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Safety and efficacy of a feed additive consisting of L-valine produced by *Escherichia coli* CCTCC M2020321 for all animal species (Kempex Holland BV)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of L-valine as a nutritional additive for all animal species. The production strain and its DNA were not detected in the final additive. Therefore, the final product does not give raise to any safety concern with regard to the genetic modification of the production strain. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concludes that L-valine produced using *Escherichia coli* CCTCC M2020321 is safe when supplemented in appropriate amounts to the diet according to the nutritional needs of the target species. The FEEDAP Panel has concerns on the use of amino acids in water for drinking for hygienic reasons, and due to the risk of imbalances when administered simultaneously via feed. The use of L-valine produced using *E. coli* CCTCC M2020321 in animal nutrition is considered safe for the consumers and for the environment. The FEEDAP Panel cannot conclude on the potential of L-valine produced using *E. coli* CCTCC M2020321 to be toxic by inhalation, irritant to the skin or eyes, or a dermal sensitiser due to the lack of data. The endotoxin activity of the additive does not represent a hazard for users handling the additive when exposed by inhalation. The additive L-valine produced by fermentation using *E. coli* CCTCC M2020321 is regarded as an efficacious source of the essential amino acid L-valine for non-ruminant nutrition. For the supplemental L-valine to be as efficacious in ruminants as in non-ruminant species, it requires protection against degradation in the rumen.

Keywords: nutritional additive, amino acid, L-valine, *Escherichia coli* CCTCC M2020321, safety, efficacy

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Kempex Holland BV,[2] for the authorisation of the additive consisting of L-valine produced by fermentation with a genetically modified strain of *Escherichia coli* (CCTCC M2020321), when used as a feed additive for all animal species (category: nutritional additives; functional group: amino acids, their salts and analogues).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 19/05/2021.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the feed additive consisting of L-valine produced by fermentation with a genetically modified strain of *E. coli* (CCTCC M2020321), when used under the proposed conditions of use (see Section 3.1.6).

1.2. Additional information

L-Valine produced by fermentation with *E. coli* CCTCC M2020321 is not authorised in the European Union.

The FEEDAP Panel has issued several scientific opinions on the safety and efficacy of L-valine produced by fermentation using different strains of *Corynebacterium glutamicum* or *E. coli* (EFSA, 2008a,b; EFSA FEEDAP Panel, 2013, 2014a,b, 2015a,b, 2019a,b, 2020a,b, 2021). L-Valine is authorised for use in feed[3,4,5,6,7,8,9] and in food.[10,11,12] L-Valine and DL-valine are also authorised as sensory additives, belonging to the functional group flavouring compounds (FLAVIS No 17.028 and 17.023, respectively).[13]

The Cosmetic Ingredient Review (CIR) Expert Panel (2012) issued a safety assessment of alpha amino acids as used in cosmetics.

The European Pharmacopoeia (2020) has a monograph dedicated to L-valine (01/2017:0796).

