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Safety and efficacy of L-arginine produced by fermentation with *Corynebacterium glutamicum* KCCM 80182 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, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Lieve Herman, Boet Glandorf, Maria Saarela, Montserrat Anguita, Jaume Galobart, Orsolya Holczknecht, Jordi Tarrés-Call, Elisa Pettenati, Fabiola Pizzo and Paola Manini

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-arginine produced by fermentation with a genetically modified strain of *Corynebacterium glutamicum* KCCM 80182 when used as a nutritional additive in feed and water for drinking for all animal species categories. Viable cells of the production strain and its recombinant DNA were not detected in the additive. The product L-arginine, manufactured by fermentation with *C. glutamicum*, KCCM 80182, does not give rise to any safety concern with regard to the production strain. L-Arginine produced using *C. glutamicum* KCCM 80182 is considered safe for the target species. The FEEDAP Panel has concerns regarding the safety of the simultaneous oral administration of L-arginine via water for drinking and feed. L-Arginine produced using *C. glutamicum* KCCM 80182 is safe for the consumer and for the environment. The additive is not hazardous by inhalation, is not a skin sensitiser, but is corrosive to skin and eyes. The product under assessment is considered an efficacious source of the amino acid L-arginine for all animal species. For L-arginine to be as efficacious in ruminants as in non-ruminant species, it requires protection against microbial degradation in the rumen.

Keywords: nutritional additive, amino acids, L-arginine, genetically modified production strain, *C. glutamicum* KCCM 80182, Safety, efficacy

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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.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of 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 authorisation of the product L-arginine (L-arginine feed grade) produced by fermentation with *Corynebacterium glutamicum* KCCM 80182, 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: flavouring compounds).

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 10 October 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-arginine (L-arginine feed grade) produced by fermentation with *C. glutamicum* KCCM 80182, when used under the proposed conditions of use (see Section 3.1.3.2).

1.2. Additional information

L-Arginine (minimum content of 98% on dry matter basis) produced by *C. glutamicum* strains ATCC 13870, KCTC 10423BP or KCCM 80099 is currently authorised as a nutritional feed additive for all animals without any restrictions by Commission Regulation (EC) No 1139/2007,[3] Commission Implementing Regulation (EU) 2016/972[4] and Commission Implementing Regulation (EU) 2018/129,[5] respectively. L-Arginine produced by chemical synthesis or product hydrolysis is authorised for use in feed as flavouring (EU Flavour Information System (FLAVIS) Number [17.003]) by Commission Implementing Regulation (EU) 2018/249.[6] The product L-arginine produced by the genetically modified strain of *C. glutamicum* KCCM 80182 has not been previously authorised as a feed additive in the European Union (EU).

[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 1139/2007 of 1 October 2007 concerning the authorisation of L-arginine as a feed additive. OJ L 256, 2.10.2007, p. 11.
[4] Commission Implementing regulation (EU) 2016/972 of 17 June 2016 concerning the authorisation of L-arginine produced by *Corynebacterium glutamicum* KCTC 10423BP as feed additive for all animal species. OJ L 161, 18.6.2016, p. 18.
[5] Commission Implementing regulation (EU) 2018/129 of 25 January 2018 concerning the authorisation of L-arginine produced by *Corynebacterium glutamicum* KCCM 80099 as feed additive for all animal species. OJ L 22, 26.1.2018, p. 21.
[6] Commission Implementing Regulation (EU) 2018/249 of 15 February 2018 concerning the authorisation of taurine, beta-alanine, L-alanine, L-arginine, L-aspartic acid, L-histidine, D,L-isoleucine, L-leucine, L-phenylalanine, L-proline, D, L-serine, L-tyrosine, L-methionine, L-valine, L-cysteine, glycine, monosodium glutamate and L-glutamic acid as feed additives for all animal species and L-cysteine hydrochloride monohydrate for all species except cats and dogs OJ L 53, 23.2.2018, p. 134.

L-Arginine is authorised for use in food for nutritional[7] and flavourings[8] purposes, for use in cosmetics[9] and as a veterinary medicinal product.[10,11]

L-Arginine is described in a monograph of the European Pharmacopoeia (MG 07/2014:0806) (PhEur, 2014).

The EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued three opinions on the safety and efficacy of L-arginine produced by genetically modified strains of *C. glutamicum* for all animal species (EFSA, 2007a; EFSA FEEDAP Panel, 2016, 2017a) and an opinion on the safety and efficacy of L-arginine produced by fermentation with *Escherichia coli* NITE BP-02186 for all animal species (EFSA FEEDAP Panel, 2018c). The FEEDAP Panel issued one opinion on the safety and efficacy of the use of amino acids (chemical group 34) when used as flavourings for all animal species (EFSA FEEDAP Panel, 2014).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) issued an opinion on the safety evaluation of certain food additives prepared by the 63rd meeting of this committee (WHO, 2006) that included L-arginine.

