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Safety and efficacy of L-threonine produced by fermentation with *Corynebacterium glutamicum* KCCM 80118 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 with *Corynebacterium glutamicum* KCCM 80118 when used as nutritional additive in feed and water for drinking for all animal species and categories. The product under assessment is L-threonine produced by fermentation with a genetically modified strain of *C. glutamicum* (KCCM 80118). The production strain and its recombinant DNA were not detected in the additive. The product L-threonine, manufactured by fermentation with *C. glutamicum*, KCCM80118 does not give rise to any safety concern with regard to the production strain. L-Threonine produced using *C. glutamicum* KCCM 80118 is considered safe for the target species. The FEEDAP Panel has concerns regarding the safety of the simultaneous oral administration of L-threonine via water for drinking and feed. L-Threonine produced using *C. glutamicum* KCCM 80118 is safe for the consumer. The additive is not a skin or eye irritant and is not a skin sensitiser. Although the workers can be exposed by inhalation, the results of an acute inhalation study showed that risk of adverse effects by inhalation is low. L-Threonine produced using *C. glutamicum* KCCM 80118 is safe for the environment. The product under assessment is considered an efficacious source of the amino acid L-threonine for all animal species. For L-threonine to be as efficacious in ruminants as in non-ruminant species, it requires protection against degradation in the rumen.

Keywords: nutritional additive, amino acid, L-threonine, safety, efficacy, genetically modified microorganism

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1. Introduction

1.1. Background and Terms of Reference

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

The European Commission received a request from CJ Europe GmbH[2] for authorisation of the product L-threonine, feed grade, produced by fermentation with *Corynebacterium glutamicum* KCCM 80118 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 20 April 2018.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product L-threonine produced by fermentation with *C. glutamicum* KCCM 80118 when used as nutritional additive in feed and water for drinking for all animal species under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

L-Threonine produced by eight different strains of *Escherichia coli* (minimum content of 98% on dry matter 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 of *C. glutamicum* KCCM 80118, 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 01/2008:1049) (Ph. Eur., 2016).

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).

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] CJ Europe GmbH, Ober der Roeth 4, 65824 Schwalbach am Taunus, Germany.
[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 L-threonine produced by fermentation with *C. glutamicum* KCCM 80118 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 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-threonine 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 by fermentation with *C. glutamicum* KCCM 80118 is in line with the principles laid down in Regulation (EC) No 429/2008[10] and the relevant guidance documents: Guidance on nutritional additives (EFSA FEEDAP Panel, 2012a), 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), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017d), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use (EFSA GMO Panel, 2011), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b).

3. Assessment

The subject of the present assessment is L-threonine (minimum 98.5%) produced by fermentation with a genetically modified strain of *C. glutamicum* (KCCM 80118). It is intended to be used as nutritional additive (functional group amino acids, their salts and analogues) to feed and water for drinking in all animal species and categories.

Under EU conditions, L-threonine seems to be the second most limiting amino acid, after L-lysine, in pigs and the third most limiting, after the sulfur amino acids and L-lysine, in poultry.

3.1. Characterisation

3.1.1. Characterisation of the production organism

The additive under assessment is produced by a genetically modified strain of *C. glutamicum*, which has been deposited [redacted]

[8] FEED dossier reference: FAD-2018-0003.

[9] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2018-0003_lthreonine.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.

[redacted]

3.1.1.1. Information relating to the genetically modified microorganism

Characteristics of the recipient microorganism

[redacted]

Characteristics of the donor organism

[redacted]

Description of the genetic modification process

[redacted]

3.1.2. Manufacturing process

[redacted]

3.1.3. Characterisation of the 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 Da. The molecular formula of L-threonine is $C_4H_9NO_3$. The structural formula is given in Figure 1.

