COMMUNITY REFERENCE LABORATORY FOR ANIMAL PROTEINS IN FEEDINGSTUFFS

Walloon Agricultural Research Centre – CRA-W (Belgium)

Annual report 2007 activities

Public version

0 Launching of the CRL

0.1 Organisation of the CRL

Organisation is completed; daily routine fine tuning is always on going.

0.2 Recruitment of the CRL personnel

Recruitment of a new technician at the molecular laboratory. Replacement of Murielle Wiedig who worked up to end of January by Julie Hulin (starting at 16th of March 2007).

Replacement of the ICT position of Benoît Leroux, by Daniele Bonsignori who started at the CRL-AP on the 8th of April 2007.

Another job description was made in spring 2007 for a new collaborator responsible for the micrographs collection project for the NRLs and the sample databank management activities. Camino Belinchón Crespo was first in the selection and started working at CRL-AP begin of October 2007.

0.3 Training of the personal.

In the Molecular biology laboratory, the new technician was trained to substitute the one previously recruited but leaving the CRL in January 2007.

A LightCycler LC480 (Roche) was acquired during the period. The personal of the CRL was trained to use this new equipment. Samples were analysed in parallel on the new thermocycler in order to compare the data obtained with the equipment commonly used and with the LC480.

Technicians were also trained to the use of the new Zeiss AxioImager research

microscope. Specific training on dedicated microscopic techniques (UV, DIC, Phase…) are realised when needs be. One more technician was trained for the NIR microscopy and imaging techniques.

Camino Belinchón Crespo was trained on the Zeiss AxioImager microscope, image analysis theory and databank management. She revealed to be rapidly operational, although numerous technical problems linked to the image analysis software, so that a first diffusion of micrographs was provided to NRLs at the end of the year.

1 Scientific advice and support to the European Commission (32.5 p/m)

1.1 Provide scientific and technical assistance to the European Commission in relation to the development of EC feed legislation. (6 p/m)

1.1.1 Submission of a report on the potential of the methods to quantify PAP in feedingstuffs

1.1.2 Submission of an updated version of the report on the status of the methods for the species-specific detection of MBM in feed

Review of available methods for routine analysis, state of the art March 2007.

1.2 Upon the request of the European Commission or in order to fulfil his role as Community reference laboratory, participate to international fora/committees relating to the detection of animal protein in feedstuffs (EFSA, WHO/FAO, JRC, etc) with eventual presentations to prepare for it. As up to 2 European or international missions/year are foreseen in support to DG Sanco and/or CRL activities, this means one for the six months period of 2006. (1 p/m)

1.2.1 Preparation and participation to international meeting/fora

1.2.2 Report/minutes following completion of the mission

Participation to the AOCS Annual Conference and Workshop in Québec City Canada from the 13th to the 19th of May 2007. The CRL-AP head of microscopy participates to the AOCS meeting and attended the Advanced Agricultural Microscopy Course (focused on animal ingredients detection in feeds). This training was a unique opportunity to learn about microscopic protocols used in USA and Canada and to compare those methods with our EU ones. Among other important topics, such as the learning of new alternative

methods being used in USA and Canada according to their way of MBM processing, qualified contacts were established with the managing team of the former American Association of Feed Microscopists, recently incorporated in the AOCS Microscopy Division. Promising contacts were also established with the Canadian Food Inspection Agency, willing to have closer cooperation with the CRL-AP.

Presentation of the results of the CRL-AP Interlaboratory Study 2006 to the Member States was made at EC headquarters in Brussels on the 8th of May 2007.

Participation to the EFSA working group for preparation of the "Opinion of the scientific panel on biological hazards on certain aspects related to the feeding of meat and bone meal to farm animals". This includes as well the reading the documentation provided by EFSA as attending the meeting and preparation of draft texts for the opinion. Meetings for this working group happened on: 23rd of April (Parma), 21st of June (Parma), 30th of July (Brussels), 3rd of October 2007 (Brussels).

At the end of June 2007, the CRL was also contacted to take part to a second working group of EFSA which will have to prepare the "Opinion of the European Food Safety Authority on a TSE risk assessment of the use of bovine blood in feeds for fish, in consideration of a report produced by the European Animal Protein Association". Meetings for this working group happened on : 6th of July (Brussels), 3rd of September (Brussels), 4th of October (Brussels).

1.3 Upon the request of the European Commission or in order to fulfil his role as Community reference laboratory, participate to meetings for the standardisation of analytical methods relating to the detection of animal protein in feedstuffs and their implementation (CEMA, ISO/CEN, OIE, IAG, etc). Up to 3 European missions/year are foreseen in support to DG Sanco and/or CRL activities, this means one (as it is a starting year) for the six months period of 2006. (1 p/m)

1.3.1 Participation to the CEMA, CEN and IAG meetings

1.3.2 Reports/minutes of the meetings

Participation to the IAG Annual meeting and workshop

Futtermittelmikroskopie in Potsdam – Germany from 18th to 21st of June 2007. Oral communication on the CRL-AP Interlaboratory Study 2006 results, with emphasis on the quantification challenge for the next future. General discussion and presentation of the NRL network and associated duties and expected tasks. The IAG meeting was also the opportunity to present CRL-AP activities to other accredited official laboratories aside NRLs. Contacts with researchers were established a.o. on quantification and molecular biological approach for detection of PAP in feed.

Invited by EFPRA to present the CRL-AP activities at their annual conference from the 6th – 8th June 2007, Marbella, Spain.

Participation to the IAG Autumn meeting in Hamburg – Germany on 26th of September 2007. Oral communication on the possible enhancement for the quantification method based on stereology derived counting method has been presented. The presentation included partial preliminary study results realised at the CRL-AP on different grids even as a theoretical presentation of elementary key concepts for the method.

1.4 To actively participate to technical and scientific support of the European Commission in the context of incidents or crises linked to incorrect use of animal proteins. (6 p/m)

1.4.1 Provide technical and scientific support

1.4.2 Submission of the report on the technical and scientific support provided

Analysis of samples on the request of NRLs:

- Analysis of 3 samples of fishmeal from Member State #1 (March 07)

- Analysis of 1 sample of fishmeal from Member State #2 (May 07)

- Analysis of 2 samples of fishmeal from Member State #3 (May 07)

- Analysis of 1 sample from Member State #4 (July 07)

- Analysis of 2 samples of fishmeal from Member State #3 (June 07)

- Analysis of 1 sample of fishmeal from Member State #3 (July 07)

- Analysis of 3 samples of fishfeed, blood mean, feather meal from Member State #2 (July 07)

- Analysis of 1 sample of fishmeal from Member State #5 (July 07)

- Analysis of 2 samples of fishmeal from Member State #6 (October 07)

- Analysis of 5 samples of fishmeal from Member State #6 (October 07)

- Analysis of 5 samples of fishmeal from Member State #6 (November 07)

As announced in the first half-year report, the tendency of increase in the number of request of analysis from the NRLs has been confirmed during the second half of 2007. A total of 26 official analyses have been realised at present for the NRLs.

1.5 To keep at CRL the highest standard possible of technical skill, scientific awareness and quality management under accreditation (ISO17025, later on maybe even ISO9001) on analytical methods for detection, quantification and identification of animal proteins in feed ingredients and in feedingstuffs. To maintain and extend the accreditation scope of the CRL lab. (12 p/m)

1.5.1 Maintain of the accreditation scope

1.5.2 Extend of the accreditation scope

1.5.3 Initiate the accreditation procedure for the organisation of interlaboratory studies

During the first half year of 2007, CRA-W has been newly accredited by Belac for the microscopic method on the detection of animal proteins in feedingstuffs. This is a consistent extension of the CRA-W accreditation.

CRA-W is working on controls to standardize the PCR test for the detection of cattle MBM in feed in order to allow its in-house validation and its accreditation in 2008. The molecular biology analyses done within the framework of the CRL are integrated within the existing quality assurance system of the Department Quality of Agricultural products. The maintenance of the existing quality assurance systems also benefits to the CRL activities (e.g. balances are checked, micropipettes are checked and recalibrated if necessary, real-time PCR devices are checked,…) even if the PCR analyses done for the CRL are not yet accredited as such.

CRA-W subscribed to a proficiency test for the detection of protein in animal feed by PCR organised by the VLA (4 sets of 5 samples to analyse per year). Two sets of samples were received and were successfully analysed in July and

October 07.

