Safety and efficacy of a feed additive consisting of L-lysine monohydrochloride and L-lysine sulfate produced by fermentation with Corynebacterium glutamicum CGMCC 17927 for all animal species (Barentz Animal Nutrition B.V.)

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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 monohydrochloride and L-lysine sulfate produced by Corynebacterium glutamicum CGMCC 17927, when used as a nutritional additive in feed and water for drinking for all animal species. The active substance is L-lysine, and it was produced in two different forms: monohydrochloride (HCl) or sulfate salts. The production strain was genetically modified. Neither viable cells nor recombinant DNA of the production strain were detected in the final products. Therefore, the Panel concluded that the additives did not pose any safety concern regarding the production strain. The use of L-lysine HCl and L-lysine sulfate produced by the strain C. glutamicum CGMCC 17927 in supplementing feed to compensate for L-lysine deficiency in feedingstuffs was safe for the target species. The FEEDAP Panel identified risks of nutritional imbalances and hygienic concerns for amino acids when administered simultaneously in feed and in water for drinking. The use of both forms of L-lysine produced by fermentation using C. glutamicum CGMCC 17927 in animal nutrition was considered safe for the consumers and for the environment. Exposure of users through inhalation to L-lysine HCl and L-lysine sulfate produced with C. glutamicum CGMCC 17927 was considered very likely. In absence of data, the FEEDAP Panel could not conclude on the potential of both forms of the additive to be irritant for skin and eyes or to be dermal sensitisers. L-lysine HCl and L-lysine sulfate were considered as efficacious sources 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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Keywords: nutritional additive, amino acid, lysine monohydrochloride, lysine sulfate, safety, efficacy

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003\(^1\) 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 Barentz Animal Nutrition B.V.\(^2\) for the authorisation of the additive consisting of L-lysine monohydrochloride (HCl) and L-lysine sulfate produced by fermentation with *Corynebacterium glutamicum* CGMCC 17927, when used as feed additives 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 1 December 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 L-lysine HCl and L-lysine sulfate produced by fermentation with *C. glutamicum* CGMCC 17927, when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

L-Lysine HCl and L-lysine sulfate produced by fermentation with *C. glutamicum* CGMCC 17927 are currently not authorised in the European Union. L-Lysine produced using different microbial strains is currently authorised for its use in all animal species as a nutritional additive and as a sensory additive.\(^3\)

L-Lysine is authorised for use in food,\(^4\) cosmetics\(^5\) and as a veterinary medicinal product.\(^6,7\)

L-Lysine hydrochloride is described in monograph 01/2008:0930 of the European Pharmacopoeia (PhEur 10th edition, 2019).

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 fermentation using different strains of *C. glutamicum*, *Escherichia coli* and *Corynebacterium casei* for all animal species.

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\(^1\) 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.
\(^2\) Barentz Animal Nutrition B.V, Hanzestraat 3, 7,006 RH Doetinchem, The Netherlands.
\(^3\) See the European Union Register of Feed Additives, available online https://ec.europa.eu/food/system/files/2021-12/animal-feed_additives_eu-register_1831-03.pdf
\(^4\) 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 (EU) 2019/701 of 5 April 2019 establishing a glossary of common ingredient names for use in the labelling of cosmetic products. OJ L 121, 8.5.2019, pp. 1–370.
\(^6\) 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 121, 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. OL 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 dossier in support of the authorisation request for the use of L-lysine HCl and L-lysine sulfate produced by fermentation with *C. glutamicum* CGMCC 17927 as feed additives.

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 HCl and L-lysine sulfate produced by fermentation with *C. glutamicum* CGMCC 17927 in animal feed. The Executive Summary of the EURL report can be found in Annex A.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-lysine monohydrochloride and L-lysine sulfate produced by fermentation with *C. glutamicum* CGMCC 17927 is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019a).

3. Assessment

L-lysine HCl (≥ 78.8% L-lysine on a dry matter [DM] basis) and L-lysine sulfate (≥ 55% L-lysine on a DM basis) produced by fermentation with a genetically modified strain of *C. glutamicum* (CGMCC 17927) are intended to be used as a nutritional feed additive (functional group: amino acids, their salts and analogues) in feed and water for drinking for all animal species. The active substance of both forms of the additive is L-lysine.

3.1. Characterisation

3.1.1. Characterisation of the production microorganism

The production strain is a genetically modified strain of *C. glutamicum* that is deposited in the China General Microbiological Culture Collection Center (CGMCC) with accession number CGMCC 17927.

