

ADOPTED: 23 June 2021

doi: 10.2903/j.efsa.2021.6706

Safety and efficacy of a feed additive consisting of L-lysine sulfate produced by *Corynebacterium glutamicum* KCCM 80227 for all animal species (Daesang Europe BV)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Montserrat Anguita, Rosella Brozzi, Jaume Galobart, Elisa Pettenati and Jordi Tarrés-Call

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 *Corynebacterium glutamicum* KCCM 80227 as a nutritional feed additive for all animal species. The FEEDAP Panel concluded that the production strain *C. glutamicum* KCCM 80227 qualifies for the qualified presumption of safety (QPS) approach to safety assessment and is not detected in the final product. L-Lysine sulfate produced using *C. glutamicum* KCCM 80227 does not pose any safety concern 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 *C. glutamicum* KCCM 80227 is safe for the consumers, users and for the environment. 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 in non-ruminant species, it would require protection against degradation in the rumen.

© 2021 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

Keywords: nutritional additive, amino acids, L-lysine sulfate, safety, *Corynebacterium glutamicum* KCCM 80227, efficacy

Requestor: European Commission

Question number: EFSA-Q-2020-00708

Correspondence: feedap@efsa.europa.eu

Panel members: Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa and Ruud Woutersen.

Legal notice: Relevant information or parts of this scientific output have been blackened in accordance with the confidentiality requests formulated by the applicant pending a decision thereon by the European Commission. The full output has been shared with the European Commission, EU Member States and the applicant. The blackening will be subject to review once the decision on the confidentiality requests is adopted by the European Commission.

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>.

Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output: Pier Sandro Coconcelli, Debora Glandorf, Lieve Herman, Miguel Prieto Maradona, Maria Saarela, Gloria López Gálvez.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, 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, Anguita M, Brozzi R, Galobart J, Pettenati E and Tarrés-Call J, 2021. Safety and efficacy of a feed additive consisting of L-lysine sulfate produced by *Corynebacterium glutamicum* KCCM 80227 for all animal species (Daesang Europe BV). *EFSA Journal* 2021;19(7):6706, 14 pp. <https://doi.org/10.2903/j.efsa.2021.6706>

ISSN: 1831-4732

© 2021 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

This is an open access article under the terms of the [Creative Commons Attribution-NoDerivs](https://creativecommons.org/licenses/by/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.



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



Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference.....	4
1.2. Additional information.....	4
2. Data and methodologies.....	5
2.1. Data.....	5
2.2. Methodologies.....	5
3. Assessment.....	5
3.1. Characterisation.....	5
3.1.1. Characterisation of the production microorganism.....	5
3.1.2. Manufacturing process.....	6
3.1.3. Characterisation of the active substance/additive.....	6
3.1.4. Physical properties of the additive.....	7
3.1.5. Stability and homogeneity.....	7
3.1.6. Conditions of use.....	8
3.2. Safety.....	8
3.2.1. Safety for the target species, consumers and the environment.....	8
3.2.1.1. Conclusions on safety for the target species, consumer and environment.....	9
3.2.2. Safety for the user.....	9
3.2.2.1. Effects on the respiratory system.....	9
3.2.2.2. Effects on skin and eyes.....	9
3.2.2.3. Conclusions on safety for the user.....	9
3.3. Efficacy.....	9
3.4. Post-market monitoring.....	10
4. Conclusions.....	10
5. Documentation provided to EFSA/Chronology.....	10
References.....	10
Abbreviations.....	13
Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of the Analysis for L-lysine sulfate produced by <i>Corynebacterium glutamicum</i> KCCM 80227; and L-lysine sulfate produced by fermentation with <i>Escherichia coli</i> CGMCC 7.398.....	14

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 Daesang Europe BV² for the authorisation of L-lysine sulfate produced by *Corynebacterium glutamicum* KCCM 80227, when used as a feed additive for all animal species (category: nutritional additives; functional group: amino acids, their salts and analogues).

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 19 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 L-lysine sulfate produced by *C. glutamicum* KCCM 80227, when used under the proposed conditions of use (see **Section 3.1.6**).

1.2. Additional information

The subject of the assessment is a product consisting of L-lysine sulfate produced by fermentation with a non-genetically modified strain of *C. glutamicum* (KCCM 80277), intended for use as a nutritional additive (functional group: amino acids, their salts and analogues) for all animal species. This feed additive has not been authorised in the EU. Nevertheless, L-lysine produced using different microbial strains is currently authorised for its use in all animal species as a nutritional additive.^{3,4,5,6}

L-Lysine is authorised for use in food,⁷ cosmetics⁸ and as a veterinary medicinal product.^{9,10}

EFSA has issued several opinions on the safety and efficacy of L-lysine and/or its salts produced by fermentation with different strains of *C. glutamicum* (EFSA, 2007a; EFSA FEEDAP Panel, 2015b, 2016b, 2019a–e, 2020a,b,c), *Corynebacterium casei* (EFSA FEEDAP Panel, 2020d) or *Escherichia 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.

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 Daesang Europe BV, Van Heuven Goedhartlaan 935, 1181 LD, Amsterdam. The Netherlands.

³ Commission implementing regulation (EU) 2019/1964 of 26 November 2019 concerning the authorisation of L-lysine base, liquid, L-lysine monohydrochloride, liquid, L-lysine monohydrochloride, technically pure, and L-lysine sulfate as feed additives for all animal species. OJ L 307 28.11.2019, p. 3.

⁴ Commission implementing regulation (EU) 2020/997 of 9 July 2020 concerning the authorisation of L-lysine base, liquid, L-lysine sulfate and L-lysine monohydrochloride, technically pure, as feed additives for all animal species. OJ L 221 10.7.2020, p. 90.

⁵ Commission implementing regulation (EU) 2020/1798 of 30 November 2020 concerning the authorisation of L-lysine monohydrochloride produced by *Corynebacterium glutamicum* DSM 32932 and L-lysine sulfate produced by *Corynebacterium glutamicum* KFCC 11043 as feed additives for all animal species. OJ L 402, 1.12.2020, p. 39.

⁶ Commission implementing regulation (EU) 2017/439 of 13 March 2017 concerning the authorisation of L-lysine sulfate produced by *Escherichia coli* as a feed additive for all animal species. OJ L 67, 14.3.2017, p. 70.

⁷ 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.

⁸ 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.

⁹ Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. OJ L 15, 20.1.2010, p. 1.

¹⁰ 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.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier¹¹ in support of the authorisation request for the use of L-lysine sulfate produced by *C. glutamicum* KCCM 80227 as a feed additive.

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 L-lysine sulfate produced by *C. glutamicum* KCCM 80227 in animal feed. The Executive Summary of the EURL report can be found in Annex A.¹²

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-lysine sulfate produced by *C. glutamicum* KCCM 80227 is in line with the principles laid down in Regulation (EC) No 429/2008¹³ 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 product subject of this application is L-lysine sulfate produced by fermentation with a non-genetically modified strain of *C. glutamicum* (KCCM 80227). The applicant is requesting the authorisation as a nutritional feed additive, under the functional group 'amino acids, their salts and analogues,' to be used in feed for all animal species.

3.1. Characterisation

3.1.1. Characterisation of the production microorganism

The additive is produced by a non-genetically modified strain of *C. glutamicum* which is deposited in the Korean Culture Collection of Microorganisms with accession number KCCM 80227.¹⁴

The genome of the production strain was sequenced and used for identification purposes. The taxonomic identification of the production strain KCCM 80227 as *C. glutamicum* was confirmed

.¹⁵

The production strain is derived from

The susceptibility of the production strain to the battery of antibiotics recommended by the FEEDAP Panel (EFSA FEEDAP Panel, 2018) was tested by broth microdilution following the method of the

¹¹ FEED dossier reference: FAD-2020-0082.

¹² The full report is available on the EURL website: <https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2020-00820085-lysine-sulfate.pdf>

¹³ 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.

¹⁴ Technical dossier/Section II/Annex 2.2.1.2.a.

¹⁵ Technical dossier/Section II/Annex 2.2.1.2.c.

¹⁶ Technical dossier/Section II/Annex 2.2.1.2.b.

Clinical and Laboratory Standards Institute (CLSI). All the minimum inhibitory concentration (MIC) values were below the corresponding cut-off values for *Corynebacterium* and other Gram-positives (EFSA FEEDAP Panel, 2018). Therefore, the production strain is considered susceptible to all relevant antibiotics.¹⁷

The WGS of the production strain was interrogated for the presence of antimicrobial resistance (AMR) genes

No hits of concern were identified.¹⁸

3.1.2. Manufacturing process

L-Lysine sulfate is produced by fermentation using *C. glutamicum* KCCM 80227 as a production microorganism.

¹⁹

The applicant declared that no antimicrobial compounds are used in the manufacturing process of the additive.²⁰

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 $C_{12}H_{28}N_4O_4 \cdot H_2SO_4$ and the molecular structure is given in Figure 1. The theoretical content of lysine in the pure lysine sulfate is 75%.

