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Safety and efficacy of L-lysine sulfate produced by fermentation using *Corynebacterium glutamicum* KFCC 11043 as a feed additive for all animal species

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Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on L-lysine sulfate produced by fermentation using *Corynebacterium glutamicum* KFCC 11043 when used as a nutritional additive in feed for all animal species. The active substance is L-lysine. The production strain qualifies for the qualified presumption of safety (QPS) approach to safety assessment and was not detected in the final product. L-Lysine sulfate produced using *C. glutamicum* KFCC 11043 does not pose any safety concern associated with the production strain. L-Lysine sulfate produced by *C. glutamicum* KFCC 11043 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* KFCC 11043 is safe for the consumer and for the environment. From the results of studies on the safety for the user of L-lysine sulfate produced by a different production strain, it was possible to conclude on the safety for the user of the product under assessment. L-Lysine sulfate produced by *C. glutamicum* KFCC 11043 is considered non-toxic by inhalation, non-irritant to skin or eyes and it is not a skin sensitiser. 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.

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1. Introduction

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

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 a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from CJ Europe GmbH² for authorisation of the product L-lysine sulfate produced by fermentation using *Corynebacterium glutamicum* KFCC 11043 when used as additive in feed 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). The particulars and documents in support of the application were considered valid by EFSA as of 7 May 2019.

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 product L-lysine sulfate produced by fermentation using *C. glutamicum* KFCC 11043, when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

L-Lysine produced by fermentation using different production strains is currently authorised for its use in all animal species as a nutritional additive.³ No maximum content in feedingstuffs is established in the European Union (EU).

L-Lysine is authorised for use in food,⁴ cosmetics⁵ and as a veterinary medicinal product.^{6,7}

The scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has published several opinions on the safety and efficacy of L-lysine and/or its salts produced by *C. glutamicum* for all animal species (EFSA, 2007a; EFSA FEEDAP Panel 2015a, 2016b, 2019a–e, 2020a,b); and three opinions on the safety and efficacy of concentrated liquid L-lysine (base), concentrated liquid L-lysine monohydrochloride and/or L-lysine monohydrochloride produced by *Escherichia coli* for all animal species (EFSA FEEDAP Panel, 2013, 2014, 2015a–c, 2016a, 2017a).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier⁸ in support of the authorisation request for the use of L-lysine sulfate produced by fermentation using *C. glutamicum* KFCC 11043 as a feed additive.

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

² CJ Europe GmbH. Ober der Roeth 4, 65824 Schwalbach am Taunus, Germany.

³ Commission Directive 88/485/EEC of 26 July 1988 amending the Annex to Council Directive 82/471/EEC concerning certain products used in animal nutrition. OJ L 239, 30.8.88, pp. 36–39.

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 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.

⁷ 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.

⁸ FEED dossier reference: FAD-2019-0015.

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 and peer-reviewed scientific papers 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 fermentation using *C. glutamicum* KFCC 11043 in animal feed. The executive summary of the EURL report can be found in Annex A.⁹

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-lysine sulfate produced by fermentation using *C. glutamicum* KFCC 11043 is in line with the principles laid down in Regulation (EC) No 429/2008¹⁰ 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, 2018a), 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 the assessment of the safety of feed additives for the user (EFSA FEEDAP Panel, 2012), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019f) and Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b).

3. Assessment

The current application is for the authorisation of L-lysine sulfate (minimum 55% lysine on dry matter basis) produced by fermentation with a strain of *C. glutamicum* (KFCC 11043). This product is intended to be used as a nutritional additive (functional group: amino acids, their salts and analogues) in feed for all animal species. The active substance of the additive is L-lysine.

3.1. Characterisation of the production organism

The additive is produced by fermentation with a strain of *C. glutamicum* which was deposited [REDACTED] with accession number KFCC 11043. [REDACTED] The taxonomic identification of the production strain was confirmed [REDACTED]

The production strain was generated from [REDACTED]

The susceptibility of the production strain to relevant antibiotics was tested against the list of antimicrobials described for 'Corynebacterium and other Gram+' in the Guidance on characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a). All measured minimum inhibitory concentration (MIC) values were lower than the cut off values specified in the Guidance. [REDACTED]

The WGS of the production strain was interrogated for the presence of antimicrobial resistance (AMR) genes [REDACTED] No relevant hits were identified.

⁹ The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2019-0014-lysine.pdf

¹⁰ Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

3.1.1. Manufacturing process

3.1.2. 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 $[\text{NH}_2\text{-(CH}_2\text{)}_4\text{-CH(NH}_2\text{)-COOH}]_2 \text{SO}_4$ and the molecular structure is given in Figure 1. The theoretical content of lysine in the lysine sulfate is 75%.

