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Safety and efficacy of L-tryptophan produced by fermentation using *Escherichia coli* CGMCC 7.267 for all animal species

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 Kos Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Noël Dierick, Gerhard Flachowsky, Boet Glandorf, Lieve Herman, Alberto Mantovani, Maria Saarela, Robert John Wallace, Montserrat Anguita, Jordi Tarrés-Call and Fernando Ramos

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 L-tryptophan produced by fermentation with a genetically modified strain of *Escherichia coli* CGMCC 7.267 when used as a nutritional additive in feed and water for drinking for all animal species and categories. The production strain *E. coli* CGMCC 7.267 is safe for the production of L-tryptophan. No viable cells or DNA of the production strain were detected in the additive under assessment. The use of L-tryptophan produced using *E. coli* CGMCC 7.267 in supplementing feed to compensate for tryptophan deficiency in feedingstuffs is safe for non-ruminant target species. However, excess doses would create amino acid imbalances with negative consequences on animal performance. The use of unprotected L-tryptophan in feed poses safety concerns for ruminants. The use of L-tryptophan produced by fermentation with *E. coli* CGMCC 7.267 in animal nutrition is considered safe for the consumers and for the environment. The endotoxin activity in the product and its dusting potential indicate an inhalation risk for the user. In the absence of data, the FEEDAP Panel cannot conclude on the potential of the additive to be irritant to skin and eyes or to be a skin sensitiser. The additive L-tryptophan produced using *E. coli* CGMCC 7.267 is regarded as an effective source of the amino acid L-tryptophan. 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*

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Correspondence: feedap@efsa.europa.eu

Panel members: Giovanna Azimonti, Vasileios Bampidis Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Kos 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.

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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 Welding GmbH & Co. KG[2] for authorisation of the product L-tryptophan, when used as a feed additive for target 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 4 October 2017.

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-tryptophan produced by fermentation with a strain of *Escherichia coli* (CGMCC 7.267), when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

L-Tryptophan (minimum 98%) produced by fermentation using a genetically modified strain of *E. coli* (CGMCC 7.267) is the subject of the present assessment. L-Tryptophan produced by this bacterial strain has 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 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,b,c,d,e). The Panel on Dietetic Products, Nutrition and Allergies (NDA) of EFSA 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), 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) and concluded that doses ≥ 250 mg/day may represent a risk of adverse health effects for children, adolescents and adults.

[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] Welding GmbH & Co. KG. Esplanase 39, 20354 Hamburg, Germany.
[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, 5.4.6006. 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, p. 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 L-tryptophan 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 and experts' knowledge, 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-tryptophan produced using *E. coli* CGMCC 7.267 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 L-tryptophan 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 L-tryptophan produced by fermentation with a genetically modified strain of *E. coli* (CGMCC 7.267). It is intended to be used as a nutritional additive (functional group: amino acids, their salts and analogues) in feed and/or in water for drinking in all animal species and categories.

3.1. Characterisation

3.1.1. Characterisation of the production organism

The additive is produced by a genetically modified strain of *E. coli*, which is deposited in the China General Microbiological Culture Collection Center with deposition number CGMCC 7.267.[10] A bioinformatic analysis of the whole genome sequence [redacted]

The susceptibility of the production strain to relevant antibiotics was demonstrated [redacted] following the requirements of the guidance on microorganisms used as feed additive or as production organisms (EFSA FEEDAP Panel, 2018a,b) [redacted] The minimum inhibitory concentration (MIC) values for the production strain for all antibiotics tested were below or equal to cut-off values defined by the Panel.

No added antimicrobial resistance genes were found in the genome of the production strain by bioinformatic analysis [redacted]

[7] FEED dossier reference: FAD-2017-0038.
[8] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2017-0038-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/Annex 2.2.1.2a.
[redacted]

[redacted]

3.1.2. Manufacturing process

L-Tryptophan is produced [redacted]

The applicant declared that no antibiotics are used in the production process of the product under assessment.[15]

3.1.3. Characterisation of the active substance/additive

L-Tryptophan (International Union of Pure and Applied Chemistry (IUPAC) name: (2*S*)-2-amino-3-(1*H*-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.