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Kempex Holland B.V. Zeelandsedijk 15, 5408SL, Volkel, The Netherlands.
[3] Commission Implementing Regulation (EU) No 848/2014 of 4 August 20144/Amended by Commission Implementing.Regulation (EU) No 2015/1114 of 9 July 2015. OJ L 182, 10.07.2015, p. 18.
[4] Commission Implementing Regulation (EU) No 1236/2014 of 18 November 2014/Amended by Commission Implementing Regulation (EU) No 2015/1114 of 9 July 2015. OJ L 182, 10.07.2015, p. 18.
[5] Commission Implementing Regulation (EU) No 2015/1114 of 9 July 2015, OJ L 182, 10.07.2015, p. 18.
[6] Commission Implementing Regulation (EU) 2020/1797 of 30 November 2020, OJ L 402, 01.12.2020, p. 36.
[7] Commission Implementing Regulation (EU) 2019/1289 of 31 July 2019, OJ L 203 01.08.2019, p. 2.
[8] Commission Implementing Regulation (EU) 2021/719 of 30 April 2021, OJ L 151, 03.05.2021, p. 12.
[9] Commission Implementing Regulation (EU) 2021/2077 of 26 November 2021, OJ L 426, 29.11.2921, p. 5.
[10] Regulation (EC) No 403/2009 of 14 May 2009/Amended by Commission Implementing Regulation (EU) No 848/2014 of 4 August 2014/Amended by Commission Implementing Regulation (EU) No 2015/1114 of 9 July 2015; Commission Implementing Regulation (EU) No 848/2014 of 4 August 20144/Amended by Commission Implementing Regulation (EU) No 2015/1114 of 9 July 2015; Commission Implementing Regulation (EU) No 1236/2014 of 18 November 2014/Amended by Commission Implementing Regulation (EU) No 2015/1114 of 9 July 2015; Commission Implementing Regulation (EU) No 2015/1114 of 9 July 2015; and Commission Implementing Regulation (EU) 2019/1289 of 31 July 2019.
[11] Commission Directive 2006/141/EC on infant formulae and follow-on formulae, OJ L 401, 30.12.2006, pp. 1–33.
[12] Commission Directive 2001/15/EC of 15 February 2001 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. OJ L 52, 22.2.2001, pp. 1–7.
[13] Commission Implementing Regulation (EU) 2018/249 of 15 February 2018. OJ 53, 23.02.2018, p. 134.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[14] in support of the authorisation request for the use of L-valine produced by fermentation with a genetically modified strain of *E. coli* (CCTCC M2020321) as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, and other scientific reports to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the L-valine produced by fermentation with *E. coli* CCTCC M2020321 in animal feed. The Executive Summary of the EURL report can be found in Annex A.[15]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-valine produced by fermentation with *E. coli* CCTCC M2020321 is in line with the principles laid down in Regulation (EC) No 429/2008[16] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019c).

3. Assessment

L-Valine (≥ 98%) produced by fermentation with a genetically modified strain of *E. coli* (CCTCC M2020321) is intended to be used as a nutritional feed additive (functional group: amino acids, their salts and analogues) in feed and water for drinking for all animal species.

3.1. Characterisation

3.1.1. Characterisation of the production organism

The additive is produced by a genetically modified strain of *E. coli* which is deposited in the China Centre for Type Culture Collection with accession number CCTCC M2020321.[17]

A bioinformatic analysis of the whole genome sequence (WGS) of the production strain confirmed its identity as an *E. coli* K-12 derivative.[18] [redacted] *E. coli* K12 is well-characterised and its safety (non-pathogenicity) has been documented (Gorbach, 1978). The strain has been shown to be ineffective in colonising the human gut (Smith, 1975) and the genome of strains MG1655 and W3110 have been fully sequenced [redacted].

No plasmids were identified [redacted].

[14] FEED dossier reference: FAD-2021-0032.
[15] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2021-0032-valine.pdf
[16] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.
[17] Technical dossier/Section II/Annex 2.2.1.2a.
[18] Technical dossier/Section II/Annex 2.2.1.2c.

The susceptibility of the production strain to the battery of antibiotics recommended in the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b) was tested by broth microdilution (except for fosfomycin which was tested by an agar dilution method) following the method of the Clinical and Laboratory Standards Institute (CLSI). All the minimum inhibitory concentration (MIC) values were below the corresponding cut-off values for Enterobacteriaceae (EFSA FEEDAP Panel, 2018b). Therefore, the production strain is considered susceptible to all relevant antibiotics.[19]

The WGS data of the production strain was interrogated for the presence of antimicrobial resistance (AMR) genes [redacted].[18] No genes of concern were identified.

The WGS of the production strain was also interrogated for the presence of genes coding for toxins and virulence factors [redacted], and therefore not considered of safety concern.

3.1.1.1. Information related to the genetic modification

Characterisation of the recipient or parental microorganism

[redacted].[20]

Characterisation of the donor organisms[21]

[redacted].

