IAG section Feed Microscopy

Newsletter 2015

Contents

It is a tradition as one of the first topics during the annual conference of IAG section Feed Microscopy to ask all the participants to reflect briefly on the special samples and requests of the past year. This year, at the conference in Oldenburg, several members mentioned two issues: the presence of muscle fibres in bakery products (two institutes) and the presence of whole seed *Ambrosia* in soy bean meal (three institutes). These issues have different aspects. The presence of exclusively muscle fibres raises the problem of identification, whereas a dedicated detection method is of particular interest. *Ambrosia* remains of interest: how can whole seeds show up in a processed matrix? The entire *Ambrosia* issue would have limited importance if these seeds would not be able to germinate. These and other subjects discussed this year enlighten the possibilities and limits of the control of feed by microscopy, and leads to further discussion about opportunities of microscopic analysis.

The board of IAG section Feed Microscopy will invite you to read further in this Newsletter. Interesting information is presented, although important questions remain. The show will go on.

Board: dr. I. Paradies-Severin, Germany, Inge.Paradies-Severin@LWK-Niedersachsen.de; dr. G. Frick, Switzerland, genevieve.frick@agroscope.admin.ch; dr. J. Vancutsem, Belgium, jeroen.vancutsem@favv.be; dr. L. van Raamsdonk, The Netherlands, leo.vanraamsdonk@wur.nl; dr. R. Weiss, Austria, roland.weiss@ages.at.

Editing newsletter: L. van Raamsdonk, RIKILT, Wageningen.

Website: www.iag-micro.org

Presidents address

Dear colleagues and members,

it is a great pleasure for me to present to you in this delivery of our IAG newsletter some of the highlights of the very busy and engaged work we performed during 2015.

For the annual IAG meeting 2015 we were invited to Oldenburg, Germany. The meeting took place in June at LUFA Nord-West, Institute for Feedingstuff Analysis.

Main discussion points of the meeting were the detection of animal constituents in feedingstuff with regard to EURL-AP / IAG interactions.

The results of 3 IAG ring tests organised by RIKILT were introduced to the audience (IAG ring test “Animal Protein 2015”; IAG ring test “Composition 2015” and IAG ring test “Ambrosia in Bird Feed 2014”. You’ll find the summaries of the ring test reports in this newsletter.

Planned ring tests for 2016 are announced in this newsletter.

Another topic was the information on 3D microscopy in feedingstuff analysis by a presentation of the HIROX company and the colleagues had the opportunity to follow a practical performance.

In October the IAG autumn meeting took place in Krefeld by invitation of the Federal Chemical and Veterinary Institute (CVUA) of North-Rhine-Westphalia, D.

Topics were the presentation of first results of the IAG ring test on Ambrosia in Bird Feed 2015 organised by ALP Posieux, CH. A summary of the ring test report will be available in the IAG newsletter delivery 2016.

Other discussion points focussed again on the detection of animal constituents, but also the microscopic detection of undesired and forbidden substances were under discussion.

The engaged contribution of all participants in very open discussions during our meetings served as useful and important platform for knowledge exchange on theoretical and practical aspects of our microscopic work.

The annual IAG meeting 2016 will take place in Copenhagen. We are invited by our colleagues from the Danish Veterinary and Food Administration. The meeting date is June, 07-09, 2016.

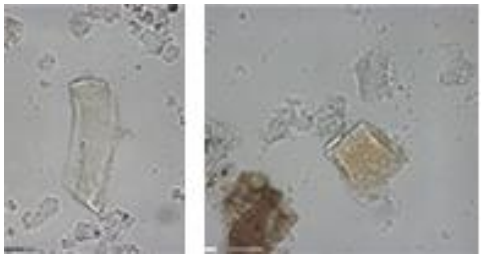
I’m looking forward to continue our successful work in the frame of the IAG section feedstuff microscopy in 2016.

Enjoy the reading of this newsletter!

Yours sincerely

I. Paradies-Severin

Highlight: microdissection



Muscle fibre from sediment (left) and from flotate (right)

Since June 2013 the total feed ban of processed animal proteins (PAPs) was partially lifted. Now it is possible to mix fish feed with PAPs from non-ruminants (pig and poultry). To guarantee that fish feed, which contains non-ruminant PAPs, is free of ruminant PAPs, it has to be analysed with a ruminant PCR assay to comply with the total ban of feeding PAPs from ruminants. However, PCR analysis cannot distinguish between ruminant DNA, which originates from proteins such as muscle and bones, and ruminant DNA, which comes from feed materials of animal origin such as milk products or fat. Thus, there is the risk of obtaining positive ruminant PCR signals based on these materials. The technique of microdissection was developed at AGES for separating individual muscle fibres. A recently published paper describes the development of the combination of two analysis methods, micro-dissection and PCR, to eliminate the problem of 'false-positive' PCR signals. With micro-dissection, single particles can be isolated and subsequently analysed with PCR.