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[12] in support of the authorisation request for the use of L-arginine feed grade produced by fermentation with *C. glutamicum* KCCM 80182 as an additive in feed and in water for drinking.

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-arginine in animal feed. The Executive Summary of the EURL report can be found in Annex A.[13]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-arginine feed grade produced by fermentation with *C. glutamicum* KCCM 80182 is in line with the principles laid down in Regulation (EC) No 429/2008[14] and the relevant guidance documents: Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008), Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use (EFSA GMO Panel, 2011), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the

[7] Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009, OJ L 181, 29.6.2013, p. 35.

[8] Commission Implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1.

[9] Commission Decision of 9 February 2006 amending Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products (2006/257/EC). OJ L 97, 5.4.2006, p. 1.

[10] 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.

[11] 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.

[12] FEED dossier reference: FAD-2018-0045.

[13] The full report is available on the EURL website: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports

[14] 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.

assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017d), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012) and Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b).

3. Assessment

The subject of the present assessment is L-arginine (≥ 98% on dry matter basis) produced by fermentation with a genetically modified strain of *C. glutamicum* (KCCM 80182). It is intended to be used as a nutritional additive (functional group amino acids, their salts and analogues) and as a sensory additive (functional group 'flavouring compounds') in feed and water for drinking for all animal species and categories.

L-Arginine is considered as a non-essential amino acid for most adult mammalian species including humans, but it is classified as essential for birds, fish, possibly reptiles and also for strict carnivores. For mammalian neonates, it is also considered to be essential.

3.1. Characterisation

3.1.1. Characterisation of the production organism

[redacted]

3.1.1.1. Information relating to the production strain

Characteristics of the recipient or parental microorganism

[redacted]

Characteristics of the donor organism

[redacted]

Description of the genetic modification process

[redacted]

[redacted]

[redacted]

3.1.2. Manufacturing process

[redacted]

3.1.3. Characterisation of the additive

L-Arginine (International Union of Pure and Applied Chemistry (IUPAC) name: (*S*)-2-amino-5-guanidinopentanoic acid; synonym 2-amino-5-guanidinovaleric acid, a compound identified with the Chemical Abstracts Service (CAS) No 74-79-3, the European Inventory of Existing Commercial Chemical Substances (EINECS) No 200-811-1) and the FLAVIS Number [17.003]. It has a molecular mass of 174.2 Da. The molecular formula of L-arginine is $C_6H_{14}N_4O_2$. The structural formula is given in Figure 1.

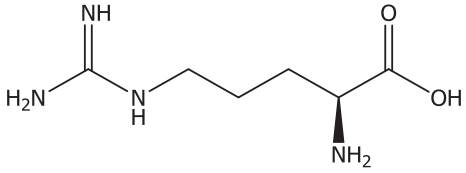

Figure 1: Molecular structure of L-arginine

The additive contains by specification ≥ 98% L-arginine on 'as is' (≥ 98.5% L-arginine on a dry matter basis), ≤ 0.5% water and ≤ 0.1% ash. The analysis of five batches showed an average value of arginine of 99.5% 'as is' (range 99.4–99.6).[21] The loss on drying was 0.29% (range 0.26–0.32%). Consequently, the amount of unidentified material was lower than 0.3% on a dry matter basis.

The specific optical rotation was measured in three batches of the final product and the average was + 27.2° (range + 27.1° to + 27.3°),[22] which is within the range established for L-arginine in the European Pharmacopoeia (+ 26.9° to + 27.9°) and demonstrates the identity of the L-enantiomer.

Three batches of L-arginine feed grade were analysed for chemical impurities. Heavy metals, (lead, cadmium and mercury) and arsenic were below the limits of detection (LODs).[23] In the same batches, polychlorinated dibenzodioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) and coplanar dioxin-like polychlorinated biphenyls (co-planar PCBs) were found below the LOD.[24] Aflatoxins (B1, B2, G1, G2), ochratoxin A, zearalenone, deoxynivalenol, fumonisins B1 and B2 were below the corresponding LOD.[25] In the same batches, nitrofurans (furazolidone, furaltadone, nitrofurazone and nitrofurantoin) and nitrofuran metabolites were below the corresponding LODs.[26] A multiresidue pesticide analysis showed that none of the 358 pesticides was present in the three batches.[27]