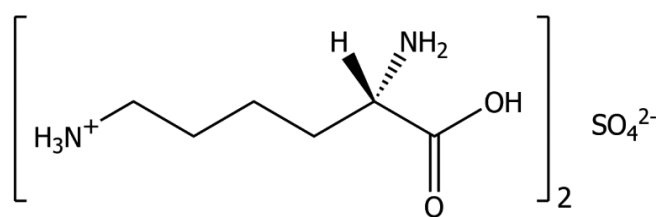


Figure 1: Molecular structure of L-lysine sulfate

L-Lysine sulfate contains by specification $\geq 52.0\%$ lysine and $\geq 17.3\%$ sulfate on a dry matter (DM) basis, being the water content $\leq 4\%$.

Analytical data to confirm the specifications were provided for five batches of the additive,²¹ showing the following average values: 59.4% lysine (58.9–60.0%) on DM basis; 20.2% sulfate (18.9–21.0%) on DM basis; 3.4% water (3.2–3.6%).

Three additional batches were analysed for quantitative composition and on a DM basis total lysine (%) ranged 62.4–62.8%, sulfate ranged 21.6–21.8%, amino acids other than lysine represented 3.4–3.5%, sugars 1.2–1.7%, lactic acid 0.2% and cadaverine 0.027%. Moisture ranged 1.3–1.5%. The amount of identified material on a DM basis was 90–91%.²²

Three batches of the additive were analysed for undesirable substances. Heavy metals (cadmium, lead and mercury) and arsenic concentrations showed the following values: cadmium and mercury were below the limit of detection (LOD), lead ranged from 0.8 to 0.9 mg/kg and arsenic ranged from 0.1 to 0.2 mg/kg.²³

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).²⁴ The calculated (upper bound) levels of dioxins were

¹⁷ Technical dossier/Section II/Annex 2.2.2.2.

¹⁸ Technical dossier/Section II/Annex 2.2.1.2c.

¹⁹ Technical dossier/Section II and supplementary information May 2021/FAD-2020-0082 SIN 300321 answers.

²⁰ Technical dossier/Section II/Annex 2.3.1b.

²¹ Technical dossier/Section II/Annex 2.1.3a batch to batch variation and Supplementary information May 2021/FAD-2020-0082 Sin 300321 answers. Analytical method for lysine is stated to be in accordance with Commission Regulation (EC) 152/2009.

²² Technical dossier/Section II/Annexes 2.1.3b1 to b3 impurities and Supplementary information May 2021/FAD-2020-0082 Sin 300321 answers. Analytical method for amino acids is stated to be in accordance with Commission Regulation (EC) 152/2009.

²³ Technical dossier/Section II/Annex 2.1.4a. LOD in mg/kg was < 0.002 for cadmium and mercury.

²⁴ Technical dossier/Section II/Annexes 2.1.4b1 to b3 dioxins. LOQ in ng/kg for dioxins, dioxin like PCBs and non-dioxin like PCBs ranged from 0.04 to 20, depending on the substance considered.

0.137 ng WHO-PCDD/F-TEQ/kg; and the calculated (upper bound) sum of dioxins and dioxin-like PCBs were 0.269 ng WHO-PCDD/F-PCB-TEQ/kg (in all three batches).

The analysis of mycotoxins showed values below the LOD for ochratoxin A, zearalenone and fumonisins ($B_1 + B_2 + B_3$).²⁵ Aflatoxins (not further identified) ranged from 0.7 to 1.2 $\mu\text{g}/\text{kg}$, deoxynivalenol (DON) ranged 876 – 2,750 $\mu\text{g}/\text{kg}$ and citrinin ranged from < LOD (15 $\mu\text{g}/\text{kg}$) – 76 $\mu\text{g}/\text{kg}$. The FEEDAP Panel notes that the levels of DON are considered high.

Microbiological contamination was analysed by determination of Enterobacteriaceae, *Escherichia coli*, *Salmonella* spp., yeasts and filamentous fungi. None could be detected in 25-g samples.²⁶

The detected amounts of the above-described undesirable substances do not raise safety concerns.

The presence of viable cells of the production strain in the final product was analysed in three batches of the additive L-lysine sulfate analysed in triplicate. Each of the 10 g samples was diluted in 90 mL 0.9% NaCl and two 5 mL subsamples filtrated onto each of two 0.45- μm filters which were then cultivated on LB agar with 5% glucose, supplemented with 256 mg/L fosfomycin and 300 mg/L cycloheximide (selective medium to avoid growth of contaminants). Cultivation was at 30°C and up to 12 days. Appropriate positive and negative controls were included. Nine colonies were obtained from the 9 samples tested. All colonies were tested with a species-specific polymerase chain reaction (PCR) targeting the *rpoB* gene (817 bp fragment). None were *C. glutamicum*. Therefore, the final product does not contain cells of the production strain.²⁷