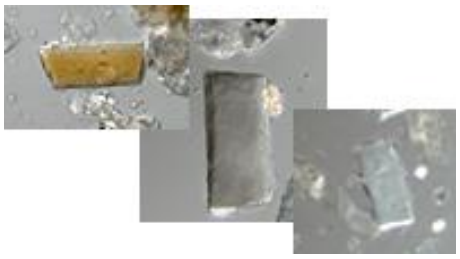
Axmann, S., Adler, A., Brandstettner, A.J., Spadinger, G., Weiss, R., Strnad, I., 2015. Species identification of processed animal proteins (PAPs) in animal feed containing feed materials from animal origin. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2015;32(7):1089-98. doi: 10.1080/19440049.2015.1036321. Epub 2015 May 11.

A special case of the application of micro-dissection is presented in the next section of the Newsletter.

Crumbs of Bread. A cooperation between Microscopy, Microdissection and PCR at AGES

In spring 2015 an official control sample was tested for animal proteins according to Commission Regulation (EC) 152/2009 as amended by regulation (EU) 51/2013.

The first determination showed no bones in the sediment, but only **some muscle fibres** were found **in the flotate**. Since the low number of particles (< 5) was found in the first examination a second and third determination were conducted in order to exclude an internal contamination. Due to insufficient sample material no new ground sample was investigated. The second and third determination confirmed the result of the first determination and so the following result for the microscopic analysis was written:



Muscle fibres from flotate

„As far as microscopic discernible on average more than 5 particles derived from animals were detected per determination in the submitted sample. The particles were identified as muscle fibres. There is no possibility to distinguish between terrestrial animals and fish."

As agreed with the Department of Control and Surveillance (responsible for the feed control) further **investigations by microdissection and PCR** were carried out to identify the origin of the found muscle fibres.

Microdissection at AGES

So following sample set of 12 PCR-tubes was prepared by microdissection and analysed by PCR for their DNA:

- 2 tubes of the ground sample of bread crumbs
- 10 tubes with single muscle fibres isolated by microdissection

Result of PCR: The bread crumb sample was found positive for ruminants, poultry and pig. The PCR of single muscle fibres isolated from microdissection showed the following results:

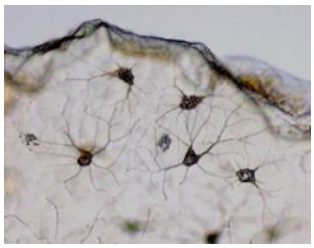
- 1 particle positive for ruminants
- 4 particles positive for pigs
- 2 particles positive for poultry
- 3 particles may derive from another species which have not been tested so far or are not from animal origin

Additionally, a subsample was sent for analyses to the EURL-AP, confirming only the results for PCR. Pictures of encountered muscle fibres were also confirmed by the EURL-AP.

CONCLUSION: The sample was clearly confirmed as positive by PCR for terrestrial animals, although only muscle fibres were found by microscopic analysis.

The present case showed the importance of the combination of microscopy, microdissection and PCR as well as the significance of the network of European laboratories.

Ring test animal proteins 2015: Abstract of report



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 - Wageningen UR, The Netherlands. The aim of the ring study was to provide the participants information on the performance of the local implementation of the detection method for their local quality systems. A further aim was to gather information about the application of the microscopic method. The current 2015 version of the IAG ring test for animal proteins is the first one in the IAG series of ring tests applying the full new method for microscopy as published in Regulation (EC) 51/2013 amending Annex VI of Regulation (EC) 152/2009 together with accompanying SOPs.

Two matrices have been used in the design of the study. Samples A and C were based on a pig feed containing whey powder and consequently were positive for ruminant DNA. Samples B and D were based on a cattle feed. Two different types of fish meal were added to samples A and D at a level of 0.1%, and a frequently tested ruminant meat and bone meal (MBM) was added to sample B at a level of 0.1%. All participants were requested to determine the presence or absence of land animal and/or fish, and to indicate the type of material found. The participants were asked to report the amount of sediment found (the fraction containing minerals and bones, if present) before and after applying the actual analyses and to answer questions on a series of parameters of the microscopic method. Of the 48 participants 42 sets of results were returned with results using the microscopic method.

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. The results are analysed in two ways: numbers below LOD (between 1 and 5 particles per determination cycle inclusive) have been considered positive and as alternative considered as negative. The choice to consider these number positive was based on the principle that any particle correctly identified as of animal origin is apparently present, and it allows a way to compare the present results with those of previous years.

