SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 23 January 2019

doi: 10.2903/j.efsa.2019.5611

Safety and efficacy of L-valine produced using *Corynebacterium glutamicum* CGMCC 11675 for all animal species

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Abstract

The product subject of this assessment is L-valine produced by fermentation with a strain of *Corynebacterium glutamicum* (CGMCC 11675). It is intended to be used in feed and water for drinking for all animal species and categories. Owing to the uncertainties regarding the possible genetic modification of the original production strain, the FEEDAP Panel cannot conclude on the safety of the additive L-valine produced with *C. glutamicum* CGMCC 11675 for the target species, the consumers, the users and the environment. The FEEDAP Panel has concerns on the safety for the target animals of the simultaneous oral administration of valine-containing additives via feed and water for drinking. In the absence of data, the FEEDAP Panel cannot conclude on the potential of L-valine produced with *C. glutamicum* CGMCC 11675 to be toxic by inhalation, irritant to skin or eyes, or on its potential to be a dermal sensitiser. The product is considered an efficacious source of the amino acid L-valine for all animal species. The supplemental L-valine requires protection against rumen degradations in order to be as efficacious in ruminant as in non-ruminant species.

Keywords: nutritional additives, amino acids and their salts and analogues, L-valine, genetically modified microorganisms, safety, efficacy

Requestor: European Commission

Question number: EFSA-Q-2016-00379

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Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output: Montserrat Anguita, Jaume Galobart and Paola Manini.

Legal notice: Relevant information or parts of this scientific output have been blackened in accordance with the confidentiality requests formulated by the applicant pending a decision thereon by the European Commission. The full output has been shared with the European Commission, EU Member States and the applicant. The blackening will be subject to review once the decision on the confidentiality requests is adopted by the European Commission.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Sanz Y, Villa RE, Woutersen R, Costa L, Dierick N, Flachowsky G, Mantovani A, Wallace J, Tarrés-Call J and Ramos F, 2019. Scientific Opinion on the safety and efficacy of L-valine produced using *Corynebacterium glutamicum* CGMCC 11675 for all animal species. EFSA Journal 2019;17(3):5611, 13 pp. https://doi.org/10.2903/j.efsa.2019.5611

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

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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 a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Agri Nutrition BV[2] for authorisation of the product L-valine (≥98.5%) produced by fermentation using *Corynebacterium glutamicum* CGMCC 11675, when used as a feed additive for all animal species (category: nutritional additive; 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). The particulars and documents in support of the application were considered valid by EFSA as of 16 August 2016.

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 product L-valine (≥98.5%) produced by fermentation using *C. glutamicum* CGMCC 11675 for all animal species, when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

L-Valine (minimum 98.5%) produced by fermentation using *C. glutamicum* CGMCC 11675 for all animal species is the object of the present assessment. It is proposed as nutritional feed additive, (functional group: amino acids, their salts and analogues) to feed and water for drinking in all animal species and categories. The product under assessment, L-valine (minimum 98.5%) produced by fermentation using *C. glutamicum* CGMCC 11675, has not been previously authorised in the European Union (EU).

The Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued 6 opinions (EFSA 2008a,b; EFSA FEEDAP Panel, 2013, 2014a, 2015a,b, 2019) on the safety and efficacy of L-valine produced by fermentation using different strains of *Escherichia coli* or *C. glutamicum* as a nutritional additive. The safety of L-valine when used as food flavouring was assessed by Joint FAO/WHO Expert Committee on Food Additives (JECFA; WHO, 2006), by the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) (EFSA, 2008c) and by the FEEDAP Panel (EFSA FEEDAP Panel, 2014b) when used as feed flavouring for animal nutrition. The FEEDAP Panel issued one opinion on the safety and efficacy of the use of amino acids (chemical group 34) when used as flavourings for all animal species (EFSA FEEDAP Panel, 2014b).

L-Valine produced by *C. glutamicum* strains KCCM 80058 or DSM 25202 and L-valine produced by *E. coli* strains NITE SD 00066 or NITE BP-01755 is currently authorised as a nutritional additive for all animal species without restrictions by Commission Implementing Regulation (EU) 848/2014[3], Commission Implementing Regulation (EU) 1236/2014[4] and Commission Implementing Regulation (EU) 1114/2015[5].