The genome of the production strain was sequenced and used for identification purposes. The taxonomic identification of the production strain CGMCC 17927 as *C. glutamicum* was confirmed.
The susceptibility of the production strain to the battery of antibiotics recommended in the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b) was tested by broth microdilution following the method of the 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-positive' (EFSA FEEDAP Panel, 2018b). Therefore, the production strain is considered susceptible to all relevant antibiotics.

The WGS data of the production strain was interrogated for the presence of antimicrobial resistance (AMR) genes. No hits of concern were identified in the WGS data of the production strain.

3.1.1.1. Information regarding the genetically modified microorganism

The recipient strain was...

3.1.1.2. Characteristics of the introduced sequences

3.1.1.3. Description of the genetic modification

All the genetic modifications were confirmed...
3.1.2. Manufacturing process

Both forms of the additive are produced by fermentation using *C. glutamicum* CGMCC 17927 as production strain.

3.1.3. Characterisation of the *L*-lysine monohydrochloride

*L*-Lysine monohydrochloride (IUPAC name: (2S)-2,6-diaminohexanoic acid monohydrochloride, synonym *L*-lysine hydrochloride, a compound identified with the CAS No. 657-27-2 and the EC number 211-519-9), has a molecular weight of 182.65 g/mol. The theoretical content of lysine in lysine monohydrochloride is 80%. The molecular formula is C₆H₁₂N₂O₂ \( \cdot \) HCl and the molecular structure is given in Figure 1.

![Molecular structure of L-lysine HCl.](image)

The spcific optical rotation of three batches of *L*-lysine HCl was analysed by the Food Chemicals Code (FCC) method and ranged from +20.4 to +21.4°. The analytical values fall within the reference values +18 to +21.5° and confirmed the *L*-enantiomer of lysine.

3.1.3.1. Impurities

Three batches of *L*-lysine HCl were analysed for impurities. Cadmium, lead, mercury and arsenic concentrations showed values below the corresponding limit of quantification (LOD), except for two batches for which the levels of lead were, respectively, 0.010 and 0.017 mg/kg.
Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar dioxin-like polychlorinated biphenyls (co-planar PCBs) were analysed in three batches of L-lysine HCl and found below the corresponding limit of quantification (LOQ). The calculated (upper bound) levels of dioxins and the sum of dioxins and dioxin-like-PCBs were 0.14 ng WHO-PCDD/F-TEQ/kg and 0.27 ng WHO-PCDD/F-PCB-TEQ/kg, respectively (in all three batches).  

In three batches of L-lysine HCl, the analysis of total aflatoxins (not further described) showed values below the LOD in one batch and were 0.84 and 1.37 µg/kg in the other two batches, respectively. Ochratoxin A, fumonisin and zearalenone values were found to be below the LOD in all three batches. Deoxynivalenol was below the LOD in two batches and was 1,665 µg/kg in one batch. Citrinin values ranged from 127 to 149 µg/kg.  

The detected amounts of the above-described impurities do not raise safety concerns. Microbiological contamination was analysed in three batches of L-lysine HCl (25 g samples) by determination of Escherichia coli, Enterobacteriaceae, filamentous fungi, yeasts and Salmonella spp. with negative results in all cases.  

The presence of viable cells of the production strain was tested in three batches of the final product L-lysine HCl, each batch tested in triplicate. First, 10 g samples were diluted in 90 ml 0.9% NaCl and then 10 mL of the solution were filtered through 0.45-µm mixed cellulose esters (MCE) filters. After filtration, the filters were transferred to nutrient agar and incubated for 5 days at 30°C. All relevant controls were included. No viable cells of the production strain were found in the samples tested.  

The presence of recombinant DNA from the production strain was analysed. No DNA from the production strain was detected.  

### 3.1.3.2. Physical properties  

L-Lysine HCl appears as light brown to yellow granules or powder. Density is 0.55–0.60 kg/L, melting point is 260°C. The additive is freely soluble in water.  

The dusting potential of three batches of L-lysine HCl was determined using the Stauber-Heubach method and showed values that ranged from 11.8 to 15.4 g/m³. The particle size distribution of the L-lysine monohydrochloride was analysed in three batches by laser-diffraction method; the results showed that the fractions of particles with a diameter < 50 µm was < 1%; and the fraction < 100 µm ranged 1.5–1.9%.  