[redacted]

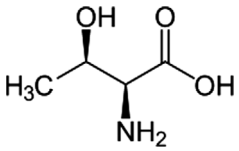

Figure 1: Structural formula of L-threonine

The additive contains by specification ≥ 98.5% L-threonine 'as is' (≥ 99.0% L-threonine on a dry matter basis), < 0.5% moisture and < 0.1% ash.[17] The analysis of five batches of the additive showed an average of threonine of 99.3% 'as is' (range 99.1–99.6%).[18] The loss on drying was 0.13% (range 0.09–0.18%) and the ash 0.02% (range 0.01–0.03%). Other amino acids (lysine, serine, glutamic acid, isoleucine and valine) represented 0.05% and sulfate was 0.01%.[19] The amount of unidentified material was lower than 1% on a dry matter basis.

The specific optical rotation was measured in three batches of the final product and the average was −28.3° (range −28.1 to −28.5°),[20] which is within the range established for L-threonine in the European Pharmacopoeia (−29.0 to −27.6°) and demonstrates the identity of the L-enantiomer.

3.1.3.1. Impurities

Five batches were analysed for heavy metals (cadmium, mercury and lead) and arsenic. Lead and arsenic were found under the limit of detection (LOD), mercury ranged from < LOD to 0.02 mg/kg and cadmium from < LOD to 0.28 mg/kg. Other metals analysed were chromium, copper, nickel and zinc and all them were under the LOD.[21] None of these amounts were considered of concern.

The microbiological quality of five batches of the product was tested by counting total bacterial count (< 10^3 colony-forming units (CFU)/g), *Salmonella* spp. (negative in 25 g), *Escherichia coli* (< 10^3 CFU/g), filamentous fungi (< 10^3 CFU/g) and yeasts (< 10^3 CFU/g).[22] Regarding mycotoxins, ochratoxin A, aflatoxins (unspecified), zearalenone and deoxynivalenol were analysed in five batches of the additive. All values were below the LOD (except zearalenone which was present at 30 μg/kg in one batch of the additive)[23] and are of no concern.

Dioxins (polychlorinated dibenzofurans (PCDF), polychlorinated dibenzo(p)dioxins (PCDD)) and dioxin-like polychlorinated biphenyls (DL-PCBs) were measured in five batches of the final product.[24] PCDD/F ranged from 0.2 to 0.3 ng WHO-TEQ/kg (upper limit). The sum of PCDD/F and dioxin-like PCB ranged from 0.3 to 0.6 ng WHO-TEQ/kg (upper limit).

[redacted]

3.1.3.2. Physical characteristics

The additive is an off-white free flowing powder. It has a pH of 4.5–7 in 10% water solution, a bulk density of 700–900 kg/m^3. Its melting point is 256°C and its solubility in water at 20°C is 97.6 g/L.[27]

[17] Technical dossier/Section II.1.3 and annex II.1.1.
[18] Technical dossier/Section II/Annex II.1.2, analytical method AOAC 999.13.
[19] Technical dossier/Section II/Annex II.1.3.
[20] Technical dossier/Section II/Supplementary information September 2018/SIN THR 180727/Annex SIN 1 specific rotation.
[21] Technical dossier/Section II/Annex II.1.4. LOD (in mg/kg) was 0.1 for lead and cadmium; 0.01 for mercury; 0.2 for chromium, copper, nickel and zinc; and 1 for arsenic.
[22] Technical dossier/Section II/Annex II.1.4 and supplementary information September 2018/SIN THR 180727/response to question 2.
[23] Technical dossier/Section II/Annex II.1.5. LOD (in μg/kg) was 5 for ochratoxin A, 0.1 for aflatoxins, 17 for zearalenone and 134 for deoxynivalenol.
[24] Technical dossier/Section II/Annex II.1.4.
[redacted]
[27] Technical dossier/Section II/Annexes II.1.1 and II.3.1.

The particle size distribution of the final product (three batches) was analysed by laser diffraction.[28] The fractions of particles having a diameter < 100, < 50 and < 10 μm ranged 33.1–35.3, 11.2–13.8, and 2.9–3.7% (v/v), respectively.

