

Project number: 871.111.01  
BAS-code: WOT-02-438-IV  
Project title: NRL-taken diervoederadditieven en nationale dossierbeoordeling/advisering  
  
Project leader: N.P. Lenis, J.J.M. Driessen

Report 2009.009

September 2009

## **Feed additives**

Annual Report 2008

of the Dutch National Reference Laboratory

A.A.M. Stolker, T. Zuidema

Business Unit: Analytical Services & Development  
Group: Veterinary Drugs

### **RIKILT - Institute of Food Safety**

Wageningen Universiteit and Research Centre  
Akkermaalsbos 2, 6708 WB Wageningen, The Netherlands  
P.O. Box 230, 6700 AE Wageningen, The Netherlands  
Tel +31 317 480 256  
Fax +31 317 417 717  
Internet [www.rikilt.wur.nl](http://www.rikilt.wur.nl)

**Copyright 2009, RIKILT – Institute of Food Safety.**

The client is allowed to publish or distribute the full report to third parties. Without prior written permission from RIKILT – Institute of Food Safety it is not allowed to:

- a) publish parts of this report;*
- b) use this report or title of this report in conducting legal procedures, for advertising, acquisition or other commercial purposes;*
- c) use the name of RIKILT – Institute of Food Safety other than as author of this report.*

The research described in this report was funded by the Dutch Ministry of Agriculture, Nature and Food Quality (WOT-02-438-IV).

**Distribution list:**

- Dutch Ministry of Agriculture, Nature and Food Quality (LNV); E.R. Deckers, T. Greutink.
- Dutch Ministry of Agriculture, Nature and Food Quality, General Inspection Service (LNV, AID); M.L. de Groot, J.A. van Wendel de Joode.
- Food and Consumer Product Safety Authority (VWA); R.M.C. Theelen, R. Herbes, H.A. van der Schee.
- Joint Research Centre of the European Commission, Institute for Reference Materials and Measurements (JRC-IRMM); C. von Holst.
- Central Veterinary Institute (CVI); N.P. Lenis.
- Medicines Evaluation Board (CBG-MEB); D.G. Vreeswijk, E. Top, B. Schat.

<p>This report from RIKILT - Institute of Food Safety has been produced with the utmost care. However, RIKILT does not accept liability for any claims based on the contents of this report.</p>
--

## Summary

This report of the National Reference Laboratory (NRL) for feed additives describes the activities employed in 2008. The main tasks of the NRL are: giving assistance to the CRL on their request and advice and support the competent authority, the Dutch Ministry of Agriculture, Nature and Food Quality - Department of Food Quality and Animal Health (LNV-VD).

### NRL activities on request of CRL

The NRL made comments (4 times) on the initial evaluation report prepared by the rapporteur laboratory. This concerned coccidiostats (3 times) and selenium enriched yeast (1 time).

An employee of the NRL actively contributed to the CRL workshop. This workshop was organized in April and was about the procedure for the evaluation of registration dossiers.

### Advices and support

The NRL gave 23 advices to the competent authority regarding temporary use exemptions of feed additives in The Netherlands. The majority of the temporary use exemptions concerns enzymes and probiotics.

The competent authority was advised 8 times regarding the status of a feed additive in relation with the analytical composition, description of the production process and/or EU regulations. Furthermore the competent authority was advised regarding the draft SANCO document 3024/2008 regarding the analytical aspects related to maximum levels of unavoidable carry-over of coccidiostats or histomonostats in non-target feed.

### Other activities

This year special attention was focused on the set up of a decision tree to find in a harmonized and in a repeatable way the answer to the question: what is the status/functionality of a new substance.

Finally, several scientific papers were written, posters presented and lectures given on analytical methods for the analysis of feed additives.

For the coming year the participation of the NRL in the dossier evaluation procedure will be continued and the NRL will advise the competent authority regarding all kind of up coming questions and temporary use exemptions. Also this year special attention will be given to the further development of a decision tree to determine the status/functionality of a new substance.



