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Safety and efficacy of L-histidine monohydrochloride monohydrate produced using *Corynebacterium glutamicum* KCCM 80179 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-histidine monohydrochloride (HCl) monohydrate produced by fermentation using *Corynebacterium glutamicum* KCCM 80179 when used as a nutritional additive (amino acid) and as a sensory additive (flavouring compound) in feed and water for drinking for all animal species. The production strain is not genetically modified. No viable cells of the production strain were detected in the final product. The use of L-histidine monohydrochloride monohydrate produced by fermentation using *C. glutamicum* KCCM 80179 is safe for the target species when used as a nutritional additive to supplement the diet in appropriate amounts to cover the requirements, depending on the species, the physiological state of the animal, the performance level, the environmental conditions, the background amino acid composition of the unsupplemented diet and the status of some essential trace elements such as copper and zinc. This conclusion would also cover the use as a sensory additive. L-Histidine HCl monohydrate produced using *C. glutamicum* KCCM 80179 supplemented at levels appropriate for the requirements of species and life stage is considered safe for the consumer. L-Histidine HCl monohydrate produced using *C. glutamicum* KCCM 80179 is not irritant to skin, is mildly irritant to eyes, and it is not a skin sensitiser. The additive does not pose a risk to users by inhalation. The use of L-histidine HCl monohydrate produced by *C. glutamicum* KCCM 80179 in animal nutrition is not expected to represent a risk to the environment. L-Histidine HCl monohydrate is considered an efficacious source of the essential amino acid L-histidine for non-ruminant animal species. For the supplemental L-histidine to be as efficacious in ruminants as in non-ruminant species, it would require protection against degradation in the rumen. It is also considered efficacious as a feed flavouring compound under the proposed conditions of use.

Keywords: nutritional additive, amino acid, L-histidine monohydrochloride monohydrate, *Corynebacterium glutamicum* KCCM 80179, feed additive, safety

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Table of contents

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

The European Commission received a request from CJ Europe GmbH[2] for authorisation of the product L-histidine monohydrochloride monohydrate (feed grade) when used as a feed additive for all animal species (category: nutritional additives; functional group: amino acids, their salts and analogues; and category: sensory additives; functional group: flavourings).

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 18 September 2018.

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-histidine monohydrochloride monohydrate produced by fermentation with *Corynebacterium glutamicum* KCCM 80179, when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

L-Histidine monohydrochloride (HCl) monohydrate produced by fermentation with a non-genetically modified strain of *C. glutamicum* (KCCM 80179) has not been assessed as a feed nutritional additive. The active substance of the product under application is L-histidine.

L-Histidine HCl monohydrate (minimum 98% on dry matter basis) produced by *Escherichia coli* ATCC 9637 is currently listed in the European Union Register of Feed Additives, and thus authorised in the European Union for use in feed for salmonids.[3] L-Histidine [EU Flavour Information System (FLAVIS) numbers 17.008] produced by chemical synthesis is currently listed in the European Union Register of Feed Additives, and thus authorised in the European Union as a feed flavouring.[4]

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) assessed L-histidine HCl monohydrate produced by *E. coli* ATCC 21318 as a nutritional feed additive (amino acid) for salmonids (EFSA, 2005, 2006a). The FEEDAP Panel assessed the safety and efficacy of L-histidine as feed flavouring (EFSA FEEDAP Panel, 2014). The EFSA's Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) evaluated L-histidine and considered it safe for use as flavours in food (EFSA, 2006b, 2008a,b; EFSA CEF Panel, 2011).

L-Histidine is authorised for use in food,[5] cosmetics[6] and as a veterinary medicinal product.[7,8]

L-Histidine HCl monohydrate is described in a monograph of the European Pharmacopoeia (2017), monograph 01/2017:0910.

