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Safety of L-tryptophan produced by fermentation with *Escherichia coli* CGMCC 7.59 for all animal species based on a dossier submitted by Feedway Europe NV

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

Abstract

L-Tryptophan is a feed additive produced by fermentation using a genetically modified strain of *Escherichia coli*. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) issued an opinion on the safety and efficacy of the product, which concluded that 'The use of L-tryptophan produced by *E. coli* CGMCC 7.59 in feed is safe for non-ruminant target species when supplemented to diets in appropriate amounts. As the metabolites of L-tryptophan produced by ruminal bacteria may be toxic to the host animal, oral administration of unprotected L-tryptophan to ruminants should be avoided. The Panel on Additives and Products or substances used in Animal Feed (FEEDAP) has concerns about the safety of L-tryptophan for target species when administered via water for drinking. . . . .In the absence of any data on sensitisation, the product should be considered a potential dermal sensitiser. The level of endotoxins present in the product and its dusting potential indicate a health risk for the user upon inhalation'. The European Commission asked EFSA to deliver an opinion on the safety of L-tryptophan, produced by an improved manufacturing process, as a nutritional additive for all animal species based on additional data submitted by the applicant on characterisation of the additive. The FEEDAP Panel reiterates its concern on the use of unprotected tryptophan to ruminants and on the safety of the amino acid L-tryptophan for target species when administered simultaneously via water for drinking. As the estimated maximum exposure to endotoxins by inhalation of the improved product is below the provisional occupational exposure limit, no risk from exposure to endotoxins for people handling the additive is expected. Concerns remain about possible dermal sensitisation.

Keywords: nutritional additive, amino acid, L-tryptophan, all animal species, safety, efficacy

Requestor: European Commission

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Summary

Following a request from the European Commission (EC), the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on the safety of L-tryptophan produced by fermentation using the genetically modified strain *Escherichia coli* CGMCC 7.59 for all animal species.

In 2015, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) adopted an opinion on the safety and efficacy of L-tryptophan produced by fermentation using *E. coli* CGMCC 7.59. In that opinion, the FEEDAP Panel concluded that 'The use of L-tryptophan produced by *E. coli* CGMCC 7.59 in feed is safe for non-ruminant target species when supplemented to diets in appropriate amounts. As the metabolites of L-tryptophan produced by ruminal bacteria may be toxic to the host animal, oral administration of unprotected L-tryptophan to ruminants should be avoided. The Panel on Additives and Products or substances used in Animal Feed (FEEDAP) has concerns about the safety of L-tryptophan for target species when administered via water for drinking. . . . . .In the absence of any data on sensitisation, the product should be considered a potential dermal sensitiser. The level of endotoxins present in the product and its dusting potential indicate a health risk for the user upon inhalation'.

The applicant provided additional information in relation to the characterisation of the additive and the production process. The FEEDAP Panel has performed the assessment of those new data following an approach in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents.

This product is fully characterised in a previous scientific opinion; the additive does not give rise to safety concerns with regard to the genetic modifications of the production strain.

In the former assessment, the FEEDAP Panel expressed its concerns about the safety of L-tryptophan for target species when administered via water for drinking. Although the applicant states that the additive will not be used in feed and in water for drinking at the same time, the FEEDAP Panel has found no reason to modify its former conclusion.

The FEEDAP Panel reiterates its concern on the use of unprotected tryptophan to ruminants.

Due to improvements at several steps of the manufacturing process, the level of endotoxins present in the product and its dusting potential have been reduced removing any health risk for the user upon inhalation. The product should be considered a potential dermal sensitiser.

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1. Introduction

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

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, Feedway Europe NV, is seeking a Community authorisation of L-tryptophan, technically pure, to be used as a nutritional additive for all animal species (Table 1).

Table 1: Description of the substances

Category of additive	Nutritional additive
Functional group of additive	Amino acids, their salts and analogues
Description	L-Tryptophan, technically pure
Target animal category	All animal species
Applicant	Feedway Europe NV
Type of request	New opinion

On 29 January 2015, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) of the European Food Safety Authority ('Authority'), in its opinion concluded that the metabolites of L-tryptophan produced by ruminal bacteria may be toxic to the host animal, therefore oral administration of unprotected L-tryptophan to ruminants should be avoided. The FEEDAP Panel has concerns about the safety of L-tryptophan for target species when administered via water for drinking due to possible amino acid imbalances. Finally, in the absence of any data on sensitisation, the product should be considered a potential dermal sensitiser. The level of endotoxins present in the product and its dusting potential indicate a health risk for the user upon inhalation.

The Commission gave the possibility to the applicant to submit complementary information in order to complete the assessment on the safety and to allow a revision of Authority's opinion.

The Commission has now received new data on the safety of L-tryptophan produced by *Escherichia coli* CGMCC 7.59.

In view of the above, the Commission asks the Authority to deliver a new opinion on the safety of L-tryptophan as nutritional additive for all animal species based on the additional data submitted by the applicant.

1.2. Additional information

L-Tryptophan was first authorised for use in animal nutrition by Directive 88/485/EEC. It is currently authorised as a nutritional additive (functional group amino acids, their salts and analogues) for use in all animal species without time limit and without maximum content in feed.

