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Safety and efficacy of L-threonine produced using *Escherichia coli* CGMCC 13325 as a feed additive for all animal species

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Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on L-threonine produced by fermentation when used as a nutritional additive in feed and water for drinking for all animal species and categories. The product under assessment is L-threonine produced using a genetically modified strain of *E. coli* CGMCC 13325. The Panel notes that three out of five batches of the additive do not comply with the minimum specification of 98.5% L-threonine on a dry matter basis proposed by the applicant. The production strain and its DNA were not detected in the final additive. Therefore, the final product does not give raise to any safety concern regarding the genetic modification of the production strain. The use of L-threonine produced using *E. coli* CGMCC 13325 in supplementing feed to compensate for threonine deficiency in feedingstuffs is safe for the target species. The FEEDAP Panel identified risks of nutritional imbalances and hygienic concerns for amino acids when administered simultaneously in feed and in water for drinking. The use of L-threonine produced by fermentation using *E. coli* CGMCC 13325 in animal nutrition is considered safe for the consumers and for the environment. There is a risk from the inhalation exposure to endotoxins for persons handling the additive. In the absence of data, the FEEDAP Panel cannot conclude on the potential of L-threonine produced using *E. coli* CGMCC 13325 to be a skin or eye irritant or a skin sensitiser. The additive under assessment is regarded as an effective source of the amino acid L-threonine for all non-ruminant species. For the supplemental L-threonine to be as efficacious in ruminants as in non-ruminant species, it requires protection against degradation in the rumen.

Keywords: L-threonine, nutritional additive, amino acid, safety, efficacy, *Escherichia coli* CGMCC 13325, genetically modified organism

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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 Kempex Holland B.V.[2] for authorisation of the product L-threonine produced using *Escherichia coli* CGMCC 13325, 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 19 May 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 product L-threonine produced using a genetically modified strain of *Escherichia coli* CGMCC 13325, when used as nutritional additive in feed and in water for drinking under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

L-Threonine produced by several different strains of *Escherichia coli* (minimum content of 98% on dry matter (DM) basis) is currently authorised as a nutritional feed additive for use in all animal species.[3] The product under assessment, L-threonine produced by the genetically modified strain *E. coli* CGMCC 13325, has not been previously authorised as feed additive in the European Union (EU).

L-Threonine is authorised for use in food,[4] cosmetics[5] and as a veterinary medicinal product.[6,7]

L-Threonine is described in a monograph of the European Pharmacopoeia (MG 01/2017:1049) (European Pharmacopoeia, 10th Edition, 2019).

The Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued ten opinions on the safety and efficacy of L-threonine produced by genetically modified strains of *E. coli* (EFSA FEEDAP Panel, 2013, 2014a,b,c,d, 2015a,b, 2016a,b, 2017a, 2018a, 2019c) or *Corynebacterium glutamicum* (EFSA FEEDAP Panel, 2019a,b).

The Joint FAO/WHO Expert Committee on Food Additives evaluated L-threonine as a food flavouring agent (JECFA; WHO, 2012).

[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] Kempex Holland B.V. Zeelandsedijk 15, 5408 SL, Volkel, The Netherlands.
[3] Commission implementing regulation (EC) 2016/1220 of 26 July 2016 concerning the authorisation of L-threonine produced by *Escherichia coli* as feed additive for all animal species. OJ L 201, 27.7.2016, p. 5.
[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 2006/141/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.
[5] Commission Decision of 9 February 2006 amending Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products. OJ L 97, 5.4.2006, pp. 1–528.
[6] Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. OJ L 15, 20.1.2010, p. 1.
[7] Regulation (EC) No 470/2009 of the European Parliament and of the Council of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and of the Council. OL L 152, 16.6.2009, p. 11.

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[8] in support of the authorisation request for the use of L-threonine produced using *E. coli* CGMCC 13325 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 L-threonine produced using *E. coli* CGMCC 13325 in animal feed. The Executive Summary of the EURL report can be found in Annex A.[9]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-threonine produced using *E. coli* CGMCC 13325 is in line with the principles laid down in Regulation (EC) No 429/2008[10] 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, 2017b), 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 target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017d), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018c) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019d).