# Contents

<b>Summary</b> .....	<b>3</b>
<b>1 Introduction</b> .....	<b>7</b>
<b>2 NRL activities</b> .....	<b>9</b>
2.1 Dossier evaluation .....	9
2.2 CRL workshop.....	9
<b>3 Advices on temporary use exemptions</b> .....	<b>11</b>
<b>4 Communications and advices</b> .....	<b>12</b>
4.1 Advices on product registration.....	12
4.2 Advice on SANCO/3024/2008 .....	12
<b>5 Other activities</b> .....	<b>13</b>
<b>6 Participating in proficiency tests</b> .....	<b>14</b>
<b>7 Posters, publications and presentation</b> .....	<b>15</b>
7.1 Posters.....	15
7.2 Other publications.....	15
7.3 Presentations.....	15
<b>8 Plan for NRL activities 2009</b> .....	<b>16</b>
<b>9 References</b> .....	<b>17</b>
Annex I CRL Workshop 17-18 April 2008, Geel, Belgium .....	19
Annex II PDV Beslisboom ter ondersteuning beslissing voedermiddel – toevoegmiddel .....	22



# 1 Introduction

Within the EU livestock production occupies a very important place in the agriculture. Satisfactory results depend to a large extent on the use of safe and good-quality feedingstuffs. In order to protect human health, animal health and the environment, feed additives should undergo a safety assessment through a Community procedure before being placed on the market, used or processed within the Community. The applicant (producer of the additive) has to submit a dossier containing all relevant information regarding safety of the product. The details of the application procedure are described in Regulation (EC) 1831/2003. The European Food Safety Authority, established by Regulation (EC) no. 178/2002 carries out scientific assessment of feed additives (including premixes).

Within the EU, the Community Reference Laboratory (CRL) and a consortium of National Reference Laboratories (NRLs) play an important role in the evaluation and authorisation process to place a feed additive on the market. The Joint Research Centre (JRC in Geel, Belgium) is the CRL. Within the Netherlands RIKILT and RIVM are the NRLs for feed additives (Commission Regulation (EC) No 378/2005).

In EU regulation 1831/2003 the tasks and duties of the CRL are defined. It is also described in this regulation that for all tasks and duties of the CRL the NRLs may be asked by the CRL for assistance. The CRL is responsible for:

- The reception, preparation, storage and maintenance of the reference samples.
- The testing and evaluation or validation of the method for detection.
- Evaluating the data provided by the applicant for authorization to place the feed additive on the market, for the purpose of testing and evaluation or validation of the method for detection
- Submitting full evaluation reports to the Authority.

Next to these tasks the CRL shall play a role in dispute settlements between Member States concerning the analytical results of testing additives for use in animal nutrition.

Commission regulation (EC) 378/2005 describes in more details the activities and organization of CRL and NRLs. During an application evaluation process it is possible that an NRL is requested to act as the rapporteur of the application (the rapporteur laboratory) or to participate in the consortium. The rapporteur laboratory has to :

- Draft an initial evaluation report concerning the data submitted in each application and submitting it for comments to the other laboratories.
- Compile the comments received from the other laboratories and prepare a revised evaluation report.
- Submit the revised evaluation report to the CRL. The CRL submits the full evaluation report to the Authority.

For doing research on the use of a feed additive that has not yet been registered as such or for a specific application, the applicant needs to have a 'temporary use exemption' for the feed additive. These permissions are obtained by the Dutch 'Bureau Diergeneesmiddelen' under supervision of the Dutch Ministry of Agriculture, Nature and Food Quality - Department of Food Quality and Animal Health (LNV-VD). LNV-VD is the competent authority in The Netherlands for authorisation of feed additives. RIKILT advises the Dutch government regarding the temporary use exemption of specific

feed additives, more specific about issues related to the application of feed additives in feed, e.g. cross-contamination, stability and homogeneity. Furthermore on request RIKILT as a NRL advises the LNV regarding other issues concerning feed additives.

This report describes the activities employed in 2008 by RIKILT regarding the functions as described above viz:

- a. as the NRL for feed additives,
- b. advices given regarding temporary use exemptions,
- c. other advices and support of LNV.