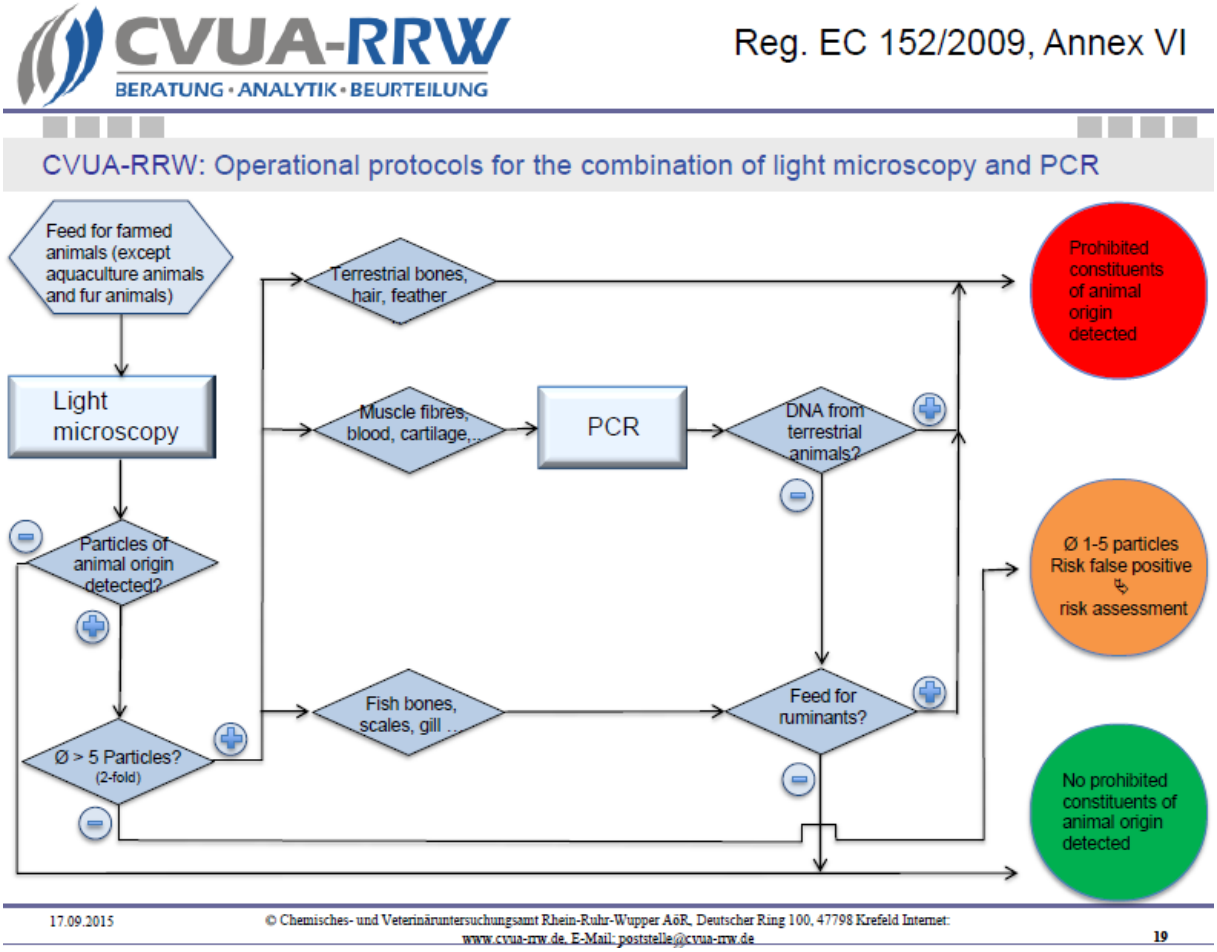
A total of 42 sets of microscopic results were returned. The participants were invited to apply the ruminant PCR as well. Twelve participants submitted both microscopic as well as PCR results, and two participants returned exclusively PCR results. Four out of 42 participants applied the wrong number of microscopic determinations, although the report form was interactive and guided the participant through the process of choosing the right number of repetitions.

Most of the specificity and sensitivity scores were at good or reasonable levels. In the presence of fish meal originating from Denmark, 10 out 42 participants erroneously recognised some particles of terrestrial animal origin (specificity 0.76). For both samples B and C, not containing fish meal, five out of 42 participants reported the presence of fish meal (specificity 0.88). Considering numbers of particles below LOD as negative, all sensitivity scores were at a level of 0.93 or higher. In contrast, applying a threshold for positive reporting consequently results in lower scores (0.88 or higher). The results indicate that the overall performance of the microscopic method is satisfactory, but applicants of the microscopic method could benefit from good and effective training and documentation in order to achieve a higher reliability in identifying particles. Samples A, B and C were correctly identified as positive for ruminant DNA by all 14 laboratories that performed ruminant PCR (sensitivity 1.0). For sample D false positive results were sent in by 2 of the 14 laboratories (specificity 0.86).

