EUROPEAN COMMISSION
DIRECTORATE GENERAL
JOINT RESEARCH CENTRE
Directorate D: Institute for Reference Materials and Measurements
European Union Reference Laboratory for Feed Additives

JRC.D.5/CvH/ZE/mds/Ares

Addendum to the EURL report for FAD-Zinc Group JRC.DG.D.6/CvH/PR/mds/ARES(2011)1156545

Upon request from DG SANTE [1], the EURL evaluated the supplementary information provided in the frame of the FAD-2010-0142 dossier [2] for the quantification of zinc chelate of amino acid hydrate in the *feed additive*. The Applicant submitted a single-laboratory validated and further verified method based on Fourier Transformed Infrared (FTIR) spectroscopy coupled with Principal Component Regression (PCR) analysis [3].

Powdered samples and calibration standards (5 to 10 mg) are subjected to infrared spectroscopy using attenuated total reflectance sampling accessory. The calibration standards (available from the Applicant upon request) are prepared from zinc sulphate and hydrolysed soya flour [3]. FTIR spectra are recorded from 1800 to 650 cm^{-1}. Nine replicate spectra per calibration standard are acquired to build the model together with six replicate spectra per sample. The statistical treatment of pre-processed spectra is performed using PCR analysis to generate the calibration and prediction models for quantification of chelated zinc content in *feed additive* samples [3]. Pilot samples containing *ca.* 40, 70 and 90% of chelated Zinc were analysed, together with several commercial batches of the *feed additive*. The following performance characteristics were reported in the frame of the validation and verification studies [3,4]: (i) a relative standard deviation for *repeatability* (RSD_r) ranging from 0.4 to 14.2%; and (ii) a bias ranging from 93 to 107 %.

Furthermore, the EURL performed a similar study on copper chelated products, and demonstrated the applicability of FTIR followed by multivariate regression methods, such as PCR or Partial Least Squares (PLS) to determine the degree of metal chelation.

Based on the performance characteristics available the EURL recommends for official control the method based on middle range FTIR followed by PCR or PLS algorithms to quantify the content of chelated zinc in the *feed additive*.

Recommended text for the registry entry (analytical method)

For the determination of chelated Zinc content in the *feed additive*:

- Fourier Transformed Infrared (FTIR) spectroscopy followed by multivariate regression methods

References

[1] Supplementary Information – DG SANTE request cf. FAD-2010-0142

[2] EURL Evaluation Report – JRC.DG.D.6/CvH/PR/mds/ARES(2011)1156545

[3] FAD-2010-0142 Supplementary information – Validation Report 210314SB

[4] FAD-2010-0142 Supplementary information – Verification Study of Bioplex Zn

Addendum
- Prepared by Zigmas Ezerskis and Piotr Robouch
- Reviewed and approved by Christoph von Holst (EURL-FA) Geel, 06/01/2016

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JRC.DG.D.6/CvH/PR/mds/ARES(2011)1156545

EURL Evaluation Report on the Analytical Methods submitted in connection with the Application for the Authorisation of Feed Additives according to Regulation (EC) No 1831/2003

Dossier related to:	**FAD-2010-0059 - CRL/100040**
	FAD-2010-0063 - CRL/100041
	FAD-2010-0072 - CRL/100074
	FAD-2010-0142 - CRL/100135
	FAD-2010-0228 - CRL/100203
Product Name:	
Active Substance(s):	**E6** **zinc acetate dihydrate** **zinc chloride anhydrous** **zinc oxide** **zinc sulphate heptahydrate** **zinc sulphate monohydrate** **zinc chelate of amino acids hydrate** **zinc chelate of glycine hydrate**
Rapporteur Laboratory:	**European Reference Laboratory for Feed Additives, IRMM, Geel, Belgium**
Report prepared by:	**Gerhard Buttinger & Piotr Robouch**
Report revised by: Date:	**Roberto Molteni (EURL-FA)** **28/10/2011**
Report approved by: Date:	**Christoph von Holst** **28/10/2011**

