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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 KCCM 80365
(FEED-2023-15218; CRL/230005)

European Union Reference Laboratory
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Dossier related to:	**FEED-2023-15218 - CRL/230005**
Name of Product:	***L-valine produced by fermentation with Corynebacterium glutamicum KCCM 80365***
Active Agent (s):	**L-valine**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA) JRC Geel, Belgium**
Report prepared by:	**María José González de la Huebra**
Report checked by: Date:	**Stefano Bellorini** **29/01/2024**
Report approved by: Date:	**Ursula Vincent** **29/01/2024**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4(1) for *L-valine produced by fermentation with Corynebacterium glutamicum* KCCM 80365 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 72 %. The *feed additive* is intended to be mixed either into *premixtures*, incorporated through complementary feed or added directly to *compound feed*. However, the Applicant did not propose any minimum or maximum content of *L-valine* in *compound feed*.

For the quantification of *valine* in the *feed additive*, *premixtures*, *compound feed* the Applicant submitted the ring-trial validated European Union (EU) method based on ion-exchange chromatography coupled to post-column derivatisation and photometric detection (IEC-VIS). The method does not distinguish between the salts of amino acids and 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 *valine* in the *feed additive*, the Applicant presented acceptable experimental data when applying the above mentioned EU method.

In the frame of this authorisation the EURL recommends for official control the ring-trial validated European Union method based on IEC-VIS for the quantification of *valine* in the *feed additive*, *premixtures*, *compound feed*.

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 KCCM 80365, 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* KCCM 80365, 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 [1,2].

According to the Applicant, *L-valine* has a minimum purity (mass fraction) of 72 % [2,3]. The *feed additive* is produced by fermentation using a genetically modified strain of *Corynebacterium glutamicum* KCCM 80365 [3], which is deposited at the Korean Culture Center of Microorganisms (KCCM) with the accession number KCCM 80365 [3].

The *feed additive* is intended to be mixed either into *premixtures*, incorporated through complementary feed or added directly to *compound feed* [4]. However, the Applicant did not propose any minimum or maximum content of *L-valine* in *compound feed* [4].

L-valine, produced by different *Corynebacterium glutamicum* strains, is already authorised as nutritional *feed additives* under Commission Implementing Regulations, namely (EU) 2019/1289 [5], (EU) 2021/2077 [6], (EU) 2021/719 [7], (EU) No 848/2014 [8].

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

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* KCCM 80365 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* and *compound feed* the Applicant proposed the ring-trial validated European Union (EU) method [10].

The EU 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 analysis of *premixtures* and *compound feed*, it does not distinguish between the salts of amino acids and cannot differentiate the amino acid enantiomers [10].

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. *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 [10].

The EU method was ring-trial validated using four different matrices for threonine, cyst(e)ine, methionine and lysine only [10]. This method was further ring-trial validated by 23 laboratories, resulting in the EN ISO 13903 method (*valine* included) [11]. The performance characteristics reported for the quantification of total *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 *valine* in the *feed additive* the Applicant presented experimental data [12] when applying the above mentioned EU method [10] demonstrating thus the suitability of the method.

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 %
[10]	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 EU method, the EURL followed the advice of several experienced National Reference Laboratories (NRLs) and recommends the use of the procedure based on the EU method also for the quantification of *valine* in the *feed additive* [9].

Based on the available data, the EURL recommends for official control the ring-trial validated European Union method based on IEC-VIS to quantify *valine* in the *feed additive*, *premixtures* and *compound feed*.

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.

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 the European Union method based on IEC-VIS for the quantification of *valine* in the *feed additive*, *premixtures* and *compound feed.*

Recommended text for the register entry (analytical method)

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

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

For the quantification of *valine* in *premixtures* and *compound feed*:

- ion-exchange chromatography coupled to 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* KCCM 80365 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://open.efsa.europa.eu/questions/EFSA-Q-2023-00439 https://webgate.ec.europa.eu/esfc/#/applications/44267

[2] *Application– Annex 1

[3] *Technical dossier, Section II: 2.1. Identity of the additive

[4] *Technical dossier, Section II: 2.5. Proposed mode of use in animal nutrition

[5] 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

[6] Commission Implementing Regulation (EU) 2021/2077 of 26 November 2021concerning 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, p. 5

[7] Commission Implementing Regulation (EU) 2021/719 of 30 April 2021 concerning the authorisation of L-valine produced by Corynebacterium glutamicum CGMCC 7.358 as a feed additive for all animal species, OJ L 232, 05.08.2014, p. 14

[8] Commission Implementing Regulation (EU) No 848/2014 of 4 August 2014 concerning the authorisation of L-valine produced by Corynebacterium glutamicum as a feed additive for all animal species and amending Regulation (EC) No 403/2009 as regards the labelling of the feed additive L-valine, OJ L 232, 05.08.2014, p. 14

[9] EURL Evaluation Reports:
https://joint-research-centre.ec.europa.eu/finrep-feed-2021-1685-valine.pdf
https://joint-research-centre.ec.europa.eu/finrep-fad-2021-0032-valine.pdf
https://joint-research-centre.ec.europa.eu/finrep_fad-2020-0033_l-valine.pdf
https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2017-0032-lvaline.pdf
https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2007-0015.pdf
https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2012-0023-L-Valine.doc.pdf
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https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2018-0066_l-valine.pdf
https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2019-0072-valine.pdf

[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] *Technical dossier, Section II: Annex_2.1_02 Batch to batch variation L- valine (VAL Pro)_Redacted_0927.pdf

*Refers to Dossier no: FEED-2023-15218

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:

- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Wageningen Food Safety Research (WFSR)[1] (NL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Instytut Zootechniki - Państwowy Instytut Badawczy, Krajowe Laboratorium Pasz, Lublin (PL)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- Thüringer Landesanstalt für Landwirtschaft (TLL). Abteilung Untersuchungswesen. Jena (DE)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)

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