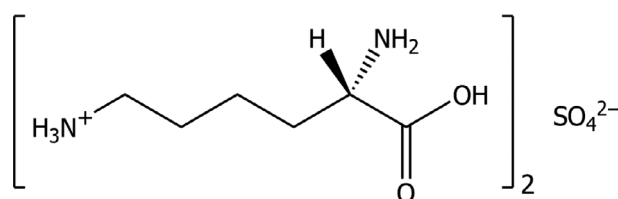


Figure 1: Molecular structure of L-lysine sulfate

L-Lysine sulfate contains by specification $\geq 55\%$ lysine on a dry matter basis and $\leq 4\%$ water.

The compositional data of five batches showed a lysine concentration of 55.1% on 'as is' basis (in all batches tested), and water content ranged 2.3–2.4%.¹⁸ On a dry matter basis, the amount of lysine was on average 56.5% (56.4–56.5%). Average content of sulfate was 20.5% (range 20.3–20.7%); 0.4% ammonium; 0.3% trehalose; sum of other amino acids 1% (0.9–1.1%, consisting on glutamic acid, leucine, phenylalanine, valine and arginine); lactic acid 0.4%; sodium 0.2%; potassium 0.7%, chloride 0.2%; phosphate 0.1% and total nucleic acids 1.6%. Ash content was 3.2% (range 3.0–3.4%).¹⁹ The total amount of identified material on a dry matter basis was about 80%.

The calculated proportion of sulfate not associated with lysine in relation to total lysine was about 1.5%.

3.1.2.1. Impurities

Impurities were analysed in three batches. The content of heavy metals (lead, cadmium and mercury) and arsenic were below the respective limits of detection (LODs).²⁰ The sum of polychlorinated dibenzodioxins and polychlorinated dibenzofurans (PCDD/PCDF) was 0.062 ng WHO TEQ (2005)/kg (88% dry matter (DM)) and the sum of PCDDs/PCDFs plus co-planar dioxin-like polychlorinated biphenyls (PCBs) was 0.12 ng WHO TEQ (2005)/kg (88% DM).²¹ Nitrofurans (furazolidone, furaltadone, nitrofurazone and nitrofurantoin) and nitrofuran metabolites (3-amino-2-oxazolidinone (AOZ), 5-methyl-morpholino-3-amino-2-oxazolidinone (AMOZ), semicarbazide (SC) and 1-aminohydantoin (AH)) were below the respective LOD.²² Pesticides (358 species) were analysed in three batches and were found $< \text{LOD}$.²³ Polyethylene glycol 400, used as antifoaming agent, was measured in three batches of the final product and in all cases the concentrations were below the limit

¹⁸ Technical dossier/Section II/Annex II.1.3 and supplementary information March 2020/Annex SIN 1 and supplementary information March 2020/03 DIN CJ L-lys G 11043. The method of analysis of lysine was HPLC with refractive index detector.

¹⁹ Technical dossier/Section II/Annex II.1.4.

²⁰ Technical dossier/Supplementary information March 2020/Annex SIN 2. LODs in $\mu\text{g/kg}$ were 1 for lead, arsenic and cadmium; 5 for mercury; 0.05 TEQ (WHO-2005) for PCDD/F and 0.13 TEQ (WHO-2005) for PCBs.

²¹ Technical dossier/Supplementary information March 2020/Annex SIN 6.

²² Technical dossier/Supplementary information March 2020/Annex SIN 2. LOD in $\mu\text{g/kg}$ were 0.09–0.15 for nitrofurans (depending on the compound considered) and 0.11–0.16 for nitrofuran metabolites (depending on the metabolite considered).

²³ Technical dossier/Supplementary information March 2020/Annex SIN 2. LOD in $\mu\text{g/kg}$ ranged 0.5–8 depending on the pesticide considered.

of quantification.²⁴ Regarding the mycotoxins content, the same batches showed aflatoxins (B1, B2, G1, G2), ochratoxin A, zearalenone, deoxynivalenol, fumonisins (B1, B2) below the LOD.²⁵

Regarding the microbiological contamination (same batches), the specifications are as follows: total plate counts < 1×10^3 colony forming units (CFU)/g, yeast and moulds < 50 CFU/g, *Salmonella* spp. absence in 25 g sample and *Escherichia coli* and coliforms absence in 1g sample; three batches of the additive analysed complied with the specification.²⁶

The amounts of the aforementioned contaminants/impurities do not raise safety concerns.