[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] Welding GmbH & Co. KG. Esplanase 39, 20354 Hamburg, Germany.
[3] Commission Regulation (EC) No 244/2007 of 7 March 2007 concerning the authorisation of L-histidine monohydrochloride monohydrate as feed additive. OJ L 73/6, 13.3.2007, p. 2.
[4] Commission List of the authorised additives in feedingstuffs published in application of Article 9th of Council Directive 70/524/EEC concerning additives in feedingstuffs (2004/C 50/01). OJ C 50/1 25.2.2004.
[5] 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.
[6] 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.
[7] 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.
[8] 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[9] in support of the authorisation request for the use of L-histidine HCl monohydrate produced by fermentation using *C. glutamicum* KCCM 80179 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 the L-histidine monohydrochloride monohydrate produced by fermentation with *C. glutamicum* KCCM 80179 in animal feed. The Executive Summary of the EURL report can be found in Annex A.[10]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-histidine monohydrochloride monohydrate produced by fermentation with *C. glutamicum* KCCM 80179 is in line with the principles laid down in Regulation (EC) No 429/2008[11] and the relevant guidance documents: Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a), Guidance on the safety of feed additives for the environment (EFSA, 2008c), 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-histidine monohydrochloride monohydrate (minimum 98% purity) produced by fermentation using a [redacted] strain of *C. glutamicum* (KCCM 80179). The product is intended to be used in feed and water for drinking for all animal species as a nutritional additive (functional group: amino acids, their salts and analogues) and as a sensory additive (functional group: flavourings compounds).

3.1. Characterisation

3.1.1. Characterisation of the production organism

The production organism *C. glutamicum* has been deposited [redacted] It is a [redacted]

The identity of the production strain as *C. glutamicum* was demonstrated [redacted].

The susceptibility of the production strain to the antibiotics listed in [redacted]

[9] FEED dossier reference: FAD-2018-0040.

[10] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2018-0040-histidine.pdf

[11] 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.

[redacted]

[redacted]

The minimum inhibitory concentrations were below to the cut off values set in such guidance.

3.1.2. Manufacturing process

L-Histidine is produced by fermentation of the production strain. [redacted]

3.1.3. Characterisation of the product/active substance

L-Histidine monohydrochloride monohydrate (International Union of Pure and Applied Chemistry (IUPAC) name (2*S*)-2-amino-3-(1*H*-imidazol-5-yl)propanoic acid;hydrate;hydrochloride, and synonyms L-α-Amino-β-(4-imidazolyl)propionic acid monohydrochloride, glyoxaline-5-alanine hydrochloride) has the Chemical Abstracts Service (CAS) No 5934-29-2 and European Inventory of Existing Commercial Chemical Substances (EINECS) No 211-438-9. The chemical formula is $C_3H_3N_2–CH_2–CH(NH_2)–COOH·HCl·H_2O$ and the molecular weight 209.63 g/mol. The structural formula is given in Figure 1.

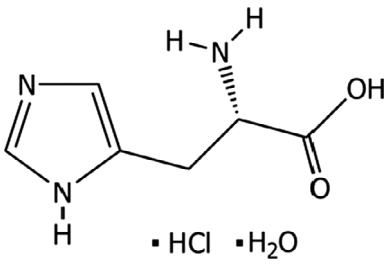

Figure 1: Molecular structure of L-histidine monohydrochloride monohydrate

According to the specification, the additive contains ≥ 98% L-histidine monohydrochloride monohydrate from which ≥ 73% is L-histidine, ≤ 10% moisture, and ≤ 1% ash.[18]

Analysis of five batches showed an average histidine content of 73.5% 'as is' (range 73.5–73.6%), a chloride content of 16.7% (range 16.6–16.9%), a moisture content of 9.1% (range 8.9–9.3%), an ash content of 0.04% (range 0.02–0.07%) and 0.01% of phosphate (in all five batches).[19] Free amino acids, ammonium, nitrate, nitrite, betaine and organic acids were not detected. The sum of the identified material on 'as is' basis was 99.4% (range 99.3–99.6%)

The specific optical rotation of the additive was measured in five batches of the final product and the average was + 10.48° (range + 10.42 to + 10.52°).[20] The measured values were within the range of the European Pharmacopoeia (+ 9.2 to + 10.6°) and confirmed the L-stereoisomer of histidine.

3.1.3.1. Impurities

Three batches of the additive were analysed for undesirable substances.

Heavy metals, (lead, cadmium and mercury) and arsenic were below the limits of detection (LODs).[21] In the same batches, polychlorinated dibenzodioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) and coplanar dioxin-like polychlorinated biphenyls (Co-planar PCBs) were found

[redacted]

[18] Technical dossier/Section II.1.3 and annex II.1.1; and supplementary information November 2018/02 SIN CJ L-HIS 80179, reply to question 1.

