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Safety of L-tryptophan produced using *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 FEEDAP Panel issued an opinion in 2019 in which they could not conclude on the safety of the additive for the target animals and for the consumer due to tryptophan-related impurities such as 1,1′-ethylidene-bis-L-tryptophan (EBT). The applicant submitted additional data and the European Commission (EC) has requested EFSA to deliver a new opinion on the safety of the additive under assessment. The manufacturing process has been adjusted to reduce the amount of tryptophan-related impurities and the analysis of three new batches of the additive indicated that levels of EBT and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA) represent no safety concerns for the target species or for the consumer.

Keywords: nutritional additive, amino acid, L-tryptophan, safety, *Escherichia coli* CGMCC 11674

Requestor: European Commission

Question number: EFSA-Q-2020-00256

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Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 9 defines the terms of the authorisation by the Commission.

The applicant Agri Nutrition BV is seeking a Community authorisation of L-tryptophan produced by fermentation using *Escherichia coli* CGMCC 11674 as feed additive to be used as amino acids their salts and analogues for all animal species (Table 1).

Table 1: Description of the substances

Category of the additive	Nutritional additive
Functional group of the additive	Amino acids, their salts and analogues
Description	L-tryptophan produced by *Escherichia coli* CGMCC 11674
Target animal category	All animal species
Applicant	Agri Nutrition BV
Type of request	New opinion

On 26 February 2019, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) of the European Food Safety Authority ('Authority'), in its opinion on the safety and efficacy of the product, could not conclude on the safety of L-tryptophan produced by *Escherichia coli* CGMCC 11674. After the discussion with the Member States on the last Standing Committee, it was suggested to check for the possibility to provide:

- A description of the manufacturing process indicating the improvements made to purify the additive; and
- Certificates of analysis of the final product indicating that the tryptophan related impurities comply with the maximum limits set in the European Pharmacopoeia, in particular the maximum content of 10 mg/kg 1,1′-ethylene-bis-L-tryptophan (EBT).

The Commission gave the possibility to the applicant to submit complementary information in order to complete the assessment and to allow a revision of Authority's opinion. The new data have been received on 23 January 2020.

In view of the above, the Commission asks the Authority to deliver a new opinion on L-tryptophan produced by *Escherichia coli* CGMCC 11674 as a feed additive for all animal species based on the additional data submitted by the applicant.

1.2. Additional information

L-Tryptophan produced by fermentation using *E. coli* CGMCC 11674 is not authorised in the European Union. The FEEDAP Panel adopted an opinion on that additive in 2019. In that assessment, the tryptophan related impurities such as 1,1′-ethylidene-bis-L-tryptophan (EBT) did not allow to conclude on the safety of the additive for the target species and for the consumer.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of supplementary information[2] to a previous application on the same product.[3]

[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] FEED dossier reference: FAD-2020-0003.
[3] FEED dossier reference: FAD-2016-0032.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety of L-tryptophan produced with *E. coli* CGMCC 11674 is in line with the principles laid down in Regulation (EC) No 429/2008[4] and the relevant guidance documents: Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b,) and Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c).

3. Assessment

The subject of this assessment is L-tryptophan produced by fermentation using *E. coli* CGMCC 11674 when used as a nutritional additive, functional group 'amino acids, their salts and analogues'.

In its previous assessment, the FEEDAP Panel could not conclude on the safety of the product for the target species and the consumer due to the tryptophan-related impurities as e.g. EBT (EFSA FEEDAP Panel, 2019). The applicant has now provided new information regarding the manufacturing process and the presence of impurities, which is the subject of the present opinion.

3.1. Characterisation of the active substance/additive

In its previous opinion (EFSA FEEDAP Panel, 2019), the Panel characterised the additive and the production strain. The product is specified to content ≥ 98% L-tryptophan on 'as is' basis and < 0.5% water. The European Pharmacopoeia (2019) specifies for L-tryptophan a maximum permitted content of 1,1′-ethylidene-bis-L-tryptophan (EBT – impurity A) of 10 mg/kg additive; and the sum of all other impurities (B-L, including 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid [MTCA]) of 390 mg/kg additive. In the previous assessment, three batches of the additive were analysed to determine concentrations of these impurities and analytical data showed EBT ranging from 16 to 18 mg/kg additive and MTCA from 106 to 128 mg/kg additive.

The applicant has adjusted the manufacturing process [redacted]

Analysis of three new batches obtained after modifying the manufacturing process and performed in an external laboratory by validated methods indicated that levels of EBT and MTCA were below the limit of detection and conform to the limits established by the European Pharmacopoeia.[6] The tryptophan content in those batches was on average 98% (range from 97.6% to 98.4%) on as is basis.[7] The loss on drying ranged from 0.1% to 0.2%.[8]

3.2. Safety

In the previous opinion (EFSA FEEDAP Panel, 2019), the Panel concluded that the additive could not be considered safe for the target species and for the consumer unless it complied with the maximum limits set in the European Pharmacopoeia for the tryptophan-related impurities.

The modifications implemented in the manufacturing process have reduced the concentrations of EBT and MTCA to the point that they comply with the maximum limits set in the European pharmacopoeia. L-Tryptophan produced by fermentation using *E. coli* CGMCC 11674 can be considered safe for the target species and the consumer regarding the tryptophan-related impurities.

[4] 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]

[6] Technical dossier, Annexes 2 to 4. Detection limits in mg/kg were 10 for EBT and 0.1 form MTCA. Analytical method used to measure EBT concentration was high pressure liquid chromatography coupled with ultraviolet detector (HPLC-UVD). Analytical method to determine MTCA concentration was HPLC wit fluorescence detection.

[7] Technical dossier/Supplementary information May 2020/Annex 7. Analytical method VDLUFA, in accordance with the EU official method for determination of amino acids of Regulation 152/2009 (Annex III G for determination of tryptophan).

[8] Technical dossier/Supplementary information May 2020/Annex 6.

4. Conclusions

The applicant has implemented changes in the manufacturing process that have reduced the concentration of EBT and MTCA in the final product. The additive L-tryptophan produced by fermentation using *E. coli* CGMCC 11674 can be considered safe for the target species and the consumer regarding the tryptophan-related impurities.

5. Documentation as provided to EFSA/Chronology

Date	Event
16/01/2020	Dossier received by EFSA. L-Tryptophan produced using *E. coli* for all animal species. Submitted by Agri Nutrition BV
25/02/2020	Reception mandate from the European Commission
26/03/2020	Application validated by EFSA – Start of the scientific assessment
15/04/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: Characterisation of the additive*
20/05/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
25/05/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

EBT 1,1′-ethylidene-bis-L-tryptophan
MTCA 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid