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## Safety and efficacy of L-tryptophan produced with *Escherichia coli* CGMCC 11674 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 *Escherichia coli* CGMCC 11674 when used as a nutritional additive in feed and water for drinking for all animal species and categories. The production strain did not raise safety concerns. The FEEDAP Panel cannot conclude on the safety of L-tryptophan produced by *E. coli* CGMCC 11674 for the target species and for the consumer due to the tryptophan-related impurities such as 1,1'-ethylidene-bis-L-tryptophan (EBT). The FEEDAP Panel has concerns on the safety of the simultaneous oral administration of L-tryptophan via water for drinking and feed. Using unprotected forms of tryptophan in ruminants can be a risk. The FEEDAP Panel cannot conclude on the dermal or ocular irritation and skin sensitisation of L-tryptophan produced by *E. coli* CGMCC 11674. The additive poses a risk by inhalation from the exposure to endotoxins for people handling the additive. The use of L-tryptophan produced by *E. coli* CGMCC 11674 in animal nutrition does not pose a risk to the environment. The additive under assessment is regarded as an effective source of the amino acid L-tryptophan for all non-ruminant species. If the product 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 Agri Nutrition BV<sup>2</sup> for authorisation of the product L-tryptophan produced by *Escherichia coli* CGMCC 11674, when used as a feed additive for all animal species (category: nutritional additive; 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 8 September 2016.

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 *Escherichia coli* CGMCC 11674 for all animal species, when used 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 *E. 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 *E. coli* CGMCC 11674, has not been previously authorised as feed additive 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 (FEEDAP) 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).

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).

<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> Agri Nutrition BV, Hanzestraat 1, 7006 RH Doetinchem, The Netherlands. Representing Xinjiang Fufeng Biotechnologies Co., Ltd.

<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 minimum 98% produced by *E. coli* CGMCC 11674 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 the L-tryptophan produced by *E. coli* CGMCC 11674 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 minimum 98% produced by *E. coli* CGMCC 11674 is in line with the principles laid down in Regulation (EC) No 429/2008<sup>10</sup> and the relevant guidance documents: Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017c), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018), 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) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012).

## 3. Assessment

L-Tryptophan minimum 98% produced by *E. coli* CGMCC 11674 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 product under assessment is produced by a non-genetically modified strain of *E. coli*, deposited in the China General Microbiological Culture Collection Centre (CGMCC) with accession number CGMCC 11674.<sup>11</sup>

[REDACTED]

<sup>8</sup> FEED dossier reference: FAD-2016-0032.

<sup>9</sup> The full report is available on the EURL website: [https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2016-0032\\_tryptophan.pdf](https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2016-0032_tryptophan.pdf)

<sup>10</sup> 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.

<sup>11</sup> Technical dossier/Section II/Annex 2.2.1.2.a.

[REDACTED]

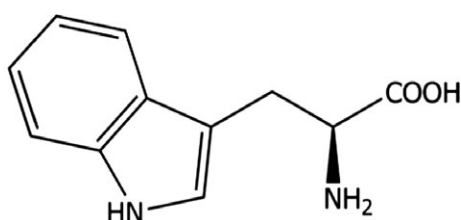
### 3.1.2. Manufacturing process

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

The applicant stated that no antibiotics are used during the production process.<sup>16</sup>

### 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-indolypropanoic acid, l- $\beta$ -3-indolyalanine) 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.



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

The L-tryptophan content of the product is specified as  $\geq 98\%$  on 'as is' basis, the other components being water ( $< 0.5\%$ ) and other amino acids (unspecified %).<sup>17</sup> The analysis of five batches of L-tryptophan showed an average content of L-tryptophan of 98.5% 'as is' (range 97.6 to 100%),<sup>18</sup> moisture was on average 0.7% (range 0.4–1.1%). Tryptophan was below the specification in two of the batches analysed and moisture was above the specification in four of the batches analysed. The amount of identified material (corresponding to tryptophan) on dry matter basis was on average 99.2% (98.3–100.6).<sup>19</sup>

The specific optical rotation measured in three batches ranged from  $-31.4$  to  $-30.8^\circ$ ,<sup>20</sup> which is within the range described in the European Pharmacopoeia (from  $-33.0$  to  $-30.0^\circ$ )<sup>21</sup> and confirms the identity of the L-enantiomer.