### 3.1.3.3. Stability and homogeneity  

The shelf life of L-lysine HCl (three batches) was studied when stored at room temperature and at 40°C in packaging paper bags free from light for 6 months. No losses were observed when kept at room temperature; when kept at 40°C losses ranged 0–1%.  

The stability of L-lysine HCl (three batches) in a vitamin-mineral premixture for piglets (containing 30,000 mg choline chloride/kg) was studied when supplemented at 4% and stored at room temperature in paper bags free from light for 6 months. No losses were observed.  

The stability of L-lysine HCl (three batches) in water for drinking was studied when supplemented at 0.16% lysine. Samples were stored at about 20°C for 48 h. Losses at the end of the storage period were 0%.  

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26 Technical dossier/Section II/Annexes 1.2.4.a-c  
27 Technical dossier/Section II/Annex 2.1.3.a. LOD in µg/kg was 0.05 for aflatoxin; 5 for ochratoxin A; 17 for zearalenone; 134 for deoxynivalenol; and 25 for fumonisn.  
28 Technical dossier/Section II/Annex 2.1.3.a and supplementary information June 2022/reply to question 9.  
29 Technical dossier/Section II/Annex 2.1.4 g and losses information July 2022/Annex 2.1.3a.  
30 Technical dossier/Section II/Annex 2.1.4 h CONFID and supplementary information July 2022/Annex Q12a CONF.  
31 Technical dossier/Section II/Annexes 2.5.2.a  
32 Technical dossier/Section II/Annexes 2.1.5.g-i  
33 Technical dossier/Section II/Annexes 2.1.5.a-c and supplementary information June 2022/Answer to Q10.  
34 Technical dossier/Section II/Annex 2.1.3.a  
35 Technical dossier/Section II/Annex 2.4.1.a and supplementary information June 2022/Annex Q11a.
The stability of L-lysine HCl (three batches) was studied in feed for chickens for fattening (mash and pelleted form) when supplemented at 0.2%. The basal diet consisted of maize, wheat, soybean meal, rapeseed meal and contained a background concentration of 0.96 g lysine/kg feed. The pelleting process was performed at 74–76°C. Samples were stored at room temperature in paper bags for 3 months. L-Lysine HCl showed losses ranging 0–4% for mash; ranging 0–10% for pelleted feed; and the pelleting process caused no loss.

The capacity for homogeneous distribution of L-lysine HCl was studied in 10 subsamples of the chicken feed described above. Total lysine was analysed and corrected by subtracting the background concentration. The coefficient of variation was 15%.

3.1.4. Characterisation of the L-lysine sulfate

L-Lysine sulfate (CAS No 60343-69-3) has a molecular weight of 390.38 g/mol. The molecular formula is $C_{12}H_{28}N_4O_4-O_4S$ and the molecular structure is given in Figure 2.

![Molecular structure of L-lysine sulfate.](image)

According to specification, L-lysine sulfate contains minimum 55% lysine on DM basis and a maximum of 4% water. The analysis of five batches of L-lysine sulfate showed an average of 58.1% lysine (range 57.6–58.5%) on a DM basis, sulfate averaged 23.1% (21.7–26.1%) and water content averaged 2.5% (range 2.4–2.6%). The amount of identified material was 81.1% (range 79.3–84.6%).

Three additional batches were submitted to a compositional analysis. Lysine represented 55.2%, sulfate 24.4%, free amino acids other than lysine 0.8%, organic acids 0.09%, ammonia 0.61%, crude ash 1.4%, sugar 0.7% and crude protein 8.4% (Kjeldahl method). This represents about 92% of identified material.

3.1.4.1. Impurities

Three batches of L-lysine sulfate were analysed for impurities. Cadmium, lead, mercury and arsenic concentrations showed values below the corresponding LOD, except for lead that ranged from 0.030 to 0.034 mg/kg.

PCDDs, PCDFs and co-planar PCBs were analysed in three batches of L-lysine sulfate and found below the corresponding LOQ. The calculated (upper bound) levels of dioxins and the sum of dioxins and dioxin-like-PCB were 0.14 ng WHO-PCDD/F-TEQ/kg and 0.27 ng WHO-PCDD/F-PCB-TEQ/kg, respectively (in all three batches).

In same three batches, the analysis of total aflatoxins showed values below the LOD in one batch, 0.33 and 0.58 µg/kg in the other two batches, respectively. Ochratoxin A values were found to be below the LOD in all three batches. Zearalenone concentration ranged from 25.5 to 42.5 µg/kg in the three batches. Deoxynivalenol ranged from 810 to 1,142 µg/kg in the three batches. Fumonisin was below the LOD in one batch, 109.6 and 298.1 µg/kg in the other two batches respectively. Citrinin values ranged from 117.5 to 146 µg/kg in the three batches. The FEEDAP Panel notes the unusual high concentrations of deoxynivalenol, fumonisin and citrinin detected.