3. Assessment

The subject of the present assessment is L-threonine (minimum 98.5%) produced by fermentation with a genetically modified strain of *E. coli* CGMCC 13325. It is intended to be used as a nutritional additive (functional group amino acids, their salts and analogues) in feed and water for drinking 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 *Escherichia coli* K-12 which is deposited in the China General Microbiological Culture Collection Center (CGMCC) with accession number CGMCC 13325.[11]

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

[redacted]

[8] FEED dossier reference: FAD-2020-0017.
[9] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2020-0017-threonine.pdf
[10] 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.
[11] Technical dossier/Section II/Annexes Section II/Annex 2.2.1.2a and Supplementary information July 2020/Annex 1.
[12] 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.

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).[13] All minimum inhibitory concentration (MIC) values were below or equal to the cut off values set in the Guidance and 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][14] This, and the fact that the production strain was not phenotypically resistant to any of the antibiotics tested, indicate that the production strain does not carry acquired antibiotic resistance genes of concern.

The WGS of the production strain was also interrogated for the presence of toxin and virulence determinant genes [redacted][14]

3.1.1.1. Information relating to the genetically modified microorganism

[redacted][15][16][17]

3.1.2. Manufacturing process

L-Threonine is produced by fermentation using *E. coli* CGMCC 13325. [redacted][18][19]

[13] Technical dossier/Section II/Annexes Section II/Annex 2.2.2.2.
[14] Technical dossier/Section II/Annexes Section II/Annex 2.2.1.2c CONFID.
[15] Technical dossier/Supplementary information October 2020/Annex 2.
[16] Technical dossier/Section II/Annexes Section II/Annex 2.2.1.2c CONFID and Annex 2.2.1.2.b CONFID.
[17] Technical dossier/Supplementary information October 2020/Annex 3.
[18] Technical dossier/Section II/Annex 2.3.1a.
[19] Technical dossier/Section II/Annex 2.3.1b

3.1.3. Characterisation of the active substance/additive

L-Threonine (International Union of Pure and Applied Chemistry (IUPAC) name: (2*S*,3*R*)-2-amino-3-hydroxybutanoic acid; synonyms: 2-amino-3-hydroxybutyric acid, α-amino-β-hydroxybutyric acid), a compound identified with the Chemical Abstracts Service (CAS) No 72-19-5 and the European Inventory of Existing Commercial Chemical Substances (EINECS) No 200-774-1, has a molecular weight of 119.12 g/mol. The molecular formula of L-threonine is $C_4H_9NO_3$. The structural formula is given in Figure 1.

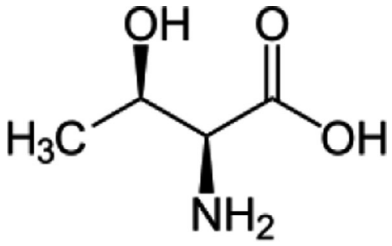

Figure 1: Structural formula of L-threonine

The additive contains by specification ≥ 98.5% L-threonine on a DM basis and < 1% moisture. Analyses of five batches showed an average of 98.3% threonine (range 97.9–98.7%)[20] and the loss on drying was on average 0.06% (range 0–0.1%).[21] Thus, the specification was not reached in three out of the five batches analysed. The amount of unidentified material is > 1%.

The specific optical rotation was measured in three batches of the final product and ranged from –27.7 to –27.8°,[22] which is within the range established for L-threonine in the European Pharmacopoeia (–27.6 to –29.0°) and demonstrates the identity of the L-enantiomer.

3.1.3.1. Impurities

Three batches were analysed for undesirable substances. Regarding heavy metals (cadmium, mercury) and arsenic,[23] all analytical values were under the respective limits of detection (LODs), except for lead that ranged from below LOD to 0.02 mg/kg. Regarding mycotoxins, ochratoxin A, zearalenone, fumonisins (sum of B1, B2 and B3), deoxynivalenol and citrinin were below the LOD, while aflatoxins (unspecified) levels ranged from below the LOD to 0.7 μg/kg.[24]

Dioxins (polychlorinated dibenzofurans (PCDF), polychlorinated dibenzo(p)dioxins (PCDD)) and dioxin-like polychlorinated biphenyls (DL-PCBs) were measured in three batches of the final product and were below the corresponding limits of quantification (LOQ). The levels of PCDD/F and the sum of PCDD/F and DL-PCB (upper limit) were calculated to be < 0.137 ng WHO-TEQ/kg and 0.269 ng WHO-TEQ/kg, respectively in all batches.[25]