EXECUTIVE SUMMARY

In the current application authorisation is sought under articles 4(1) and 10(2) for *zinc acetate dihydrate*[1], *zinc chloride anhydrous*[1], *zinc oxide*[1, 5], *zinc sulphate heptahydrate*[1], *zinc sulphate monohydrate*[1, 2, 3], *zinc chelate of amino acids hydrate*[1, 4] *and zinc chelate of glycine hydrate*[1] under the category/functional group 3(b) of "nutritional additives"/"compounds of trace elements", according to the classification system of Annex I of Regulation (EC) No 1831/2003.

According to the Applicants: - *zinc acetate dihydrate* is white solid with a minimum content of 29.6 % *total zinc*; - *zinc chloride anhydrous* is white to slightly coloured solid with a minimum content of 46 % *total zinc*; - *zinc oxide* is white to dark green or beige brownish solid with a minimum content of 72 % *total zinc*; - *zinc sulphate heptahydrate* is a white solid with a minimum content of 22 % *total zinc;* - *zinc sulphate monohydrate* is a white to cream coloured solid with a minimum content of 34 % *total zinc*; - *zinc chelate of amino acid hydrate* is beige to dark tanned solid with a minimum content of 10 % *total zinc*; and - *zinc chelate of glycine hydrate* is white to cream coloured solid with a minimum content of 24 % *total zinc*. Specifically, authorisation is sought for the use of these *feed additives* for all categories and species.

For the identification and quantification of the inorganic zinc compounds (i.e. *zinc acetate dihydrate, zinc chloride anhydrous, zinc oxide, zinc sulphate heptahydrate and zinc sulphate monohydrate*) in the *feed additive*, the EURL recommends for official control the relevant European Pharmacopoeia Monograph (1482, 0110, 0252, 0111 and 2159) methods, based on complexometric titration with 0.1 M sodium EDTA using xylenol orange triturate as indicator.

For the quantification of "amino" content in the amino zinc chelates (i.e. *zinc chelate of glycine hydrate* and *zinc chelate amino acids hydrate*), the Applicant proposed - upon request from the EURL - the Community method based on High Performance Liquid Chromatography (HPLC) combined with post-column derivatisation using ninhydrin as derivatisation agent and photometric detection at 570 nm. The EURL considers the Community method suitable for the characterisation of the amino compounds in the frame of official control.

For the *determination* of total zinc in all the *feed additives*, *premixtures* and *feedingstuffs* the Applicant submitted the CEN method (EN 15510), based on inductively coupled plasma atomic emission spectroscopy (ICP-AES). The following performance characteristics were

[1] FAD-2010-0142; [2] FAD-2010-0228; [3] FAD-2010-0059; [4] FAD-2010-0063; [5] FAD-2010-0072

reported: - a relative standard deviation of *repeatability* (RSD_r) ranging from 1.7 to 8.8 %; - a relative standard deviation for *reproducibility* (RSD_R) ranging from 5.0 to 19 %; and - a limit of quantification (LOQ) of 3 mg/kg. Furthermore, the Applicant identified an alternative CEN ring-trial validated method (CEN/TS 15621) based on ICP-AES after pressure digestion, for the determination of total zinc in the *feed additive*, *premixtures* and *feedingstuffs*. The following performance characteristics were reported for a feed for pigs, and for sheep, a rock phosphate, a mineral premix and a mineral mix, where the total zinc content ranged from 26.6 to 3618 mg/kg: - RSD_r ranging from 1.5 to 5.4 %; - RSD_R ranging from 2.7 to 22 %; and - LOQ = 1 mg/kg *feedingstuffs*. Finally, the Applicant suggested the Community method for the determination of total zinc in *feedingstuffs,* with limited method performance characteristics provided. However, the UK Food Standards Agency organised a comparative trial based on the above mentioned Community method and reported precisions (RSD_r and RSD_R) for *feedingstuffs* ranging from 1.0 to 9.5 %.

