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

**L-arginine** produced by fermentation with  
*Corynebacterium glutamicum* (*KCTC 10423BP*)  
(FAD-2014-0012; CRL/140008)



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

Dossier related to: **FAD-2014-0012 - CRL/140008**

Name of Product: ***L-arginine produced by fermentation with  
Corynebacterium glutamicum  
KCTC 10423BP***

Active Agent (s): **L-arginine**

Rapporteur Laboratory: **European Union Reference Laboratory for  
Feed Additives (EURL-FA)  
Geel, Belgium**

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Date: **19/12/2014**

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Date: **19/12/2014**

## EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *L-arginine produced by fermentation with Corynebacterium glutamicum (KCTC 10423BP)*, under the category/functional group 3(c) 'nutritional additives'/amino acids, their salts and analogues', according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for all animal species. *L-arginine* is already authorised under Commission Regulation (EC) No 1139/2007.

For the quantification of *L-arginine* in *feed additive, premixtures* and *feedingstuffs* the Applicant submitted the Community method (Commission Regulation (EC) No 152/2009). The method was further ring-trial validated resulting in EN ISO 13903:2005. The method is based on ion exchange chromatography coupled with post-column derivatisation and photometric detection (IEC-VIS). This method does not distinguish between the salts and the amino acid enantiomers and it is designed for *feedingstuffs* and *premixtures*. The following performance characteristics were reported for the quantification of total *arginine*: a relative standard deviation for *repeatability* (RSD<sub>r</sub>) ranging from 2.3 to 3.3% and a relative standard deviation for *reproducibility* (RSD<sub>R</sub>) ranging from 7.2 to 9.7%.

Even if not explicitly described in the scope of the Community method, the EURL, followed the advice of several experienced National Reference Laboratories (NRLs), recommends the Community method for the quantification of *arginine* in the *feed additive*. Furthermore, for the characterisation of the *feed additive*, the EURL identified the methods described in the "*L-arginine* monograph" of the Food Chemical Codex (FCC).

Based on the performance characteristics available, the EURL recommends for official control the ring-trial validated Community method (equivalent to the EN ISO 13903:2005) based on IEC-VIS for the quantification of *arginine* in the *feed additive, premixtures* and *feedingstuffs*, together with the "*L-arginine* monograph" of the FCC for the characterisation of the *feed additive*.

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

*L-arginine produced by fermentation with Corynebacterium glutamicum (KCTC 10423BP), nutritional additives, amino acids, their salts and analogues, all animal species*

## 1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (authorisation of a new feed additive) for *L-arginine produced by fermentation with Corynebacterium glutamicum (KCTC 10423BP)*, under the category/functional group 3(c) 'nutritional additives'/amino acids, their salts and analogues', according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for all animal species [1-2]. *L-arginine* is already authorised under Commission Regulation (EC) No 1139/2007 [3-4].

According to the Applicant, *L-arginine* is a white crystalline powder with a minimum purity of 98% [1,5]. As described in the "*L-arginine* monograph" of the Food Chemical Codex, the *feed additive* has a specific optical rotation ranging from +25.8° and +27.9° [6]. *L-arginine* is intended to be mixed either in *premixtures* or added directly to *feedingstuffs* [7]. The Applicant proposed no minimum or maximum *L-arginine* concentrations in *feedingstuffs* [1,7].

## 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 *L-arginine produced by fermentation with Corynebacterium glutamicum (KCTC 10423BP)* 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*

#### *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, mercury, aflatoxin B1 and dioxins) are available from the respective European Union Reference Laboratories [8].

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

For the quantification of *L-arginine* in *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the Community method [9-10]. This method applies for the

determination of *free* and of *total* (peptide-bound and free) amino acids, using an amino acid analyzer or High Performance Liquid Chromatography equipped with ion exchange column. Intended for *premixtures* and *feedingstuffs*, it does not distinguish between the salts and the amino acid enantiomers.

The *free* amino acids are extracted with diluted hydrochloric acid. Co-extracted nitrogenous macromolecules are precipitated with sulfosalicylic acid and removed by filtration. The solution is filtered and adjusted to pH 2.2. The amino acids are separated by ion exchange chromatography (IEC) and *free arginine* is determined after post column derivatisation with ninhydrin by spectrophotometric detection at 570 nm (Visible – VIS).