3.1.4. Physical properties of the additive

The additive appears as brown granules of characteristic odour and is partially soluble in water. Its bulk density was analysed in three batches and ranged from 610 to 620 kg/m^3 .²⁸

The dusting potential of three batches of the additive was determined using the Stauber–Heubach method and showed values ranging from < 0.1 to 1 mg/m^3 .²⁹ Particle size distribution of three batches, measured by laser diffraction, showed < 1% of particles below 500 μm of diameter.³⁰

3.1.5. Stability and homogeneity

The shelf life of the additive (three batches) was studied when stored at room temperature and at 40°C in bags (no further description) for 6 months. No losses were detected.³¹

The stability of the additive (three batches) in a vitamin–mineral premixture (containing 92 g choline chloride/kg)³² for chickens for fattening was studied when supplemented at 4% L-lysine sulfate and stored at room temperature in sealed bags protected from light for 6 months. Only one batch showed a loss of 20%.

The stability of the additive (three batches) in a complete feed for chickens for fattening (mash and pelleted form) based on wheat, soybean meal, maize and rapeseed meal containing a background of 1% lysine was studied when supplemented at 0.2% L-lysine sulfate.³³ Pelleting was performed at 73°C and, at time 0, represented a loss of 2% in only one of the 3 batches. Samples were stored at room temperature in sealed bags protected from light for 3 months. At the end of the storage period, only one batch of mash feed showed a loss of 4% while no losses were observed in the pelleted feed.³⁴

The capacity for homogeneous distribution of the additive in the pelleted feed described above was studied by analysing 10 subsamples. Total lysine (free lysine and protein-bound lysine) was analysed and the background level of lysine of the complete feed (1%) was subtracted from each sample to calculate the coefficient of variation that was 17%.³⁵

²⁵ Technical dossier/Section II/Annex 2.1.4a. LOD in $\mu\text{g}/\text{kg}$ for ochratoxin A was 5, for zearalenone was 17 and for sum of fumonisines B_1 to B_3 was 25.

²⁶ Technical dossier/Section II.6.3 and annex 2.1.4a.

²⁷ Technical dossier/Section II/Annex 2.1.4c_Absence viable cells.

²⁸ Technical dossier/Section II/Annexes 2.1.5.c1 to c3.

²⁹ Technical dossier/Section II/Annexes 2.1.5.b1 to b3 dusting potential.

³⁰ Technical dossier/Section II/Annexes 2.1.5.a1 to a3 particle size.

³¹ Technical dossier/Section II/Annex 2.1.4a.

³² Technical dossier/Section II/Annexes 2.4.1a Premix composition and annex 2.4.1b.

³³ Technical dossier/Section II/Annexes 2.4.1b and 2.4.1c feed composition.

³⁴ Technical dossier/Section II/Annexes 2.4.1b and 2.4.1c.

³⁵ Technical dossier/Section II/Annex 2.4.1b.

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 for the target species, consumers and the environment

The production strain belongs to a species, *C. glutamicum*, that qualifies for the Qualified Presumption of Safety (QPS) approach to safety assessment (EFSA, 2007b) when used for production purposes (EFSA BIOHAZ Panel, 2020). The strain was unambiguously identified as *C. glutamicum* and was shown not to harbour acquired AMR determinants for antibiotics of human and veterinary importance, thus meeting the QPS requirements. The production strain is not genetically modified. The final product does not contain viable cells of the production strain. Consequently, no safety concerns for target animal, consumers and the environment are expected from the additive concerning the production strain and/or potential fermentation residues that may be present in the final additive.

L-Lysine requirements of different non-ruminant 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.

