EUROPEAN COMMISSION
DIRECTORATE GENERAL
JOINT RESEARCH CENTRE
Directorate F – Health, Consumers and Reference Materials
European Union Reference Laboratory for Feed Additives

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

Addendum to the EURL report FAD-2010-0095-Iron Group

(JRC.D.5/CvH/PR/mds/ARES(2012)1040236)

Upon request from DG SANTE [1], the EURL evaluated the supplementary information provided in the frame of the FAD-2010-0095 dossier [2] for the quantification of iron chelate of protein hydrolysates 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 reflection. The calibration standards of chelated iron are prepared from iron (II) sulphate and hydrolysed soya flour. FTIR spectra are recorded from 1800 to 870 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 iron content in the *feed additive* samples [3].

In the frame of the validation and verification studies, the Applicant reported precisions ranging from 3.5 to 12 % for samples formulated to contain 30 or 90 % of chelated iron [3]. Similar performance characteristics were reported when analysing commercial samples from the same producer [3].

However, when applying this method to analyse batches of four similar commercial products produced by other companies, average values ranging from 22 % to 237 % chelated iron content, were obtained [4]. These results do not allow any reliable conclusion on the degree of iron chelation. This indicates that the proposed FTIR-PCR method is product specific and may not be suitable for the determination of the degree of chelation of all commercially available iron chelates of protein hydrolysates.

Based on the performance characteristics available the EURL cannot recommend for official control a reliable method to quantify the content of chelated iron in the *feed additive* containing iron chelate of protein hydrolysates.

References

[1] Supplementary Information – DG SANTE request cf. Chelate method, Ares(2017)2202334

[2] EURL Evaluation Report – JRC.D.5/CvH/PR/mds/ARES(2012)1040236

[3] FAD-2010-0095 Supplementary information:Annex_Qi_Report Chelated Fe_company(b1)

[4] FAD-2010-0095 Supplementary information:Annex_Qi_Report Chelated Fe_12samples

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Addendum
- Prepared by Zigmas Ezerskis and Piotr Robouch
- Reviewed and approved by Christoph von Holst (EURL-FA) Geel, 08/05/2017

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
European Union Reference Laboratory for Feed Additives

JRC.D.5/CvH/PR/mds/ARES(2012)

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-2010-0068 - CRL/100041** **FAD-2010-0095 - CRL/100116** **FAD-2010-0236 - CRL/100276** **FAD-2010-0295 - CRL/100359** **FAD-2010-0296 - CRL/100274** **FAD-2010-0380 - CRL/100305**
Feed Additive Name(s):	**Ferrous chelate of glycine hydrate** **Ferrous/iron chelate of amino acids hydrate** **Ferrous fumarate** **Ferric oxide** **Ferric chloride hexahydrate** **Ferrous sulfate monohydrate** **Ferrous sulfate heptahydrate** **Ferrous carbonate**
Active substance(s):	**E1 - Iron**
Rapporteur Laboratory:	**European Reference Laboratory for Feed Additives, IRMM, Geel, Belgium**
Report prepared by:	**Piotr Robouch**
Report revised by: Date:	**Stefano Bellorini (EURL-FA)** **06/09/2012**
Report approved by: Date:	**Christoph von Holst** **06/09/2012**

EXECUTIVE SUMMARY

In the current application authorisation is sought under articles 4(1) and 10(2) for *ferrous chelate of glycine hydrate*[1], *ferrous/iron chelate of amino acids hydrate*[1,2], *ferrous fumarate*[1], *ferric oxide*[3], *ferric chloride hexahydrate*[1], *ferrous sulfate monohydrate*[1,4], *ferrous sulfate heptahydrate*[1,5], *ferrous carbonate*[1,6] under the category/ functional group (3b) "nutritional additives"/"compounds of trace elements", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of these *feed additives* for all categories and species.