CRA-W also subscribed to a similar proficiency test for the detection of bone in animal feed by microscopy. The samples are analysed in parallel by PCR. Four sets of 4 samples were received (March, June, September and December) and were successfully analysed. The PCR results are in accordance with the expected results (presence of fish and/or terrestrial animals).

Three samples of a proficiency test organised by IAG-section microscopy were also successfully analysed in January 2007.

With respect to PCR, a total of 29 samples coming from proficiency tests were all correctly analysed.

Analyses were performed in parallel on the pre-existing thermocycler (ABI 5700) and on a new thermocycler (LightCycler LC 480) installed in June '07 in order to accumulate results. The aim of these analyses is to provide as soon as possible results with equivalent level of confidence on both devices.

The study initiated together with JRC-IRMM for analysing transfer of the real-time PCR method (see point 4.2.) to other thermocyclers will also be used for setting up the in-house validation dossier of the method for accreditation. It was first planned to use only two thermocyclers of CRA-W but sufficient material was prepared in December to achieve the analysis on the four thermocyclers available at CRA-W. This will provide data of great interest for comparison of the thermocyclers and integration of their use in accredited analyses (at least for three most recent thermocyclers because the reference thermocycler ABI5700 is becoming old and its use will have to be stopped – however as the method was developed on that device it had to be integrated in the study). IRMM will provide a sound statistical analysis comparing the several devices. Moreover the results will be used for defining performance parameters required for the validation dossier (here mainly the limit of detection).

1.6 On the request of DG SANCO, to perform analyses on samples with disputed results. (6 p/m)

1.6.1 Perform the requested analyses

1.6.2 Report on the analyses performed

Before performing any analyses the CRL was requested to attend the sampling process for the dispute between Member State #1 and the United States of America. The CRL-AP head of microscopy team went to Member State #1 on the 14th and 15th of February 2007 for that purpose. Comments had to be given by the CRL on the sampling process that would be used.

Analyses of which the list of numbers of reports is given under 1.6.2. were analysed in the molecular laboratory.

Due to the repeated Rapid Alert System postings, and the amount of samples yet investigated in the Member State #6-Russian dispute, on request of DG SANCO some samples of a fishmeal production line in Member State #6 were transmitted to the CRL-AP for analysis. At time of writing those analyses are performed.

1.7 Assist TAIEX with targeted assistance towards specific training/workshop for new member states or/and candidate countries. (0.5 p/m)

1.7.1 Administration of participation of candidate countries to the annual NRL network workshop.

1.7.2 Request of financial support for candidate countries to TAIEX

In the second half-year we received a asking for participating to a TAIEX on feed safety for Croatia.

Visit of the CRL-AP was organised for Ms Zadravec from the Croatian Veterinary Institute on the 26th of November. Croatia want to participate to a training session in 2008 focused on PAPs dectection by microscopy, which might be difficult to organised within a broader scope of a TAIEX. Agreement on practical modalities is not yet decided. Ms Zadravec was participating at the FEEDSAFETY conference on the 27th and 28th of November in Namur.

2 Coordination of activities of NRL network (13 p/m)

2.1 Construction, development and maintenance of CRL website (internet/intranet) to disseminate to and share information with NRLs and others stake holders. (6 p/m)

2.1.1 Information collection and validation

2.1.2 Development of the website (internet and intranet)

2.1.3 Development of the management tools of the website

2.1.4 Test of the information system and validation

The information on the website is produced by CRA-W and validated by the coordination team.

A monthly update of the website and intranet is done according to the feedback from the NRL partners and the CRL staff. The structure of the webpages has been reorganised on the public website. On intranet, libraries of newsletters (see 2.2) and micrographs (see 4.5.3) have been created.

Statistic tools have been installed in order to follow the accesses to the website and to intranet. The Google Analytics tool shows that, during the 2007 year, the website was visited 1185 times by 615 visitors coming from 59 countries, mainly in Europe; 4242 webpages were visited. Regarding the intranet, the internal MS sharepoint intranet usage tool shows that 8874 hits from 49 participants were registered during the 2007 year.

Requirement analysis has been undertaken for the internal development of a Web based encoding interface dedicated to interlaboratory studies and proficiency tests by the CRL-AP. Development of this modular encoding platform is on going. A POC is yet operating. During the second half year, the work charge of the IT team was focused on other more urgent IT development, such as the conceptualization and realization of a centralized and integrated management tool for the collection of samples, sediments, slides and the daily increasing collection of micrographs intended for the NRLs. The collaborative studies organized this year by the CRL-AP (June-July Proficiency Test and on-going November-January Interlaboratory Studies) were during that period still based on a secured Excel report form communicated on the CRL-AP Intranet to the NRLs. The project will be continued in 2008.

2.2 Prepare and send a four-months newsletter for NRLs. (2 p/m)

2.2.1 Preparation and sending of three newsletters in 2007

A framework and composition template was created in order to facilitate the production of newsletter for the NRL network.

The first CRL-AP newsletter was diffused to the NRLs on the 1st of February

2007 by posting on the Intranet. The content concerned the inauguration day report, news of the Website and Intranet, CRL-AP organigram and organisation, coordinates of all NRLs, announcements of the first CRL-AP Workshop planned in April and a second interlaboratory study for the summer period.

The second CRL-AP newsletter was diffused on the 15th of June 2007. This newsletter presents in draft the minutes of the first CRL-AP Workshop held in Gembloux in April 2007. Important information such as decision of values for the "f" factors to use through next coming interlaboratory studies are to be found in this newsletter. Final version of this second newsletter will be posted after comments on the minutes will be collected (for the 15th of July).

The third CRL-AP newsletter was posted on the 12th of December 2007. It announces the implementation of the micrographs collection for the NRLs, informs on the development of the new sample management software being developed, makes the point on the status of the quantification method enhancements and the PCR methods, reminds on the on-going Interlaboratory Study 2007 on improvement of quantification method and summarises the results of the Proficiency Test 2007 of which final report was diffused on 13th of November. It also focuses on the proven need for continuous training in microscopy and announces new training actions for 2008.

2.3 Organise and host the annual NRL meeting/workshop and produce minutes of the meeting. (1 p/m)

2.3.1 Organisation of the 1st annual CRL-AP workshop

2.3.2 Preparation of the agenda

2.3.3 Invitation of the attendees

2.3.4 Realisation of the workshop

2.3.5 Minutes of the annual workshop

Preparation of the lectures by the CRL staff

The first CRL-AP Workshop was held in Gembloux from the 16th to the 18th of April 2007. 41 Participants attended the Workshop. On all NRLs nominated at that time, only the NRL from Member State #2 could not attend the meeting.

Participation of molecular laboratory staff members to the April workshop

Minutes of the first CRL-AP Workshop were presented in the second CRL-AP newsletter on the 15th of June.

2.4 Supply information, scientific advices and protocols to NRLs, testing laboratories, detection, quantification and identification of animal proteins in feed ingredients and feedingstuffs. (3 p/m)

2.4.1 On the request of the NRLs supply of information and scientific advice

Mr Reiser from the French CEDUS (Centre d'Etude et de Documentation du Sucre) requested informations on the methods of detection in link with possible problems of beetpulp declared positive for the detection of bones coming from terrestrial animals.

The possibilities and the limitations of the main techniques of detection (microscopy, NIR microscopy, PCR and Immunology) were explained by phone to Mr Reiser.

A list of equipments as exhaustive as possible was drawn up in order to allow the French NRL to prepare the budget for a PCR lab dedicated to the detection of PAPs. The list mentioned the models and prices of equipments used in the CRL-AP as well as web sites of providers or distributors in France.

During 2007, numerous mails were received at the CRL-AP for helping some NRLs to detect the nature of certain types of particles resembling suspected terrestrial bones. Those requests came notably from Finland in August concerning the problem of identifying sea mammal's bones in fishmeal. Answer of the CRL-AP was satisfying the Finnish NRL. Again in beginning of December, the Finnish NRL asked help for the identification of unknown particles within a fishmeal : pictures where sent to the CRL-AP but those were insufficient for analysis. At present we are awaiting some samples from the Finnish NRL in order to solve the issue.

Overall, it seems clearly that a better detection method for the identification of sea mammals is expected from the NRL network.

2.5 Participate to annual CRL Directors co-ordination meeting.

2.5.1 Participation to the CRL directors co-ordination meeting

The CRL-AP also participated in the Belgian NRL formation organised on the 9th of October 2007. The Belgian NRL took over many of the training presentation supports prepared by the CRL-AP (cf 5.1.3).

2.6 Prepare the six months and annual reports of activities according to report guidelines transmitted by DG SANCO. (1 p/m)

2.6.1 Prepare and submit the 6-months report (January 2007 – June 2007) and annual report (January 2007 – December 2007)

Preparation of the previous six months report (July 2006-December 2006), preparations of the first half-year report for 2007 (January 2007 – June 2007) and yet the present report (January 2007-December 2007).

3 Interlaboratory studies and quality assurance (22 p/m)

3.1 Coordinate the preparation, reception, storage, maintenance and distribution to national reference laboratories (NRL) of samples containing animal proteins derived from different species and in particular from fish, poultry, pigs and ruminants to be used as reference materials or to carry out comparative testing. (12 p/m)

3.1.1 Definition of the needs

3.1.2 Set up of a planning of the samples to produce in the 2007-2008 period

3.1.3 Collection of the raw materials to use in the preparation of the samples

3.1.4 Control of the raw materials

3.1.5 Production of the samples

3.1.6 Test of the homogeneity of the samples produced

3.1.7 Report on the produced samples

3.1.8 Distribution of the samples

Needs were mainly collected during the first CRL-AP Annual Workshop in April.

Planning for sample production is realised.

Concerning the control on raw material : see analyses performed at point 4.5.6.

By end November 2007, the production of a 0.1% MBM adulterated mixed compound feed was launched. The production itself, its sealed vacuum bag

packing and shipment to all NRLs will still be running at the beginning of 2008. Each of the 26 NRL will thus receive a batch of 100 vacuum bags of 12g each. Production of extra reserve quantity is also planned.

3.2 Organize interlaboratory study for the determination of PAPs in feed using classical microscopy. (6 p/m)

3.2.1 Redaction of the report of the CRL-AP interlaboratoires study 2006

3.2.2 Definition (with the collaboration of the DG-Sanco) of the objectives of the ring trial to perform at the end of 2007.

3.2.3 Preparation of the interlaboratory study.

3.2.4 Invitation of the NRLs to participate.

3.2.5 Preparation and homogeneity test of the samples (cf. task 3.1)

3.2.6 Sending of the samples (Link with task 3.1.)

3.2.7 Collection of the data.

3.2.8 Redaction of the report of the CRL-AP interlaboratoires study 2007

Analysis of results from the CRL-AP Interlaboratory Study 2006 was realised in February and March 2007. The working version of the report of the CRL-AP Interlaboratory Study 2006 was diffused by the 11th of April to all NRLs as a preparation of the workshop. Final version of the document, with remarks from the workshop was made by the 8th of May 2007.

In 2007 two collaborative studies have been planned by the CRL-AP for that period, namely a proficiency test and an interlaboratory study.

The Proficiency Test 2007 was only intended to assess the skills of each NRL at detecting the presence of PAPs by the microscopic method described in EC 126/2003 Directive. A set of 4 blind samples were send to each NRL for qualitative analysis. Results were in on the 21st of August. A first version of the report was diffused to the NRL network on the 15th of October after having been presented at DG SANCO. NRLs had the opportunity to make comments on this first version. Based on the comments received, the final version was prepared and diffused on the CRL-AP Intranet and communicated to the European Commission on the 13th of November 2007. Of note the paid participation of the Canadian Food Inspection Agency (after approval by DG

SANCO) to this proficiency test : it is the very first time that a American country asked for this kind of participation, wanted for its accreditation system.

The Interlaboratory Study 2007 organised during the present period aims at evaluating at large scale the proposed improvements of the official quantification method based on the preliminary internal study made by the CRL-AP team. Announcement of this test was made to the NRLs on the 15th October. The sending of the 10 blind samples, a set of compound feed fortified with different percentages of fish meal, were send on due date the 30th of November. Each NRL requires the use of a specific eyepiece reticule, manufactured in US by a sole provider, for the counting of fishbone particles. Due to this requirement and also the fact that the provider did not respect his commitment on the delivery schedules to each NRL, the deadline for the returning of the results has been postponed, in agreement with DG SANCO, to the 21st of January 2007 instead of the initially planned 20th of December this year. Results from this interlaboratory study will thus be examined and reported on the first half-year of 2008.