Based on these acceptable method performance characteristics the EURL recommends for official control the ICP-AES CEN methods (EN 15510 and CEN/TS 15621) to determine total zinc content by in the *feed additive* and *premixtures.* As for the determination of total zinc content in *feedingstuffs*, the EURL recommends for official control the Community method based on AAS together with the above mentioned ICP-AES CEN methods.

For the quantification of total zinc in *water* the Applicant[2] submitted the ring trial validated method EN ISO 11885, based on ICP-AES. The following performance characteristics are reported: - RSD_r ranging from 1.5 to 2.4 %; - RSD_R ranging from 4.9 to 5.9 %; and LOQ = 1 µg/L. Based on these acceptable method performance characteristics the EURL recommends for official control the CEN methods (EN ISO 11885) to quantify total zinc content by ICP-AES in the *water.*

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

KEYWORDS

zinc acetate dihydrate, zinc chloride anhydrous, zinc oxide, zinc sulphate heptahydrate, zinc sulphate monohydrate, zinc chelate of amino acids hydrate, zinc chelate of glycine, all categories and species, compounds of trace elements

1. BACKGROUND

In the current application authorisation is sought under articles 4(1) and 10(2) for *zinc acetate dihydrate*[1], *zinc chloride anhydrous*[1], *zinc oxide*[1, 5], *zinc sulphate heptahydrate*[1], *zinc sulphate monohydrate*[1, 2, 3], *zinc chelate of amino acids hydrate*[1, 2] *and zinc chelate of glycine hydrate*[1] under the category of "nutritional additives" functional group 3b (compounds of trace elements) [1], according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additives* for all categories and species [1].

According to the Applicants [2, 3]:

- *zinc acetate dihydrate* is white solid with a minimum content of 29.6 % *total zinc*;

- *zinc chloride anhydrous* is white to slightly coloured solid with a minimum content of 46 % *total zinc*;

- *zinc oxide* is white to dark green or beige brownish solid with a minimum content of 72 % *total zinc*;

- *zinc sulphate heptahydrate* is a white solid with a minimum content of 22 % *total zinc*;.

- *zinc sulphate monohydrate* is a white to cream coloured solid with a minimum content of 34 % *total zinc*;

- *zinc chelate of amino acid hydrate* is beige to dark tanned solid with a minimum content of 10 % *total zinc*; and

- *zinc chelate of glycine hydrate* is white to cream coloured solid with a minimum content of 24 % *total zinc*; or a transparent liquid with a minimum content of 7 % *total zinc* [3].

These *feed additives* are intended to be mixed into *premixtures, feedingstuffs* and *water*. The Applicants suggested maximum levels ranging from 150 to 250 mg *total zinc*/kg *feedingstuffs* and from 28.6 to 125 mg *total zinc*/L *water*, similar to limits set in the previous regulations [4,5].

[1] FAD-2010-0142; [2] FAD-2010-0228; [3] FAD-2010-0059; [4] FAD-2010-0063; [5] FAD-2010-0072

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, 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 the tasks of the European Union Reference Laboratory concerning applications for authorisations of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *zinc acetate dihydrate, zinc chloride anhydrous, zinc oxide, zinc sulphate heptahydrate, zinc sulphate monohydrate, zinc chelate of amino acids hydrate and zinc chelate of glycine hydrate*, and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

3. EVALUATION

Identification /Characterisation of the feed additive

The following European Pharmocopoeia methods are recommended by the EURL for the identification of the "inorganic zinc compounds" of interest in the *feed additives*:

- Eur. Ph. monograph 1482 [6], based on selective precipitation reactions of zinc and acetate, for the identification of *zinc acetate dehydrate;*
- Eur. Ph. monograph 0110 [7], based on selective precipitation reactions of zinc and chloride for the identification of *zinc chloride anhydrous* ;
- Eur. Ph. monograph 0252 [8], based on selective precipitation reactions of zinc and a selective colour reaction, for the identification of *zinc oxide*;
- Eur. Ph. monograph 0111 and 2159 [9,10], based on selective precipitation reactions of zinc and sulphates, for the identification of *zinc sulphate heptahydrate* and *zinc sulphate monohydrate*.

