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Safety and efficacy of L-valine produced by fermentation using *Escherichia coli* KCCM 80159 for all animal species

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Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of L-valine (≥ 98.0%) produced by fermentation using a genetically modified strain of *Escherichia coli* (KCCM 80159). It is intended to be used in feed for all animal species and categories. Species identity of the production organism *E. coli* KCCM 80159 was confirmed; the strain was sensitive to antibiotics at concentrations below or equal to thresholds specified by EFSA; and no viable cells or its recombinant DNA were detected in the final product. Therefore, the final product does not give raise to any safety concern with regard to the genetic modification of the production strain. The FEEDAP Panel concludes that L-valine produced by *E. coli* KCCM 80159 is safe for the target species when supplemented in appropriate amounts to the diet according to the nutritional needs of the target species, for the consumer, for the users and for the environment. The product L-valine produced by fermentation using *E. coli* KCCM 80159 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 acids, L-valine, *Escherichia coli* KCCM 80159, 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 a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Daesang Europe BV[2] for authorisation of the product L-valine, 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). The particulars and documents in support of the application were considered valid by EFSA as of 30 October 2018.

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 (≥ 98.0%), produced by fermentation using *Escherichia coli* KCCM 80159, when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

L-Valine (minimum 98.0%) produced by fermentation using *Escherichia coli* KCCM 80159 is the object of the present assessment. It has not been previously assessed as a feed additive in the European Union. L-Valine produced by different microbial strains is authorised as a feed additive for all animal species.[3]

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, 2015a,b; EFSA FEEDAP Panel, 2019a,b) or when used as a feed flavouring compound (EFSA FEEDAP Panel, 2014b).

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[4] in support of the authorisation request for the use of L-valine 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, 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 using *E. coli* KCCM 80159 in animal feed. The Executive Summary of the EURL report can be found in Annex A.[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] Daesang Europe BV, Prof. JH Bvincklaan 3, 1183 AT Amstelveen, The Netherlands.
[3] Reg (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.
[4] FEED dossier reference: FAD-2018-0066.
[5] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2018-0066_l-valine.pdf

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of the product under assessment is in line with the principles laid down in Regulation (EC) No 429/2008[6] and the relevant guidance documents: Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on studies concerning the safety of use of feed additives for user/workers (EFSA FEEDAP Panel, 2012), Guidance on the assessment of the efficacy of feed additives (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 (minimum 98.0%) produced by *E. coli* KCCM 80159 is intended to be used as a nutritional additive functional group amino acids, their salts and analogues in feed for all animal species.

3.1. Characterisation

3.1.1. Characterisation of the production organism

L-Valine is produced by a genetically modified strain of *E. coli* which is deposited in the Korean Culture Collection of Microorganisms with accession number KCCM 80159.[7]

[redacted] the strain is considered phenotypically susceptible to [redacted] antibiotics.

The WGS of the production strain was interrogated for the presence of antimicrobial resistance (AMR) genes [redacted] the production strain does not carry acquired antibiotic resistance genes of concern.

[redacted] All [redacted] genes were [redacted] considered not to be pathogenic.

[6] 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.

[7] Technical dossier/Section II/ANNEX Section II/Annex II.2.02.

[redacted]

3.1.1.1. Information related to the genetically modified microorganism

[redacted]

3.1.2. Manufacturing process

[redacted]

The applicant stated that no antimicrobials are used during the manufacturing process of the additive[13]

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.

[redacted]

[13] Technical dossier/Section II/Annex II.1.5.

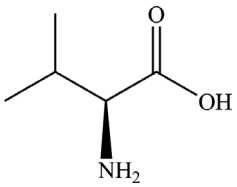

Figure 1: Molecular structure of L-valine

The additive is specified to contain ≥ 98% L-valine on dry matter basis, and < 1.5% moisture.[14]

The analysis of five batches showed an average of 99.7% valine 'as is' (range 99.6 to 99.9%) and a loss on drying of 0.2%.[15] Other amino acids ranged from 0.54 to 0.63%.