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Agri Nutrition BV, Hanzestraat 1, 7006 RH, Doetinchen. The Netherlands. Representing Xinjiang Fufeng Biotechnologies Co., Ltd.
[3] 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, 5.8.2014, p. 13.
[4] Commission Implementing Regulation (EU) No 1236/2014 of 18 November 2014 concerning the authorisation of L-valine produced by *Corynebacterium glutamicum* (DSM 25202) as a feed additive for all animal species Text with EEA relevance OJ L 332, 19.11.2014, p. 26.
[5] 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 (Text with EEA relevance) OJ L 182, 10.7.2015, p. 18.

L-Valine produced by chemical synthesis or product hydrolysis is authorised for use in feed as flavouring (EU Flavour Information System (FLAVIS) Number [17.028]) by Commission Implementing Regulation (EU) 2018/249[6].

L-Valine is authorised for use in food,[7,8] cosmetics[9] and as a veterinary medicinal product.[10] L-Valine [17.028] is currently listed in the EU database of flavouring substances[11] and is authorised for use in food as flavouring.

L-Valine has a dedicated monograph in the European Pharmacopoeia.[12]

The Panel on nutrition, dietetic products, novel food and allergy of the Norwegian Scientific Committee for Food Safety (VKM, 2016) published an opinion on the safety of L-valine in food supplements at 1,500, 1,750, 2,000, 2,250 and 2,500 mg/kg per day for the general population (ages 10 years and above) risk assessment of 'other substances' – L-leucine, L-isoleucine and L-valine, the branched chain amino acids.

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[13] in support of the authorisation request for the use of L-valine (minimum 98.5%) produced by *C. glutamicum* CGMCC 11675 as a feed additive for all animal species.

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 *C. glutamicum* CGMCC 11675 in animal feed. The Executive Summary of the EURL report can be found in Annex A.[14]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-valine (minimum 98.5%) produced by *C. glutamicum* CGMCC 11675 is in line with the principles laid down in Regulation (EC) No 429/2008[15] and the relevant guidance documents: Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008d), Technical Guidance: Microbial Studies (EFSA, 2008e), Guidance on nutritional additives (EFSA FEEDAP Panel, 2012a), Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d) and Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018).

[6] Commission Implementing Regulation (EU) 2018/249 of 15 February 2018 concerning the authorisation of taurine, beta-alanine, L-alanine, L-arginine, L-aspartic acid, L-histidine, D,L-isoleucine, L-leucine, L-phenylalanine, L-proline, D,L-serine, L-tyrosine, L-methionine, L-valine, L-cysteine, glycine, monosodium glutamate and L-glutamic acid as feed additives for all animal species and L-cysteine hydrochloride monohydrate for all species except cats and dogs OJ L 53, 23.2.2018, p. 134.

[7] Commission Regulation (EC) No 1243/2008 of 12 December 2008 amending Annexes III and VI to Directive 2006/141/EC as regards compositional requirements for certain infant formulae. OJ L 335 25, 13.12.2008, p. 18.

[8] Commission Regulation (EC) No 953/2009 of 13 October 2009 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. OJ L 269 9, 14.10.2009, 11 pp.

[9] Commission Decision of 9 February 2006 amending Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products. OJ L 97, 5.4.2006, pp. 1–528.

[10] Commission Regulation (EC) No 1931/1999, amending Annexes I, II and III of Council Regulation (EEC) No 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. OJ L 240, 10.9.1999, pp. 3–10.

[11] Commission Implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1.

[12] European Pharmacopoeia monograph 01/2008: 0796, correction 6.0.

[13] FEED dossier reference: FAD-2016-0031.

[14] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2016-0031_valine.pdf

[15] 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.

3. Assessment

L-Valine (minimum 98.5%) produced by fermentation using *C. glutamicum* CGMCC 11675 for all animal species is the object of the present assessment. It is proposed as nutritional feed additive, functional group: amino acids, their salts and analogues.

