SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 27 January 2021

doi: 10.2903/j.efsa.2021.6425

Safety and efficacy of the feed additive consisting of L-tryptophan produced by *Escherichia coli* KCCM 80210 for all animal species (Daesang Europe BV)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Pier Sandro Cocconcelli, Boet Glandorf, Lieve Herman, Miguel Prieto Maradona, Maria Saarela, Montserrat Anguita, Jaume Galobart, Orsolya Holczkencht, Paola Manini, Elisa Pettenati, Fabiola Pizzo and Jordi Tarrés-Call

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 the feed additive consisting of L-tryptophan produced by fermentation with *Escherichia coli* KCCM 80210 when used as a nutritional additive in feed for all animal species and categories. The production strain *E. coli* KCCM 80210 is safe for the production of L-tryptophan and it was not detected in the final product. The Panel notes that two out of five batches of the additive do not comply with the minimum specification of 98% L-tryptophan on a dry matter basis proposed by the applicant. The use of L-tryptophan (≥ 98%) produced by *E. coli* KCCM 80210 in supplementing feed to compensate for L-tryptophan deficiency in feedingstuffs is safe for non-ruminant target species. There may be a risk for an increased production of toxic metabolites when unprotected L-tryptophan is used in ruminants. The use of L-tryptophan produced by *E. coli* KCCM 80210 in animal nutrition raises no safety concerns to consumers of animal products and to the environment. The additive under assessment is considered a mild eye irritant. The endotoxin activity of the additive and its dusting potential indicate a risk by inhalation for the users. The additive is not a skin irritant and is not a skin sensitiser. The additive L-tryptophan is regarded as an effective source of the amino acid L-tryptophan for all non-ruminant species. In order to be as efficacious in ruminants as in non-ruminants, it should be protected from ruminal degradation.

Keywords: nutritional additive, amino acid, L-tryptophan, safety, efficacy, *Escherichia coli* KCCM 80210

Requestor: European Commission

Question number: EFSA-Q-2020-00499

Correspondence: feedap@efsa.europa.eu

Panel members: Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa and Ruud Woutersen.

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.

Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Acknowledgements: The Panel wishes to acknowledge the contribution of Yolanda Garcia Cazorla to this opinion.

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, Fašmon Durjava MF, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Cocconcelli PS, Glandorf B, Herman L, Maradona MP, Saarela M, Anguita M, Galobart J, Holczkencht O, Manini P, Pettenati E, Pizzo F and Tarrés-Call J, 2021. Scientific Opinion on the safety and efficacy of the feed additive consisting of L-tryptophan produced by *Escherichia coli* KCCM 80210 for all animal species (Daesang Europe BV). EFSA Journal 2021;19(3):6425, 17 pp. https://doi.org/10.2903/j.efsa.2021.6425

ISSN: 1831-4732

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

Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

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 feed additive consisting of L-tryptophan produced by fermentation using *Escherichia coli* KCCM 80210, 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 15 September 2020.

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-tryptophan (≥ 98%) produced by fermentation with a genetically modified strain of *E. coli* (KCCM 80210), when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The subject of the present assessment is the product consisting of L-tryptophan (minimum 98%) produced by a genetically modified strain of *E. coli* (KCCM 80210). L-Tryptophan produced by this bacterial strain had not been previously authorised as feed additive in the European Union.

L-Tryptophan (≥ 98%) produced by fermentation with specific strains of *E. coli* is currently authorised for use as a nutritional additive in the European Union, under the functional group 'amino acids, their salts and analogues'.[3]

L-Tryptophan is authorised for use in food for nutritional purposes,[4] and for use in cosmetics.[5] It is authorised for use as a veterinary medical product without maximum residue limits.[6]

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) published several opinions on the safety and efficacy of L-tryptophan produced by different strains of *E. coli* for all animal species (EFSA FEEDAP Panel, 2013, 2014a,b, 2015a,b, 2016a,b, 2017a,b, 2019a–e, 2020a,b, c). The EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) issued a scientific opinion on the substantiation of health claims related to L-tryptophan (EFSA NDA Panel, 2011).

L-Tryptophan is described in the European Pharmacopoeia, 9th Edition (2017), so that this citation matches the Reference List. Please confirm that this is correct. monograph 01/2017:1272.

The Norwegian Scientific Committee for Food Safety assessed the safety of L-tryptophan in food (VKM, 2013) supplements and energy drinks (VKM, 2016).

[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. Van Heuven Goedhartlaan 935, 1181 LD, Amsterdam (The Netherlands).
[3] Commission Implementing Regulation (EU) 2017/873 of 22 May 2017 concerning the authorisation of L-tryptophan produced by *Escherichia coli* as a feed additive for all animal species. OJ L 134, 23.5.2017, p. 14–17.
[4] Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC No 41/2009 and (EC) No 953/2009. OJ L 181, 29.6.2013, p. 35–56.
[5] Commission Decision 2006/257/EC of 9 February 2006 amending Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products. OJ L 97/1, 5.4.2006. p. 598.
[6] Commission Regulation (EC) No 37/2010 of 22 December 2009, on pharmacologically active substances and their classification regarding maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. OJ L 15, 20.1.2010, pp. 1.

