

ADOPTED: 23 March 2022

doi: 10.2903/j.efsa.2022.7246

Safety and efficacy of a feed additive consisting of L-lysine sulfate produced by *Escherichia coli* CGMCC 7.398 for all animal species (Kempex Holland B.V.)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of L-lysine sulfate produced by the genetically modified strain *Escherichia coli* CGMCC 7.398 as a nutritional feed additive for all animal species. Neither the production strain nor its recombinant DNA were detected in the final product. The additive does not pose any safety concerns associated with the production strain. The additive under assessment is considered safe for the target species. When using L-lysine sulfate, the background sulfur/sulfate content in the compound feed should be taken into account. L-lysine sulfate produced by *E. coli* CGMCC 7.398 is safe for the consumers and for the environment. In the absence of data, the FEEDAP Panel cannot conclude on the potential of the additive under assessment to be irritant to skin or eyes, or on its potential to be a dermal sensitiser. The endotoxin activity of the additive represents a risk by inhalation for users handling the additive. The additive L-lysine sulfate is considered as an efficacious source of the essential amino acid L-lysine for non-ruminant animal species. For the supplemental L-lysine to be as efficacious in ruminants as it is in non-ruminant species, this would require protection against degradation in the rumen.

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Keywords: Lysine sulfate, amino acid, nutritional additive, *Escherichia coli* CGMCC 7.398, safety, efficacy

Requestor: European Commission

Question number: EFSA-Q-2021-00086

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Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at <https://ess.efsa.europa.eu/doi/doiweb/doisearch>.

Acknowledgments: The Panel wishes to thank the following for the support provided to this scientific output: Martina Reitano, Joana Revez and the working group on Microbiology of the FEEDAP Panel.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos MDL, Christensen H, Dusemund B, Fašmon Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Herman L, Galobart J, Pettenati E and Tarrés-Call J, 2022. Scientific Opinion on the safety and efficacy of a feed additive consisting of L-lysine sulfate produced by *Escherichia coli* CGMCC 7.398 for all animal species (Kempex Holland B.V.). EFSA Journal 2022;20(4):7246, 17 pp. <https://doi.org/10.2903/j.efsa.2022.7246>

ISSN: 1831-4732

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The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.



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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003¹ 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 feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Kempex Holland B.V.² for the authorisation of the additive consisting of L-lysine sulfate produced by *Escherichia coli* CGMCC 7.398, when used as a feed additive (category: nutritional additives; functional group: amino acids, their salts and analogues) for all animal species.

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). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 16 February 2021.

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 feed additive consisting of produced by *Escherichia coli* CGMCC 7.398, when used under the proposed conditions of use (see Section 3.1.6).

1.2. Additional information

L-lysine sulfate produced by fermentation with a genetically modified strain of *Escherichia coli* (CGMCC 7.398) is not authorised in the EU. L-lysine produced using different microbial strains is currently authorised for its use in all animal species as a nutritional additive.³

L-Lysine is authorised for use in food,⁴ cosmetics⁵ and as a veterinary medicinal product.⁶

EFSA issued several opinions on the safety and efficacy of L-lysine and/or its salts produced by fermentation by different strains of *Corynebacterium glutamicum* (EFSA, 2007; EFSA FEEDAP Panel, 2015b, 2016b, 2019a–e, 2020a,b,c, 2021a,b,c); *Corynebacterium casei* (EFSA FEEDAP Panel, 2020d); or other strains of *E. coli* (EFSA FEEDAP Panel, 2013, 2014, 2015a,b,c, 2016a, 2017a) when used in feed and/or in water for drinking for all animal species.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier⁷ in support of the authorisation request for the use of L-lysine sulfate produced by *Escherichia coli* CGMCC 7.398 as a feed additive.

¹ Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

² Kempex Holland B.V., Zeelandsedijk 15, 5408SL Volkel, The Netherlands.

³ See the European Union Register of Feed Additives, available online: https://ec.europa.eu/food/safety/animal-feed/feed-additives/eu-register_en

⁴ 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.

⁵ 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.

⁶ 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.

⁷ FEED dossier reference: FAD-2020-0085.

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-lysine sulfate produced by *Escherichia coli* CGMCC 7.398 in animal feed. The Executive Summary of the EURL report can be found in Annex A.⁸

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-lysine sulfate produced by *E. coli* CGMCC 7.398 is in line with the principles laid down in Regulation (EC) No 429/2008⁹ and the relevant guidance documents: Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b); 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, 2017c); Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017d); Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012); and Guidance for assessing the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019f).

