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JRC F.5/CvH/ZE/AS/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*
CGMCC 18932
(FEED-2021-1685; CRL/210044)**



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Dossier related to: **FEED-2021-1685 - CRL/210044**

Name of Product: ***L-Valine* produced by fermentation with
Corynebacterium glutamicum CGMCC
18932**

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

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

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Date: **26/09/2022**

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Date: **26/09/2022**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4 for *L-valine* produced by fermentation with *Corynebacterium glutamicum* CGMCC 18932, 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 98.5 %. The *feed additive* is produced by fermentation with *Corynebacterium glutamicum* CGMCC 18932.

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 Food Chemical Codex (FCC), where different tests (including the test based on an optical rotation) are used for the identification of *L-valine*.

For the quantification of *valine* in the *feed additive*, *premixtures*, *feedingstuffs* and *water* the Applicant proposed two equivalent ring-trial validated methods, namely EN ISO 13903 method (only for the *feed additive*) and the European Union (EU) method for all the matrices.

The EU method is applicable for the determination of free (synthetic and natural) and total (peptide-bound and free) amino acids, using an amino acid analyser or High Performance Liquid Chromatography equipment provided with an ion-exchange column (IEC). The method is intended for *premixtures* and *feedingstuffs*, it does not distinguish between the amino acids and their salts, or between different salts of the same amino acids, and it cannot differentiate between enantiomers. The following performance characteristics were reported in the frame of the ring-trial validation studies for the quantification of total *valine* in *feedingstuffs*: 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, the Applicant performed the analysis of *valine* in the *feed additive*, *premixtures*, *feedingstuffs* and *water* in the frame of stability and homogeneity studies by using the above mentioned EU method and demonstrating the applicability of the method for all the mentioned matrices.

Furthermore, the EURL recently recommended in the frame of several dossiers (FAD-2019-0072, FAD-2020-0033 and FAD-2021-0032) the EU method for the determination of *valine* in very similar *feed additives* and *water*. Moreover, in the frame of *L-valine* dossiers (FAD-2017-0032 and FAD-2021-0032) the EURL previously recommended

VDLUFA official method (4.11.6) or equivalent method based on ion-exchange chromatography coupled with post-column derivatisation and optical (visible or fluorescence) detection (IEC-VIS/FLD) for the quantification of *valine* in *water*. The EURL considers that these recommendations are also valid in the frame of the current application.

Based on the available data, the EURL recommends for official control (i) the ring-trial validated European Union method based on IEC-VIS to quantify *valine* in the *feed additive*, *premixtures*, *feedingstuffs* and *water*; and (ii) the analytical method described by VDLUFA (4.11.6) or equivalent method based on IEC-VIS/FLD 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* CGMCC 18932, 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 18932, 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 [1,2]. Specifically, the authorisation is sought for all animal species [2].

L-valine, produced by different microorganisms, is already authorised as nutritional *feed additive* under several Commission Implementing Regulations [3-7].

According to the Applicant, *L-valine* has a minimum purity (mass fraction) of 98.5 % [8]. The *feed additive* is produced by fermentation with *Corynebacterium glutamicum* CGMCC 18932 [9].

The *feed additive* is intended to be mixed either in *premixtures* or added directly to *feedingstuffs* or *water* for drinking [10]. However, the Applicant did not propose any minimum or maximum content of *L-valine* in *feedingstuffs* [2,10].

Note: The EURL has previously evaluated the analytical methods for the determination of *L-valine* in the frame of several dossiers [11-21].

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 *valine* in the *feed additive, premixtures, feedingstuffs* and *water* the Applicant proposed [22] two equivalent methods, namely EN ISO 13903 method [23] (only for the *feed additive*) and the European Union (EU) method [24] for all the matrices.

The EU method is applicable for the determination of free (synthetic and natural) and total (peptide-bound and free) amino acids, using an amino acid analyser or High Performance Liquid Chromatography equipment provided with an ion-exchange column (IEC). The method is intended for *premixtures* and *feedingstuffs*, it does not distinguish between the amino acids and their salts, or between different salts of the same amino acids, and it cannot differentiate between enantiomers [24].