The presence of viable cells of the production strain was investigated in three batches of the final product L-lysine sulfate [REDACTED]

[REDACTED] No colonies were detected.

3.1.2.2. Physical characteristics

As regards the physical properties, the product under assessment consists of pale-brown free-flowing granules. It has a bulk density of 650–850 kg/m³, a solubility in water of 36% at 30°C.²⁸ The pH measured in ten batches ranged from 3.5 to 4.3.²⁹ Dusting potential was measured in three batches (Stauber–Heubach method) and ranged from 0.201 to 0.262 g/m³ (w/v).³⁰ Particle size distribution was measured by sieving (three batches) and the percentage of particles < 300 µm diameter ranged from 0.4% to 1.7% (w/w).³¹

3.1.2.3. Stability and homogeneity

No data were provided in the technical dossier with the additive under assessment, but studies were submitted by the applicant using a L-lysine sulfate produced by a different strain of *C. glutamicum* (KCCM 80183). As the production process is the same, the composition and the physical characteristics of the products are very similar,³² the FEEDAP Panel considers that the results of such studies can be applicable to the product under assessment and are described below.

The shelf life of three batches of a L-lysine sulfate produced by *C. glutamicum* KCCM 80183 was tested when stored in sealed bags (3 ply paper and 2 ply polyethylene) at 25°C for 2 years. No losses were detected.³³

The stability of L-lysine sulfate (three batches) produced by *C. glutamicum* KCCM 80183 was tested when supplemented at 10% (corresponding to 5% lysine) in a vitamin/mineral premixture (without choline chloride) for chickens for fattening and stored in the sealed bags described above at 25°C for 6 months. Losses were of 1%.³⁴

The stability of a L-lysine sulfate (three batches) produced by *C. glutamicum* KCCM 80183 was tested when supplemented at 0.3% L-lysine in a compound mash feed for chickens for fattening (basal lysine content was 1.11%, feed composition not provided).³⁵ Feed was stored in the sealed bags described above at 25°C for 3 months. No losses in total lysine (protein-bound and free lysine) were observed.

The stability of a L-lysine sulfate (three batches) produced by *C. glutamicum* KCCM 80183 was tested when supplemented at 1.4% L-lysine in a compound pelleted feed for chickens for fattening (feed composition not provided)³⁶; pelleting was performed at 72°C. Feed was stored in the sealed bags described above at 25°C for three months. No losses in total lysine (protein-bound and free lysine) were observed.

²⁴ Technical dossier/Supplementary information March 2020/Annex SIN 4. Limit of quantification for PEG 400 was 4.3 mg/kg.

²⁵ Technical dossier/Supplementary information March 2020/Annex SIN 2. LOD in µg/kg were 0.1 for each aflatoxin and for ochratoxin A, 1.5 for zearalenone, 0.5 for deoxynivalenol, and 5 for each fumonisin.

²⁶ Technical dossier/Supplementary information March 2020/03_SIN CJ L-Lys G 11043 and Annex SIN 2. LOD were 10 CFU/g for total plate count and for yeast and moulds; and 1 CFU/g for coliform bacteria, *E. coli* and *Salmonella* spp.

²⁸ Technical dossier/Section II/Annex II.1.1.

²⁹ Technical dossier/Section II/Annex II.1.3 and supplementary information March 2020/Annex SIN 09.

³⁰ Technical dossier/Section II/Annex II.1.7.

³¹ Technical dossier/Section II/Annex II.1.5.

³² Technical dossier/Supplementary information March 2020/03 SIN CJ L-Lys G 11043, section 1.2.6.

³³ Technical dossier/Section II/Annex II.4.1.

³⁴ Technical dossier/Section II/Annex II.4.3.

³⁵ Technical dossier/Section II/Annex II.4.4.

³⁶ Technical dossier/Section II/Annex II.4.5.

The capacity of the additive to distribute homogeneously in feed was measured in the premixture and in the chicken feed (mash and pelleted) described above.³⁷ Ten subsamples of each matrix were analysed and the coefficients of variation were 0.9%, 2% and 2%, respectively.

3.1.3. Conditions of use

The additive is intended to be used in 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.³⁸

3.2. Safety

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 L-lysine 'per se' will not raise safety concerns for the target animals provided it is supplemented in appropriate amounts to the diets. Potential concerns would arise from the fermentation process. The production strain KFCC 11043 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*, was shown to be susceptible to the relevant antibiotics. There were also no viable cells in the final product. It can be concluded that no safety concerns for target animal, consumers and the environment would rise from the fermentation residues that may be present in the final additive.