#### 3.1.3.1. Impurities

Three batches of the final product were analysed heavy metals (lead, cadmium and mercury) and arsenic. Cadmium ranged from 0.0008 to 0.0042 mg/kg; mercury from 0.031 to 0.044 mg/kg and lead from 0.02 to 0.08 mg/kg. Arsenic was  $< 0.2$  mg/kg in all cases.<sup>22</sup> Dioxins (polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)), dioxin-like polychlorinated biphenyls (DL-PCBs) were analysed in three batches and the values ranged 0.11–0.25 ng/kg and 0.13–0.26 ng/kg, respectively.<sup>23</sup>

Analysis of the microbial contamination of the final product (six batches) indicated that *Salmonella* spp. (in 10 g), Enterobacteriaceae and *E. coli* were absent.<sup>24</sup> Three batches were analysed for yeasts

<sup>16</sup> Technical dossier/Section II/Annex 2.3.k.

<sup>17</sup> Technical dossier/Section II.2.1.3.

<sup>18</sup> Technical dossier/Section II/Annex 2.1.3a; supplementary information November 2018/Annex 1; and answers to FAD-2016-0032 SIn 110918, response to question 2. Analytical method Regulation No 152/2009, III, F.

<sup>19</sup> Technical dossier/Supplementary information November 2018/Answers to FAD-2016-0032 SIn 110918, response to questions 1 and 2 and Annexes 1 and 2.

<sup>20</sup> Technical dossier/Section II/Annex 2.1.4a and Supplementary information July 2018/Annex 4.

<sup>21</sup> European Pharmacopoeia 9th edition, monograph 01/2017:1272

<sup>22</sup> Technical dossier/Section II/Annex 2.1.4a.

<sup>23</sup> Technical dossier/Supplementary information July 2018/Annexes 6.1 to 6.3.

<sup>24</sup> Technical dossier/Section II/Annex 2.1.3a and 2.1.4a.

and filamentous fungi and the results were negative.<sup>25</sup> Mycotoxins levels were analysed in three batches. The levels of aflatoxin, ochratoxin A, zearalenone, fumonisin, deoxynivalenol and citrinin were all below the limit of detection (LOD) except ochratoxin A that showed values of 6.2 and 7.5 µg/kg in two of the three batches analysed.<sup>26</sup>

The antimicrobial activity of one batch of the final product was tested against the reference strains proposed in the Technical guidance of microbial studies (EFSA, 2008). In all cases, the minimum inhibitory concentration was > 2,500 mg/L.<sup>28</sup>

1,1'-Ethyldiene-bis-L-tryptophan (EBT) and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA), present in a specific brand of L-tryptophan produced by fermentation, were implicated in the eosinophilia–myalgia syndrome (EMS) outbreak that occurred in humans in New Mexico in 1989 (Hertzman et al., 1990). In the product under assessment, the levels of MTCA (three batches) ranged from 106 to 128 mg/kg and EBT concentrations ranged from 16 to 18 mg/kg.<sup>29</sup> As specified in the European Pharmacopoeia 9th edition (2017), the maximum permitted content of EBT (impurity A) and the sum of all other impurities (B-L, including MTCA) in L-tryptophan are 10 mg/kg and 390 mg/kg, respectively.

Overall, the data on undesirable contaminants (chemical and microbiological) do not give rise to safety concerns, except for the concentration of the tryptophan impurity EBT, which is further discussed in the safety section.

### 3.1.3.2. Physico-chemical characteristics

The additive is a white crystalline powder with a pH value (1% solution measured in three batches) ranging from 5.1 to 5.8.<sup>22</sup> The density is 500 kg/m<sup>3</sup>.<sup>31</sup>

The particle size distribution of the final product (three batches) was analysed by laser diffraction.<sup>32</sup> In all cases 100% (v/v) of the particles were < 100 µm diameter. The fractions of particles having diameters < 50 and < 10 µm diameter ranged 77–87% (v/v) and 9–15% (v/v), respectively.

The dusting potential analysed in three batches (Stauber–Heubach) ranged from 39 to 53 g/m<sup>3</sup>.<sup>33</sup>

### 3.1.3.3. Stability and homogeneity

The shelf life of three batches of the final product was studied under two different temperatures (25 and 40°C) when stored for 3 months. The samples were stored in sealed plastic bags protected from light.<sup>34</sup> Losses ranged from 0 to 2.3% at 25°C and from 0 to 1.2% at 40°C.<sup>35</sup>

The stability of the additive (three batches) was studied in a vitamin/mineral premixture also containing choline chloride (18.4 g/kg).<sup>36</sup> The supplemental rate was not described. The premixture was stored at room temperature in plastic bags protected from light for 6 months. No losses were observed.