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 procedure chosen for the determination of the *total* amino acids depends on the amino acids under investigation. *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 h. The hydrolysate is adjusted to pH 2.2. The amino acids are separated by IEC and determined by post-column derivatisation with ninhydrin and optical detection at 570 nm [24].

Table 1: The performance characteristics obtained in the frame of the ring-trial validated EN ISO 13903 method for the quantification of total *valine* in *feedingstuffs*

Ring-Trial	Matrix	<i>valine</i> content g/kg	RSD _r %	RSD _R %
[23]	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.

The EU method was ring-trial validated using four different matrices for threonine, cyst(e)ine, methionine and lysine [24]. This method was further ring-trial validated by 23 laboratories, resulting in the above mentioned EN ISO 13903 method [23]. The performance characteristics reported for the quantification of total *valine* in feed are listed in Table 1.

In addition, the Applicant performed the analysis of *valine* in the *feed additive*, *premixtures*, *feedingstuffs* and *water* in the frame of stability and homogeneity studies by using the above mentioned EU method demonstrating the applicability of the method for all the mentioned matrices [25,26].

Furthermore, the EURL recently recommended in the frame of several dossiers [19-21] the EU method for the determination of *valine* in very similar *feed additives* and *water*. Moreover, in the frame of *L-valine* dossiers [17,21] the EURL previously recommended VDLUFA official method (4.11.6) [27] or equivalent method based on ion-exchange chromatography coupled with post-column derivatisation and optical (visible or fluorescence) detection (IEC-VIS/FLD) for the quantification of *valine* in *water*. The EURL considers that these recommendations are also valid in the frame of the current application.

Based on the available data, the EURL recommends for official control (i) the ring-trial validated European Union method based on IEC-VIS to quantify *valine* in the *feed additive*, *premixtures*, *feedingstuffs* and *water*; and (ii) the analytical method described by VDLUFA (4.11.6) or equivalent method based on IEC-VIS/FLD to quantify *valine* in *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), where different tests (including the test based on an optical rotation) are used for the identification of

L-valine in the *feed additive* [28]. The EURL recommends for official control the approach of 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) for the identification of *L-valine* in the *feed additive*; (ii) the European Union method based on IEC-VIS for the quantification of *valine* in the *feed additive*, *premixtures*, *feedingstuffs* and *water*; and (iii) the analytical method described by VDLUFA (4.11.6) or equivalent method based on IEC-VIS/FLD 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 optical detection (IEC-VIS)

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

- Ion-exchange chromatography coupled with post-column derivatisation and optical 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 or IEC-VIS/FLD)

