

## SCIENTIFIC OPINION

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## Safety and efficacy of L-tryptophan produced by fermentation with *Corynebacterium glutamicum* KCCM 80176 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-tryptophan produced by fermentation with a genetically modified strain of *Corynebacterium glutamicum* (KCCM 80176) when used as a nutritional additive in feed and water for drinking for all animal species. Viable cells of the production strain and its recombinant DNA were not detected in the additive. L-Tryptophan manufactured by fermentation using *C. glutamicum* KCCM 80176 does not give rise to any safety concern with regard to the genetic modification of the production strain. The use of L-tryptophan produced using *C. glutamicum* KCCM 80176 in supplementing feed to compensate for tryptophan deficiency in feedingstuffs is safe for non-ruminant target species and in ruminants when a rumen-protected form is used. The use of L-tryptophan produced by *C. glutamicum* KCCM 80176 in animal nutrition presents no safety concerns to consumers of animal products. L-Tryptophan produced by *C. glutamicum* KCCM 80176 is considered not toxic by inhalation, not irritant to skin or eyes and not a dermal sensitiser. L-Tryptophan produced by *C. glutamicum* KCCM 80176 is safe for the environment. L-Tryptophan produced by *C. glutamicum* KCCM 80176 is regarded as an effective source of the amino acid L-tryptophan for all non-ruminant species. If L-tryptophan is intended for use in ruminants, it should be protected from ruminal degradation.

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## 1. Introduction

### 1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003<sup>1</sup> 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<sup>2</sup> for authorisation of the product L-tryptophan 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 10 July 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-tryptophan produced by fermentation with *Corynebacterium glutamicum* KCCM 80176, when used as a 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-Tryptophan (minimum content of 98% on dry matter basis) produced by fermentation with six strains of *Escherichia coli* (KCCM 11132P, DSM 25084, FERM BP-11200, FERM BP-11354, CGMCC 7.59 or CGMCC 3667) is currently authorised as a nutritional feed additive for use in all animal species.<sup>3</sup> The product under assessment, L-tryptophan produced by fermentation with *C. glutamicum* KCCM 80176, has not been previously authorised in the European Union (EU).

L-Tryptophan is authorised for use in food,<sup>4</sup> cosmetics<sup>5</sup> and as a veterinary medicinal product.<sup>6,7</sup>

L-Tryptophan is described in the European Pharmacopoeia (2017), monograph 01/2017:1272.

The EFSA Panel on Additives and Products or Substances used in Animal Feed published several opinions on the safety and efficacy of L-tryptophan produced by different strains of *E. coli* for all animal species (EFSA FEEDAP Panel, 2013, 2014a,b, 2015a,b, 2016a,b, 2017a,b, 2019a,b,c,d).

The Panel on Dietetic Products, Nutrition and Allergies (NDA) of EFSA issued a scientific opinion on the substantiation of health claims related to L-tryptophan (EFSA NDA Panel, 2011). The Panel on Nutrition, Dietetic Products, Novel Food and Allergy of the Norwegian Scientific Committee for Food Safety (VKM) published an opinion on histidine, methionine, S-adenosylmethionine and tryptophan added to foods and drinks and in food supplements (VKM, 2013) and another on L-tryptophan in food supplements and energy drinks (VKM, 2016) and concluded that doses  $\geq 250$  mg/day may represent a risk of adverse health effects for children, adolescents and adults.

<sup>1</sup> 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.

<sup>2</sup> CJ Europe GmbH, Ober der Roeth 4, 65824 Schwalbach am Taunus, Germany.

<sup>3</sup> Commission implementing regulation (EU) 2017/873 of 22 May 2017 concerning the authorisation of L-tryptophan produced by *Escherichia coli* as feed additive for all animal species. OJ L 134, 23.5.2017, p. 14.

<sup>4</sup> 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.

<sup>5</sup> 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.

<sup>6</sup> 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.

