SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 2 July 2019

doi: 10.2903/j.efsa.2019.5783

Safety and efficacy of L-histidine monohydrochloride monohydrate produced using *Corynebacterium glutamicum* KCCM 80172 for all animal species

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Maryline Kouba, Mojca Kos Durjava, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Lucio Costa, Francesco Cubadda, Noël Dierick, Gerhard Flachowsky, Boet Glandorf, Lieve Herman, Alberto Mantovani, Maria Saarela, Kettil Svensson, Luca Tosti, Robert John Wallace, Montserrat Anguita, Jordi Tarrés-Call and Fernando Ramos

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 80172 when used as a nutritional additive in feed and water for drinking for all animal species. The production strain is genetically modified. The production strain and its recombinant DNA were not detected in the final product. L-Histidine HCl monohydrate manufactured by fermentation using *C. glutamicum* KCCM 80172 does not give rise to any safety concern regarding the genetic modification. The use of L-histidine HCl monohydrate produced by fermentation using *C. glutamicum* KCCM 80172 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. L-Histidine HCl monohydrate produced using *C. glutamicum* KCCM 80172 supplemented at levels appropriate for the requirements of the target species is considered safe for the consumer. L-Histidine HCl monohydrate produced using *C. glutamicum* KCCM 80172 is 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. The use of L-histidine HCl monohydrate produced by *C. glutamicum* KCCM 80172 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.

Keywords: nutritional additive, amino acid, L-histidine monohydrochloride monohydrate, *Corynebacterium glutamicum* KCCM 80172, feed additive, safety

Requestor: European Commission

Question number: EFSA-Q-2018-00438

Correspondence: feedap@efsa.europa.eu

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

Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output: Jaume Galobart, Niovi Kordali, Paola Manini, Elisa Pettenati, Konstantinos Sofianidis.

Legal notice: Relevant information or parts of this scientific output have been blackened in accordance with the European Commission decision on the confidentiality requests formulated by the applicant. The full output has been shared with the European Commission, EU Member States and the applicant.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), BampidisV, 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, Svensson K, Tosti L, Wallace RJ, Anguita M, Tarrés-Call J and Ramos F, 2019. Scientific Opinion on the safety and efficacy of L-histidine monohydrochloride monohydrate produced using *Corynebacterium glutamicum* KCCM 80172 for all animal species. EFSA Journal 2019;17(7):5783, 20 pp. https://doi.org/10.2903/j.efsa.2019.5783

ISSN: 1831-4732

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

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 when used as a feed additive for all animal species (category: nutritional additives; functional group: amino acids, their salts and analogues).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). The particulars and documents in support of the application were considered valid by EFSA as of 3 July 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 80172, 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 genetically modified strain of *C. glutamicum* (KCCM 80172) 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 content of 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/FLAVIS No 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 EFSA FEEDAP Panel (2014) assessed the safety and efficacy of L-histidine as a feed flavouring. 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 Pharmacopeia (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] CJ Europe GmbH, Ober der Roeth 4, 65824 Schwalbach am Taunus, 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 monohydrochloride monohydrate produced by fermentation with *C. glutamicum* KCCM 80172 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 80172 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 80172 is in line with the principles laid down in Regulation (EC) No 429/2008 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 by a genetically modified strain of *C. glutamicum* (KCCM 80172). 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).

3.1. Characterisation

3.1.1. Characterisation of the production organism

The additive is produced by a genetically modified strain of *C. glutamicum,* which is deposited [redacted] The identity of the strain was confirmed as *C. glutamicum* by [redacted]

The susceptibility of the recipient strain and the production strain was tested against [redacted] The minimum inhibitory concentration (MIC) values were equal or below the cut-off values established for that species.

3.1.1.1. Information relating to the genetically modified microorganism

Characteristics of the recipient or parental microorganism

The recipient strain is [redacted]

[9] FEED dossier reference: FAD-2018-0013.

[10] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2018-0013_histidinehcl.pdf

[redacted]

Characterisation of the donor organism

All the genes used in the genetic modification are derived from [redacted]

Description of the genetic modification process

The recipient strain [redacted]

3.1.2. Manufacturing process

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

The applicant stated that [redacted]

3.1.3. Characterisation of the active substance/additive

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.

[redacted]

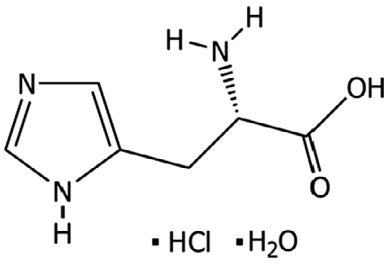

Figure 1: Molecular structure of L-histidine monohydrochloride monohydrate

The additive is specified to contain ≥ 98% L-histidine HCl monohydrate, ≤ 10% moisture and ≤ 1% ash.[17]

Analysis of five batches showed an average histidine content of 73.6% 'as is' (range 73.5–73.6), a chloride content of 16.7%, a moisture content of 8.6% and an ash content of 0.15%.[18] Other components analysed were phosphate 0.2% (0.01–0.03%) and ammonium (detected in only three batches at 0.01%).[19] The sum of the identified material on 'as is' basis was 99% (99.0–99.1%).

The specific optical rotation of the additive was measured in five batches of the final product and the average was +10.5°(range +10.4 to +10.6°).[20] It is within the range of the European Pharmacopoeia (+9.2 to +10.6°) and confirms the L-stereoisomer of histidine.