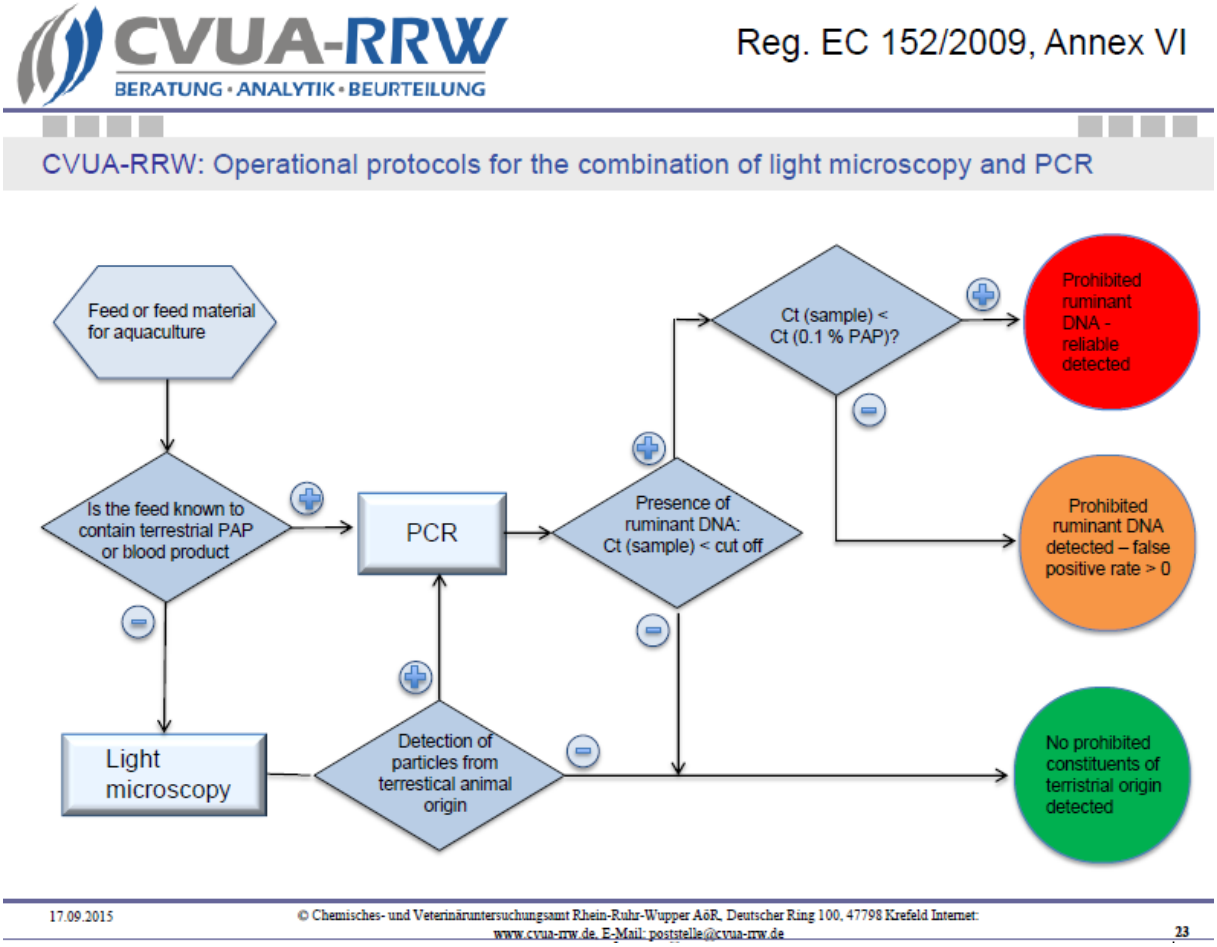
Full reference: L.W.D. van Raamsdonk, N. van de Rhee, I.M. Scholtens, T.W. Prins, J.J.M. Vliege, V. Pinckaers, 2015. *IAG ring test animal proteins 2015.* Wageningen, RIKILT Wageningen UR (University & Research centre), RIKILT report 2015.016. 31 blz.; 10 tab.; 12 ref.

Reflections on Operational schemes for analysing animal proteins

During the interesting and fruitful Autumn meeting of IAG section Feed Microscopy in Krefeld (Germany), interesting reflections on the operational schemes for the analysis of animal proteins in feed were presented by CVUA-RRW. The operational schemes address the combined application of microscopy and PCR. The procedure in Regulation (EC) 152/2009 (amended by Regulation (EC) 51/2013) requires to repeat the analysis of up to 6 slides if between 1 and 5 (inclusive) fragments were found. In specific cases a second repetition is required. In a lot of analyses a confirmation is required after a positive result. In the current procedures for the analysis of animal proteins a positive result means any number of particles of 6 or higher, and in that case a second analysis (first repetition) is not demanded. This major point of view is reflected in the scheme for terrestrial farmed animals. Another issue is the identification of muscle fibres, blood, cartilage, which can be related to accompanying bone fragments, but will not necessarily do so. This issue is addressed by including a PCR step when necessary.