The microbiological quality of the product was tested by counting *Salmonella* spp. (in 25 g samples), Enterobacteriaceae, *E. coli* and yeasts. These were not detected in three batches analysed.[26]

The endotoxin activity of the additive was measured in three batches and was found < 30,000 IU/g in all batches.[27]

The presence of viable cells of the production strain in the final product was tested in three batches of the additive.[28] [redacted]

[20] Technical dossier/Section II/Annex 2.1.3 and supplementary information July 2020/Sin 120620 Answers. Analytical method for L-threonine was VDLUFA III, 4.11.1.
[21] Technical dossier/Supplementary information July 2020/Annex 2.
[22] Technical dossier/Section II/Annex 2.1.5d.
[23] Technical dossier/Section II/Annex 2.1.3. LOD in mg/kg was 0.002 for cadmium and mercury, and 0.01 for arsenic and lead.
[24] Technical dossier/Section II/Annex 2.1.3. LOD in μg/kg was 0.1 for aflatoxins, 5 for ochratoxin A, 15 for citrinin, 17 for zearalenone, 25 for fumonisins and 134 for DON.
[25] Technical dossier/Section II/Annex 2.1.4a-c.
[26] Technical dossier/Section II/Annex 2.1.3 and supplementary information July 2020/Annex 2 and Sin 120620 Answers. Methods were according to VDLUFA. LOD was 1 CFU/25 g sample.
[27] Technical dossier/Supplementary information July 2020/Annexes 3a to 3c. Analytical method gel clot: limit test according to European Pharmacopoeia 2.6.14 method A.
[28] Technical dossier/Section II/Annex 2.1.4d.

[redacted] No cells of the production strain were found in three independent batches of the final product.

The presence of DNA from the production strain was tested in three batches of the additive in triplicate.[29] [redacted] No DNA of the production strain was detected.

3.1.3.2. Physical characteristics

The additive is a solid powder, with a density of 600–800 kg/m^3, and its solubility in water at 25°C is 205 g/L.[30]

The dusting potential (three batches) of the final product (Stauber–Heubach method) ranged from 1,700 to 2,000 mg/m^3.[31] The particle size distribution of the final product (three batches) was analysed by laser diffraction. The fractions of particles having a diameter < 100, < 50 and < 10 μm ranged 63–65%, 34–39% and 3–7% (v/v), respectively.[32]

3.1.3.3. Stability and homogeneity

The shelf life of the additive was studied when stored in sealed bags protected from light at 25°C for 12 months and at 40°C for 6 months. Losses at the end of the storage period ranged from 1% to 6% at 25°C and from 1% to 2% at 40°C.[33]

The stability of the additive (three batches) in a vitamin–mineral premixture was studied when supplemented at 4%. The samples were stored in sealed plastic bags, protected from light, at room temperature for 6 months. No losses were observed.[34]

The stability of the additive (three batches) in a complete feed (mash and pelleted) when supplemented at 0.2% was studied. The basal diet consisted on barley and wheat and had a basal threonine content of 0.01%. Pelleting was performed at 78°C and 0.07–0.14 MPa steam pressure and represented a threonine loss ranging from 0% to 6%, depending on the batch considered. The samples were stored at room temperature in sealed plastic bags protected from light for 3 months. No losses were observed in mash or pelleted complete feed.[35]

The stability of the additive (three batches) in water for drinking was studied when supplemented at 0.2%. Samples were stored at room temperature for 48 h. No losses were observed.[33]

The capacity of the additive to distribute homogeneously in feed was studied in 10 subsamples of the pelleted feed described above. The coefficient of variation was 3%.[36]

3.1.4. Conditions of use

It is proposed that L-threonine will be used in feeds for all animal species to achieve an adequate amino acid profile and to meet the L-threonine requirements. It can be added directly to complete or complementary feed, or via a premixture. It is also proposed to use the additive in water for drinking. 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, the water intake and the amino acid composition of the unsupplemented diet.

