Adopted: 12 March 2024

DOI: 10.2903/j.efsa.2024.8707

SCIENTIFIC OPINION

Safety and efficacy of a feed additive consisting of L-tryptophan (produced with *Escherichia coli* CGMCC 7.460) for all animal species (Kempex Holland B.V.)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) | **Vasileios Bampidis** | **Giovanna Azimonti** | **Maria de Lourdes Bastos** | **Henrik Christensen** | **Mojca Durjava** | **Birgit Dusemund** | **Maryline Kouba** | **Marta López-Alonso** | **Secundino López Puente** | **Francesca Marcon** | **Baltasar Mayo** | **Alena Pechová** | **Mariana Petkova** | **Fernando Ramos** | **Roberto Edoardo Villa** | **Ruud Woutersen** | **Lieve Herman** | **Montserrat Anguita** | **Matteo Lorenzo Innocenti** | **Jordi Tarrés-Call** | **Elisa Pettenati**

Correspondence: feedap@efsa.europa.eu

Abstract

Following a request from the European Commission, EFSA 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* CGMCC 7.460 when used as a nutritional additive in feed and water for drinking for all animal species and categories. The production strain is not genetically modified. Viable cells of the production strain were not detected in the final additive. The additive does not give rise to any safety concern regarding the production strain. The use of L-tryptophan (≥ 98%) produced with *E. coli* CGMCC 7.460 to supplement feed 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 EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has concerns on the safety of the simultaneous oral administration of L-tryptophan via water for drinking and feed due to possible amino acid imbalances and hygienic reasons. The use of L-tryptophan produced with *E. coli* CGMCC 7.460 in animal nutrition raises no safety concerns to consumers of animal products and to the environment. In the absence of data, the FEEDAP Panel cannot conclude on the potential of the additive to be irritant to skin or eyes, or on its potential to be a dermal sensitiser. The endotoxin activity of the additive in combination with the high dusting potential may represent a risk of exposure by inhalation to endotoxins for users. The additive L-tryptophan is regarded as an effective source of the amino acid L-tryptophan for all non-ruminant species. To be as efficacious in ruminants as in non-ruminants, it should be protected from ruminal degradation.

KEYWORDS

amino acid, efficacy, *Escherichia coli* CGMCC 7.460, L-tryptophan, nutritional additive, safety

CONTENTS

1 | INTRODUCTION

1.1 | Background and Terms of Reference

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

The European Commission received a request from Kempex Holland B.V.[2] for the authorisation of the additive consisting of L-tryptophan (produced with *Escherichia coli* CGMCC 7.460), 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 dossier was received on 27 January 2023 and the general information and supporting documentation are available at https://open.efsa.europa.eu/questions/EFSA-Q-2023-00048. The particulars and documents in support of the application were considered valid by EFSA as of 12 July 2023.

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 (produced with *E. coli* CGMCC 7.460), when used under the proposed conditions of use (see **Section 3.1.6**).

1.2 | Additional information

L-Tryptophan produced with *E. coli* CGMCC 7.460 has not been previously authorised as a feed additive in the European Union.

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[3] in support of the authorisation request for the use of L-tryptophan (produced with *E. coli* CGMCC 7.460) as a feed additive.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 12 July 2023 to 12 October 2023 for which the received comments were considered for the assessment.

In accordance with Article 38 of the Regulation (EC) No 178/2002[4] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[5] a non-confidential version of the dossier has been published on Open.EFSA.

According to Article 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 22 January to 12 February 2024 for which no comments were received.

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

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substance in animal feed.[6]

[1]Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

[2]Kempex Holland B.V., Zeelandsedijk 15, 5408 SL, Volkel, The Netherlands.

[3]Dossier reference: FEED-2022-12430.

[4]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.

[5]Decision https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements

[6]Evaluation report received on 11/09/2023 and available on the EU Science Hub https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-tryptophan (produced with *E. coli* CGMCC 7.460) is in line with the principles laid down in Regulation (EC) No 429/2008[7] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019), EFSA statement on the requirements for whole-genome sequence analysis of microorganisms intentionally used in the food chain (EFSA, 2021) and Guidance on the assessment of the safety of feed additives for the users (EFSA FEEDAP Panel, 2023).

