2006 WORK PROGRAMME FOR COMMUNITY REFERENCE LABORATORY

"Detection of animal protein in feedingstuffs"

CRL-AP

Walloon Agricultural Research Centre – CRA-W (Belgium)

Report of 2006 activities

PUBLIC VERSION

0 Launching of the CRL

0.1 Organisation of the CRL.

CRL is organised.

0.2 Recruitment of the CRL personal.

Still ongoing for the replacement of personal that leaved the CRL (3 persons).

0.3 Training of the personal.

About 90 % of the forecasted training of the CRL staff has been completed.

0.4 CRL inauguration.

The CRL inauguration took place November 8th (14:00-17:00).

0.5 List of the NRLs or Member state representatives designated by their authorities if the NRLs for some member states are not yet designated[1].

A first list with the nominated NRLs has been transmitted to the CRL-AP

[1] The same remark is to be considered each time NRLs are considered, it might temporarily be representatives of the member states designates by their authorities as long as no NRL for a specified member State has been chosen.

on 21th September. Additional nominated NRLs have been transmitted to the CRL along the end of 2006.

A list has been published in by January 2007 and refers to 23 NRLs. Malta and Romania did not yet designate their NRL. Bulgaria and Lithuania have designated NRLs, but some data information are still lacking. In order to collect the relevant information an Inquiry has been sent to all the NRLs.

1 Scientific advice and support to the European Commission

1.1 Provide scientific and technical assistance to the European Commission in relation to the development of EC feed legislation.

1.1.1 Submission of a report on the potential of classical microscopy

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

Relevant updated information has been collected and additional new information will be gathered. An update of the review of the methods has been made on the basis of the document published by von Holst et al. in 2004 (Report has been made). It is proposed that this text replaces or completes the existing one on the DG-Sanco web-site (http://ec.europa.eu/food/food/biosafety/bse/bse52_en.pdf).

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.

1.2.1 Preparation and participation to international meeting/fora

1.2.2 Report/minutes following completion of the mission

CRL-AP has been invited to participate to the CEN/JRC workshop that took place in Brussels 30th November, 5th Workshop of the Community Reference Laboratory for Feed Additives Authorisation with Laboratories of the Consortium of National Reference Laboratories .

Participation to the EFSA working group "BSE in Fish" (two meetings one in Parma on 19 October 2006 and one in Gembloux on 22 November 2006). It was a request of EFSA to try to organize one of the meetings in Gembloux in order to give the opportunity to the participants of the working group to have a visit of the CRL-AP facilities. The aim of the working group was to outline an opinion on the "assessment of health risks of feeding of ruminants with fishmeal in relation to the risk of TSE". Presence of someone of the CRL-AP was asked to highlight the analytical aspects of detection of processed animal proteins.

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.

1.3.1 Participation to the CEMA, CEN and IAG meetings

1.3.2 Reports/minutes of the meetings

Presentation of the CRL-AP activities at CEN TC327 meeting (London, 28 June).

Presentation of the CRL-AP activities at the CEMA meeting (Brussels, 7 September).

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.

1.4.1 Provide technical and scientific support

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

In August, the CRL-AP, on the request of the EC, has been contacted by one Member State in order to analyse a series of samples giving positive results for the presence of avian DNA (PCR method). Several analyses (PCR, classical microscopy, NIR microscopy, immunoassays) have been performed by CRL-AP. The Member State identified a possible problem in their analyses of fish meals. With microscopic analyses the fish meals were correct but when performing PCR with an avian target, positive signals appeared on batches especially from countries like Peru or Morocco.

Member State lab (NRL) sent samples to the CRL-AP. Out of these ones, 19 were submitted to classical microscopy, NIRM microscopy and within these ones 5 were submitted to PCR analyses. The CRL-AP has no global avian target for PCR but a chicken target. On explicit request of the NRL lab this target was tested on a limited set of samples for which NRL had a positive result (one negative sample for NRL was however also included for comparison); These same samples were analysed with the available commercial immunoassay test kits. From the whole set of results no clear evidence of other animal proteins could be found in these fish meals. The hypothesis given by the CRL-AP is that the PCR test developed by the NRL lab is probably reacting with avian DNA originating from avian droppings contaminating the fish meal. NRL lab confirmed that their test was validated on fish meals coming from sites where it is not left outside and so risk for contamination by avian droppings is very limited. In Peru and Morocco the fish meal may be stored outside and contamination by avian droppings is possible. (Report has been made)

In December, the CRL-AP, on request of another NRL and with the agreement of DG SANCO, has performed PCR method in order to determine the presence of bovine DNA in suspicious blood samples (3 samples were analyzed). (Report has been made)

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.

1.5.1 Maintain of the accreditation scope

Maintenance of the quality assurance system in the Molecular biology laboratory. Even if the PCR analyses are not carried out under accreditation they benefit from the already existing Quality assurance system and its maintenance (e.g. checking of balances, calibration of micropipettes, temperature verifications on thermocyclers,…).

Time has also been spent for comparison of real-time PCR in order to have a new device and to take into account the problem of transfer of the PCR technique to this new device

1.5.2 Preparation of the agenda for the methods to be accredited

1.5.3 Extend of the accreditation scope

CRA-W has prepared all the procedures and documents required to be accredited for the determination of animal meal by optical microscopy. On 24-25th October, CRA-W had a first internal audit in the framework of its ISO17025. Minor remarks were formulated in the audit report. In January 2007, CRA-W has been audited by BELAC, the Belgian Organisation for Accreditation. Only few minor remarks and suggestions have been noted.

The extension of the scope in order to include the determination of animal meal by optical microscopy is thus achieved.

Moreover, CRA-W has initiated the procedures to accredit its RT-PCR method (and commercial immunological methods) for species specific detection of DNA.

1.6 On the request of DG SANCO, to perform analyses on samples with disputed results.

1.6.1 Perform the requested analyses

1.6.2 Report on the analyses performed

In December, one Member State authorities asked the CRL-AP for help in counter expertise of samples of feed from USA where presence of MBM was detected by a Member State Lab (NRL) and confirmed for a second time by an expert laboratory from another MS. The implication of the US Embassy in the conflict required the agreement of the EC for the CRL-AP intervention. A Member of the CRL-AP will go to the MS for checking the sampling process under control of the conflict parties.

1.7 Assist TAIEX with targeted assistance towards specific training/workshop for new member states or/and candidate countries (i.e. Romania and Bulgaria).

1.7.1.1 Administration of participation of Romania and Bulgaria to the annual NRL network workshop.

1.7.1.2 Request of financial support for Romania and Bulgaria to TAIEX

No activity has been undertaken in 2006 under task.

2 Coordination of activities of NRL network

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

2.1.1 Hardware and software selection

2.1.2 Analysis of the information system to be built

2.1.3 Information collection and validation

2.1.4 Development of the website (internet and intranet)

2.1.5 Dispatching of the login and password to the NRLs or Member state representatives designated by their authorities if the NRLs for some member states is not yet designated (sse task 0.5)

2.1.6 Development of the management tools of the website

2.1.7 Test of the information system and validation

2.1.8 Maintenance and update of the information system

Development of the CRL-AP Website is fulfilled. Maintenance is provided even as content updates. The CRL-AP intranet has been developed too and is in production since mid December. Access to the intranet has been granted by way of user ID and password to all NRLs members (i.e. head of NRL and contact person for CRL-AP Interlaboratory Study 2006).

Ease of use is appreciated, and all the documents related to the first Interlaboratory study have been communicated through the sole intranet platform. Management tools are still in progress.

2.2 Prepare and send a four-months newsletter for NRLs.

2.2.1 Prepare a framework for the newsletter content

2.2.2 Setting up of the distribution list

2.2.3 Preparation and sending of the first newsletter at the end of 2006

Framework has been realised, the first newsletter has been prepared by end of 2006. Due to the simultaneous Interlaboratory study, the edition of this newsletter has been postponed by the end of January 2007. The first newsletter has thus been diffused by posting on the intranet for the whole NRL community.

2.3 Organise and host the annual NRL meeting/workshop and produce minutes of the meeting.

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

No meeting/workshop is forecasted in 2006 for the CRL-AP. Preparation for the first workshop in 2007 has however been initiated by end of 2006.

2.4 Supply information, scientific advices and protocols to NRLs, testing laboratories, detection, quantification and identification of animal proteins in feed ingredients and feedingstuffs.

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

Since the sending of the samples for the Interlaboratory Study 2006, some information and explanation have been formulated by participants (mainly by mail, and about 5 phone calls). All requests were immediately treated to the full satisfaction of the asking persons.