[29] Technical dossier/Section II/Annex 2.1.4e CONFID.
[30] Technical dossier/Supplementary information July 2020/Sin 120620 Answers.
[31] Technical dossier/Section II/Annexes 2.1.5a-c.
[32] Technical dossier/Section II/Annex 2.1.4a2, Annex 2.1.4b2 and Annex 2.1.4c2.
[33] Technical dossier/Section II/Annex 2.1.3.
[34] Technical dossier/Section II/Annexes 2.4.1a and b and supplementary information July 2020/Sin 120620 Answers.
[35] Technical dossier/Section II/Annexes 2.4.1b-c and supplementary information July 2020/Sin 120620 Answers.
[36] Technical dossier/Section II/Annex 2.4.1b.

3.2. Safety

3.2.1. Safety of the production microorganism

[redacted] The genetic modifications performed to obtain the production strain *E. coli* CGMCC 13325 have the purpose to increase the production of L-threonine. None of the introduced modifications raise a safety concern. The production strain does not carry acquired antimicrobial resistance genes. The production strain and its DNA were not detected in the final additive. Therefore, the final product does not give raise to any safety concern with regard to the genetic modification of the production strain.

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

The L-threonine 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 (> 98% L-threonine on a DM basis). The endotoxin activity was below 30,000 IU/g. Considering a worst-case scenario in which endotoxin activity was up to 30,000 IU/g, these results would be low when compared with ca. 1×10^6 IU/g commonly found in feedingstuffs (Cort et al., 1990). Therefore, at the usual conditions of use of the additive in feed, the oral intake of endotoxins added by the additive would be not relevant compared with the background in feed.

Concerns on the use of the additive would not derive from the L-threonine, which is considered safe but may arise from residues of the fermentation process/production strain remaining in the final product. Although the amount of identified material exceeds 1% on a DM basis, the production strain and the ingredients used in the fermentation medium are considered safe. The production strain was identified as an *E. coli* K12 derivative and no safety concerns related to the genetic modification process were noted. The production strain was susceptible to antimicrobials of clinical human and veterinary relevance, and no viable cells or DNA of the production strain were found in the final product. Consequently, no safety concerns for target animals, consumers of products derived from animals fed the additive and the environment would rise from the fermentation residues that may be present in the final additive.

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 hygienic reasons (EFSA FEEDAP Panel, 2010)

The absorption and metabolic fate of L-threonine in the organism were described in a previous opinion (EFSA FEEDAP Panel, 2013). The amino acid L-threonine, 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-threonine in animal nutrition, which is thus considered safe for the consumer.

The amino acid L-threonine is a physiological and natural component of animal and plant proteins. 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 product L-threonine in animal nutrition would not lead to any localised increase in its concentration in the environment. The use of L-threonine produced by *E. coli* CGMCC 13325 as a feed additive does not pose a risk to the environment.

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

The use of L-threonine produced using *E. coli* CGMCC 13325 in supplementing feed to compensate for threonine deficiency in feedingstuffs is safe for the target species. The FEEDAP Panel has concerns on the safety of the simultaneous oral administration of L-threonine via water for drinking and feed due to possible amino acid imbalances.

The use of L-threonine produced by fermentation using *E. coli* CGMCC 13325 in animal nutrition is considered safe for the consumers and for the environment.

3.2.3. Safety for user

No studies were provided by the applicant to support the safety of L-threonine produced using *E. coli* CGMCC 13325 for users and workers.[30]

3.2.3.1. Safety for the respiratory system

The additive has a dusting potential up to 2,000 mg/m^3 and the particle size distribution shows a proportion of particles having diameters below 100 μm of up to 65%. Thus, the users/workers can be exposed by inhalation.

The bacterial endotoxin activity (analysed in three batches) was < 30,000 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), is described in Appendix A. The threshold for the quantity of inhaled endotoxins per working day is 900 IU, derived from the provisional occupational exposure limits given by the Dutch Expert Committee on Occupational Safety (Health Council of the Netherlands, 2010) and the UK Health and Safety Executive (HSE, 2013). Based on calculations of the content of endotoxins in dust considering an endotoxin activity of 30,000 IU/g (as a worst-case scenario), the estimated exposure would be 33,330 IU per 8-h working day, indicating a risk by inhalation due to exposure to endotoxins for people handling the additive.

3.2.3.2. Conclusions on safety for the user

There is a risk from the inhalation exposure to endotoxins for persons handling the additive. In absence of data, the FEEDAP Panel cannot conclude on the potential of L-threonine produced using *E. coli* CGMCC 13325 to be a skin or eye irritant or a skin sensitiser.