For the quantification of the *total arginine*, the sample is hydrolysed with hydrochloric acid (6 mol/l) containing 1g phenol/l for 23 hours. The hydrolysate is adjusted to pH 2.2. The amino acids are separated by IEC and *total arginine* is determined after post column derivatisation with ninhydrin by spectrophotometric detection at 570 nm.

The Community method was further ring-trial validated by twenty-three laboratories for the determination of total *arginine* and resulted in the standard method EN ISO 13903:2005 [11]. The reported performance characteristics are listed in Table 1.

Even if not explicitly described in the scope of the Community method, the EURL, followed the advice of several experienced National Reference Laboratories (NRLs), recommends the use of the above described Community method (equivalent to the EN ISO 13903:2005) for the quantification of *arginine* in the *feed additive* [12-16].

Based on the performance characteristics available, the EURL recommends for official control the ring-trial validated Community method (equivalent to the EN ISO 13903:2005), based on IEC-VIS to quantify *arginine* in *premixtures* and *feedingstuffs*.

**Table 1:** Method performance characteristics reported in EN ISO 13903:2005 for the determination of total *arginine* [11]

Matrix	Arginine (g/kg)	RSD <sub>r</sub> (%)	RSD <sub>R</sub> (%)
poultry meal	43.5	3.3	9.7
broiler finisher feed	12.8	2.3	8.6
broiler starter feed	15.7	2.7	8.2
corn	4	3.3	9.5
fishmeal	34	3.0	7.2

RSD<sub>r</sub> and RSD<sub>R</sub> - relative standard deviation for *repeatability* and *reproducibility*, respectively

Furthermore, for the characterisation of *arginine* in the *feed additive*, the EURL recommends the "*L-arginine* monograph" of the Food Chemical Codex (FCC), where identification is based on infrared absorption in combination with the analysis of the optical rotation, while quantification is based on titration with perchloric acid (0.1N) [6].

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 FCC method for the characterisation of *L-arginine* in the *feed additive* based on infrared absorption and optical rotation; and
- the Community method based on ion exchange chromatography coupled with post-column derivatisation and photometric detection (IEC-VIS), to quantify *arginine* in *feed additive*, *premixtures* and *feedingstuffs*.

##### ***Recommended text for the register entry (analytical method)***

For the characterisation of *L-arginine* in *feed additive*:

- Food Chemical Codex "L-arginine monograph"

For the quantification of *arginine* in *feed additive*:

- ion exchange chromatography coupled with post-column derivatisation and photometric detection (IEC-VIS)

For the quantification of *arginine* in *premixtures* and *feedingstuffs*:

- ion exchange chromatography coupled with post-column derivatisation and photometric detection (IEC-VIS) – Commission Regulation (EC) No 152/2009

#### **5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL**

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *L-arginine* 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] \*Application, Proposal of Registry Entry – Annex A
- [2] \*Application, Reference SANCO/G1: Forw. Appl. 1831/0018-2014
- [3] \*Technical dossier, Section II: 2.1.2 Proposal for classification
- [4] Commission Regulation (EC) No 1139/2007 of 1 October 2007 concerning the authorisation of L-arginine as a feed additive, O.J. L 256/11, 02.10.2007
- [5] \*Technical dossier, Section II: 2.1.3 Qualitative and quantitative composition
- [6] Food Chemical Codex monograph "*L-Arginine*", FCC 7 (2010), p. 69
- [7] \*Technical dossier, Section II: 2.5.1 Proposed mode of use in animal nutrition
- [8] 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
- [9] \*Technical dossier, Section II: 2.6.1 Methods of analysis for the active substance
- [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, O.J. L 54, 26.02.2009 (Annex III, F)
- [11] EN ISO 13903:2005 - *Animal feeding stuffs – Determination of amino acids content*
- [12] FAD-2007-0015, L-valine, Ref. D08/FSQ/CvH/GS/D2008(5731) – 28/02/2008  
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- [16] FAD-2014-0015, L-valine produced by *Escherichia coli* K-12 (NITE BP-01755), Ref. JRC.D.5/SFB/CvH/SB/mds/ Ares(2014)2983094 - 11/09/2014  
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\*Refers to Dossier no: FAD-2014-0012

## 7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European Union 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.



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## 8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft, Freistaat Sachsen, Nossen<sup>1</sup> (DE)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen, Jena (DE)

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