The dusting potential (three batches) of the final product (Stauber–Heubach method) ranged from 0.5 to 1.7 g/m^3.[29]

3.1.3.3. Stability and homogeneity

The shelf-life of the additive was studied when stored in sealed brown glass at 25°C and 40°C for 6 months. No losses were observed.[30]

The stability of the additive (three batches) in a vitamin–mineral premixture containing choline chloride (4%) was studied when supplemented at 5%. The samples were stored in aluminium vacuum bags at 25°C for 6 months. No losses were observed.[31]

The stability of the additive (three batches) in a complete feed for chicken for fattening when supplemented via premixture at 0.4% was studied. The basal diet consisted of maize, soybean meal and wheat. The samples were stored at 25°C in aluminium vacuum bags for 3 months. Losses ranged from 0 to 5%.[32]

The stability of the additive (three batches) in water for drinking was studied when supplemented at 0.05%. Samples were stored at 25 and 40°C for 48 h. No losses were observed.[33]

The capacity of the additive to distribute homogeneously in feed was studied in the premixture described above, in the mash feed described above and in a different pelleted feed.[34] The pelleted feed was supplemented with 0.2% L-threonine and conditioned at 72°C, pelleted at 82°C and dried at 60–65 °C. The coefficients of variation were 3, 2 and 4%, respectively.

3.1.3.4. Physico-chemical incompatibilities

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

3.1.4. Conditions of use

It is proposed that L-threonine will be used in feeds to achieve an adequate amino acid profile and to meet the L-threonine requirements for all animal species. It can be added directly to feedingstuffs or complementary feedingstuffs, 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 and the amino acid composition of the unsupplemented diet.[35]

3.2. Safety

3.2.1. Safety of the genetic modification

The recipient strain belongs to a species, *C. glutamicum*, which is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007) when used for production purposes (EFSA BIOHAZ Panel, 2019).

[redacted]

There are no safety concerns related to the genetic modification.

[28] Technical dossier/Section II/Annexes II.1.6 and II.1.7.
[29] Technical dossier/Section II/Annex II.1.7.
[30] Technical dossier/Section II/Annex II.4.1.
[31] Technical dossier/Section II/Annex II.4.2 and Supplementary information September 2018/SIN THR 180727/SIN CJE L-thr.
[32] Technical dossier/Section II/Annex II.4.3 and Supplementary information September 2018/SIN THR 180727/SIN CJE L-thr.
[33] Technical dossier/Section II/Annex II.4.4.
[34] Technical dossier/Section II/Annexes II.4.5 to II.4.7.
[35] Technical dossier/Section II.5.1.

3.2.2. Safety for the target species

Concerns from the use of the additive may arise from residues of the fermentation process/ production strain remaining in the final product. The additive is highly purified (> 99%), is produced by fermentation using a genetically modified strain that belongs to a species that qualifies for the QPS approach for safety assessment and the genetic modification raised no concerns. Therefore, the FEEDAP Panel concludes that L-threonine produced by *C. glutamicum* KCCM 80118 is safe for the target species provided that it is supplemented in appropriate amounts to the diets. Due to the risk of nutritional imbalances and hygienic reasons, associated to the use of amino acids via water for drinking (EFSA FEEDAP Panel, 2010), the FEEDAP Panel has concerns on the safety of the simultaneous oral administration of threonine-containing additives via feed and water for drinking.

3.2.2.1. Conclusions on safety for the target species

L-Threonine produced using *C. glutamicum* KCCM 80118 is considered safe for the target species when supplemented in appropriate amounts to the diet. The FEEDAP Panel has concerns regarding the safety of the simultaneous administration of L-threonine via feed and water for drinking.

3.2.3. Safety for the consumer

The amino acid L-threonine, supplemented to feed, will be incorporated into proteins of tissues and/or products of animal origin and any potential excess will be metabolised and excreted as urea/uric acid and carbon dioxide. Therefore, the composition of tissues and products of animal origin will not be affected by the use of L-threonine in animal nutrition.

The product under assessment is produced by fermentation using a *C. glutamicum* strain which fulfils the qualifications for the QPS approach to safety assessment and the genetic modification raised no concerns. Therefore, the FEEDAP Panel concludes that the use of L-threonine produced by *C. glutamicum* KCCM 80118 in animal nutrition is safe for the consumer.