[19] Technical dossier/Section II/Annex II.1.4. Analytical method for histidine was HPLC with C18 column using potassium phosphate buffer as mobile phase.

[20] Technical dossier/Section II/Annex II.1.2.

[21] Technical dossier/Section II/Annex II.1.2. LOD (in mg/kg) were 1 for lead; 0.5 for cadmium and arsenic; and 0.1 for mercury.

below the LOD.[22] Aflatoxins (B1, B2, G1, G2), ochratoxin A, zearalenone, deoxynivalenol, fumonisins B1 and B2 were below the corresponding LOD.[23]

In the same batches, nitrofurans (furazolidone, furaltadone, nitrofurazone and nitrofurantoin) and nitrofuran metabolites were below the corresponding LODs.[24] A multiresidue pesticide analysis showed that none of the 358 pesticides was present in the three batches.[25]

Analysis of microbial contamination of the final product (three batches) indicated that *Salmonella* spp. (25 g sample), *E. coli*, and coliforms were absent whereas total bacterial count, was $< 10^3$ colony forming unit (CFU)/g; and yeasts and filamentous fungi were < 50 CFU/g.[20]

The presence of viable cells of the production strain in the final product was tested in three batches of the additive. [redacted] no bacterial growth was observed.

3.1.3.2. Physical properties

L-Histidine monohydrochloride monohydrate is an off-white, odourless, colourless and crystalline powder, with a bulk density of 550–750 kg/m^3, melting point at 254°C, pH 3.5–4.5 (1% solution in water) and a water solubility of 56.6 g/L at 25°C.[27]

No information on the dusting potential of the additive under assessment was submitted. The particle size distribution (three batches analysed by sieving) showed that the fraction of particles < 125 μm ranged from 15 to 20% (w/w).[28]

3.1.3.3. Stability and homogeneity

The applicant submitted data on stability of an L-histidine HCl monohydrate produced by a different strain of *C. glutamicum* (KCCM 80172). These data related to shelf life studies, stability in premixtures and feedingstuffs, in water for drinking and studies on the capacity of the additive to distribute homogeneously in feed (EFSA FEEDAP Panel, 2019).[29] As the characteristics of the additives were similar and the production process was the same, the FEEDAP Panel considered the results of such studies applicable to the stability and the capacity to distribute homogeneously in feed of the additive under assessment. Regarding the shelf life, no losses were observed after storage at 25 or 40°C for 6 months. In a premixture with choline chloride, the losses ranged 4–6% after 6 months storage. In a mash feed, losses from 0 to 2% were observed after 3 months. No losses were observed in water for drinking stored at 25 and 40°C for 48 h. The coefficient of variation in a premixture was 5% and in a pelleted feed for chickens for fattening 6%.

The applicant provided a new stability study in feedingstuffs. The stability of three batches of the additive was studied in a pelleted feed for chicken for fattening (feed ingredients not described, only the nutritional composition) when supplemented at 0.4%.[30] The additive was added via a premixture that was prepared containing 16% L-histidine HCl monohydrate. The feed was pelleted at 72°C and dried at 60–65°C for 10 min. Feed processing (pelleting) represented a loss ranging from 3% to 8%. Pelleted samples were then kept at 25 ± 2°C packed in polyethylene bags for 3 months. At the end of the stability period, only one batch showed a loss of 16%.

3.1.3.4. Physicochemical incompatibilities in feed

No physicochemical incompatibilities in feed are expected with other additives, medicinal products or other feed materials.

[22] Technical dossier/Section II/Annex II.1.2. LOD (in μg/kg) was 0.05 for PCDD/F and 0.13 for Co-PCBs.
[23] Technical dossier/Section II/Annex II.1.2. LOD (in μg/kg) was 0.1 for aflatoxins (B1, B2, G1, G2) and ochratoxin A; 1.5 for zearalenone; 0.5 for deoxynivalenol and 5 for fumonisins B1 and B2.
[24] Technical dossier/Section II/Annex II.1.3. LOD (in μg/kg) was 0.09-0.15 for nitrofurans and 0.11-0.16 for nitrofuran metabolites.
[25] Technical dossier/Section II/Annex II.1.4. LOD (in μg/kg) was 0.5-8.
[redacted]
[27] Technical dossier/Section II.2.2.1 and Annex II.3.1.
[28] Technical dossier/Section II.2.2.1/Annex II.1.5.
[29] Technical dossier/Section II/Annexes II.4.1, II.4.3, II.4.4., II.4.2 and II.4.5.
[30] Technical dossier/Supplementary information December 2018/Annex Sin 2 CJ.