This year within the project special attention was focused on the set up of a decision tree to find in a harmonized and in a repeatable way the answer to the question: What is the status/functionality of a feed additive.

This report also presents the activities performed by the NRL to keep up expertise on the analysis of feed additives like participation in proficiency tests and presenting (posters, abstracts, publications) analytical research.



## 2 NRL activities

### 2.1 Dossier evaluation

In 2008 the NRL made comments (4 times) on the initial evaluation report prepared by the rapporteur laboratory.

The advices were described in evaluation reports and included the following additives:

- Maxiban G160: modification of authorisation for establishment of Maximum Residue Limits: quantification of marker residue 4,4'-dinitrocarbanilide DNC in liver by an LC-MS/MS method.
- Cycostat 66G: modification of authorisation for establishment of Maximum Residue Limits in chicken for fattening and turkeys: quantification of robenidine residues in muscle, skin/fat, liver and kidney by HPLC-UV and LC-MS/MS methods.
- Avatec 15 G: modification of authorisation, extension of the use for other feeds, namely for pheasants, partridges, quails, guinea fowl, ducks, geese: evaluation of an HPLC-UV method for the determination of the active substance lasalocid A sodium in the feed additive and of an HPLC-FLU method for the determination of the active substance lasalocid A sodium in premixtures and feedingstuffs.
- Selsaf (Selenium enriched yeast): new authorisation; evaluation of the quantification of total selenium in feedingstuffs for all animal species by FAAS, ICP-AES and HGAAS methods.

Some general remarks can be made regarding the evaluation of the analytical procedures. A general remark is the applicability of the submitted analytical method for residues in products of animal origin as a confirmatory method. Within the EU document 2002/657/EC the criteria for a confirmatory method are clearly described however it happens several times that the proposed method is based on UV detection or on specific MS detection (detecting only one precursor and one transition ion). Although these techniques are very useful for quantitative purposes the methods do not fulfill the confirmation criteria, in other words, not enough identification points are collected for using the method as a confirmatory method.

Furthermore, a general remark is that several times the analytical methods proposed by the rapporteur are not included in the dossier and only a very limited description without specific references is included. Consequently the traceability (and availability) as an official method is not shown.

Another general remark is that several times methods have not been validated for the specific feeds for which registration is sought.

For Selsaf the question was raised if a method should have been included in the dossier for the specific determination of seleno-methionine.

### 2.2 CRL workshop

In 2008 an employee of RIKILT participated to the following workshop:

- CRL-Workshop April 16-17, JRC-IRMM in Geel, Belgium.

T. Zuidema actively contributed to this workshop in discussions regarding the evaluation of registration dossiers. According to feed additive regulation 1831/2003 a huge number of additives have to be re-evaluated before November 7, 2010. Annex I presents sheets of the CRL workshop.

Furthermore the workshops included a discussion concerning the new dossier evaluation guidelines. A concept of an evaluation protocol - developed by a working group and the CRL - is open for discussion and comments. The concept has also been sent to FEFANA (EU Association of feed additives and premixtures operators) and during the workshop distributed to the NRL representatives. After all the comments have been collected the final version of the protocol will be established (planning final version 2009).

The expert working group on 'Analytical methods for coccidiostats' chaired by J. de Jong from RIKILT organized a meeting between CRL feed additives and CRL residues of veterinary drugs for coccidiostats in Berlin. Both CRLs will formally work together to set up analytical method requirements for the analysis of coccidiostats. A first draft regarding this issue is available however there are some points of discussion regarding the method characteristics like LOQ, repeatability and within-lab reproducibility.

### **3 Advices on temporary use exemptions**

In 2008 the number of requests for temporary use exemptions (TUE) was not significantly different from the number in 2007. In 2008 the number of TUEs was 23. The majority of TUEs concerns enzymes, probiotics, coccidiostats/histomonostats or amino acids. A general critical item in some TUE's was the analytical method needed to monitor the non-intended 'carry over' of the feed additive from one production badge to the next production badge. Therefore it is very helpful that the EU came up with Commission Directive 2009/8/EC describing maximum levels of unavoidable carry-over of coccidiostats or histomonostats in nontarget feed (see also section 4.2).