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[7] in support of the authorisation request for the use of the product consisting of L-tryptophan (minimum 98.0%) produced by fermentation using *E. coli* KCCM 80210 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 product consisting of L-tryptophan produced by fermentation using *E. coli* KCCM 80210 in animal feed. The Executive Summary of the EURL report can be found in Annex A.[8]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of the additive under assessment is in line with the principles laid down in Regulation (EC) No 429/2008[9] 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 identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017c), 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, 2017d), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017e), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b) and Guidance for assessing the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019f).

3. Assessment

The product subject of this application is an additive consisting of L-tryptophan (≥ 98%) produced by fermentation with a genetically modified strain of *Escherichia coli* (KCCM 80210). It is proposed to be used as nutritional additive (functional group: amino acids, their salts and analogues) in feed for all animal species and categories.

3.1. Characterisation

3.1.1. Characterisation of the production organism

The additive is produced by a genetically modified derivative of *E. coli* K-12 which is deposited in the Korean Culture Collection of Microorganisms (KCCM) with accession number KCCM 80210.[10]

A bioinformatic analysis of the whole genome sequence (WGS) of the production strain confirmed its identity as an *E. coli* K-12 derivative.[11] This was based [redacted] *E. coli* K-12 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 its genome (MG1655 and W3110) has been fully sequenced (Hayashi et al., 2006).

[7] FEED dossier reference: FAD-2020-0038.
[8] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2020-0038-tryptophan.pdf
[9] 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.
[10] Technical dossier/Section II/Annexes Section II/Annex 2.2.1.2a CONFID.
[11] Technical dossier/Section II/Annexes Section II/Annex 2.2.1.2c CONFID, Annex 2.2.1.2d CONFID and Annex 2.2.1.2e CONFID.

[redacted][12]

The production strain was tested for its susceptibility to all the antimicrobials listed for 'Enterobacteriaceae' in the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a,b).[13] All minimum inhibitory concentration (MIC) values were below or equal to the cut-off values set in the Guidance and, therefore, the strain is considered susceptible to those antibiotics.

The WGS of the production strain was interrogated for the presence of antimicrobial resistance (AMR) genes [redacted][12] This, and the fact that the production strain was not phenotypically resistant to any of the antibiotics tested, suggests that the production strain does not carry antibiotic resistance genes of concern.

The WGS of the production strain was also interrogated for the presence of toxin and virulence factor genes [redacted][12]

3.1.1.1. Information regarding to the genetically modified microorganism

Characterisation of the recipient microorganism

[redacted][14]

Characteristics of the introduced sequences

[redacted][14]

[redacted][14]

Description of the genetic modification

[redacted][14]

[redacted][14]

[redacted][12]

[redacted]

[12] Technical dossier/Section II/Annexes Section II/Annex 2.2.1.2c CONFID.
[13] Technical dossier/Section II/Annexes Section II/Annex 2.2.2.2.
[14] Technical dossier/Section II/Annexes Section II/Annex 2.2.1.2b CONFID.

3.1.2. Manufacturing process

L-Tryptophan is produced by fermentation using *E. coli* KCCM 80210. [redacted][15]

[redacted][16]

3.1.3. Characterisation of the product/active substance

L-Tryptophan (International Union of Pure and Applied Chemistry (IUPAC) name: (2S)-2-amino-3-(1H-indol-3-yl) propanoic acid; synonyms: (S)-α-amino-1-H-indole-3-propanoic acid, L-α-aminoindole-3-propionic acid,-l-α-amino-3-indolepropionic acid, 2-amino-3-indolylpropanoic acid, L-β-3-indolylalanine) has the Chemical Abstracts Service (CAS) No 73-22-3 and European Inventory of Existing Commercial Chemical Substances (EINECS) No 200-795-6. The chemical formula is $C_{11}H_{12}N_2O_2$, the molecular weight is 204.23 g/mol. The structural formula is given in Figure 1.

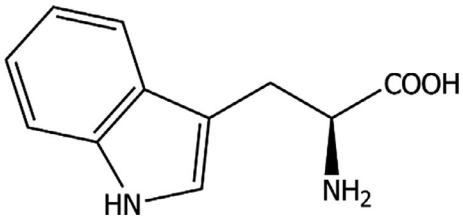

Figure 1: Structural formula of L-tryptophan

According to the specification, the product contains ≥ 98% L-tryptophan on a DM basis and ≤ 1% moisture.

The analysis of five batches of the additive showed an average content of tryptophan of 98.4% on 'as is' basis (range 97.3–99.7%),[17] moisture was 0.1% and ash ranged 0.02–0.06%.[18] On a dry matter basis, the tryptophan content was on average 98.5% (97.4–99.8%), corresponding to the amount of identified material. Two out of the five analysed batches did not reach the specification of minimum 98% tryptophan on a DM basis.