Analysis of microbial contamination of the same three batches indicated that total bacterial count was $< 10^3$ colony forming unit (CFU)/g, yeasts and filamentous fungi were $< 5 \times 10^1$ CFU/g, *Salmonella* spp., *E. coli* and coliforms were absent in 25 g.[22]

[redacted]

[21] Technical dossier/Section II/Annex II.1.2.
[22] Technical dossier/Section II/Annex II.1.3.
[23] Technical dossier/Section II/Annex II.1.3. LODs (in mg/kg) were 1 for arsenic, lead and cadmium, 5 for mercury.
[24] Technical dossier/Section II/Annex II.1.3. LOD (in μg/kg) was 0.05 for PCDD/F and 0.13 for co-PCBs.
[25] Technical dossier/Section II/Annex II.1.3. 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.
[26] 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.
[27] Technical dossier/Section II/Annex II.1.4. LOD (in μg/kg) was 0.5–8.

[redacted]

The additive is a white odourless crystalline powder, with a bulk density of 400–600 kg/m^3, pH is specified to range from 10.5 to 12.0 (1% solution in water) and a water solubility of 50–60 g/L at 25°C.[30] The particle size distribution (three batches, analysed by sieving) of the product under assessment showed that the amount of particles < 150 μm, < 88 μm and < 75 μm ranged from 64.6–65.4%, from 48.5–49.0% and 20.3–24.2%, respectively.[31] The dusting potential (three batches) of the final product (Stauber-Heubach method) ranged from 1.5 to 2.6 g/m^3.[32]

3.1.3.1. Stability and homogeneity

No information on the shelf-life, stability (in premixtures and feedingstuffs) and capacity of the additive to distribute homogeneously in feed of the additive under assessment was provided. The applicant provided data performed with an authorised L-arginine originating from a different strain (*C. glutamicum* KCCM 80099).[33] As the production process is the same and the product characteristics are very similar,[34] the FEEDAP Panel considers that the results of those studies can be applicable to the product under assessment.

When the stability and homogeneity properties of L-arginine produced using *C. glutamicum* KCCM 80099 were assessed previously (EFSA FEEDAP Panel, 2017a), the only concern was high losses (up to 17% at 25°C and up to 33% at 40°C) in vitamin–mineral premixtures containing choline chloride,[35] indicating incompatibility with one or more constituents of the feed premixtures.

L-Arginine was shown to be stable for 36 months when stored in plastic bags at 25°C and for 6 months at 40°C,[36] in mash and pelleted feed when stored at 25 or 40°C for 3 months[37] and in water for drinking for 48 h (at 25 or 40°C).[38] The capacity of the additive to distribute homogeneously in feed[39] and in water for drinking[40] was also demonstrated.

The applicant provided additional data on three batches which showed that L-arginine is stable during feed processing (pelleting at 72°C).[41]

3.1.3.2. Physicochemical incompatibilities in feed

No physicochemical incompatibilities in feed are expected with medicinal products or feed materials. High losses in premixtures indicate incompatibility with one or more constituents of the feed premixture.

3.1.4. Conditions of use

L-Arginine is intended to be used in feed or water for drinking for all animal species and categories without maximum content in feed or time of administration. The additive can be added directly to feedingstuffs or complementary feedingstuffs, or via premixtures.[42] No inclusion levels are proposed as the requirements in quantitative terms depend on the species, the physiological state of the animal,

[redacted]

[30] Technical dossier/Section II.2.2.1 and Annex II.3.1.
[31] Technical dossier/Section II.2.2.1/Annex II.1.5.
[32] Technical dossier/Supplementary information December 2018/Annex SIN02CJ L-Arg-Dusting potential report KCCM 80182.
[33] Technical dossier/Section II/Annexes II.4.1 to II.4.7.
[34] Technical dossier/Section II/Annex II.1.5.
[35] Technical dossier/Section II/Annex II.4.3. Method according to the EU official method (Reg EC 152/2009).
[36] Technical dossier/Section II/Annex II.4.1 and Annex II.4.2. Method according to the EU official method (Regulation (EC) 152/2009).
[37] Technical dossier/Section II/Annex II.4.4. Method according to the EU official method (Reg EC 152/2009).
[38] Technical dossier/Section II/Annex II.4.5.
[39] Technical dossier/Section II/Annex II.4.6.
[40] Technical dossier/Section II/Annex II.4.7.
[41] Technical dossier/Supplementary information December 2018/Annex SIN03CJ L-Arg-Pellet stability_KCCM 80182.
[42] Technical dossier/Section II.5.1

the performance level and the environmental conditions, and the amino acid composition of the unsupplemented diet.