3.1. Characterisation

3.1.1. Characterisation of the production organism

L-Valine is produced by a strain of *C. glutamicum* which is deposited in the Chinese General Microbiological Culture Collection Centre (CGMCC) with deposition number CGMCC 11675.[16] The applicant initially stated that the production strain was genetically modified without providing any information on the genetic modification. When details on the genetic modification process were requested, the applicant sent a statement saying that the strain has not been genetically modified, with no details regarding the origin, techniques used and the steps followed to select/obtain the strain.[17]

The production strain was identified as belonging to species *C. glutamicum* by bacterial 16S rRNA gene sequencing and by its morphological and physiological characteristics.[18] [redacted]

Analytical evidence was provided on the susceptibility of the production strain to the antimicrobials listed in the Guidance on the characterisation of microorganisms used as feed additives or as production organisms. [redacted] None of the minimum inhibitory concentrations exceeded the cut-off values established in the guidance (EFSA FEEDAP Panel, 2018).

3.1.2. Manufacturing process

[redacted]

The applicant stated that no antibiotics are used during the production process.[22]

3.1.3. Characterisation of the additive

L-Valine (International Union of Pure and Applied Chemistry (IUPAC)) name: (2*S*)-2-amino-3-methylbutanoic acid; synonyms: α-aminoisovaleric 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.

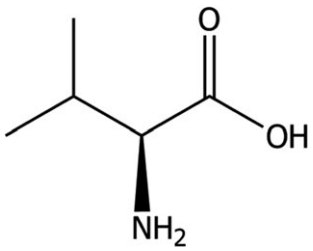

Figure 1: Molecular structure of L-valine

[16] Technical dossier/Section II/Annex 2.2.1.2a.
[17] Technical dossier/Supplementary information July 2017/Annex 8 and supplementary information April 2018/Annex Q1.
[18] Technical dossier/Section II/Annex 2.2.1.2b.
[redacted]
[22] Technical dossier/Section II/Annex 2.3g.

The additive is specified to contain ≥ 98.5% L-valine 'as is', < 0.3% moisture and an undetermined fraction of other amino acids.[23] Compliance with the specification was confirmed in four batches. Valine content was 100% on a dry matter basis in all four batches, only traces of moisture (0.1%) were detected.[24]

The specific optical rotation (European Pharmacopoeia method) measured in eight batches ranged from +27.9 to +28.7° and thus within the range set in the European Pharmacopoeia for the L-enantiomer of valine (+25.6 to +29.0°).[25]

Impurities

Three batches of the final product were analysed for heavy metals and arsenic. In all cases, cadmium was 0.001 mg/kg, lead ranged from 0.005 to 0.09 mg/kg, mercury from 0.002 to 0.07 mg/kg and arsenic was < 0.2 mg/kg in all cases. The microbial contamination was studied in three batches of the additive: total plate count ranged from 20 to 220 colony forming units (CFU)/g, yeasts and moulds had 20 CFU/g in all cases, *Salmonella* spp. was not detected in 10 g samples, *E. coli* and *Staphylococcus aureus* were not detected in 1 g samples.[26] As regards mycotoxin concentrations (three batches analysed), aflatoxin B1 was < 1.7 μg/kg, ochratoxin A < 5 μg/kg, zearalenone < 17 μg/kg, fumonisin < 25 μg/kg and deoxinivalenol < 134 μg/kg. Citrinin ranged from < 15 to 76 μg/kg.[27] Polychlorinated dibenzodioxins/dibenzofurans (PCDD/F) and dioxin-like polychlorinated biphenyls (DL-PCBs) were measured in three batches of the additive. The sum of PCDD/F and DL-PCBs ranged from 0.059 to 0.099 WHO-toxic equivalent (TEQ) pg/g (upper bound) based on 88% dry matter.[28] These impurities do not represent a safety concern.

The antimicrobial activity of one batch of the additive was tested against the reference strains proposed in the Guidance of microbial studies (EFSA, 2008d). In all cases, the minimum inhibitory concentration was > 2,500 mg/L which would correspond to a concentration in feed of 0.25%.[29] No antimicrobial activity was detected.