The scheme for aquafeed includes a PCR analysis as confirmation when any bone particles were found. An interesting aspect is the comparison of the Ct value of the sample with that of a reference sample (0.1% w/w).

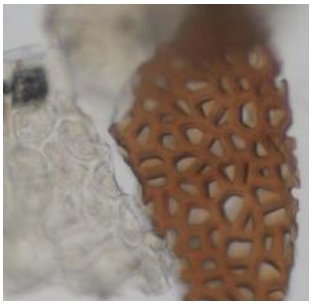


All together the schemes provide interesting points of view and show how individual labs can deal with practical problems.

Topic and schemes provided by mrs. Renate Krull-Wöhrmann, CVUA-RRW, Krefeld.

Note from the board of IAG section Feed stuff Microscopy. The Newsletter is meant to be a medium for communication. In this framework space is provided for viewpoints which are not necessarily adopted by the board. In the case of control of the feed ban on animal proteins legislation together with SOPS exists which has to be followed.

Ring test botanic composition 2015: Abstract of report



A ring test was organized for the microscopic determination of botanic composition in animal feed 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 - Wageningen UR, The Netherlands. The aim of the ring study was to provide the participants information on the performance of the local implementation of the method for composition analysis of feed.

The sample was based on a pig feed produced at a pilot plant dedicated to produce animal protein free test feeds. The sample was distributed with the request to produce a correct declaration of the ingredients of the sample. The results were analysed using the IAG model for uncertainty limits. Shares of ingredients in the feed formulation outside the limits of the model were indicated as under- or over-estimations.

A total of 25 sets of results were returned. The percentage of under- or over-estimations was 20.4% for the seven main ingredients. In the overview of results all three wheat ingredients and all three soy products were pooled to one ingredient each. There is a general overestimation, also for the ingredient (wheat products) with the highest share (51.7%). The maximum overestimation for soy products (share 11.5%) and for beetpulp (share 5.0%) is 32% in both cases. In addition to the usual ingredients which cannot be detected using a microscope, such as fat and molasse, the pig feed contained bakery by-products and whey powder up to a total of 8.4%. Overestimation can be more serious for samples in which a higher share of microscopically undetectable ingredients is present than expected. After adjusting the composition for these ingredients, the share of overestimations was lower.

The analysis of composition in terms of ingredients is important for detecting economic fraud and for monitoring feed safety. Composition analysis and label control of feed is regulated in Regulation (EC) 767/2009. In a broader view, composition analysis in the entire food chain can improve the effect of monitoring actions. The new legislation on food labelling (Regulation (EC) 1169/2011), effective from December 13th 2014, obliges to provide more detailed information to customers on composition and related topics.

The current results indicate that feed ingredients can be identified and shares can be estimated successfully. Besides a proper method, maintenance and dissemination of expertise of technicians are vital for a good performance. An evaluation of the IAG uncertainty model can help to improve its application.

Full reference: L.W.D. van Raamsdonk, N. van de Rhee, V. Pinckaers, J.J.M. Vliege, 2015. *IAG ring test composition 2015.* Wageningen, RIKILT Wageningen UR (University & Research centre), RIKILT report 2015.017. 24 blz.; 3 tab.; 5 ref.

IAG ring test 2014 for botanic impurities in bird feed

The ring test botanic impurities was aimed at the detection of undesirable substances of botanic origin and of *Ambrosia* seeds as specified in Directive 2002/32/EC.

The test comprised of 2 samples of bird feed. It was organised in Autumn 2014, and reported in June 2015.

Two samples of 200 grams of bird feed were used as matrix. Composition of the bird feed:

40-50% canary seed, *Phalaris canariensis*
20-15% proso millet seed, *Panicum miliaceum*
15-10% shelled oat, *Avena sativa*
10-5% rape seed, *Brassica rapa*
5-3% flax seed, *Linum usitatissimum*
2-1% niger seed, *Guizotia abyssinica*

The bird feed was examined on excessive contamination with prohibited seeds. Sample A was spiked with **three *Ambrosia* seeds** and sample B with **five *Datura* seeds**. Every jar with 200 grams was individually spiked and the specific weight of the portion was stored for later analysis of the recovery.

The participants were requested to report any presence of seeds of species as listed in Directive 2002/32/EC. The report form allowed to indicate the number of seeds and the total weight of the seeds found per species. 25 participants returned their results.

All participants were able to detect the spiked seeds. In the case of *Ambrosia* (sample A) the lower limit was the recovery of 1 seed. The highest reported number was 5, indicating that the bird feed was not completely free of *Ambrosia* seeds. For *Datura* the number of reported seeds ranged from 5 to 13, clearly indicating a still remaining contamination of the original bird feed.