3.1.3.1. Impurities

Five batches of the additive were analysed for undesirable substances. As regards heavy metals, lead, chromium, copper, nickel and zinc were below the limit of detection (LOD), cadmium ranged from < LOD to 0.3 mg/kg and mercury ranged from 0.014 to 0.041 mg/kg. Arsenic was also below the limit of detection.[21] The same batches were analysed for dioxins (polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F)) and sum of PCDD/F and dioxin-like polychlorinated biphenyls (PCBs) and the values were 0.16 ng TEQ-WHO/kg and 0.30 ng TEQ-WHO/kg, respectively (in all batches).[22]

In reference to the microbial contamination, five batches were analysed. The determination of bacterial counts of *Salmonella*, were negative. Yeasts, filamentous fungi, *E. coli* and total bacterial count gave values < 10^3 colony forming units (CFU)/g.[22]

Regarding the mycotoxin content, analytical data of three batches showed levels of aflatoxins (B1, B2, G1, G2), ochratoxin A, zearalenone and deoxynivalenol below the limit of quantification (LOQ).[23]

No viable cells of the production strain were detected in three batches of the final product [redacted]

The absence of recombinant DNA from the production strain was confirmed in three batches of L-histidine.[redacted]

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.[26]

[17] Technical dossier/Section II.1.3.
[18] Technical dossier/Section II/Annex II.1.4. Histidine analysed by HPLC with C18 column using potassium phosphate buffer as mobile phase.
[19] Technical dossier/Section II/Table II.1.4a.
[20] Technical dossier/Section II/Annex II.1.2.
[21] Technical dossier/Section II/Annex II.1.6. LOD (in mg/kg) were 1 for lead and arsenic; 0.2 for chromium, cooper, nickel and zinc; and 0.1 for cadmium.
[22] Technical dossier/Section II/Annex II.1.6.
[23] Technical dossier/Section II/Annex II.1.5. LOQ (in μg/kg) were 1 for aflatoxins and ochratoxin A; < 10 for zearalenone and < 20 for deoxynivalenol.
[redacted]
[26] Technical dossier/Section II.2.2.1 and Annex II.3.1.

The dusting potential was analysed (Stauber–Heubach method) in three batches and ranged from 0.05 to 0.17 g/m^3.[27] Concerning the particle size, three batches were analysed by laser diffraction.[28] The fractions below 100, 50 and 10 μm diameter (v/v) were 0.5–2.3%, 0.0–0.9% and 0.00–0.01%, respectively.

3.1.3.3. Stability and homogeneity

The shelf life of the additive (three batches) was studied when stored at 25°C and at 40°C in sealed brown glass bottles for 6 months. No losses were observed.[29]

The stability of the additive (three batches) in a vitamin/mineral premixture (containing 40,000 mg choline chloride/kg) supplemented with 5% L-histidine HCl monohydrate was studied when stored in sealed containers at 25°C for 6 months. Losses ranged from 4 to 6%.[30]

The stability of the additive (three batches) was studied in mash feed for chicken for fattening (basal diet consisted of maize, soy extraction meal, wheat and vegetable fatty acids) when supplemented at 0.4%. The samples were kept at 25°C in aluminium vacuum bags for 3 months. Losses ranged from 0 to 2%.[31] No information was provided on the stability of the additive during feed processing.

The stability of the additive (three batches) was studied when solved in water for drinking at a concentration of 0.05% and stored at 25°C and 40°C for 48 h. No losses were detected.[32]

The capacity of one batch of the additive to distribute homogeneously in the premix described above (inclusion rate 5%) was studied in 10 subsamples. The coefficient of variation (CV) was 5%.[33]

The capacity of one batch of the additive to distribute homogeneously in a pelleted feed for chickens for fattening (inclusion rate 0.2%) was studied in 10 subsamples. The CV was 5%.[34]

The capacity of one batch of the additive to distribute homogeneously in a mash feed for fish (inclusion rate 0.4%) was studied in 10 subsamples. The CV was 6%.[35]

3.1.3.4. Physico-chemical incompatibilities in feed

No physico-chemical incompatibilities in feed are expected with other additives, medicinal products or other feed materials.

3.1.4. Conditions of use

According to the applicant, the additive can be added directly in a compound feed, through complementary feed or through premixtures or water for drinking[36] 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.[37]

3.2. Safety

3.2.1. Safety aspects of the production organism

The recipient strain belongs to a species, [redacted] that is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment when used for production purposes (EFSA, 2007; EFSA BIOHAZ Panel, 2019a).

The production strain *C. glutamicum* KCCM 80172 [redacted]

[27] Technical dossier/Section II/Annex II.1.8.
[28] Technical dossier/Section II/Annex 2.1.7.
[29] Technical dossier/Section II/Annex II.4.1 and supplementary information September 2018/02 SIN CJE.
[30] Technical dossier/Section II/Annex II.4.3.
[31] Technical dossier/Section II/Annex II.4.4 and supplementary information September 2018/02 SIN CJE.
[32] Technical dossier/Section II/Annex II.4.2.
[33] Technical dossier/Section II/Annex II.4.5.
[34] Technical dossier/Section II/Annex II.4.7.
[35] Technical dossier/Section II/Annex II.4.6.
[36] Technical dossier/Section II.4.1.3.
[37] Technical dossier/Section II/II.5.1.

The identity of the strain has been established, the strain is susceptible to the relevant antimicrobials and there are no safety concerns related to the genetic modification, therefore, the production strain is presumed safe.

3.2.2. Safety for the target species

3.2.2.1. Absorption, distribution, metabolism and excretion of histidine

L-Histidine released during enzymatic digestion is rapidly absorbed via different sodium-dependent and -independent transport systems in the brush border membrane of the small intestine (Munck and Munck, 1994; Massey et al., 1998).

Once absorbed, L-histidine is used in the body for tissue protein synthesis and for the formation of free dipeptides, e.g. carnosine, anserine and balenine. Only carnosine is fully effective as a precursor of L-histidine, while the methylated dipeptides have no histidine sparing activity.

L-Histidine is a glucogenic amino acid; its main catabolism in the inner compartment (intermediary system) proceeds via deamination to urocanate, 4-imidazolone-5-propionate and *N*-formiminoglutamate. The formimino group transfer to tetrahydrofolate forms glutamate which is then converted to α-ketoglutarate in the tricarboxylic acid cycle. A minor part of 4-imidazolone-5-propionate can be oxidised to hydantoin propionate and excreted as such in the urine.

L-Histidine is also a precursor for histamine, produced by the enzyme histidine decarboxylase, present in, e.g. brain and gut tissues and in gut microbiota. Histamine mediates a variety of cellular actions such as gastric acid secretion, vasodilation, as a mediator of many inflammatory and allergic reactions and as a neurotransmitter in the brain.

3.2.2.2. Animal requirements

L-Histidine, or 2-Amino-3-(1*H*-imidazol-4-yl) propanoic acid, is a (conditionally) essential amino acid. Adult men and animals, in contrast to young children and young animals, can partly meet their needs for L-histidine by endogenous synthesis. The enzyme catalysing the final step in the biosynthesis of L-histidine, histidinol dehydrogenase, has indeed been described in tissues of e.g. pig, mouse, fowl, duck and cattle (Kriengsinyos et al., 2002; Onodera, 2003; WHO, 2007, VKM, 2016). To date the indispensability of L-histidine in healthy adult species remains unresolved. Histidine is considered essential in dairy cows (NRC, 2001).

It is generally considered that histidine is different from other essential amino acids in that substantial quantities exist in haemoglobin and in the form of free imidazole derivatives (dipeptides) in animal muscle tissues such as carnosine (β-alanyl histidine), anserine (β-alanyl-1-methyl histidine) and balenine/ophidine (β-alanyl-3-methyl histidine) leading to the difficulty of establishing conclusive indispensability. Furthermore, these dipeptides have anti-inflammatory and osmolytic/antioxidant effects; it is believed that they interact with oxygen radicals and lipid peroxidation products to prevent membrane damage (Buttery and D'Mello, 1994; Onodera, 2003).

Depending on genetics, sex and physiological stage, L-histidine requirements for pigs range, according to the National Research Council (NRC, 2012), from 0.20% (gestating sows) to 0.50% (post-weaned piglets). In poultry, L-histidine requirements range between 0.15 and 0.25% (layers), between 0.25 and 0.35% (chickens for fattening) and between 0.25 and 0.60% (turkeys) (NRC, 1994). The requirement for fish for L-histidine regarding the different fish species were set by NRC (2011) between 0.5 to 1.0% in feed. Estimated requirements for rat range from 0.08% (maintenance) to 0.28% (growth and female reproduction); those for mice 0.2% and those for guinea pig 0.36% (NRC, 1995). The requirements for rabbits are 0.3% for growth (NRC, 1997). In the intensive farming of Atlantic salmon (*Salmo salar* L.), the occurrence of production-related disorders such as cataract poses a potential threat for fish welfare. The cataract-mitigating ability of L-histidine has been attributed to the occurrence of the histidine imidazole *N*-acetyl-histidine (NAH) that is synthesised in the lens (Remo et al., 2014; Taylor et al., 2015). The dietary L-histidine requirement to minimise this risk of cataract development in intensive farming of diploid and triploid Atlantic salmon is estimated to be 1.4–1.7% in the diet (Remo et al., 2014; Taylor et al., 2015).

3.2.2.3. L-Histidine content in feedingstuffs

Cereals contain between 0.25 and 0.50% histidine (lowest values for barley and maize), legume seeds (soybean, lupin, pea, beans, lentils) between 0.5 and 1.0% histidine, and protein-rich extracted feed materials, concentrates and isolates (e.g. soybean, potato) and animal derived products (e.g. blood and fish meal) between 1.0 and 5.1% histidine (except whey: 0.25%) (National Research Council (NRC),

2012). Low crude protein (CP) diets for pigs and poultry, especially when they are based on maize and barley, may need additional histidine. In maize/soybean diets, L-histidine may become the sixth limiting amino acid, after L-lysine, DL-methionine, L-threonine, L-tryptophan and L-valine.

L-Histidine has been implicated as a limiting amino acid in cattle, particularly in dairy cows receiving grass silage supplemented with barley or oats (Schwab et al., 2005). Feather meal as a protein supplement also has a histidine content less than half of other protein supplements (Schwab et al., 2005). Rumen bacterial protein also has a lower histidine content than cereals and most protein supplements (O'Connor et al., 1993).

Literature data and current knowledge indicate that L-histidine supplementation in complete feeds varies from 0.05% (laying hens and gestating sows) to 0.35% (chickens for fattening and early weaned piglets). As already reported above, in order to avoid nutrition related cataracts (diets low in blood meal and/or fish meal), higher amounts of histidine (up to twice the requirements) in diets for Atlantic salmon are normally present.

3.2.2.4. Experimental data in animals

Safety data in the target species are normally not required for highly purified amino acids. However, the FEEDAP Panel considers that excesses of L-histidine in the diet are not well tolerated, most probably due to (i) amino acid imbalances, (ii) interactions with trace elements (e.g. Cu, Zn) in the gut, and (iii) microbial production of histamine in the gut/rumen (Ahrens, 1967; Aoyama and Kato, 2000; Aoyama et al., 2001; Xu et al., 2003; Glover and Wood, 2008; Golder et al., 2012; Khan and Abidi, 2014). Brain histidine and histamine are involved in the regulation of feed intake. Reduction in feed intake in response to excess dietary histidine intake may be probably triggered by the elevation of brain histidine and histamine levels (Sheiner et al., 1985).

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. Nearly all the studies found referred to requirements for L-histidine and not to excesses nor tolerance for L-histidine. The following paragraphs include data from references retrieved in this literature search and other relevant references.

As reviewed by Garlick (2004), high dietary histidine levels have been shown to result in potentially serious adverse effects in both animals (e.g. hyperlipidemia, hypercholesterolemia, enlarged liver) and humans (e.g. increase in urinary zinc, headache, weakness). In fact, there is evidence from studies in experimental animals and humans that intakes of high levels of histidine can alter copper and zinc metabolism and cause deficiencies of the free forms of these metal ions due to increased excretion (VKM, 2016; National Research Council (NRC), 2011).