When used as sensory additive, the maximum proposed use level is 25 mg/kg complete feed, in compliance with regulation (EU) 2018/249.[43]

3.2. Safety

3.2.1. Safety aspects of the production strain

The recipient organism belongs to a species, *C. glutamicum*, which is considered by EFSA to be suitable for the QPS approach to safety assessment (EFSA, 2007b) for production purposes (EFSA BIOHAZ Panel, 2019).

The production strain *C. glutamicum* KCCM 80182 [redacted]

None of the affected traits raise safety concerns. [redacted]

The identity of the production strain has been established, the strain is susceptible to the relevant antibiotics and the genetic modification raises no concerns, therefore the production strain is presumed safe for the production of L-arginine.

3.2.2. Safety for the target species

Concerns from the use of the additive may arise from residues of the fermentation process/ production strain remaining in the final product. The additive is highly purified (> 99%), is produced by fermentation using a genetically modified strain that qualifies for the QPS approach for safety assessment and the genetic modification raised no concerns. Therefore, the FEEDAP Panel concludes that L-arginine produced by *C. glutamicum* KCCM 80182 is safe for the target species provided that it is supplemented in appropriate amounts to the diets. Due to the risk of nutritional imbalances and hygienic reasons, associated to the use of amino acids via water for drinking (EFSA FEEDAP Panel, 2010), the FEEDAP Panel has concerns on the safety of the simultaneous oral administration of arginine-containing additives via feed and water for drinking.

The use of L-arginine as a feed flavouring agent is proposed at the maximum level of 25 mg/kg complete feed.[44] The FEEDAP Panel considers that such inclusion level is unlikely to have any impact on the arginine-lysine ratio when the amino acid composition of the diet is sufficiently balanced.

3.2.2.1. Conclusions on safety for the target species

L-Arginine produced by *C. glutamicum* KCCM 80182 is considered safe for target species when supplemented to diets in appropriate amounts according to the nutritional needs of the animals. The FEEDAP Panel has concerns on the safety of the simultaneous oral administration of L-arginine via feed and water for drinking.

3.2.3. Safety for the consumer

The amino acid L-arginine, supplemented to feed, will be incorporated into proteins of tissues and/or products of animal origin and any potential excess will be metabolised and excreted as urea/uric acid and carbon dioxide. Therefore, the composition of tissues and products of animal origin will not be affected by the use of L-arginine in animal nutrition.

The product under assessment is produced by fermentation using a *C. glutamicum* strain which fulfils the qualifications for the QPS approach to safety assessment and the genetic modification raised no concerns. Therefore, the FEEDAP Panel concludes that the use of L-arginine produced by *C. glutamicum* KCCM 80182 in animal nutrition is safe for the consumer.

[43] OJ L 53, 23.2.2018, p. 134.
[44] Technical dossier/Section III.1.1.

3.2.4. Safety for the user

The applicant submitted an acute inhalation toxicity study, an eye irritation test, a skin irritation test and a dermal sensitisation test, all performed with L-arginine produced by a different production strain (*C. glutamicum* KCCM 80099) as test item[45] and evaluated by the FEEDAP Panel in a previous opinion (EFSA FEEDAP Panel, 2017a). As the product characteristics are very similar, the production process is the same and the strains qualify for the QPS approach of safety assessment, the FEEDAP Panel considers that the conclusion of these studies are applicable to the product under assessment.

3.2.4.1. Conclusions on safety for the user

The product L-arginine produced by *C. glutamicum* KCCM 80182 is corrosive to skin and eyes but is not a skin sensitiser. There is a potential for user exposure by inhalation, however, an acute inhalation toxicity test did not indicate a hazard by inhalation when handling the additive.

3.2.5. Safety for the environment

Viable cells of the production strain 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.

The amino acid L-arginine is a physiological and natural component of the proteins of living organisms. When consumed, it will be absorbed, and the non-absorbed fraction will be incorporated into the intestinal microbial mass and excreted as such. The absorbed L-arginine will be incorporated into body protein or excreted as urea/uric acid and as carbon dioxide.

L-Arginine produced using *C. glutamicum* KCCM 80182 is safe for 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-arginine is well established in the scientific literature (Schuhmacher, 2002).

In beef or dairy cattle fed a variety of diets, L-arginine has not been identified to be limiting (Schwab et al., 2005). The rapid degradation of L-arginine by ruminal microorganisms has been described in a previous opinion (EFSA FEEDAP Panel, 2016). Consequently, for the supplemental L-arginine to be as efficacious in ruminants as in non-ruminant species, it requires protection against degradation in the rumen.