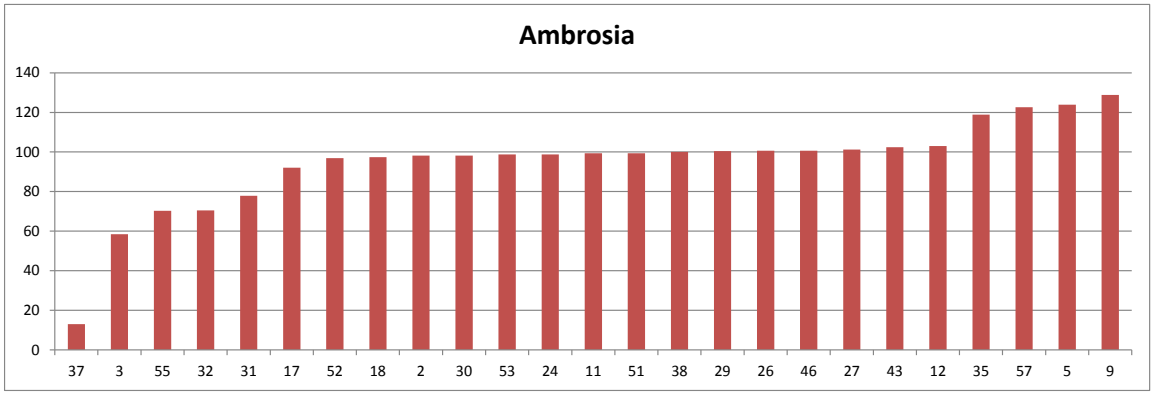


Figure 1. Recovery of Ambrosia seeds in sample A, in % w/w. X-axis: participant number.

The 16 out of 27 participants who reported three seeds for *Ambrosia* in sample A showed a recovery between 92% and 103% w/w (Figure 1). All participants reported the spiked number of five *Datura* seeds (sample B), or an excess of this number. Besides the spiked species, rarely *Crotalaria* seeds and ergot sclerotia were found as well.

RIKILT Wageningen UR; L.W.D. van Raamsdonk, N. van de Rhee, V. Pinckaers, J.J.M. Vliege.

Ambrosia in soybean meal. A case story at AGES

In spring 2015, an official control sample of soybean meal was tested for botanical impurities and the result was approx. 7 times the limit of 50 ppm for *Ambrosia artemisiifolia*. The analysts found whole seeds of *Ambrosia*, most of them looked like peeled.

After this, further investigations of one whole soybean and two soybean meal samples were carried out to identify the source of contamination. The analysis obtained following results:

- Soybean meal: One of the two samples was found positive for *Ambrosia* (4 seeds).
- Whole soybean seeds: Positive for *Ambrosia* (7 seeds).

After discussions with experts and feed producers it's still unclear, if the Ambrosia seeds really run through the production process or are removed by the cleaning and peeling step, but will be reapplied after the extraction process for the correction of the protein content. It can be assumed that the seeds of *Ambrosia* are going to be peeled within this process and still able to germinate. This has already been shown in a previous project of AGES where about 15 % of *Ambrosia* seeds found in bird feed and sunflower seeds were able to sprout.

Read more about this publication:

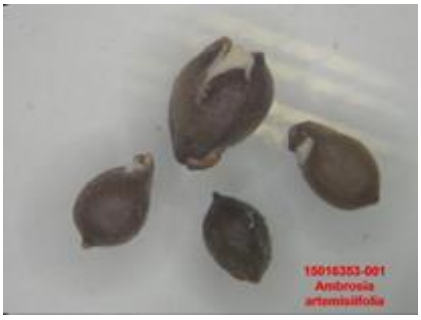
https://www.dafne.at/prod/dafne_plus_common/attachment_download/abe84b6a4f22a01cf822e915d284ddd2/Endbericht-RAGWEED-ProjektNr-100198.pdf

Due to these project results the collected seeds were also tested for their germination ability:

- Soybean meal: None of the 4 *Ambrosia* seeds out of the positive sample was germinating.
- Whole soy bean seeds: one out of 7 *Ambrosia* seeds was sprouting.

Further investigations on botanical impurities, especially for *Ambrosia* and its germination are planned in future.