The additive (three batches) was added at a level of 0.3% to a complete mash feed for piglet for fattening based on barley, soybean meal and wheat containing 0.21% tryptophan.<sup>37</sup> The mash feed

<sup>25</sup> Technical dossier/Supplementary information July 2018/Annex 2b.

<sup>26</sup> Technical dossier/Section II/Annex 2.1.3a. LOD in µg/kg were 1.7 for aflatoxins, 5 for ochratoxin A, 15 for citrinin, 17 for zearalenone, 25 for fumonisin and 134 for deoxynivalenol.

<sup>28</sup> Technical dossier/Section II/Annex 2.2.2.2.

<sup>31</sup> Technical dossier/Section II/Table 2.1.5a.

<sup>32</sup> Technical dossier/Section II/Annexes 2.1.5 a, b and c.

<sup>33</sup> Technical dossier/Section II/Annexes 2.4.3 a, b and c.

<sup>34</sup> Technical dossier/Supplementary information July 2018/Answers to FAD-2016-0032 Sin 141016, answer to question 13.

<sup>35</sup> Technical dossier/Section II/Annex 2.1.3a.

<sup>36</sup> Technical dossier/Section II/Annex 2.1.3a and Annex 2.4.1 feed composition. Supplementary information July 2018/Answer to question 14 and annex 2.b.

<sup>37</sup> Technical dossier/Section II/Annex 2.1.3a and Annex 2.4.1 feed composition. Supplementary information July 2018/Answers to FAD-2016-0032/answer to question 15.

was conditioned at 45°C and the pelleting was done at 60°C. Samples of mash and pelleted feed were subsequently stored in paper bags at room temperature and protected from light. No loss of tryptophan was observed during storage of the mash feed. In pelleted feed two batches showed a loss of 4%.

The stability of the product under assessment (three batches) was studied when diluted to a concentration of 1% in water for drinking and solutions were stored at room temperature for 24 h.<sup>38</sup> Only one batch showed a loss of tryptophan of 1%.

The homogeneous distribution of one batch of the additive when supplemented at 0.3% inclusion level in the mash pelleted feed described above was studied by analysing 10 subsamples.<sup>39</sup> The coefficient of variation of the mean was 2.2%.

#### 3.1.3.4. Physico-chemical incompatibilities in feed

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

#### 3.1.4. Conditions of use

The current application of L-tryptophan is as a nutritional additive to feed for all animal species and categories without maximum content in feed or time of administration. According to the applicant, the additive can be added directly to compound feedingstuffs or via premixtures. It can be added to water for drinking.<sup>40</sup> No inclusion levels are proposed as the requirements in quantitative terms depend on the species, the physiological state of the animal, the performance level and the environmental conditions, as well as the amino acid composition of the unsupplemented diet.

### 3.2. Safety

#### 3.2.1. Safety for the target species

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. The additive contains on average 99.2% tryptophan and < 1% unidentified material on a dry matter basis. The production strain has been identified at strain level as a *E. coli* K-12 derivative, no genes of concern have been identified and the production strain is sensitive to the antimicrobials listed in the FEEDAP guidance of 2018. The endotoxin activity [REDACTED]. These values are compared with ca. 1,000 IU/mg commonly found in feedingstuffs (Cort et al., 1990). Therefore, at the usual conditions of use of the additive in feed, the endotoxins added by the additive would be insignificant compared with the background in feed.

However, the additive contains concentrations of EBT (up to 18 mg/kg) higher than the limit (< 10 mg/kg) set for this impurity by the European Pharmacopoeia. The applicant performed a literature search to demonstrate that the high levels of EBT in the additive do not represent a safety concern for the target species.<sup>41</sup> The database platforms Scopus and Web of Science were searched, and no restrictions were applied. The search string was 'eosinophilia AND syndrome AND tryptophan AND EBT.' From 51 hits retrieved only 6 were considered relevant (Belongia et al., 1992; Love et al., 1993; Sato et al., 1995; Takagi et al., 1995; Adachi et al., 2000; Simat et al., 2003). The Panel notes that there is uncertainty on the association of EBT and other tryptophan-related substances with adverse effects observed in humans (see Section 3.2.2) and on the relevance of these effects for the target species. In the absence of reliable data on the target species, the FEEDAP Panel concludes that the additive cannot be considered safe for the target species unless it complies with the maximum limits set in the European Pharmacopoeia for the related impurities.

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).

<sup>38</sup> Technical dossier/Section II.2.4.1 and Annex 2.1.3a.

<sup>39</sup> Technical dossier/Section II.2.4.2 and Annex 2.1.3a. Supplementary information July 2018/Answers to FAD-2016-0032/answer to question 16.

<sup>40</sup> Technical dossier/Section II.2.5.1.

<sup>41</sup> Technical dossier/Supplementary information January 2009/Answers to FAD-2016-0032 Sin.



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

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

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

The FEEDAP Panel cannot conclude on the safety of L-tryptophan produced by *E. coli* CGMCC 11674 for the target species due to the tryptophan related impurities as e.g. EBT.

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

Using unprotected forms of tryptophan in ruminants can be a risk.

### 3.2.2. Safety for the consumer

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 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-tryptophan in animal nutrition

The product under assessment is produced by fermentation. Concerns for the consumer would derive not from the amino acid itself, which will be incorporated into proteins, but from possible residues from fermentation. The additive contains on average 99.2% tryptophan and < 1% unidentified material on a dry matter basis. No concerns have been identified regarding the production strain and in the manufacturing process. Nevertheless, the concentrations of EBT ranged from 16 to 18 mg/kg.

The applicant performed a literature search to demonstrate that the high levels of EBT in the additive do not represent a safety concern for the consumer (see Section 3.2.2). Despite *in vivo* data useful to estimate consumer exposure to EBT are not available, *in vitro* data show that EBT can compete with tryptophan in the protein synthesis and can be incorporated into proteins (Sidransky et al., 1994; Buss et al., 1996a,b; Sidransky, 2002). The limited information provided showed that the occurrence of EMS in humans has been related to several impurities (EBT, MTCA and tryptophan-related substances). However, there is uncertainty on the association of EBT and/or other tryptophan-related substances and EMS in humans (Committee on Toxicity (COT), 2004). In the absence of toxicological data showing a dose–response relationship between exposure to EBT and the adverse effects observed in humans, an effect level could not be identified and a health based guidance value could not be derived for EBT. Therefore, the FEEDAP Panel considers it appropriate to take a precautionary approach and concludes that the product cannot be considered safe for consumers unless it complies with the maximum limits set in the European Pharmacopoeia for the related impurities.

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

The FEEDAP Panel cannot conclude on the safety of L-tryptophan produced by *E. coli* CGMCC 11674 for the consumer due to the tryptophan related impurities as e.g. EBT.

### 3.2.3. Safety for the user

No studies were submitted to support the safety of the additive under assessment for user/workers.

#### 3.2.3.1. Effects on the respiratory system

The additive under assessment is a fine powder (all particles have a diameter < 100 µm). The fractions of particles having diameters < 50 and < 10 µm diameter ranged 77–87% (v/v) and 9–15% (v/v), respectively. The dusting potential ranged from 39 to 53 g/m<sup>3</sup>. Therefore, inhalation exposure of users is likely.

The endotoxin activity in the additive was [REDACTED]. 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 (2012), is described in Appendix A. The health based recommended threshold for the quantity of inhaled endotoxins per working day is 900 IU, derived from provisional occupational

exposure limits given by the Dutch Expert Committee on Occupational Safety (DECOS) (Health Council of the Netherlands, 2010) and the Health and Safety Executive (HSE, 2013). Based upon the calculation of the potential endotoxin content in dust (Wallace et al., 2016), the inhalation exposure could be up to 8,068 endotoxin IU per 8-h working day, indicating a risk from the exposure to endotoxins for people handling the additive.

### 3.2.3.2. Effects on the skin and eyes

In the absence of data, the FEEDAP Panel cannot conclude on the irritation potential of the additive under assessment for skin or eyes, and on its potential to be a dermal sensitiser.

### 3.2.3.3. Conclusions on safety for the user

The FEEDAP Panel cannot conclude on the potential dermal or ocular irritation and skin sensitisation of the product under assessment. The product under assessment poses a risk from the exposure to endotoxins for people handling the additive.

### 3.2.4. 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 excreted not as such, but as urea/uric acid, indole-related compounds and carbon dioxide. The use of the product, L-tryptophan, in animal nutrition would not lead to any localised increase in the concentration in the environment. It is concluded that the use of the product L-tryptophan produced by *E. coli* CGMCC 11674 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 L-tryptophan is well established in the scientific literature. The additive under assessment 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 (EFSA FEEDAP Panel, 2014b). The FEEDAP Panel reiterates that, if the product L-tryptophan is intended for use 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>42</sup> and Good Manufacturing Practice.