<sup>7</sup> 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. OJ 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<sup>8</sup> in support of the authorisation request for the use of L-tryptophan produced by fermentation using *C. glutamicum* KCCM 80176 as an additive for feed and water for drinking in all animal species.

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-tryptophan produced by fermentation with *C. glutamicum* KCCM 80176 in animal feed. The Executive Summary of the EURL report can be found in Annex A.<sup>9</sup>

### 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-tryptophan produced by fermentation with *C. glutamicum* KCCM 80176 is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017d), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017e), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008) and Guidance on the characterisation of microorganisms used as feed additives or as production strain (EFSA FEEDAP Panel, 2018).

## 3. Assessment

L-Tryptophan produced by fermentation using a genetically modified strain of *C. glutamicum* is intended to be used as a nutritional feed additive under the functional group 'amino acids, their salts and analogues' in feed and water for drinking for all animal species.

### 3.1. Characterisation

#### 3.1.1. Characterisation of the production organism

The additive is produced by a genetically modified strain of *C. glutamicum* [REDACTED]

The applicant tested the susceptibility of the recipient and production strains against the list of antibiotics proposed for *Corynebacterium* in the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018). [REDACTED]

##### 3.1.1.1. Information relating to the genetically modification

[REDACTED] *C. glutamicum* is recommended for the qualified presumption of safety (QPS) status with the qualification for production purposes.

<sup>8</sup> FEED dossier reference: FAD-2018-0033.

<sup>9</sup> The full report is available on the EURL website: [https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2018-0033\\_tryptophan.pdf](https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2018-0033_tryptophan.pdf)

### 3.1.1.2. Characteristics of the donor organisms

[REDACTED]

### 3.1.1.3. Description of the genetic modification

[REDACTED]

- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]

[REDACTED]

### 3.1.2. Manufacturing process

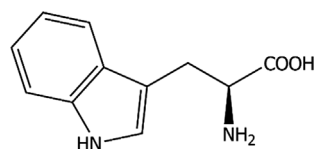
The dossier contains information on the production process. [REDACTED]

[REDACTED]

### 3.1.3. Characterisation of the active substance/additive

L-Tryptophan (International Union of Pure and Applied Chemistry (IUPAC) name: (2S)-2-amino-3-(1H-indol-3-yl) propanoic acid; synonyms: (S)- $\alpha$ -amino-1-H-indole-3-propanoic acid, L- $\alpha$ -aminoindole-3-propionic acid, L- $\alpha$ -amino-3-indolepropionic acid, 2-amino-3-indolylpropanoic acid, L- $\beta$ -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<sub>11</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub>, the molecular weight is 204.23 g/mol. The structural formula is given in Figure 1.

[REDACTED]



**Figure 1:** Structural formula of L-tryptophan

According to the specification, the product contains  $\geq 98\%$  L-tryptophan 'as is', and  $\leq 1\%$  moisture.

The analysis of five batches of L-tryptophan showed an average content of L-tryptophan of 98.4% on 'as is' basis (range 98.35–98.42%),<sup>19</sup> moisture ranged from 0.41 to 0.43%, ammonium ranged between 0.06 and 0.08%, glutamic acid ranged from 0.11 to 0.15%, valine ranged from 0.03 to 0.05%, lysine ranged from 0.01 to 0.02% and ash ranged between 0.12 and 0.19%.<sup>20</sup> On a dry matter basis, the average tryptophan was 98.8% (range 98.76–98.83%); the amount of identified material was, on average, 99.2% (range 99.1–99.3%).