Bacterial endotoxin activities were measured (*Limulus* amebocyte lysate chromogenic endotoxin quantitation kit) in three batches of the additive and ranged from 0.006 to 0.034 IU/mg.[30]

[redacted]

3.1.3.1. Physico-chemical characteristics

The additive is a white crystalline powder. The pH value measured in eight batches of the additive (1% solution at 21°C) ranged from 5.7 to 6.2.[32] The density is 0.5 kg/L.[33]

The particle size distribution of the additive (three batches) was analysed by laser diffraction.[34] The fractions of particles having diameters < 100, < 50 and < 10 μm diameter ranged 74–79% (v/v), 55–61% (v/v) and 24–29% (v/v), respectively. The dusting potential analysed in three batches (Stauber–Heubach) ranged from 11 to 17 g/m^3.[35]

3.1.3.2. Stability and homogeneity

The shelf life of three batches of the additive was studied under two different temperatures (25 and 40°C) when stored in sealed plastic bags protected from light for 3 months. Only one of the three batches showed losses: 0.5% at 25°C and 0.7% at 40°C.[36]

[23] Technical dossier/Section 2.1.3.
[24] Method assessed by the EURL (email exchange).
[25] Technical dossier/Section II/Annexes 2.1.3a and 2.1.4b.
[26] Technical dossier/Section II/Annex 2.1.4b.
[27] Technical dossier/Section II/Annex 2.1.4a.
[28] Technical dossier/Supplementary information July 2017/Annex 5.
[29] Technical dossier/Section II/Annex 2.2.2.2.
[30] Technical dossier/Section II/Annex 2.1.4d.
[redacted]
[32] Technical dossier/Section II/Annex 2.1.3a and 2.1.4b; Supplementary information July 2017/Answer to question 6.
[33] Technical dossier/Section II/Table 2.1.5a.
[34] Technical dossier/Section II/Annexes 2.1.5a, b and c.
[35] Technical dossier/Section II/Annexes 2.4.3a, b and c.
[36] Technical dossier/Section II.2.4.1/Annex 2.1.4a and Supplementary information July 2017/Answers Sin 161014 final.

The additive (three batches) was incorporated at 10% into a vitamin/mineral premixture also containing choline chloride.[37] The premixtures were stored at room temperature in closed plastic bags protected from light for 6 months. Only one batch showed a loss of 1%.

The additive under assessment (three batches) was added at a level of 0.5% to a complete mash feed based on barley, soybean meal and wheat.[38] The mash feed was conditioned at 45°C and the pelleting was done at 65°C. The pelleting process caused a loss of valine of 8% and 10% in two of the batches tested. Samples of mash and pelleted feed were subsequently stored (packaging and temperature unknown) for 3 months. Losses of 7% were observed in meal (2 batches) whereas no losses were observed in pelleted feed.

The homogeneous distribution of one batch of the additive when supplemented at 0.5% inclusion level in mash pelleted feed was studied by analysing 10 subsamples.[39] The coefficient of variation of the mean was 1.3%.

The stability of the additive (three batches) was studied when diluted to a concentration of 1% in water for drinking and solutions were stored at room temperature for 24 h.[40] A loss of 4% valine was observed in one batch.

3.1.4. Conditions of use

The additive is intended to be used for all animal species and categories with no minimum or maximum content specified. It is intended to be added to feedingstuffs via premixtures or directly into complete feed and complementary feed. The additive is also proposed for the use in water for drinking. No inclusion levels are 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 unsupplemented diet. No withdrawal period is proposed.

3.2. Safety

Corynebacterium glutamicum is regarded as qualified presumption of safety (QPS) only when used as a production organism (EFSA, 2007; EFSA BIOHAZ Panel, 2019). The identity of the production strain has been established as *C. glutamicum* and the absence of antimicrobial resistance has been proven. Therefore, the strain qualifies for the QPS approach for safety assessment. However, uncertainty remains regarding the possible genetic modification of the strain and, therefore, the eventual presence of recombinant DNA in the additive. In the absence of such information/evidence, it is not possible to assess the origin and concerns of the sequences that might have been introduced in the strain.

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. However, due to the risk of nutritional imbalances and hygienic reasons, associated to the use of amino acids via water for drinking (EFSA FEEDAP Panel, 2010), the FEEDAP Panel has concerns on the safety of the simultaneous oral administration of valine-containing additives via feed and water for drinking.

The amino acid L-valine, supplemented to feed, will be incorporated into proteins of tissues and/or products of animal origin and any 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 animals and plants. It is not excreted as such (but as urea/uric acid and carbon dioxide). The use of L-valine in animal nutrition would not lead to any localised increase in the concentration of L-valine or its metabolites in the environment.

The additive is highly purified and is produced by fermentation using a strain which qualifies for the QPS approach for safety assessment. Uncertainty remains regarding the possible genetic modification of the strain and, therefore, the eventual presence of recombinant DNA in the additive. In the absence of such information/evidence, it is not possible to assess the origin and concerns of the sequences that might have been introduced in the strain. The FEEDAP Panel cannot conclude on the safety of the additive L-valine produced by *C. glutamicum* CGMCC 11675 for the target species, consumer and the environment.

[37] Technical dossier/Section II.2.4.1/Annexes 2.1.4a and 2.4.1; Supplementary information July 2017/Annex 11 92209 and Answers SIn 161014 final.
[38] Technical dossier/Section II.2.4.1/Annexes 2.1.4a and Annex 2.4.1; Supplementary information July 2017/Answers SIn 161014 final. feed composition.
[39] Technical dossier/Section II.2.4.2/Annex 2.1.4a.
[40] Technical dossier/Section II.2.4.1/Annex 2.1.4a.

3.2.1. Safety for the user

The additive under assessment is a fine powder with a high dusting potential (up to 17 g/m^3). Therefore, inhalation exposure of users is likely.

No studies were submitted to support the safety of the product for user/workers. In the absence of data, the FEEDAP Panel cannot conclude on the potential of the L-valine to be toxic by inhalation, irritant to skin or eyes, or on its potential to be a dermal sensitiser. Furthermore, owing to the uncertainties regarding the possible genetic modification of the original production strain, the FEEDAP Panel cannot conclude on the safety of the additive L-valine produced by *C. glutamicum* CGMCC 11675 for the users.

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 is regarded as an efficacious source of the essential amino acid L-valine for non-ruminant nutrition.

In ruminants, the amino acid valine has been implicated as being present at lower than optimum levels in microbial protein leaving the rumen (O'Connor et al., 1993; Schwab et al., 2005). Thus, when requirements for more limiting essential amino acids, usually L-methionine, L-lysine and L-histidine, have been met, L-valine supplementation could be beneficial. Free L-valine is rapidly degraded by ruminal microbiota, with an estimated half-life in the rumen of 2.1 h (Chalupa, 1976). Broderick and Balthrop (1979) found that 45% of free L-valine added to ruminal digesta *in vitro* remained after 3 h. Accordingly, only small amounts of dietary L-valine provided to ruminants would be expected to reach the abomasum intact and be absorbed. Therefore, measures, such as encapsulation, would ensure a more efficient delivery of L-valine beyond the rumen, and only limited nutritional benefit may be derived from dietary supplementation with the unprotected, free amino acid.

The additive L-valine produced by *C. glutamicum* CGMCC 11675 is regarded as an efficacious source of the amino acid L-valine for all animal species. For the supplemental L-valine to be as efficacious in ruminants as in non-ruminant species, protection against degradation in the rumen is required.

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[41] and Good Manufacturing Practice.

4. Conclusions

Owing to the uncertainties regarding the possible genetic modification of the original production strain, the FEEDAP Panel cannot conclude on the safety of the additive L-valine produced with *C. glutamicum* CGMCC 11675 for the target species, the consumers, the users and the environment.

In the absence of data, the FEEDAP Panel cannot conclude on the potential of the L-valine produced with *C. glutamicum* CGMCC 11675 to be toxic by inhalation, irritant to skin or eyes, or on its potential to be a dermal sensitiser.

The FEEDAP Panel has concerns on the safety for the target animals of the simultaneous oral administration of valine-containing additives via feed and water for drinking.

The product is considered an efficacious source of the amino acid L-valine for all animal species. The supplemental L-valine requires protection against rumen degradation in order to be as efficacious in ruminant as in non-ruminant species.

