IAG Meeting
Copenhagen 07.06. – 09.06.2016

- Opening and Welcome by Dr. N. Ellermann (FVST) and Dr. I. Paradies-Severin (President of IAG)
- Welcome from FVST
- Presentation of the participants and activities of 2015/2016
- Presentation of new participants (Irish Equine Center and ALcontrol AS)
- Information on the annual EURL-AP workshop and activities

- Concerning the technical lectures will be referred to the proceedings of our meeting.

Ring Tests

→for detailed information look at the evaluation of the particular ring test

IAG Ring Test 2015: Ambrosia in Bird Feed - POSIEUX (CH)

The ring test for botanic impurities was aimed at the detection of *Ambrosia* seeds as specified in Directive 2002/32/EC.
The test comprised of 2 samples of bird feed. It was organised in September 2015, and presented at this meeting.

Two samples of 200 grams of bird feed were spiked with four *Ambrosia* seeds (sample A) or with one *Ambrosia* seed (sample B). Every jar with 200 grams was individually spiked with this dedicated amount of seeds after a cleaning step before.
The participants were requested to report any presence of Ambrosia seeds 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.

31 participants took part in the ring test and showed excellent results for sample A (71% correct results) and sample B (87% correct results).
Details for sample A showed that 10 participants found less than the 4 spiked seeds of Ambrosia (2 or 3 seeds), but 8 participants found more than 4 seeds which indicates the possibility of a contamination of sample A.
Details for sample B showed that only 4 participants didn't find any seeds of Ambrosia.
Contaminations of both samples with insects have also been reported by some of the participants.

There was also a look at the sieving of the sample in fractions of 1,5mm and 4,0mm – especially for the participants who found less than the spiked Ambrosia seeds (5 used the recommended sieves, 1 used sieves not specified, 4 didn't use sieves) but no influence can be reported.

IAG Ring Test 2016: Animal Proteins - RIKILT (NL)

The annual ring test for the detection of animal proteins in animal feed of the IAG - International Association for Feedingstuff Analysis, Section Feedingstuff Microscopy was organized by 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 2016 version of the IAG ring test for animal proteins facilitated the full scenario with the methods for microscopy and PCR as published in Regulation (EC) 51/2013 amending Annex VI of Regulation (EC) 152/2009 together with accompanying SOPs.
All four samples were based on an artificial feed mimicking a formulation for ruminant feed. Two samples were labelled as fish feed (B and D), which was effectuated by adding 2% of a general fish meal. Adulteration was achieved by adding 0.1% pig MBM (B), 0.1% ruminant MBM (D) and a combination of 0.1% ruminant MBM and 0.1% fish meal (C). This combination of different spikes allowed the diverse application of the detection methods.
Forty eight participants enrolled for the ring test, of which 45 submitted microscopic results. Of these, 20 participants applied the combination of microscopic and PCR analysis. Three participants submitted exclusively PCR results.

Microscopy

All participants were requested to determine the presence or absence of land animal and/or fish, to indicate the type of material found and the method used.
Incorrect positive results (positive deviations) were expressed in a specificity score and incorrect negative results (negative deviations) were expressed in a sensitivity score. The optimal score is 1.0. The results are analysed in two ways: numbers below threshold (between 1 and 5 particles per determination cycle inclusive) have been considered positive and as alternative considered negative. By comparing both ways of analysis it is possible on one hand to compare the results with those from previous ring trials (there these numbers were considered positive based on the legal principle of zero tolerance), and on the other hand to compare it to the official method (where numbers between 1 and 5 are considered negative).
Most of the specificity and sensitivity scores for microscopy were at good or reasonable levels. In the combination of fish meal (0.1%) and ruminant MBM (0.1%) the detection of fish material was sub optimal. Considering numbers of particles below threshold negative, the sensitivity scores show a considerable drop. 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.

PCR

The specificity (samples A and B) and sensitivity (samples C and D) scores for PCR were between 0.87 and 0.91.

L.W.D. van Raamsdonk, N. van de Rhee, I.M. Scholtens, T.W. Prins, J.J.M. Vliege, V.G.Z. Pinckaers, 2016. *IAG ring test animal proteins 2016.* Wageningen, RIKILT Wageningen UR (University & Research centre), RIKILT report 2016.016.

IAG Ring Test 2016: Composition - RIKILT (NL)

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.
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 Feedingstuff Analysis, Section Feedingstuff 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 an artificially produced feed mimicking a ruminant feed, and distributed without label information. The participants were requested 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 overestimations.
A total of 25 sets of results was returned. The percentage of under- or over-estimations was 28.6% for the seven main ingredients. In the overview of results, the two declared wheat ingredients and the two declared corn products were pooled to one ingredient each. This was necessary since some participants declared a general ingredient ("wheat" and "corn") and others a specific type (gluten or bran). The use of the original declarations would result in an extra number of non-matching estimations without precise justification. The share of the citrus pulp, in the presence of an equal amount of beet pulp, was underestimated or not detected in 44% of the results. Citrus pulp as such is recognisable as feed ingredient. Still almost three quarter of all estimations appeared to be correct in the ranges of the uncertainty model. This result shows that visual inspection of the composition of a sample can be used for label control and this method can support traceability of ingredients in case of an incidence.
The current results indicate that specific formulations can influence the precision of the estimation of the composition of the feed. The current lack of a complementary system for (chemical) proximate analysis could be a drawback for the overall approach of supporting traceability, necessary for fighting food fraud and for supporting feed safety.