The specific optical rotation was measured in five batches of the additive and was on average –32.3° (range –32.0 to –32.4°),[18] which is within the range described in the European Pharmacopoeia (–30 to –33°) for this amino acid and confirms the identity of the L-enantiomer.[19]

3.1.3.1. Undesirable substances

Five batches of the additive were analysed for heavy metals (cadmium, lead and mercury) and arsenic. All values were below the respective limit of detection (LOD) except for lead which ranged from 0.03 to 0.06 mg/kg.[20] 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).[21] The calculated (upper bound) levels of dioxins and the sum of dioxins and dioxin-like-PCBs were calculated to be 0.137 ng WHO-PCDD/F-TEQ/kg and 0.269 ng WHO-PCDD/F-PCB-TEQ/kg, respectively (in all three batches). In relation to mycotoxins, aflatoxins (not specified), ochratoxin A, zearalenone, fumonisins (B1, B2 and B3), deoxynivalenol and citrinin were analysed in three batches of the additive. All analytical values

[15] Technical dossier/Section II/Annex 2.3.1a Confid.
[16] Technical dossier/Section II/Annex 2.3.1.b.5.
[17] Technical dossier/Section 2.6.1 and Annex II.6.1. Spontaneous submission of additional information, Q2. Supplementary information November 2021/Annex 2 TRP feed grade. Tryptophan analysed by European Pharmacopoeia 9.0, 2.2.56, method I.
[18] Technical dossier/Section II/Annexes 2.1.3a to e.
[19] European Pharmacopoeia monograph 1/2017:1272.
[20] Technical dossier/Section II/Annex 2.1.4a. LOD in mg/kg were 0.01 for arsenic and lead, and 0.002 for cadmium and mercury.
[21] Technical dossier/Section II/Annex 2.1.4b1 to d1. LOQ in ng/kg ranged 0.04–2 for PCDD/Fs; and ranged 1–20 for PCBs, depending on the substance considered.

were found below the LOD except for citrinin in one batch (15.6 μg/kg).[22] The detected amounts of these undesirable substances do not raise safety concerns.

Microbial contamination was analysed in three batches. *Salmonella* spp. was absent in 25 g samples. In 1 g samples, *E. coli* and *Enterobacteriaceae* were not detected; and yeast and moulds ranged 510–700 colony-forming units (CFU)/g.[23]

The concentration of 1,1′-ethylidene-bis-L-tryptophan (EBT) was < 10 mg/kg in all three batches analysed. The concentration of 1-methyl-1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acid (MTCA) was measured in three batches and was 1 mg/kg in all cases.[24]

Endotoxin activity was measured in three batches (*Limulus* amebocyte lysate assay) and ranged from 1,720 to 5,180 EU/g.[25]

The presence of viable cells of the production strain in the final product was tested in three batches of the additive.[26] [redacted] No growth was detected.

The presence of DNA from the production strain was tested in three batches of the additive in triplicate.[27] [redacted] No DNA of the production strain was detected.

3.1.3.2. Physical-chemical properties

The additive is described as a white to yellowish white odourless crystalline powder. It has a solubility in water of 10.6 g/L (20°C)[28] and a density of 550–650 kg/m^3.

Dusting potential was analysed in three batches (Stauber-Heubach method) and the values ranged from 0.6 to 0.8 g/m^3.[29] Particle size distribution of the final product was measured in three batches and the fractions of particles below 11, 52 and 105 μm diameter ranged 21–25, 64–74 and 76–86%, respectively.[30]

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 bags protecting from light and air exchange for 6 months.[31] Losses observed at both temperatures ranged from 0% to 2% depending on the batch considered.

The stability of the additive (three batches) in a vitamin mineral premixture containing choline chloride (30,000 mg/kg) was studied when supplemented at an inclusion rate of 4%.[32] The samples were stored at room temperature in bags protecting from light and air exchange for 6 months. Losses observed ranged from 3% to 10%.

The stability of the additive (three batches) was studied in a complete feed for chickens for fattening, when supplemented at 0.2%. The basal diet consisted of wheat, maize and soybean meal.[33] Mash and pelleted feed were tested after storage at room temperature in bags protecting from light and air exchange for 3 months. Pelleting temperature was about 70°C. Pelleting the mash feeds produced loses up to 2%. After 3 months storage, only a loss of 2% was observed in one of the batches for each mash and pelleted feed.