3 | ASSESSMENT

The L-tryptophan under assessment is produced by fermentation with a non-genetically modified strain of *E. coli* (CGMCC 7.460) and it is intended to be used as a nutritional additive (functional group: amino acids, their salts and analogues) in feed and water for drinking[8] for all animal species.

3.1 | Characterisation

3.1.1 | Characterisation of the production organism

The production strain is a non-genetically modified derivative of *E. coli* K12 [redacted], deposited in the China General Microbiological Culture Collection Center with accession number CGMCC 7.460.[9] The production strain was obtained from [redacted].[10]

The taxonomic identification of the production strain as *E. coli* K12 derivative was confirmed [redacted].[11] *E. coli* K-12 is well characterised, its safety (non-pathogenicity) has been documented (Gorbach, 1978) and its ineffectiveness in colonising the human gut is reported (Smith, 1975).

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, 2018b).[12] 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 all relevant antimicrobials.

The WGS data of the production strain were interrogated for the presence of antimicrobial resistance (AMR) genes [redacted].[13] The search evidenced [redacted] above the thresholds set by EFSA (EFSA, 2021), [redacted] (EFSA BIOHAZ Panel, 2023), and therefore, it can be concluded that no hits of concern were identified.

The WGS data of the production strain were also interrogated for the presence of toxin and virulence determinant genes [redacted].[14] [redacted]. No hits of concern were identified.

[7]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.

[8]RFI 240129 Q3 Statement conditions of use.

[9]2.2.1.2.a Certificate of deposition CGMCC 7.460.

[10]2.2.1.2.b Mutation screening method of tryptophan producing strain.

[11]2.2.1.2.c Bioinformatic report T1446R1451 rev.

[12]2.2.2.2_MIC_T1446R1432_2022.

[13]2.2.1.2.c Bioinformatic report T1446R1451 rev.

[14]2.2.1.2.c Bioinformatic report T1446R1451 rev.

3.1.2 | Manufacturing process

L-Tryptophan is produced by fermentation with *E. coli* CGMCC 7.460.[15] [redacted].

The applicant declared that no antimicrobials are used in the manufacturing process.[16]

3.1.3 | Characterisation of the additive

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.

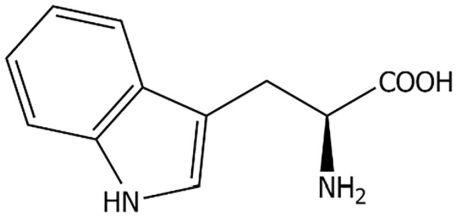

FIGURE 1 Structural formula of L-tryptophan.

According to the specification, the product contains ≥ 98% L-tryptophan on a dry matter (DM) basis and ≤ 0.5% water.

The analysis of five batches of the additive showed an average content of L-tryptophan of 99.5% on 'as is' basis (range 98.7%–101.1%).[17] Moisture was 0.3% and ash ranged 0.2%–0.3%. On a DM basis, the L-tryptophan content was on average 99.8% (99.2%–101.5%), the amount of unidentified material in DM basis was < 1%.

The specific optical rotation was measured in three batches of the additive and ranged from −30.8 to −31.9°.[18] This range is within the reference values established for L-tryptophan in the European Pharmacopoeia (−30.0 to −33.0°) and confirmed the L-enantiomer of tryptophan.

Three batches of the additive were analysed for the presence of arsenic (< limit of quantification (LOQ) to 0.071 mg/kg), cadmium (< LOQ), mercury (< LOQ) and lead (< LOQ to 0.011 mg/kg).[19] Microbiological contamination was analysed in the same three batches and included *Enterobacteriaceae*, *E. coli*, *Salmonella* spp., yeasts and filamentous fungi and none were detected in 25-g samples.[20]

Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar dioxin-like polychlorinated biphenyls (PCBs) were analysed in three batches and found below the corresponding limit of quantification (LOQ).[21] The calculated upper bound concentrations were 0.121 ng WHO-PCDD/F-TEQ/kg for the sum of dioxins and 0.237 ng WHO-PCDD/F-PCB-TEQ/kg for the sum of dioxins and dioxin-like-PCBs (all expressed in 88% dry matter).

