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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 fermentation with
Corynebacterium glutamicum CGMCC 7.358
(FAD-2019-0072; CRL/190041)

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-2019-0072 - CRL/190041**
Name of Product / Feed Additive:	***L-valine produced by fermentation with Corynebacterium glutamicum CGMCC 7.358***
Active Agent (s):	**L-valine**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
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Report checked by: Date:	**Stefano Bellorini** **17/04/2020**
Report approved by: Date:	**Christoph von Holst** **20/04/2020**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4(1) for *L-valine produced by fermentation with Corynebacterium glutamicum CGMCC 7.358*, under the category/functional groups 3(c) 'nutritional additives'/'amino acids, their salts and analogues', according to Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisation is sought for all animal species.

According to the Applicant, *L-valine* has a minimum purity (mass fraction) of 98 %. The *feed additive* is intended to be mixed either in *premixtures* or added directly to *feedingstuffs* or *water* for drinking. However, the Applicant did not propose any minimum or maximum content of *L-valine* in *feedingstuffs*.

For the characterisation of the *feed additive*, the EURL found 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*, *feedingstuffs* and *water* the Applicant submitted the ring-trial validated European Union 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 %. In addition, in the frame of the batch-to-batch analysis and for the stability studies of *L-valine* in the *feed additive* and *water*, the Applicant presented acceptable experimental data when applying the above mentioned European Union method.

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*, and (ii) the ring-trial validated European Union method based on IEC-VIS for the quantification of *valine* in the *feed additive*, *premixtures*, *feedingstuffs* and *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 CGMCC 7.358, nutritional additives, amino acids, all animal species

1. BACKGROUND

In the current application an authorisation is sought under Article 4(1) (authorisation of a new feed additive) for *L-valine produced by fermentation with Corynebacterium glutamicum CGMCC 7.358*, under the category/functional groups 3(c) 'nutritional additives'/'amino acids, their salts and analogues', according to Annex I of Regulation (EC) No 1831/2003 [1]. Specifically, the authorisation is sought for all animal species [1,2].

L-valine, produced by different microorganisms, is already authorised as nutritional *feed additive* under Commission Implementing Regulations, namely (EU) 2015/1114 [3] and (EU) 2019/1289 [4].

According to the Applicant, *L-valine* has a minimum purity (mass fraction) of 98 % [2,5]. The *feed additive* is produced by fermentation using a non-genetically modified strain of *Corynebacterium glutamicum CGMCC 7.358* [5], which is deposited in the "China General Microbiological Culture Collection Center (CGMCC)" under the deposition number 7.358 [5].

The *feed additive* is intended to be mixed either in *premixtures* or added directly to *feedingstuffs* or *water* for drinking [5]. However the Applicant did not propose any minimum or maximum content of *L-valine* in *feedingstuffs* [2,5].

Note: The EURL has previously evaluated the analytical methods for the determination of *L-valine* in the frame of several dossiers [6-14].

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* and their suitability to be used for official controls in the frame of the authorisation were evaluated.

3. EVALUATION

Description of the analytical methods for the determination of the active substance in the feed additive, premixtures, feedingstuffs and when appropriate water (section 2.6.1 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the quantification of *L-valine* in the *feed additive* the Applicant proposed [5] the EN ISO 17180 method without submitting corresponding experimental proofs of the extension of scope, while for the quantification *L-valine* in *premixtures*, *feedingstuffs* and *water* the Applicant submitted the ring-trial validated European Union method [15].

The European Union method applies for the determination of free (synthetic and natural) and of total (peptide-bound and free) amino acids, using an amino acid analyser 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 [15].

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 determined by post-column derivatisation with ninhydrin and photometric 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. The oxidation is performed at 0 °C with a performic acid/phenol mixture. An excess of oxidation reagent is decomposed with sodium disulfite. The oxidised or non-oxidised sample is hydrolysed with hydrochloric acid (6 mol/l) for 23 hours. The hydrolysate is adjusted to pH 2.2. The amino acids are separated by IEC and determined by post-column derivatisation with ninhydrin and photometric detection at 570 nm [15].

The European Union method was ring-trial validated using four different matrices for threonine, cyst(e)ine, methionine and lysine only [15]. This method was further ring-trial validated by twenty-three laboratories, resulting in the EN ISO 13903 method (*valine* included) [16]. The performance characteristics reported for the quantification of total *L-valine* in feed are listed in Table 1.

In addition, in the frame of the batch-to-batch analysis and for the stability studies of *L-valine* in the *feed additive* and *water*, the Applicant presented experimental data [5] when applying the above mentioned European Union method [15].

Table 1: Method performance characteristics obtained in the frame of ring-trial validation of EN ISO 13903 method for the quantification of total *valine* in *feed*

Ring-Trial	Matrix	*valine* content g/kg	RSD_r %	RSD_R %
[16]	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, RSD_R - relative standard deviation for *repeatability* and *reproducibility,* respectively

Furthermore, even if not explicitly stated in the scope of the European Union method, the EURL follows the advice of several experienced National Reference Laboratories (NRLs) and recommends the use of the procedure based on the European Union method also for the quantification of *valine* in the *feed additive* [13,14]. Based on the available data, the EURL recommends for official control the ring-trial validated European Union method based on IEC-VIS to quantify *L-valine* in the *feed additive*, *premixtures*, *feedingstuffs* and *water*.

Methods of analysis for the determination of the residues of the additive in food (section 2.6.2 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

An evaluation of corresponding methods of analysis is not relevant for the present application.

Identification/Characterisation of the feed additive (section 2.6.3 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

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 [17].

The EURL recommends for official control the Food Chemical Codex for the identification of *L-valine* in 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, 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*, and (ii) the European Union method based on IEC-VIS for the quantification of *valine* in the *feed additive*, *premixtures*, *feedingstuffs* and *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* and *water*:

– 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)

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 CGMCC 7.358* 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, Reference SANTE/E5: Forw. Appl. 1831-0074-2019 & Annex I – submission number 1571923010445-2470

[2] *Application, Proposal of Registry Entry – Annex A

[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, OJ L 182, 10.7.2015

[4] Commission Implementing Regulation (EU) 2019/1289 of 31 July 2019 concerning the authorisation of L-valine produced by Corynebacterium glutamicum KCCM 11201P as a feed additive for all animal species, OJ L 203, 01.08.2019

[5] *Technical dossier, Section II: Identify, characterisation and conditions of use of the additive; methods of analysis

[6] FAD-2007-0032, L-valine produced by fermentation with Corynebacterium glutamicum (KCCM11201P), Ref. Ares(2018)1317378 - 09/03/2018 https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2017-0032-lvaline.pdf

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[12] 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

[13] FAD-2017-0032, L-valine produced by fermentation with Corynebacterium glutamicum (KCCM11201P), Ref. Ares(2018)1317378 - 09/03/2018 https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2017-0032-lvaline.pdf

[14] FAD-2018-0066, L-valine produced by fermentation with Escherichia coli K12 KCCM 80159 (C001), Ref. Ares(2019)540948 - 30/01/2019 https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2018-0066_l-valine.pdf

[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

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

*Refers to Dossier no: FAD-2019-0072

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

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- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Wageningen Food Safety Research[1] (WFSR) (NL)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft. Geschäftsbereich 6 — Labore Landwirtschaft, Nossen (DE)
- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)
- Instytut Zootechniki — Państwowy Instytut Badawczy, Krajowe Laboratorium Pasz, Lublin (PL)

[1] Name and address according to according COMMISSION IMPLEMENTING REGULATION (EU) 2015/1761: RIKILT Wageningen UR, Wageningen.