3. Assessment

The current application is for the authorisation of L-lysine sulfate ($\geq 55\%$ on dry matter [DM] basis) produced by fermentation with a genetically modified strain of *Escherichia coli* (CGMCC 7.398). The L-lysine sulfate is to be used in feed for all animal species as a nutritional additive (functional group 'amino acids, their salts and analogues').

3.1. Characterisation

3.1.1. Characterisation of the production organism

L-Lysine is produced by a genetically modified strain of *Escherichia coli* which is deposited in the China General Microbiological Culture Collection Center (CGMCC) with accession number CGMCC 7.398.¹⁰

The taxonomic assignment was performed

[REDACTED]

However, only the [REDACTED] is not sufficient to taxonomically assign the production strain to the *E. coli* K12 lineage.

The applicant submitted a bioinformatic analysis of the WGS data which showed that the production strain has a [REDACTED] a well-known K12 derivative. Overall, considering the whole data set available, it can be concluded that the production strain belongs to the *E. coli* K12 lineage. *E. coli* K-12 is well-characterised, its safety (non-pathogenicity) has been documented (Gorbach, 1978) and its ineffectiveness in colonising the human gut is reported (Smith, 1975). The genome of its derivatives (MG1655 and W3110) has been fully sequenced (Hayashi et al., 2006).

According to the applicant, no plasmids were identified in the WGS-based analysis.¹² However, the methodology followed to establish the presence/absence of plasmids was not reported.

The production strain was tested for its susceptibility to the eight antimicrobials listed for 'Enterobacteriaceae' recommended by EFSA (EFSA FEEDAP Panel, 2018).¹³ All minimum inhibitory

⁸ The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2020-00820085_en#files

⁹ 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.

¹⁰ Technical dossier/Section II/Annex 2.2.1.2a.

¹¹ Technical dossier/Section II/Annexes Sct_II/Annex 2.2.1.2.b and 2.2.1.2.c.

¹² Technical dossier/Section II/Annexes Sct_II/Annex 2.2.1.2.b.

¹³ Technical dossier/Section II/Annex 2.2.2.2.

concentration (MIC) values were equal or fell below the EFSA cut-off values, and therefore, the strain is considered susceptible to the relevant antimicrobials.

The WGS data of the production strain were interrogated for the presence of antimicrobial resistance (AMR) genes [redacted]¹⁴ The search [redacted]

[redacted] did not identify any hit.

The WGS data of the production strain were queried for the presence of genes coding for toxins and virulence factors [redacted]¹⁵

[redacted] Therefore, the production strain was shown not to harbour genes encoding for known pathogenicity factors.

3.1.1.1. Information relating to the genetically modified microorganism

The parental strain is reported as [redacted]¹⁶

[redacted]¹⁷

3.1.2. Manufacturing process

L-Lysine is produced by fermentation using *E. coli* CGMCC 7.398. [redacted]¹⁸

The applicant declared that no antimicrobials are used in the manufacturing process.¹⁹

3.1.3. Characterisation of the active substance/additive

L-Lysine sulfate (CAS No 60343-69-3) has a molecular weight of 390.38 g/mol. The molecular formula is $C_{12}H_{28}N_4O_4 \cdot O_4S$ and the molecular structure is given in Figure 1. The theoretical content of lysine in the pure lysine sulfate is 75%.

¹⁴ Technical dossier/Supplementary information October 2021/FAD-2020-0085 Sin 300421 Answers, reply to Q2.

¹⁵ Technical dossier/Supplementary information October 2021/FAD-2020-0085 Sin 300421 Answers, reply to Q3.

¹⁶ Technical dossier/Technical dossier/Annex 2.2.1.2.e. and Supplementary information October 2021/FAD-2020-0085 Sin 300421 Answers, reply to Q4.

¹⁷ Technical dossier/Section II/Annexes 2.2.1.2g and 2.2.1.2f and Supplementary information October 2021/FAD-2020-0085 Sin 300421 Answers, reply to Q5 and Annex 2.

¹⁸ Technical dossier/Supplementary information October 2021/ FAD-2020-0085 Sin 300421 Answers.

¹⁹ Technical dossier/Section II/Annex 2.3.1b.