[22] Technical dossier/Section II/Annex 2.1.4a. LOD in μg/kg were 1.7 for aflatoxins, 5 for ochratoxin A, 17 for zearalenone, 25 for the sum of the three fumonisins, 134 for DON and 15 for citrinin.
[23] Technical dossier/Section II/Annexes 2.1.3a to e and annex 2.1.4a.
[24] Technical dossier/Section II/Annexes 2.1.4g1 to g3 for EBT and 2.1.4h1 to h3 for MTCA. EBT Analysed according to European Pharmacopoeia 10th Edition, monograph 1272; MTCA analysed by HPLC with fluorescent detector.
[25] Technical dossier/Section II/Annex 2.1.4i.
[26] Technical dossier/Section II/Annex 2.1.4.e.
[27] Technical dossier/Section II/Annex 2.1.4f CONFID.
[28] Technical dossier/Section II/Annex 2.5.2.6.
[29] Technical dossier/Section II/Annexes 2.1.5a to c.
[30] Technical dossier/Section II/Annexes 2.1.4b2, c2 and d2.
[31] Technical dossier/Section II.4.1 and Annex 2.1.4a.
[32] Technical dossier/Section II.4.1, annex 2.1.4b received as spontaneous submission of supplementary information, and premixture composition in annex 2.4.1.
[33] Technical dossier/Section II.4.1, annex 2.1.4b, and feed composition in annex 2.4.1c.

One of the pelleted feeds for chicken for fattening described above was used to study the capacity of the additive to distribute homogeneously in feed.[34] Total tryptophan was analysed in 10 subsamples. The coefficient of variation was 2%.

3.1.4. Conditions of use

L-Tryptophan is intended to be used in feeds to achieve an adequate amino acid profile and to meet the L-tryptophan requirements for all animal species. It can be added directly to complete/complementary feedingstuffs or via premixtures. 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 strain

[redacted] The genetic modifications performed to obtain the production strain KCCM 80210 have the purpose to increase the production of L-tryptophan. None of the introduced modifications raise a safety concern. The production strain is free of all antibiotic resistance genes used during the genetic modification process. 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.

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

The L-tryptophan requirements of the target animal species and the safety of this essential amino acid in non-ruminant and ruminant nutrition were summarised in previous opinions of the EFSA FEEDAP Panel (2013, 2015a).

The additive is highly purified (> 97.4% tryptophan and about 2% unidentified material on a dry matter basis) and is produced by fermentation using a strain that is considered safe. Concerns on the use of the additive would not derive from the L-tryptophan, which is considered safe but may arise from residues of the fermentation process/production strain remaining in the final product.

The endotoxin activity was up to 5,180 IU/g. These values are very low when compared with ca. 1,000,000 IU/g 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. Since the production strain was identified as an *E. coli* K12 derivative, the genetic modifications performed are considered safe, it was susceptible to antimicrobials of clinical human and veterinary relevance, and no viable cells and DNA of the production strain were found in the final product, L-tryptophan produced with *E. coli* KCCM 80210 is safe for non-ruminant target species when used to supplement the diet in appropriate amounts to satisfy the animal requirements.

The FEEDAP Panel reiterates that ruminal metabolism of unprotected L-tryptophan may result in the production of toxic quantities of 3-methylindole (skatole), which causes pulmonary disease (fog fever; emphysema) in cattle and goats (Hammond et al., 1979). Consequently, only a protected form of L-tryptophan should be used in ruminants (EFSA FEEDAP Panel, 2013).

The absorption and metabolic fate of L-tryptophan in the organism were described in a previous opinion (EFSA FEEDAP Panel, 2013). The amino acid L-tryptophan, supplemented to feed, will be incorporated into proteins of tissues and/or products of animal origin and any of its potential excess will be metabolised and excreted. Therefore, the composition of tissues and products of animal origin will not be affected by the use of L-tryptophan in animal nutrition. EBT and MTCA present in a specific brand of L-tryptophan produced by fermentation were implicated in the eosinophilia–myalgia syndrome outbreak that occurred in humans in New Mexico in 1989 (Hertzman et al., 1990). The concentrations of EBT were < 10 mg/kg additive and those of MTCA were 1 mg/kg and do not represent a safety concern according to the European Pharmacopoeia 9th edition (2017) that established a maximum permitted content of EBT (impurity A) and the sum of all other impurities (B-L, including MTCA) in L-tryptophan as 10 mg/kg and 390 mg/kg, respectively.

[34] Technical dossier/Section II/Annexes 2.4.1b and annex 2.1.4a received as spontaneous submission of supplementary information.

The amino acid L-tryptophan is a physiological and natural component of animals and plants. When given to animals, it is not excreted as such, but as urea/uric acid, indole-related compounds and carbon dioxide. The use of the product L-tryptophan in animal nutrition would not lead to any localised increase in the concentration in the environment. The use of L-tryptophan produced by *E. coli* KCCM 80210 as a feed additive does not represent a risk to the environment.

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

The use of L-tryptophan produced using *E. coli* KCCM 80210 to supplement feed to compensate for tryptophan deficiency in feedingstuffs is safe for non-ruminant species. There may be a risk for an increased production of toxic metabolites when unprotected tryptophan is used in ruminants.

The use of L-tryptophan produced by fermentation using *E. coli* KCCM 80210 in animal nutrition is considered safe for the consumers and for the environment.