[redacted]

[14] Technical dossier/Section 2.3.
[15] Technical dossier/Section II/Annex 2.3m.

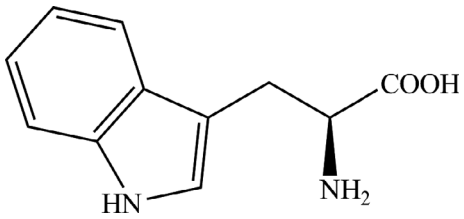

Figure 1: Structural formula of L-tryptophan

The L-tryptophan content of the product is specified as ≥ 98%, the other components being water (≤ 0.5%), and undefined substances (< 1%).[16]

The analysis of five batches of the additive showed an average content of L-tryptophan of 99.4% on 'as is' basis (range 98–100%), water 0.1–0.5%.[17] On a dry matter basis, the amount of identified material was on average 99.7% (range 98.1–100%).

The specific optical rotation of the additive (three batches) ranged –31.2 to –31.8°,[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. Impurities

Three batches of the final product were analysed for heavy metals (lead, cadmium and mercury) and arsenic. Lead and cadmium were below the limit of detection (LOD) (except one batch that had a lead content of 0.02 mg/kg),[20] mercury ranged from 0.004 to 0.009 mg/kg and arsenic from 0.03 to 0.06 mg/kg.

Mycotoxins were analysed in three batches of the final product. Zearalenone, fumonisin (unspecified), deoxinivalenol and citrinin showed concentrations below the limit of quantification (LOQ); ochratoxin A ranged from 16 to 29 μg/kg and aflatoxins (unspecified) ranged from below LOQ to 4.2 μg/kg.[21]

Dioxins (polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)) and dioxin-like polychlorinated biphenyls (PCBs) were analysed in three batches of the final product. The sum of dioxins and dioxin like PCBs (WHO-PCDF/D-TEQ) was < 0.14 ng/kg L-tryptophan.[22] Non-dioxin like PCBs were < 0.13 ng/kg TEQ in each batch. The sum of PCDF/D and PCBs was < 0.27 ng/kg L-tryptophan in each batch.

Analysis of microbial contamination of the final product (three batches) indicated that *Salmonella* spp. was absent (in 25-g samples); Enterobacteriaceae, *E. coli,* yeasts and filamentous fungi were not detected in 1 g samples.[23]

The endotoxin activity (three batches analysed by *Limulus* Amoebocyte Lysate test) ranged from 1.35 to 4.56 IU/mg.[24]

The concentrations of 1,1′-ethylidene-bis-L-tryptophan (EBT) and 1-methyl-1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acid (MTCA) were measured in three batches of the additive. EBT was ≤ 10 mg/kg in all cases. MTCA concentrations ranged from 1 to 2 mg/kg.[25]

Viable cells of the production strain were not detected [redacted]

[redacted]

[16] Technical dossier/Section 2.1.3.
[17] Technical dossier/Section II/Annex 2.1.3b and Supplementary information August 2018/Annex Q5b. Tryptophan analysed by method ISO 13903.
[18] Technical dossier/Supplementary information August 2018/Annex Q7.
[19] European Pharmacopoeia monograph 1/2017:1272.
[20] Technical dossier/Section II/Annex 2.1.4a. LOD in mg/kg were 0.01 for lead and 0.002 for cadmium.
[21] Technical dossier/Section II/Annex 2.1.4a. LOQ (in μg/kg) was 1.7 for aflatoxins, 17 for zearalenone, 25 for fumonisin, 134 for deoxynivalenol and 15 for citrinin.
[22] Technical dossier/Section II/Annex 2.1.4g.
[23] Technical dossier/Section II/Annex 2.1.4a and supplementary information August 2018/Annex Q8.
[24] Technical dossier/Section II/Annex 2.1.4e.
[25] Technical dossier/Supplementary information August 2018/Annexes Q9a to Q9d (analysed using the European Pharmacopoeia method).

[redacted]

[redacted] However, since the total of the colonies was not checked for confirmation, uncertainty remained on the presence of the production strain.