Since L-arginine [17.003] is used in food as a flavouring compound, and their function in feed is essentially the same as that in food no further demonstration of efficacy is necessary.

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[46] and Good Manufacturing Practice.

4. Conclusions

Viable cells of the production strain and its recombinant DNA were not detected in the final product. The additive L-arginine, manufactured using the production strain *C. glutamicum* KCCM 80182, does not rise to any safety concern regarding the production strain.

L-Arginine produced by *C. glutamicum* KCCM 80182 is considered safe for the target species when supplemented in appropriate amounts to the diet. The FEEDAP Panel has concerns on the safety of the simultaneous oral administration of L-arginine via feed and water for drinking.

The use of L-arginine produced by *C. glutamicum* KCCM 80182 in animal nutrition is safe for the consumer.

The additive is not hazardous by inhalation, is not a skin sensitiser, but is corrosive to skin and eyes.

L-Arginine produced using *C. glutamicum* KCCM 80182 is safe for the environment.

[45] FAD-2016-0037/Technical dossier/Section III/Annex III.3.2-4.

[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.

The additive is an effective source of arginine for all species. For the supplemental L-arginine to be as efficacious in ruminants as in non-ruminant species, it requires protection against microbial degradation in the rumen.

Documentation provided to EFSA/Chronology

Date	Event
19/07/2018	Dossier received by EFSA. L-Arginine with *Corynebacterium glutamicum* KCCM 80182 for all animal species. Submitted by CJ Europe GmbH.
03/08/2018	Reception mandate from the European Commission
10/10/2018	Application validated by EFSA – Start of the scientific assessment
15/11/2018	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, conditions of use*
06/12/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
14/12/2018	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 production strain, impurities*
18/01/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
06/02/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation of the production strain, impurities*
12/02/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
11/01/2019	Comments received from Member States
18/01/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
10/02/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
03/04/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ATCC	American type culture collection
CAS	Chemical Abstracts Service
CFU	colony forming unit
CJ	Cheil Jedang
CV	coefficient of variation
DM	dry matter

EINECS	European Inventory of Existing Commercial Chemical Substances
EURL	European Union Reference Laboratory
FCC	Food Chemical Codex
FEEDAP	FEEDAP Panel on additives and products or substances used in animal feed
FLAVIS	EU Flavour Information System (FLAVIS
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
JECFA	Joint FAO/Who Expert Committee on Food Additives
KCCM	Korean Culture Centre of Microorganisms
LOD	limit of detection
MIC	minimum inhibitory concentration
PCR	polymerase chain reaction assay
pH	hydrogen potential
PCBs	polychlorinated biphenyls
PCDDs	polychlorinated dibenzodioxins
PCDFs	polychlorinated dibenzofurans
QPS	qualified presumption of safety
WHO	World Health Organization

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for L-arginine produced by fermentation with *Corynebacterium glutamicum* KCCM 80182

In the current application, authorisation is sought under Article 4(1) for *L-arginine produced by fermentation with Corynebacterium glutamicum KCCM 80182*, under the category/functional groups 2 (b) 'sensory additives/flavouring compounds' and 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-arginine* has a minimum purity (mass fraction) of 98%. The *feed additive* is intended to be added directly into *feedingstuffs* or through *premixtures* and *water* for drinking. However, the Applicant did not propose any minimum or maximum content of *L-arginine* in *feedingstuffs*.

For the quantification of *L-arginine* 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 did not present a verification study and therefore the EURL cannot recommend the method for official control purposes.

For the quantification of the *L-arginine* content in *premixtures* and *feedingstuffs*, the Applicant submitted 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 Community method was further ring-trial validated by 23 laboratories for the determination of total *arginine* in feed and resulted in the equivalent standard method EN ISO 13903:2005. The following performance characteristics were reported for the quantification of total *arginine*: RSDr ranging from 2.3 to 3.3% and RSDR ranging from 7.2% to 9.7%.

Based on the performance characteristics available, the EURL recommends for official control the ring-trial validated Community method based on IEC-VIS to quantify *arginine* in *premixtures* and *feedingstuffs*.

The Applicant provided no experimental data to determine *arginine* in *water.* Nevertheless, as concluded in the previous EURL reports and specified in the corresponding legislation, the EURL recommends the Community method for official control for the quantification of *arginine* in the *feed additive* and *water.*

In addition, the EURL identified the "L-arginine monograph" of the Food Chemical Codex (FCC) for the identification of the *feed additive*.