3.2.3. Safety for the user

The applicant provided an *in vivo* acute inhalation toxicity study, a dermal irritation study, an eye irritation study and a dermal sensitisation study testing the additive under assessment.

3.2.3.1. Effects in the respiratory system

The highest measured dusting potential of the additive under assessment was 0.8 g/m^3 and the fraction of particles having a diameter < 52 μm ranged from 64% to 74% (see Section 3.1.3.2). Hence, the users can be exposed to the additive by inhalation.

In an acute inhalation toxicity study in rats performed in accordance with OECD Guideline 403 and consistent with good laboratory practices (GLP), the additive showed an inhalation median lethal concentration (LC_{50}) greater than 5.13 mg/L air and the test item required no classification.[35]

Users can suffer from occupational respiratory disease depending on the level of endotoxins in air and dust (Rylander, 1999; Thorn, 2001). The bacterial endotoxin activity (analysed in three batches) ranged from 1,720 to 5,180 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 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) (HCN, 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 2,300 endotoxin IU per working day, indicating that inhalation exposure to endotoxins for persons handling the additive is above the recommended threshold.

3.2.3.2. Effects on skin and eyes

The skin irritation potential of the additive was tested in a valid study in rabbits performed according to OECD guideline 404 and consistent with GLP, which showed that it is not a skin irritant and has no corrosive effect on skin.[36]

The eye irritation potential of the additive was tested in a valid study in rabbits performed according to OECD guideline 405 and consistent with GLP, which showed that according to the results obtained and under the assayed experimental conditions, the test item was classified as mild irritant to eyes.[37]

In a valid skin sensitisation study following local lymph node assay (LLNA) performed in mice according OECD guideline 429 and compliant with GLP, it was found that the additive does not have to be classified as a skin sensitiser.[38]

3.2.3.3. Conclusions on safety for the user

L-Tryptophan produced using *E. coli* KCCM 80210 is not a skin irritant and it is not a skin sensitiser but is mild irritant to eyes. The endotoxin activity of the additive and its dusting potential indicate a risk by inhalation for the users.

[35] Technical dossier/Section III/Annex 3.3.1.1.
[36] Technical dossier/Section III/Annex 3.3.1.2a.
[37] Technical dossier/Section III/Annex 3.3.1.2b.
[38] Technical dossier/Section III/Annex 3.3.1.2c.

3.3. Efficacy

Efficacy studies are not required for amino acids naturally occurring in the proteins of plants and animals. The nutritional role of the amino acid L-tryptophan is well established in the scientific literature. The additive feed grade L-tryptophan is regarded as an effective source of the amino acid L-tryptophan.

The efficacy of this essential amino acid in non-ruminant and ruminant nutrition was summarised in a previous opinion of the EFSA FEEDAP Panel (2014b). The FEEDAP Panel reiterates that, if the product L-tryptophan is used in ruminants, it should be protected from ruminal degradation.

Overdosing of supplemental L-tryptophan may increase skatole and indole in the hind gut resulting in boar taint of pork (Zamaratskaia and Squires, 2008).

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[39] and Good Manufacturing Practice.

4. Conclusions

The Panel notes that two out of five batches of the additive do not comply with the minimum specification of 98% L-tryptophan on a dry matter basis as proposed by the applicant.

The use of L-tryptophan (≥ 98%) produced using *E. coli* KCCM 80210 to supplement feed to compensate for L-tryptophan deficiency in feedingstuffs is safe for non-ruminant species. There may be a risk for an increased production of toxic metabolites when unprotected L-tryptophan is used in ruminants.

The use of L-tryptophan produced by fermentation using *E. coli* KCCM 80210 in animal nutrition is considered safe for the consumers and for the environment.

L-Tryptophan produced using *E. coli* KCCM 80210 is not a skin irritant and it is not a skin sensitiser but is mild irritant to eyes. The endotoxin activity of the additive and its dusting potential indicate a risk by inhalation for the users.

The product L-tryptophan produced by fermentation using *E. coli* KCCM 80210 is regarded as an effective source of the amino acid L-tryptophan for all non-ruminant species. In order to be as efficacious in ruminants as in non-ruminants, it should be protected from ruminal degradation.

5. Recommendation(s)

The FEEDAP Panel recommends that the specification of the additive includes limits for L-tryptophan-related impurities in compliance with the European Pharmacopeia.

6. Documentation as provided to EFSA/Chronology

Date	Event
29/05/2020	Dossier received by EFSA. L-tryptophan produced by fermentation with *Escherichia coli* KCCM 80210 for all animal species. Submitted by Daesang Europe BV.
07/07/2020	Reception mandate from the European Commission
15/09/2020	Application validated by EFSA – Start of the scientific assessment
21/09/2020	Reception of spontaneous supplementary information from the applicant – *Issues: Characterisation of the additive*
26/11/2020	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, manufacturing process*
27/11/2017	Reception of supplementary information from the applicant - Scientific assessment re-started
27/11/2020	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
15/12/2020	Comments received from Member States
27/01/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

[39] 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.