Documentation provided to EFSA

1) L-Valine produced by fermentation using *C. glutamicum*. August 2016. Submitted by Agri Nutrition BV.
2) L-Valine produced by fermentation using *C. glutamicum*. Supplementary information. July 2017. Submitted by Agri Nutrition BV.
3) L-Valine produced by fermentation using *C. glutamicum*. Supplementary information. April 2018. Submitted by Agri Nutrition BV.

[41] 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.

4) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for L-valine produced by *Corynebacterium glutamicum* CGMCC 11675.
5) Evaluation of the method of analysis to determine valine in the additive (batch to batch variation) by the EURL (email).
6) Comments from Member States.

Chronology

Date	Event
24/5/2016	Dossier received by EFSA
2/6/2016	Reception mandate from the European Commission
16/8/2016	Application validated by EFSA – Start of the scientific assessment
14/10/2016	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: Production process, characterisation of the additive, characterisation of the production microorganism, stability and capacity to distribute homogeneously in feed, safety for the user*
16/11/2016	Comments received from Member States
16/11/2016	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
25/7/2017	Reception of supplementary information from the applicant – Scientific assessment re-started
25/10/2017	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 production microorganism, characterisation of the additive*
5/4/2018	Reception of supplementary information from the applicant – Scientific assessment re-started
3/9/2018	Request by email to the EURL to assess the method of analysis to determine valine in the additive
5/11/2018	EURL assessment of the method of analysis to determine valine in the additive received by email
23/1/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

Broderick GA and Balthrop JE, 1979. Chemical inhibition of amino acid deamination by ruminal microbes in vitro. Journal of Animal Science, 49, 1101–1111.

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Abbreviations

AFC	EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food
ANI	average nucleotide identity
BIOHAZ	EFSA Panel on Biological Hazards
CAS	Chemical Abstracts Service
CFU	colony-forming unit
CGMCC	Chinese General Microbiological Culture Collection Centre
DL-PCB	dioxin-like polychlorinated biphenyls
EINECS	European Inventory of Existing Commercial chemical Substances
EURL	European Union Reference Laboratory
FCC	Food Chemical Codex
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FLAVIS	The EU Flavour Information System
FL-no	FLAVIS number
IEC	ion exchange chromatography
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
PCDD	polychlorinated dibenzodioxin
PCDF	polychlorinated dibenzofuran
QPS	qualified presumption of safety
RSDr	relative standard deviation for repeatability
RSDR	relative standard deviation for reproducibility
TEQ	toxic equivalent
VKM	Norwegian Scientific Committee for Food and Environment
WHO	World Health Organization

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method s of Analysis for L-valine produced by *Corynebacterium glutamicum* CGMCC 11675

In the current application, authorisation is sought under Article 4(1) for L-valine produced by *Corynebacterium glutamicum* CGMCC 11675, 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 feed additive under Commission Implementing Regulation (EU) 2015/1114 amending Regulation (EC) No 403/2009 and Implementing Regulation (EU) No 848/2014 and (EU) 1236/2014.

For the quantification of L-valine in feed additive, premixtures, feedingstuffs and water the Applicant submitted the ring-trial validated Community method (Commission Regulation (EC) No 152/2009). The method was further ring-trial validated by CEN resulting in EN ISO 13903:2005. The method is based on ion exchange chromatography coupled with post-column derivatisation and photometric detection (IEC-VIS). This method does not distinguish between the salts and the amino acid enantiomers and is designed for feedingstuffs and premixtures. The following performance characteristics were reported for the quantification of total valine: 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 identified the 'L-arginine monograph' of the Food Chemical Codex (FCC) for the characterisation of the feed additive. Since the Applicant provided no experimental data to determine valine in water', the EURL is neither able to evaluate nor to recommend a method for official control to determine valine in water.

Based on the performance characteristics available, the EURL recommends for official control the Community method (equivalent to the EN ISO 13903:2005 method) based on IEC-VIS for the quantification of valine in the feed additive, premixtures and feedingstuffs together with the 'L-valine monograph' of the FCC for the characterisation of 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) is not considered necessary.