Description of the genetic modification[21]

[redacted]

[19] Technical dossier/Section II/Annex 2.2.2.2.
[20] Technical dossier/Section II/Annex 2.2.1.2.b.
[21] Technical dossier/Section II/Annex 2.2.1.2b and Annex 2.2.1.2c.
[22] Technical dossier/Section II/Annex 2.2.1.2b.

[redacted].[23]

3.1.2. Manufacturing process

L-Valine is produced by fermentation using *E. coli* CCTCC M2020321 as a production strain. [redacted].[24] [redacted].[25]

3.1.3. Characterisation of the active substance/additive

L-Valine (International Union of Pure and Applied Chemistry (IUPAC)) name: (2*S*)-2-amino-3-methylbutanoic acid; synonyms: α-amino isovaleric acid, 2-amino-3-methylbutyric acid, a compound identified by Chemical Abstracts Service (CAS) No 72-18-4 and European Inventory of Existing Commercial Chemical Substances (EINECS) No 200-773-6, has a molecular weight of 117.15 g/mol; the molecular formula is $C_5H_{11}NO_2$ and its structural formula is given in Figure 1.

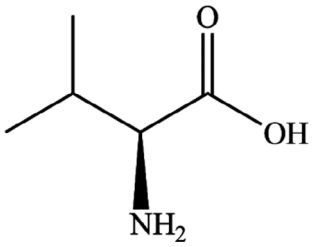

Figure 1: Molecular structure of L-valine

The additive is specified to contain ≥ 98% L-valine on a dry matter (DM) basis and ≤ 2.0% moisture.

The analysis of five batches showed an average of 99.7% valine (range 99.2–100%) on DM.[26] Moisture was on average 0.004% (range 0–0.2%). Residue on ignition was analysed in three batches and represented < 0.5%.[27]

The specific optical rotation measured in three batches ranged from + 27.1 to + 27.7° which fall within the reference range (+ 26.5 to + 29.0°) set in the European Pharmacopoeia (10th edition) (2020) and confirms the L-enantiomer of valine.[28]

Three batches of the additive were analysed for impurities. Concentrations of arsenic, cadmium and mercury were below the limit of detection.[29] Lead was < 0.01 mg/kg in two batches and 0.038 mg/kg in one batch. Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar dioxin-like polychlorinated biphenyls (Co-planar PCBs) were analysed in three batches and found below the corresponding limit of quantification (LOQ). The calculated (upper bound) levels of dioxins and the sum of dioxins and dioxin-like-PCBs were 0.14 ng WHO-PCDD/F-TEQ/kg and 0.27 ng WHO-PCDD/F-PCB-TEQ/kg, respectively (in all three batches).[30] The analysis of mycotoxins (aflatoxins, ochratoxin A, zearalenone, deoxynivalenol) showed values below the respective LODs.[31]

The detected amounts of the above described contaminants do not raise safety concerns.

[23] Technical dossier/Section II/Annex 2.2.1.2c, section 6.
[24] Technical dossier/Section II/Annex 2.3.1a CONFID and Supplementary information October 2021/Annex 1 CONFID.
[25] Technical dossier/Section II/Annex 2.3.1b.
[26] Technical dossier/Section II/Annex 2.1.3a. Analytical method to determine L-valine in the additive was stated to be according to Commission Regulation 152/2009 Appendix III.
[27] Technical dossier/Section II/Annex 2.1.5g, h and i.
[28] Technical dossier/Section II/Annex 2.1.5d, e, and f. European Pharmacopoeia 2.6.14 method A.
[29] Technical dossier/Section II/Annex 2.1.3a. LOD in mg/kg were 0.01 for arsenic and lead; 0.002 for cadmium and mercury.
[30] Technical dossier/Section II/Annex 2.1.4a, 4b and 4c. LOQ in ng/kg ranged from 0.04 to 20 depending on the parameter considered.
[31] Technical dossier/Section II/Annex 2.1.3a. LOD in μg/kg were 17 for zearalenone; 0.05 for aflatoxin; 5 for ochratoxin, 25 for fumonisin; 134 for deoxynivalenol; and 15 for citrinin.