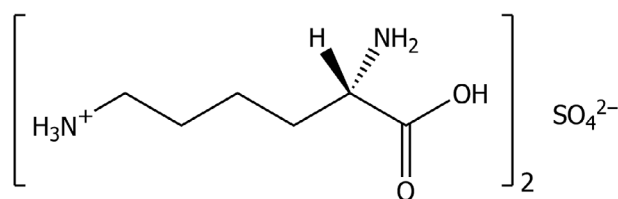


Figure 1: Molecular structure of L-lysine sulfate

L-Lysine sulfate under assessment contains by specification $\geq 55.0\%$ lysine and $\geq 18.0\%$ sulfate on a dry matter basis, with a water content $\leq 4.0\%$.

Analytical data to confirm the specifications were provided for five batches of the additive, showing the following average values: 56.1% lysine (55.7–56.9%) on DM basis; 23.2% sulfate (21.6–24.2%) on DM basis; 2.6% water (2.1–3.1%).²⁰ The identified material is about 79% of the additive on a DM basis.

The applicant provided compositional data of additional five batches,²¹ indicating that lysine averaged 58.1% (range 57.5–58.7%) on 'as is' basis; sulfate averaged 19.7% (range 19.4–20.1%); amino acids other than lysine (not further described) averaged 8.3% (7.9–8.6%); residue on ignition averaged 3.4% (range 3.1–3.7%); loss on drying averaged 3.2% (range 2.9–3.4%); reducing sugars were on average 2.4% (range 1.9–2.8%); crude fibre represented 2.0% (range 1.9–2.3%); crude fat represented 1.0% (range 0.9–1.2%); and ammonium represented 0.95% (range 0.91–0.94%). The calculated total identified material was about 99.1% based on DM basis.

Three batches of the additive were analysed for cadmium, lead, mercury and arsenic concentrations. Results showed values for mercury below the limit of detection (LOD), lead ranged from 0.03 to 0.04 mg/kg, cadmium was 0.004 mg/kg and arsenic ranged from 0.04 to 0.05 mg/kg.²²

Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar dioxin-like polychlorinated biphenyls (Co-planar PCBs) were analysed in three batches and found below the corresponding limit of quantification (LOQ).²³ The calculated (upper bound) levels of dioxins and the sum of dioxins and dioxin-like-PCBs were 0.137 ng WHO-PCDD/F-TEQ/kg and 0.269 ng WHO-PCDD/F-PCB-TEQ/kg, respectively (in all three batches).

The same three batches were analysed for mycotoxins.²⁴ Aflatoxins ranged from 1.6 to 2.0 $\mu\text{g}/\text{kg}$, zearalenone ranged from 64 to 113 $\mu\text{g}/\text{kg}$, ochratoxin A ranged from 25 to 26 $\mu\text{g}/\text{kg}$, deoxynivalenol ranged from 200 to 260 $\mu\text{g}/\text{kg}$ and citrinin ranged from 21 to 22 $\mu\text{g}/\text{kg}$. Fumonisin (B1+B2+B3) were below the limit of detection of 25 $\mu\text{g}/\text{kg}$.

The detected amounts of the above described impurities do not raise safety concerns.

Microbiological contamination was analysed in five batches for *Enterobacteriaceae*, *Escherichia coli*, *Salmonella* spp., yeasts and filamentous fungi and none were detected in 25-g samples.²⁵

Endotoxin activity was analysed (Ph Eur 2.6.14, method A) in three batches of the final product and in all cases the result was < 30 IU/mg.²⁶

The applicant provided two sets of data regarding the possible presence of viable cells of the production strain *E. coli* CGMCC 7.398 in the final product. In the first set of data,²⁷ three batches of the additive were tested in triplicate. Samples of 10 g were diluted into 90 mL of sterile water, and then, a total of 10 mL (corresponding to 1 g of the original sample) were plated on non-selective Luria Bertani (LB) agar and incubated at 37°C for 48 h. Positive controls were included. No colonies of the production strain were found in three independent batches of the final product. In a second analysis,²⁸ the presence of the production strain was tested in nine batches of the product under assessment. A sample of 10 g was collected from each of the nine batches and diluted in 90 mL of sterile water.

²⁰ Technical dossier/Section II/Annex 2.1.3a and supplementary information October 2021/FAD-2020-0085 Sin 300421 Answers. Analytical method was stated to be in accordance with Commission Regulation No 152/2009 Appendix III.