The specific optical rotation measured in three batches ranged from +28.1 to +28.9° which fall within the range set in the European Pharmacopoeia (+25.6 to +29.0°) and confirm the L-enantiomer of valine.[16]

3.1.3.1. Undesirable substances

Five batches of the additive were analysed for heavy metals (cadmium, lead and mercury) and arsenic (results expressed on an 'as is' basis). Lead was ≤ 5 mg/kg, arsenic ≤ 2 mg/kg, cadmium < 0.01 mg/kg and mercury < 0.005 mg/kg in all batches.[17] Polychlorinated dibenzodioxins/dibenzofurans (PCDDs/Fs) and dioxin-like polychlorinated biphenyls (PCBs) were analysed in three batches. PCDD/F ranged from 0.0601 to 0.0616 ng WHO-PCDD/F-TEQ/kg and PCB ranged from 0.0366 to 0.0375 ng/kg.

In relation to mycotoxins, aflatoxins (B_1, B_2, G_1 and G_2), ochratoxin A, zearalenone, deoxynivalenol, zearalenone, T-2 toxin, HT-2 toxin and fumonisins (B_1 and B_2) were analysed in three batches of the additive and found below the limit of quantification (LOQ).[18] The detected amounts of these undesirable substances do not raise safety concerns. As regards the microbial contamination, *Salmonella* spp. was negative in 25-g samples. *E. coli* was not detected, yeast and moulds were < $2x10^2$ colony forming units (CFU)/g and total aerobic bacterial count ≤10^5 CFU/g.[19]

The antimicrobial activity of the additive was tested against *Enterococcus hirae* ATCC 10541, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 9027, *E. coli* ATCC 25922 and *Candida albicans* ATCC 10231.[20] No antimicrobial activity was detected.

Endotoxin activity was measured in three batches of the final product (*Limulus* amoebocyte assay according to the EurPh) and the values ranged from 21.7 to 61.4 international units (IU)/g.[20]

The applicant provided two sets of data regarding the presence of viable cells of the production strain in the final product. [redacted]. No growth was detected.

The presence of DNA from the production strain was investigated [redacted]

[14] Technical dossier/Section II.1.3 and Annex II.1.2.
[15] Technical dossier/Section II/Annex II.1.1. Valine analysed according to European Pharmacopoeia 2.2.56, method I.
[16] Technical dossier/Section II/Annex II.1.3.
[17] Technical dossier/Section II/Annexes II.1.1 and II.1.3.
[18] Technical dossier/Section II/Annexes II.1.1 and II.1.3. LOQ (in μm/kg) was 0.1 for aflatoxins (B1, B2, G1 and G2); 0.2 for ochratoxin A; 10 for zearalenone, T-2 toxin and HT-2 toxin; and 20 for deoxynivalenol and fumonisins B1 and B2.
[19] Technical dossier/Section II/Annex II.1.1.
[20] Technical dossier/Annex II.1.3.
[redacted]

[redacted] No DNA from the production strain was detected in the samples.

3.1.3.2. Physico-chemical properties

The additive is a white crystalline powder with a solubility in water (at 20°C) of 57 g/L, a pH ranging from 5 to 7 (solution of 5 g in 100 mL),[23] and a tapped density (measured in three batches) ranging from 765 to 787 kg/m^3.[16]

The dusting potential was analysed (Stauber–Heubach method) in three batches of the final product. The values ranged from 0.3 to 1.01 g/m^3.[24]

The particle size distribution of three batches of the final product was measured (laser diffraction). One of the batches had no particles < 100 μm diameter. In the other two, the fractions of particles with diameters < 100, < 50 and < 10 μm ranged 3–4%, 1–2%, and 0 to < 1%, respectively. In all three batches, most of the particles had a size between 200 and 750 μm diameter.[16]

3.1.3.3. Stability and homogeneity

The shelf life of the additive (three batches) was tested at 25°C and 40°C when stored in paper bags with one-ply polyethylene inner for 2 years.[25] Losses up to 1% were observed after storage at 25°C and up to 1.5% after storage at 40°C.