A long-term toxicity and carcinogenicity study of L-histidine was carried out in rats (Ikezaki et al., 1996). Groups of 50 males and 50 female rats were given L-histidine HCl monohydrate in their diet at concentrations of 0 (control), 1.25 and 2.5% for 104 weeks. The 2.5% group showed significant depression of body weight gain in comparison with the control group. There was no significant increase in the incidence of malignant tumours in the exposed groups compared to the control group. From the study, it was concluded that, under the experimental conditions, L-histidine was not carcinogenic in rats.

Studies in target species

A study was carried out with young pigs (10–20 kg body weight (BW)) under a feeding regimen of histidine deficient diets (19% protein, 0.22% histidine) (Izquierdo et al., 1988); the diet was then supplemented with 0.06, 0.12 or 0.18% L-histidine and offered on an *ad libitum* basis for 3 weeks. It was observed impaired growth and feed/gain ratio in pigs fed diet exceeding 0.34% total histidine (corresponding to 0.12% histidine supplementation). However, Wessels et al. (2016) reported no differences in performances in a piglet study (8–25 kg BW) in which animals were fed a low protein diet (14% protein, 0.23% histidine) based on wheat, maize and barley and supplemented with L-histidine to contain 0.26–0.38% total histidine for 6 weeks. These authors noted also that the diets administered in the study by Izquierdo et al. (1988) contained large quantities of maize starch and feather meal and that the dietary concentration of lysine was not analysed.

In poultry, the addition of 1% L-histidine to a diet (zinc deficient or not) for 3 weeks, did not negatively affect the growth (Finch, 1971).

The ruminal metabolism of L-histidine has not been well investigated. However, findings support that histamine, produced through ruminal microbial decarboxylation of histidine (originating from free and protein bound histidine in the feed), might be associated with sub-acute acidosis in the rumen (Ahrens, 1967; Aschenbach and Gäbel, 2000; D'Mello, 2003; Golder et al., 2012).

Dietary histidine requirement of Indian catfish (*Heteropneustes fossilis*) fry was found to be 1.6% in a trial that lasted 12 weeks; depressed growth and poor feed conversion were more frequently noted on the diets below and beyond this level (Khan and Abidi, 2014). An 8-week feeding trial was conducted by Ahmed (2013) to determine the dietary histidine requirement of adult *H. fossilis*. Maximum live weight gain occurred at 0.55% dietary histidine level. Higher levels (up to 0.75%) decreased feed intake and growth. In a study with grass carp fed diets containing between 0.5 and 1.8% total histidine for 10 weeks, best performance was noted at a histidine concentration of 1.05% in the diet; higher amount negatively affected growth and feed to gain (F/G) ratio (Gao et al., 2016). Michelato et al. (2017) reported that when feeding juvenile tilapia, diets containing between 0.4 and 1.15% total histidine on dry matter (DM) (92%) for 14 weeks, optimal performances were obtained with 0.82% histidine; higher amounts negatively affected growth and F/G ratio. No effects were noted on the biochemical and haematological parameters investigated.

Although there is limited evidence from the published literature on the effects of supplementing histidine levels above the requirements, the FEEDAP Panel considers that 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, 2010), identified risks of nutritional imbalances and hygienic concerns for amino acids when administered in water for drinking.

3.2.2.5. Conclusions on the safety for the target species

The use of L-histidine monohydrochloride monohydrate produced by fermentation using *C. glutamicum* KCCM 80172 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.

3.2.3. Safety for the consumer

The product under assessment is produced by fermentation using a strain *C. glutamicum* (KCCM 80172) which fulfils the QPS qualifications for production purposes (EFSA BIOHAZ Panel, 2019a). 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 Panel, 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.[38]

Histamine is a biogenic amine that can be synthesised endogenously from histidine by an L-histidine decarboxylase. Histamine can be metabolised either extracellularly by a diamino oxidase (DAO) present in the gut mucosa, or intracellularly by a histamine-*N*-methyltranferase (HNMT). The existence of both pathways has been confirmed in rainbow trout (Shiozaki et al., 2003). The resulting products act as regulators of the respective enzymes. An impaired histamine degradation may cause symptoms similar to the ones seen in allergic reactions (Maintz and Novak, 2007). As regards the exogenous production of histamine, many different bacterial species are known to possess histidine decarboxylase and have

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

the ability to convert free histidine into histamine (An and Ben-Bigirey, 1998; Özogul and Özogul, 2006; Kanki et al., 2004; Emborg et al., 2006; Dalgaard et al., 2008).

The applicant provided two literature reviews to address the potential for deposition in edible tissues or products: one on deposition of histidine in target species [Histidine AND diet AND (different animal species…) AND concentration AND (muscle OR flesh OR tissue)] which resulted in 55 hits; and one specifically on histamine in fish [Histamine AND concentration AND fish AND (muscle OR flesh OR tissue)] which resulted in 60 hits. Several platform databases (Livivo, Toxnet, Ovid, Web of Knowledge, Google Scholar) were searched. Finally, 31 and 19 papers, respectively, were selected. From those, only the scientific papers considered as relevant by the FEEDAP Panel are quoted below.

3.2.3.1. Histidine intake and histidine deposition in animal tissues or products

In weaned rats fed for 2 weeks 0.1% (deficient), 0.3% or 0.8% (overdosing) histidine in the diet, the histidine content in muscle increased linearly with the dietary intake, average being 18.4, 59.4 and 101.3 mg/kg, respectively. The histidine increase in muscle was more marked than in kidney and comparable to that in brain (Lee et al., 1981).

In dairy cows, the milk protein and the mammary uptake of histidine from plasma was not significantly influenced by abomasal or duodenal infusions with up to 6.5 g histidine/day for 14 days (Korhonen et al., 2000; Vanhatalo et al., 1999).