3.3 Audit NRLs, coordinate training on methods of analysis and assist staff from NRLs if comparative testing reveals limited experience. Up to 3 European missions/year are foreseen in support to DG SANCO and/or CRL activities. (2 p/m)

3.3.1 On the basis of the results of the interlaboratoires studies, organisation and plannification of the audit

3.3.2 Report of the audits

No activities were planned in 2007.

3.4 To help to develop, extend and keep in the NRLs the highest standard of technical skill and quality management under accreditation on analytical methods for detection, quantification and identification of animal proteins in feed ingredients and in feedingstuffs. (2 p/m)

3.4.1 Definition of the needs of the NRLs

3.4.2 Preparation of programme, in consultation with the Commission, including actions to be undertaken

3.4.3 Provide the requested help to the NRLs

The CRL-AP has helped numerous NRLs by realising on their requests analyses (cf. 1.4.2.).

4 Development of analytical methods and tools (35 p/m)

4.1 Contribute to the development of new methods of analysis and improvement of existing methods of analysis. (1 p/m)

4.1.1 Update of the list of national, European and international initiatives conducted in order to develop methods to detect, quantify and identify MBM in feed

4.1.2 Establishment/maintain of contact with the laboratory in charge of the development in order to be frequently informed about the progress of their development

4.1.3 Summary report on the progress of the different initiatives (link with task 1.1.2.)

4.1.4 Definition of the potential support of the CRL to these initiatives

4.1.5 Establishment of the needs in the development of methods

As a consequence of the results from the CRL-AP Interlaboratory Study 2006 which demonstrated the inapplicability of the quantitative method as described in EC/126/2003 Directive, research for proposing a new method (or at least enhancement of actual method) has been started. The testing and tuning of this new method, according to the NRL network consensus, is based on a stereological derived method (grid or point counting). Developments are still in progress and the method should be validated through a next interlaboratory study planned by the end of 2007.

4.2 Contribute to the development of complementary analytical methods necessary to assure the correct implementation of official methods and explorative or alternative methods. (2 p/m)

4.2.1 Specification of the needs

4.2.2 Report of the results of the tests

Through the JRC-IRMM, the CRL-AP was contacted by a German company (Intergenic-Chipron GmbH) to test an array developed for the detection of animal species. A set of DNAs extracted from representative well-known samples was provided to the Laboratory to test the performances of the array in blind. The conclusion of this first evaluation on a limited number of samples is that the method needs to be improved before a further and more thorough assessment.

Information was obtained on a PCR method for the detection of bovine DNA in feed developed by the "Departamento de Nutrición, Bromatología y Tecnología de los Alimentos – Universidad Complutense de Madrid (Spain)". The results presented at the Feedsafety Conference of Namur (November 2007) are promising and the assessment of the method will be considered in 2008 as contacts were made with this research group.

Collaboration with the JRC-IRMM was initiated to characterise the CRA-W fish targets and to evaluate whether quantitative PCR analyses on fishmeal are realistic. Eleven samples of fish coming from species present in common fishmeal were analysed by PCR. A set of 27 reference samples were also bought to the Federal Research Centre for Nutrition and Food - Department of Fish Quality, Hambourg (Germany) and shared with the JRC-IRMM.

A study with the JRC-IRMM was initiated to transfer the real-time PCR method developed in CRA-W to other instruments and to the laboratory of IRMM-FSQ. The experimental design and the materials were prepared in November and December 2007. The results of the study will be completed in January 2008.

The samples analysed by gas chromatography, came from the feed database in CRA-W and were composed by pure vegetal feeds (vegetal meal), feeds contaminated by "animal origin proteins", pure and mixture animal fats.

The analysis of fatty acid profiles showed that the samples qualified as "vegetal", even if they did not contain animal particle, they presented some ruminant fatty acid specific peaks (C18:1 trans). Therefore the formulas of the compound feed showed that animal fats and/or whey powder have been added to the product.

We analysed animal fats in order to construct models that could be used to discriminate fats on basis of their FA profile. The use of unsupervised techniques (PCA) on FA content of pure fats allowed us to separate tallow chicken and fish samples from the others. The results of the study have been published in a poster presented during the "FEED-SAFETY International Conference" held in Namur on 27th and 28th November 2007.