The detected amounts of the above-described impurities do not raise safety concerns.
Microbiological contamination was analysed for three batches of l-lysine sulfate (25 g samples) by determination of *E. coli*, Enterobacteriaceae, filamentous fungi, yeasts and *Salmonella* spp. which were not detected in any sample.  

The presence of viable cells of the production strain was tested in three batches of the final product.  

First, 10 g samples were diluted in 390 ml 0.9% NaCl and then five 8 ml subsamples were filtered through 0.45-µm MCE filters. After filtration, the filters were transferred to a selective medium (*Corynebacterium* agar containing 128 mg/L fosfomycin (CAF)) and incubated for 5 days at 30°C. All relevant controls were included. Two colonies were found in the non-spiked samples which were tested negative by PCR using specific primers of the production strain, amplifying an 859-bp amplicon. No viable cells of the production strain were found in the samples tested.

The presence of recombinant DNA from the production strain was analysed in three batches.  

No DNA from the production strain was detected.

### 3.1.4.2. Physical properties

l-Lysine sulfate appears as a yellow or brown powder, density is 0.60–0.66 kg/L. The additive is soluble in water.

The dusting potential of three batches of l-lysine sulfate was determined using the Stauber–Heubach method and showed values that ranged from 51.5 to 67.4 g/m³. The particle size of the dust was analysed in three batches by the laser diffraction method; the results showed that no particles were found of < 100 µm diameter.

### 3.1.4.3. Stability and homogeneity

The shelf life of l-lysine sulfate (three batches) was studied when stored at room temperature and at 40°C in packaging paper bags free from light for 6 months. Losses at the end of the storage period ranged 1–2% when kept at room temperature; and ranged 0–1% when kept at 40°C.

The stability of l-lysine sulfate (three batches) in vitamin–mineral premixture for piglets (containing 30,000 mg choline chloride/kg) was studied when supplemented at 4% and stored at room temperature in paper bags free from light for 6 months. Losses at the end of the storage period ranged from 0 to 2%.

The stability of l-lysine sulfate (three batches) in water for drinking was studied when supplemented at 0.11% lysine. Samples were stored at about 20°C for 48 h. Losses at the end of the storage period were 0%.

The stability of l-lysine sulfate (three batches) was studied in feed for chickens for fattening (mash and pelleted form) when supplemented at 0.2%. The basal diet consisted of maize, wheat, soybean meal, rapeseed meal and contained a background concentration of 0.96 g lysine/kg feed. The pelleting process was performed at 74–76°C. Samples were stored at room temperature in paper bags for 3 months. l-Lysine sulfate showed losses ranging 0–6% for mash and pelleted feed, respectively, and the pelleting process caused a loss of 2%.

The capacity for homogeneous distribution of l-lysine sulfate was studied in 10 subsamples of the chicken feed described above. Total lysine was analysed and corrected by subtracting the background concentration. The coefficient of variation was 20%.
3.1.5. Conditions of use

Both forms of the additive are intended to be used in feed for all species and can be added directly in compound feed, 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, the water intake and the amino acid composition of the unsupplemented diet.

Both forms of the additive are intended to be used in water for drinking. No inclusion levels in water were proposed by the applicant.

3.2. Safety

3.2.1. Safety of the production organism

The production strain belongs to a species, *C. glutamicum*, that is suitable for the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007) when used for production purposes (EFSA BIOHAZ panel, 2020). The production strain *C. glutamicum* CGMCC 17927 was developed to increase the production of L-lysine. The genes inserted during the genetic modification do not raise safety concerns and the production strain does not carry acquired antimicrobial resistance genes. The production strain and its DNA were not detected in the final products. Therefore, the final products do not pose any safety concern as regards the production strain.