3.3. Efficacy

Efficacy studies are not required for amino acids which naturally occur in the proteins of plants and animals. The nutritional role of L-threonine is well established in the scientific literature. Since most of the studies have been performed with supplemental L-threonine, the product L-threonine, technically pure, is regarded as an effective source of the amino acid L-threonine.

The efficacy of L-threonine for both non-ruminant and ruminant species was described in previous opinions (EFSA FEEDAP Panel, 2013, 2014a). Supplemental L-threonine 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[37] and Good Manufacturing Practice.

4. Conclusions

The Panel notes that three out of five batches of the additive do not comply with the minimum specification of 98.5% L-threonine on a dry matter basis as proposed by the applicant.

The production strain and its DNA were not detected in the final additive. Therefore, the final product does not give raise to any safety concern regarding the genetic modification of the production strain.

The use of L-threonine produced using *E. coli* CGMCC 13325 in supplementing feed to compensate for threonine deficiency in feedingstuffs is safe for the target species. The FEEDAP Panel identified risks of nutritional imbalances and hygienic concerns for amino acids when administered simultaneously in feed and in water for drinking.

The use of L-threonine produced by fermentation using *E. coli* CGMCC 13325 in animal nutrition is considered safe for the consumers and for the environment.

There is a risk from the inhalation exposure to endotoxins for persons handling the additive. In the absence of data, the FEEDAP Panel cannot conclude on the potential of L-threonine produced using *E. coli* CGMCC 13325 to be a skin or eye irritant or a skin sensitiser.

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

The additive under assessment is regarded as an effective source of the amino acid L-threonine for all non-ruminant species. For the supplemental L-threonine to be as efficacious in ruminants as in non-ruminant species, it requires protection against degradation in the rumen.

5. Documentation as provided to EFSA/Chronology

Date	Event
27/02/2020	Dossier received by EFSA. L-Threonine produced using *E. coli* for all animal species. Submitted by Kempex Holland BV
06/05/2020	Reception mandate from the European Commission
19/05/2020	Application validated by EFSA – Start of the scientific assessment
12/06/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterization of the additive, characterization of the production stain, safety for the user*
27/07/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
19/08/2020	Comments received from Member States
19/08/2020	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
09/09/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterization of the production strain*
09/10/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
18/11/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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, A-Physiology Pathology, Clinical Medicine, 37, 130–137.

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 2010;8(12):1956, 9 pp. 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 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-threonine produced by *Escherichia coli* (FERM BP-10942) for all animal species based on a dossier submitted by Ajinomoto Eurolysine SAS. EFSA Journal 2013;11(7):3319, 19 pp. https://doi.org/10.2903/j.efsa.2013.3319

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-threonine (ThreAMINO) produced by *Escherichia coli* K-12 (DSM25086) for all animal species and categories based on a dossier by Evonik Industries A.G. EFSA Journal 2014;12 (2):3564, 2 pp. https://doi.org/10.2903/j.efsa.2014.3564

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-threonine produced by *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):3674, 3 pp. https://doi.org/10.2903/j.efsa.2014.3674

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014c. Scientific Opinion on the safety and efficacy of L-threonine produced by *Escherichia coli* for all animal species, based on a dossier submitted by HELM AG on behalf of Star Lake Bioscience Co. EFSA Journal 2014;12(6):3726, 14 pp. https://doi.org/10.2903/j.efsa.2014.3726

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014d. Scientific Opinion on the safety and efficacy of L-threonine produced by *Escherichia coli* (FERM BP-11383) for all animal species, based on a dossier submitted by Ajinomoto Eurolysine S.A.S. EFSA Journal 2014;12(10):3825, 14 pp. https://doi.org/10.2903/j.efsa.2014.3825

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-threonine produced by *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):4051, 17 pp. https://doi.org/10.2903/j.efsa.2015.4051

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-threonine produced by *Escherichia coli* strains NRRL B-30843, DSM26131, KCCM11133P or DSM 25085 for all animal species based on a dossier submitted by AMAC EEIG. EFSA Journal 2015;13(9):4236, 34 pp. https://doi.org/10.2903/j.efsa.2015.4236