The specific optical rotation of three batches of the final product ranged from  $-31.2$  to  $-31.7^\circ$ ,<sup>21</sup> which is within the range described in the European Pharmacopoeia ( $-30$  to  $-33^\circ$ ) for this amino acid and confirms the identity of the L-enantiomer.<sup>22</sup>

### 3.1.3.1. Impurities

Three batches of the final product were analysed for heavy metals (lead, cadmium and mercury) and arsenic. All analytical values were below the respective limits of detection (LOD).<sup>23</sup>

Dioxins (polychlorinated dibenzodioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (Co-planar PCBs) were analysed in three batches of the final product. Both parameters were found below the LOD.<sup>24</sup> In the same batches, nitrofurans (furazolidone, furaltadone, nitrofurazone and nitrofurantoin) and nitrofuran metabolites were below the corresponding LODs.<sup>25</sup> A multiresidue pesticide analysis (358 pesticides) showed that none of them was present.<sup>26</sup>

Analysis of microbial contamination of the final product (three batches) indicated that *Salmonella* spp. (25 g samples),<sup>27</sup> *E. coli*, and coliforms were absent whereas total bacterial count was  $< 10^3$  colony forming unit (CFU)/g; and yeasts and filamentous fungi were  $< 5 \times 10^1$  CFU/g.<sup>21</sup>

Mycotoxins were analysed in three batches of the final product. Aflatoxins (B1, B2, G1 and G2), ochratoxin A, zearalenone, deoxynivalenol and fumonisins (B1 and B2) concentrations were below the corresponding LOD.<sup>28</sup>

1,1'-Ethylidene-bis-L-tryptophan (EBT) and 1-methyl-1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acid (MTCA), present in a specific brand of L-tryptophan produced by fermentation, were implicated in the eosinophilia–myalgia syndrome outbreak that occurred in humans in New Mexico in 1989 (Hertzman et al., 1990). The concentrations of EBT and of MTCA were analysed in three batches of the final product and were  $< 3$  mg/kg in all cases.<sup>29,30</sup> The maximum permitted content of EBT (impurity A) and the sum of all other impurities (B–L, including MTCA) in L-tryptophan, as specified by the European Pharmacopoeia (2017), is 10 mg/kg and 390 mg/kg, respectively.

Three batches of the additive were tested for the presence of viable cells.

<sup>19</sup> Technical dossier/Section II/Annex II.6.1. Method HPLC with C18 column using potassium phosphate buffer as mobile phase.

<sup>20</sup> Technical dossier/Section II/Annex II.1.2. Analysis of L-tryptophan performed by HPLC.

<sup>21</sup> Technical dossier/Section II/Annex II.1.3.

<sup>22</sup> European Pharmacopoeia monograph 1/2015:1272.

<sup>23</sup> Technical dossier/Section II/Annex II.1.3. LOD in  $\mu\text{g}/\text{kg}$  were 5 for mercury and 1 for cadmium, lead and arsenic.

<sup>24</sup> Technical dossier/Section II/Annex II.1.3. LOD (in  $\mu\text{g}/\text{kg}$ ) was 0.05 for PCDD/F and 0.13 for Co-PCBs.

<sup>25</sup> Technical dossier/Section II/Annex II.1.3. LOD (in  $\mu\text{g}/\text{kg}$ ) was 0.09–0.15 for nitrofurans and 0.11–0.16 for nitrofuran metabolites.

<sup>26</sup> Technical dossier/Section II/Annex II.1.4. LOD (in  $\mu\text{g}/\text{kg}$ ) was 0.5–8 depending on the pesticide considered.

<sup>27</sup> Technical dossier/Supplementary information October 2018/SIN CJ L-trp C.gI80176/Reply to question 2.

<sup>28</sup> Technical dossier/Section II/Annex II.1.3. LOD (in  $\mu\text{g}/\text{kg}$ ) was 0.1 for aflatoxins (B1, B2, G1, G2) and ochratoxin A; 1.5 for zearalenone; 0.5 for deoxynivalenol and 5 for fumonisins B1 and B2.

<sup>29</sup> Technical dossier/Section II/Annex II.1.3 and supplementary information October 2018/SIN CJ L-trp C.gI80176/Reply to question 2. Method according to European Pharmacopoeia 2.2.29.

<sup>30</sup> Technical dossier/Supplementary information October 2018/Annex SIN 02 MTCA. LOD was 3 mg/kg. Method HPLC according to European Pharmacopoeia 2.2.29.



[REDACTED] Viable cells of the production strain were not detected.

Three batches of L-tryptophan were tested for the presence of DNA. [REDACTED]

[REDACTED] No DNA was detected in any of the batches.

### 3.1.3.2. Physical characteristics

The product under assessment is a solid pale brownish odourless crystalline granular product. The pH in 10% solution at 20°C ranges from 4.5 to 7.0. The bulk density ranges from 350 to 500 kg/m<sup>3</sup>. Its water solubility is 10.6 g/L at 20°C.<sup>33</sup>

No analytical data were submitted on the dusting potential of the additive under assessment. The particle size distribution (three batches analysed by sieving) showed that the fraction of particles < 125 µm and < 88 µm ranged from 55 to 69% and from 34 to 38%, respectively.<sup>34</sup>

### 3.1.3.3. Stability and homogeneity

The applicant provided one study on the shelf life of the additive under assessment. The rest of the studies on stability in premixtures, feedingstuffs, and water for drinking, and the capacity of the additive to distribute homogeneously in pelleted feed and in premixtures were performed using L-tryptophan originating from different production strains (*E. coli* KCCM 80135 or *E. coli* KCCM 80152).<sup>35</sup> As the manufacturing process is the same and the characteristics of the additives very similar, the FEEDAP Panel considers that the results of those stability studies can be applied to the stability and homogeneity of the additive under assessment.

The shelf-life of three batches of the additive under assessment was tested when stored at 25 ± 2°C and at 40 ± 2°C for 6 months.<sup>36</sup> The packaging was not described. No losses were observed.

The stability of three batches of L-tryptophan produced by *E. coli* KCCM 80135 in a vitamin–mineral premixture (containing 4% choline chloride) at a supplementation rate of 0.5% was tested when stored at 25 ± 2°C in aluminium bags for 6 months. The losses observed ranged from 0.4 to 2.2% after the 6-month period.<sup>37</sup>

The stability of three batches of L-tryptophan produced by *E. coli* KCCM 80135 in a complete feed for chickens for fattening (mash feed based on maize, soybean meal and wheat), at a supplementation rate of 0.4%, was tested after storage at 25 ± 2°C in aluminium bags for 3 months. After the 3-month period, the observed losses ranged from 0.5 to 4.6%.<sup>38</sup> No data were provided on the stability of the additive during feed processing and pelleting.

The stability of three batches of L-tryptophan produced by *E. coli* KCCM 80135 in a pelleted feed for chicken for fattening (16% crude protein, 6% crude fat, 3% crude fibre) at a supplementation rate of 0.2% was tested after storage at 25°C for 3 months. Pelleting was performed at 72°C and pellets were subsequently dried at 60–65°C. Losses detected ranged from 0 to 7% depending on the batch considered.<sup>39</sup>

The stability of three batches of L-tryptophan produced by *E. coli* KCCM 80152 in water for drinking (three batches) was measured at a concentration of 0.5 g/L when stored at 25 and 40°C for 48 h. Losses were < 1% in both cases.<sup>40</sup>

The capacity of L-tryptophan produced by *E. coli* KCCM 80135 to distribute homogeneously in the premixture described above was studied by analysing 10 subsamples. The coefficient of variation (CV) was 5.7%.<sup>41</sup>

<sup>33</sup> Technical dossier/Section II/Annex II.1.1.

<sup>34</sup> Technical dossier/Section II/Annex II.1.5.

<sup>35</sup> Technical dossier/Supplementary information October 2018/SIN CJ L-trp Cg180176/Reply to question 1.

<sup>36</sup> Technical dossier/Section II/Annex II.4.09.

<sup>37</sup> Technical dossier/Section II/Annex II.4.03.

<sup>38</sup> Technical dossier/Section II/Annex II.4.04.

<sup>39</sup> Technical dossier/Supplementary information October 2018/Annex SIN 3.

<sup>40</sup> Technical dossier/Section II/Annex II.4.05. VDLUFA 4.11.2 method.

<sup>41</sup> Technical dossier/Section II/Annex II.4.07.