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

The 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 previous opinions (EFSA FEEDAP Panel, 2013, 2014). No safety concerns for ruminants would arise from ruminal lysine metabolism (EFSA FEEDAP Panel, 2013, 2014). Safety concerns from the additive could derive from the residues of the fermentation process/production strain remaining in the final product. The production strain qualifies for the QPS approach to safety assessment when used for production purposes. It can be concluded that no safety concerns for target animals, consumers and the environment would arise from the fermentation residues that may be present in the final additive. The use of the amino acid *per se* will not raise safety concerns for the target animals, provided that it is supplemented in appropriate amounts to satisfy the nutritional requirements of the animals in L-lysine-deficient diets. However, due to the risk of nutritional imbalances and hygienic reasons, associated to the use of amino acids via water for drinking (EFSA FEEDAP Panel, 2010), the FEEDAP Panel has concerns on the safety of the use of the amino acid via water for drinking.

There is a high inherent content of sulfate in L-lysine sulfate which could be a safety concern for the target species, depending on the supplementation level and the tolerance of the target species. The EFSA FEEDAP Panel (2019a) has 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 National Research Council (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. Consequently, no negative effects are expected at normal use levels for the target species provided that the total S intake complies with the recommendations of established scientific bodies.

The 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. Potential concerns for consumers might arise from the fermentation process. However, the production strain is considered safe, and no impurities of concern were identified (see Section 3.2.1).

The amino acid L-lysine is a physiological and natural component of animals and plants. When supplemented to feed it will be incorporated into proteins of tissues and/or products of animal origin and any potential excess will be catabolised and excreted as urea/uric acid and carbon dioxide. The use of amino acids in water for drinking, when given in addition to complete diets with a well-balanced amino acid profile, would disturb the nitrogen balance and increase nitrogen excretion via urine. The
use of \( \text{L-lysine} \) in animal nutrition would not lead to localised increase in the concentration of \( \text{L-lysine} \) or its metabolites in the environment.

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

The use of \( \text{L-lysine HCl} \) and \( \text{L-lysine sulfate} \) produced by \( \text{C. glutamicum CGMCC 17927} \) in supplementing feed to compensate for lysine deficiency in feedingstuffs is safe for the target species. The FEEDAP Panel has concerns on the use of amino acids in water for drinking for hygienic reasons, and due to the risk of imbalances when administered simultaneously via feed.

The use of both forms of \( \text{L-lysine} \) produced with \( \text{C. glutamicum CGMCC 17927} \) in animal nutrition is considered safe for the consumer and for the environment.

#### 3.2.3. Safety for the user

No specific information was submitted to support the safety for the user regarding the potential of the additive to be toxic by inhalation, irritant to skin or eyes or to be a skin sensitiser.

Considering the dusting potentials of both formulations (up to 15 g/m\(^3\) and 67 g/m\(^3\) for \( \text{L-lysine HCl} \) and \( \text{L-lysine sulfate} \), respectively), the FEEDAP Panel considered that the exposure through inhalation is very likely.

### 3.2.3.1. Conclusions on safety for the user

Exposure through inhalation to \( \text{L-lysine HCl} \) and \( \text{L-Lysine sulfate} \) produced with \( \text{C. glutamicum CGMCC 17927} \) is considered very likely.

In the absence of data, the FEEDAP Panel cannot conclude on the potential of both forms of the additive to be irritant for skin and eyes or to be dermal sensitisers.

### 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 \( \text{L-lysine} \) is well established in the scientific literature. The efficacy of \( \text{L-lysine} \) for both non-ruminant and ruminant species was described in previous opinions (EFSA FEEDAP Panel, 2013, 2014). In general, the products \( \text{L-lysine HCl} \) and \( \text{L-lysine sulfate} \) are considered as efficacious sources of the essential amino acid \( \text{L-lysine} \) for non-ruminant animal species. For the supplemental \( \text{L-lysine} \) 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 Regulation\(^{54}\) and Good Manufacturing Practice.

### 4. Conclusions

\( \text{L-lysine HCl} \) and \( \text{L-lysine sulfate} \) produced by the strain \( \text{Corynebacterium glutamicum CGMCC 17927} \) do not pose any safety concern as regards the production strain.

The use of both forms of the additive under assessment in supplementing feed to compensate for \( \text{L-lysine} \) deficiency in feedingstuffs is safe for the target species. The FEEDAP Panel identified risks of nutritional imbalances and hygienic concerns for amino acids when administered simultaneously in feed and in water for drinking.

The use of both forms of \( \text{L-lysine} \) produced by fermentation using \( \text{C. glutamicum CGMCC 17927} \) in animal nutrition is considered safe for the consumers and for the environment.

Exposure of users through inhalation to \( \text{L-lysine HCl} \) and \( \text{L-Lysine sulfate} \) produced with \( \text{C. glutamicum CGMCC 17927} \) is considered very likely. In the absence of data, the FEEDAP Panel cannot conclude on the potential of both forms of the additive to be irritant for skin and eyes or to be dermal sensitisers.