3.1.4. Conditions of use

According to the applicant, the additive can be added directly in compound feed, through complementary feed or through premixtures and is aimed for all animal species. 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, in particular on the amino acid composition of the unsupplemented diet.[31]

The additive can be also used in water for drinking.[32]

L-Histidine monochloride monohydrate is proposed to be as a feed flavouring in feed or in water for drinking at a typical inclusion rate of 5 mg/kg, being the maximum recommended level of inclusion 25 mg/kg.

3.2. Safety

3.2.1. Safety for the target species

The absorption, distribution, metabolism and excretion of L-histidine; its essentiality in animal nutrition; the requirements for the main food producing animal species, the histidine content in different feedingstuffs, and the consequences of excesses of histidine supplementation in animal nutrition were considered in a previous opinion of the EFSA FEEDAP Panel (2019).

Tolerance studies are not normally required for highly purified amino acids. Such tolerance studies with a certain indispensable amino acid will inevitably result in amino acid imbalances with depression of feed intake and hence impaired performance. No tolerance studies were submitted, and no use levels were proposed when used as a nutritional additive by the applicant. The applicant based the safety of the additive on the fact that L-histidine HCl monohydrate is produced by a *C. glutamicum* that qualifies for the qualified presumption of safety (QPS) assessment, and has a high purity, with > 99% identified material.

On request of EFSA, the applicant provided a literature search for studies to address the safety for target animals. Several platform databases (Livivo, Toxnet, Ovid, Web of Knowledge, Google Scholar) were searched using the following search syntax: Histidine AND diet* AND (Chick* OR Birds OR Turkey OR Calf OR Calv* OR cow* OR rumen OR pig* OR fish OR salmon). The full text search for histidine in 'title + keywords' gave in total 658 hits. This was narrowed down to the term histidine in publication title: 89 hits. The search syntax "trace mineral" OR copper OR Zinc provided 23 hits. The same dataset assessed in a previous opinion (EFSA FEEDAP Panel, 2019) was submitted. Nearly all the studies found referred to requirements for L-histidine and not to excesses nor tolerance for L-histidine.

Although there is limited evidence from the published literature on the effects of supplementing histidine levels above the requirements, the FEEDAP Panel considers that, as with other amino acids, adverse effects might occur with levels of histidine in feeds exceeding the requirements, depending on the balance with other amino acids and the status of some essential trace elements such as copper and zinc.

The FEEDAP Panel, in its previous statement (EFSA FEEDAP Panel, 2010), identified risks of nutritional imbalances and hygienic concerns for amino acids when administered in water for drinking.

Since the levels proposed for the use of L-histidine monohydrochloride monohydrate as flavouring (up to 25 mg/kg complete feed) are substantially lower than the animal requirements, the FEEDAP Panel considers L-histidine monohydrochloride monohydrate produced with *C. glutamicum* KCCM 80179 is safe when used as a flavouring compound.

3.2.1.1. Conclusions on the safety for the target species

The use of L-histidine monohydrochloride monohydrate produced by fermentation using *Corynebacterium glutamicum* KCCM 80179 is safe for the target species when used as a nutritional additive to supplement the diet in appropriate amounts to cover the requirements, depending on the species, the physiological state of the animal, the performance level, the environmental conditions, the background amino acid composition of the unsupplemented diet and the status of some essential trace elements such as copper and zinc. This conclusion would also cover the use as sensory additive.

[31] Technical dossier/Section II/II.5.1.
[32] Technical dossier/Section II.4.1.3.