The capacity of an L-tryptophan produced by *E. coli* KCCM 80135 to distribute homogeneously in the pelleted complete feed for chickens for fattening described above was studied analysing 10 subsamples. The CV was 2.9%.<sup>42</sup>

#### 3.1.3.4. Physicochemical incompatibilities

No physicochemical incompatibilities in feed are expected with other additives, medical products or feed materials.

#### 3.1.4. Conditions of use

It is proposed that L-tryptophan will be used in feeds to achieve an adequate amino acid profile and to meet the L-tryptophan requirements for all animal species. It can be added directly to feedingstuffs/complementary feedingstuffs or via premixtures. The additive is also proposed for use 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.<sup>43</sup>

### 3.2. Safety

#### 3.2.1. Safety of the production organism

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

[REDACTED]

[REDACTED] no viable cells of the production strain were detected in the additive and there are no safety concerns related to the genetic modification, therefore, the production strain is presumed safe.

#### 3.2.2. Safety for the target species

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

Tolerance studies are not normally required for highly purified amino acids. Such tolerance studies with a certain indispensable amino acid will inevitably result in amino acid imbalances, with depression of feed intake and hence impaired performance and increased nitrogen excretion. This is also the case for the product under application which contains 98.8% tryptophan and less than 1% unidentified material on a dry matter basis. The recipient strain belongs to a species, *C. glutamicum*, which is considered by EFSA to be suitable for the QPS approach to safety assessment for production purposes (EFSA 2007; EFSA BIOHAZ Panel, 2019a). The identity of the production strain has been established as *C. glutamicum* and the absence of antimicrobial resistance has been proven. Given the high purity of the product, the FEEDAP Panel considers that the use of L-tryptophan produced with *C. glutamicum* KCCM 80176 is safe for non-ruminant target species when used to supplement the diet in appropriate amounts.

The FEEDAP Panel reiterates (2015a) 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., 1978). Consequently, using unprotected forms of tryptophan in ruminants can be a risk.

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 use via water for drinking.

<sup>42</sup> Technical dossier/Section II/Annex II.4.06.

<sup>43</sup> Technical dossier/Section II.5.1.

### 3.2.2.1. Conclusions on safety for the target species

The use of L-tryptophan produced using *C. glutamicum* KCCM 80176 in supplementing feed to compensate for tryptophan deficiency in feedingstuffs is safe for non-ruminant target species and in ruminants when a rumen-protected form is used. There may be a risk for an increased production of toxic metabolites when unprotected tryptophan is used in ruminants.

The FEEDAP Panel has concerns on the safety of the simultaneous oral administration of L-tryptophan via water for drinking and feed.

### 3.2.3. Safety for the consumer

The absorption and metabolic fate of L-tryptophan were described in a previous opinion (EFSA FEEDAP Panel, 2013).

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

The product under assessment is produced by fermentation. Concerns for the consumer would not derive from the amino acid itself, which will be incorporated into proteins, but from possible residues from fermentation. The production strain meets the QPS qualifications. Considering that (1) the product originating from *C. glutamicum* KCCM 80176 is highly purified (98.8% L-tryptophan and < 1% unidentified material on a dry matter basis); (2) the concentrations of EBT and MTCA are < 3 mg/kg additive; therefore, the use of L-tryptophan produced using *C. glutamicum* KCCM 80176 in animal nutrition is considered safe for the consumer.

#### 3.2.3.1. Conclusions on safety for the consumer

The use of L-tryptophan produced by fermentation with *C. glutamicum* KCCM 80176 in animal nutrition raises no safety concerns to consumers of animal products.