The applicant provided a second set of information. The presence of the production strain was tested in three batches of the additive in triplicate. [redacted] Therefore, the absence of viable cells of the production strain could not be established.

The applicant provided a third set of information. The presence of viable cells of the production strain in the final product was investigated in three batches of the final product in triplicate. [redacted]

Considering the results of those three tests, the FEEDAP Panel concludes that the data indicate that no viable cells of the production strain are present in the additive.

The absence of recombinant DNA of the production strain was confirmed in three samples [redacted]

3.1.3.2. Physical characteristics

The product under assessment is a light yellow crystalline powder with light odour.[30] It has a pH of 5.7 in 10% solution in water at 20°C, and a density of 600–650 kg/m^3.[31]

The particle size distribution (analysed in three batches of the additive by laser diffraction) showed that all particles were < 100 μm diameter. The percentages of particles having a diameter < 50 and < 10 μm ranged from 83% to 94% and from 16% to 32% (v/v), respectively.[32] The dusting potential (the same three batches analysed by the Stauber–Heubach method) ranged from 5.9 to 29.2 g/m^3.[33]

3.1.3.3. Stability and homogeneity

The shelf life of three batches of the additive (packed in sealed plastic bags protected from light) was tested at 25°C during 12 months (standard conditions) and at 40°C during 6 months (accelerated conditions).[34] Losses were up to 4% and 3% under the standard and accelerated conditions, respectively.

The stability of three batches of the additive in a vitamin mineral premixture (with choline chloride 16 g/kg)[35] at a supplementation rate of 10% was studied when stored (packed in sealed plastic bags protected from light) at room temperature for 6 months. The losses observed ranged from 1% to 5% after the 6-month period.

The stability of the additive in a complete feed for piglets (based on barley, heat treated soybeans and soybean meal, background tryptophan content: 0.19%), at a supplementation rate of 0.45%, was tested (three additive's batches) after storage in sealed plastic bags protected from light, at room temperature for 3 months.[36] Mash and pelleted feed were examined. The pelleting temperature was 55–60°C and the pelleting process showed no losses of tryptophan. After the 3-month period, the observed losses in total tryptophan content in mash feed and in pelleted feed were negligible (up to 1.5% in mash feed and up to 3% in pelleted feed).

[redacted]

[30] Technical dossier/Section II/Annex 2.5.2.
[31] Technical dossier/Section 2.1.5.
[32] Technical dossier/Section II/Annex 2.1.5.
[33] Technical dossier/Section II/Annexes 2.4.3a to 2.4.3c.
[34] Technical dossier/Section II/Annex 2.1.4a and supplementary information August 2018/Answers to SIn.
[35] Technical dossier/Section II/Annex 2.4.1a and 2.4.1c.
[36] Technical dossier/Section II/Annex 2.4.1b and 2.4.1c and supplementary information August 2018/Answers to SIn.

The stability of three batches of the additive in water for drinking was tested at a concentration of 0.5% when stored at room temperature for 24 h[37]; no losses were observed. The FEEDAP Panel notes that the minimum duration of the stability of the additive in water for drinking should last a minimum of 48 h.

The capacity of the additive to distribute homogeneously in a pelleted feed for piglets supplemented with 0.5% tryptophan was studied by analysing 10 subsamples. The coefficient of variation was 2%.[38]

3.1.4. Physico-chemical 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-tryptophan will 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 feedingstuffs or complementary feed 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 unsupplemented diet. The additive is also proposed to be used via water for drinking. The applicant recommends that special care is taken to avoid amino acids unbalance if it is added to feed and to water for drinking at the same time.[39]

3.2. Safety

3.2.1. Safety aspects of the genetic modification

The recipient organism *E. coli* K-12 MG1655 is considered to be safe (see Section 3.1.1). The production strain CGMCC 7.267 [redacted] traits do not raise any safety concern and therefore the production strain CGMCC 7.267 is considered to be safe.