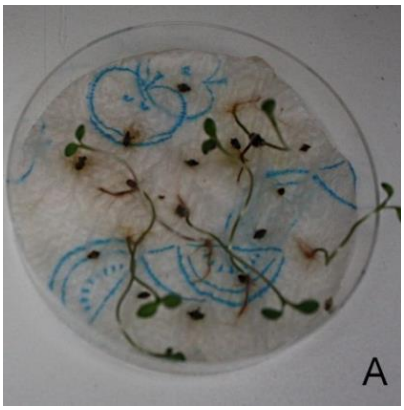

Ambrosia found in the soybean meal


Ambrosia found in the soybean meal


Ambrosia found in the whole soybean seeds

In the brief round among the members during our annual conference, some other reports of *Ambrosia* seeds were given. One member reflected on the presence of *Ambrosia* seeds in crushed, toasted sunflower seeds, with amounts exceeding the fixed limit. These seeds appeared to be no more viable. These findings give rise to question on the origin of these seeds in processed materials, and on the need to enforce the presence of *Ambrosia* seeds in compound feed materials when these seeds are not viable.

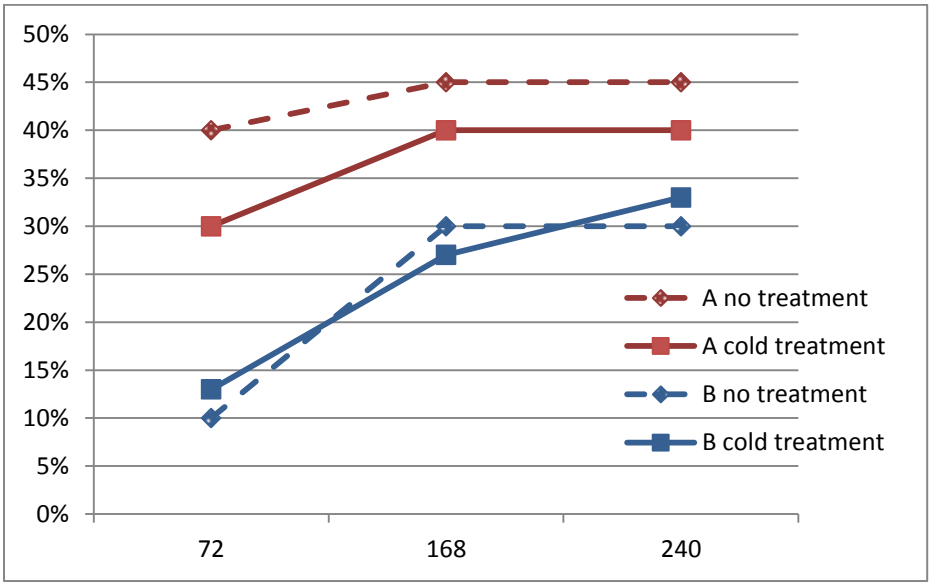
Ambrosia seed germination



In 2011 a germination study of *Ambrosia* seeds was carried out by RIKILT Wageningen UR. Seeds of two populations were each divided in two groups; two groups received a cold treatment for two weeks. All four groups were monitored afterwards for germination. Germination percentages up to 45% were found after one week.

Ambrosia species, exotic annual invasive plants originating from northern America, are a recognised cause of allergenic response. A major way of dispersal is the presence in whole seed bird feed, followed by spilling on the feeding locations. It is important to know for eradication of populations of *Ambrosia* whether seeds produced by local plants can survive winter and are able to germinate in the next year. Seeds collected from a German and from a Swiss population were used to raise series of plants in the botanic garden of Wageningen University. All plants produced seeds abundantly. Seeds, obviously produced in local Dutch circumstances were collected and used for germination experiments.

Sets of 20 seeds (group B cold treatment: 15) were selected and used for the experiments as such or after cold treatment for two weeks at minus 20 degrees Celsius. The results are presented in the graph. All four groups showed a germination percentage after one week (168 hours) between 27% and 45%. Extended germination periods of up to 240 hours did not result in higher germination percentages.



The results show that local *Ambrosia* populations in Dutch circumstances can produce germinable seeds and, hence, can maintain themselves. The *Ambrosia* seeds do not need a cold period, and occasional severe winter conditions will not prevent seeds to germinate.

Source: RIKILT and Natuurkalender; http://www.natuurkalender.nl/nieuwsitems/2011-08_ambrosia3.asp

Prohibited materials – which can be detected microscopically?

Regulation (EC) 767 / 2009, Annex III lists a range of prohibited materials for which microscopic detection is or might be part of the enforcement. The list includes the following categories, indicated by a summarised description:

Chapter 1: Prohibited materials

1. Faeces, urine and separated digestive tract content, irrespective of any form of treatment or admixture.
2. Hide treated with tanning substances, including its waste.
3. Seeds and other plant-propagating materials which, after harvest, have undergone specific treatment with plant-protection products.
4. Wood, including sawdust or other materials derived from wood, which has been treated with wood preservatives.
5. All waste obtained from the various phases of the urban, domestic and industrial waste water, irrespective of any further processing of such waste and irrespective also of the origin of the water.
6. Solid urban waste, such as household waste.
7. Packaging from the use of products from the agri-food industry, and parts thereof.