Microbiological contamination of the final product was analysed in three batches by determination of *E. coli*, *Salmonella* spp., Enterobacteriaceae, yeasts and filamentous fungi which were not detected in 25-g samples.[32]

Endotoxin activity (lipopolysaccharides, LPS) was measured in three batches of the final product (European Pharmacopoeia 2.6.14, Method A) and the analytical results indicated levels > 30 and < 300 IU/g.[33]

The presence of viable cells of the production strain was tested in three batches of the final product.[34] Samples of 10 g of each batch (triplicate samples) were dissolved in 290 mL of 0.9% sterile NaCl from which 30 mL (corresponding to 1 g of the original sample) were passed through a 0.45-μm mixed cellulose esters filter, and the filter was cultured on Luria broth (LB) agar containing 32 mg/L vancomycin and incubated at 30°C for 2 days. Positive controls with samples from each of the three batches and 0.9% NaCl spiked with the production strain were included. No growth was detected in any of the three batches tested.

The presence of DNA from the production strain was analysed [redacted] Three batches of the additive were analysed in triplicate.[35] [redacted] No DNA of the production strain was detected in any of the three L-valine batches analysed.

3.1.4. Physical properties of the additive

The additive is a white crystalline powder and has a solubility in water of 88.5 g/L at 25°C.[36] The dusting potential (Stauber–Heubach method) of three batches of the additive ranged100-200 mg/m^3.[37] The particle size distribution was measured in three batches of the additive by laser diffraction. The fraction of particles < 11 μm ranged from 1% to 2%; that < 52.5 μm ranged from 4% to 6% and the fraction < 105 μm ranged from 8% to 14% (v/w).[38]

3.1.5. Stability and homogeneity

The shelf-life of the additive (three batches) was studied when stored for 6 months (temperature and packaging were not described). No losses were observed at the end of the storage period.[39]

The stability of the additive (three batches) in vitamin mineral premixture for weaned piglets (containing 30,000 mg choline chloride) was studied when supplemented at 4% and stored at room temperature in paper bags for 6 months. Losses at the end of the storage period ranged from 4% to 12%.[40]

The stability of the additive (three batches) in feed (mash and pellet form) for chicken for fattening was studied when supplemented at 0.3%, stored at room temperature in paper bags for 3 months. The basal diet consisted of wheat, soybean meal, rapeseed meal, soybean oil and contained a background concentration of 0.89% valine. The pelleting conditions were not described. No losses were observed at the end of the storage in the mash or pelleted feed.[41]

The stability of the L-valine (three batches) in water for drinking was studied when supplemented at 0.2%. Samples were stored at room temperature for 48 h. There were no losses at the end of the storage period.[42]

[32] Technical dossier/Section II/Subsection II.6.3. LOD was one colony forming unit/25 g sample.
[33] Technical dossier/Section II/Annex 2.1.4d, e, f.
[34] Technical dossier/Section II/Annex 2.1.4g and clarifications received via email on 24/11/2021.
[35] Technical dossier/Section II/Annex 2.1.4h.
[36] Technical dossier/Supplementary information October 2021/Annex 2 SDS.
[37] Technical dossier/Section II/Annex 2.1.5d, e, f.
[38] Technical dossier/Section II/Annex 2.1.5a, b, c.
[39] Technical dossier/Supplementary information October 2021/Annex 3.
[40] Technical dossier/Section II/Annex 2.4.1b and Supplementary information October 2021/Annex 4.
[41] Technical dossier/Section II/Annex 2.a.1b and Supplementary information October 2021/Annex 4.
[42] Technical dossier/Section II/Annex 2.1.3a and Supplementary information October 2021/FAD-2021-0032 Sin 210819 Answers.

The capacity for homogeneous distribution of the additive in feed was studied in ten subsamples of pelleted feed. Total valine was analysed and the background concentration of valine in feed was subtracted. The resulting coefficient of variation was 13%.[43]

3.1.6. Conditions of use

L-Valine is intended to be used in feeds and water to achieve an adequate amino acid profile and to meet the L-valine requirements for all animal species. It can be added directly to complete feed, water, complementary feed or it can be supplemented via a premixture. No inclusion levels have been proposed, as the requirements, in quantitative terms, depend on the species, the physiological state of the animal, the performance level, the environmental conditions and the amino acid composition of the un-supplemented diet.