### 3.2.4. Safety for the user

The applicant provided an acute inhalation toxicity test, an eye irritation test, a skin irritation test and a dermal sensitisation test. Those studies tested an L-tryptophan produced by different production strains (*E. coli* KCCM 11132P in the case of the acute inhalation and the dermal sensitisation tests; and *E. coli* KCCM 80135 in the case of the eye and skin irritation tests).<sup>44</sup> As the purity and physical characteristics of the test item including particle size distribution are very similar to the ones of the product under assessment,<sup>45</sup> and the production process is the same (*E. coli* KCCM 80135) or very similar (*E. coli* KCCM 11132P), the FEEDAP Panel considers that the results of the toxicological studies performed with L-tryptophan originating from *E. coli* KCCM 11132P or *E. coli* KCCM 80135 can be used to support the safety for the user of L-tryptophan produced with *C. glutamicum* KCCM 80176.

From an acute toxicity inhalation study, there were no indications that the product under assessment is toxic by inhalation. The additive is not considered irritant for skin or eyes and is not considered a skin sensitiser.

#### 3.2.4.1. Conclusions on safety for the user

L-Tryptophan produced using *C. glutamicum* KCCM 80176 is considered not toxic by inhalation, not irritant to skin or eyes and not a dermal sensitiser.

### 3.2.5. Safety for the environment

The amino acid L-tryptophan is a physiological and natural component of animals and plants. When given to animals, it is not excreted as such, but as urea/uric acid, indole-related compounds and carbon dioxide. The use of amino acids in water for drinking, when given in addition to complete diets with a well-balanced amino acid profile, would disturb the nitrogen balance and increase nitrogen excretion via urine. The use of the product L-tryptophan in animal nutrition would not lead to any localised increase in the concentration in the environment.

<sup>44</sup> Technical dossier/Supplementary information October 2018/Sin CJ L-Trp C. gl 80176.

<sup>45</sup> FAD-2017-0052/Technical dossier/Section III/Annex III.3.07.

Viable cells of the production strain and its DNA was confirmed not to be present in the final product. The final product does not pose any environmental safety concern associated with the genetic modification of the production strain.

The use of L-tryptophan produced by *C. glutamicum* KCCM 80176 as a feed additive does not represent a risk to the environment.

### 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.

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

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

### 3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation<sup>46</sup> and Good Manufacturing Practice.

## 4. Conclusions

Viable cells of the production strain and its recombinant DNA were not detected in the additive. L-Tryptophan manufactured by fermentation using *C. glutamicum* KCCM 80176 does not give rise to any safety concern with regard to the genetic modification of the production strain.

The use of L-tryptophan produced using *C. glutamicum* KCCM 80176 in supplementing feed to compensate for tryptophan deficiency in feedingstuffs is safe for non-ruminant target species and in ruminants when a rumen-protected form is used.

The use of L-tryptophan produced by *C. glutamicum* KCCM 80176 in animal nutrition presents no safety concerns to consumers of animal products.

L-Tryptophan produced by *C. glutamicum* KCCM 80176 is considered not toxic by inhalation, not irritant to skin or eyes and not a dermal sensitiser.

L-Tryptophan produced by *C. glutamicum* KCCM 80176 is safe for the environment.

L-Tryptophan produced by *C. glutamicum* KCCM 80176 is regarded as an effective source of the amino acid L-tryptophan for all non-ruminant species. If L-tryptophan is intended for use in ruminants, it should be protected from ruminal degradation.

## 5. Recommendations

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

## Chronology

| Date       | Event   |
|------------|---|
| 30/05/2018 | Dossier received by EFSA. Feed grade L-tryptophan produced with <i>Corynebacterium glutamicum</i> KCCM 80176. Submitted by CJ Europe GmbH   |
| 29/05/2018 | Reception mandate from the European Commission  |
| 10/07/2018 | Application validated by EFSA – Start of the scientific assessment  |
| 06/09/2018 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation of the production microorganism, characterisation of the additive, stability of the additive.</i> |

<sup>46</sup> Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 October 2003 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

| Date       | Event  |
|------------|--|
| 10/10/2018 | Comments received from Member States   |
| 19/10/2018 | Reception of supplementary information from the applicant - Scientific assessment re-started   |
| 09/11/2018 | Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives   |
| 13/11/2018 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended <i>Issues: Characterisation of the additive.</i>  |
| 06/12/2018 | Reception of supplementary information from the applicant - Scientific assessment re-started   |
| 19/12/2018 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended <i>Issues: Characterisation of the additive and characterisation of the production microorganism.</i> |
| 19/02/2019 | Reception of supplementary information from the applicant - Scientific assessment re-started   |
| 16/05/2019 | Opinion adopted by the FEEDAP Panel. End of the Scientific assessment  |