²¹ Technical dossier/Supplementary information October 2021/Annex 7. Analytical method for determination of lysine was EU No. 152/2009.

²² Technical dossier/Section II/Annex 2.1.3a. LOD of mercury in mg/kg was 0.002.

²³ Technical dossier/Section II/Annexes 2.1.4a to c. LOQ in ng/kg ranged 0.04 to 2 for PCDD/F and 5 to 20 for dioxin-like PCBs.

²⁴ Technical dossier/Supplementary information October 2021/Annex 8.

²⁵ Technical dossier/Section II/Annex 2.1.3a and Section II.6.3.

²⁶ Technical dossier/Section II/Annexes 2.1.4.d to 2.1.4.f. Gel clot limit test.

²⁷ Technical dossier/Section II/Annex 2.2.1.2.b and Supplementary information October 2021/FAD-2020-0085_SIn_300421 Answers.

²⁸ Technical dossier/Supplementary information October 2021/FAD-2020-0085_SIn_300421 Answers and Annex 3.

Subsequently, 10 mL was plated on LB media and incubated at 37°C for 48 h. As above, positive controls were included. The results did not show growth.

The presence of recombinant DNA from the production strain was tested in three batches of the additive in triplicate.²⁹ The starting material was 1 gram, DNA was extracted using a suitable method and primers targeted the junction of the deletion of the *maeA* with an expected amplicon size of 580 bp. Positive and negative controls were included. The limit of detection of samples spiked with genomic DNA of the production strain was 1 ng/g of additive. No recombinant DNA of the production strain was detected.

3.1.4. Physical properties of the additive

The additive appears as brown granules, soluble in water. The reported bulk density was 600 kg/m³.³⁰

The dusting potential of three batches of the additive was determined using the Stauber-Heubach method and showed values ranging from 100 to 300 mg/m³.³¹

Particle size distribution of three batches (laser diffraction) showed no particles below 100 µm diameter, being 98% of the particles above 500 µm of diameter.³²

3.1.5. Stability and homogeneity

The shelf-life of the additive was studied in three batches when stored in plastic bags at 25°C for 24 months or at 40°C for 12 months. No losses of lysine were observed at the end of the storage periods.³³

The stability of three batches of the additive was studied in a vitamin/mineral premixture (containing 0.75% choline chloride) for pigs for fattening when supplemented at 4.3% with lysine sulfate (corresponding to 2.4% lysine) and stored at about 25°C in plastic bags for 6 months. No losses were observed at the end of the storage period.³⁴

The stability of three batches of the additive was studied in a complete starter feed for chicken for fattening (mash and pelleted form) based on maize and soybean meal, when supplemented at 0.3% with L-lysine sulfate (corresponding to 0.17% lysine).³⁵ The total lysine in the supplemented diet was 1.17%. Pelleting was performed at 80°C and it represented no loss of lysine. Samples were stored at 20–30°C in plastic bags for 3 months. At the end of the storage period, no losses were observed in mash or in pelleted feed.

The stability of three batches of the additive was studied in a complete grower feed for pigs for fattening (mash and pelleted form) based on maize, wheat bran and soybean meal, when supplemented at 0.14% with L-lysine sulfate (corresponding to 0.08% lysine).³⁶ The total lysine in the supplemented diet was 0.75%. Pelleting was performed at 80°C and it represented a loss of lysine < 1%. Samples were stored at 20–30°C in plastic bags for 3 months. At the end of the storage period, no losses were observed in mash or in pelleted feed.

The stability of three batches of the additive was studied in a complete grower feed for laying hens (mash and pelleted form) based on maize, wheat bran and soybean meal, when supplemented at 0.09% with L-lysine sulfate (corresponding to 0.05% lysine).³⁷ The total lysine in the supplemented diet was 0.95%. Pelleting was performed at 80°C and it represented no loss of lysine. Samples were stored at 20–30°C in plastic bags for 3 months. At the end of the storage period, no losses were observed in mash or in pelleted feed.

The capacity for homogeneous distribution of the additive in the compound feed for laying hens (mash form) described above was studied in 10 subsamples. Total lysine was analysed and the coefficient of variation (CV) was 0.6%.³⁸ When the background lysine of the diet (0.9%) was subtracted to each subsample, the CV was 8%.