References

Cort N, Fredriksson G, Kindahl H, Edqvist LE and Rylander R, 1990. A clinical and endocrine study on the effect of orally administered bacterial endotoxin in adult pigs and goats. Journal of Veterinary Medicine Series A, 37, 130–137.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. https://doi.org/10.2903/j.efsa.2012.2539

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific opinion on the safety and efficacy of L-tryptophan produced by *Escherichia coli* (FERM BP-11200) for all animal species based on a dossier submitted by Ajinomoto Eurolysine SAS. EFSA Journal 2013;11(10):3368, 2 pp. https://doi.org/10.2903/j.efsa.2013.3368

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-tryptophan produced by *Escherichia coli* (FERM BP-11354) for all animal species, based on a dossier submitted by Ajinomoto Eurolysine S.A.S. EFSA Journal 2014;12(10):3826, 13 pp. https://doi.org/10.2903/j.efsa.2014.3826

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014b. Scientific Opinion on the safety and efficacy of L-tryptophan technically pure produced by fermentation with *Escherichia coli* for all animal species, based on a dossier submitted by HELM AG on behalf of Global Bio-Chem Technology. EFSA Journal 2014;12(5):3673, 2 pp. https://doi.org/10.2903/j.efsa.2014.3673

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015a. Scientific Opinion on the safety and efficacy of L-tryptophan produced by *Escherichia coli* CGMCC 7.59 for all animal species based on a dossier submitted by HELM AG on behalf of Meihua Holdings Co. Ltd. EFSA Journal 2015;13(2):4015, 17 pp. https://doi.org/10.2903/j.efsa.2015.4015

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015b. Scientific Opinion on the safety and efficacy of L-tryptophan, technically pure, produced by *Escherichia coli* strains DSM 25084, KCCM 11132P or SARI12091203 for all animal species based on a dossier submitted by AMAC EEIG. EFSA Journal 2015;13(9):4238, 29 pp. https://doi.org/10.2903/j.efsa.2015.4238

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016a. Scientific opinion on the safety of L-tryptophan produced by fermentation using *Escherichia coli* CGMCC 3667, for all animal species based on a dossier submitted by GBT Europe GmbH. EFSA Journal 2016;14(1):4343, 13 pp. https://doi.org/10.2903/j.efsa.2016.4343

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016b. Scientific opinion on the safety of L-tryptophan produced by fermentation with *Escherichia coli* CGMCC 7.59 for all animal species based on a dossier submitted by Feedway Europe NV. EFSA Journal 2016;14(5):4444, 9 pp. https://doi.org/10.2903/j.efsa.2016.4444

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimont i G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wester P, Costa LG, Dierick N and Wallace RJ, 2017a. Scientific opinion on the safety of L-tryptophan technically pure, produced by fermentation with *Escherichia coli* strains DSM 25084, KCCM 11132P and SARI12091203 for all animal species based on a dossier submitted by FEFANA Asbl. EFSA Journal 2017;15(2):4712, 11 pp. https://doi.org/10.2903/j.efsa.2017.4712

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Glandorf B, Herman L, Kärenlampi S, Aguilera J and Cocconcelli PS, 2017b. Scientific opinion on the safety of L-tryptophan technically pure, produced by *Escherichia coli* CGMCC 3667, for all animal species based on a dossier submitted by GBT Europe GmbH. EFSA Journal 2017;15(3):4705, 7 pp. https://doi.org/10.2903/j.efsa.2017.4705

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J and Innocenti ML, 2017c. Guidance on the identity, characterisation and conditions of use of feed additives. EFSA Journal 2017;15(10):5023, 12 pp. https://doi.org/10.2903/j.efsa.2017.5023

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2017d. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017;15(10):5021, 19 pp. https://doi.org/10.2903/j.efsa.2017.5021

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Dujardin B, Galobart J and Innocenti ML, 2017e. Guidance on the assessment of the safety of feed additives for the consumer. EFSA Journal 2017;15(10):5022, 17 pp. https://doi.org/10.2903/j.efsa.2017.5022

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Anguita M, Brozzi R and Galobart J, 2018a. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal 2018;16(3):5206, 24 pp. https://doi.org/10.2903/j.efsa.2018.5206

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2018b. Guidance on the assessment of the efficacy of feed additives. EFSA Journal 2018;16(5):5274, 25 pp. https://doi.org/10.2903/j.efsa.2018.5274

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, Glandorf B, Herman L, Mantovani A, Saarela M, Wallace RJ, Anguita M, Tarrés-Call J and Ramos F, 2019a. Scientific Opinion on the safety and efficacy of L-tryptophan produced by fermentation with *Escherichia coli* CGMCC 7.248 for all animal species. EFSA Journal 2019;17(2):5601, 16 pp. https://doi.org/10.2903/j.efsa.2019.5601

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, de Lourdes Bastos M, 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, 2019b. Scientific Opinion on the safety and efficacy of L-tryptophan produced with *Escherichia coli* CGMCC 11674 for all animal species. EFSA Journal 2019;17(3):5642, 17 pp. https://doi.org/10.2903/j.efsa.2019.5642