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 18932 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] *Forwarding of applications for authorisation of feed additives in accordance with Regulation (EC) No 1831/2003 – E-Submission Food Chain platform –
<https://webgate.ec.europa.eu/esfc/#/applications/2452>
<https://open.efsa.europa.eu/questions/EFSA-Q-2021-00566>
- [2] *Application, Annex 1
- [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] Commission Implementing Regulation (EU) 2020/1797 of 30 November 2020 concerning the authorisation of L-valine produced by *Escherichia coli* KCCM 80159 as a feed additive for all animal species, OJ L 402, 1.12.2020
- [6] Commission Implementing Regulation (EU) 2021/719 of 30 April 2021 concerning the authorisation of L-valine produced by *Corynebacterium glutamicum* CGMCC7.358 as a feed additive for all animal species, OJ L 151, 3.5.2021
- [7] Commission Implementing Regulation (EU) 2021/2077 of 26 November 2021 concerning the authorisation of L-valine produced by *Corynebacterium glutamicum* CGMCC 7.366 as a feed additive for all animal species, OJ L 426, 29.11.2021
- [8] *Technical dossier, Section II: 2.1.3. Qualitative and quantitative composition (active substance/agent, other components, impurities, batch to batch variation)
- [9] *Technical dossier, Section II: 2.2.2.2 Microorganisms
- [10] *Technical dossier, Section II: 2.5.1. Proposed mode of use in animal nutrition
- [11] FAD-2007-0015, L-valine, Ref. D08/FSQ/CvH/GS/D2008(5731) – 28/02/2008
https://joint-research-centre.ec.europa.eu/publications/fad-2007-0015_en
- [12] FAD-2012-0023, L-valine, Ref. JRC.D.5/SFB/CvH/SB/mds/Ares(2013)181751 – 12/02/2013
https://joint-research-centre.ec.europa.eu/publications/fad-2012-0023_en
- [13] FAD-2012-0028, L-valine, Ref. JRC.D.5/SFB/CvH/SB/mds/Ares(2013)998581 – 02/05/2013
https://joint-research-centre.ec.europa.eu/publications/fad-2012-0028_en
- [14] FAD-2011-0053, L-valine, Ref. JRC.D.5/SFB/CvH/SB/mds/Ares(2013)2914464 – 21/08/2013
https://joint-research-centre.ec.europa.eu/publications/fad-2011-0053_en
- [15] FAD-2014-0015, L-valine, Ref. Ares(2014)2983094 – 11/09/2014
https://joint-research-centre.ec.europa.eu/publications/fad-2014-0015_en

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- [16] FAD-2016-0031, L-valine produced by *Corynebacterium glutamicum* CGMCC 11675, Ref. Ares(2016)6444701 – 16/11/2016
https://joint-research-centre.ec.europa.eu/publications/fad-2016-0031_en
- [17] FAD-2017-0032, L-valine produced by fermentation with *Corynebacterium glutamicum* (KCCM11201P), Ref. Ares(2018)1317378 – 09/03/2018
https://joint-research-centre.ec.europa.eu/publications/fad-2017-0032_en
- [18] FAD-2018-0066, L-valine produced by fermentation with *Escherichia coli* K12 KCCM 80159 (C001), Ref. Ares(2019)540948 – 30/01/2019
https://joint-research-centre.ec.europa.eu/publications/fad-2018-0066_en
- [19] FAD-2019-0072, L-valine produced by fermentation with *Corynebacterium glutamicum* CGMCC 7.358, Ref. Ares(2020)2131128 – 20/04/2020
https://joint-research-centre.ec.europa.eu/publications/fad-2019-0072_en
- [20] FAD-2020-0033, L-valine produced by fermentation with *Corynebacterium glutamicum* CGMCC 7.366, Ref. Ares(2020)4619250 – 04/09/2020
https://joint-research-centre.ec.europa.eu/publications/fad-2020-0033_en
- [21] FAD-2021-0032 L-valine produced by fermentation with *Escherichia coli* CCTCC M2020321, Ref. Ares(2021)4558248 - 14/07/2021
https://joint-research-centre.ec.europa.eu/publications/fad-2021-0032_en
- [22] *Technical dossier, Section II: 2.6.1. Methods of analysis for the active substance
- [23] EN ISO 13903:2005 - Animal feeding stuffs – Determination of amino acids content
- [24] 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)
- [25] *Technical dossier, Section II: 2.4.1. Stability
- [26] *Technical dossier, Section II: 2.4.1. Homogeneity
- [27] VDLUFA MB III 4.11.6 Bestimmung von Lysin, Methionin und Threonin in Aminosäurehandelsprodukten und Vormischungen
- [28] Food Chemical Codex monograph "L-valine", FCC 7 (2010), p. 1072
- *Refers to Dossier no: FEED-2021-1685

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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- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
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- Thüringer Landesanstalt für Landwirtschaft (TLL). Abteilung Untersuchungenwesen. Jena (DE)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft. Geschäftsbereich 6 — Labore Landwirtschaft, Nossen (DE)

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² Name and address according to COMMISSION IMPLEMENTING REGULATION (EU) 2015/1761: Elintarviketurvallisuusvirasto/Livsmedelssäkerhetsverket (Evira), Helsinki/Helsingfors.