As regards chicken for fattening, Kopeć et al. (2013) observed that the supplementation of L-histidine 0.18% in diet for 6 weeks (total histidine in diet 0.76% and 0.68% in starter and grower diet, respectively) led to a significant (+30%) increase of the histidine dipeptide carnosine in breast muscle compared to unsupplemented controls (total histidine content in diet 0.57% and 0.53% in starter and grower diet, respectively). The same supplementation level (0.18%) did not cause an increase of histidine itself or carnosine in the breast muscle of turkeys for fattening fed the experimental diets for 9 weeks: the total histidine content in diets during the feeding period ranged 0.58–0.49% in controls and 0.78–0.63% in the supplemented group (Kopeć et al., 2016).

In salmon, the use of high histidine levels (background plus supplementation: 1.4%, 1.6% or 1.8%) for 13 weeks in order to prevent cataracts led to a dose- and time-related increase of free histidine (plus anserine) in flesh (up to 4 μmol/g at top dose at 13 weeks, corresponding to 620 mg histidine/kg flesh). On the other hand, the deposition was very low, if any, and not significant at lower histidine levels (1.0% or 1.2%) hinting to a mechanism for deposition characterised by a threshold and a steep dose-response (Remo et al., 2014). Also in another trial on cataract prevention in salmon, free histidine content in muscle raised sharply from 0.13 μmol/g (in lower histidine level of 1% in feed, corresponding to 20 mg histidine/kg muscle) to 0.70 μmol/g (in middle histidine level of 1.3% in feed, corresponding to 108 mg histidine/kg muscle) and 1.9 μmol/g (higher histidine level of 1.7% in feed, corresponding to 295 mg histidine/kg muscle) (Waagbø et al., 2010).

In juvenile yellowtail (*Seriola quinqueradiata*, used as an edible finfish in the Pacific area) supplemented for 6 weeks with crystalline histidine (2.5%, background histidine content 1.6%), the deposition of histidine in muscle was significantly higher, by a 1.5-factor, than in unsupplemented control group (63 vs 42 μmol/kg) (Ogata, 2002).

3.2.3.2. Histamine concentration in animal tissues or products in relation to dietary histidine

As review by the EFSA BIOHAZ Panel (2011), the histamine concentration in raw meat of mammals and birds is considered lower compared to fish flesh. Histamine poisoning is also related to fermented food of animal origin. The threshold for adverse health effects (acute reference dose) is 50 mg histamine per consumption for healthy individuals, and below detectable limits for individuals with histamine intolerance.

Limited data are available on the relationship between dietary histidine and histamine concentration in animal tissues.

In the study in rats of Lee et al. (1981) described above, histamine content in muscle increased by twofold in the two higher histidine dietary levels compared to the lower one, averaging 4.3 and 4.5 μg/kg vs. 2.5 μg/kg, respectively. Levels of histamine in muscles were much higher (by 1–2 magnitude orders) than in brain or kidney at all dietary levels; however, contrary to histidine, the histamine concentration in muscle increased until reaching an apparent plateau (around 4.5 μg/kg) from the intermediate to high dietary level.

The FEEDAP Panel considers that histamine food poisoning is mainly associated with the consumption of fish. Regarding fish species, none of the studies mentioned above analysed histamine

content in edible tissues or products (Remo et al., 2014; Ogata, 2002). Nevertheless, it is known that trout may retain histamine (mainly in muscle and liver) given orally by intragastric administration (Shiozaki et al., 2003). Farmed shrimp *Neomys japonica* shows increased body retention of histamine upon direct administration of 2% histamine or more in feed; the same feed levels of histamine, however, did not increase tissue retention in *Neomysis awatschensis* (Yang et al., 2010).

The scarce evidence seems to suggest that supplementation of fish with histidine increases histidine deposition in fish flesh. The data reviewed by the Food and Agriculture Organization (FAO) of the United Nations indicate that the levels of histidine in salmonids are comparatively lower than in other wild sea fish species (FAO, 2013, 2018). Compared with scombroid fish which have free histidine levels ranging from approximately 5,000 to 20,000 mg/kg flesh, most species in the Salmonidae family have less than 1,000 mg/kg histidine (FAO, 2013). The Panel notes that it is unclear if salmonids exposed to dietary concentrations of histidine to prevent cataract were used in these studies. Thus, most members of the Salmonidae family have somewhere between 10 and 200 times less free histidine than scombroid fish. The FEEDAP Panel assumes than the levels of histidine reported in the FAO report would reflect histidine supplementation of salmonids under current aquaculture conditions.

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,[39] 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 improper 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.

3.2.3.3. Conclusions on the safety for the consumer

L-Histidine HCl monohydrate produced using *C. glutamicum* KCCM 80172 supplemented at levels appropriate for the requirements of the target species is considered safe for the consumer.

3.2.4. Safety for the user

3.2.4.1. Effects on the respiratory system

The analytical data on particle size distribution of the additive indicate that only 0.5–2.3% of the particles had a diameter < 100 μm, and the dusting potential was up to 0.17 g/m^3.

In an acute inhalation toxicity test performed in accordance with OECD Guideline 403, 10 RccHan™: WIST strain rats (5 males and 5 females) were exposed (nose only exposure system) to a dust atmosphere containing 5 g of the additive/m^3 for 4 h and were subsequently observed for 2 weeks.[40] The signs observed after exposure (decreased respiratory rate, hunched posture, piloerection, wet fur) had disappeared one day after exposure. No relevant effects were seen on body weight. No mortality occurred.

Although transient adverse effects were observed at an exposure 29X, the maximum dusting potential measured in the additive, they do not indicate a hazard by inhalation when handling the

[39] According to the Commission Regulation (EC) No 2073/2005, in particular fish species of the families Scombridae, Clupeidae, Engraulidae, Coryfenidae, Pomatomidae *and* Scombresosidae.
[40] Technical dossier/Section III/Annex III.3.3.

additive. The inhalation exposure will be very limited and taking into account the limited inhalation toxicity of histidine, the risk is considered low.