3.2.4. Safety for the user

3.2.4.1. Effects on the respiratory system

The additive has a dusting potential up to 1.7 g/m^3 and the fraction of particles having a diameter < 10 μm are up to 3.7% (v/v). Consequently, workers may be exposed by inhalation.

In an acute inhalation toxicity study performed in accordance with OECD Guideline 403,[36] 10 RccHan TM:WIST strain rats (five males and five females) were exposed to a dust atmosphere (5.2 mg/L) for 4 h. All dust was composed by particles of respirable size (≤ 10 um). Transient clinical signs (hunched posture, irregular respiration rate, piloerection, red discharge from nose and eyes) were noted immediately after the treatment and lasted for up to 2 days post-treatment in two animals. No mortality was observed and no macroscopic abnormalities were found at necropsy. The inhalation exposure to 5.2 mg/L of respirable dust, a concentration 10 to 3-fold higher than the dusting potential of the additive, induced only transient clinical signs.

3.2.4.2. Effects on skin and eyes

In an *in vitro* eye irritation/corrosion test (bovine corneal opacity and permeability test) in accordance with OECD Guideline 437,[37] the additive did not cause any increase of corneal opacity or permeability. The negative and positive controls performed as expected.

In an *in vitro* skin irritation test (human skin model test) in accordance with OECD Guideline 439,[38] the additive did not show irritant potential. The positive and negative controls performed as expected.

In a skin sensitisation test (local lymph node assay) in accordance with OECD Guideline 429,[39] the test item was considered to be a non skin-sensitiser.

[36] Technical dossier/Section III/Annex III.3.2.
[37] Technical dossier/Section III/Annex III.3.3.
[38] Technical dossier/Section III/Annex III.3.4.
[39] Technical dossier/Section III/Annex III.3.5.

3.2.4.3. Conclusions on safety for the user

The additive is not a skin or eye irritant and is not a skin sensitiser. Although the workers can be exposed by inhalation, the results of an acute inhalation study showed that risk of adverse effects by inhalation is low.

3.2.5. Safety for the environment

The production organism and its DNA were not detected in the final product. The final product does not pose any environmental safety concern associated with the genetic modification of the production strain. L-Threonine produced using *C. glutamicum* KCCM 80118 is safe for the environment.

The amino acid L-threonine is a physiological and natural component of the proteins of living organisms. When consumed, it will be absorbed, and the non-absorbed fraction will be incorporated into the intestinal microbial mass and excreted as such. The absorbed L-threonine will be incorporated into body protein or excreted as urea/uric acid and as carbon dioxide.

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[40] and Good Manufacturing Practice.

4. Conclusions

The production strain and its recombinant DNA were not detected in the final products. The product L-threonine, manufactured by fermentation with *Corynebacterium glutamicum* KCCM80118, does not give rise to any safety concern to the production strain.

L-Threonine produced using *C. glutamicum* KCCM 80118 is considered safe for the target species when supplemented in appropriate amounts to the diet. The FEEDAP Panel has concerns regarding the safety of the simultaneous administration of L-threonine via feed and water for drinking.

L-Threonine produced using *C. glutamicum* KCCM 80118 is safe for the consumer.

The additive is not a skin or eye irritant and is not a skin sensitiser. Although the workers can be exposed by inhalation, the results of an acute inhalation study showed that risk of adverse effects by inhalation is low.

L-Threonine produced using *C. glutamicum* KCCM 80118 is safe for the environment.

The product 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.

Documentation provided to EFSA

1) Feed grade L-threonine produced by fermentation with *Corynebacterium glutamicum* KCCM 80118. February 2018. Submitted by CJ Europe GmbH.
2) Feed grade L-threonine produced by fermentation with *Corynebacterium glutamicum* KCCM 80118. Supplementary information. July 2018. Submitted by CJ Europe GmbH.
3) Feed grade L-threonine produced by fermentation with *Corynebacterium glutamicum* KCCM 80118. Supplementary information. December 2018. Submitted by CJ Europe GmbH.