In addition, there is a high inherent content of sulfate in this product. These levels of sulfate could be a safety concern for the target species (EFSA FEEDAP Panel, 2015b) depending on the supplementation level and the tolerance of the target species. The applicant performed an extensive literature search using different databases (Web of Knowledge, MedPharmFacts [LIVIVO and Ovid], ETH Library Search Portal and Google scholar). Key words used included terms on toxicity, excess, poisoning, tolerance or safe.³⁹ No limits were applied to the searches. Studies where sulfur or sulfate (in its different forms) had been administered to the diet of food producing animals were selected. From those sources, 20 scientific papers were selected for cattle, 13 for sheep and goats, 10 for swine, 6 for poultry, 1 for horses and 6 for laboratory animals (mice and rats).

With regard to the high 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, as established by NRC (2005) and set in ruminant diets at 3 g S/kg DM (diet rich in concentrate) and at 5 g S/kg DM (diet rich in roughage) and in non-ruminant diets at 4 g S/kg DM. Also, the contribution of S/sulfate present in water for drinking to the total S intake should be considered, especially when the content is high.⁴⁰

More than half of the papers selected by the applicant were published before year 2000, others did not report sulfate/sulfur content in the basal diet, or total sulfate/sulfur in the diet, or feed intake (or water intake when sulfate was present/added in water for drinking), or were addressing topics already described in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2019a and its related Appendix A). The rest of the studies selected by the applicant (Nichols et al. (2013), Morine et al. (2014), Drewnoski et al., 2014 and Hartman et al. (2017) in cattle; Morrow et al. (2013) in lambs) confirm the statement by NRC (2005) as 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 of established scientific bodies.

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.

³⁷ Technical dossier/Section II/Annexes II.4.3 to II.4.5.

³⁸ Technical dossier/Supplementary information March 2020/03 SIN CJ L-lys G 11043, section 1.2.7.

³⁹ Technical dossier/Supplementary information March 2020/03 SIN CJ L-lys G 11043, section 2.

⁴⁰ See appendix A of EFSA FEEDAP Panel (2019a–e, 2020a,b).

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. Safety for user

No studies were provided to support the safety for the user performed with the additive under assessment. The applicant provided an acute inhalation study, a skin irritation study, an eye irritation study and a skin sensitisation study performed using a L-lysine sulfate produced by another strain of *C. glutamicum* (KCCM 80183) (having a purity of 55% lysine) as a test item. As the physical characteristics (dusting potential, particle size distribution, purity) are very similar and the production process is the same, the FEEDAP Panel considers that the results of those studies testing L-lysine sulfate produced using of *C. glutamicum* KCCM 80183 (described in the following sections) are applicable to the L-lysine sulfate under assessment.

3.2.1.1. Effects on the respiratory system

The highest dusting potential of the additive analysed was 0.26 g/m³ and the fraction of particles with a diameter < 300 µm diameter ranged from 0.4% to 1.7%. Users may be exposed by inhalation.

In an acute inhalation toxicity test performed in accordance with OECD Guideline 403,⁴¹ the single exposure acute inhalation LC₅₀ of the substance tested was greater than 5.27 g/m³ in rat.

3.2.1.2. Effects on skin and eyes

An *in vitro* study (cytotoxicity in reconstructed human epidermal cultures) was carried out according to OECD guideline 439.⁴² The results indicated that the test substance was non-irritant to skin.

In an acute eye irritation study using the bovine corneal opacity and permeability assay in accordance with OECD 437,⁴³ the test substance was considered as non-irritant to eyes (falling under 'no category' of the United Nations Global Harmonised System).

A local lymph node assay (LLNA) was performed using female CBA/Ca mice, in accordance with OECD Guideline 429.⁴⁴ The test substance was not considered a skin sensitiser.

3.2.1.3. Conclusions on safety for the user

From the results of studies on the safety for the user of L-lysine sulfate produced by a different production strain, it was possible to conclude on the safety for the user of the product under assessment. L-lysine sulfate produced by *C. glutamicum* KFCC 11043 is considered non-toxic by inhalation, non-irritant to skin or eyes and it is 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 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 Regulation⁴⁵ and Good Manufacturing Practice.

⁴¹ Technical dossier/Section III/Annex III.3.3.

⁴² Technical dossier/Section III/Annex II.3.5.

⁴³ Technical dossier/Section III/Annex II.3.4.

⁴⁴ Technical dossier/Section III/annex II.3.6.

⁴⁵ 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.