According to the Applicants *ferrous chelate of glycine hydrate* is a green-gray free-flowing powder with a minimum content of 17 % *total iron*, *ferrous/iron chelate of amino acids hydrate* is a brown free-flowing powder with a minimum content of 10 % *total iron*, *ferrous fumarate* is white reddish powder with a minimum content of 30 % *total iron*, *ferric oxide* is a red brown powder with a minimum content of 56% *total iron*, *ferric chloride hexahydrate* is a yellow brown solid aggregate with a minimum content of 59 % *total iron*, *ferrous sulfate monohydrate* consists of beige to gray free-flowing granules with a minimum content of 29 % *total iron*, *ferrous sulfate heptahydrate* is a blue-green crystalline powder with a minimum content of 18 % *total iron* and *ferrous carbonate* is a brown powder with a minimum content of 37 % *total iron*. These *feed additives* are intended to be mixed into *premixtures, feedingstuffs* and/or *water*(*). The Applicants suggested maximum levels ranging from 250 to 1250 mg *total iron* /kg *feedingstuffs* and from 100 to 2273 mg *total iron* /L *water*, similar to limits set in the previous regulations.

(*) not for *ferric oxide, ferrous carbonate* and *ferrous chelate of amino acids hydrate*.

For the identification and quantification of the inorganic iron compounds (i.e. *ferrous fumarate*, *ferric chloride hexahydrate* and *ferrous sulphate mono* and *heptahydrate*) in the *feed additive*, the EURL recommends for official control the relevant titrimetric methods described in the European Pharmacopoeia Monographs 0083, 0902 and 1515. As for the identification of *ferrous carbonate* and *ferric oxide* the EURL recommends using X-ray diffraction.

For the determination of *ferric oxide* (also know as *iron oxide red)* in the *feed additive* the internationally recognised FAO JECFA monograph for food additives is recommended by Commission Directive 2008/128/EC, laying down specific purity criteria concerning colours for use in foodstuffs. Identification is based on solubility in solvents, while quantification is based on digestion and iodometric titration.

[1]FAD-2010-0095; [2]FAD-2010-0068; [3]FAD-2010-0236; [4]FAD-2010-0295; [5]FAD-2010-0296; [6]FAD-2010-0380

For the quantification of "amino" content in the amino iron chelates (i.e. *ferrous chelate of glycine hydrate* and *ferrous/iron chelate amino acids hydrate*), the Applicant proposed the Community method based on ion exchange chromatography combined with post-column ninhydrin derivatisation 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.

Furthermore, the EURL identified the generic European Pharmacopoeia methods for the "identification reactions of ions and functional groups", such as carbonate, chloride and sulfate in the *feed additives*. Finally, the EURL recommends crystallographic techniques, such as X-Ray diffraction for the characterisation of crystalline structures of *ferric oxide, ferric chloride hexahydrate, ferrous carbonate and ferrous sulfate mono* and *heptahydrate*.

For the *quantification* of total iron in the *feed additives*, *premixtures* and *feedingstuffs* the Applicants submitted three ring trial validated CEN methods: EN 6869, based on atomic absorption spectrometry (AAS), EN 15510, based on inductively coupled plasma atomic emission spectroscopy (ICP-AES) and CEN/TS 15621, based on ICP-AES after pressure digestion. Precisions ranging from 2 to 16 % were reported, together with limits of quantification (LOQ) ranging from 1 to 5 mg/kg *feedingstuffs*. Furthermore, the EURL identified the comparative trial organised by the UK Food Standards Agency, based on the Community method for the determination of iron in *feedingstuffs*, in which precisions ranging from 1.0 to 9.5 % were reported.

For the quantification of total iron in *water* the Applicant (FAD-2010-0095) submitted the ring trial validated method EN ISO 11885, based on ICP-AES. The following performance characteristics were reported: a relative standard deviation for *repeatability* (RSD_r) ranging from 1.5 to 2.4 %, a relative standard deviation for *reproducibility* (RSD_R) ranging from 4.9 to 5.9 %, and LOQ = 1 µg/L.

Based on the available performance characteristics the EURL recommends for official control all the above mentioned CEN methods together with the Community method to quantify total iron content in the *feed additives, premixtures, feedingstuffs* and/or *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

E1, *ferrous chelate of glycine hydrate, ferrous/iron chelate of amino acids hydrate, ferrous fumarate, ferric oxide, ferric chloride hexahydrate, ferrous sulfate monohydrate, ferrous sulfate heptahydrate, ferrous carbonate,* all categories and species, compounds of trace elements

1. BACKGROUND

In the current application authorisation is sought under articles 4(1) (new use in water) and 10(2) (authorisation of an existing product) for *ferrous chelate of glycine hydrate*[1], *ferrous/iron chelate of amino acids hydrate*[1,2], *ferrous fumarate*[1], *ferric oxide*[3], *ferric chloride hexahydrate*[1], *ferrous sulfate monohydrate*[1,4], *ferrous sulfate heptahydrate*[1,5], *ferrous carbonate*[1,6] under the category/ functional group (3b) "nutritional additives"/"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 these *feed additives* for all categories and species [1].