²⁹ Technical dossier/Section II/Annex 2.2.1.2.b and Supplementary information October 2021/FAD-2020-0085_SIn_300421 Answers, Annex 4 and Annex 5.

³⁰ Technical dossier/Section II.1.5.

³¹ Technical dossier/Section II/Annexes 2.1.5b to d.

³² Technical dossier/Section II/Annex 2.1.5a.

³³ Technical dossier/Section II/Annex 2.4.1a.

³⁴ Technical dossier/Section II/Annex 2.4.1c and d 2.4.1b.

³⁵ Technical dossier/Section II/Annexes 2.4.1e and 2.4.1d for feed composition.

³⁶ Technical dossier/Section II/Annexes 2.4.1f and 2.4.1d for feed composition.

³⁷ Technical dossier/Section II/Annexes 2.4.1g and 2.4.1d for feed composition.

³⁸ Technical dossier/Section II/Annex 2.4.1h and supplementary information October 2021/FAD-2020-0085 Sin 300421 Answers.

3.1.6. Conditions of use

The additive is intended to be used in complete feed for all animal species, directly or through complementary feed or via premixtures. No proposed inclusion levels are provided, as the optimal daily allowance in quantitative terms depends 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 of the production microorganism

L-lysine is produced by a genetically modified strain of *E. coli*. The parental strain of the production strain is considered to be safe. The data provided allow to confirm that the production strain belongs to the *E. coli* K12 lineage. The genes introduced during the genetic modification do not raise safety concerns. The production strain was shown susceptible to all relevant antimicrobials and does not harbour genes encoding for AMR of concern or known pathogenicity factors. In addition, no viable cells or recombinant DNA of the production strain were detected in the final product. It can be concluded that the final product does not give rise to any safety concern with regard to the production strain *E. coli* CGMCC 7.398.

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

L-Lysine requirements of different nonruminant species and animal categories, the absorption and metabolic fate of L-lysine, the tolerance to L-lysine excess and the lysine to arginine antagonism have been described in detail in a previous opinion (EFSA FEEDAP Panel, 2013). No safety concerns for ruminants would arise from ruminal lysine metabolism (EFSA FEEDAP Panel, 2014). The use of the amino acid 'per se' will not raise safety concerns for the target animals provided it is supplemented in appropriate amounts to the diets.

Safety concerns from the additive could derive from the residues of the fermentation process/production strain remaining in the final product. The production strain was identified as an *E. coli* K12 derivative, and no safety concerns related to the genetic modification process were noted. The production strain does not harbour genes encoding for AMR of concern or known pathogenicity factors, and its viable cells and recombinant DNA were not detected in the final product. Therefore, no safety concerns for target animal, consumers and the environment are expected from the additive concerning the production strain and the fermentation residues that may be present in the final additive.

With regard to the high intrinsic content of sulfate in L-lysine sulfate, the EFSA FEEDAP Panel (2019a–e, 2020a,b) already concluded that the formulation of the complete feed should carefully take into account the maximum tolerable level of total sulfur (S), as established by NRC (2005) and set in ruminant diets at 3 g S/kg DM (diet rich in concentrate) or 5 g S/kg DM (diet rich in roughage); and in non-ruminant diets at 4 g S/kg DM. Also, the contribution of sulfur/sulfate present in water for drinking to the total sulfur intake should be considered, especially when the content is high.

To support the safety of the sulfate contained in the additive, the applicant provided the same data as in a previous opinion (EFSA FEEDAP Panel, 2021c): a tolerance study in chickens for fattening administered with manganese sulfate (unpublished study),³⁹ and a literature search in Scopus database to support the safety of the sulfur content of the additive for the target species.⁴⁰ The same seven references were considered relevant. Consequently, no negative effects are to be expected at normal use levels for the target species provided that the total sulfur intake complies with the recommendations established by scientific bodies. When using L-lysine sulfate, the background sulfur/sulfate content in the compound feed should be taken into account.

Absorption, distribution, metabolism and excretion of L-lysine were described in a previous scientific opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2013). The use of the amino acid L-lysine itself in animal nutrition is considered safe for consumers.

The amino acid L-lysine is a physiological and natural component of animals and plants. It is not excreted as such (but as urea/uric acid and carbon dioxide). The use of L-lysine in animal nutrition

³⁹ Technical dossier/Section III/Annex 3.1.1.b.