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016a. Scientific opinion on the safety of L-threonine produced by fermentation using *Escherichia coli* CGMCC 3703, for all animal species based on a dossier submitted by GBT Europe GmbH. EFSA Journal 2016;14(1):4344, 13 pp. https://doi.org/10.2903/j.efsa.2016.4344

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016b. Scientific opinion on the safety of L-threonine, technically pure, produced by fermentation with *Escherichia coli* CGMCC7.58 for all animal species based on a dossier submitted by Feedway Europe NV. EFSA Journal 2016;14 (5):4470, 11 pp. https://doi.org/10.2903/j.efsa.2016.4470

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, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wester P, Costa L, Dierick N, Leng L, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Tarrés-Call J and Wallace RJ, 2017a. Scientific Opinion on the safety and efficacy of L-threonine produced by fermentation with *Escherichia coli* CGMCC 11473 for all animal species. EFSA Journal 2017;15(7):4939, 16 pp. https://doi.org/10.2903/j.efsa.2017.4939

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, 2017b. 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, 2017c. 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, 2017d. 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), 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, Cocconcelli PS, Costa L, Dierick N, Flachowsky G, Glandorf B, Herman L, Kärenlampi S, Mantovani A, Saarela M, Anguita M, Tarrés-Call J and Wallace RJ, 2018a. Scientific Opinion on the safety and efficacy of L-threonine produced by fermentation using *Escherichia coli* CGMCC 7.232 for all animal species. EFSA Journal 2018;16(10):5458, 17 pp. https://doi.org/10.2903/j.efsa.2018.5458

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, 2018b. 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, 2018c. 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, Mantovani A, Wallace RJ, Tarrés-Call J and Ramos F, 2019a. Scientific Opinion on the safety and efficacy of L-threonine produced by fermentation with *Corynebacterium glutamicum* ■■■■■ for all animal species. EFSA Journal 2019;17(2):5602, 13 pp. https://doi.org/10.2903/j.efsa.2019.5602

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, Costa L, Dierick N, Flachowsky G, Glandorf B, Herman L, Mantovani A, Saarela A, Anguita M, Tarrés-Call J and Wallace RJ, 2019b. Scientific Opinion on the safety and efficacy of L-threonine produced by fermentation with *Corynebacterium glutamicum* ■■■■■ for all animal species. EFSA Journal 2019;17(3):5603, 14 pp. https://doi.org/10.2903/j.efsa.2019.5603

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, Cocconcelli PS, Glandorf B, Herman L, Saarela M, Anguita M and Mayo B, 2019c. Safety of L-threonine produced by fermentation with *Escherichia coli* CGMCC 11473 as a feed additive for all animal species. EFSA Journal 2019;17(11):5885, 8 pp. https://doi.org/10.2903/j.efsa.2019.5885

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, 2019d. 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

European Pharmacopoeia, 10th Edition, 2019. European Directorate for the Quality of Medicines and Health, Monograph 01/2017:1049.

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.

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.

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

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

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

WHO (World Health Organization), 2012. Evaluation of certain food additives: seventy-sixth report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO technical report series No 974.

Abbreviations

AMR	antimicrobial resistance
BIOHAZ	EFSA Panel on Biological Hazards
CAS	Chemical Abstracts Service
CFU	colony forming unit
CGMCC	China General Microbiological Culture Collection Center
DL-PCB	dioxin-like polychlorinated biphenyls
DM	dry matter
EINECS	European Inventory of Existing Commercial chemical Substances
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
IEC-VIS/FLD	ion-exchange chromatography coupled to post-column derivatisation and optical (visible or fluorescence) detection
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
OECD	Organisation for Economic Co-operation and Development
PCDD	polychlorinated dibenzodioxin
PCDF	polychlorinated dibenzofuran
RSDr	relative standard deviation for repeatability
RSDR	relative standard deviation for reproducibility
WGS	whole genome sequence

Appendix A – Safety of endotoxin activity for the user

The effects of the 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 likely 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, equal to = 800 s/day. With an uncertainty factor of 2, maximum inhalation exposure would occur for 2 × 800 = 1,600 s (0.444 h per 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 endotoxin content of the product (the dusting potential of the product, expressed in g/m^3; 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 used 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 the simple multiplication a × b. This resulting value is further used for calculation of potential inhalation exposure by users to endotoxin 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-threonine produced by *E. coli* CGMCC 13325, including consideration of using filter half mask (FF P2 or FF P3) as a preventative measure