Upon request from the EURL, the Applicants confirmed [11] using the Community method of analysis for amino-acids [12] to quantify the "amino acid" content in the amino zinc chelates (i.e. *zinc chelate of glycine hydrate* and *zinc chelate amino acids hydrate*). Additionally, the Applicant suggests [3] to quantify the total zinc content (described in the next section) for the characterisation of the above mentioned products.

Qualitative and quantitative composition of impurities in the additive

When required by EU legislation, analytical methods for official control of undesirable substances in the additive (e.g. arsenic, cadmium, lead and dioxins) are available from the respective European Union Reference Laboratories [13].

Description of the analytical methods for the determination of the active substance in feed additive, premixtures and feedingstuffs

For the quantification of the "inorganic zinc compounds" under investigation in the *feed additive (i.e. zinc acetate dehydrate, zinc chloride anhydrous*, *zinc oxide, zinc sulphate heptahydrate* and *zinc sulphate monohydrate*) the EURL recommends for official control the European Pharmacopoeia Monograph methods mentioned above (1482, 0110, 0252, 0111 and 2159) [6-10]. All these methods are based on the complexometric titration with 0.1 M EDTA using xylenol orange triturate as indicator.

For the quantification of "amino" content in the amino zinc chelates (i.e. *zinc chelate of glycine hydrate* and *zinc chelate amino acids hydrate*), the Applicant proposed [11] - upon request from the EURL - the Community method [12] based on High Performance Liquid Chromatography (HPLC) combined with post-column derivatisation using ninhydrin as derivatisation agent and photometric detection at 570 nm. The EURL considers the Community method suitable for the characterisation of the amino compounds in the frame of official control.

For the quantification of <u>total zinc</u> in all the *feed additives* of concern, in *premixtures* and *feedingstuffs* the Applicants (FAD-2010-0142 & FAD-2010-0059) submitted the ring trial validated CEN method EN 15510 [14], based on inductively coupled plasma atomic emission spectroscopy (ICP-AES). For the determination of total zinc, a test portion of the sample is ashed and dissolved in hydrochloric acid (in the case of organic feedingstuffs) or wet digested with hydrochloric acid (in the case of mineral compounds). The following performance characteristics were reported for a complete feed for pigs, a complete feed for sheep, a rock phosphate, a mineral premix and two different mineral mixtures, where the <u>total zinc</u> content ranged from 27.4 to 3826 mg/kg:

- a relative standard deviation of *repeatability* (RSD_r) ranging from 1.7 to 8.8 %$^{(*)}$
- a relative standard deviation for *reproducibility* (RSD_R) ranging from 5.0 to 19 %$^{(*)}$;
- a limit of quantification of 3 mg/kg.

(*) the highest precision values were obtained for mineral mixes

Applicants (FAD-2010-0059 & FAD-2010-0063) submitted an alternative CEN ring-trial validated method (CEN/TS 15621) based on ICP-AES after pressure digestion [15], for the quantification of total zinc in the *feed additive*, *premixtures* and *feedingstuffs*. The total zinc concentration is determined using external calibration or standard addition technique. The following performance characteristics were reported for a feed for pigs, and for sheep, a rock phosphate, a mineral premix and a mineral mix, where the total zinc content ranged from 26.6 to 3618 mg/kg: - RSD_r ranging from 1.5 to 5.4 %; - RSD_R ranging from 2.7 to 22 %; and - LOQ = 1 mg/kg *feedingstuffs,* suitable for low total zinc contents.