According to the Applicants [2, 3]:

- *ferrous chelate of glycine hydrate* is a green-gray free-flowing powder with a minimum content of 17 % *total iron*;
- *ferrous/iron chelate of amino acids hydrate* is a brown free-flowing powder with a minimum content of 10 % *total iron*;
- *ferrous fumarate* ($FeC_4H_2O_4$) is white reddish powder with a minimum content of 30 % *total iron*;
- *ferric oxide* (Fe_2O_3) is a red brown powder with a minimum content of 56% *total iron* (FAD-2010-0236);
- *ferric chloride hexahydrate* ($FeCl_3$, $6H_2O$) is a yellow brown solid aggregate (prill) with a minimum content of 59 % *total iron*;
- *ferrous sulfate monohydrate* ($FeSO_4$, H_2O) consists of beige to gray free-flowing granules with a minimum content of 29 % *total iron*;
- *ferrous sulfate heptahydrate* ($FeSO_4$, $7H_2O$) is a blue-green crystalline powder with a minimum content of 18 % *total iron*; and
- *ferrous carbonate* ($FeCO_3$) is a brown powder with a minimum content of 37 % *total iron*.

[1]FAD-2010-0095; [2]FAD-2010-0068; [3]FAD-2010-0236; [4]FAD-2010-0295; [5]FAD-2010-0296; [6]FAD-2010-0380

These *feed additives* are intended to be mixed into *premixtures, feedingstuffs* and *water*[*]. The Applicants suggested maximum levels ranging from 250 to 1250 mg *total iron* /kg *feedingstuffs* and from 100 to 2273 mg *total iron* /L *water*, similar to limits set in the previous regulations [4,5].

(*) not for *ferric oxide, ferrous carbonate* and *ferrous chelate of amino acids hydrate*.

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 *ferrous chelate of glycine hydrate, ferrous/iron chelate of amino acids hydrate, ferrous fumarate, ferric oxide, ferric chloride hexahydrate, ferrous sulfate monohydrate, ferrous sulfate heptahydrate, ferrous carbonate*, and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

3. EVALUATION

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 [6].

Identification /Characterisation of the feed additives

For the characterisation of *ferrous sulfate heptahydrate* in the *feed additive* the Applicant (FAD-2010-0296, [3[e]]) submitted two dedicated methods: - the CEN standard method (EN 889) based on the titration with potassium dichromate solution [7]; and - the European Pharmacopoeia monograph 0083, based on the iron and sulfate reactions and on the titration with ammonium and cerium nitrates [8]. According to the Applicant (FAD-2010-0295, [3[d]]) these methods also apply to the characterisation of *ferrous sulfate monohydrate* in the *feed additive*, applying a correction factor taking into account the difference in water content.

Upon request from the EURL, the Applicant (FAD-2010-0095) confirmed [9] using the Community method of analysis for amino-acids [10] to quantify the "amino acid" content in the amino iron chelates (i.e. *ferrous chelate of glycine hydrate* and *ferrous chelate amino acids hydrate*). This method is based on ion exchange chromatography combined with post-column ninhydrin derivatisation and photometric detection at 570 nm.

Furthermore, the EURL identified the following European Pharmacopoeia methods for the characterisation of the "inorganic iron compounds" of interest in the *feed additives*:

- Eur. Ph. monograph 0902 [11], based on titration with cerium sulfate for the identification of *iron fumarate*;
- Eur. Ph. monograph 1515 [12], based on titration with sodium thiosulfate for the identification of *ferric chloride hexahydrate*, together with
- the generic Eur. Ph. monograph 20301 [13] for the "identification reactions of ions and functional groups", such as carbonate, chloride and sulfate.

Additionally, the EURL already evaluated and recommended (cf. FAD-2010-0204) the internationally recognised FAO JECFA monograph for food additives [23] for the determination of *ferric oxide* in the *feed additive*, as recommended by Commission Directive 2008/128/EC, laying down specific purity criteria concerning colours for use in foodstuffs. The identification is based on solubility in solvents, while quantification is based on digestion in hydrochloric acid and hydrogen peroxide with an iodometric titration.