3.2. Safety

3.2.1. Safety of the production microorganism

The recipient strain is an *E. coli* K-12 derivative which is considered to be safe. The genetic modifications performed to obtain the production strain CCTCC M2020321 had the purpose to increase the production of L-valine. None of the introduced modifications raise a safety concern and the production strain does not carry acquired AMR genes. The production strain and its DNA were not detected in the final additive. Therefore, the final product does not give raise to any safety concern with regard to the genetic modification of the production strain.

3.2.2. Safety for the target species, consumers and the environment

L-Valine requirements of different species (non-ruminant and ruminant) and animal categories, absorption and metabolic fate of L-valine, and tolerance to L-valine excess in the diet were described in previous opinions (EFSA FEEDAP Panel, 2013, 2014a).

The additive is highly purified (contains > 98% L-valine and < 1% unidentified material on DM basis). The use of the amino acid 'per se' will not raise safety concerns for the target animals provided it is supplemented in appropriate amounts to the diets. The endotoxin activity ranges from 30 to 300 IU/g. These values are compared with ca. 1,000 IU/mg commonly found in feedingstuffs (Cort et al., 1990). Therefore, at the usual conditions of use of the additive in feed, the endotoxins added by the additive would be insignificant compared with the background in feed. Safety concerns from the additive could derive from the residues of the fermentation process/production strain remaining in the final product. As the production strain does not raise safety concerns (see Section 3.2.1), L-valine produced with *E. coli* CCTCC M2020321 is safe for the target species when used to supplement the diet in appropriate amounts to satisfy the animal requirements.

The FEEDAP Panel has concerns on the use of amino acids in water for drinking for hygienic reasons, and due to the risk of imbalances when administered simultaneously via feed (EFSA FEEDAhP Panel, 2010).

The amino acid L-valine, supplemented to feed, will be incorporated into proteins of tissues and/or products of animal origin and any of their potential excess will be metabolised and excreted as urea/ uric acid and carbon dioxide. Therefore, the composition of tissues and products of animal origin will not be affected by the use of L-valine in animal nutrition.

The amino acid L-valine is a physiological and natural component of the proteins of living organisms. When consumed, it will be absorbed, and the non-absorbed fraction will be incorporated into the intestinal microbial mass and excreted as such. The use of amino acids in water for drinking, when given in addition to complete diets with a well-balanced amino acid profile, would disturb the nitrogen balance and increase nitrogen excretion via urine. The use of the additive in animal nutrition would not lead to any localised increase in the concentration of L-valine or its metabolites in the environment.

3.2.2.1. Conclusions on safety for the target species, consumers and the environment

L-Valine produced using *E. coli* CCTCC M2020321 is safe for the target species when supplemented in appropriate amounts to the diet according to the nutritional needs of the target species. The

[43] Technical dossier/Section II/Annex 2.4.1b.

FEEDAP Panel has concerns on the use of amino acids in water for drinking for hygienic reasons, and due to the risk of imbalances when administered simultaneously via feed.

The use of L-valine produced by *E. coli* CCTCC M2020321 in animal nutrition is considered safe for the consumers and for the environment.

3.2.3. Safety for the user

No studies were submitted to support the safety of the additive for the user.

The additive under assessment is a powder with a dusting potential up to 200 mg/m^3 and containing a fraction of inhalable particles (< 105 μm diameter) up to 14% (v/w). Therefore, exposure of users by inhalation is possible.

