	glycerol	no	40%
18	15	chem.sed.funnel	TCE	yes	yes	medium	5%	glycerol/water		
19	10	beaker (flat bottom)	TCE	no	no	small	90%	mineral oil	no	100%
20	10	conical champagne glass	TCE	no	yes	small	70%	paraffin oil	no	
21	10	chem.sed.funnel	TCE	yes	yes	large	100%	glycerol	no	40%

	Amount	Glassware *)	Agent	Staining	Binocular	Size	Sed. used	Embedding	ARIES	F-factor
22	10	chem.sed.funnel	TCE	yes	yes	small	80%	paraffin oil	no	60%
23	10	chem.sed.funnel	TCE	yes	yes	small	100%	NOA	no	
24	10	chem.sed.funnel	TCE	no	no	large	100%	paraffin oil	no	40%
25	10	chem.sed.funnel	TCE	yes	yes	small	100%	NOA	no	40%
26	20	beaker (flat bottom)	TCE	no	yes	small		immersion oil	no	25%
27	10	chem.sed.funnel	TCE	no	yes	medium	10%	paraffin oil	no	
28	10	beaker (flat bottom)	TCE	no	yes	small	60%	paraffin oil	no	
29	10	chem.sed.funnel	TCE	no	yes	small	60%	paraffin oil	no	
30	10	beaker (flat bottom)	TCE	no	yes	small		immersion oil	no	60%
31	5	special conical glass with cock	TCE	yes	yes	small		glycerol	no	
32	10		TCE	no	yes	small	75%	glycerol	no	60%
33	10	chem.sed.funnel	TCE	no	no	small		immersion oil	no	
34										
35	10	conical champagne glass	TCE	no	yes	large	50%	immersion oil	no	50%
36	35	beaker (flat bottom)	TCE	no	no	medium	100%	paraffin oil	no	55%
37	10	chem.sed.funnel	TCE	yes	yes	small		immersion oil	yes	
38	10	chem.sed.funnel	TCE	yes	yes	small	75%	paraffin oil	yes	40%
39	10	chem.sed.funnel	TCE	no	yes	large	40-73%	paraffin oil	no	60%
40	10	chem.sed.funnel	TCE	yes	yes	large	100%	glycerol	no	25%
41	5	conical champagne glass	TCE	yes	yes	small	100%	glycerol	no	40%
42	10	conical champagne glass	TCE	no	yes	medium	100%	immersion oil	no	
43	10	chem.sed.funnel	TCE	yes	yes	small	80%	paraffin oil	no	40%
44	10	chem.sed.funnel	TCE	no	no	large	75%	paraffin oil	no	
45	10	chem.sed.funnel	TCE	no	yes	small	60%	xylol		
46	10	beaker (flat bottom)	TCE	no	no	small	100%	glycerol	no	20%, 40%

	Amount	Glassware *)	Agent	Staining	Binocular	Size	Sed. used	Embedding	ARIES	F-factor
47	10	centrifugation tube	TCE	yes	yes	large	100%	paraffin oil		
48	10	special conical glass with cock	TCE	yes	yes	small	0.2%	NOA	no	40%
49	10	evaporation dish	chloroform	yes	yes	small	5%	NOA	no	
50	10	conical champagne glass	TCE	no	yes	small	50%	immersion oil	no	60%
51	10	chem.sed.funnel	TCE	no	yes	large	10%	glycerol	no	
52	10	chem.sed.funnel	TCE	no	no	small	100%	NOA	no	40%
53	10	chem.sed.funnel	TCE	yes	yes	small	100%	paraffin oil	no	40%
54	10	special conical glass with cock		no	yes	small	1%	paraffin oil		
55	10	chem.sed.funnel	TCE	yes	no	small	25%	NOA		35%
56	10	chem.sed.funnel	TCE	yes	no	small		glycerol	no	
57	10	chem.sed.funnel	TCE	no/yes	yes	medium		NOA/glycerol	no	40%