The discussion on the participation of visual or microscopic examination for the detection of these materials was raised at the autumn meeting in Krefeld and led to interesting discussions.

The listed substances are basically treated products or waste. In some cases it is possible to detect the used material directly by microscopy (packaging materials), but in other cases it is basically impossible to determine if the material was first treated or not (example: sawdust treated with preservatives, hide treated with tanning substances). Microscopy alone is not sufficient, but could be useful to detect and announce a suspicion and to trigger other analysis. Alternatively, microscopy can be used after chemical analysis has been carried out with a positive result to search for a source of the contamination.

Many microscopists are trained on these materials. It is one of the main goals of our section to support all efforts for combination of methods. In those cases where treatment is indicated, neither the detection of the vector nor the detection of the chemical substance (hide and tanning substance, seed and protection agent, wood and preservative) on itself is sufficient. Cooperation and combination of results is a fruitful approach. A statement on this subject might be useful for all microscopists confronted with this question.

Scheme of ring tests 2016

The IAG section Feeding stuff Microscopy organizes annually several ring tests for the evaluation of composition or detection of prohibited constituents in animal feed. The presidium of the IAG section Feeding stuff Microscopy and RIKILT have agreed to organize together the 2016 ring test for the following situations:

- Test IAG-2016-A. Detection of the presence of animal proteins in a set of four samples. This test was already organised by RIKILT in previous years (see abstract in this Newsletter). Targeted protocol: Regulation (EC) 152/2009, consolidated version of February 12, 2013. Cost for participation: € 230.
- Test IAG-2016-B. Declaration of the composition of a compound feed (one sample). This test was organised in 2014 by RIKILT as well (see abstract in this Newsletter). RIKILT will continue the organisation for the year 2015. Targeted protocol: IAG method A2. Cost for participation: € 50.
- Test IAG-2016-C. Detection and quantification of packaging material in two samples of ground bakery products. Targeted protocol: IAG method A4. Cost for participation: € 120.

The single sample for the composition test will be part of the animal protein test. On behalf of the IAG section Feeding stuff Microscopy, RIKILT will invite you for participation in these ring tests. RIKILT will encourage you to subscribe to all four tests, although this is not mandatory. Participation in all three test would cost € 450; in this case a discount of 10% will be granted, resulting in a total cost of € 405 for the total set of four tests.

The samples for test IAG-2016-A and IAG-2016-B will be sent around late February or early March 2016. Also a questionnaire will be sent by E-mail, together with instructions and relevant documentation on protocols. A time slot of four weeks is planned for the analyses of the samples by every participant. This means that late March or early April all results are expected to be returned to RIKILT. The samples of test IAG-2016-C will be send late August and results needs to be reported in October. All results are intended to be reported at the annual meeting of the IAG working group Microscopy in Copenhagen (Denmark) in June 2016 (tests A and B) or in 2017 (tests C). The final reports will be published later in either 2016 or 2017. All communications of the evaluation will be fully anonymous.

If you are interested to participate in one or more ring tests, please return the application form, which accompanies this newsletter, to nastasja.vanderhee@wur.nl or leo.vanraamsdonk@wur.nl. Subscription closes Thursday February 25th, 2016. You are requested to make a payment after receiving the invoice from RIKILT. Make sure that the reference number, your name and your institute's name are mentioned upon payment. This information is necessary to avoid loss of payments that cannot be linked to participating institutes.

Closing remark.

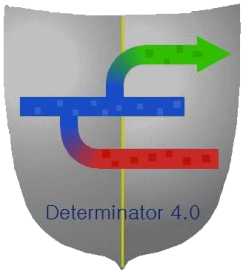
Two issues from the actuality concerning (feed) microscopy got attention in this newsletter: the presence of animal proteins in bakery products intended for feeding, and the presence of (germinable) *Ambrosia* seeds in several feed materials. It is interesting to find out that these *Ambrosia* seeds are even found in ground (!) soy bean meal and crushed (!), toasted sunflower seeds.

In all cases where a certain hazard, or infringement with legal regulations, is encountered, the first question for starting a risk assessment is to collect data on frequency. Are these indications to be considered as incidences, or might a higher frequency occur?

It would be informative to have further data on occurrence of several identified hazards. With this prospect we wish you a happy and prosperous 2016.

Board of IAG section Feed Microscopy.

Latest news: Determinator



Last year much effort was put in the improvement of the platform for decision support systems, Determinator. A new version Determinator 4.0 will be placed on the website www.determinator.wur.nl in the next few weeks (official launch: late December 2015). The most notable improvement is a smooth procedure for installation of the system and loading of data modules.

Determinator is a platform for determination of all kinds of materials relevant for animal feeding or for human food in a broad perspective, which can provide support and background documentation in the process of visual identification. Current published data modules included Starch, Ragwort and Pollen. Other modules are in preparation. A Developer is available for the production of dedicated modules.