If animal protein from meat and bone meal are totally banned as feed ingredients for cattle, milk powder and milk by-products can still be used. The CRL-AP started to investigate methods to determine the lactose content of feedingstuffs. An enzymatic method using difference in pH (differential pHmetry) was already investigated. The confirmation with an HPLC method on "real life" samples will be finished by mid 2008.

An HPLC method was developed by Prof Gianfranco Piva to assess the Molecular weight distribution of hydrolysed proteins. (CRL-AP Technical note 2006-01 ver00). At the present stage, the recommended column and standards used for the establishment of the calibration curve have been ordered. Some standards are still missing. As soon as they are available, this method will be checked and evaluated.

The CRL-AP was contacted by a company of Gainesville (Florida, USA) as they wanted us to consider the ELISA kit they developed (April). The company claims to be able to detect ruminant material and porcine material (two different tests) in feed at a sensitivity of 0.1%. As for any test submitted in this way to the CRL-AP, a set of blind samples was sent to the company and to sent back results. The results (May) were partly disappointing: aspecific reactions in presence of DCP and absence of detection of 0.1 % MBM (consisting of pig and cattle) in presence of fishmeal. The company asked again to consider their kit in October . The CRL-AP asked first in what sense they had improved the results but no further reply was given on this demand of the CRL-AP.

4.3 Coordination of evaluation studies on alternative methods. As soon as they become available, methods specifically detecting ruminant, pig or poultry proteins should be evaluated. (12 p/m)

4.3.1 Transfer of validated PCR methods to the CRL

4.3.2 Organisation/planning validation study of the NEOGEN test kit

4.3.3 Submission of the report of the evaluation of the NEOGEN test kit

Collaboration with JRC-IRMM on a comparison of PCR methods of the CRL, of TNO and VLA – contribution to publication of the data.

A meeting was organised in Gembloux (05th of November) with the TNO to initiate a PCR working group gathering the CRL, JRC-IRMM, TNO and VLA. The organisation of this working group is mentioned in the Newsletter of December.

4.4 Performing CRL available methods or adapting them on outbreak material to make them available for the NRLs network. (6 p/m)

4.4.1 Preparation of the framework for the transmission of methods to NRLs network

4.4.2 On the basis of the results of the validation of the PCR and immunological dipstick methods (see tasks 1.5.3.2. and 1.5.3.3.) preparation of CRL protocol to apply these methods in the NRLs labs

4.5 Construction and extension of the sample bank with a special focus on the animal meals of one single species origin (e.g. fish, poultry, pig, bovine, sheep) from different processes. Test, packaging and storage of the new samples as well as production of microscopic image representative of the particles making up the samples collected and selected to be included in the CRL sample bank. (14 p/m)

4.5.1 Establishment of the specification for the CRL sample bank

4.5.2 List of the priority needs regarding the materials to include in the sample bank

4.5.3 Production of informatics tools for the appropriate management of the samples

4.5.4 Collection/production of samples of animal meals of one single species origin (e.g. fish, poultry, pig, bovine, sheep)

4.5.5 Collection/production of samples of compound feeds free of MBM

4.5.6 Test of the samples collected

4.5.7 Preparation of the samples for the storing

4.5.8 Storing of the samples

4.5.9 Maintenance of the sample bank

Specifications on a reference micrographs collection are almost readily defined. The collection of micrographs was started as soon as the new collaborator was engaged (September 2007).

Reflections on a management system for the sample databank for the NRL network allowed the IT team to start the development of a centralised sample bank management system (cf text hereunder).

A centralized and integrated management tool for the collection of samples, sediments, slides and the daily increasing collection of micrographs intended for the NRLs has been developed by the IT team. A first version of the system, called SMS for Sample Management Suite, was yet operational by beginning of December 2007. This version is only limited to the encoding interfaces for the samples, sediments, slides and micrographs. Further development including the consultation screens, the search screens, the interfaces for listings creations such as the automatic updates of the micrographs collection files for the NRL network will continue in 2008. The achievement of this centralized management system will improve the reliability of the traceability within the CRL-AP and saves a lot of time-consuming activities such as the search of materials with specified features and the establishment of listings.

Collection of single species fish samples for testing specificity of the fish PCR probes was realised.

