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Safety of L-threonine produced by fermentation with *Escherichia coli* CGMCC 11473 as a feed additive for all animal species

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Abstract

The L-threonine under assessment is produced by fermentation with a genetically modified strain of *Escherichia coli* and it is intended to be used as a nutritional additive for all animal species. In 2017 the Panel on Additives and products or Substances used in Animal Feed (FEEDAP) of EFSA issued an opinion on the safety and efficacy of the product. In that assessment, the Panel could not conclude on the safety of the additive for the target species, consumers and the environment due to the lack of data regarding the characterisation of the production strain and the resulting product. The applicant provided additional data on the identity of the production strain, the genetic modification, the susceptibility to antibiotics and the absence of cells and recombinant DNA of the production strain in the final product. The recipient strain is safe and the genetic modification does not raise concerns. Moreover, viable cells or DNA of the production strain were not detected in the final product. With this new information the FEEDAP Panel concluded that L-threonine produced by *E. coli* CGMCC 11473 is safe for the all animal species, the consumers and the environment. The FEEDAP Panel could not conclude on the potential of the additive to be irritant to skin and eyes or on the skin sensitisation potential. It was concluded that there is a risk of exposure to endotoxins by inhalation for persons handling the additive.

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1. Introduction

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

The applicant, Agri Nutrition B.V., is seeking a Community authorisation of L-threonine produced by *Escherichia coli* CGMCC 11473 as a 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 additive	Nutritional additives
Functional group of additive	Amino acids, their salts and analogues
Description	L-threonine produced by <i>Escherichia coli</i> CGMCC 11473
Target animal category	All animal species
Applicant	AgriNutrition B.V.
Type of request	New opinion

On 5 July 2017, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority (Authority), in its opinion on the safety of the product, could not conclude on the safety of L-threonine produced by *E. coli* CGMCC 11473 in all animal species, under the condition of use as proposed by the applicant, concerning the target species, consumers and the environment. After the discussion with the Member States on the Standing Committee, it was suggested to check for the possibility to demonstrate the safety of the additive.

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 28 August 2018.

In view of the above, the Commission asks the Authority to deliver a new opinion on L-threonine produced by *E. coli* CGMCC 11473 as a feed additive for all animal species based on the additional data submitted by the applicant.

1.1. Additional information

L-Threonine produced by *E. coli* CGMCC 11473 is not authorised in the European Union (EU). The FEEDAP Panel adopted an opinion on the additive produced by the genetically modified strain of *E. coli* CGMCC 11473 in 2017. The data provided in that assessment did not allow the Panel to characterise the genetic modification of the production strain or to characterise other aspects relevant to establish the safety of the additive. Moreover, uncertainty remained on the possible presence of cells from the production strain and its recombinant DNA in the product. Consequently, the Panel could not conclude on the safety of the additive for the target species, consumers and the environment.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of supplementary information¹ to a previous application on the same product.²

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety of L-threonine produced with *E. coli* CGMCC 11473 is in line with the principles laid down in Regulation (EC) No 429/2008³ and the relevant guidance documents: Guidance on nutritional additives (EFSA FEEDAP Panel, 2012) and Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018).

¹ FEED dossier reference: FAD-2018-0051.

² FEED dossier reference: FAD-2016-0003.

³ 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.

3. Assessment

The additive under assessment is produced by a genetically modified strain of *E. coli* and contains by specification $\geq 98.5\%$ L-threonine, $< 1\%$ moisture and $< 1.4\%$ other substances. It is intended to be used in feed and water for drinking and for all animal species, but the levels to be included were not proposed. The product was assessed in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2017), but the information provided regarding the production strain and the final additive did not allow the Panel to characterise it in full.

In particular, the susceptibility of the production strain to relevant antimicrobials was not established. The strain was claimed to be genetically modified but the parental/recipient strain and the origin of the genes inserted were not indicated, and the genetic modifications could not be assessed. Moreover, no data were provided to assess the possible presence of viable cells of the production strain in the product and the data regarding the presence of recombinant DNA did not allow to draw conclusions. Owing to this lack of information, the Panel could not conclude on the safety aspects regarding the use of this additive for target animals, consumers and the environment.