## 4. Conclusions

The FEEDAP Panel cannot conclude on the safety of L-tryptophan produced by *E. coli* CGMCC 11674 for the target species and for the consumer due to the tryptophan-related impurities as e.g. EBT.

The FEEDAP Panel has concerns on the safety of the simultaneous oral administration of L-tryptophan via water for drinking and feed. Using unprotected forms of tryptophan in ruminants can be a risk.

The FEEDAP Panel cannot conclude on the dermal or ocular irritation and skin sensitisation of L-tryptophan produced by *E. coli* CGMCC 11674. The additive poses a risk by inhalation from the exposure to endotoxins for people handling the additive.

The use of L-tryptophan produced by *E. coli* CGMCC 11674 in animal nutrition does not pose a risk to the environment.

The additive under assessment is regarded as an effective source of the amino acid L-tryptophan for all non-ruminant species. If the product L-tryptophan is intended for use in ruminants, it should be protected from ruminal degradation.

<sup>42</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.

## Documentation provided to EFSA

- 1) L-Tryptophan produced by *E. coli* for all animal species. August 2016. Submitted by Agri Nutrition BV.
- 2) L-Tryptophan produced by *E. coli* for all animal species. Supplementary information. July 2018. Submitted by Agri Nutrition BV.
- 3) L-Tryptophan produced by *E. coli* for all animal species. Supplementary information. November 2018. Submitted by Agri Nutrition BV.
- 4) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for L-Tryptophan Produced by *Escherichia coli* CGMCC 11674.
- 5) Comments from Member States.

## Chronology

Date	Event
24/05/2016	Dossier received by EFSA
02/06/2016	Reception mandate from the European Commission
08/09/2016	Application validated by EFSA – Start of the scientific assessment
14/11/2016	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: manufacturing process, characterisation of the additive and of the production microorganism, stability and homogeneity and safety for the user.</i>
08/12/2016	Comments received from Member States
04/01/2017	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
13/07/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
11/09/2018	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 additive, safety for the target species and for the consumer.</i>
27/11/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
26/02/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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## Abbreviations

CAS	Chemical Abstracts Service
CFU	colony forming unit
CGMCC	China General Microbiological Culture Collection Center
COT	Committee on Toxicity
CV	coefficient of variation
DL-PCB	dioxin-like polychlorinated biphenyls
DECOS	Dutch Expert Committee on Occupational Safety
DM	dry matter
EBT	1,1'-ethylidene-bis-L-tryptophan
EINECS	European Inventory of Existing Commercial Chemical Substances
EMS	eosinophilia–myalgia syndrome
EURL	European Union Reference Laboratory
FCC	Food Chemical Codex
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
GMO	EFSA Panel on Genetically Modified Organisms
HPLC-FD	High-Performance Liquid Chromatography equipment coupled with fluorescence detection
HSE	Health and Safety Executive

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
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
PCDD	polychlorinated dibenzodioxin
PCDF	polychlorinated dibenzofuran
RSDr	relative standard deviation for repeatability
RSDR	relative standard deviation for reproducibility
VKM	Norwegian Scientific Committee for Food Safety
TEQ	toxic equivalent
WHO	World Health Organization
█	█

## Appendix A – Calculation of exposure to endotoxins

### Calculation of maximum acceptable levels of exposure from feed additives.

The probable exposure time according to EFSA guidance (EFSA FEEDAP Panel, 2012) for additives added in premixtures assumes a maximum of 40 periods of exposure per day, each comprising 20 seconds =  $40 \times 20 = 800$  s/day. With an uncertainty factor of 2, maximum inhalation exposure would occur for  $2 \times 800 = 1,600$  s = 0.444 h/day. Again, assuming a respiration volume of 1.25 m<sup>3</sup>/h, the inhalation volume providing exposure to potentially endotoxin-containing dust would be  $0.444 \times 1.25 = 0.556$  m<sup>3</sup>/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<sup>3</sup>.

### Calculation of endotoxin content of dust.

Two key measurements are required to evaluate the potential respiratory hazard associated with the endotoxin content of the product (the dusting potential of the product, expressed in g/m<sup>3</sup>, and the endotoxin activity of the dust, determined by the *Limulus* amoebocyte lysate assay (expressed in IU/g)). If data for the dust are not available, the content of endotoxins of the product can be taken instead. If the content of endotoxins of the relevant additive is a IU/g and the dusting potential is b g/m<sup>3</sup>, then the content of endotoxins of the dust, c IU/m<sup>3</sup>, is obtained by simple multiplication,  $a \times b$ . This resulting value is further used for calculation of the potential inhalatory exposure of users to endotoxins from the additive under assessment (Table A.1) (EFSA FEEDAP Panel, 2012).