A meeting was organised on 05th of October 2007 with CCL for reception of a set of "pure" species PAPs originating from several renderers throughout Europe. Tests done by CCL (some with the method developed by CRA-W) gave evidence that not all samples were completely pure.

49 animal meals and 22 fish meals were analysed by PCR to check whether they contain material from one single species.

DNAs coming from 25 samples provided by the CCL were also extracted and are currently analysed.

34 compound feeds and raw materials were analysed by PCR to check whether they are free of MBM.

13 samples of fat were also analysed by PCR.

5 Workshops/trainings (3 p/m)

5.1 Provide specific workshop for the benefit of NRLs for the correct application of the 126/2003/EC directive to detect animal proteins in feed (Classical microscopy) and any new directive linked to the detection, identification and quantification of animal proteins in feed. (2 p/m)

5.1.1 Organisation of a workshop for the quantification of PAP using the 126/2003/EC method

5.1.2 Invitation of the attendees

5.1.3 Preparation and submission of the minutes of the workshop

Standard trainings on the qualitative detection of PAP in feed have been prepared and compiled on powerpoint presentations. Improvement of those presentations is still ongoing in order to extend the expertise of training participants. A working book has been realised to and a set of sample materials (pure compounds and feeds) and sediments has been elaborated and tested.

Based on this work, two training sessions have yet been organised. According to the priorities, and for some member states considering the results obtained from the CRL-AP Interlaboratory Study 2006, the participating countries were :

- ***Member State #7 (2 persons) and Member State #5 (1 person) : 4th and 5th of June 2007***
- ***Member State #8 (2 persons) and Member State #9 (1 person) : 7th and 8th of June 2007***
- ***Member State #1 (2 persons), Member State #10 (1 person) and Member State #11 (1 person) : 17th and 18th July 2007***

Certificates of participation were given to each participant. In the future similar training will be planned for all NRLs either they a good performing or underperforming: it appears from NRLs that continuous formation is important as a refreshment of their knowledge and an opportunity to submit and discuss on specific cases.

Training requests have been received from the Estonian NRL at the end of October, from the French NRL at the end of October (specifically dedicated to

intense training of a recently hired NRL technician but also with discussion on PCR issues for a next coming implementation of the real time PCR technique in France). All those trainings will be planned in 2008.

The Romanian authorities have asked this year to have an on-site microscopy training in Bucharest, agreement has been given by DG SANCO and this will be realised in January 2008. Accordingly a common decision from CRL-AP, DG Sanco and the NRL from Member State #1, an on-site training has been decided this year but will occur in 2008.

5.2 Provide specific workshop for the benefit of NRLs for the detection, identification and/or quantification of PAPs in feed according new validated method.

No activities forecasted in 2007

5.3 Provide specific workshop of experts from candidate member states for the correct application of the 126/2003/EC directive to detect animal proteins in feed (Classical microscopy) and any new directive linked to the detection, identification and quantification of animal proteins in feed. (0.5 p/m)

5.3.1 Organisation of a workshop for Romania and Bulgaria for the correct application of 126/2003/EC directive. This specific workshop will be combined with the workshop forecasted in 5.1.

5.3.2 Preparation and submission of the minutes of the workshop

5.4 Provide training through dissemination tools like CD's or DVD's. Development of analytical support and libraries for the training and the maintenance of the skill of laboratories performing classical microscopy or other validated method. (0.5 p/m)

5.4.1 Definition of the needs

5.4.2 According to the outputs of 5.4.1., dissemination of CD's including the description of the method and the support to perform the classical microscopy method described in 126/2003/EC.

5.4.3 Definition of the action plan for the 2007-2011 period

In 2007, in the framework of the CRL-AP Interlaboratory Study on quantification improvement, a written protocol of the quantification method

was prepared and diffused through the NRL network by the intranet. An extended PowerPoint presentation on this counting based quantification method was also posted on the intranet for the benefit of the NRL network. In order to avoid any possible confusions or errors of computation and to have a unique way of applying the quantitative method, a calculation tool based on a protected Excel files was also provided to the NRLs.

On proposal of the CRL-AP to the NRL network, a CD of ARIES was provided to all NRLs. Most of them received it during the CRL-AP workshop organised in April. During the workshop a dedicated introduction and training session on the right utilisation of ARIES was given to the NRLs.