4. Conclusions

The production strain *C. glutamicum* KFCC 11043 qualifies for the QPS approach to safety assessment and was not detected in the final product. L-Lysine sulfate produced using *C. glutamicum* KFCC 11043 does not pose any safety concern associated with the production strain.

L-Lysine sulfate produced by *C. glutamicum* KFCC 11043 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* KFCC 11043 is safe for the consumer, user and for the environment.

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 as provided to EFSA/Chronology

Date	Event
22/02/2019	Dossier received by EFSA. L-lysine sulphate produced by fermentation with <i>Corynebacterium glutamicum</i> KFCC 11043. Submitted by CJ Europe GmbH
20/03/2019	Reception mandate from the European Commission
07/05/2019	Application validated by EFSA – Start of the scientific assessment
08/07/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: Characterisation of the production organism, characterization of the additive, safety for the target species, safety for the user</i>
07/08/2019	Comments received from Member States
08/09/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
12/03/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
01/07/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

AH	1-aminohydantoin
AMOZ	5-methyl-morpholino-3-amino-2-oxazolidinone
AOZ	3-amino-2-oxazolidinone
CAS	Chemical Abstracts Service
CFU	colony forming unit
CV	coefficient of variation
DM	dry matter
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on additives and products or substances used in animal feed
IEC-VIS/FLD	ion exchange chromatography coupled to visible or fluorescence detection
LLNA	local lymph node assay
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
PCB	polychlorinated biphenyls
PCDD/F	polychlorinated dibenzodioxins/dibenzofurans
RSDr	relative standard deviation for repeatability
RSDR	relative standard deviation for reproducibility
SC	semicarbazide
TEQ	toxic equivalents
VDLUFA	Association of German agricultural analytic and research institutes
WHO	World Health Organization

Annex A – Executive Summary of the EURL on analytical methods to detect L-lysine sulfate produced by fermentation with *Corynebacterium glutamicum* KFCC 11043

In the current application authorisation is sought under Article 4(1) (authorisation of a new feed additive) for L-lysine sulphate produced by fermentation with *Corynebacterium glutamicum* KFCC11043, 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. Authorisation is sought for all animal species.

According to the Applicant, L-lysine sulphate contains a minimum of 55% (w/w) of L-lysine.

For the quantification of lysine in the feed additive the Applicant submitted a slightly modified protocol of the European Union method dedicated for the determination of amino acids in feed. However, the EURL previously evaluated lysine dossiers and recommended for the quantification of lysine in the feed additives and premixtures (containing more than 10% lysine) the ring-trial validated method EN ISO 17180:2013 based on ion exchange chromatography coupled to visible or fluorescence detection (IEC-VIS/FLD). This standard method does not distinguish between the salts of amino acids and it cannot differentiate between enantiomers. It applies for products containing more than 10% of amino acid. The following performance characteristics are reported: 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%. In addition, the EURL identified the generic European Pharmacopoeia monograph on sulphates (Ph. Eur. 20301) for the identification of sulphate in L-lysine sulphate.

For the quantification of lysine in premixtures and feedingstuffs the Applicant suggested using the ring-trial validated VDLUFA 4.11.6 method. However, the EURL previously evaluated lysine dossiers and recommended for the quantification of lysine in premixtures and feedingstuffs the ring-trial validated European Union method (Commission Regulation (EC) No 152/2009) based on IEC coupled with photometric detection (IEC-VIS). This method, designed only for the analysis of amino acids in premixtures and feedingstuffs, does not distinguish between the salts and the amino acid enantiomers. The following performance characteristics were reported for the quantification of total lysine: RSDr ranging from 2.1 to 2.8% and RSDR ranging from 3 to 6.7%.

The Applicant did not provide any experimental data to determine lysine in water. Nevertheless, as concluded in previous EURL's amino acids reports, the IEC-VIS procedure described in the European Union method (or similar ones e.g. VDLUFA Method 4.11.6.) is considered fit for purpose for the determination of lysine in this matrix.

In the frame of this authorisation the EURL recommends for official control (i) the European Pharmacopoeia monograph (Ph. Eur. 01/2008:20301) for the identification of the sulphate ion in L-lysine sulphate; (ii) the ring-trial validated method EN ISO 17180:2013 based on ion exchange chromatography coupled to visible or fluorescence detection (IEC-VIS/FLD) to quantify free lysine in the feed additive and premixtures (containing more than 10% lysine); (iv) the ring-trial validated Community method based on IEC-VIS for the quantification of lysine in premixtures, feedingstuffs and water.

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) is not considered necessary.