The stability of the additive (three batches) in a vitamin–mineral premixture containing choline chloride (300 mg/kg) was studied when supplemented at an inclusion rate of 0.27%.[26] The samples were stored in plastic bags at 25°C for 6 months. A loss of 5% was seen in only one of the batches.

The stability of the additive (three batches) was studied in a complete feed for pigs for fattening, another for fish and a third one for chickens for fattening (a different batch of additive was used for each species) when supplemented at 0.3%.[27] The basal diet of the complete feed for pig for fattening consisted on barley, wheat and soybean meal; that of fish on wheat, fish meal and soybean meal; and the one of chicken for fattening on maize, soybean meal and wheat.[28] Mash and pelleted feed were tested after storage at 25°C in plastic bags for 3 months. No losses were observed in the fish feed. A loss of 19% was observed in the mash feed for chickens for fattening (no loss in pelleted feed). Regarding the pig feed, a loss of 5% was observed in the pelleted feed (no loss in mash feed).

The feedingstuffs (mash form) described above were used to study the capacity of the additive to distribute homogeneously in feed.[29] Free valine was analysed in 10 subsamples (9 in the case of fish feed) of each feed. The coefficients of variation were 3.4%, 4.5% and 4% for pig, fish and chicken for fattening mash feeds, respectively.

3.1.4. Physicochemical incompatibilities

No physico-chemical incompatibilities in feed are expected with other additives, medicinal products or feed materials.

3.1.5. Conditions of use

It is proposed that L-valine will be used in feeds 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 or complementary feedingstuffs, or via 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.

[23] Technical dossier/Section II/Annex II.2.1.
[24] Technical dossier/Section II/Annex II.1.4.
[25] Technical dossier/Section II/Annex II.4.1.
[26] Technical dossier/Section II/Annex II.4.3.
[27] Technical dossier/Section II/Annex II.4.2.
[28] Technical dossier/Supplementary information March 2019/Annex section II/Annex II.4.5.
[29] Technical dossier/Section II/Annex II.4.4.

3.2. Safety

3.2.1. Safety of the production microorganism

[redacted]. None of the introduced modifications raise a safety concern. 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 of L-valine for the target species, the consumer 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 contains ≥ 98% L-valine in a dry matter basis and < 1% unidentified material. The level of endotoxins in the product (up to 61.4 IU/g, see Section 3.1.3) is negligible in comparison with that observed in other feedingstuffs (Wallace et al., 2016) and is therefore of no concern for the target species. Safety concerns from the additive may derive either from the amino acid or from the residues of the fermentation process/production strain remaining in the final product. The recipient strain is considered safe, the genetic modification does not raise safety concerns, and no viable cells or DNA of the production strain were found in the final product, consequently no safety concerns for target animal, consumers and the environment would rise from the fermentation residues that may be present in the final additive.

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 additive does not pose any environmental safety concern associated with the production strain. 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 FEEDAP Panel concludes that L-valine produced by *E. coli* KCCM 80159 is safe for the target species when supplemented in appropriate amounts to the diet according to the nutritional needs of the target species, for the consumer and for the environment.

3.2.3. Safety for the user

The applicant provided an acute inhalation toxicity study, an acute eye irritation study, and acute skin irritation study and a skin sensitisation study performed using the additive under assessment as test item.

3.2.3.1. Effects on the respiratory system

The additive under assessment is a powder with a moderate dusting potential (up to 1 g/m^3) and contains a fraction of particles < 100 and < 50 μm diameter up to 4 and 2%, respectively (see Section 3.1.3.2). Therefore, inhalation exposure of users is likely.

An acute inhalation study was performed in compliance with OECD Guideline No 436.[30] A group of three male and three female CRL (WI) BR Wistar rats was exposed nose-only to an atmosphere containing 5.05 mg/L L-valine for 4 h, followed by a 14-day observation period. No adverse effects (no relevant clinical signs, or mortality, or pathology) were observed. The 4-hour median concentration (LC_{50}) was derived to be higher than 5.05 mg/L.

The additive contains endotoxin activity levels up to 61 IU/g. The scenario used to estimate the exposure of persons handling the additive to endotoxins in the dust, based on the EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012, 2014b) is described in the Appendix A. The health based

[30] Technical dossier/Section III/Annex III.3.8.

