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

JRC F.5/CvH/SB/BK/Ares

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 fermentation with
Corynebacterium glutamicum (KCCM11201P)
(FAD-2017-0032; CRL/170022)

European Union Reference Laboratory
Feed Additives

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-2017-0032 - CRL/170022**
Name of Product:	***L-valine produced by fermentation with Corynebacterium glutamicum (KCCM11201P)***
Active Agent (s):	**L-valine**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
Report prepared by:	**Stefano Bellorini**
Report checked by: Date:	**Zigmas Ezerskis (EURL-FA)** **09/03/2018**
Report approved by: Date:	**Christoph von Holst** **09/03/2018**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *L-valine* produced by fermentation with *Corynebacterium glutamicum KCCM11201P*, 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 nutritional *feed additive* under Commission Regulation (EC) No 403/2009 as last amended by Commission Implementing Regulation (EU) 2015/1114.

For the characterisation of the *feed additive*, the EURL identified the "L-valine monograph" of the Food Chemical Codex (FCC) where identification is based on infrared absorption.

For the quantification of *L-valine* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated Community method based on ion exchange chromatography coupled with post-column derivatisation and photometric detection (IEC-VIS). The method does not distinguish between the salts of amino acids and it cannot differentiate between enantiomers. The following performance characteristics were reported for the quantification of total *valine* in feed: a relative standard deviation for *repeatability* (RSD_r) ranging from 1.7 to 3.8 % and a relative standard deviation for *reproducibility* (RSD_R) ranging from 8.8 to 16.1 %.

For the quantification of *L-valine* in *water* the Applicant did not submit any suitable method. However, in the frame of the stability studies, the Applicant presented experimental data obtained when analysing *valine* with the VDLUFA official method (4.11.6) based on IEC coupled with post-column derivatisation and optical detection (IEC-VIS/FD). The results are considered sufficient to demonstrate the suitability of the procedure for the analysis of the amino acid in *water*. Furthermore, according to the experience of NRLs, other similar ring trial validated methods designed for the analysis of amino acids and based on similar analytical procedures are fit for purpose

Based on the performance characteristics available, the EURL recommends for official control: (i) the "*L-valine* monograph" of the FCC based on infrared absorption for the identification of *L-valine* in the *feed additive*; (ii) the Community method based on IEC-VIS for the quantification of *valine* in the *feed additive*, *premixtures* and *feedingstuffs*; and (iii) the IEC-VIS/FD method based on the procedure described by VDLUFA (4.11.6) or equivalent to quantify *valine* in *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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

KEYWORDS

L-valine produced by fermentation with *Corynebacterium glutamicum (KCCM11201P)*, nutritional additives, amino acids, their salts and analogues, all animal species

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (new *feed additive*) for *L-valine* produced by fermentation with *Corynebacterium glutamicum KCCM11201P*, 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 [1,2]. Authorisation is sought for all animal species. *L-valine*, produced by different microorganisms, is already authorised as nutritional feed additive under Commission Regulation (EC) No 403/2009 as last amended by Commission Implementing Regulation (EU) 2015/1114 [3,4].

According to the Applicant, *L-valine* is a white crystalline powder with a minimum purity of 98 % [5,6]. The *feed additive* is produced by fermentation with a specific strain derived from *Corynebacterium glutamicum* [5]. The production strain is registered in the "Korean Culture Collection of Microorganisms" (KCCM) with deposition number KCCM11201P [7].

L-valine is intended to be mixed either in *premixtures* or added directly to *feedingstuffs* or *water* for drinking [8]. The Applicant did not propose a minimum or maximum *L-valine* content in *feedingstuffs* [1].

Note: The EURL has previously evaluated the analytical methods for the determination of *L-valine* in the frame of six dossiers [9-14].

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* produced by fermentation with *Corynebacterium glutamicum KCCM11201P* 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 [15].

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

The EURL found the "*L-valine* monograph" of the Food Chemical Codex (FCC) for the characterisation of *L-valine* in the *feed additive*, where identification is based on infrared absorption while quantification is based on titration with perchloric acid [16].

For the quantification of *L-valine* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated Community method [17-18]. 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 High Performance Liquid Chromatography equipment provided with an ion exchange (IE) column. The method is 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 non-oxidised 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 non-oxidised 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 [19]. 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* [19]

Matrix	*valine content* (g/kg)	RSD_r (%)	RSD_R (%)
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_r and RSD_R - relative standard deviation for *repeatability* and *reproducibility,* respectively.