## 4 Communications and advices

One of the tasks of the NRL is to communicate with the Competent Authority on issues regarding the control and use of feed additives. The communication and advice activities employed by the NRL in 2008 are described below.

### 4.1 Advices on product registration

In The Netherlands the Advising Committee for Registration of Feed Additives ('Adviescommissie Product Registratie' (APR)) advises the competent authority about issues related to the registration or use of feed additives. In 2008 RIKILT was asked 8 times by E-mail to give advice. .

The majority of the advices concerns information regarding the status of the product in relation with the analytical compositions, description of the production processes and the EU regulations. Among others, advice was given about the question if a nanostructured clay with a claim to bind mycotoxins could be categorised under the functional group of "Binders, anti-caking agents and coagulants".

Another advice was about the interpretation of Regulation (EC) 1831/2003 for the maximum content of sodium bisulphite and sodium metabisulphite where it was advised not to take other sources of SO<sub>2</sub> into account for the calculation of this maximum content.

### 4.2 Advice on SANCO/3024/2008

RIKILT was asked by the competent authority to give their opinion about the draft SANCO document 3417/2008 'amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels of unavoidable carry-over of coccidiostats or histomonostats in non-target feed'. Comments were sent by the NRL to the competent authority (by e-mail as requested). These comments mainly concerned the availability of validated methods of analysis for 1 % and 3 % carry-over in non-target feeds. It was concluded that there is a need for further method development and international harmonisation (CEN) of methods of analysis

## 5 Other activities

This year a discussion was started between the NRL, LNV, VWA (Food and Consumer Product Safety Authority) and the PDV (Productschap Diervoeders) regarding the development of a decision tree. There is a need for a harmonized and repeatable approach to determine the status/functionality of a new substance. Sometimes there is a discussion if a new substance is a feed additive, a feed material or a veterinary drug. The functionality of a new substance has influence on the legal requirements that have to be fulfilled to bring the product on the market, viz. for a feed additive the data that are necessary for the registration dossier. Therefore there is a need for a decision tree describing the determination of the functionality of a new substance in a harmonized and repeatable way.

In the first meeting the points of discussion are: function of the decision tree (which questions have to be answered) taking into account the EU definitions. The PDV is already using a prototype of a decision tree (see Annex II) and this prototype was used as an example. Furthermore the PDV introduced relevant cases. Finally it was decided that the prototype of the decision tree and the cases introduced by the PDV will be used as starting points for the new decision tree. The first draft of the new decision tree will be discussed in a follow-up meeting in 2009.

## 6 Participating in proficiency tests

Due to the scope of the NRL task assigned, RIKILT participated in proficiency tests organized by The Agricultural Laboratories Quality Service (KDLL, Zeist, the Netherlands).

In 2008 RIKILT participated in the following proficiency tests:

- Nicarbazin in feed (RIKILT method RSV A0846); z-scores for four different feed samples -0.2; 0; -0.9; -1
- Salinomycine in feed ; This analyte is not within the scope of the accredited RIKILT method (RSV A0846) however, the sample was analyzed and retrospective evaluated for the concentration of salinomycine. Concentration was within the tolerance interval.

## 7 Posters, publications and presentation

### 7.1 Posters

Posters presented at the Euro Residue VI Conference on Residues of Veterinary Drugs in Food; Egmond aan Zee; 19-21 May, 2008. Page numbers refer to the proceedings of the Euro Residue Conference (Proceedings of the Euro Residue VI; Residues of Veterinary Drugs in Food Edited by: L.A. Van Ginkel and A.A. Bergwerff; ISBN 978-90-804925-3-0).