**Table A.1:** Estimation of user exposure to endotoxins from the additive L-tryptophan produced by *Escherichia coli* CGMCC 11674, including consideration of using a filter mask FF P2 or FF P3 as a preventative measure

Calculation	Identifier	Description	Amount	Source
	a	Endotoxin content IU/g product	■	Technical dossier
	b	Dusting potential (g/m <sup>3</sup> )	53	Technical dossier
$a \times b$	c	Endotoxin content in the air (IU/m <sup>3</sup> )	14,522	
	d	No of premixture batches made/working day	40	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012)
	e	Time of exposure (s) per production of one batch	20	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012)
$d \times e$	f	Total duration of daily exposure/worker (s)	800	
	g	Uncertainty factor	2	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012)
$f \times g$	h	Refined total duration of daily exposure/worker (s)	1600	
$h/3$	i	Refined total duration of daily exposure (h)	0.44	
	j	Inhaled air (m <sup>3</sup> ) per eight-hour working day	10	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012)
$j/8 \times i$	k	Inhaled air during exposure (m <sup>3</sup> )	0.56	
$c \times k$	l	<b>Endotoxin inhaled (IU) during exposure per eight-hour working day</b>	<b>8,068</b>	
	m	Health-based recommended exposure limit of endotoxin (IU/m <sup>3</sup> ) per eight-hour working day	90	Health Council of the Netherlands (2010)
$m \times j$	n	<b>Health-based recommended exposure limit of total endotoxin exposure (IU) per eight-hour working day</b>	<b>900</b>	

Calculation	Identifier	Description	Amount	Source
I/10		Endotoxins inhaled (IU) per eight-hour working day reduced by filter mask FF P2 (reduction factor 10)	807	
I/20		Endotoxins inhaled (IU) per eight-hour working day reduced by filter mask FF P3 (reduction factor 20)	403	



## **Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis of L-Tryptophan Produced by *Escherichia coli* CGMCC 11674**

In the current application, authorisation is sought under Article 4(1) for L-tryptophan produced by *Escherichia coli* CGMCC 11674, under the category/functional group 3(c) 'nutritional additives/'amino acids, their salts and analogues', according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for all animal species. L-Tryptophan is already authorised as a feed additive under Commission Directive 88/485/EEC of 26 July 1988 amending the Annex to Council Directive 82/471/EEC concerning certain products used in animal nutrition.

For the quantification of L-tryptophan in premixtures, feedingstuffs and water, the Applicant submitted the ring-trial validated Community method (Commission Regulation (EC) No 152/2009). This High-Performance Liquid Chromatography equipment coupled with fluorescence detection (HPLC-FD) method applies for the determination of free (synthetic and natural) and of total (peptide-bound and free) amino acid in feedingstuffs only. The following performance characteristics were reported for the quantification of tryptophan: a relative standard deviation for repeatability (RSDr) ranging from 0.8 to 1.9% and a relative standard deviation for reproducibility (RSDR) ranging from 1.5 to 6.3%.

For the quantification of L-tryptophan in the feed additive, the Applicant submitted the ring-trial validated EN ISO 13904:2005 based on HPLC-FD and specifically designed for feedingstuffs. The standard was further ring-trial validated to assess the performance characteristics of the analytical method for the determination of the amino acid in feed additives and premixtures thus resulting in the recent standard EN ISO 13904:2016. The following performance characteristics were reported for the quantification of free tryptophan: RSDr ranging from 0.5 to 5.3% and RSDR ranging from 1.0 to 9.5%.

Based on the performance characteristics presented, the EURL recommends for official control (i) the ring-trial validated EN ISO 13904:2016 based on reversed phase HPLC-FD, to determine tryptophan in feed additives and premixtures and (ii) the Community method, based on reversed phase HPLC-FD, to determine tryptophan in feedingstuffs. In addition, the EURL identified the 'L-tryptophan monograph' of the Food Chemical Codex (FCC) for the characterisation of the feed additive.

Since the Applicant provided no experimental data to determine L-tryptophan in water, the EURL is neither able to evaluate nor to recommend a method for official control. 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.