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## Abbreviations

|        |  |
|--------|--|
| CAS    | Chemical Abstracts Service   |
| CFU    | colony forming unit  |
| CV     | coefficient of variation   |
| DM     | dry matter   |
| EBT    | 1,1'-Ethylidene-bis-L-tryptophan                                       |
| EINECS | European Inventory of Existing Commercial Chemical Substances          |
| EURL   | European Union Reference Laboratory                                    |
| FCC    | Food Chemical Codex  |
| FEEDAP | EFSA Panel on Additives and Products or Substances used in Animal Feed |
| FLD    | fluorescence detection   |
| HPLC   | high-performance liquid chromatography                                 |
| IEC    | ion exchange chromatography  |
| IUPAC  | International Union of Pure and Applied Chemistry                      |
| LOD    | limit of detection   |
| MTCA   | 1-methyl-1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acid           |
| MIC    | minimum inhibitory concentration                                       |
| NDA    | EFSA Panel on Dietetic Products, Nutrition and Allergies               |
| PCB    | polychlorinated biphenyl   |
| PCDD   | polychlorinated dibenzodioxin  |
| PCDFs  | polychlorinated dibenzofuran   |
| QPS    | qualified presumption of safety  |
| RH     | relative humidity  |
| VKM    | Norwegian Scientific Committee for Food Safety                         |
| WHO    | World Health Organization  |



## **Annex A – Executive summary of the evaluation report of the EURL on the analytical methods to detect L-tryptophan produced by fermentation with *Corynebacterium glutamicum* KCCM 80176**

In the current application authorisation is sought under Article 4(1) for L-tryptophan produced by fermentation with *Corynebacterium glutamicum* KCCM80176, 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. According to the Applicant, the product has a minimum purity of 98% and it is intended to be mixed either in premixtures or added directly to feedingstuffs or water for drinking. However, the Applicant did not propose a minimum or maximum L-tryptophan content in feedingstuffs.

For the quantification of L-tryptophan in the feed additive the Applicant submitted a single-laboratory validated analytical method based on High Performance Liquid Chromatography (HPLC) and photometric detection. Furthermore, for the quantification of L-tryptophan in premixtures and feedingstuffs the Applicant submitted the ring-trial validated method VDLUFA 4.11.6, based on ion exchange chromatography (IEC) coupled with post-column derivatisation and optical detection which has been specifically designed for the determination of free lysine, methionine and threonine in feed additive and concentrated premixtures. The method is not applicable to the determination of tryptophan in premixtures and feedingstuffs.

However, the EURL previously evaluated and recommended (i) the ring-trial validated EN ISO 13904:2016 method based on HPLC and fluorescence detection (HPLC-FLD) for the quantification of L-tryptophan in feed additive and premixtures (containing more than 2% of tryptophan); and (ii) the ring-trial validated Community method based on HPLC-FLD for the quantification of L-tryptophan in feedingstuffs. Based on the performance characteristics available, the EURL recommends for official control these two ring-trial validated methods to quantify tryptophan in the feed additive, premixtures and/or feedingstuffs. Furthermore, in the frame of the stability and homogeneity studies, the Applicant presented experimental data obtained analysing tryptophan in water with a slightly modified version of the VDLUFA official method 4.11.2 based on HPLC-FLD and dedicated for the determination of tryptophan in feed. The results presented are considered sufficient to demonstrate the suitability of the method for the analysis of the amino acid in water. Hence, the EURL recommends for official control this method to quantify tryptophan in water.

In addition, the EURL identified the "L-tryptophan monograph" of the Food Chemical Codex (FCC) for the identification of the feed additive.

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