The same three batches were analysed for mycotoxins.[22] Aflatoxins (not specified) ranged from 1.6 to 2.3 µg/kg, ochratoxin A, zearalenone, deoxynivalenol, fumonisins (B1 + B2 + B3) and citrinin were below the limit of detection (LOD) of the corresponding methods.

The concentration of 1,1'-ethylidene-bis-L-tryptophan (EBT) and 1-methyl-1,2,3,4-tetrahydro-beta-carboline-3-carboxyl ic acid (MTCA), which are formed during the biotechnological manufacturing process, was measured in three batches of the additive and values were, respectively, < 10 mg/kg (LOD) and < 0.1 mg/kg (LOD) in all cases.[23] The levels of both compounds were below the maximum permitted content of EBT (impurity A) and the sum of all other impurities (B-L, including MTCA) in L-tryptophan which are 10 mg/kg and 390 mg/kg, respectively (European Pharmacopoeia 11th edition (2023)).

The FEEDAP Panel considers that the microbial contamination and the amounts of the above-detected impurities do not raise safety concerns.

[15] 2.3.1 a Flow chart of L-Tryptophan manufacturing process dc and 2.3.1.c Amount of each used in the main fermentation Trp.

[16] 2.3.1.b Statement letter Absence Antibiotics.

[17] 2.1.3 BtB 132447. Method of analysis in accordance with Commission Regulation 152/2009 Appendix III, method G and F.

[18] Optical rotation tryptophan.

[19] 2.1.3 BtB 132447 and Sect_2.6. LOQ in mg/kg were: 0.01 for arsenic and lead; 0.002 for cadmium and mercury.

[20] 2.1.3 BtB 132447 and Sect_2.6.

[21] 2.1.4.a_Dioxins. Upper bound concentrations are calculated on the assumption that all values of the different congeners below the limit of quantification are equal to the limit of quantification. TEQ = toxic equivalency factors for dioxins, furans and dioxin-like PCBs established by WHO in 2005 (van den Berg et al., 2006).

[22] 2.1.3 BtB 132447 and RFI 240129 Q1 Statement LOD ochratoxin. LOD were, respectively, 0.003 mg/kg for lead and arsenic; 0.0006 mg/kg for mercury and cadmium; 134 µg/kg for deoxynivalenol; 17 µg/kg for zearalenone; 25 µg/kg for fumonisins; 1.8 for aflatoxins; 2.8 for ochratoxin A; 15 µg/kg for citrinin.

[23] 2.1.4.d EBT MTCA. EBT Analysed according to European Pharmacopoeia (2017) monograph 01/2017:1272; MTCA analysed by HPLC with fluorescent detector.

Endotoxin activity was analysed in three batches of the final product, and in all cases, the result was < 300 IU/g.[24]

The presence of viable cells of the production strain was investigated in three batches of the additive, each tested in triplicate.[25] Controls were included. [redacted]. No viable cells of the production strain were detected in any of the samples.

3.1.4 | Physical properties of the additive

The additive appears as white to yellowish crystals or a crystalline powder.[26] The reported bulk density was 300 kg/m^3 and the solubility in water was 11.4 g/L (at 25°C).[27]

The dusting potential of three batches of the additive was determined using the Stauber-Heubach method and showed values ranging from 10.1 to 12.9 g/m^3.[28]

3.1.5 | Stability and homogeneity

The shelf-life of the additive (three batches) was tested at room temperature when stored in typical packaging bags, protected from light or moisture, for 6 months.[29] No losses were observed.

The stability of the additive (three batches) in a premixture for piglets was studied when supplemented at an inclusion rate of 4%.[30] The samples were stored at room temperature in paper bags protecting from light, for 6 months. Losses observed ranged from 8% to 14%.