2.5 Participate to annual CRL Directors co-ordination meeting.

2.5.1 Participation to the CRL directors co-ordination meeting

Presentation of the CRL-AP activities at CRL directors co-ordination meeting (Brussels, 4th July).

2.6 Prepare the six months and annual reports of activities according to report guidelines transmitted by DG SANCO.

2.6.1 Prepare a framework of the six months and annual report

2.6.2 Prepare and submit the first annual report (July 2006 – December 2006).

Preparation of a September status on the CRL-AP activities as well as expenditure.

Preparation of the 2007 CRL-AP working programme.

Preparation of the 2007 budget.

3 Ring trials, comparative testing and quality assurance

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.

3.1.1 Definition of the needs

3.1.2 Set up of a procedure to prepare spiked samples at different level and including different species origin

3.1.3 Set up of a protocol to test the homogeneity of the samples produced

3.1.4 Set up of a planning of the samples to produce in the 2006-2007 period

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

3.1.6 Control of the raw materials

3.1.7 Production of the samples

3.1.8 Test of the homogeneity of the samples produced

3.1.9 Report on the produced samples

3.1.10 Distribution of the samples

The homogeneity protocol has been set up. Collection and test of several pure animal meals and feedingstuffs have been done.

Classical microscopy, PCR, immunological, NIR-microscopy and NIR imaging analyses performed on raw material (about 150 samples).

Summary of results produced.

Preparation of the samples for the CRL-AP 2006 interlaboratory study has been completed.

3.2 Organize interlaboratory studies for the determination of PAPs in feed using classical microscopy.

3.2.1 Definition (with the collaboration of the DG-Sanco) of the objectives of the ring trial to perform at the end of 2006. (with the evaluation of the data end 2006- beginning 2007).

3.2.2 Preparation of the ring trial.

3.2.3 Invitation of the NRLs to participate.

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

3.2.5 Collection of the data.

The first CRL-AP Interlaboratory Study has been organised and performed by NRLs. 19 Samples were sent to the participants by beginning of December 2006. Results were expected by 22nd of January 2007 from all participants. Analyses of results are in progress.

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.

No activities forecasted in 2006 (starting year).

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.

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

No requests.

4 Development of analytical methods and tools

4.1 Contribute to the development of new methods of analysis and improvement of existing methods of analysis.

4.1.1 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 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

Contacts with several institutes have been established in order to start this activity.

On August 25th meeting with the JRC-IRMM and DG-Sanco the proposal of a PCR working group gathering CRA-W (BE), VLA (UK) and TNO (NL) has been initiated.

4.2 Contribute to the development of complementary analytical methods necessary to assure the correct implementation of official methods and explorative or alternative methods.

4.2.1 Specification of the needs

4.2.2 Report on the available methods to detect milk and milk by-products in feed

4.2.3 Report on the available methods to detect animal fats in feed

4.2.4 Report on the available methods to identify the species origin of the fats

4.2.5 Test of the promising methods

4.2.6 Report of the results of the tests

In collaboration with JRC-IRMM, the possibility of assessing the quantification of fish meal in feed has been discussed. The CRL-AP fish targets have been cloned in plasmids for that perspective.

CRA-W is looking for a combination between NIRM and PCR but this task is outside the CRL activities.

Bibliographic study has been initiated in order to look for the most suitable methods to detect milk by-products and animal fats in feedingstuffs; to control the molecular weight of hydrolised proteins. (Reports have been made)

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.

4.3.1 Definition of the pre-requisite for the test of alternative methods by the CRL

4.3.2 According to tasks 4.1.1. and 4.3.1., organisation of an evaluation study

4.3.3 Planning of the evaluation study

On August 23rd and 25th meetings with the JRC-IRMM and DG-Sanco have been organised in order to study the status of the immunological and PCR methods.

On September 12th a phone conference with JRC-IRMM and NEOGEN company has been organised in order to discuss the latest developments/results reached with the NEOGEN test kit.

4.4 Performing CRL available methods or adapting them on outbreak material to make them available for the NRLs network.

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

No activity has been undertaken under task.

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.

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

As decided during the negotiation of the CRL-AP 2006 budget, the starting of this task has been postponed to 2007

5 Workshops/trainings

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.

No activities forecasted in 2006 (starting year)

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 2006 (starting year)

5.3 Provide specific workshop of experts from canditate 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.

No activities forecasted in 2006 (starting year)

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.

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

Contacts have been initiated with RIKILT (NL) in order to purchase a copy of ARIES to all the NRLs.