3.2.4.2. Effects on the eyes and skin

An *in vitro* skin irritation study (cytotoxicity in reconstructed human epidermal cultures)[41] was carried out according to OECD guideline 439 and method B.46 as described in Commission Regulation (EC) No 761/2009.[42] After 15 min exposure to approximately 10 mg of the additive (26.3 mg/cm^2) and 48-h incubation, the viability of the exposed tissues was 94%. The results indicate that the additive under assessment is not irritant to skin.

In an acute eye irritation study using the bovine corneal opacity and permeability assay in accordance with OECD 437 and method B47 of Commission Regulation (EC) No 440/2008,[43] a concentration of 20% w/v of the product under assessment in saline solution was applied for 4 h to bovine corneas.[44] The controls performed as expected. Although the *in vitro* irritancy score obtained in the test (5.6) did not allow a prediction on the eye irritation of the test substance, according to the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM, 2006) irritation classification scheme, it corresponds to a mildly irritant substance.

A local lymph node assay (LLNA) was performed using female CBA/Ca mice, in accordance with OECD Guideline 429.[45] No proliferation response was elicited by the treatment. No clinical signs, mortality or changes in body weight were observed. The product under assessment is not considered a skin sensitiser

3.2.4.3. Conclusions on the safety for the user

L-Histidine HCl monohydrate produced using *C. glutamicum* KCCM 80172 is 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.

3.2.5. 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 80172 in animal nutrition is not expected to represent a risk to the environment.

The production organism and its DNA were not detected in the final product. The final product does not pose any environmental safety concern associated with the genetic modification of the production strain.

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 HCl monohydrate is well established in the scientific literature (National Research Council (NRC), 1994, 1998, 2011, 2012).

In general, the product 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.

[41] Technical dossier/Section III/Annex III.3.5.
[42] Commission Regulation (EC) No. 761/2009 of 23 July 2009 amending, for the purpose of its adaptation to technical progress, Regulation (EC) No 440/2008 laying down tests methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). OJ L 220/1, 24.8.2009.
[43] Commission Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). OJ L 142, 31.5.2008, p. 1
[44] Technical dossier/Section III/Annex III.3.4.
[45] Technical dossier/Section III/Annex III.3.6.

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[46] and Good Manufacturing Practice.

4. Conclusions

The production strain and its recombinant DNA were not detected in the final products. L-Histidine HCl monohydrate manufactured by fermentation using *C. glutamicum* KCCM 80172 does not give rise to any safety concern regarding the production strain and its genetic modification.

The use of L-histidine monohydrochloride monohydrate produced by fermentation using *C. glutamicum* KCCM 80172 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.

L-Histidine HCl monohydrate produced using *C. glutamicum* KCCM 80172 supplemented at levels appropriate for the requirements of the target species is considered safe for the consumer.

L-Histidine HCl monohydrate produced using *C. glutamicum* KCCM 80172 is 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.

The use of L-histidine HCl monohydrate produced by *C. glutamicum* KCCM 80172 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.

Chronology

Date	Event
02/03/2018	Dossier received by EFSA. L-histidine monhohydrochloride monohydrate feed grade from *Corynebacterium glutamicum*. Submitted by CJ Europe GmbH.
22/05/2018	Reception mandate from the European Commission
09/07/2018	Application validated by EFSA – Start of the scientific assessment
06/08/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: Manufacturing process, characterisation of the production strain and of the additive, safety for target species, safety for the consumer, safety for the user and efficacy*
03/09/2018	Request to the European Union Reference Laboratory (EURL) for feed additives to check the adequacy of the method to detect histidine in the additive.
21/09/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
09/10/2018	Comments received from Member States
29/10/2018	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.*
05/11/2018	Reply of the EURL on the adequacy of the method to detect histidine in the additive.
09/11/2018	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
06/12/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
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

[46] 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.

References

Ahmed I, 2013. Dietary amino acid L-histidine requirement of fingerling Indian catfish, *Heteropneustes fossilis* (Bloch), estimated by growth and whole body protein and fat composition. Journal of Ichthyology, 29, 602–609.

Ahrens FA, 1967. Histamine, lactic acid and hypertonicity as factors in the development of rumenitis in cattle. American Journal of Veterinary Research, 28, 1335–1342.

An H and Ben-Bigirey B, 1998. Scombrotoxin poisoning. In: Millar I, Gray D and Strachan N (eds.). Microbiology of Seafoods. Chapman Hall Ltd, London. pp. 68–69.

Aoyama Y and Kato C, 2000. Suppressive effect of excess dietary histidine on the expression of hepatic metallothionein-1 in rats. Bioscience Biotechnology Biochemistry, 64, 588–591.

Aoyama Y, Kato C and Sakakibara S, 2001. Expression of metallothionein in the liver and kidney of rats is influenced by excess dietary histidine. Comparative Biochemistry and Pathology Part C, 128, 339–347.

Aschenbach J and Gäbel G, 2000. Effect and absorption of histamine in sheep rumen: significance of acidotic epithelial damage. Journal of Animal Science, 78, 464–470.

Buttery P and D'Mello J, 1994. Amino acid metabolism in farm animals. In: D'Mello J (ed.). Amino Acids in Farm Animal Nutrition. Cab International, Wallingford, UK. pp. 1–61.

Dalgaard P, Emborg J, Kjolby A, Sorensen N and Ballin N, 2008. Histamine and biogenic amines - formation and importance in seafood. In: Borrensen T (ed.). Improving Seafood Products for the Consumer. Woodhead Publishing Ltd., Cambridge, UK. pp. 292–324.