- Performance evaluation of hormone and veterinary drug residue screening by ultra performance chromatography coupled to time-of-flight and orbitrap mass spectrometry, Ed van der Heeft, Paul Zomer, Linda A.M. Stolker and Michel W.F. Nielen, pages 565-569
- Development and validation of a confirmatory method for the analysis of avilamycin in animal feed, Ilse Zwartjes, Tina Zuidema, Frédérique van Holthoorn, Jacob de Jong, pages 729-732
- Development of an LC-MS/MS method for the determination of aminoglycosides in animal feed, Payam Aqai, Bjorn Berendsen, Tina Zuidema and Linda Stolker, pages 733-738
- S. Weigel and M.W.F. Nielen and J. de Jong,; CONFIDENCE in Food and Feed: a new European Research Project; BioCop Open Day, The latest Advances in Chemical Food Analysis Safety, 3 September 2008, York (UK)

### 7.2 Other publications

The following articles were published in a peer reviewed Journal:

- E. van der Heeft, Y.J.C. Bolck, B. Beumer, A.W.J.M. Nijrolder, A.A.M. Stolker and M.W.F. Nielen;  
Full-scan Accurate Mass Selectivity of Ultra Performance Liquid Chromatography Combined with Time-of-Flight and Orbitrap Mass Spectrometry in Hormone and Veterinary Drug Residue Analysis. Source: J. Am. Soc. Mass Spectrom. In press
- I. Pol-Hofstad, W. Driessen-van Lankveld, M. Tomassen, J. de Jong and H. van Egmond;  
Collaborative study of a microbiological screening method (three-plate) for the banned antimicrobial growth promoters tylosin, virginiamycin, spiramycin, zinc bacitracin and avoparcin in animal feed; Food Additives & Contaminants Part A, 25 (2008) 1465-1474

### 7.3 Presentations

- J. de Jong, S. Weigel and M.W.F. Nielen; CONFIDENCE in Food and Feed: a new European Research Project; 1st MoniQA International Conference „Increasing Trust in Rapid Analysis for Food Quality and Safety“, 8 – 10 October 2008, Rome

## **8 Plan for NRL activities 2009**

The participation of the NRL in the dossier evaluation procedure will be continued for the coming year. The NRL will also advise the competent authority regarding all kind of up coming questions and temporary use exemptions.

An employee of the NRL (W. Beek) will be trained by the CRL in the evaluation of registration dossiers using the new evaluation criteria. The training will take place in May 2009.

Furthermore Dr. J. de Jong of the NRL will participate in the workshops organized by the CRL (June 11+12) and he is invited to give a guest lecture regarding CEN procedures for harmonization of analytical methods for feedingstuffs.

Also this year special attention will be paid to the further development of a Decision Tree to obtain a harmonized and repeatable protocol to establish the status/functionality of a new substance. In 2008 the first meeting between different parties (NRL, LNV-VD, VWA and the PDV) was organized to start with setting up such a protocol. In 2009 this activity will be continued and hopefully finalized.



## 9 References

Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003. On additives for use in animal nutrition, L 268 page 29-43.


Commission Regulation (EC) No 378/2005 of 4 March 2005 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the duties and tasks of the Community Reference Laboratory concerning applications for authorisations of feed additives.


SANCO/3024/2008 Draft Amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels of unavoidable carry-over of coccidiostats or histomonostats in non-target feed.


COMMISSION DIRECTIVE 2009/8/EC of 10 February 2009 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels of unavoidable carry-over of coccidiostats or histomonostats in nontarget feed, L 40 page 19-25.



## Annex I CRL Workshop 17-18 April 2008, Geel, Belgium

 **JRC**  
EUROPEAN COMMISSION

 **itm**  
Institute for Reference and Research Materials




**Community Reference Laboratory for Feed Additives**  
**Introduction and overview of the activities in 2007**


Christoph von Holst

*CRL workshop 16-17 April 2008*

 **JRC**  
EUROPEAN COMMISSION


**The CRL team**

 **itm**  
Institute for Reference and Research Materials



Dalia Garalevičienė left in November 2007 to the European Chemicals Agency (Helsinki)

*Dalia Garalevičienė*



New colleague: Piotr Robouch

*Piotr Robouch*


- Overview of **activities in 2007**
- Update on the activities of the **working group meeting**
  - Probiotics
  - Coccidiostats
- **High number of dossiers:** How do we get prepared for 2010?
  - **Experiences** from the evaluation of about 65 dossiers
  - **Industry approach**
  - What do we expect from the **NRLs?**
- Update on the **new guidelines** including **concept of verification**
- Scientific discussion
  - New CEN method for **trace elements**
  - Presentation of **EFSA's carry-over** study in respect to coccidiostats
  - Methods for the detection of **coccidiostats in tissue**