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 on the calculation of the potential endotoxin content in dust, the inhalation exposure could be up to 34 endotoxin IU per working day, indicating thus no risk related to the inhalation exposure to endotoxins for persons handling the additive.

3.2.3.2. Effects on skin and eyes

In a dermal irritation/corrosion test conducted in accordance with OECD Guideline No 404, 0.5 g of L-valine was applied to the skin of three New Zealand White rabbits for 4 h.[31] Clinical signs, body weight and adverse effects at the application site were surveyed over 72 h. There were no observations of irritancy to the skin of any rabbit; thus, it was concluded that the substance is non-irritant to the skin.

In an eye irritation test conducted in accordance with OECD Guideline No 405, 0.1 g of L-valine was instilled to one of the conjunctival sacs of three New Zealand White rabbits.[32] Clinical signs, body weight and adverse effects at the application site were surveyed over 72 h. The exposure caused initially slight conjunctival irritant effects (slight conjunctival redness and discharge 1 h after the treatment in all rabbits, reduced to slight conjunctival redness in one rabbit at 24 h post administration) which were fully reversible within 48 h. Thus, it was concluded that the substance is non-irritant to the eye.

A skin sensitisation test (local lymph node assay) was conducted in accordance with OECD Guideline No 429.[33] 24 CBA/Ca Ola Hsd female mice (4/group) were locally exposed to concentrations of 2.5%, 1% or 0.5% (w/v) of the test item formulated in aqueous 1% (w/v) Pluronic PE 9200. A positive control received 2.5% α-hexylcinnamaldehyde in acetone–olive oil mixture (AOO), and there was a control group for the vehicle of the positive control (AOO) and another for the vehicle of the test item (Pluronic PE 9200). No significantly increased lymphoproliferation was noted for L-valine at the applied test concentrations. No dose-related response was observed. It was concluded that the additive under assessment was not a skin sensitiser.

3.2.3.3. Conclusions on safety for the user

L-Valine produced using *E. coli* KCCM 80159 is not irritant to the skin or eyes, is not a skin sensitiser and is not toxic 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* KCCM 80159 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 ruminant metabolism would reduce the delivery of the amino acid to the abomasum, and therefore, measures to ensure a more efficient delivery 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[34] and Good Manufacturing Practice.

4. Conclusions

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.

[31] Technical dossier/Section III/Annex III.3.10.
[32] Technical dossier/Section III/Annex III.3.9.
[33] Technical dossier/Section III/Annex 3.11.
[34] 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 FEEDAP Panel concludes that L-valine produced by *E. coli* KCCM 80159 is safe for the target species when supplemented in appropriate amounts to the diet according to the nutritional needs of the target species, for the consumer, for the users and for the environment.

The product L-valine produced by fermentation using *E. coli* KCCM 80159 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.

Chronology

Date	Event
06/09/2018	Dossier received by EFSA. L-Valine produced using *Escherichia coli* KCCM 80159 for all animal species. Submitted by Daesang Europe BV
19/09/2018	Reception mandate from the European Commission
30/10/2018	Application validated by EFSA – Start of the scientific assessment
30/01/2019	Comments received from Member States
30/01/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
08/02/2019	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 strain, characterisation of the additive, manufacturing process.*
08/03/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
15/03/2019	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.*
02/10/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
18/03/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014a. Scientific Opinion on the safety and efficacy of L-valine (ValAMINO®) produced by Corynebacterium glutamicum (DSM 25202) for all animal species, based on a dossier submitted by Evonik Industries AG. EFSA Journal 2014;12 (7):3795, 14 pp. https://doi.org/10.2903/j.efsa.2014.3795