⁴⁰ Technical dossier/Section III/Annex 3.1.1.a and related annexes.

would not lead to any localised increase in the concentration of L-lysine or its metabolites in the environment.

3.2.2.1. Conclusions on the safety for the target species, consumer and environment

L-Lysine sulfate produced by *E. coli* CGMCC 7.398 is considered safe for the target species. When using L-lysine sulfate, the background sulfur/sulfate content in the compound feed should be taken into account. L-Lysine sulfate produced by *E. coli* CGMCC 7.398 is safe for the consumers and for the environment.

3.2.3. Safety for the user

No specific data on inhalation toxicity, skin/eye irritation or dermal sensitisation were submitted to support the safety of the additive under assessment for the user. The highest measured dusting potential of the additive was 300 mg/m³ but no particles of a diameter below 100 µm were detected. The exposure of the user/worker to the additive is expected to be limited.

Users can suffer from occupational respiratory disease depending on the level of endotoxins in air and dust (Rylander et al., 1999; Thorn, 2001). The bacterial endotoxin activity (analysed in three batches) was reported to be < 30 EU/mg. A worst-case scenario was assumed considering that at least one of the three batches could contain 29 EU/mg. 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 (EFSA FEEDAP Panel, 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) (HCN, 2010) and the UK Health and Safety Executive (HSE, 2013). Based upon the calculation of the potential endotoxin content in dust, the inhalation exposure is calculated as 4,833 endotoxin IU per working day, indicating a risk of exposure by inhalation to endotoxins for persons handling the additive.

3.2.3.1. Conclusions on safety for the user

In the absence of data, the FEEDAP Panel cannot conclude on the potential of the additive to be irritant to skin or eyes, or on its potential to be a dermal sensitiser. The endotoxin activity of the additive represents a risk of exposure by inhalation to endotoxins for persons handling the additive.

3.3. Efficacy

Efficacy studies are not required for amino acids naturally occurring in proteins of plants and animals. The nutritional role of the amino acid L-lysine is well established in the scientific literature. In general, L-lysine sulfate is considered as an efficacious source of the essential amino acid L-lysine for non-ruminant animal species. For the supplemental L-lysine to be as efficacious in ruminants as it is in non-ruminant species, it would require protection against degradation in the rumen.

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 Regulation⁴¹ and Good Manufacturing Practice.

4. Conclusions

L-Lysine sulfate is produced by the genetically modified strain *Escherichia coli* CGMCC 7.398. Neither the production strain nor its recombinant DNA were detected in the final product. The additive does not pose any safety concern associated with the production strain.

L-Lysine sulfate produced by *Escherichia coli* CGMCC 7.398 is considered safe for the target species. When using L-lysine sulfate, the background sulfur/sulfate content in the compound feed should be taken into account.

The use of L-lysine sulfate produced by *Escherichia coli* CGMCC 7.398 as a feed additive is considered safe for the consumer and for the environment.

⁴¹ Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

In the absence of data, the FEEDAP Panel cannot conclude on the potential of the additive to be irritant to skin or eyes, or on its potential to be a dermal sensitiser. The endotoxin activity of the additive represents a risk by inhalation for users handling the additive.

The additive L-lysine sulfate is considered as an efficacious source of the essential amino acid L-lysine for non-ruminant animal species. For the supplemental L-lysine to be as efficacious in ruminants as it is in non-ruminant species, it would require protection against degradation in the rumen.

5. Documentation provided to EFSA/Chronology

Date	Event
04/11/2020	Dossier received by EFSA. L-lysine sulfate produced by <i>Escherichia coli</i> CGMCC 7.398 for all animal species. Submitted by Kempex Holland BV.
09/11/2020	Reception mandate from the European Commission
16/02/2021	Application validated by EFSA – Start of the scientific assessment
14/04/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
30/04/2021	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, characterisation of the strain, composition of the additive, purity, safety for the user.</i>
17/05/2021	Comments received from Member States
26/10/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
23/03/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

CAS	Chemical Abstracts Service
CFU	colony-forming unit
CV	coefficient of variation
DM	dry matter
EINECS	European Inventory of Existing Chemical Substances
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
IUPAC	International Union of Pure and Applied Chemistry
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
WHO	World Health Organization

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, 2015a).

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 s = $40 \times 20 = 800$ 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 m³/h, the inhalation volume providing exposure to potentially endotoxin-containing dust would be $0.444 \times 1.25 = 0.556$ m³/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/m³.