D'Mello JPF, 2003. Amino acids as multifunctional molecules. In: JPF D'Mello (ed.). Amino Acids in Animal Nutrition. CABI International: Wallingford, Oxon, UK. pp. 1–14.

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), 2007. Opinion of the Scientific Committee on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. EFSA Journal 2007;5(12):587, 16 pp. https://doi.org/10.2903/j.efsa.2007.587

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;14(9):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), 2019a. 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, 2019a. 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: http://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), 2017a. 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), 2017c. 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

Emborg J, Dalgaard P and Ahrens P, 2006. *Morganella psychrotolerans* sp. nov., a histamine producing bacterium isolated from various seafoods. International Journal of Systematic Evolutive Microbiology, 56, 2473–2479.

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

Finch B, 1971. Tibia and intestine alkaline phosphatase activity and zinc status of the chick as related to dietary protein source and supplements of zinc and histidine. Thesis Master of Science, Montana State University, Bozeman, Montana, USA. 42 pp.

Gao Y-J, Liu Y-J, Chen X-Q, Yang H-J, Li X-F and Tian L-X, 2016. Effects of graded levels of histidine on growth performance, digested enzymes activities, erythrocyte osmotic fragility and hypoxia-tolerance of juvenile grass carp *Ctenopharyngodon idella*. Aquaculture, 452, 388–394.

Garlick P, 2004. The nature of human hazards associated with excessive intake of amino acids. Journal of Nutrition, 134, 1633S–1639S.

Glover C and Wood C, 2008. Absorption of copper and copper-histidine complexes across the apical surface of freshwater rainbow trout intestine. Journal of Comparative Physiology B, 178, 101–109.

Golder H, Celi P, Rabies A, Heuer C, Bramley E, Miller D, King R and Lean I, 2012. Effects of grain, fructose and histidine on ruminal pH and fermentation products during an induced subacute acidosis protocol. Journal of Dairy Science, 95, 1971–1982.

Harvey PW, Hunsaker HA and Allen KG, 1981. Dietary L-histidine-induced hypercholesterolemia and hypocupremia in the rat. Journal of Nutrition, 111, 639–647.

Ikezaki S, Nishikawa A, Furukawa F, Enami T, Mitsui M, Tanakamaru Z, Kim HC, Lee IS, Imazawa T and Takahashi M, 1996. Long-term toxicity/carcinogenicity study of L-histidine monohydrochloride in F344 rats. Food and Chemical Toxicology, 34, 687–691.

Izquierdo O, Wedekind K and Baker D, 1988. Histidine requirement of the young pig. Journal of Animal Science, 66, 2886–2892.

Kanki M, Yoda T, Ishibashi M and Tsukamoto T, 2004. *Photobacterium phosphoreum* caused a histamine fish poisoning incident. International Journal of Food Microbiology, 92(1), 79–87.
Khan M and Abidi S, 2014. Dietary histidine requirement of Singhi *Heteropneustes fossilis* fry (Bloch). Aquaculture, 45, 1341–1354.
Kopeć W, Jamroz D, Wiliczkiewicz A, Biazik E, Pudlo A, Hikawczuk T, Skiba T and Korzeniowska M, 2013. Influence of different histidine sources and zinc supplementation of broiler diets on dipeptide content and antioxidant status of blood and meat. British Poultry Science, 54, 454–465. https://doi.org/10.1080/00071668.2013.793295
Kopeć W, Wiliczkiewicz A, Jamroz D, Biazik E, Pudlo A, Hikawczuk T, Skiba T and Korzeniowska M, 2016. Antioxidant status of turkey breast meat and blood after feeding a diet enriched with histidine. Poultry Science, 95, 53–61. https://doi.org/10.3382/ps/pev311
Korhonen M, Vanhatalo A, Varvikko T and Huhtanen P, 2000. Responses to graded postruminal doses of histidine in dairy cows fed grass silage diets. Journal of Dairy Science, 83, 2596–2608.
Kriengsinyos W, Rafii M, Wykes L, Ball R and Pencharz P, 2002. Long-term effects of histidine depletion on whole body protein metabolism in healthy adults. Journal of Nutrition, 132, 3340–3348.
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 Vet, 80, 365–372. https://doi.org/10.2754/avb201180040365
Lee NS, Fitzpatrick D, Meier E and Fisher H, 1981. Influence of dietary histidine on tissue histamine concentration, histidine decarboxylase and histamine methyltransferase activity in the rat. Agents and Actions, 11, 307–311.
Maintz L and Novak N, 2007. Histamine and histamine intolerance. The American Journal of Clinical Nutrition, 85, 1185–1196. https://doi.org/10.1093/ajcn/85.5.1185
Massey K, Blakeslee C and Pitkow H, 1998. A review of physiological and metabolic effects of essential amino acids. Amino Acids, 14, 271–300.
Michelato M, Zaminhan M, Boscolo W, Nogaroto V, Vicari M, Artoni R, Furuya V and Furuya W, 2017. Dietary histidine requirement of Nile tilapia juveniles based on growth performance, expression of muscle-growth-related genes and haematological responses. Aquaculture, 467, 63–70.
Munck I and Munck B, 1994. Amino acid transport in the small intestine. Physiological Research, 43, 335–345.
National Research Council (NRC), 1994. Nutrient requirements of poultry. 9th revised edition. The National Academies Press, Washington, DC. 176 pp.
National Research Council (NRC), 1995. Nutrient Requirements of Laboratory Animals. 4th revised edition. The National Academies Press, Washington, DC. 192 pp.
National Research Council (NRC), 1997. Nutrient Requirements of Rabbits. 2nd revised edition. The National Academies Press, Washington, DC. 30 pp.
National Research Council (NRC), 1998. Nutrient Requirements of Swine. 10th revised edition. The National Academies Press, Washington, DC. https://doi.org/10.17226/6016
National Research Council (NRC), 2001. Nutrient requirement of dairy cattle. 7th revised edition. The National Academies Press, Washington, DC, 381 pp.
National Research Council (NRC), 2011. Nutrient Requirements of Fish and Shrimp. The National Academies Press, Washington, DC, USA. 376 pp.
National Research Council (NRC), 2012. Nutrient Requirements of Swine. 11th revised edition. National Academy Press, Washington, DC. 178 pp.
O'Connor JD, Sniffen CJ, Fox DG and Chalupa W, 1993. A net carbohydrate and protein system for evaluating cattle diets: IV. Predicting amino acid adequacy. Journal of Animal Science, 71, 1298–1311.
Ogata HY, 2002. Muscle buffering capacity of yellowtail fed diets supplemented with crystalline histidine. Journal of Fish Biology, 61, 1504–1512. https://doi.org/10.1006/jfbi.2002.2169
Onodera R, 2003. Essentiality of histidine in ruminants and other animals including human beings. Asian-Australasian Journal of Animal Science, 16, 445–454.
Özogul F and Özogul Y, 2006. Biogenic amine content and biogenic amine quality indices of sardines (*Sardina pilchardus*) stored in modified atmosphere packaging and vacuum packaging. Food Chemistry, 99, 574–578.
Remo S, Hevroy E, Olsvik P, Fontanillas R, Breck O and Waagbo R, 2014. Dietary histidine requirement to reduce the risk and severity of cataracts is higher than the requirement for growth in Atlantic salmon smolts, independently of the lipid source. British Journal of Nutrition, 111, 1759–1772.
Schwab CG, Huhtanen P, Hunt CW and Hvelplund T, 2005. Nitrogen requirements of cattle. In: Pfeffer E and Hristov AN (eds.). Nitrogen and Phosphorus Nutrition of Cattle. CABI International, Wallingford, Oxfordshire, UK. pp. 13–70.
Sheiner J, Morris P and Andersen H, 1985. Food intake suppression by histidine. Pharmacology Biochemistry and Behavior, 23, 721–726.
Shiozaki K, Nakano T, Yamaguchi T and Sato M, 2003. Metabolism of exogenous histamine in rainbow trout (*Oncorhynchus mykiss*). Fish Physiology and Biochemistry, 29, 289–295.
Taylor J, Waagbo R, Diez-Padrisa M, Campbell P, Walton J, Hunter D, Matthew C and Migaud H, 2015. Adult triploid Atlantic salmon (*Salmo salar*) have higher dietary histidine requirements to prevent cataract development in seawater. Aquaculture Nutrition, 21, 18–32.