- List of dossiers prepared and sent on-time to EFSA


Product/Additive Name	Active Substance	Rapporteur Laboratory
Quantum Phytase	6-phytase	CRL-FA
Toyocerin (sows)	Bacillus cereus var. toyoi	CRL-FA
Ronozyme_Biofeed	6-phytase	CRL-FA
L-Arginine	L-arginine	CRL-FA
Availa Cr	Chromium-methionine chloride	CREAA
Sorbiflore (piglets)	L. Rhamnosus, L. Farciminis	CRL-FA
Bioplus 2B (sows)	B. subtilis, B. licheniformis	CRL-FA
Natugrain Wheat	Endo-1,4-beta-xylanase □	LYNGBY
Panaferd-AX	Astaxanthin	CRL-FA
CLINACOX 0.5%	Diclozauril	RIKILT
Danisco Xylanase	Endo-1,4 beta-xylanase	CRL-FA
BiominC5 (chicken)	P. acidilactici, E. Faecium, B. animalis ssp. Animalis, L. reuteri, L. salivarius ssp. Salivarius	CRL-FA
Lantharenol	Lantharanum carbonate	CREAA
Carophyl® Stay-Pink	Astaxanthin dimethylsuccinate	CRL-FA

Product/Additive Name	Active Substance	Rapporteur Laboratory
Calsporin (chicken)	Bacillus subtilis	CRL-FA
Safizym_X (ducks)	Endo-1,4 beta-xylanase	CRL-FA
Borvital (sows)	E. faecium	CRL-FA
Avizyme 1505	Endo-1,4-beta-xylanase, subtilisin	CRL-FA
Natuphos® (ducks)	Endo-1,4-beta-xylanase	LYNGBY
CreAminoTM	Guanidinoacetic acid	UKZUZ
Coxidin 25%	Monensin sodium	CRL-FA
Toyocerin (Turkeys)	Bacillus cereus var. toyoi	CRL-FA
Mintrex-Zn	Zn chelate of hydroxy analogue of	CREAA
Mintrex-Mn	Mn chelate of hydroxy analogue of	CREAA
Mintrex-Cu	Cu chelate of hydroxy analogue of	CREAA
Biosaf Sc47 (pigs)	S. cerevisiae	CRL-FA
Avizyme 1505	Endo-1,4-beta-xylanase, subtilisin	CRL-FA
Danisco Xylanase	Endo-1,4 beta-xylanase	CRL-FA

- Reports of 28 dossiers, on-time reported to EFSA
- Quite different analytical methodology



**JRC**  
 EUROPEAN COMMISSION


**What did we achieve in 2007? cont.**




5

- **Organisation of working group** meetings in the field of microorganism, trace elements, coccidiostats, verification study
- Adaptation of the **fee to be paid** by the applicant:
  - Old regime: 3000 Euro, 1500 to CRL and rapporteur
  - **New regime: 6000 Euro, 2000 to CRL and 4000 to rapporteur**
- The Commission issued the new dossier guideline
  - Official food and feed Regulation 882/2004: criteria for methods
  - Verification concept proposed by the CRL/NRLs

  
 New Commission dossier guideline


**JRC**  
 EUROPEAN COMMISSION

**What did we achieve in 2007? cont.**



6

- Authorisation of notified products
  - *Regulation 1831/2003, Art. 10.2: An application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and **within a maximum of seven years after the entry** (8.11.2010) into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC. **A detailed calendar listing in order of priority the different classes of additives to be re-evaluated may be adopted in accordance with the procedure referred to in Article 22(2).***
- Initiating discussions with EFSA/Commission/Industry on concepts how to cope with the high number of applications to be expected by 2010 (Priority list, joint applications...)
- Getting ready for 2010 and the time after (Poster)

# Annex II PDV Beslisboom ter ondersteuning beslissing voedermiddel – toevoegmiddel