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/m³, 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/m³, then the content of endotoxins of the dust, c IU/m³, 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-lysine sulfate produced by fermentation with a genetically modified strain of *E. coli* (CGMCC 7.398) considering a worst-case scenario of endotoxin activity up to 29 IU/mg of final product

Calculation	Identifier	Description	Amount	Source
	a	Endotoxin content IU/g product	29,000	Technical dossier
	b	Dusting potential (g/m ³)	0.3	Technical dossier
$a \times b$	c	Endotoxin content in the air (IU/m ³)	8,700	
	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)	1,600	
$h/3,600$	i	Refined total duration of daily exposure (h)	0.44	
	j	Inhaled air (m ³) 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 ³)	0.56	
$c \times k$	l	Endotoxin inhaled (IU) during exposure per 8-h working day	4,833	
	m	Health-based recommended exposure limit of endotoxin (IU/m ³) per eight- hour working day	90	Health Council of the Netherlands (2010)
$m \times j$	n	Health-based recommended exposure limit of total endotoxin exposure (IU) per 8-h working day	900	

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Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for L-lysine sulfate produced by fermentation with *Corynebacterium glutamicum* KCCM 80227; and L-lysine sulfate produced by fermentation with *Escherichia coli* CGMCC 7.398

In the current applications, authorisations are sought under Article 4(1) for L-lysine sulfate produced by fermentation with *Corynebacterium glutamicum* KCCM 80227 and L-lysine sulfate produced by fermentation with *Escherichia coli* CGMCC 7.398, 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. The authorisations are sought for all animal species.

According to the Applicants, the feed additives contain a minimum of 52% (w/w) of L-lysine in L-lysine sulfate produced by fermentation with *Corynebacterium glutamicum* KCCM 80227 and a minimum of 55% (w/w) of L-lysine in L-lysine sulfate produced by fermentation with *Escherichia coli* CGMCC 7.398.

The feed additives are intended to be added directly into feedingstuffs or through premixtures. However, the Applicants did not propose any minimum or maximum content of L-lysine sulfate in feedingstuffs.

For the identification of the sulfate in L-lysine sulfate, the EURL found the European Pharmacopoeia monograph (Ph. Eur. 01/2008:20301).

For the quantification of lysine in the feed additive and premixtures, the Applicants proposed the ring-trial validated method EN ISO 17180:2013 based on ion-exchange chromatography (IEC) coupled with optical (visible – VIS or fluorescence – FLD) detection which is dedicated for the determination of lysine, methionine and threonine in commercial amino acid products and premixtures containing more than 10% of amino acid. The method does not distinguish between the amino acids and their salts, or between different salts of the same amino acids, and it cannot differentiate between enantiomers. The following performance characteristics were reported in the frame of the ring-trial validation studies for the quantification of free lysine: a relative standard deviation for repeatability (RSDr) ranging from 0.7% to 1.7%; and a relative standard deviation for reproducibility (RSDR) ranging from 1.5% to 2.5%.

For the quantification of lysine in feedingstuffs, the Applicants proposed the European Union (EU) method dedicated for the determination of amino acids in feed. This method is designed for the quantification of free (synthetic and natural) and of total (peptide-bound and free) amino acids in premixtures and feedingstuffs, using an amino acid analyser or ion-exchange chromatography (IEC) coupled with post-column derivatisation and optical (VIS) detection. The method does not distinguish between the amino acids and their salts, or between different salts of the same amino acids, and it cannot differentiate between enantiomers. The following performance characteristics were reported in the frame of the ring-trial validation studies for the quantification of total lysine: RSDr ranging from 2.1% to 2.8% and RSDR ranging from 3.0% to 6.7%.

Based on the performance characteristics available, the EURL recommends for official control (i) the European Pharmacopoeia monograph (Ph. Eur. 01/2008:20301) for the identification of the sulfate in L-lysine sulfate; (ii) the ring-trial validated method EN ISO 17180:2013 based on ion-exchange chromatography coupled with optical (visible or fluorescence) detection (IEC-VIS/FLD) to quantify free lysine in the feed additive and premixtures (containing more than 10% lysine); and (iii) the ring-trial validated European Union (EU) method based on IEC-VIS for the quantification of lysine in premixtures and feedingstuffs.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.