3.2.2. Safety for the consumer

The product under assessment is produced by fermentation using a strain *C. glutamicum* (KCCM 80179) which fulfils the QPS qualifications for production purposes (EFSA BIOHAZ Panel, 2019). Therefore, the FEEDAP Panel considers that no safety concerns would derive from the fermentation process. The additive contains 98.9% L-histidine HCl monohydrate and the amount of unidentified material is < 1%.

The FEEDAP, however, is aware that the intake of histamine, a metabolic by-product of histidine, through fish flesh following microbial spoilage is a serious concern for consumers (EFSA BIOHAZ Panel, 2011). Histamine poisoning from fish flesh has been called 'scombroid' poisoning because of the edible fish species (e.g. tuna, mackerel) more liable to histamine formation due to the high content of histidine in their flesh. However, bacterial spoilage may induce histamine formation also in other teleost species, including diverse freshwater species such as trout and carp (Křížek et al., 2011) and catfishes (Widjaja et al., 2010). Commission Regulation (EC) No 2073/2005 sets a maximum limit of 200 mg histamine/kg flesh for sea fishery products (raw fish at the point of the first sale) of fish species associated with a high amount of histidine, in particular fish species of the families: Scombridae, Clupeidae, Engraulidae, Coryfenidae, Pomatomidae *and* Scombresosidae.[33] The FEEDAP Panel considers that histamine food poisoning is mainly associated with the consumption of fish.

The Applicant provided two literature searches described and assessed in a previous opinion (EFSA FEEDAP Panel, 2019). The scarce evidence suggests that increasing dietary levels of histidine resulted in increased concentrations of histidine in edible tissues of food-producing animals. Although histidine is a precursor of histamine, the main factors influencing histamine formation in fish are storage time, temperature, pH, hygienic conditions (e.g. bacterial contamination) or starter cultures of fermented foods, which have been reviewed in previous publications (EFSA BIOHAZ Panel, 2011; FAO, 2013, 2018; Technical report EFSA, 2017).

As pointed out by FAO (2018), 'the available evidence highlights that under appropriate time × temperature control, and within the sensory shelf-life of the product, histamine development in Salmonidae to the levels that cause scombroid fish poisoning is unlikely to occur'.

In view of the above, the FEEDAP Panel considers that supplementing the diets of salmonids with histidine to cover the requirements is unlikely to result in the increase of histamine formation, provided that appropriate handling and storage of fish are ensured. Although there is no evidence from other aquaculture species, the Panel considers that the above conclusions can be extrapolated to other commonly farmed fish. For fish species associated with high levels of histidine in flesh,[34] the Panel notes that supplemental histidine may increase histidine concentration in fish flesh and the possibility to have higher levels of histamine in fish flesh following unproper storage. However, there are limits established for histamine to protect the consumer, in particular for Scombroid fish species.

In the absence of histamine poisoning records associated with raw mammal or poultry edible tissues and products, the FEEDAP Panel considers it unlikely that supplementation of feed with histidine to cover animal requirements will increase the risk of histamine poisoning upon consumption of such raw edible tissues and products from mammals and birds, provided that appropriate handling and storage are ensured.

3.2.2.1. Conclusions on the safety for the consumer

L-Histidine HCl monohydrate produced using *C. glutamicum* KCCM 80179 supplemented at levels appropriate for the requirements of target species and life stage is considered safe for the consumer.

3.2.3. Safety for the user

The analytical data on particle size distribution of the additive indicate that 15–20% (w/w) of the particles had a diameter < 125 μm, and no data on dusting potential were available.

The applicant provided an acute inhalation toxicity test, an eye irritation test, a skin irritation test and a dermal sensitisation test. Those studies tested an L-histidine monohydrochloride monohydrate produced by fermentation with a different production strain *C. glutamicum* KCCM 80172 (Batch H20170425, purity

[33] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ 22.12.2005, L 338/1 26 pp.

[34] According to the Commission Regulation (EC) No 2073/2005, in particular fish species of the families Scombridae, Clupeidae, Engraulidae, Coryfenidae, Pomatomidae *and* Scombresosidae.

99.48%, year 2017).[35] As the purity and physical characteristics of the test item are very similar to the ones of the product under assessment and the production process is the same, the FEEDAP Panel considers that the results of the toxicological studies performed with L-histidine monohydrochloride monohydrate originating from *C. glutamicum* KCCM 80172 can be used to support the safety for the user of L-histidine monochloride monohydrate produced with *C. glutamicum* KCCM 80179.