The applicant has provided additional information on the physical properties of the additive and on the production process.

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[1] following a previous application on the same product.[2]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the characterisation and safety of L-tryptophan produced by *E. coli* CGMCC 7.59 is in line with the principles laid down in Regulation (EC) No 429/2008[3] and the relevant guidance documents: Guidance on nutritional additives (EFSA FEEDAP

[1] FEED dossier reference: FAD-2015-0031.
[2] FEED dossier reference: FAD-2010-0287.
[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.

Panel, 2012a) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b).

3. Assessment

L-Tryptophan (minimum content of L-tryptophan 98%, 'as is' basis) was first authorised for use in animal nutrition by Directive 88/485/EEC. It is currently included in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 concerning additives in feedingstuffs.

The current application is for the authorisation of L-tryptophan produced by a genetically modified (GM) strain of *E. coli* (CGMCC 7.59), a derivative of *E. coli* K-12. It is intended to be used in all animal species as a nutritional additive (functional group amino acids, their salts and analogues) to feed and to water for drinking. The additive does not give rise to safety concerns with regard to the genetic modifications of the production strain.

3.1. Characterisation

This product has been characterised in a previous scientific opinion (EFSA FEEDAP Panel, 2015).

Additional data have been submitted on the physical properties of the additive and on the manufacturing process.

3.1.1. Impurities[4]

The endotoxin activity in three batches of the former product ranged from 0.36 to 1.12 IU/mg.[5] The applicant stated that an improvement had been performed in the downstream process to reduce the amount of bacterial endotoxins in the final product. The filtration by ceramic membrane has been modified (from a former pore size of 300 kDa to the current smaller size of 20 kDa) and an additional technique has been applied. New data have been submitted on the bacterial endotoxin activity of the product, measured in three batches. The values ranged from 0.33 to 0.67 IU/mg (European Pharmacopoeia 2.6.14 method).[6]

3.1.2. Physical properties

The applicant stated that the production process has been modified to increase the crystal size of the final product and consequently decrease its dustiness. The evaporation temperature has been lowered (from 75 to 72°C), the evaporation rate reduced (from 2.5 to 2.0 m^3/h), the evaporation time prolonged (from 12 to 15 h) and the cooling time extended (from 3 to 5 h).

New analytical data have been provided to characterise the particle size and dustiness of the new final product. The particle size distribution (three batches analysed by laser diffraction) had a fraction of 97.8% (v/v) particles < 100 μm, of 73.7% (v/v) of particles < 50 μm and 13.5% (v/v) of particles < 10 μm of diameter.[7] In the former product (six batches analysed by sieving), about 4% (w/w) of particles had a diameter < 50 μm.[8] The dusting potential (three batches analysed by Stauber–Heubach) ranged from 0.81 to 1.21 g/m^3.[9] The dusting potential of the product assessed in January 2015 (three batches) ranged from 3.9 to 4.2 g/m^3.[10]

3.2. Safety

In its previous assessment the FEEDAP Panel, concluded that the additive is safe for the consumer and the environment, but expressed some concerns regarding safety for the target animals and the user (EFSA FEEDAP Panel, 2015).

[4] This section has been amended following the applicable provisions on confidentiality.
[5] Technical dossier FAD-2010-0287/ Supplementary information, December 2014/Attachments 1–4.
[6] Technical dossier/Technology improvement on production process, Supplementary information on endotoxin levels and Attachments 1–3. According to the European Pharmacopoeia, monograph 01/2010:50110, the International Unit of endotoxin is defined as the specific activity of a defined mass of the World Health Organization International Standard. One international unit (IU) of endotoxin is equal to one endotoxin unit (EU).
[7] Technical dossier/Supplementary information February 2016/Attachment 5.
[8] Technical dossier FAD-2010-0287/Section II.1.5 and Annex II.1.2.
[9] Technical dossier/Supplementary information February 2016/Annex 5.
[10] Technical dossier FAD-2010-0287/Supplementary information January 2013/Attachment 8.

3.2.1. Safety for the target species

In the former assessment, it was concluded that 'the FEEDAP Panel has concerns about the safety of L-tryptophan for target species when administered via water for drinking'. Although the applicant states that the additive will not be used in feed and in water for drinking at the same time, the FEEDAP Panel has found no reason to modify its former conclusion.

In its previous opinion, the Panel expressed concerns on the use of unprotected forms of L-tryptophan in ruminants as the metabolites of L-tryptophan produced by ruminal bacteria may be toxic to the host animals (EFSA FEEDAP Panel, 2015). No information has been provided by the applicant in this regard and the FEEDAP Panel does not see a need to reconsider its conclusion.

3.2.2. Safety for the user

3.2.2.1. Effects on the skin

In its former assessment, the FEEDAP Panel concluded that 'the product should be considered a potential dermal sensitiser'. No new data have been submitted that could justify a change in that conclusion.

3.2.2.2. Effects on the respiratory system

Although a previous assessment was performed in January 2015 (EFSA FEEDAP Panel, 2015), the technological improvement in the manufacturing process makes necessary to re-assess the relevance of the bacterial endotoxin activities found in the final product for the user.