Besides a proper method description and up-to-date descriptions of ingredients, well developed skills of technicians are vital for a good performance.

L.W.D. van Raamsdonk, N. van de Rhee, V. Pinckaers, J.J.M. Vliege, 2016. *IAG ring test composition 2016.* Wageningen, RIKILT Wageningen UR (University & Research centre), RIKILT report 2016.014.

IAG Ring Test 2015: Ergot sclerotia in unground rye - RIKILT (NL)

Ergot alkaloids are recognised as serious toxic substances, which caused a series of outbreaks in the past. In the EU, enforcement is implemented by visual detection and quantification of ergot sclerotia produced by moulds of the genus *Claviceps*.
On behalf of the IAG section Feedstuff Microscopy, RIKILT organised a ring test for the visual detection of ergot sclerotia in two unground rye samples in September 2015. In this report the results from the ring test for ergot in rye 2015 are presented.
The ring test "Ergot sclerotia in rye" was designed to test the capability to visually detect sclerotia or parts thereof at relatively high levels. One sample was based on a level of approx. 400 ppm, and the second sample contained an amount of approx. 1000 ppm (EU legal limit for feeds and ingredients: 1000 ppm = 1 gram/kg = 0.1%). An amount of approx. 250 grams of rye grains was chosen as sample size. All samples were individually spiked. Thirty participants enrolled for the ring test. Participants were requested to report the number of recovered (fragments of) sclerotia and the total weight per sample. The percentage of recovery for every sample was calculated. A dedicated IAG method as well as other (lab internal) methods were allowed for application. Principally, methods are based on sieving (preferably with a mesh size of 0.5 mm), examination of every particle (grain) in the fraction with whole grains or particles larger than 0.5 mm, selection of sclerotia fragments supported by documentation, and weighing the final selection of sclerotia and fragments thereof.
The average recovery for both samples was approx. 97%. All results except one were between the expected recovery limits (80 – 110 % w/w). Supporting data from an intralaboratory validation study of the IAG method showed trueness at different low spike levels between 98 and 105% w/w. Limit of detection was established at 7 ppm. It can be concluded that examination by visual detection of sclerotia is a valuable indicator of the expected presence of ergot alkaloids. The results of this study provide the data for a partial validation of the method of IAG for the examination of whole kernel cereal samples.

L.W.D. van Raamsdonk, N. van de Rhee, J.J.M. Vliege, V.G.Z. Pinckaers, 2016. *IAG ring test visual detection of ergot sclerotia in rye 2015.* Wageningen, RIKILT Wageningen UR (University & Research centre), RIKILT report 2016.013.

Decided IAG Ring tests for the year 2017

- Animal Proteins – RIKILT (Wageningen; NL) 4 samples
- Open Declaration – RIKILT (Wageningen; NL) 2 samples (one blind, one labelled)
- Botanical Impurities – RIKILT (Wageningen; NL): asking members for response by email until end of the year

- Packaging Material – RIKILT (Wageningen; NL) in autumn 2016, presenting results at the annual meeting 2017

Method Reading

It was decided to install a working group of interested colleagues who should work continuously on the actualizing of IAG methods (actualizing every 5 years, pictures in methods, validation etc.). The coordinator of method revision is Roland Weiss. The other members of this group are Bezabe Allain Arbe (Germany), Lotte Houghs (Denmark), Gabriele Russ (Germany), Igor Ujcic-Vrhovnik (Slovenia), Pascal Veys (Belgium) and also the scientific officer Leo van Raamsdonk (Netherlands).
It is planned to start with the adaption of IAG Method A5" Method for the Determination of Ambrosia (Ambrosia artemisiifolia L.) in non-pelleted Animal Feedingstuff" also for pelleted feed, for more detailed information and pictures of the seeds, the validation of the method (data from the IAG ring test) and including the Tetrazoliumtest at the beginning of 2017.

Important informations and decisions

IAG Board matters:

- Decision that Inge Paradies-Severin (Germany) will continue to be IAG president for one more year -> Election of the new IAG president at the annual meeting 2017
- Change of Secretary: Genevieve Frick (Switzerland) instead of Roland Weiss (Austria)
- Installation of the working group for actualizing the IAG-methods see "Method Reading"
- Installation of the scientific officer: Leo van Raamsdonk (NL): also responsible for the IAG-ringtests
- New Webmaster: Jerome Vancutsem (Belgium)

It also was decided to prepare a new draft of the current Rules and Regulations. Extra items: mission and scope of the IAG section, tasks of board members, procedure for board elections and activities (e.g. Newsletter, Website, Ring Tests, Methods...)

International AssociationforFeedingstuff Analysis
IAG – Internationale Arbeitsgemeinschaft für Futtermitteluntersuchung
SectionFeedingstuffMicroscopy – Sektion Futtermittelmikroskopie

AGES

For our annual conference 2017 we are invited to our colleagues from Sweden and the meeting will take place from 13. to 15. 06.2017 in Uppsala.

Foreseen topics: Results of the IAG ring tests 2017
IAG method revision
IAG affairs (Election of new President, board)
and much more...

Many thanks to the organizer team from FVST Copenhagen!!

Secretary:

Roland Weiss

President

Dr. Inge Paradies-Severin