\(^{54}\) Regulation (EC) No 183/2005 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.
5. Documentation provided to EFSA/Chronology

| Date       | Event                                                                 |
|------------|------------------------------------------------------------------------|
| 23/03/2021 | Dossier received by EFSA. L-lysine monohydrochloride and L-lysine sulfate produced by fermentation with Corynebacterium glutamicum CGMCC 17927. Submitted by Barentz Animal Nutrition B.V |
| 25/05/2021 | Reception mandate from the European Commission                          |
| 01/12/2021 | Application validated by EFSA – Start of the scientific assessment       |
| 22/02/2022 | Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives |
| 09/03/2022 | Comments received from Member States                                   |
| 18/03/2022 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. Issues: characterisation of the production strain, characterisation of the additive, manufacturing process, safety for the user |
| 07/06/2022 | Reception of supplementary information from the applicant – Scientific assessment re-started |
| 27/09/2022 | Opinion adopted by the FEEDAP Panel. End of the Scientific assessment   |

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Abbreviations

AMR antimicrobial resistance
CAS Chemical Abstracts Service
CFU colony forming unit
CLSI Clinical and Laboratory Standards Institute
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
JECFA The Joint FAO/WHO Expert Committee on Food Additives
LOD limit of detection
LOQ limit of quantification
MCE mixed cellulose esters
MIC minimum inhibitory concentration
OECD Organisation for Economic Co-operation and Development
PCB polychlorinated biphenyl
PCDD polychlorinated dibenzodioxin
PCDF polychlorinated dibenzofuran
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 monohydrochloride and L-lysine sulphate produced by fermentation with Corynebacterium glutamicum CGMCC 17927

In the current application an authorisation is sought under Article 4(1) for L-lysine monohydrochloride and L-lysine sulphate produced by fermentation with Corynebacterium glutamicum CGMCC 17927, 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 authorisation is sought for all animal species.

According to the Applicant, the minimum L-lysine content for L-lysine monohydrochloride is 78.8% (w/w) while the content of L-lysine for L-lysine sulphate is 55.0% (w/w).

The two forms of the feed additive are intended to be added directly into feedingstuffs or through complementary feed, premixtures and water without any recommended inclusion levels. Furthermore, no minimum or maximum contents have been proposed by the Applicant.

For the quantification of lysine in the feed additive, premixtures, feedingstuffs and water the Applicant proposed the ring-trial validated method EN ISO 13903:2005. This method is equivalent to the ring-trial validated European Union method (Commission Regulation (EC) No 152/2009) based on ion-exchange chromatography (IEC) coupled with post-column derivatisation and optical (visible) (VIS) detection. 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 3.0 to 6.7%.

However, the EURL is aware of the ring-trial validated method EN ISO 17180:2013 based on ion-exchange chromatography (IEC) coupled with post-column derivatisation and optical (visible or fluorescence) detection (IEC-VIS/FLD). It applies for products containing more than 10% of amino acid and, as the previous method, does not distinguish between the salts of amino acids and cannot differentiate between enantiomers. 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%. Furthermore, the EURL identified the “L-lysine monohydrochloride monograph” of the Food Chemical Codex (FCC) for the identification of L-lysine monohydrochloride in the feed additive and the generic European Pharmacopoeia monograph (Ph. Eur. 20,301) for the identification of sulphate ions in the feed additive.

Based on the performance characteristics available, the EURL recommends for official control (i) the “L-lysine monohydrochloride monograph” of the Food Chemical Codex (FCC) for the identification of L-lysine monohydrochloride in the feed additive; (ii) the European Pharmacopoeia monograph (Ph. Eur. 01/2008:20301) for the identification of the sulphate ions in the feed additive (L-lysine sulphate); (iii) the ring-trial validated method EN ISO 17180:2013 based on ion-exchange chromatography coupled with post-column derivatisation and optical detection (IEC-VIS/FLD) to quantify free lysine in the feed additives and premixtures (containing more than 10% lysine); (iv) the European Union method based on ion-exchange chromatography coupled with post-column derivatisation and optical detection (IEC-VIS) for the quantification of lysine in premixtures and feedingstuffs; and (v) the ion-exchange chromatography coupled with post-column derivatisation and optical detection (IEC-VIS/FLD) or coupled with post-column derivatisation and optical detection (IEC-VIS) methods for the quantification of lysine in 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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.