Users can suffer from occupational respiratory disease depending on the level of endotoxins in air and dust (Rylander et al., 1999; Thorn, 2001). The highest bacterial endotoxin activity analysed (three batches) was 300 IU/g. The exposure of persons handling the additive to endotoxins in the dust was calculated (Appendix A). The health-based recommended threshold for the quantity of inhaled endotoxins per working day is 900 IU, derived from provisional occupational exposure limits given by the Dutch Expert Committee on Occupational Safety (DECOS) (Health Council of the Netherlands, 2010) and the UK Health and Safety Executive (HSE, 2013). Based upon the calculation of the potential endotoxin content in dust, the inhalation exposure is calculated as 33 endotoxin IU per working day, indicating no risk of exposure by inhalation to endotoxins for persons handling the additive.

3.2.3.1. Conclusions on safety for the user

In the absence of data, the FEEDAP Panel cannot conclude on the potential of the additive to be toxic by inhalation, irritant to skin or eyes, or on its potential to be a dermal or a respiratory sensitiser. The endotoxin activity of the additive does not represent a hazard for users handling the additive when exposed by inhalation.

3.3. Efficacy

Efficacy studies are not required for amino acids that occur naturally in plant and animal proteins. The nutritional role of the amino acid L-valine is well established in the scientific literature. The product L-valine produced by fermentation using *E. coli* CCTCC M2020321 is regarded as an efficacious source of the essential amino acid L-valine for non-ruminant nutrition. The Panel indicated in a previous opinion (EFSA FEEDAP Panel, 2013) that ruminal degradation would reduce the delivery of the amino acid to the abomasum, and that protective measures should be considered.

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[44] and Good Manufacturing Practice.

4. Conclusions

The production strain and its DNA were not detected in the final additive. The final product does not give raise to any safety concern with regard to the genetic modification of the production strain.

L-Valine produced using *E. coli* CCTCC M2020321 is safe for the target species when supplemented in appropriate amounts to the diet according to the nutritional needs of the target species. The FEEDAP Panel has concerns on the use of amino acids in water for drinking for hygienic reasons, and due to the risk of imbalances when administered simultaneously via feed and water.

The use of L-valine produced using *E. coli* CCTCC M2020321 in animal nutrition is considered safe for the consumers and for the environment.

The FEEDAP Panel cannot conclude on the potential of L-valine produced using *E. coli* CCTCC M2020321 to be toxic by inhalation, irritant to the skin or eyes, a dermal or a respiratory sensitiser due to the lack of data. The endotoxin activity of the additive does not represent a hazard for users handling the additive when exposed by inhalation.

[44] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

The additive L-valine produced by fermentation using *E. coli* CCTCC M2020321 is regarded as an efficacious source of the essential amino acid L-valine for non-ruminant nutrition. For the supplemental L-valine to be as efficacious in ruminants as in non-ruminant species, it requires protection against degradation in the rumen.

5. Documentation provided to EFSA/Chronology

Date	Event
19/03/2021	Dossier received by EFSA. L-Valine produced by fermentation with *E. coli* CCTCC M2020321 for all animal species. Submitted by Kempex Holland B.V.
31/03/2021	Reception mandate from the European Commission
19/05/2021	Application validated by EFSA – Start of the scientific assessment
19/08/2021	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation of the additive, stability, manufacturing process, safety for the user*
14/07/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
20/10/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
27/01/2022	Clarification received via e-mail
	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

No Member States comments were received for this dossier.

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Abbreviations

AMR	antimicrobial resistance
CAS	Chemical Abstracts Service
CFU	colony-forming unit
CIR	Cosmetic Ingredient Review
CLSI	Clinical and Laboratory Standards Institute
CV	coefficient of variation
DECOS	Dutch Expert Committee on Occupational Safety
DM	dry matter
EINECS	European Inventory of Existing Chemical Substances
EURL	European Union Reference Laboratory
FCC	Food Chemical Codex
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FLAVIS	The EU Flavour Information System
FL-no	FLAVIS number
HSE	UK Health and Safety Executive
IEC	ion-exchange chromatography
IUPAC	International Union of Pure and Applied Chemistry
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LB	Luria broth
LOD	limit of detection
LOQ	limit of quantification
LPS	lipopolysaccharides
MIC	minimum inhibitory concentration
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzodioxins
PCDF	polychlorinated dibenzofurans
RSDr	relative standard deviation for repeatability
RSDR	relative standard deviation for reproducibility
TEQ	toxic equivalent
WHO	World Health Organization