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, Glandorf B, Herman L, Mantovani A, Saarela M, Wallace RJ, Anguita M, Tarrés-Call J and Ramos F, 2019c. Scientific Opinion on the safety and efficacy of L-tryptophan produced by fermentation with Escherichia coli KCCM 80152 for all animal species. EFSA Journal 2019;17(5):5695, 16 pp. https://doi.org/10.2903/j.efsa.2019.5695

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, de Lourdes Bastos M, 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, Glandorf B, Mantovani A, Wallace RJ, Anguita M, Manini P, Tarrés-Call J and Ramos F, 2019d. Scientific Opinion on the safety and efficacy of L-tryptophan produced by fermentation with *Corynebacterium glutamicum* KCCM 80176 for all animal species. EFSA Journal 2019;17(6):5729, 15 pp. https://doi.org/10.2903/j.efsa.2019.5729

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, 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, Glandorf B, Herman L, Leng L, Mantovani A, Saarela M, Wallace RJ, Anguita M, Tarrés-Call J and Ramos F, 2019e. Scientific Opinion on the safety and efficacy of L-tryptophan produced by fermentation with *Escherichia coli* KCCM 80135 for all animal species. EFSA Journal 2019;17(5):5694, 18 pp. https://doi.org/10.2903/j.efsa.2019.5694

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, 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, Ramos F, Sanz Y, Villa RE, Woutersen R, Brock T, Knecht J, Kolar B, Beelen P, Padovani L, Tarrés-Call J, Vettori MV and Azimonti G, 2019f. Guidance on the assessment of the safety of feed additives for the environment. EFSA Journal 2019;17(4):5648, 78 pp. https://doi.org/10.2903/j.efsa.2019.5648

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, Kos Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Sanz Y, Villa RE, Woutersen R, Dierick N, Flachowsky G, Glandorf B, Herman L, Mantovani A, Saarela M, Wallace RJ, Anguita M, Tarrés-Call J and Ramos F, 2020a. Scientific Opinion on the safety and efficacy of l-tryptophan produced by fermentation using *Escherichia coli* CGMCC 7.267 for all animal species. EFSA Journal 2020;18(2):6013, 17 pp. https://doi.org/10.2903/j.efsa.2020.6013

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, Kos Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Sanz Y, Villa RE, Woutersen R, Cubadda F, Dierick N, Glandorf B, Herman L, Mantovani A, Saarela M, Anguita M, Pettenati E, Tarrés-Call J and Ramos F, 2020b. Scientific opinion on the safety and efficacy of l-tryptophan produced by fermentation with *Escherichia coli* KCCM 10534 for all animal species. EFSA Journal 2020;18(4):6071, 18 pp. https://doi.org/10.2903/j.efsa.2020.6071

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, Ramos F, Sanz Y, Villa RE, Woutersen R, Anguita M, Galobart J, Holczknecht O, Manini P, Pettenati E, Pizzo F and Tarrés-Call J, 2020c. Scientific Opinion on the safety of l-tryptophan produced using *Escherichia coli* CGMCC 11674 for all animal species. EFSA Journal 2020;18(6):6168, 6 pp. https://doi.org/10.2903/j.efsa.2020.6168

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2011. Scientific Opinion on the substantiation of health claims related to L-tryptophan and maintenance of normal sleep (ID 596, 1671), enhancement of mood (ID 596), contribution to normal cognitive function (ID 596), and contribution to the maintenance or achievement of a normal body weight (ID 604) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2011;9(4):2073, 16 pp. https://doi.org/10.2903/j.efsa.2011.2073

European Pharmacopoeia, 9th Edition, 2017. European Directorate for the Quality of Medicines and Health, Monograph 01/2017:1272.

Gorbach SL, 1978. Risk assessment of recombinant DNA experimentation with *Escherichia coli* K12. Proceedings from a workshop at Falomouth. Massachusetts. Journal of Infectious Diseases, 137, 613–714.

Hammond AC, Bradley BJ, Yokoyama MT, Carlson JR and Dickinson EO, 1979. 3-Methylindole and naturally occurring acute bovine pulmonary edema and emphysema. American Journal of Veterinary Research, 40(10), 1398–1401.

Hayashi K, Morooka N, Yamamoto Y, Fujita K, Isono K, Choi S, Ohtsubo E, Baba T, Wanner BL, Mori H and Horiuchi T, 2006. Highly accurate genome sequences of Escherichia coli K-12 strains MG1655 and W3110. Molecular Systems Biology, 2(2006), 0007.

HCN (Health Council of the Netherlands), 2010. Endotoxins, 2010. Health-based recommended occupational exposure limit. Publication No 2010/04OSH. Health Council of the Netherlands, The Hague, 100 pp.

Hertzman PA, Blevins WL, Mayer J, Greenfield B, Ting M and Gleich GJ, 1990. Association of the eosinophiliamyalgia syndrome with the ingestion of tryptophan. New England Journal of Medicine, 322, 869–873.