The stability of the additive (three batches) was studied in a complete feed, when supplemented at 0.2%. The basal diet consisted of [redacted].[31] Mash and pelleted feed were tested after storage at room temperature in paper bags protecting from light, for 3 months. Pelleting the mash feeds at 73°C–75°C resulted in no losses. After 3 months storage, losses ranged between 5% and 7.3% and between 4.8% and 4.9% for mash and pelleted feed, respectively.

The stability of the additive (three batches) in water for drinking was studied when supplemented at an inclusion rate of 0.2%.[32] The samples were stored at 20°C for 48 h. Losses observed ranged from 1.5% to 2.9%.

The pelleted feed described above was used to study the capacity of the additive to distribute homogeneously in feed.[33] Total tryptophan (protein-bound + free tryptophan) was analysed in 10 subsamples and resulted in a coefficient of variation (CV) of 2%. When the background content of tryptophan in the complete feed (protein bound tryptophan, 0.23%) was subtracted from the total tryptophan amount of each individual subsample, the resulting CV was 5%.

3.1.6 | Conditions of use

L-Tryptophan is intended to be used in complete feed for all animal species, directly or through complementary feed, premixtures or water. 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 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, 2015).

Safety concerns on the use of the additive may derive from the amino acid itself, L-tryptophan, and/or on the residues/metabolites derived from the fermentation process.

[24] 2.1.4.b Endotoxins. Gel clot limit test.
[25] 2.1.3 BtB 132447 and 2.1.4.c Absence viable cells T1446R1452_2022.
[26] 2.1.5.b CoAs GBT optical rotation 12.22-L-Tryptophan (COA-5 Batches).
[27] Sect_2.1–2.2.
[28] 2.1.5.a Dust Tryptophan.
[29] 2.1.3 BtB 132447.
[30] 2.4.1.a stability feed premix homogeneity 132,563 and 2.4.1.b CONFID Premix264Piglet_Eng.
[31] 2.4.1.a stability feed premix homogeneity 132,563 and 2.4.1.c CONFID Feed composition RDS_5260.
[32] 2.1.3 BtB 132447.
[33] 2.4.1.a stability feed premix homogeneity 132563.

The additive is produced by fermentation with an *E. coli* K12 derivative, which has been shown not to harbour any gene of concern. Moreover, the resulting product is highly purified (≥ 98% tryptophan and < 1% unidentified material on a DM basis). The endotoxin activity was below 300 IU/g. This value is very low when compared with ca. 1,000,000 IU/g commonly found in feedingstuffs (Cort et al., 1990).

For non-ruminant species, the FEEDAP Panel considers that the use of the additive is safe when added to supplement the diets with appropriate amounts to satisfy 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). Finally, the FEEDAP Panel reiterates its 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). Moreover, it may result in an increased nitrogen excretion via urine. Therefore, the FEEDAP Panel has concerns on the safety of the simultaneous oral administration of tryptophan-containing additives via feed and water for drinking.

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 analysed concentrations of EBT in L-tryptophan produced with *E. coli* CGMCC 7.460 were shown to be < 10 mg/kg additive and those of MTCA < 1 mg/kg (see Section 3.1.3). Therefore, the Panel considers that the use of the additive in animal species is safe for the consumer.

The amino acid L-tryptophan is a physiological and natural component of animals and plants. When consumed, it will be absorbed, and the non-absorbed fraction will be incorporated into the intestinal microbial mass and excreted as such. The use of amino acids in water for drinking, when given in addition to complete diets with a well-balanced amino acid profile, would disturb the nitrogen balance and increase nitrogen excretion via urine. The use of the additive L-tryptophan in animal nutrition would not lead to any localised increase in the concentration in the environment. The use of L-tryptophan produced with *E. coli* CGMCC 7.460 as a feed additive does not represent a risk to the environment.