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

The L-tryptophan requirements of the target animal species and the safety of the use 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 (> 99% tryptophan on average and less than 1% 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 of the additive ranged from 1.35 to 4.56 IU/mg. These values are very low compared with ca. 1,000 IU/mg commonly found in feedingstuffs (Cort et al., 1990). Therefore, at the usual conditions of use of the additive in feed, the endotoxins added by the additive would be insignificant compared to the background in feed. Since the identity of the production strain has been established as an *E. coli* K12 derivative, it is susceptible to the relevant antimicrobials used in human and veterinary medicine; and there are no viable cells or recombinant DNA of the production strain in the final product, L-tryptophan produced by *E. coli* CGMCC 7.267 is considered safe for non-ruminant species provided that it is supplemented in appropriate amounts to satisfy the animal requirements of the diets.

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.

[37] Technical dossier/Section II/Annex 2.1.4a.
[38] Technical dossier/Section II/Annex 2.4.1c.
[39] Technical dossier/Section 2.5.1.

The FEEDAP Panel recommended in a previous statement that amino acids, their salts and analogues should generally not be used in water for drinking because of the risk of imbalances and for hygiene reasons (EFSA FEEDAP Panel, 2010).

The absorption and metabolic fate of L-tryptophan 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 their 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. 1,10-ethylidene-bis-L-tryptophan (EBT) and 1-methyl-1,2,3,4-tetrahydro-betacarboline-3-carboxylic acid (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 and MTCA were $\leq$ 10 mg/kg additive and $<$ 2 mg/kg additive, respectively, and do not represent a safety concern, according 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 and 390 mg/kg, respectively.

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 amino acids in water for drinking, when given in addition to complete diets with a well-balanced amino acid profile, might disturb the nitrogen balance and increase nitrogen excretion via urine. The use of the product L-tryptophan in animal nutrition would not lead to any localised increase in the concentration in the environment. The production strain and its recombinant DNA were not detected in the final product. The product does not pose any environmental safety concern associated with the genetic modification of the production strain.

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

The use of L-tryptophan produced using *E. coli* CGMCC 7.267 in supplementing feed to compensate for 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 tryptophan is used in ruminants. The use of L-tryptophan produced by fermentation with *E. coli* CGMCC 7.267 in animal nutrition is considered safe for the consumers and for the environment.

3.2.3. Safety for user

No studies have been submitted to support the safety of the additive for users.

3.2.3.1. Effects on the respiratory system

Dusting potential up to 29.2 g/m^3 was measured. The additive contains a significant fraction of particles $<$ 50 (up to 94% v/v) and $<$ 10 μm (up to 32% v/v). In addition, the endotoxin activity measured was up to 4.56 IU/mg.

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, 2012EFSA FEEDAP Panel, 2014b) is described in the 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 on the calculation of the potential endotoxin content in dust, the inhalation exposure could be up to 73,973 endotoxin IU per working day, indicating thus an inhalation risk to endotoxins for persons handling the additive.

3.2.3.2 Conclusions on safety for the user

The level of endotoxins in the product and its dusting potential indicate an inhalation risk for the user. In the absence of data, the FEEDAP Panel cannot conclude on the potential of the additive to be irritant to skin and eyes or to be a skin sensitiser.

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 L-tryptophan is regarded as an effective source of the amino acid L-tryptophan.

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

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.

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[40] and Good Manufacturing Practice.

4. Conclusions

The production strain *E. coli* CGMCC 7.267 is safe for the production of L-tryptophan. No viable cells or DNA of the production strain were detected in the additive under assessment.

The use of L-tryptophan produced using *E. coli* CGMCC 7.267 in supplementing feed to compensate for 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 tryptophan is used in ruminants.

The use of L-tryptophan produced by fermentation with *E. coli* CGMCC 7.267 in animal nutrition is considered safe for the consumers and for the environment.

The endotoxin activity in the product and its dusting potential indicate an inhalation risk for the user. In absence of data, the FEEDAP Panel cannot conclude on the potential of the additive to be irritant to skin and eyes or to be a skin sensitiser.

The additive L-tryptophan produced using *E. coli* CGMCC 7.267 is regarded as an effective source of the amino acid L-tryptophan. In order to be as efficacious in ruminants as in non-ruminants, it should be protected from ruminal degradation.