Furthermore, the Community method [16] was suggested by Applicant (FAD-2010-0228) for the quantification of total zinc in *feedingstuffs*. The sample is brought into solution in hydrochloric acid after destruction of organic matter, if any. Zinc is then determined after appropriate dilution by AAS. No method performance characteristics are reported in the Regulation, except an LOQ of 20 mg/kg *feedingstuffs*. However, the UK Food Standards Agency recently organised a ring-trial [17] based on the above mentioned Community method, using samples such as dog biscuits, layer pellets, beef nuts, sow rolls or rabbit pellets. Precisions (RSD_r and RSD_R) ranging from 1.0 to 9.5 % were reported for samples containing total zinc levels ranging from 93 to 199 mg/kg *feedingstuffs*.

Based on the acceptable method performance characteristics presented, the EURL recommends for official control the ICP-AES CEN methods (EN 15510 or CEN/TS 15621) to quantify total zinc content in *feed additives* and *premixtures.* As for the quantification of total zinc content in *feedingstuffs*, the EURL recommends for official control the Community method based on AAS together with the above mentioned CEN methods (EN 15510 or CEN/TS 15621).

For the quantification of total zinc in *water* the Applicant (FAD-2010-0228) submitted the ring trial validated CEN method EN ISO 11885 [18], based on inductively coupled plasma optical emission spectroscopy (ICP-OES). The total zinc concentration is determined using external calibration or standard addition technique. The following performance characteristics were reported for drinking water, surface water (filtered) and waste water, where the total zinc content ranged from 124 to 1251 µg/L: - RSD_r ranging from 1.5 to 2.4 %; - RSD_R ranging from 4.9 to 5.9 %; and - LOQ = 1 µg/L.

Based on these acceptable method performance characteristics the EURL recommends for official control the ICP-AES CEN method (EN ISO 11885) to quantify total zinc content in the *water.*

The EURL is aware of several ring-trial validated methods (Community method [12], ISO [19], AOAC [20] and VDLUFA [21]) dedicated to the determination of "amino acids" in *premixtures* and *feedingstuffs*. However, these methods are not relevant for official control when the monitoring of total zinc content is required, as set in previous legislations [4,5].

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control:

- the European Pharmacopoeia Monograph 1482 for the identification and the quantification of *zinc acetate dihydrate* in *feed additive;*
- the European Pharmacopoeia Monograph 0110 for the identification and the quantification of *zinc chloride anhydrous* in *feed additive;*
- the European Pharmacopoeia Monograph 0252 for the identification and the quantification of *zinc oxide* in *feed additive;*
- the European Pharmacopoeia Monograph 0111 for the identification and the quantification of *zinc sulphate heptahydrate* in *feed additive;*
- the European Pharmacopoeia Monograph 2159 for the identification and the quantification of *zinc sulphate monohydrate* in *feed additive;*
- the Community method for the quantification of amino acid content in the *feed additives* (*zinc chelate of glycine hydrate* and *zinc chelate amino acids hydrate*), using High-Performance Liquid Chromatography (HPLC) coupled to post column derivatisation and photometric detection;
- the CEN methods EN 15510 and CEN/TS 15621 for the quantification of total zinc content by ICP-AES in the *feed additive* and *premixtures;*
- the Community method based on AAS and the above mentioned ICP-AES CEN methods (EN 15510 or CEN/TS 15621) for the quantification of total zinc in the *feedingstuffs;*
- the CEN methods EN ISO 11885 for the quantification of total zinc content by ICP-AES in *water*

Recommended text for the register entry (analytical method)

For the quantification of the *zinc acetate dihydrate* in the *feed additive*:

- titration with sodium edetate (European Pharmacopoeia Monograph 1482)

For the quantification of the *zinc chloride anhydrous* in the *feed additive*:

- titration with sodium edetate (European Pharmacopoeia Monograph 0110)

For the quantification of the *zinc oxide* in the *feed additive*:

- titration with sodium edetate (European Pharmacopoeia Monograph 0252)

For the quantification of the *zinc sulphate heptahydrate* in the *feed additive*:

- titration with sodium edetate (European Pharmacopoeia Monograph 0111)

For the quantification of the *zinc sulphate monohydrate* in the *feed additive*:

- titration with sodium edetate (European Pharmacopoeia Monograph 2159)

For the quantification of amino acid content in the *feed additives*
(*zinc chelate of glycine hydrate* and *zinc chelate amino acids hydrate*):

- ion exchange chromatography method with post-column derivatisation and UV or fluorescence detection: Commission Regulation (EC) No 152/2009 (Annex III, F)

For the quantification of <u>total zinc</u> in the *feed additive* and *premixtures*:

- EN 15510: Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES), or
- CEN/TS 15621: Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES) after pressure digestion.

For the quantification of <u>total zinc</u> in the *feedingstuffs*:

- Regulation (EC) No 152/2009 - Atomic Absorption Spectrometry (AAS); or
- EN 15510: Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES); or
- CEN/TS 15621: Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES) after pressure digestion.

For the quantification of <u>total zinc</u> in the *water*:

- EN ISO 11885: Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-AES).

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *zinc acetate dihydrate, zinc chloride anhydrous, zinc oxide, zinc sulphate heptahydrate, zinc sulphate monohydrate, zinc chelate of amino acids hydrat and zinc chelate of glycine hydrate* have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

6. REFERENCES

[5] *Application, Reference SANCO D2/WT/eu (2011)470063

[6] *Application, Proposal for Register Entry – Annex A

[7] *Technical dossier, Section II: Identity, characterisation and conditions of use of the additive; Methods of analysis

[8] Commission Regulation (EC) No 1334/2003 amending the conditions of authorisation of a number of additives in feedingstuffs belonging to the group of trace elements

[9] Commission Regulation (EC) No 479/2006 as regards the authorisation of certain additives belonging to the group compounds of trace elements

[10] European Pharmacopoeia Monograph 1482

[11] European Pharmacopoeia Monograph 0110

[12] European Pharmacopoeia Monograph 0252

[13] European Pharmacopoeia Monograph 0111

[14] European Pharmacopoeia Monograph 2159

[15] *Supplementary information

[16] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (cf. Annex III-F)

[17] Commission Regulation (EC) No 776/2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards to Community Reference Laboratories

[18] EN 15510:2007 – *Animal feeding stuffs – Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum, arsenic, lead and cadmium by ICP-AES*

[19] CEN/TS 15621:2007 – *Animal feeding stuffs – Determination of cadmium, sodium, phosphorus, magnesium, potassium, sulphur, iron, zinc, copper, manganese, cobalt and molybdenum after pressure digestion by ICP-AES*

[20] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (cf. Annex IV-C)

[21] *Supplementary Information - Food Standards Agency – Information Bulletin on Methods of Analysis and Sampling for Foodstuffs, No 102; March 2010

[22] EN ISO 11885:2009 – *Water quality – Determination of selected elements by inductively coupled plasma optical emission spectrometry (ICP-OES – ICP-AES)*

[23] EN ISO 13903:2005 - *Animal feeding stuffs – Determination of amino acids content*

[24] AOAC Official Method 999:13 – Lysine, Methionine and Threonine in Feed Grade Amino Acids and Premixes

[25] Bestimmung von Lysin, Methionin und Threonin in Aminosäurenhandelsprodukten und Vormischungen – 4.11.6, Methodenbuch III, 5. Erg. 2004, VDLUFA – Verlag, Darmstadt.

* Refers to Dossier No. FAD-2010-0142

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European Reference Laboratory for Feed Additives, IRMM, Geel, Belgium. This report is in accordance with the opinion of the consortium of National Reference Laboratories as referred to in Article 6(2) of Commission Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009.

8. ACKNOWLEDGEMENTS

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- Państwowy Instytut Weterynaryjny, Puławy (POL)
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia i Pesca, Generalitat de Catalunya,Cabrils (ES)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)