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Abbreviations

AFC EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food

AMR	antimicrobial resistance
AOO	acetone–olive oil mixture
CAS	Chemical Abstracts Service
CFU	colony forming unit
CG	chemical group
CV	coefficient of variation
DECOS	Dutch Expert Committee on Occupational Safety
DM	dry matter
EINECS	European Inventory of Existing Commercial Chemical Substances
EU	Endotoxin Unit, equivalent to an international unit of endotoxin activity (IU)
EURL	European Union Reference Laboratory
FCC	Food Chemical Codex
HSE	UK Health and Safety Executive
IEC-VIS	ion exchange chromatography coupled with post-column derivatisation and photometric detection
IUPAC	International Union of Pure and Applied Chemistry
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
KCCM	Korean Culture Collection of Microorganisms
LC_{50}	lethal concentration, median
LOQ	limit of quantification
MIC	minimum inhibitory concentration
OECD	Organisation for Economic Co-operation and Development
PCB	polychlorinated biphenyl
PCDD/F	polychlorinated dibenzodioxin/dibenzofuran
RH	relative humidity
RSDr	relative standard deviation for *repeatability*
RSDR	relative standard deviation for *reproducibility*
SCAN	Scientific Committee on Animal Nutrition
TEQ	toxic equivalents
WGS	whole genome sequence
WHO	World Health Organization

Appendix A – Safety for the user

The effects of endotoxin inhalation and the exposure limits have been described in a previous opinion (EFSA FEEDAP Panel, 2015).

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/m3, then the content of endotoxins of the dust, *c* IU/m3, is obtained by simple multiplication, $a \times b$. This resulting value is further used for calculation of the potential inhalatory 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 *Escherichia coli* KCCM 80159, including consideration of using a filter mask FF P2 or FF P3 as a preventative measure

Calculation	identifier	Description	Amount	Source
	a	Endotoxin content IU/g product	61.4	Technical dossier
	b	Dusting potential (g/m^3)	1.01	Technical dossier
$a \times b$	*c*	Endotoxin content in the air (IU/m^3)	62	
	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)
$d \times e$	*f*	Total duration of daily exposure/worker (s)	800	
	g	Uncertainty factor	2	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012)
$f \times g$	*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 eight-hour working day	10	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012)
$j/8 \times i$	*k*	Inhaled air during exposure (m^3)	0.56	
$c \times k$	*l*	**Endotoxin inhaled (IU) during exposure per eight-hour working day**	**34**	
	m	Health-based recommended exposure limit of endotoxin (IU/m^3) per eight-hour working day	90	Health Council of the Netherlands, 2010
$m \times j$	*n*	**Health-based recommended exposure limit of total endotoxin exposure (IU) per eight-hour working day**	**900**	
l/10		Endotoxins inhaled (IU) per eight-hour working day reduced by filter mask FF P2 (reduction factor 10)	3	
l/20		Endotoxins inhaled (IU) per eight-hour working day reduced by filter mask FF P3 (reduction factor 20)	2	

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Health Council of the Netherlands, 2010. Endotoxins. Health-based recommended occupational exposure limit. Publication no 2010/04OSH, 100 pp.

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 fermentation using *Escherichia coli* KCCM 80159 in animal feed

In the current application authorisation is sought under Article 4(1) for *L-valine produced by fermentation with Escherichia coli* K12 KCCM 80159 (C001), 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. Authorisation is sought for all animal species.

According to the Applicant, *L-valine* has a minimum purity (mass fraction) of 98%. *L-valine* is intended to be added directly into *feedingstuffs* or through *premixtures*. 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 identified the "L-valine monograph" of the Food Chemical Codex (FCC) where identification is based on infrared absorption.

For the quantification of *L-valine* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated Community method based on ion exchange chromatography coupled with post-column derivatisation and photometric detection (IEC-VIS). The method does not distinguish between the salts of amino acids and it cannot differentiate between enantiomers. The following performance characteristics were reported for the quantification of total *valine* in feed: a relative standard deviation for *repeatability* (RSDr) ranging from 1.7 to 3.8% and a relative standard deviation for *reproducibility* (RSDR) ranging from 8.8 to 16.1%.

In the frame of this authorisation the EURL recommends for official control (i) the "L-valine monograph" of the Food Chemical Codex (FCC) based on infrared absorption for the identification of *L-valine* in the *feed additive*; and (ii) the ring-trial validated Community method based on IEC-VIS for the quantification of *valine* in the *feed additive*, *premixtures* and *feedingstuffs*.

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.