The bacterial endotoxin activity (the three new batches) is up to 0.67 IU/mg.[11] The dusting potential ranges from 0.81 to 1.21 g/m^3.

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 (2012b), 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 455 endotoxin IU per 8-h working day, indicating no risk from the exposure to endotoxins for people handling the additive.

3.2.2.3. Conclusions on safety for the user

The level of endotoxins present in the product and its dusting potential indicate no health risk for the user upon inhalation. In the absence of any data on sensitisation, the product should be considered a potential dermal sensitiser.

4. Conclusions

In the absence of new data, the Panel reiterates their concerns on target animal safety with regard to the use of the additive in water and the use of unprotected forms in ruminants.

Due to improvements at several steps of the manufacturing process, the level of endotoxins present in the product and its dusting potential have been reduced removing any health risk for the user upon inhalation. The product should be considered a potential dermal sensitiser.

Documentation provided to EFSA

1) L-Tryptophan produced by *Escherichia coli* for all animal species. October 2015. Submitted by Feedway Europe NV.
2) L-Tryptophan produced by *Escherichia coli* for all animal species. Supplementary information. November 2015. Submitted by Feedway Europe NV.
3) L-Tryptophan produced by *Escherichia coli* for all animal species. Supplementary information. February 2016. Submitted by Feedway Europe NV.

[11] Technical dossier/Supplementary information on endotoxin levels and Attachments 1–3.

References

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Guidance for the preparation of dossiers for nutritional additives. EFSA Journal 2012;10(1):2535, 14 pp. doi:10.2903/j.efsa.2012.2535

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. doi:10.2903/j.efsa.2012.2539

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015. Scientific Opinion on the safety and efficacy of L-tryptophan produced by *Escherichia coli* CGMCC 7.59 for all animal species based on a dossier submitted by HELM AG on behalf of Meihua Holdings Co. Ltd. EFSA Journal 2015;13(2):4015, 17 pp. doi:10.2903/j.efsa.2015.4015

Health Council of the Netherlands, 2010. Endotoxins, 2010. Health-based recommended occupational exposure limit. Publication No 2010/04OSH. Health Council of the Netherlands, The Hague, 100 pp.

HSE (Health and Safety Executive), 2013. Occupational hygiene implications of processing waste at materials recycling facilities (MRFs). RR977 Research Report, HSE, London, UK, 41 pp.

Wallace RJ, Gropp J, Dierick N, Costa LG, Martelli G, Brantom PG, Bampidis V, Renshaw D and Leng L, 2016. Risks associated with endotoxins in feed additives produced by fermentation. Environmental Health, 15, 1–7.

Abbreviations

CGMCC	China general microbiological culture collection center
DECOS	Dutch Expert Committee on occupational safety
EC	European Commission
FEEDAP Panel	EFSA Panel on Additives and Products or Substances used in Animal Feed
GM	genetically modified
HSE	British Health and Safety Executive
IU	International unit of endotoxin activity. One IU corresponds to one EU

Appendix A – Safety for the user

The effects of endotoxin inhalation and the exposure limits have been described in a previous opinion (EFSA FEEDAP Panel, 2015).

Calculation of maximum acceptable levels of exposure from feed additives

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

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^3, 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^3, then the content of endotoxins of the dust, *c* IU/m^3, is obtained by simple multiplication, *a* × *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, 2012b).

Table A.1: Estimation of user exposure to endotoxins from the additive L-tryptophan produced by *Escherichia coli* CGMCC 7.59, 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	671	Technical dossier
	b	Dusting potential (g/m^3)	1.21	Technical dossier
a × *b*	*c*	Endotoxin content in the air (IU/m^3)	811.9	
	d	No of premixture batches made/working day	40	EFSA FEEDAP Panel (2012b)
	e	Time of exposure (s) per production of one batch	20	EFSA FEEDAP Panel (2012b)
d × *e*	*f*	Total duration of daily exposure/worker (s)	800	
	g	Uncertainty factor	2	EFSA FEEDAP Panel (2012b)
f × *g*	*h*	Refined total duration of daily exposure/ worker (s)	1,600	
h/3 600	*i*	Refined total duration of daily exposure (h)	0.44	
	j	Inhaled air (m^3) per 8-h working day	10	EFSA FEEDAP Panel (2012b)
j/8 × *i*	*k*	Inhaled air during exposure (m^3)	0.56	
c × *k*	*l*	**Endotoxin inhaled during exposure per 8-h working day**	**455**	
	m	Health-based recommended exposure limit of endotoxin (IU/m^3) per 8-h working day	90	Health Council of the Netherlands (2010)
m × *j*	*n*	**Health-based recommended exposure limit of total endotoxin exposure (IU) per 8-h working day**	**900**	
l/10		Endotoxins inhaled (IU) per 8-h working day reduced by filter mask FF P2 (reduction factor 10)	45	
l/20		Endotoxins inhaled (IU) per 8-h working day reduced by filter mask FF P3 (reduction factor 20)	23	