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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-valine** produced by  
Corynebacterium glutamicum CGMCC 11675  
(*FAD-2016-0031; CRL/160003*)





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Dossier related to: **FAD-2016-0031 - CRL/160003**

Feed Additive: ***L-valine*** produced by *Corynebacterium glutamicum* CGMCC 11675

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

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

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Date: **16/11/2016**

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Date: **16/11/2016**

## EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *L-valine produced by Corynebacterium glutamicum CGMCC 11675*, 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-valine* is already authorised as *feed additive* under Commission Implementing Regulation (EU) 2015/1114 amending Regulation (EC) No 403/2009 and Implementing Regulation (EU) No 848/2014 and (EU) 1236/2014.

For the quantification of *L-valine* in *feed additive*, *premixtures*, *feedingstuffs* and *water* the Applicant submitted the ring-trial validated Community method (Commission Regulation (EC) No 152/2009). The method was further ring-trial validated by CEN 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 is designed for *feedingstuffs* and *premixtures*. The following performance characteristics were reported for the quantification of total *valine*: a relative standard deviation for *repeatability* (RSD<sub>r</sub>) ranging from 1.7 to 3.8 % and a relative standard deviation for *reproducibility* (RSD<sub>R</sub>) ranging from 8.8 to 16.1 %.

In addition, the EURL identified the "L-arginine monograph" of the Food Chemical Codex (FCC) for the characterisation of the *feed additive*. Since the Applicant provided no experimental data to determine *valine* in *water* the EURL is neither able to evaluate nor to recommend a method for official control to determine *valine* in *water*.

Based on the performance characteristics available, the EURL recommends for official control the Community method (equivalent to the EN ISO 13903:2005 method) based on IEC-VIS for the quantification of *valine* in the *feed additive*, *premixtures* and *feedingstuffs* together with the "L-valine 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-valine produced by Corynebacterium glutamicum CGMCC 11675, 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-valine produced by Corynebacterium glutamicum CGMCC 11675*, 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-valine* is already authorised as *feed additive* under Commission Implementing Regulation (EU) 2015/1114 amending Regulation (EC) No 403/2009 and Implementing Regulation (EU) No 848/2014 and (EU) 1236/2014 [3].

According to the Applicant, the product is a white powder with a minimum purity of 98.5 % [4]. The *feed additive* is produced by fermentation with a genetically modified strain of *Corynebacterium glutamicum*. The production strain is deposited in the "Chinese General Microbiological Culture Collection Centre" (CGMCCC) with reference *Corynebacterium glutamicum* CGMCCC 11675 [5]. *L-valine* is intended to be mixed either in *premixtures* or added directly to *feedingstuffs* or *water* for drinking [6]. The Applicant did not propose minimum or maximum *L-valine* content in *feedingstuffs* [1,6].

Note: The EURL has previously evaluated the analytical methods in the frame of five *valine* related dossiers [7-11].

## 2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761, 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-valine* 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 [12].

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

For the characterisation of *L-valine* in the *feed additive*, the EURL found the "L-valine monograph" of the Food Chemical Codex (FCC), where identification is based on infrared absorption in combination with the analysis of the optical rotation (ranging from +26.6° to +29.0°), while quantification is based on titration with perchloric acid (0.1 N) [13].

For the quantification of *L-valine* in the *feed additive, premixtures* and *feedingstuffs* the Applicant submitted a ring-trial validated Community method [14-15]. This method applies for the determination of *free* (synthetic and natural) and of *total* (peptide-bound and free) amino acids, using an amino acid analyzer or a High Performance Liquid Chromatograph equipped with an ion exchange (IE) column. Intended for *premixtures* and *feedingstuffs*, it does not distinguish between the salts of amino acids and it cannot differentiate 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 valine* is determined after post-column derivatisation with ninhydrin by spectrophotometric detection at 570 nm (Visible – VIS).

The procedure chosen for the determination of the total amino acids depends on the amino acids under investigation. *L-valine* can be determined in either oxidised or unoxidised samples. Oxidation is performed at 0 °C with a performic acid/phenol mixture. The excess of the oxidation reagent is decomposed with sodium disulfite. The oxidised or unoxidised sample is hydrolysed with hydrochloric acid (6 mol/l) containing 1 g phenol/l for 23 hours. The hydrolysate is adjusted to pH 2.2. The amino acids are separated by IEC and *total valine* is determined by post-column derivatisation with ninhydrin and photometric detection at 570 nm. The Community method was further ring-trial validated by twenty-three laboratories for the determination of total *valine* and resulted in the standard method EN ISO 13903:2005 [16]. The reported performance characteristics are listed in Table 1.

**Table 1:** Method performance characteristics reported in EN ISO 13903:2005 for the determination of total *valine* [16]

Matrix	valine content (g/kg)	RSD <sub>r</sub> (%)	RSD <sub>R</sub> (%)
poultry meal	28.2	3.2	12.8
broiler finisher feed	9.2	3.8	12.7
broiler starter feed	11.1	1.7	8.8
corn	3.8	2.4	16.1
fishmeal	27.8	2.3	11.2

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

Even if not explicitly stated in the scope of the Community method, the EURL follows the advice of several experienced National Reference Laboratories (NRLs) and recommends the use of this Community method for the quantification of *valine* in the *feed additive* [11].

However, since the Applicant provided no experimental data to demonstrate the applicability of the Community method for the determination of *valine* in *water* the EURL is neither able to evaluate nor to recommend a method for official control to determine *valine* in *water*.

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 method) based on IEC-VIS to quantify *valine* in *feed additive*, *premixtures* and *feedingstuffs*.

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 Food Chemical Codex "L-valine" monograph based on infrared absorption and optical rotation to identify *L-valine* in the *feed additive*; and the ring-trial validated Community method, using ion exchange chromatography (IEC) coupled with post-column derivatisation and photometric detection, to quantify *valine* in *feed additive*, *premixtures* and *feedingstuffs*.

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

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

- Food Chemical Codex "L-valine monograph"

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

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

For the quantification of *valine* 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-valine produced by fermentation with Corynebacterium glutamicum CGMCCC 11675*" 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. 1831/0031-2016
  - [3] Commission Implementing Regulation (EU) 2015/1114 of 9 July 2015 concerning the authorisation of L-valine produced by *Escherichia coli* as a feed additive for all animal species and amending Regulation (EC) No 403/2009 and Implementing Regulations (EU) No 848/2014 and (EU) No 1236/2014, O.J. L 182/18, 10.07.2015
  - [4] \*Application: Annex 1
  - [5] \*Technical dossier, Section II: 2.2.1.2 Micro-organisms
  - [6] \*Technical dossier, Section II: 2.5.1 Proposed mode of use in animal nutrition
  - [7] FAD-2007-0015, L-valine, Ref. D08/FSQ/CvH/GS/D2008(5731) – 28/02/2008  
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  - [12] 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
  - [13] Food Chemical Codex monograph "*L-Valine*", FCC 7 (2010), p. 1072
  - [14] \*Technical dossier, Section II: II.6.1 Methods of analysis for the active substance
  - [15] 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)
  - [16] EN ISO 13903:2005 - Animal feeding stuffs – Determination of amino acids content
- \*Refers to Dossier no: FAD-2016-0031

## 7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the European Union Reference Laboratory for Feed Additives, JRC, 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 (EU) 2015/1761.



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## 8. ACKNOWLEDGEMENTS

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