3.2.1.1 | *Conclusions on the safety for the target species, consumers and the environment*

The use of L-tryptophan produced with *E. coli* CGMCC 7.460 to supplement feed is safe for non-ruminant species. There may be a risk for an increased production of toxic metabolite skatole when unprotected tryptophan is used in ruminants. The FEEDAP Panel has concerns on the safety of the simultaneous use of L-tryptophan via water for drinking and feed due to possible amino acid imbalances and hygienic reasons.

The use of L-tryptophan produced by fermentation with *E. coli* CGMCC 7.460 in animal nutrition is considered safe for the consumers and for the environment.

3.2.2 | Safety for the user

No specific data on inhalation toxicity, skin/eye irritation or dermal sensitisation were submitted to support the safety of the additive under assessment for the user. The highest measured dusting potential of the additive was 12.9 g/m^3 (see Section 3.1.4). Hence, the users can be exposed to the additive by inhalation.

The bacterial endotoxin activity (analysed in three batches) was < 300 IU/g in any batch tested. The scenario used to estimate the exposure of users to endotoxins in the dust was based on the EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012) and considered a worst-case scenario in which the endotoxin activity was set at 300 IU/g. 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 considering an endotoxin activity of 300 IU/g, the estimated inhalation exposure would be 2150 endotoxin IU per working day, indicating that inhalation exposure to endotoxins for users may be above the recommended threshold.

In the absence of data, the FEEDAP Panel cannot conclude on the potential of the additive to be irritant to skin or eyes, or on its potential to be a dermal sensitiser. The endotoxin activity of the additive in combination with the high dusting potential may represent a risk of exposure by inhalation to endotoxins for users.

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 (2014). Supplemental L-tryptophan is degraded by ruminal microbiota if not given in a protected form.

3.4 | Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[34] and good manufacturing practice.

4 | CONCLUSIONS

The use of L-tryptophan produced with *E. coli* CGMCC 7.460 in feed is safe for non-ruminant target species. There may be a risk for an increased production of the toxic metabolite skatole when unprotected tryptophan is used in ruminants. The FEEDAP Panel has concerns on the safety for the target species resulting from the simultaneous oral administration of L-tryptophan via water for drinking and feed due to possible amino acid imbalances and hygienic reasons.

The use of L-tryptophan produced by fermentation with *E. coli* CGMCC 7.460 in animal nutrition is considered safe for the consumers and for the environment.

In the absence of data, the FEEDAP Panel cannot conclude on the potential of the additive to be irritant to skin or eyes, or on its potential to be a dermal sensitiser. The endotoxin activity of the additive in combination with the high dusting potential may represent a risk of exposure by inhalation to endotoxins for users.

The feed additive consisting of L-tryptophan produced by fermentation with *E. coli* CGMCC 7.460 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.

ABBREVIATIONS

DECOS	Dutch Expert Committee on Occupational Safety
DM	Dry matter
EBT	1,1'-ethylidene-bis-L-tryptophan
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
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
PCBs	polychlorinated biphenyls
PCDDs	Polychlorinated dibenzo-p-dioxins
PCDFs	polychlorinated dibenzofurans
WGS	whole-genome sequence

ACKNOWLEDGEMENTS

The Panel wishes to thank the following for the support provided to this scientific output (in alphabetical order of the last name): FEEDAP Working Group of Microbiology and Jaume Galobart.

CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

European Commission

QUESTION NUMBER

EFSA-Q-2023-00048

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

PANEL MEMBERS

Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Mojca Durjava, Birgit Dusemund, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, 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 EFSA. The full output has been shared with the European Commission, EU Member States (if applicable) and the applicant. The blackening may be subject to review once the decision on the confidentiality requests is adopted by EFSA and in case it rejects some of the confidentiality requests.

REFERENCES

Cort, N., Fredriksson, G., Kindahl, H., Edqvist, L. E., & 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 (European Food Safety Authority). (2021). EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain. *EFSA Journal*, *19*(7), 6506. https://doi.org/10.2903/j.efsa.2021.6506

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Chemaly, M., De Cesare, A., Hilbert, F., Lindqvist, R., Nauta, M., Nonno, R., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Cocconcelli, P. S., Suarez, J. E., … Herman, L. (2023). Statement on how to interpret the QPS qualification on 'acquired antimicrobial resistance genes'. *EFSA Journal*, *21*(10), 8323. https://doi.org/10.2903/j.efsa.2023.8323

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances Used in Animal Feed). (2010). Scientific opinion on the use of feed additives authorised/applied for use in feed when supplied via water. *EFSA Journal*, *8*(12), 1956. https://doi.org/10.2903/j.efsa.2010.1956

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*, *10*(1), 2539. 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*, *11*(10), 3368. https://doi.org/10.2903/j.efsa.2013.3368

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2014). 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*, *12*(5), 3673. https://doi.org/10.2903/j.efsa.2014.3673

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-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*, *13*(2), 4015. https://doi.org/10.2903/j.efsa.2015.4015

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Innocenti, M. L. (2017a). Guidance on the assessment of the safety of feed additives for the consumer. *EFSA Journal*, *15*(10), 5022. 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, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Innocenti, M. L. (2017b). Guidance on the identity, characterisation and conditions of use of feed additives. *EFSA Journal*, *15*(10), 5023. 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, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Martino, L. (2017c). Guidance on the assessment of the safety of feed additives for the target species. *EFSA Journal*, *15*(10), 5021. 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, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Martino, L. (2018a). Guidance on the assessment of the efficacy of feed additives. *EFSA Journal*, *16*(5), 5274. https://doi.org/10.2903/j.efsa.2018.5274

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Galobart, J. (2018b). Guidance on the characterisation of microorganisms used as feed additives or as production organisms. *EFSA Journal*, *16*(3), 5206. https://doi.org/10.2903/j.efsa.2018.5206

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis, V., 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., Ramos, F., Sanz, Y., Villa, R. E., Woutersen, R., Brock, T., de Knecht, J., … Azimonti, G. (2019). Guidance on the assessment of the safety of feed additives for the environment. *EFSA Journal*, *17*(4), 5648. 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, M. L., Christensen, H., Durjava, M., Dusemund, B., Kouba, M., López-Alonso, M., López Puente, S., Marcon, F., Mayo, B., Pechová, A., Petkova, M., Ramos, F., Villa, R. E., Woutersen, R., Brantom, P., Chesson, A., … Galobart, J. (2023). Guidance on the assessment of the safety of feed additives for the users. *EFSA Journal*, *21*(11), e8469. https://doi.org/10.2903/j.efsa.2023.8469

European Pharmacopoeia, 11th Edition. (2023). *European Directorate for the Quality of Medicines and Health*.

Gorbach, S. L. (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, A. C., Bradley, B. J., Yokoyama, M. T., Carlson, J. R., & Dickinson, E. O. (1979). 3-Methylindole and naturally occurring acute bovine pulmonary edema and emphysema. *American Journal of Veterinary Research*, *40*(10), 1398–1401.

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

Hertzman, P. A., Blevins, W. L., Mayer, J., Greenfield, B., Ting, M., & Gleich, G. J. (1990). Association of the eosinophilia myalgia 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* (p. 41). HSE.

Smith, H. W. (1975). Survival of orally administered *E. Coli* K-12 in alimentary tract of man. *Nature*, *255*, 500–502.

Van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., … Peterson, R.E. (2006). The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicological Sciences*, *93*(2), 223–241. https://doi.org/10.1093/toxsci/kfl055

How to cite this article: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis, V., Azimonti, G., Bastos, M. L., Christensen, H., Durjava, M., Dusemund, B., Kouba, M., López-Alonso, M., López Puente, S., Marcon, F., Mayo, B., Pechová, A., Petkova, M., Ramos, F., Villa, R. E., Woutersen, R., Herman, L., Anguita, M., … Pettenati, E. (2024). Safety and efficacy of a feed additive consisting of L-tryptophan (produced with *Escherichia coli* CGMCC 7.460) for all animal species (Kempex Holland B.V.). *EFSA Journal*, *22*(4), e8707. https://doi.org/10.2903/j.efsa.2024.8707