Consequently, L-Histidine HCl monohydrate produced using *C. glutamicum* KCCM 80179 is considered not irritant to skin, is a mildly irritant to eyes, and it is not a skin sensitiser. The additive does not pose a risk to users by inhalation.

3.2.4. Safety for the environment

The amino acid L-histidine is a physiological and natural component of animal and plant proteins. It is not excreted as such (but as urea/uric acid and carbon dioxide). The use of L-histidine in animal nutrition would not lead to any localised increase in its concentration in the environment. 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 L-histidine HCl monohydrate produced by *C. glutamicum* KCCM 80179 in animal nutrition is not expected to represent a risk to the environment.

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-histidine monohydrochloride monohydrate is well established in the scientific literature (NRC, 1994, 1998, 2011, 2012)

In general, the product L-histidine monohydrochloride monohydrate is considered as an efficacious source of the essential amino acid L-histidine for non-ruminant animal species. For the supplemental L-histidine to be as efficacious in ruminants as in non-ruminant species, it would require protection against degradation in the rumen.

As L-histidine is used in food as a flavouring compound, it is expected that it can provide a similar function in feed and no further demonstration of efficacy is necessary.

3.4. Post-marketing 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[36] and Good Manufacturing Practice.

4. Conclusions

The use of L-histidine monohydrochloride monohydrate produced by fermentation using *C. glutamicum* KCCM 80179 is safe for the target species when used as a nutritional additive to supplement the diet in appropriate amounts to cover the requirements, depending on the species, the physiological state of the animal, the performance level, the environmental conditions, the background amino acid composition of the unsupplemented diet and the status of some essential trace elements such as copper and zinc. This conclusion would also cover the use as a sensory additive.

L-Histidine HCl monohydrate produced using *C. glutamicum* KCCM80179 supplemented at levels appropriate for the requirements of species and life stage is considered safe for the consumer.

L-Histidine HCl monohydrate produced using *C. glutamicum* KCCM80179 is considered not irritant to skin, is mildly irritant to eyes, and it is not a skin sensitiser. The additive does not pose a risk to users by inhalation.

The use of L-histidine HCl monohydrate produced by *C. glutamicum* KCCM80179 in animal nutrition is not expected to represent a risk to the environment.

L-Histidine HCl monohydrate is considered an efficacious source of the essential amino acid L-histidine for non-ruminant animal species. For the supplemental L-histidine to be as efficacious in ruminants as in non-ruminant species, it would require protection against degradation in the rumen. It is also considered efficacious as a feed flavouring compound under the proposed conditions of use.

[35] FAD-2017-0052/Technical dossier/Section III/Annex III.3.07.

[36] 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.

Chronology

Date	Event
20/06/2018	Dossier received by EFSA: L-histidine monhohydrochloride monohydrate feed grade from *Corynebacterium glutamicum*. Submitted by CJ Europe GmbH.
06/07/2018	Reception mandate from the European Commission
18/09/2018	Application validated by EFSA – Start of the scientific assessment
22/10/2018	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 additive, safety for target species, safety for the consumer and safety for the user.*
18/12/2018	Comments received from Member States
06/12/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
20/12/2018	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
15/01/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended *Issues: Safety for the target species and for the consumer*
11/02/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
02/07/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

EFSA (European Food Safety Authority), 2005. Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on the safety and the bioavailability of product L-Histidine monohydrochloride monohydrate for salmonids. EFSA Journal 2005;3(4):195, 10 pp. https://doi.org/10.2903/j.efsa.2005.195

EFSA (European Food Safety Authority), 2006a. Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on L-histidine monohydrochloride monohydrate as feed additive for use in salmonids. EFSA Journal 2006;4(11):407, 5 pp. https://doi.org/10.2903/j.efsa.2006.407

EFSA (European Food Safety Authority), 2006b. Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on the Flavouring Group Evaluation 26 (FGE.26). Consideration of Amino acids from chemical group 34 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). EFSA Journal 2006;8(12):373, 48 pp. https://doi.org/10.2903/j.efsa.2006.373