Based on the performance characteristics available, the EURL recommends for official control the ring-trial validated Community method based on IEC-VIS to quantify *valine* in *premixtures* and *feedingstuffs*.

Furthermore, 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 the procedure described in the Community method also for the quantification of *valine* in the *feed additive* [14].

For the determination of *L-valine* in *water* the Applicant did not submit any suitable method [17]. However, in the frame of the stability studies, the Applicant presented experimental data obtained when analysing *valine* with the VDLUFA official method which is actually designed for the analysis of lysine, methionine and threonine in *feed* [20-22]. This method is based on IEC coupled with post-column derivatisation and optical detection (VIS or fluorescence detection - FD). The results presented are considered sufficient to demonstrate the suitability of the procedure for the analysis of the amino acid in *water*. Furthermore, according to the experience of NRLs, other similar ring trial validated methods designed for the analysis of amino acids and based on similar analytical procedures are fit for purpose [18-19,23]. Hence, the EURL recommends this method or equivalent for official control for the determination of *L-valine* in *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 as last amended by Regulation (EU) 2015/1761) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control (i) the "*L-valine* monograph" of the Food Chemical Codex (FCC) based on infrared absorption for the identification of *L-valine* in the *feed additive*; (ii) the Community method based on IEC-VIS

for the quantification of *valine* in the *feed additive*, *premixtures* and *feedingstuffs*; and (iii) the analytical method described by VDLUFA (4.11.6) or equivalent based on IEC-VIS/FD to quantify *valine* in *water*.

Recommended text for the register entry (analytical method)

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

- Food Chemical Codex "L-valine monograph"

For the quantification of *valine* in the *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 (Annex III, F)

For the quantification of *valine* in *water*:

- ion exchange chromatography coupled with post-column derivatisation and optical detection (IEC-VIS/FD)

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 KCCM11201P* 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 SANTE/E5: FORW. APPL. 1831-0022-2017

[3] Commission Regulation (EC) No 403/2009 of 14 May 2009 concerning the authorisation of a preparation of L-valine as a feed additive, OJ L 120, 15.5.2009

[4] 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, OJ L 182, 10.7.2015

[5] *Technical dossier, Section II: II.1 Identity of the additive

[6] *Technical dossier, Section II: II.2.1.1 Chemical substances

[7] *Technical dossier, Section II: II.2.2.2 Microorganism

[8] *Technical dossier, Section II: II.5.1 Proposed mode of use in animal nutrition

[9] FAD-2007-0015, L-valine, Ref. D08/FSQ/CvH/GS/D2008(5731) – 28/02/2008 https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2007-0015.pdf

[10] FAD-2012-0023, L-valine, Ref. JRC.D.5/SFB/CvH/SB/mds/Ares(2013)181751 – 12/02/2013 https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2012-0023-L-Valine.doc.pdf

[11] FAD-2012-0028, L-valine, Ref. JRC.D.5/SFB/CvH/SB/mds/Ares(2013)998581 - 02/05/2013 https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2012-0028-L-Valine.doc_.pdf

[12] FAD-2011-0053, L-valine, Ref. JRC.D.5/SFB/CvH/SB/mds/Ares(2013)2914464 - 21/08/2013 https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2011-0053-L-Valine.doc_.pdf

[13] FAD-2014-0015, L-valine, Ref. Ares(2014)2983094 - 11/09/2014 https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2014-0015-l_valine.pdf

[14] FAD-2016-0031, L-valine produced by Corynebacterium glutamicum CGMCC 11675 Ref. Ares(2016)6444701 - 16/11/2016 https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2016-0031_valine.pdf

[15] 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

[16] Food Chemical Codex monograph "L-valine", FCC 7 (2010), p. 1072

[17] *Technical dossier, Section II: II.6.1 Methods of analysis and reference samples

[18] 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)

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

[20] VDLUFA MB III 4.11.6 Bestimmung von Lysin, Methionin und Threonin in Aminosäurenhandelsprodukten und Vormischungen

[21] *Technical dossier, Section II: II.4.1.3 Stability of the additive used in water

[22] * Technical dossier, Section II: Annex_II_4_05

[23] EN ISO 17180:2013 - Animal feeding stuffs – Determination of lysine, methionine and threonine in commercial amino acid products and premixtures

*Refers to Dossier no: FAD-2017-0032

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.

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft. Geschäftsbereich 6 — Labore Landwirtschaft, Nossen (DE)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Fødevarestyrelsens Laboratorie Aarhus (kemisk) (DK)
- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)
- Thüringer Landesanstalt für Landwirtschaft (TLL). Abteilung Untersuchungswesen. Jena (DE)