5. Recommendation

It is recommended that the specification of the additive complies with the European Pharmacopeia with regard to L-tryptophan impurities.

Documentation as provided to EFSA/Chronology

Date	Event
06/07/2017	Dossier received by EFSA. L-Tryptophan produced using *Escherichia coli* CGMCC 7.267. Submitted by Welding GmbH & Co. KG
31/07/2017	Reception mandate from the European Commission
04/10/2017	Application validated by EFSA – Start of the scientific assessment
07/12/2017	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 strain, characterisation of the additive, stability and safety for the user*
08/01/2018	Comments received from Member States
02/02/2018	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
28/08/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
26/10/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – *Issues: Characterisation of the additive*
04/01/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
12/02/2019	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 additive*
12/04/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
25/04/2019	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*
09/12/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
28/01/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

[40] 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.

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Abbreviations

CAS	Chemical Abstracts Service
CGMCC	China General Microbiological Culture Collection Center
DECOS	Dutch Expert Committee on Occupational Safety
DM	dry matter
EBT	1,1′-ethylidene-bis-L-tryptophan
EINECS	European Inventory of Existing Commercial Chemical Substances
EURL	European Union Reference Laboratory
FCC	Food Chemical Codex
FD	fluorescence detection
HPLC	high performance liquid chromatography
HSE	UK Health and Safety Executive
IUPAC	International Union of Pure and Applied Chemistry
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
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzodioxin
PCDF	polychlorinated dibenzofuran
TEQ	toxic equivalent
VKM	Norwegian Scientific Committee for Food Safety
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 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/m^3, then the content of endotoxins of the dust, c IU/m^3, is obtained by simple multiplication, a × b. This resulting value is further used for calculation of the potential exposure by inhalation 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* CGMCC 7.267, 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	4,560	Technical dossier
	b	Dusting potential (g/m^3)	29.2	Technical dossier
a × *b*	*c*	Endotoxin content in the air (IU/m^3)	133,152	
	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 eight-hour 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 eight-hour working day**	**73,973**	
	m	Health-based recommended exposure limit of endotoxin (IU/m^3) per eight-hour working day	90	HCN (2010)

Calculation	Identifier	Description	Amount	Source
$m \times j$	n	**Health-based recommended exposure limit of total endotoxin exposure (IU) per eight-hour working day**	**900**	
//10		Endotoxins inhaled (IU) per eight-hour working day reduced by filter mask FF P2 (reduction factor 10)	7,397	
//20		Endotoxins inhaled (IU) per eight-hour working day reduced by filter mask FF P3 (reduction factor 20)	3,699	

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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 methods of analysis for L-tryptophan produced using *Escherichia coli* CGMCC 7.267

In the current application authorisation is sought under Article 4(1) for L-tryptophan produced by *Escherichia coli* CGMCC 7.267, 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-Tryptophan is already authorised as feed additive under Commission Directive 88/485/EEC.

For the quantification of L-tryptophan in the feed additive, premixtures, feedingstuffs and water the Applicant submitted the ring-trial validated Community method. This method can be applied for the determination of the amino acid in feedingstuffs only, using High Performance Liquid Chromatography (HPLC) coupled with fluorescence detection (FD). However, the EURL previously evaluated (i) the ring-trial validated Community method for the quantification of L-tryptophan in feedingstuffs; and (ii) the ring-trial validated EN ISO 13904:2016 method for the quantification of L-tryptophan in feed additive and premixtures (containing more than 2% of tryptophan). Based on the performance characteristics available, the EURL recommends for official control these two ring-trial validated methods to quantify tryptophan in the feed additive, premixtures and/or feedingstuffs. In addition, the EURL identified the "L-tryptophan monograph" of the Food Chemical Codex (FCC) for the identification of the feed additive.

In the frame of the stability studies, the Applicant presented experimental data obtained analysing tryptophan in water with the VDLUFA official method based on HPLC-FD for the determination of tryptophan in feed. The results presented are considered sufficient to demonstrate the suitability of the method for the analysis of the amino acid in water. Hence the EURL recommends for official control this method to quantify tryptophan in water.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.