EFSA (European Food Safety Authority), 2008a. Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on the Flavouring Group Evaluation 79 (FGE. 79). Consideration of amino acids and related substances evaluated by JECFA (63rd meeting). EFSA Journal 2008;6 (11):870, 46 pp. https://doi.org/10.2903/j.efsa.2008.870

EFSA (European Food Safety Authority), 2008b. Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) on amino acids from chemical group 34 Flavouring Group Evaluation 26, Revision 1. EFSA Journal 2008;6(8):790, 51 pp. https://doi.org/10.2903/j.efsa.2008.790

EFSA (European Food Safety Authority), 2008c. Guidance for assessing the safety of feed additives for the environment. EFSA Journal 2008;6(10):842, 28 pp. https://doi.org/10.2903/j.efsa.2008.842

EFSA (European Food Safety Authority), 2017. Assessment of the incidents of histamine intoxication in some EU countries. EFSA supporting publication 2017:EN-1301, 37 pp. https://doi.org/10.2903/sp.efsa.2017.en-1301

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2011. Scientific opinion on risk based control of biogenic amine formation in fermented foods. EFSA Journal 2011;9(10):2393, 93 pp. https://doi.org/10.2903/j.efsa.2011.2393. Available online: www.efsa.europa.eu/efsajournal

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordonez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, Hilbert F, Lindqvist R, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Cocconcelli PS, Fernandez Escamez PS, Maradona MP, Querol A, Suarez JE, Sundh I, Vlak J, Barizzone F, Correia S and Herman L, 2019. Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 9: suitability of taxonomic units notified to EFSA until September 2019. EFSA Journal 2019;17(1):5555, 46 pp. https://doi.org/10.2903/j.efsa.2019.5555

EFSA CEF Panel (Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2011. Scientific Opinion on Flavouring Group Evaluation 96 (FGE.96): Consideration of 88 flavouring substances considered by EFSA for which EU production volumes/anticipated production volumes have been submitted on request by DG SANCO. Addendum to FGE. 51, 52, 53, 54, 56, 58, 61, 62, 63, 64, 68, 69, 70, 71, 73, 76, 77, 79, 80, 83, 84, 85 and 87. EFSA Journal 2011;9(12):1924, 60 pp. https://doi.org/10.2903/j.efsa.2011.1924

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances Used in Animal Feed), 2010. Scientific opinion on the use of feed additives authorised/applied for use in feed when supplied via water. EFSA Journal 2010;8(12):1956, 9 pp. https://doi.org/10.2903/j.efsa.2010.1956. Available online: www.efsa.europa.eu/efsajournal

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), 2014. Scientific Opinion on the safety and efficacy of the use of amino acids (chemical group 34) when used as flavourings for all animal species. EFSA Journal 2014;12(5):3670, 19 pp. https://doi.org/10.2903/j.efsa.2014.3670

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, 2017a. 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), 2017b. 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, 2017b. 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, 2017c. 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), 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, Glandorf B, Herman L, Karenlampi S, Aguilera J, Anguita M, Brozzi R and Galobart J, 2018a. 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), 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, 2018b. Guidance on the assessment of the efficacy of feed additives. EFSA Journal 2018;16(5):5274, 25 pp. https://doi.org/10.2903/j.efsa.2018.5274

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, Glandorf B, Herman L, Mantovani A, Saarela M, Wallace RJ, Anguita M, Tarrés-Call J and Ramos F, 2019. Safety and efficacy of L-histidine monohydrochloride monohydrate produced using *Corynebacterium glutamicum* KCCM80172 for all animal species. EFSA Journal 2019;17(7):5783, 18 pp. https://doi.org/10.2903/j.efsa.2019.5783

European Pharmacopeia (PhEur), 2017. L-Histidine monohydrochloride monohydrate, Monograph 01/2017:0910, 9th Edition. Strasbourg, France. Council of Europe (COE) – European Directorate for the Quality of Medicines.

