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Safety of L-lysine sulfate produced by fermentation with *Escherichia coli* CGMCC 3705 for all animal species

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Abstract

In 2015, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion on the safety and efficacy of L-lysine sulfate produced by fermentation with *Escherichia coli* CGMCC 3705, when used as a nutritional additive for all animal species. The Panel concluded that a maximum supplementation of complete feed up to 1% L-lysine sulfate was considered safe for all animal species; its use in animal feed does not pose a risk to the consumer or the environment; L-Lysine sulfate was not considered a skin or eye irritant or a skin sensitiser, but should be considered a hazard by inhalation. The applicant originally proposed a specification of minimum 55% lysine. However, based on the data provided in the technical dossier, the FEEDAP Panel recommended that the additive should be specified to a L-lysine content $\geq$ 65%. The applicant seeks authorisation for L-lysine sulfate with a minimum content of 55% L-lysine. The Commission gave the applicant the possibility of submitting complementary information to complete its assessment. The additional data on the characterisation of the additive containing $\geq$ 55% L-lysine showed that $>$ 99% of the product is comprised of known constituents, which do not give rise to safety concerns at the concentrations present. Therefore, the FEEDAP Panel considers that the conclusions of the previous opinion are applicable to the product L-lysine produced with *E. coli* CGMCC 3705 containing $\geq$ 55% L-lysine. Specifically, the FEEDAP Panel concludes that a maximum supplementation of complete feed up to 1% L-lysine sulfate is considered safe for all animal species; that the use in animal feed does not pose a risk to the consumer or the environment; and that L-Lysine sulfate is not considered a skin or eye irritant or a skin sensitiser, but should be considered a hazard by inhalation.

Keywords: nutritional additive, amino acids, L-lysine sulfate, safety, genetically modified microorganism

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Summary

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 the safety of L-lysine sulfate produced with a genetically modified strain of *Escherichia coli* (CGMCC 3705).

In 2015, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion on the safety and efficacy of L-lysine sulfate produced by fermentation with *E. coli* CGMCC 3705, when used as a nutritional additive for all animal species. The FEEDAP Panel concluded that L-lysine sulfate, feed grade, does not give rise to any safety concern with regard to the genetic modification of the production strain. A maximum supplementation of complete feed up to 1% L-lysine sulfate was considered safe for all animal species. The Panel also concluded that the use in animal feed of L-lysine sulfate produced by *E. coli* CGMCC 3705 does not pose a risk to the consumer or the environment. L-Lysine sulfate was not considered a skin or eye irritant or a skin sensitiser, but should be considered a hazard by inhalation. The applicant originally proposed a specification of minimum 55% lysine. However, based on the data provided in the technical dossier, the FEEDAP Panel recommended that the additive should be specified to a L-lysine content $\geq$ 65% (at a water content of the additive $\leq$ 1.5%).

The applicant seeks authorisation for L-lysine sulfate with a minimum content of 55% L-lysine. The Commission gave the applicant the possibility of submitting additional information to allow the FEEDAP Panel to complete its assessment. For this reason, additional data have been provided on the qualitative and quantitative composition of five batches of L-lysine sulfate containing minimum 55% L-lysine produced by the manufacturing process described in the former opinion.

The additional data on the characterisation of the additive produced with *E. coli* CGMCC 3705 and containing $\geq$ 55% L-lysine are the subject of this assessment. Although the active substance does not reach 95% of the product on a dry matter basis, > 99% of the product has been shown to be comprised of known constituents, which do not give rise to safety concerns at the concentrations present.

Therefore, the FEEDAP Panel considers that the conclusions of the previous opinion are applicable to the product L-lysine produced with *E. coli* CGMCC 3705 containing $\geq$ 55% L-lysine. Specifically, the FEEDAP Panel concludes that a maximum supplementation of complete feed up to 1% L-lysine sulfate is considered safe for all animal species and categories. The Panel also concludes that the use in animal feed of L-lysine sulfate produced by *E. coli* CGMCC 3705 does not pose a risk to the consumer or the environment. L-Lysine sulfate is not considered a skin or eye irritant or a skin sensitiser, but should be considered a hazard by inhalation.

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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, GBT Europe GmbH, is seeking a Community authorisation of L-lysine sulfate produced by fermentation with *Escherichia coli* CGMCC 3705 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-Lysine sulfate produced by fermentation with *Escherichia coli* CGMCC 3705
Target animal category	All animal species
Applicant	GBT Europe GmbH
Type of request	New opinion

On 16 June 2015, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ("Authority"), in its opinion could not conclude on the safety of the additive with the L-lysine content proposed by the applicant (≥55%) and recommended a L-lysine content ≥65% (at a water content of the additive ≤1.5%).

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.

In view of the above, the Commission asked the Authority to deliver a new opinion for L-lysine sulfate produced by fermentation with *E. coli* CGMCC 3705 as a nutritional additive for all animal species based on the additional data submitted by the applicant.

1.2. Additional information

The active substance, L-lysine, is produced by a genetically modified strain of *E. coli* (CGMCC 3705). L-Lysine is currently authorised for its use in all animal species as a nutritional additive (functional group amino acids, their salts and analogues).[1] No maximum content in feedingstuffs is established in the European Union (EU).