Calculation	Identifier	Description	Amount	Source
	a	Endotoxin content IU/g product	30,000	Technical dossier
	b	Dusting potential (g/m^3)	2	Technical dossier
a × b	c	Endotoxin content in the air (IU/m^3)	60,000	
	d	No of premixture batches made/working day	40	EFSA FEEDAP Panel (2012)
	e	Time of exposure (s)/production of one batch	20	EFSA FEEDAP Panel (2012)
d × e	f	Total duration of daily exposure/worker (s)	800	
	g	Uncertainty factor	2	EFSA FEEDAP Panel (2012)
f × g	h	Refined total duration of daily exposure (s)	1,600	
h/3,600	i	Refined total duration of daily exposure (h)	0.44	
	j	Inhaled air (m^3)/8-h working day	10	EFSA FEEDAP Panel (2012)
j/8 × i	k	Inhaled air during exposure (m^3)	0.56	
c × k	l	**Endotoxin inhaled (IU) during exposure/8-h working day**	**33,333**	
	m	Health-based recommended exposure limit of endotoxin (IU/m^3)/8-h working day	90	Health Council of the Netherlands (2010)
m × j	n	**Health-based recommended exposure limit of total endotoxin exposure (IU)/8-h working day**	**900**	
l /10		Endotoxins inhaled (IU)/8-h working day reduced by filter half mask FF P2 (reduction factor 10)	3,333	
l /20		Endotoxins inhaled (IU)/8-h working day reduced by filter half mask FF P3 (reduction factor 20)	1,667	

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.2012.253

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

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-threonine produced by fermentation using *Escherichia coli* CGMCC 13325

In the current application an authorisation is sought under Article 4(1) for L-threonine produced by fermentation with *Escherichia coli* CGMCC 13325, 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 as active substance a minimum of 98.5% (w/w) of L-threonine.

L-threonine is intended to be mixed either in premixtures or added directly into feedingstuffs or water. However, the Applicant did not propose a minimum or maximum L-threonine content in feedingstuffs.

For the identification of L-threonine in the feed additive, the EURL recommends the Food Chemical Codex "L-threonine monograph". For the quantification of threonine in the feed additive and premixtures the Applicant submitted the ring-trial validated method EN ISO 17180:2013 based on ion-exchange chromatography coupled to post-column derivatisation and optical (visible or fluorescence) detection (IEC-VIS/FLD). The method is dedicated for the determination of free lysine, methionine and threonine in commercial amino acid products and premixtures containing more than 10% of the amino acid. The following performance characteristics were reported for the quantification of threonine: a relative standard deviation for repeatability (RSDr) ranging from 0.7 to 1.4% and a relative standard deviation for reproducibility (RSDR) ranging from 1.9 to 2.3%.

Based on the performance characteristics available, the EURL recommends for official control the EN ISO 17180:2013 method based on IEC-VIS/FLD for the quantification of threonine in the feed additive and premixtures (containing more than 10% threonine).

For the quantification of threonine in feedingstuffs and water the Applicant submitted the ring-trial validated European Union (EU) method (Commission Regulation (EC) No 152/2009). The method is based on ion-exchange chromatography coupled to photometric detection (IEC-VIS) and is dedicated for the analysis of free and total amino acids in premixtures and feedingstuffs. The following performance characteristics were reported for the quantification of total threonine: RSDr ranging from 1.9 to 2.7% and RSDR ranging from 3.8 to 5.2%.

The Applicant provided no experimental data demonstrating the applicability of the EU method for the quantification of threonine in water, however this method has been previously included in Commission Implementing Regulation (EU) 2016/1220, authorising another L-threonine product, for official control to quantify threonine in water. In addition, in the frame of previous dossiers for already authorised L-threonine products, namely FAD-2018-0035 and FAD-2018-0037, the EURL recommended AOAC (999.13) and VDLUFA (4.11.6) methods based on IEC-VIS/FLD for official control to quantify threonine in water.

Based on the performance characteristics available, the EURL recommends for official control the ring-trial validated European Union method, based on IEC-VIS to quantify threonine in premixtures, feedingstuffs and water. In addition, the EURL recommends for official control AOAC (999.13) and VDLUFA (4.11.6) methods based on IEC-VIS/FLD for the quantification of threonine 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.