** the indications of the parameters are short names for the full descriptions as presented in Annex B.*

Annex VII
Results: presence of MBM, microscopic detection

Lab nr	Fish				MBM				Feather meal
	A	B	C	D	A	B	C	D	D
1	no	no	yes	no	no	no	yes	yes	no
2	no	no	yes	yes	yes	no	yes	yes	no
3	no	no	yes	no	yes	no	yes	yes	no
4	no	no	yes	no	yes	no	yes	yes	
5	yes	no	yes	no	yes	no	no	yes	
6	no	no	yes	no	yes	no	yes	yes	
7	no	no	yes	no	yes	no	yes	yes	
8	no	no	yes	no	yes	no	yes	yes	no
9	no	no	yes	no	yes	no	yes	yes	no
10	no	no	yes	no	yes	no	yes	yes	no
11	no	no	yes	no	yes	no	yes	yes	no
12	no	no	yes	yes	yes	no	yes	yes	yes
13	no	no	yes	no	yes	no	yes	yes	
14	no	no	yes	no	yes	no	yes	yes	no
15	no	no	yes	no	yes	no	yes	no	
16	no	no	yes	no	yes	no	yes	yes	no
17	no	yes	yes	yes	yes	no	yes	yes	
18	no	no	yes	yes	yes	no	yes	yes	
19	no	no	yes	no	yes	no	yes	yes	yes
20	no	no	yes	no	yes	no	yes	yes	yes
21	no	no	yes	no	yes	no	yes	yes	
22	yes	no	yes	no	yes	no	yes	yes	no
23	no	no	yes	no	yes	no	yes	yes	no
24	no	no	yes	no	yes	no	yes	yes	no
25	no	no	yes	no	yes	no	yes	yes	no
26	no	no	yes	no	yes	no	yes	yes	no
27	no	no	yes	no	yes	no	yes	yes	no
28	no	no	yes	no	yes	no	yes	yes	no
29	no	no	yes	yes	yes	no	yes	yes	
30	no	no	yes	no	yes	no	yes	yes	no
31	no	no	no	no	yes	no	yes	yes	yes
32	no	no	yes	no	yes	no	yes	yes	no
33	yes	no	yes	no	yes	no	yes	yes	no
34									
35	no	no	yes	no	yes	no	yes	yes	yes
36	no	no	yes	no	yes	no	yes	yes	

Lab nr	Fish				MBM				Feather meal
	A	B	C	D	A	B	C	D	D
37	no	no	yes	yes	yes	no	yes	yes	
38	no	no	yes	no	yes	no	yes	yes	no
39	no	no	yes	no	yes	no	yes	yes	no
40	no	no	yes	no	yes	no	yes	yes	
41	no	no	yes	no	yes	no	yes	yes	no
42	yes	no	yes	yes	yes	no	yes	yes	yes
43	no	no	yes	no	yes	no	yes	yes	no
44	no	no	yes	no	yes	no	yes	yes	no
45	no	no	yes	no	yes	no	yes	yes	yes
46	no	no	yes	no	yes	no	yes	yes	yes
47	yes	no	yes	no	yes	no	no	yes	
48	no	no	yes	no	yes	no	yes	yes	yes
49	no	no	yes	no	yes	no	yes	yes	yes
50	no	no	yes	no	yes	no	yes	yes	yes
51	no	no	yes	no	yes	no	yes	yes	yes
52	no	no	yes	no	yes	no	yes	yes	yes
53	no	no	yes	no	yes	no	yes	yes	no
54	no	no	yes	no	yes	no	no	yes	
55	no	no	yes	no	yes	no	yes	yes	
56	no	no	yes	no	yes	no	yes	yes	no
57	no	no	yes	no	yes	no	yes	yes	no

Annex VIII
Results: sediment and quantification

Lab nr	Amount of sediment (mg)				Amount MBM (%)		
	A	B	C	D	A	C	D
1	59	53	326	53		2.00%	0.10%
2	43	38	303	40		0.75%	0.03%
3	27	40	1209	12			
4	43.6	50	1550.7	51.8			
5	85	78	700	68			
6	50	47	1286	44	0.03%	1.10%	0.01%
7	162.8	113.3	1366.4	139.4			
8	63	60	1481	62	0.03%	0.10%	0.04%
9	60	63	1473	63			
10	87	92	1400	166	0.02%	0.10%	0.02%
11	55	56	1427	59	0.05%	0.20%	0.10%
12	30	40	1120	40	1.00%	0.75%	0.55%
13	30	29	530	16	0.05%	0.20%	0.02%
14	54	50	1408	51	1.00%	0.75%	0.08%
15							
16	50		1189	50	0.36%	2.14%	0.23%
17	50	41	841	47	0.13%	1.05%	0.06%
18	142	127	2278	117			
19	87.7	60.9	1465.6	61.3	0.02%	0.49%	1.00%
20	130	60	1450	60	0.10%	0.10%	0.10%
21	70.2	60	1646	82	0.14%	0.40%	0.12%
22	94	88	1465	104	0.06%	0.35%	0.03%
23	62	48	1413	84			
24	138	115	807	116	0.10%	0.20%	0.05%
25	45.3	39.2	1138.6	43.8	0.25%	0.13%	0.17%
26	77	61	2158	65	0.03%	0.56%	0.03%
27	98	109	1402	135			
28	41	63	429	63			
29							
30	59.5	55.9	1454.5	56.5	0.08%	1.00%	0.04%
31	159.2	36.6	735.2	78.6			
32	72	83	1463	93	0.50%	2.00%	0.10%
33	72	59	1423	106			
34							
35	104	108	1530	104	0.25%	1.00%	0.55%
36	130	126	3961	120	0.34%	0.73%	0.04%

Lab nr	Amount of sediment (mg)				Amount MBM (%)		
	A	B	C	D	A	C	D
37	85	80	1459	80			
38	46	39	1480	42	0.21%	0.10%	0.05%
39	55	45	1362	52	0.16%	1.26%	0.07%
40							
41	60	60	1470	60	0.05%	0.05%	0.05%
42	38.5	41.3	1274.6	40.1	0.20%	1.00%	0.60%
43	28	38	1229	40	0.12%	0.06%	0.10%
44	74	46	1490	59			
45	36	35	800	33	0.08%	0.08%	0.08%
46	60	70	1405	60	0.04%	0.25%	0.02%
47	57	87	1234	128			
48	36	27	1004	34	0.14%	0.70%	0.06%
49	119.7	72.3	1696.1	282.3			
50	150	300	1200	"1500"	0.05%	1.00%	0.10%
51	56	54	1438	62			
52	71	64	1518	58	0.40%	0.10%	0.25%
53	66.7	58.2	1268.5	57.8	0.50%	1.00%	0.10%
54							
55	60	60	1500	60	0.01%	0.28%	0.004%
56	42.9	42.7	1288.1	44.4			
57	40	35	1118	36	0.32%	2.67%	0.09%

Annex IX
Results: presence of MBM, DNA detection

Lab	MBM				amount MBM			
	A	B	C	D	A	C	method	target
	0.05%	0	1%	1% (avian)				
19	yes		yes					Bovine
	yes		yes	yes				Pig
			yes	yes				Avian
33	yes	yes	yes	no			EURL-AP	Cattle

Annex X
Results: presence of MBM, protein detection

Lab	MBM				amount MBM			
	A	B	C	D	A	C	Method	target
	0.05%	0	1%	1% (avian)				
33	no	no	yes	no			Melisa-TEK	Ruminant
	no	no	no	no			Reveal	Ruminant

RIKILT - Institute of Food Safety is part of the international knowledge organisation Wageningen UR (University & Research centre). RIKILT conducts independent research into the safety and quality of food. The institute is specialised in detecting and identifying substances in food and animal feed and determining the functionality and effect of those substances.

RIKILT advises national and international governments on establishing standards and methods of analysis. RIKILT is available 24 hours a day and seven days a week in cases of incidents and food crises.

The research institute in Wageningen is the National Reference Laboratory (NRL) for milk, genetically modified organisms, and nearly all chemical substances, and is also the European Union Reference Laboratory (EU-RL) for substances with hormonal effects.

RIKILT is a member of various national and international expertise centres and networks. Most of our work is commissioned by the Dutch Ministry of Economic Affairs, Agriculture and Innovation and the new Dutch Food and Consumer Product Safety Authority. Other parties commissioning our work include the European Union, the European Food Safety Authority (EFSA), foreign governments, social organisations, and businesses.

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