The applicant has provided additional information on the characterisation of the additive, in particular the quantitative and qualitative composition of the additive with an L-lysine content ≥ 55%.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of additional information[2] in support of the authorisation request for the use of L-lysine sulfate produced by fermentation with *E. coli* CGMCC 3705 as a feed additive, following a previous application on the same product.[3]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety of L-lysine sulfate produced by fermentation with *E. coli* CGMCC 3705 is in line with the principles laid down in Regulation (EC) No 429/2008[4] and the relevant guidance documents: Guidance on nutritional additives (EFSA FEEDAP Panel, 2012a).

[1] 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. OJ L 239, 30.8.1988, p. 36–39.
[2] FEED dossier reference: FAD-2016-0074.
[3] FEED dossier reference: FAD-2013-0045.
[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.

3. Assessment

The product under application is L-lysine sulfate with a content of L-lysine ≥ 55%. It is produced by fermentation with a genetically modified strain of *Escherichia coli* (CGMCC 3705). It is intended to be used in all animal species as nutritional additive (functional group amino acids, their salts and analogues) in feed.

In 2015, the FEEDAP Panel issued an opinion on the safety and efficacy of L-Lysine sulfate produced with *E. coli* CGMCC 3705 (EFSA FEEDAP Panel, 2015). In that opinion, the FEEDAP Panel concluded that the product L-lysine sulfate (≥ 65%) manufactured by fermentation with *E. coli* CGMCC 3705, does not give rise to any safety concern with regard to the genetic modification of the production strain. A maximum supplementation of complete feed up to 1% L-lysine sulfate was considered safe for all animal species and categories. The Panel also concluded that the use in animal feed of L-lysine sulfate produced by *E. coli* CGMCC 3705 does not pose a risk to the consumer or the environment and is regarded as an efficacious source of the amino acid L-lysine for all animal species. L-Lysine sulfate is not considered a skin or eye irritant or a skin sensitiser, but should be considered a hazard by inhalation. The applicant originally proposed a specification of minimum 55% lysine; however, based on the data provided in the technical dossier, the FEEDAP Panel recommended that the additive should be specified to a L-lysine content ≥ 65% (at a water content of the additive ≤ 1.5%). The applicant is now requesting that the product is specified at minimum 55% and has provided additional data on the qualitative and quantitative composition of five batches of L-lysine sulfate. The applicant states that no changes have been introduced in the manufacturing process.[5]

3.1. Characterisation of the product

In the previous assessment, the average analytical L-lysine content of five pilot batches was 66.4% as is; the average sulfate content was 20% as is, and the amount of identified material was on average 100.4% on dry matter basis (EFSA FEEDAP Panel, 2015).

The applicant requests the additive is specified to contain ≥ 55% L-lysine and ≤ 4% moisture. To support this, data on the composition of five new batches were provided. The average lysine content of these batches was 57.8% (range 56.7–58.6%) as is, and the moisture was, on average, 2% (range 0.2–2.5%). The mean sulfate content was 22.2% (range 20.6–23.2%). Amino acids other than lysine amounted to, on average, 8.4% (8.2–8.5%). Minor constituents were crude ash (range 2.9–3.3%), total dietary fibre (range 1.7–4.5%), starch (range 0.3–0.4%), total sugars (range < 1–1.3%), organic acids (range 1.6–1.7%), biogenic amines (range 0.23–0.25%), betaine (0.31%), crude fat (range 0.18–0.24%), nitrate (range < 0.001%) and nitrite (< 0.001–0.0006%).

The amount of identified material in these five batches was on average 99.5% (98.3–101.3%) on a dry matter basis.

3.2. Safety

In its previous opinion, the FEEDAP Panel concluded that L-lysine sulfate was safe for the target species (with a maximum supplemental level of 1% lysine sulfate), consumers, users (except that it should be considered a hazard by inhalation) and the environment. The FEEDAP Panel is assessing now if the reduction in the minimum specification has an impact on the previous conclusions.

The new data provided by the applicant on the composition showed that the product contains on average 57.8% L-lysine (range 56.7–58.6%) as is and that the amount of unidentified substances is below 0.5% on average on dry matter. The new data does not raise issues that were not addressed in the previous opinion. The FEEDAP Panel considers that the conclusions of the previous opinion are applicable to the product L-lysine produced with *E. coli* CGMCC 3705 containing a minimum of 55% lysine.

4. Conclusions

The data provided shows that the product L-lysine sulfate produced with *E. coli* CGMCC 3705 contains on average 57.8% L-lysine (range 56.7–58.6%) as is and contains on average 99.5% identified material on dry matter basis without toxicological concern. The FEEDAP Panel considers that the conclusions of the previous opinion are applicable to the product under assessment.

[5] Technical dossier/Additional information FAD-2013-0045 and written confirmation product identity.

Therefore, the FEEDAP Panel concludes that a maximum supplementation of complete feed up to 1% L-lysine sulfate is considered safe for all animal species and categories. The Panel also concludes that the use in animal feed of L-lysine sulfate produced by *E. coli* CGMCC 3705 does not pose a risk to the consumer or the environment. L-Lysine sulfate is not considered a skin or eye irritant or a skin sensitiser, but should be considered a hazard by inhalation.

Recommendation

The description of L-lysine sulfate should contain the statement 'produced by fermentation with *Escherichia coli* CGMCC 3705'.

Documentation provided to EFSA

1) Dossier name. December 2016. Submitted by GBT Europe GmbH.

References

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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-lysine sulphate produced by fermentation with *Escherichia coli* CGMCC 3705 for all animal species. EFSA Journal 2015;13(7):4155, 22 pp. doi:10.2903/j.efsa.2015.4155

Abbreviations

CGMCC	China General Microbiological Culture Collection Center
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
GBT	Global Bio Chem Technology