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 submitted a tolerance study in chickens for fattening administered with manganese sulfate (unpublished study),³⁶ and performed a literature search in Scopus database to support the safety of the sulfur content of the additive for the target species.³⁷ Time limits were set to retrieve publications posterior to 2008. The search strings are described in the dossier and involved the tolerance of sulfate to poultry, cattle and pigs. The search process was poorly reported (e.g. the use of Boolean operators, the inclusion and exclusion criteria, and the selection methodology were not described). From 511 references retrieved, seven were considered of relevance by the applicant. One of these studies (Bobeck et al., 2013) was already considered in the previous review (EFSA FEEDAP Panel, 2019a). Five of the remaining studies could not be further considered: (i) Conly et al. (2012), did not report basal content of sulfate in feed of chickens for fattening; and high concentration of manganese (up to 5,400 mg/kg feed) administered in parallel could have contributed to the adverse effect observed as the maximum tolerable level (MTL) for poultry established by the National Research Council in 2005 was 2,000 mg manganese/kg feed (NRC, 2005); (ii) similarly, Hamdi et al. (2018), tested copper sulfate levels in chickens for fattening but the concentration of copper (up to 300 mg/kg) could have influenced the adverse effects observed as the MTL established for NRC for poultry is 250 mg copper/kg feed (NRC, 2005); (iii) Li et al. (2019), compared relative bioavailability of L-lysine sulfate vs L-lysine monohydrochloride in growing pigs and tested relatively low levels of sulfate (0.8 g/kg feed); (iv) Faulkner et al. (2016) were administering a relatively low level of sulfur (about 17 mg S/kg feed) in dairy cows and the study lasted only 16 days; (v) Cherdthong et al. (2014), tested the effects of feeding blocks containing calcium sulfate in cattle (Thai breed) performance but the sulfate intake was unclear. The remaining two studies (Fohr et al. (2014), inducing an experimental diarrhoea in weaned pigs using about 5,660 mg sulfur/kg feed; and the unpublished tolerance study of manganese sulfate in chickens for fattening testing up to 3,340 mg sulfate/kg feed) confirmed the statement of NRC

³⁶ Technical dossier/Section III/Annex 3.1.1b.

³⁷ Technical dossier/Section III/Annex 3.1.1a. The search was performed on August 2020.

(2005) specified above. 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 would not lead to any localised increase in the concentration of L-lysine or its metabolites in the environment.

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

L-Lysine sulfate produced by *C. glutamicum* KCCM 80227 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 *C. glutamicum* KCCM 80227 is safe for the consumer and for the environment.

3.2.2. Safety for the user

3.2.2.1. Effects on the respiratory system

The dusting potential of the additive is low (up to 1 mg/m³, see Section 3.1.4) and the particle size distribution indicates that the fraction of particles < 500 µm diameter is < 1%. The exposure of the user/worker to the additive is expected to be limited.

The applicant provided an acute inhalation toxicity study testing L-lysine sulfate produced by a different strain of *C. glutamicum* that had been evaluated in a previous opinion (EFSA FEEDAP Panel 2016b). As the production strains share a common origin (*C. glutamicum* ATCC 13032), and the product characteristics and the manufacturing process are similar, the FEEDAP Panel considers that the results of this study are applicable to the L-lysine under assessment: 'The lack of respiratory toxicity indicates that there is no significant concern regarding user safety for the L-lysine sulfate product'.

3.2.2.2. Effects on skin and eyes

The applicant submitted an acute skin irritation/corrosion test and an acute eye irritation/corrosion test performed with L-lysine sulfate produced by *C. glutamicum* KCCM 80227.

In an acute dermal irritation/corrosion test performed in accordance with OECD TG 404 and GLP compliant,³⁸ the additive under assessment did not show skin irritation or corrosive effects.

In an acute eye irritation/corrosion test performed in accordance with OECD TG 405 and GLP compliant,³⁹ the additive under assessment did not show eye irritation or corrosive effects.

The applicant provided a dermal sensitisation test testing L-lysine sulfate produced by a different strain of *C. glutamicum* that had been evaluated in a previous opinion (EFSA FEEDAP Panel, 2016b). As the production strains share a common origin (*C. glutamicum* ATCC 13032), and the product characteristics and the manufacturing process are similar, the FEEDAP Panel considers that the results of this study are applicable to the L-lysine under assessment: 'The lack of dermal sensitisation indicates that there is no significant concern regarding user safety for the L-lysine sulfate product'.

3.2.2.3. Conclusions on safety for the user

L-Lysine sulfate produced by *C. glutamicum* KCCM 80227 is considered non-toxic by inhalation, not irritant to skin or eyes, and not a skin sensitiser.

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. The efficacy of L-lysine for both non-ruminant and ruminant species was described in two previous EFSA

³⁸ Technical dossier/Section II/Supplementary information May 2021/Annex 2.

³⁹ Technical dossier/Section II/Supplementary information May 2021/Annex 3.

opinions (EFSA FEEDAP Panel, 2013, 2014). 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 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 Regulation⁴⁰ and Good Manufacturing Practice.

4. Conclusions

L-Lysine sulfate produced using *C. glutamicum* KCCM 80227 does not pose any safety concern associated with the production strain.

L-Lysine sulfate produced by *C. glutamicum* KCCM 80227 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 *C. glutamicum* KCCM 80227 is safe for the consumers, the users and the environment.

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 in non-ruminant species, it would require protection against degradation in the rumen.