Vanhatalo A, Huhtanen P, Toivonen V and Varvikko T, 1999. Response of dairy cows fed grass silage diets to abomasal infusions of histidine alone or in combinations with methionine and lysine. Journal of Dairy Science, 82, 2674–2685.

VKM, 2016. Report from the Norwegian Scientific Committee for Food Safety (VKM). Risk assessment of "other substances" – L-histidine.

Waagbø R, Tröße C, Koppe W, Fontanillas R and Breck O, 2010. Dietary histidine supplementation prevents cataract development in adult Atlantic salmon, *Salmo salar* L., in seawater. British Journal of Nutrition, 104, 1460–1470. https://doi.org/10.1017/S0007114510002485

Wessels A, Kluge H, Mielenz N, Corrent E, Bartelt J and Stangl G, 2016. Estimation for the leucine and histidine requirements for piglets fed a low protein diet. Animal, 10, 1803–1811.

WHO, 2007. Protein and amino acid requirements in human nutrition: report of a joint FAO/WHO/UNU expert consultation (WHO technical report series; no. 935), Geneva, Switzerland.

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

Xu H, Sakakibara S, Morifuji M, Salamatulla Q and Aoyama Y, 2003. Excess of dietary histidine decreases the liver copper level and serum alanine aminotransferase activity in Long-Evans Cinnamon rats. British Journal of Nutrition, 90, 573–579.

Yang X, Wang J, Fan P, Zhao L, Cheng Y, Wu X and Zeng Ch, 2010. Survival, growth, sexual maturity and tissue histamine accumulation of the mysis, *Neomysis awatschensis* and *N. japonica nakazawa*, fed histamine supplemented diets. Aquaculture, 302, 256–260. https://doi.org/10.1016/j.aquaculture.2010.02.006

Abbreviations

AFC	Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
BW	body weight
CAS	Chemical Abstracts Service
CFU	colony forming unit
CP	crude protein
CV	coefficient of variation
DAO	diamino oxidase
DM	dry matter
EINECS	European Inventory of Existing Commercial Chemical Substances
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
F/G	feed to gain
FLAVIS	Flavour Information System
HNMT	histamine-*N*-methyltranferase
HPLC-UV	high-performance liquid chromatography coupled with ultraviolet detection
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
IEC-VIS	ion exchange chromatography coupled with photometric detection
IUPAC	International Union of Pure and Applied Chemistry
LLNA	local lymph node assay
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
NAH	*N*-acetyl-histidine
OECD	Organisation for Economic Co-operation and Development
PCB	polychlorinated biphenyl
PCDD/F	polychlorinated dibenzo-*p*-dioxins and dibenzofurans
QPS	qualified presumption of safety
RH	relative humidity
Rrec	recovery rate
RSDip	relative standard deviations for intermediate precision
RSDr	relative standard deviations for repeatability
TEQ	toxic equivalent
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 80172

In the current application authorisation is sought under Article 4 for L-histidine monohydrochloride monohydrate produced by fermentation with *Corynebacterium glutamicum* KCCM 80172, 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 relative standard deviations 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, from further analytical data presented by the Applicant, the EURL calculated a RSDr of 0.1%.

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. Even if within the scope of the Community method, validation data for the determination of histidine in premixtures and feedingstuffs are not presented. The Community method was further ring-trial validated by twenty-three laboratories for the determination of total histidine in feed and resulted in the equivalent standard method EN ISO 13903:2005. 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) is not considered necessary.