FAO (Food and Agriculture Organization of the United Nations), 2013. Joint FAO/WHO Expert Meeting on the Public Health Risks of Histamine and Other Biogenic Amines from Fish and Fishery Products. Available online: http://www.fao.org/fileadmin/user_upload/agns/news_events/Histamine_Final_Report.pdf

FAO (Food and Agriculture Organization of the United Nations), 2018. Joint FAO/WHO Literature review. Available online: http://www.fao.org/3/CA1207EN/ca1207en.pdf

Křížek M, Vácha F, Vejsada P and Pelikánová T, 2011. Formation of biogenic amines in fillets and minced flesh of three freshwater fish species stored at 3 & #xB0;C and 15 & #xB0;C. Acta Veterinaria Brno, 80, 365–372. https://doi.org/10.2754/avb201180040365

NRC (National Research Council), 1994. Nutrient requirements of poultry. 9th revised edn. The National Academies Press, Washington, DC, 176 pp.

NRC (National Research Council), 1998. Nutrient Requirements of Swine. 10th revised edn. Washington, DC: The National Academies Press. https://doi.org/10.17226/6016

NRC (National Research Council), 2011. Nutrient requirements of fish and shrimp. The National Academies Press, Washington, DC, USA, 376 pp.

NRC (National Research Council), 2012. Nutrient requirements of swine. 11th revised edn. National Academy Press, Washington, DC, 178 pp.

Widjaja WP, As AA, Bakar FA, Saari NB, Ishak ZB and Harith HH, 2010. Amino acids and biogenic amines determination in *Mystus nemurus*. Journal of Food Processing and Preservation, 35, 342–348. https://doi.org/10.1111/j.1745-4549.2009.00473.x

Abbreviations

AFC	Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
CAS	Chemical Abstracts Service
CFU	colony forming unit
Co-planar PCB	coplanar dioxin-like polychlorinated biphenyl
CV	coefficient of variation
DM	dry matter
EINECS	European Inventory of Existing Commercial Chemical Substances
EURL	European Union Reference Laboratory
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
FLAVIS	EU Flavour Information System
HPLC-UV	high-performance liquid chromatography coupled with ultraviolet detection
IEC-VIS	ion exchange chromatography coupled with photometric detection
IUPAC	International Union of Pure and Applied Chemistry
KCCM	Korean Culture Centre of Microorganisms
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
OECD	Organisation for Economic Co-operation and Development
PCDD	polychlorinated dibenzodioxin
PCDF	polychlorinated dibenzofuran
QPS	qualified presumption of safety
RH	relative humidity
Rrec	recovery rate
RSDip	relative standard deviation for intermediate precision
RSDr	relative standard deviation for repeatability
WHO	World Health Organization
VKM	Norwegian Scientific Committee for Food Safety

Annex A – Evaluation report of the analytical methods submitted in connection with the application for authorisation of L-histidine monohydrochloride monohydrate produced by fermentation with *Corynebacterium glutamicum* KCCM 80179

In the current application, authorisation is sought under Article 4 for L-histidine monohydrochloride monohydrate produced by fermentation with *Corynebacterium glutamicum* KCCM 80179, 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-histidine monohydrochloride monohydrate has a minimum purity (mass fraction) of 98%. The feed additive is intended to be added directly into feedingstuffs or through premixtures. However, the Applicant did not propose any minimum or maximum content of L-histidine monohydrochloride monohydrate in feedingstuffs.

For the quantification of L-histidine monohydrochloride monohydrate in the feed additive, the Applicant submitted an in-house validated analytical method based on reversed-phase high-performance liquid chromatography coupled with ultraviolet detection (HPLC-UV). The Applicant reported in the frame of the validation study a relative standard deviation for repeatability (RSDr) and intermediate precision (RSDip) ranging from 0.1 to 2.1% and a recovery rate (Rrec) ranging from 98 to 102%. Furthermore, the EURL calculated a RSDr of 0.3% from further analytical data presented by the Applicant.

For the quantification of L-histidine in premixtures and feedingstuffs, the EURL identified the ring-trial validated Community method (Commission Regulation (EC) No 152/2009) based on ion exchange chromatography coupled with photometric detection (IEC-VIS). This method, designed 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 histidine: RSDr ranging from 2.4 to 7.0% and RSDR ranging from 13 to 23%.

Based on the performance characteristics available, the EURL recommends for official control the in-house validated method based on HPLC-UV to quantify L-histidine monohydrochloride monohydrate in the feed additive and the ring-trial validated Community method based on IEC-VIS to quantify histidine 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.