5. Documentation provided to EFSA/Chronology

Date	Event
19/10/2020	Dossier received by EFSA. L-Lysine sulfate produced by <i>C. glutamicum</i> KCCM 80227 for all animal species. Submitted by Daesang Europe B.V. (represented by Regal B.V.)
26/10/2020	Reception mandate from the European Commission
19/02/2021	Application validated by EFSA – Start of the scientific assessment
	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: manufacturing process, characterisation of the additive.</i>
14/04/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
10/05/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
20/05/2021	Comments received from Member States
23/06/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

- Bobeck E, Payne RL, Kerr BJ and Persia ME, 2013. Supplemental lysine sulfate does not negatively affect the performance of broiler chicks fed dietary sulfur from multiple dietary and water sources. *Journal of Applied Poultry Research*, 22, 461–468.
- Cherdthong A, Wanapat M, Wongwungchun W and Yeekeng Y, 2014. Effect of feeding feed blocks containing different levels of urea calcium sulphate mixture on feed intake, digestibility and rumen fermentation in Thai native beef cattle fed on rice straw. *Animal Feed Science and Technology*, 198, 151–157.
- Conly AK, Conly R, Poureslami R, Koutsos EA, Batal AB, Jung B, Beckstead R and Peterson DG, 2012. Tolerance and efficacy of tribasic manganese chloride in growing broiler chickens. *Poultry Science*, 91, 1633–1640.
- EFSA (European Food Safety Authority), 2007a. Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on a request from the European Commission on the safety and efficacy of L-lysine sulphate (Vitalys[®] Liquid and Vitalys[®] Dry) for all animal species. *EFSA Journal* 2007;5(9):522, 26 pp. <https://doi.org/10.2903/j.efsa.2007.522>
- EFSA (European Food Safety Authority), 2007b. Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. *EFSA Journal* 2007;5(12):587, 16 pp. <https://doi.org/10.2903/j.efsa.2007.587>

⁴⁰ Regulation (EC) No 1831/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

- EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Hilbert F, Lindqvist R, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Cocconcelli PS, Fernández Escámez PS, Maradona MP, Querol A, Suarez JE, Sundh I, Vlaskovic J, Barizzzone F, Correia S and Herman L, 2020. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA (2017–2019). EFSA Journal 2020;18(2):5966, 56 pp. <https://doi.org/10.2903/j.efsa.2020.5966>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. <https://doi.org/10.2903/j.efsa.2012.2539>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific Opinion on the safety and efficacy of concentrated liquid L-lysine (base), concentrated liquid L-lysine monohydrochloride and L-lysine monohydrochloride produced by *Escherichia coli* (FERM BP-10941) for all animal species, based on three dossiers submitted by Ajinomoto Eurolysine SAS. EFSA Journal 2013;11(10):3365, 22 pp. <https://doi.org/10.2903/j.efsa.2013.3365>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014. Scientific Opinion on the safety and efficacy of concentrated liquid L-lysine (base), concentrated liquid L-lysine monohydrochloride and L-lysine monohydrochloride technically pure produced using *Escherichia coli* (FERM BP-11355) for all animal species based on a dossier submitted by Ajinomoto Eurolysine S.A.S. EFSA Journal 2014;12(11):3895, 19 pp. <https://doi.org/10.2903/j.efsa.2014.3895>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015a. 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. <https://doi.org/10.2903/j.efsa.2015.4155>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015b. Scientific Opinion on the safety and efficacy of L-lysine monohydrochloride, technically pure, produced with *Escherichia coli* CGMCC 3705 and L-lysine sulphate produced with *Corynebacterium glutamicum* CGMCC 3704 for all animal species, based on a dossier submitted by HELM AG. EFSA Journal 2015;13(7):4156, 25 pp. <https://doi.org/10.2903/j.efsa.2015.4156>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015c. Scientific Opinion on the safety and efficacy of L-lysine monohydrochloride produced by fermentation with *Escherichia coli* for all animal species based on a dossier submitted by HELM AG on behalf of Meihua Holdings Group Co. Ltd. EFSA Journal 2015;13(3):4052, 16 pp. <https://doi.org/10.2903/j.efsa.2015.4052>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016a. Scientific opinion on the safety of L-lysine monohydrochloride produced by fermentation with *Escherichia coli* CGMCC 7.57 for all animal species based on a dossier submitted by Feedway Europe NV. EFSA Journal 2016;14(5):4471, 9 pp. <https://doi.org/10.2903/j.efsa.2016.4471>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016b. Scientific opinion on the safety and efficacy of concentrated liquid L-lysine (base), L-lysine monohydrochloride and L-lysine sulphate produced using different strains of *Corynebacterium glutamicum* for all animal species based on a dossier submitted by AMAC/EEIG. EFSA Journal 2016;14(3):4346, 3 pp. <https://doi.org/10.2903/j.efsa.2016.4346>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wester P, Costa L, Dierick N, Leng L and Wallace RJ, 2017a. Scientific opinion on the safety of L-lysine sulfate produced by fermentation with *Escherichia coli* CGMCC 3705 for all animal species. EFSA Journal 2017;15(2):4714, 7 pp. <https://doi.org/10.2903/j.efsa.2017.4714>
- EFSA FEEDAP Panel (EFSA Panel on additives and products or substances used in animal feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J and Innocenti ML, 2017b. Guidance on the identity, characterisation and conditions of use of feed additives. EFSA Journal 2017;15(10):5023, 12 pp. <https://doi.org/10.2903/j.efsa.2017.5023>
- EFSA FEEDAP Panel (EFSA Panel on additives and products or substances used in animal feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2017c. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017;15(10):5021, 19 pp. <https://doi.org/10.2903/j.efsa.2017.5021>
- EFSA FEEDAP Panel (EFSA Panel on Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Dujardin B, Galobart J and Innocenti ML, 2017d. Guidance on the assessment of the safety of feed additives for the consumer. EFSA Journal 2017;15(10):5022, 17 pp. <https://doi.org/10.2903/j.efsa.2017.5022>

- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rycken G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Anguita M, Brozzi R and Galobart J, 2018. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal 2018;16(3):5206, 24 pp. <https://doi.org/10.2903/j.efsa.2018.5206>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kouba M, Kos Durjava M, Lopez-Alonso M, Lopez Puente S, Marcon F, Mayo B, Pechova A, Petkova M, Sanz Y, Villa RE, Woutersen R, Costa L, Dierick N, Flachowsky G, Glandorf B, Herman L, Karenlampi S, Leng L, Mantovani A, Wallace RJ, Aguilera J, Tarres-Call J and Ramos F, 2019a. Scientific Opinion on the safety of concentrated L-lysine (base), L-lysine monohydrochloride and L-lysine sulfate produced using different strains of *Corynebacterium glutamicum* for all animal species based on a dossier submitted by FEFANA asbl. EFSA Journal 2019;17(1):5532, 24 pp. <https://doi.org/10.2903/j.efsa.2019.5532>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bampidis V, Bastos ML, Christensen H, Dusemund B, Kouba M, Kos Durjava M, Lopez-Alonso M, Lopez Puente S, Marcon F, Mayo B, Pechova A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Costa L, Dierick N, Flachowsky G, Glandorf B, Herman L, Karenlampi S, Mantovani A, Aguilera J, Anguita M, Tarres-Call J and Wallace RJ, 2019b. Scientific opinion on the safety and efficacy of L-lysine monohydrochloride and concentrated liquid L-lysine (base) produced by fermentation using *Corynebacterium glutamicum* strain NRRL B-50775 for all animal species based on a dossier submitted by ADM. EFSA Journal 2019;17(1):5537, 18 pp. <https://doi.org/10.2903/j.efsa.2019.5537>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kouba M, Kos M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Sanz Y, Villa RE, Woutersen R, Costa L, Dierick N, Flachowsky G, Mantovani A, Wallace RJ, Tarrés-Call J and Ramos F, 2019c. Scientific Opinion on the safety and efficacy of L-lysine monohydrochloride and L-lysine sulfate produced using *Corynebacterium glutamicum* CCTCC M 2015595 for all animal species. EFSA Journal 2019;17(3):5643, 19 pp. <https://doi.org/10.2903/j.efsa.2019.5643>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Sanz Y, Villa RE, Woutersen R, Costa L, Cubadda F, Dierick N, Flachowsky G, Mantovani A, Wallace RJ, Tarres-Call J and Ramos F, 2019d. Scientific Opinion on the safety and efficacy of L-lysine monohydrochloride and concentrated liquid L-lysine (base) produced by fermentation using *Corynebacterium glutamicum* strain KCCM 10227 for all animal species. EFSA Journal 2019;17(5):5697, 15 pp. <https://doi.org/10.2903/j.efsa.2019.5697>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Costa L, Cubadda F, Dierick N, Flachowsky G, Glandorf B, Herman L, Mantovani A, Anguita M, Tarrés-Call J and Ramos F, 2019e. Scientific Opinion on the safety and efficacy of L-lysine monohydrochloride and concentrated liquid L-lysine (base) produced by fermentation using *Corynebacterium glutamicum* strains NRRL-B-67439 or NRRL B-67535 for all animal species. EFSA Journal 2019;17(11):5886, 22 pp. <https://doi.org/10.2903/j.efsa.2019.5886>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Bastos M, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Brock T, de Knecht J, Kolar B, van Beelen P, Padovani L, Tarrés-Call J, Vettori MV and Azimonti G, 2019f. Guidance on the assessment of the safety of feed additives for the environment. EFSA Journal 2019;17(4):5648, 78 pp. <https://doi.org/10.2903/j.efsa.2019.5648>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, de Lourdes Bastos M, Christensen H, Dusemund B, Kos 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, Cocconcelli PS, Glandorf B, Herman L, Maradona MP, Saarela M, Anguita M, Galobart J, Holczknecht O, Manini P, Pettenati E, Pizzo F and Tarrés-Call J, 2020a. Scientific Opinion on the safety and efficacy of L-lysine monohydrochloride produced by fermentation with *Corynebacterium glutamicum* DSM 32932 for all animal species. EFSA Journal 2020;18(4):6078, 14 pp. <https://doi.org/10.2903/j.efsa.2020.6078>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kos Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Sanz Y, Villa RE, Woutersen R, Cocconcelli PS, Dierick N, Glandorf B, Herman L, Mantovani A, Prieto Maradona M, Saarela M, Wallace RJ, Anguita M, Pettenati E, Tarrés-Call J and Ramos F, 2020b. Scientific Opinion on the safety and efficacy of L-lysine monohydrochloride and L-lysine sulfate produced using *Corynebacterium glutamicum* CGMCC 7.266 for all animal species. EFSA Journal 2020;18(2):6019, 17 pp. <https://doi.org/10.2903/j.efsa.2020.6019>

- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kouba M, Fošman Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Cocconcelli PS, Glandorf B, Herman L, Prieto Maradona M, Saarela M, Anguita M, Galobart J, Holczkencht O, Manini P, Pettenati E, Pizzo F and Tarrés-Call J, 2020c. Scientific Opinion on the safety and efficacy of L-lysine sulfate produced by fermentation using *Corynebacterium glutamicum* KFCC 11043 as a feed additive for all animal species. EFSA Journal 2020;18(7):6203, 14 pp. <https://doi.org/10.2903/j.efsa.2020.6203>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kouba M, Fošman Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Cocconcelli PS, Glandorf B, Herman L, Prieto Maradona M, Saarela M, Anguita M, Galobart J, Holczkencht O, Manini P, Pettenati E, Pizzo F and Tarrés-Call J, 2020d. Scientific Opinion on the safety and efficacy of concentrated liquid L-lysine (base) and L-lysine monohydrochloride produced by fermentation with *Corynebacterium casei* KCCM 80190 as feed additives for all animal species. EFSA Journal 2020;18(11):6285, 16 pp. <https://doi.org/10.2903/j.efsa.2020.6285>
- Faulkner MJ, Wenner BA, Solden LM and Weiss WP, 2016. Source of supplemental dietary copper, zinc, and manganese affects fecal microbial relative abundance in lactating dairy cows. Journal of Dairy Science, 100, 1037–1044.
- Fohr JR, Tokach MD, Dritz SS, DeRouchey JM, Goodband RD and Nelssen JL, 2014. The effects of sodium sulfate in the water of nursery pigs and the efficacy of non-nutritive feed additives to mitigate those effects. Journal of Animal Science, 92, 3624–3635.
- Hamdi M, Sola-Oriol D, Franco Rossello R and Durosoy S, 2018. Including copper sulphate or dicopper oxide in the diet of broiler chickens affects performance and copper content in the liver. Animal Feed Science and Technology, 237, 89–97.
- Li Q, Gould SA, Kyaw Htoo JK, González-Vega JC and Patience JF, 2019. Bioavailability of L-lysine sulfate relative to L-lysine HCl for growing–finishing pigs. Translational Animal Science, 3, 1254–1262.
- NRC (National Research Council), 2005. Mineral tolerance of animals, 2nd revised edition. National Academies Press, Washington DC, USA.

Abbreviations

AMR	antimicrobial resistance
CAS	Chemical Abstracts Service
CFU	colony forming unit
DM	dry matter
DON	deoxynivalenol
EINECS	European Inventory of Existing Chemical Substances
EURL	European Union Reference Laboratory
FAO	Food Agricultural Organization
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FLD	fluorescence detection
IEC	ion-exchange chromatography
IUPAC	International Union of Pure and Applied Chemistry
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
OECD	Organisation for Economic Co-operation and Development
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzodioxin
PCDF	polychlorinated dibenzofuran
PCR	polymerase chain reaction
RSDr	relative standard deviation for repeatability
RSDR	relative standard deviation for reproducibility
TEQ	toxic equivalents
VIS	visible detection
WHO	World Health Organization

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of the Analysis for L-lysine sulfate produced by *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.