[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.

4) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for L-threonine produced by fermentation with *Corynebacterium glutamicum* KCCM 80118.
5) Comments from Member States.

Chronology

Date	Event
1/2/2018	Dossier received by EFSA
1/2/2018	Reception mandate from the European Commission
20/4/2018	Application validated by EFSA – Start of the scientific assessment
18/6/2018	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*
4/7/2018	Request of additional 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, impurities.*
20/7/2018	Comments received from Member States
20/7/2018	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
27/7/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
29/10/2018	Request of additional 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*
12/12/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
22/1/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. EFSA Journal 2007;5(12):587, 16 pp.

EFSA (European Food Safety Authority), 2008. Technical Guidance of the Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) for assessing the safety of feed additives for the environment. EFSA Journal 2008;6(10):842, 28 pp. https://doi.org/10.2903/j.efsa.2008.842

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Abbreviations

BIOHAZ	EFSA Panel on Biological Hazards
CAS	Chemical Abstracts Service
CFU	colony-forming unit
DL-PCB	dioxin-like polychlorinated biphenyls
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	Ion Exchange Chromatography coupled with photometric detection
IEC-VIS/FLD	IEC coupled with post-column derivatisation and Visible or Fluorescence Detection
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KCCM	Korean Culture Collection of Microorganisms
LOD	limit of detection
OECD	Organisation for Economic Co-operation and Development
PCDD	polychlorinated dibenzodioxin
PCDF	polychlorinated dibenzofuran
PCR	polymerase chain reaction
QPS	qualified presumption of safety
RSDr	relative standard deviation for repeatability
RSDR	relative standard deviation for reproducibility
TEQ	Toxic equivalent
WHO	World Health Organization

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 with *Corynebacterium glutamicum* KCCM80118

In the current application, authorisation is sought under Article 4(1) for *L-threonine* produced by *Corynebacterium glutamicum* KCCM 80118, 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-threonine* is already authorised as feed additive under Commission Implementing Regulation (EU) 2016/1220.

For the quantification of *L-threonine* in the *feed additive, premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated Community method (Commission Regulation (EC) No 152/2009) based on Ion Exchange Chromatography coupled with photometric detection (IEC-VIS). This method, designed only for the analysis of *premixtures* and *feedingstuffs,* does not distinguish between the salts and the amino acid enantiomers. The following performance characteristics were reported for the quantification of total *threonine:* relative standard deviation for repeatability (RSDr) ranging from 1.9 to 2.7%, and relative standard deviation for reproducibility (RSDR) ranging from 3.8 to 5.2%.

For the quantification of *L-threonine* in the *feed additive*, the European Union Reference Laboratory (EURL) identified the ring-trial validated method EN ISO 17180:2013 based on IEC coupled with post-column derivatisation and Visible or Fluorescence Detection (IEC-VIS/FLD). The following performance characteristics are reported: RSDr ranging from 0.7 to 1.4%; and RSDR ranging from 1.9 to 2.3%. In addition, the EURL identified the 'L-threonine monograph' of the Food Chemical Codex (FCC) for the identification of *L-threonine* in the *feed additive.*

Within the dossier, the Applicant presented experimental data obtained analysing *threonine* in *water* with the AOAC official method 999.13 based on IEC-VIS/FLD. The results presented are considered sufficient to demonstrate the suitability of the procedure for the analysis of the amino acid in *water.* Hence, the EURL recommends for official control this method to quantify *threonine* in *water.*

In the frame of this authorisation, the EURL recommends for official control (i) the '*L-threonine* monograph' of the FCC based on infrared absorption for the identification of *L-threonine* in the *feed additive*; (ii) the ring-trial validated method EN ISO 17180:2013 based on IEC-VIS/FLD to quantify free *threonine* in the *feed additive* and *premixtures* (containing more than 10% threonine); (iii) the Community method based on IEC-VIS for the quantification of *threonine* in *premixtures* and *feedingstuffs*; and (iv) the analytical method described by AOAC (999.13) based on IEC-VIS/FLD to quantify *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) is not considered necessary.