Finally, the EURL recommends crystallographic techniques, such as X-Ray diffraction for the characterisation of crystalline structures of *ferric oxide, ferric chloride hexahydrate, ferrous carbonate and ferrous sulfate monohydrate* and *heptahydrate*.

Several Applicants suggested quantifying the total iron content in the *feed additives* using the analytical techniques described hereafter.

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

Three different ring-trial validated methods were submitted by the Applicants for the quantification of total iron in the *feed additives, premixtures* and *feedingstuffs*:

- Applicants (FAD-2010-0095, -0236 and -0380) submitted the CEN method EN 15510 [14], based on Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). 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);

- Applicants (FAD-2010-0068 and -0380) submitted the CEN method (CEN/TS 15621), based on ICP-AES after pressure digestion [15]; while
- Applicant (FAD-2010-0095) submitted the CEN (EN ISO 6869), based on Atomic Absorption Spectrometry (AAS) [16].

In addition, the Community method [17] prescribes an AAS method for the quantification of total iron in *feedingstuffs*. The sample is brought into solution in hydrochloric acid after destruction of organic matter, if any. Iron is then determined after appropriate dilution by AAS. No method performance characteristics are reported, except a limit of quantification (LOQ) of 20 mg/kg *feedingstuffs*. However, the UK Food Standards Agency recently organised a ring-trial [18] based on the above mentioned Community method, using samples such as dog biscuits, layer pellets, beef nuts, sow rolls or rabbit pellets.

The performance characteristics reported by all the methods mentioned above are summarised in Table 1.

For the quantification of total iron in *water* the Applicant (FAD-2010-0095) submitted the ring trial validated CEN ISO method (EN ISO 11885), based on ICP-AES [19]. The total iron 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 iron content ranged from 196 to 880 µg/L: - a relative standard deviation for repeatability (RSD_r) ranging from 1.7 to 1.9 %; - a relative standard deviation for reproducibility (RSD_R) ranging from 4.7 to 5.9 %; and - a limit of quantification (LOQ) of 1 µg/L.

Table 1: Performance characteristics for the quantification of total iron in *premixtures* and *feedingstuffs*

	EN 15510 [14]	EN 15621 [15]	EN 6869 [16]	LGC [18]
Method	ICP-AES	ICP-AES	AAS	AAS
Content (mg/kg)	293 – 8182	325 – 8550	79 – 31000	197 – 340
RSD_r (%)	2.4 – 4.8	1.9 – 5.2	0.9 – 16	2.3 – 9.5
RSD_R (%)	5.1 – 10	8 – 16	6.0 – 24	5.3 – 9.5
LOQ (mg/kg)	3	1	5	20

RSD_r and RSD_{ip}: relative standard deviation for *repeatability* and *intermediate precision*;
LOQ: limit of quantification

Based on the acceptable method performance characteristics presented, the EURL recommends for official control all the above mentioned ring-trial validated methods for the quantification of total iron in the *feed additives*, *premixtures*, *feedingstuffs* and *water*.

The EURL is aware of several ring-trial validated methods (Community method [10], ISO [20], AOAC [21] and VDLUFA [22]) 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 iron content is required, as set in previous regulations [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 0083 and the CEN method (EN 889) for the identification and the quantification of *ferrous sulfate mono and hepta-hydrate* in *feed additive;*
- the European Pharmacopoeia Monograph 0902 for the identification and the quantification of *ferrous fumarate* in *feed additive;*
- the European Pharmacopoeia Monograph 1515 for the identification and the quantification of *ferric chloride hexahydrate* in the *feed additive;*
- the generic European Pharmacopoeia Monograph 20301 for the "identification reactions of ions and functional groups", such as carbonate, chloride and sulfate;
- X-Ray diffraction for the characterisation of crystalline structures of *ferric oxide, ferric chloride hexahydrate, ferrous carbonate* and *ferrous sulfate mono* and *heptahydrate*;
- the Community method for the quantification of amino acid content (*iron chelate of glycine hydrate* and *iron chelate amino acids hydrate*) in the *feed additives,* based on ion exchange chromatography combined with post-column ninhydrin derivatisation and photometric detection;
- the CEN methods (EN 6869, 15510 and CEN/TS 15621) for the quantification of total iron content in the *feed additive* and *premixtures;*
- the Community method based on AAS and the above mentioned CEN methods (EN 6869, 15510 or 15621) for the quantification of total iron in the *feedingstuffs;* and
- the CEN ISO method (EN ISO 11885) for the quantification of total iron content by ICP-AES in *water.*

Recommended text for the register entry (analytical method)

For the quantification of the *ferrous sulfate mono* and *heptahydrate* in the *feed additive*:

- titration with ammonium and cerium nitrate (Ph. Eur Monograph 0083); or
- titrate with potassium dichromate (EN 889)

For the quantification of the *ferrous fumarate* in the *feed additive*:

- titration with cerium sulfate (Ph. Eur Monograph 0902)

For the quantification of the *ferric chloride hexahydrate* in the *feed additive*:

- titration with sodium thiosulfate (Ph. Eur Monograph 1515)

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

- ion exchange chromatography combined with post-column ninhydrin derivatisation and photometric detection (Commission Regulation (EC) No 152/2009, Annex III, F)

For the "identification reactions of ions and functional groups", such as iron, carbonates, chlorides and sulfates in *feed additive:*

– European Pharmacopoeia Monograph 2.3.1*;*

For the crystallographic characterisation of *feed additives*, such as *ferric oxide, ferric chloride hexahydrate, ferrous carbonate and ferrous sulfate mono and heptahydrate:*

– X-Ray diffraction

For the quantification of total iron in the *feed additive* and *premixtures*:

- Atomic Absorption Spectrometry, AAS (EN ISO 6869); or
- Inductively Coupled Plasma – Atomic Emission Spectrometry, ICP-AES (EN 15510); or
- Inductively Coupled Plasma – Atomic Emission Spectrometry after pressure digestion, ICP-AES (CEN/TS 15621)

For the quantification of total iron in the *feedingstuffs*:

- Atomic Absorption Spectrometry, AAS (Commission Regulation (EC) No 152/2009, Annex IV-C); or
- Atomic Absorption Spectrometry, AAS (EN ISO 6869); or
- Inductively Coupled Plasma – Atomic Emission Spectrometry, ICP-AES (EN 15510) or
- Inductively Coupled Plasma – Atomic Emission Spectrometry after pressure digestion, ICP-AES (CEN/TS 15621)

For the quantification of total iron in *water*:

- Inductively Coupled Plasma – Atomic Emission Spectrometry, ICP-AES (EN ISO 11885).

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *ferrous chelate of glycine hydrate, ferrous/iron chelate of amino acids hydrate, ferrous fumarate, ferric oxide, ferric chloride hexahydrate, ferrous sulfate monohydrate, ferrous sulfate heptahydrate, ferrous carbonate,* 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

[1] [a,b,c,d,e,f] Application, Reference SANCO G1/WT/ci 359135(2012)

[2] [a,b,c,d,e,f] Application, Proposal for Register Entry – Annex A

[3] [a,b,c,d,e,f] Technical dossier, Section II: (i) Identity, (ii) characterisation of the active substance; (iii) conditions of use of the additive; (iv) Methods of analysis

[4] 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

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

[6] 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

[7] EN 889:2004 - *Chemicals used for treatment of water intended for human consumption. Iron (II) sulfate*

[8] European Pharmacopoeia Monograph 01/2010:0083

[9] Supplementary information – see FAD-2010-0142

[10] 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)

[11] European Pharmacopoeia Monograph 01/2008:0902

[12] European Pharmacopoeia Monograph 01/2008:1515

[13] European Pharmacopoeia Monograph 01/2008: 20301

[14] 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*

[15] 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*

[16] EN ISO 6869:2000 - *Animal feeding stuffs - Determination of the contents of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc - Method using atomic absorption spectrometry*

[17] 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)

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

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

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

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

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

[23] FAO JEFCA Iron oxides monographs (2008) http://www.fao.org/ag/agn/jecfa-additives/specs/monograph5/additive-238-m5.pdf

[a] Refers to Dossier No. FAD-2010-0068
[b] Refers to Dossier No. FAD-2010-0095
[c] Refers to Dossier No. FAD-2010-0236
[d] Refers to Dossier No. FAD-2010-0295
[e] Refers to Dossier No. FAD-2010-0296
[f] Refers to Dossier No. FAD-2010-0380

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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- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft, Labore Landwirtschaft, Leipzig (DE)