HSE (Health and Safety Executive), 2013. Occupational hygiene implications of processing waste at materials recycling facilities (MRFs). RR977 Research Report, HSE, London, UK, 41 pp.

Rylander R, 1999. Health effects among workers in sewage treatment plants. Occupational Environmental Medicine, 56, 354–357.

Smith HW, 1975. Survival of orally administered E. coli K-12 in alimentary tract of man. Nature, 255, 500–502.

Thorn J, 2001. The inflammatory response in humans after inhalation of bacterial endotoxin: a review. Inflammatory Response, 50, 254–261.

VKM, 2013. Risk assessment of Opinion of histidine, methionine, S-adenosylmethionine and tryptophan. Opinion of the Panel on Nutrition, dietetic products, Novel Food an Allergy of the Norwegian Scientific Committee for Food Safety. ISBN: 978-82-8259-079-2, Oslo, Norway.

VKM, 2016. Risk assessment of "other substances" – L-tryptophan. Opinion of the Panel on Nutrition, dietetic products, Novel Food an Allergy of the Norwegian Scientific Committee for Food Safety. ISBN: 978-82-8259-193-5, Oslo, Norway.

Zamaratskaia G and Squires E, 2008. Biochemical, nutritional and genetic effects on boar taint in entire male pigs. Animal, 2, 1–14.

Abbreviations

CAS	Chemical Abstracts Service
CFU	colony-forming unit
CV	coefficient of variation
DM	dry matter
EBT	1,1′-ethylidene-bis-L-tryptophan
EURL	European Union Reference Laboratory
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
MTCA	1-methyl-1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acid
RH	relative humidity
WHO	World Health Organization
VKM	Norwegian Scientific Committee for Food Safety
WGS	Whole genome sequence

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, 2015a,b).

Calculation of maximum acceptable levels of exposure from feed additives

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/m3, 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 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-tryptophan produced by *Escherichia coli* KCCM 80210, 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	5,180	Technical dossier
	b	Dusting potential (g/m^3)	0.8	Technical dossier
a × b	c	Endotoxin content in the air (IU/m^3)	4,144	
	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**	**2,300**	
	m	Health-based recommended exposure limit of endotoxin (IU/m^3) per 8-h working day	90	HCN, 2010
m × j	n	**Health-based recommended exposure limit of total endotoxin exposure (IU) per 8-h working day**	**900**	

Calculation	Identifier	Description	Amount	Source
I/10		Endotoxins inhaled (IU) per 8-h working day reduced by filter mask FF P2 (reduction factor 10)	230	
I/20		Endotoxins inhaled (IU) per 8-h working day reduced by filter mask FF P3 (reduction factor 20)	115	

References

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. https://doi.org/10.2903/j.efsa.2012.2539

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015. Scientific Opinion on the safety and efficacy of L-lysine monohydrochloride produced by fermentation with Escherichia coli for all animal species based on a dossier submitted by HELM AG on behalf of Meihua Holdings Group Co. Ltd. EFSA Journal 2015;13(3):4052, 16 pp. https://doi.org/10.2903/j.efsa.2015.4052

HCN (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-tryptophan produced by fermentation using *Escherichia coli* KCCM 80210

In the current application, an authorisation is sought under Article 4(1) for L-tryptophan produced by fermentation with *Escherichia coli* KCCM 80210 (W008), 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. The authorisation is sought for all animal species.

According to the Applicant, the feed additive contains L-tryptophan with a minimum purity of 98% (w/w). The feed additive is intended to be mixed either in premixtures or added directly to feedingstuffs. However, the Applicant did not propose a minimum or maximum L-tryptophan content in feedingstuffs.

For the determination of tryptophan in the feed additive and premixtures, the Applicant submitted the ring-trial validated EN ISO 13904:2016 method based on high-performance liquid chromatography with fluorescence detection (HPLC-FLD). The method is dedicated for the determination of free tryptophan in commercial products and premixtures (containing more than 2% (w/w) of tryptophan) and for the determination of free and total tryptophan in feedingstuffs.

For the determination of tryptophan in feedingstuffs, the Applicant submitted a ring-trial validated European Union (EU) method, where the procedure to determine tryptophan in feedingstuffs is identical to the one described in the above-mentioned EN ISO 13904:2016 method. The EU method is applicable for the determination of free (synthetic and natural) and total (peptide-bound and free) amino acid using an HPLC method with fluorescence detection (FLD). The method does not distinguish between the amino acid enantiomers.

Based on the performance characteristics available, the EURL recommends for official control the above-mentioned two ring-trial validated methods based on HPLC-FLD to determine tryptophan in the feed additive, premixtures and/or feedingstuffs.

In addition, for the identification of the feed additive, the EURL recommends the 'L-tryptophan monograph' of the Food Chemical Codex (FCC), where different tests (including one based on optical rotation) are described.

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.