Appendix A – Calculation of user's exposure to lipopolysaccharides

The probable exposure time according to EFSA guidance (EFSA FEEDAP Panel, 2012) for additives added in premixtures assumes a maximum of 40 periods of exposure per day, each comprising 20 s = 40 × 20 = 800 s/day. With an uncertainty factor of 2, maximum inhalation exposure would occur for 2 × 800 = 1,600 s = 0.444 h/day. Again, assuming a respiration volume of 1.25 m^3/h, the inhalation volume providing exposure to potentially endotoxin-containing dust would be 0.444 × 1.25 = 0.556 m^3/day. This volume should contain no more than 900 IU endotoxin, so the dust formed from the product should contain no more than 900/0.556 = 1,619 IU/m^3.

Calculation of endotoxin content of dust

Two key measurements are required to evaluate the potential respiratory hazard associated with the endotoxin content of the product (the dusting potential of the product, expressed in g/m^3, and the endotoxin activity of the dust, determined by the *Limulus* amoebocyte lysate assay (expressed in IU/g)). If data for the dust are not available, the content of endotoxins of the product can be taken instead. If the content of endotoxins of the relevant additive is a IU/g and the dusting potential is b g/m^3, then the content of endotoxins of the dust, c IU/m^3, is obtained by simple multiplication, a × b. This resulting value is further used for calculation of the potential inhalation exposure of users to endotoxins from the additive under assessment (Table A.1) (EFSA FEEDAP Panel, 2012).

Table A.1: Estimation of user exposure to endotoxins from the additive L-valine produced by fermentation with a genetically modified microorganism (GMO) *E. coli* CCTCC M2020321

Calculation	identifier	Description	Amount	Source
	a	Endotoxin content IU/g product	300	Technical dossier
	b	Dusting potential (g/m^3)	0.2	Technical dossier
a × b	c	Endotoxin content in the air (IU/m^3)	60	
	d	No of premixture batches made/ working day	40	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012)
	e	Time of exposure (s) per production of one batch	20	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012)
d × e	f	Total duration of daily exposure/ worker (s)	800	
	g	Uncertainty factor	2	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012)
f × g	h	Refined total duration of daily exposure/worker (s)	1,600	
h/3,600	i	Refined total duration of daily exposure (h)	0.44	
	j	Inhaled air (m^3) per 8-h working day	10	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012)
j/8 × i	k	Inhaled air during exposure (m^3)	0.56	
c × k	l	**Endotoxin inhaled (IU) during exposure per 8-h working day**	**33**	
	m	Health-based recommended exposure limit of endotoxin (IU/m^3) per 8-h working day	90	Health Council of the Netherlands (2010)
m × j	n	**Health-based recommended exposure limit of total endotoxin exposure (IU) per 8-h working day**	**900**	

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Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for L-valine produced by fermentation with Escherichia coli CCTCC M2020321

In the current application an authorisation is sought under Article 4(1) for L-valine produced by fermentation with *Escherichia coli* CCTCC M2020321, 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 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 feedingstuffs the Applicant submitted the ring-trial validated European Union (EU) method based on ion-exchange chromatography (IEC) coupled with post-column derivatisation and optical (visible – VIS) detection. This method applies for the determination of free (synthetic and natural) and total (peptide-bound and free) amino acids. The EU 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 for the quantification of total valine in feedingstuffs: a relative standard deviation for repeatability (RSDr) ranging from 1.7% to 3.8% and a relative standard deviation for reproducibility (RSDR) ranging from 8.8% to 16.1%.

In addition, the EURL recently evaluated and recommended in the frame of two dossiers (FAD-2019-0072 and FAD-2020-0033) the EU method for the determination of valine in very similar feed additives and water. Furthermore, in the frame of another L-valine dossier (FAD-2017-0032), the EURL previously evaluated and 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.

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 ring-trial validated 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 IECVIS/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.