The applicant has provided supplementary information to address the limitations regarding the characterisation of the production strain and the additive.

3.1. Characterisation of the production strain

The additive is produced by a strain of *E. coli* K-12 which is deposited in the Chinese General Microbiological Culture Collection Centre with accession number CGMCC 11473.⁴

The applicant provided new data regarding the susceptibility of the production strain to all antibiotics recommended by EFSA for Enterobacteriaceae (EFSA FEEDAP Panel, 2018), tested by broth microdilution. All minimum inhibitory concentration values were equal or below the corresponding cut-off values defined by the FEEDAP Panel. Therefore, the production strain is considered to be susceptible to the relevant antibiotics.

They are all considered to be housekeeping genes in *E. coli* K-12, and under normal circumstances, do not contribute to antibiotic resistance. This, suggest that the production strain does not carry acquired antibiotic resistance genes of concern.

3.1.1. Characterisation of the genetic modification

According to the newly submitted data, all genes inserted into the recipient strain were derived from *E. coli*. The genes include some involved in the synthesis of L-threonine

⁴ Technical dossier FAD-2016-0003/Section II/Annex 2.2.1.2.a and 2.2.1.2.b.

⁵ Technical dossier FAD-2018-0051/Annex 4.

[Redacted text]

Regarding the genetic modification, it has been explained that five different combinations of the genes mentioned above were constructed. These gene combinations were used to develop the several gene cassettes utilised [Redacted text]

[Redacted text]

[Redacted text]

[Redacted text]

3.1.2. Absence of production strain in the additive

The applicant provided three sets of data regarding the presence of viable cells of the production strain in the additive. In the first set of data, [Redacted text]

[Redacted text]

The Panel acknowledges the limitations found in the three sets of data but considering the results of all the tests done concludes that the data indicate that no viable cells of the production strain are present in the additive.

The presence of DNA from the production strain was investigated in two sets of experiments. [Redacted text]

[Redacted text]

[Redacted text]

Therefore, it can be concluded that no viable cells or recombinant DNA of the production strain were detected in the final additive.

3.2. Safety

3.2.1. Safety of the genetic modification

The recipient organism *E. coli* K-12 is considered to be safe. The genetic modification confers increased L-threonine biosynthesis capacity. No genes of concern were introduced into the recipient strain and no genes encoding antimicrobial resistance remained in the production strain. The product L-threonine produced by fermentation with *E. coli* CGMCC 11473 is considered to be safe with regard to the genetic modification of the production strain.

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

In the previous opinion, the FEEDAP Panel concluded that 'The genetic modification cannot be assessed. Uncertainty remains on the possible presence of cells from the production strain and/or their recombinant DNA in the product, including possible genes conferring antimicrobial resistance. Therefore, the FEEDAP Panel cannot conclude on the safety of the product L-threonine, produced by fermentation with *E. coli* CGMCC 11473 for the target species, consumers and the environment'.

The supplementary information submitted by the applicant allowed the Panel to conclude that the recipient strain is safe, the genetic modification raises no safety concerns and that no viable cells or DNA of the production strain were detected in the final product. The newly submitted data allow to address the limitation expressed by the Panel in the previous assessment regarding the production strain.

Considering the above, the FEEDAP Panel concludes that the additive is safe for the target species consumers of products derived from animals fed the additive and for the environment.

4. Conclusions

The recipient strain is considered safe and the genetic modifications raise no safety concerns. No viable cells or recombinant DNA were detected in the final additive. The Panel concludes that L-threonine produced with *E. coli* CGMCC 11473 is safe for the target species, consumers and the environment.

Documentation as provided to EFSA/Chronology

Date	Event
27/07/2018	Dossier received by EFSA. L-threonine produced by Escherichia coli CGMCC 11473 for all animal species. Submitted by Agri Nutrition B.V.
07/09/2018	Reception mandate from the European Commission
07/09/2018	Application validated by EFSA – Start of the scientific assessment
26/10/2018	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>
03/01/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
12/02/2019	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>
10/04/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
21/05/2019	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>
26/06/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
04/10/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

- CGMCC Chinese General Microbiological Culture Collection Centre
FEEDAP EFSA Panel on Additives and Products or Substances used in Animal Feed