SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 14 May 2019

doi: 10.2903/j.efsa.2019.5720

Assessment of the application for renewal of authorisation of L-arginine produced by fermentation using *Corynebacterium glutamicum* NITE SD 00285 for all animal species

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Abstract

L-Arginine is an amino acid that is conditionally essential for mammalian neonates, some strict carnivores, birds and fish. The subject of this opinion is a request for renewal of authorisation of L-arginine produced by a strain of *Corynebacterium glutamicum*. The strain designation has changed to its new deposition number, NITE SD 00285, but the strain is otherwise unchanged from the previous opinion. It is not genetically modified and possesses no antibiotic resistance of safety concern. Minor changes in downstream processing following fermentation have been made. The FEEDAP Panel notes that two out of five batches did not meet the specification of the current authorisation (minimum 98% on a dry matter basis). The FEEDAP Panel concludes that L-arginine produced by fermentation to *C. glutamicum* NITE SD 00285 remains safe for the target species, consumers of products from animals fed the additive and the environment under the approved conditions of authorisation. The additive is considered as irritant to skin, corrosive to eyes and poses a risk by inhalation.

Keywords: nutritional additive, amino acids, L-arginine, *Corynebacterium glutamicum* NITE SD 00285, safety

Requestor: European Commission

Question number: EFSA-Q-2016-00783

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Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output: Montserrat Anguita, Rosella Brozzi, Jaume Galobart and Orsolya Holcznecht.

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), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Costa L, Dierick N, Flachowsky G, Mantovani A, Tarrés-Call J and Wallace J, 2019. Scientific Opinion on the assessment of the application for renewal of authorisation of L-arginine produced by fermentation using *Corynebacterium glutamicum* NITE SD 00285 for all animal species. EFSA Journal 2019;17(6):5720, 17 pp. https://doi.org/10.2903/j.efsa.2019.5720

ISSN: 1831-4732

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

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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 14(1) of that Regulation lays down that an application for renewal shall be sent to the Commission at the latest 1 year before the expiry date of the authorisation.

The European Commission received a request from Kyowa Hakko Europe GmbH[2] for renewal of the authorisation of the product L-arginine produced with *Corynebacterium glutamicum* NITE SD 00285, 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 14(1) (renewal of the authorisation). The particulars and documents in support of the application were considered valid by EFSA as of 25/01/2017.

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 of the product L-arginine produced by *C. glutamicum* NITE SD 00285 for all animal species, when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

The current authorisation is for L-arginine 98% produced by *C. glutamicum* ATCC 13870 for all animal species.[3] The applicant is requesting the renewal of the authorisation for the product L-arginine produced by fermentation using *C. glutamicum* NITE SD 00285. This strain was derived from the parental strain ATCC 13870 (the one authorised) by classical mutagenesis, and it was the strain evaluated in the previous application.[4] The authorisation mentioned the parental strain ATCC 13870 and not the actual production strain.

The EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued several opinions on the safety and efficacy of the product containing L-arginine produced by fermentation using either *C. glutamicum* strains (ATCC-13870, KCTC 10423BP, KCCM 80099, KCCM 10741P and KCCM 80182) or *Escherichia coli* strains (NITE BP-02186) for all animal species (EFSA, 2007a; EFSA FEEDAP Panel, 2016, 2017a, 2018a,b, 2019a).

L-Arginine (98%) produced by *C. glutamicum* KCTC 10423BP, KCCM 80099, KCCM 10741P and *E. coli* NITE BP-02186 are also authorised as nutritional feed additives for all animals without any restrictions (Commission implementing Regulations (EU) 2016/972[5], 2018/129[6], and 2019/12).[7]

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 European Pharmacopoeia has a dedicated monograph (07/2014:0806) for arginine (European Pharmacopeia, 2017).

[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] Kyowa Hakko Europe GmbH, Am Wehrhahn 50, D-40211 Dusseldorf, 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, 11, 2.10.2007, p. 3.
[4] FEED dossier reference: FAD-2006-0009.
[5] 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, 18.6.2016, p. 3.
[6] Commission implementing regulation (EU) 2018/129 of 25 January 2018 concerning the authorization of L-arginine produced by *Corynebacterium glutamicum* KCCM 80099 as feed additive for all animal species. OJ L 22/21, 26.1.2018, p. 3.
[7] Commission implementing regulation (EU) 2019/12 of 3 January 2019 concerning the authorisation of L-arginine as a feed additive for all animal species. OJ L 2/21, 4.1.2019. p. 6.

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[8] in support of the authorisation request for the use of L-arginine produced by fermentation with *C. glutamicum* NITE SD 00285 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, to deliver the present output.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment are valid and applicable for the current application.[9]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety of L-arginine produced by fermentation using *C. glutamicum* NITE SD 00285 is in line with the principles laid down in Regulation (EC) No 429/2008[10] and the relevant guidance documents: Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2013), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018c) and the Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012).

3. Assessment

The additive L-arginine is currently authorised by Commission Regulation (EC) No 1139/2007[11] as additive in feed for all animal species, under the category of nutritional additives and the functional group of amino acids, their salts and analogues when produced by *C. glutamicum* ATCC 13870 and containing a minimum of 98% L-arginine. [redacted]

In the framework of the renewal of the authorisation, the applicant has applied for the renewal of L-arginine [redacted] as a nutritional additive in feed for all animal species, although no changes in the production strain have taken place since the previous assessment.[14]

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 active substance/additive

The product L-arginine is authorised to have ≥ 98% arginine on dry matter basis. [redacted]

[8] FEED dossier reference: FAD-2016-0071.

[9] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2006-0009.pdf

[10] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1–65.

[11] Commission Regulation (EC) No 1139/2007 of 1 October 2007 concerning the authorisation of L-arginine as feed additive. OJ L 256, 2.10.2007, p. 11–13.

[redacted]

[14] Technical dossier/Supplementary information July 2017/L-Arg response, reply to question 6.

[redacted]

The Panel considers that the changes introduced to the production process since the 2007 authorisation are not expected to have an effect on the safety of the additive.

The applicant provided data on batch to batch variation of five batches of the additive. Average content of arginine 'as is' was 83.2% (ranging from 82.1 to 84.1%).[17] Moisture was on average 15.1% (ranging from 14.6% to 16.4%). This resulted in an average arginine content of 98% (ranging from 96.4% to 99.2%) on a dry matter basis. Two of the five batches analysed did not comply with the specification.

The product was confirmed to be the L-enantiomer of arginine from its specific optical rotation in three batches (range +26.9 to +27.2°, compared to European Pharmacopoeia values of +25.5 to +28.5° (European Pharmacopeia, 2017)).[18]

Heavy metals and arsenic were analysed in five batches of the final product. Cadmium and lead were below the limit of detection (LOD).[19] Mercury ranged from LOD to 0.03 mg/kg and arsenic was also under LOD.[20] Polychlorinated dibenzodioxins and dibenzofurans (PCDD/F) and dioxin-like polychlorinated biphenyls (PCBs) were analysed in three batches of the final product. PCDD/F and sum of PCDD/F and PCBs were in all cases < 0.1 ng toxic equivalents (TEQ)/kg (LOD).[21]

Microbial contamination was studied in three batches. *Salmonella* spp. were absent in 25 g samples. Total aerobic count ranged from $< 10^3$ to 8×10^3 colony forming units (CFU)/g. Yeasts and filamentous fungi were < 100 CFU/g and Enterobacteriaceae < 10 CFU/g.[22] Three batches were analysed for mycotoxins (aflatoxin B1, B2, G1, G2; fumonisin B1, B2; ochratoxin, deoxynivalenol (DON) and zearalenone). All analytical values were below the LOD.[23]

The taxonomic classification of the strain has been confirmed as belonging to the species *C. glutamicum* [redacted] The production strain has been deposited in the Biological Resource Centre of the National Institute of Technology and Evaluation of Japan (NBRC) with the access number NITE SD 00285.[25]

The susceptibility of the production strain to the antimicrobials listed in the guidance on characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018c) was tested. [redacted]

No viable cells were detected in three batches of the final product when 20 µL of 100 g/L product suspended in sterile water were plated on an appropriate growth medium, at 37°C for 48 h.[27]

The additive is a solid, white, odourless powder, with a pH of 10.5–12 (10% solution).[28] The bulk density, measured in three batches, ranged from 555 to 625 kg/m^3 untapped and from 769 to 833 kg/m^3 when tapped.[29]

[redacted]

[17] Technical dossier/Supplementary information September 2018/Annex A, analytical method was EU official method (Regulation 152/2009).
[18] Technical dossier/Sin July 2017/Annex A. European Pharmacopoeia (9th Edition) monograph 07/2014:0806.
[19] Technical dossier/Supplementary information July 2017/Annex C. LOD (in mg/kg) of cadmium and mercury was < 0.01, the one of lead < 0.05, and the one of arsenic < 0.1.
[20] Technical dossier/Section II/Annex 2.3.
[21] Technical dossier/Supplementary information July 2017/Annex B and supplementary information September 2018/Applicant response and Annex B.
[22] Technical dossier/Supplementary information July 2017/Annex B.
[23] Technical dossier/Section II/Annex 2.5. LOD (in µg/kg) was 0.5 for each aflatoxin, 1 for ochratoxin, 10 for zearalenone, 20 for each fumonisin and 100 for the DON.
[redacted]
[25] Technical dossier/Section II/Annex 2.1.
[redacted]
[27] Technical dossier/Supplementary information July 2017/Annex F.
[28] Technical dossier/Section II/Annex 2.22.
[29] Technical dossier/Section II/Annex 2.10.

The dusting potential was analysed in three batches (Stauber–Heubach) and ranged from 2.3 to 2.8 g/m^3.[30]

Three batches of the final product were analysed by laser diffraction to determine the particle size distribution.[31] The fraction of particles having a diameter of less than 100 or 50 μm ranged from 0 to 32% and from 0 to 10%, respectively. No particles were found < 10 μm.

3.1.2. Stability and homogeneity

Shelf life and stability in premixtures for piglet and sows feed were already assessed in the former opinion. The new data submitted on the stability were the following:

Stability in premixtures

The stability of the additive (three batches) was studied in a vitamin/mineral premixture for chickens for fattening containing choline chloride (15 g/kg). Arginine was supplemented at 20%. Samples were stored in polyethylene bottles at ambient temperature for 3 months. Losses ranged from 0 to 3.4%.

The stability of the additive (one batch) was studied when supplemented at 20% in a vitamin mineral premixture (containing choline chloride) for salmon feed 'without vegetal carrier or supplemented protein sources'.[32] Samples were packaged in polyethylene bottles and stored at ambient temperature for six months. No losses were observed.

Stability in feedingstuffs

The stability of the additive (one batch) was studied when supplemented at 1.1% in complete feed for piglets (with and without calcium propionate), and in complete feed for sows.[33] Basal diets consisted on wheat and corn (piglet feed) or wheat, rye and wheat middlings (sow feed). Temperature of conditioning was about 60°C. Pelleting was performed at 1.8 bar and about 73°C. The pelleting process represented about 6% arginine loss in sow feed (not observed in piglet feed). No losses were observed after 12 months' storage. Certificates of analysis were not provided. Total arginine was measured. Loss of added L-arginine was calculated by subtracting the arginine content of the basal feed.

[redacted]

The stability of the additive (one batch) was studied in a meal, kernel and pelleted feed for salmon (containing choline chloride).[32] The basal diet contained wheat, soya protein concentrate, fish oil, sunflower meal and rapeseed oil. Total arginine (3.73%) was analysed and the free arginine was calculated as before. Supplemented at 1% in feed and addition of 13.5% oil coated to the kernel. To achieve this, 1.16% arginine was added to the dry meal mix. Finished dry mash was conditioned at 75°C and 2 bar pressure and extruded at 110–130°C and 20 bar pressure. Losses during feed processing were observed after extrusion and were of 5%. Samples were stored in polyethylene bottles at room temperature for 3 months. The observed loss in mash, kernel and pelleted feed after 3 months storage was 21.6%, 12.7% and 0.6%, respectively.

Homogeneity

[redacted]

The capacity of the additive (one batch) to distribute homogeneously in a feed for salmon (kernel and pellet) was studied in 10 subsamples. Total arginine was analysed and the free arginine was calculated as above. The coefficient of variation was 13% in kernel feed and 16% in pelleted feed.[32]

[30] Technical dossier/Section II/Annex 2.9.
[31] Technical dossier/Section II/Annex 2.8.
[32] Technical dossier/Section II/Annex 2.20.
[33] Technical dossier/Section II/Annex 2.18.
[redacted]

3.1.3. Conditions of use

The current application is for L-arginine as a nutritional additive to feed for all animal species and categories without maximum content in feed or time of administration. No inclusion levels are proposed as the requirements in quantitative terms depend on the species, the physiological state of the animal, the performance level and the environmental conditions, as well as the amino acid composition of the unsupplemented diet.[35]

3.2. Safety

In the previous opinion, the FEEDAP Panel concluded that L-arginine under assessment was considered safe for the target species, consumer and the environment. It was considered an irritant to persons handling the additive.

C. glutamicum is regarded by EFSA to be suitable for the Qualified Presumption of Safety (QPS) approach to safety assessment only when used as production organism (EFSA, 2007b; EFSA BIOHAZ Panel, 2019b). This approach requires the identity of the strain to be conclusively established and evidence that it does not show acquired resistance to antibiotics of human and veterinary importance. The identity of the production strain has been established as *C. glutamicum* and the absence of acquired resistance was demonstrated so the qualifications were met. Consequently, the production strain NITE SD 00285 used for production purposes is presumed safe for the target species, consumers of products derived from animals fed the additive and the environment.

The applicant conducted two literature searches. One was not further considered due to the poor reporting of the process.[36] The second literature search was performed using the Web of Science platform of databases.[37] The time span covered was January 2006 to June 2018. Titles and abstracts were screened to eliminate obvious irrelevant scientific papers. The full text was then submitted to inclusion criteria: arginine supplemented in diet and end points relevant to safety (performance, blood parameters, production and reproduction). Exclusion criteria were non-dietary administration; induced infection, stress or other response; administration only as a blend with other substances; basal diets with nutritional imbalances; studies on receptor response; acute administration to induce blood flow or physiological response only. The search terms used were 'arginine' in combination with different animal/groups of animal species and synonyms related to these species (e.g. for poultry, synonyms were chicken, duck quail, breeder, pullet, hen (including laying) and turkey). The Panel notes that the search applied did not included specific terms for the production strain or addressing safety other than target species. The references of this literature search are listed in Appendix A.

As a result of the literature review conducted by the applicant, 94 full papers were submitted and 89 were considered relevant. Most of the papers concerned the effects of different supplementation levels on animal performance (e.g. weight gain, feed conversion efficiency, reproductive parameters) but in none of these publications adverse events or safety issues concerning L-Arginine produced using *C. glutamicum* NITE SD 00285 the additive were reported.

With regard to the user safety, the Panel notes that the high dusting potential (up to 2.8 g/m^3), indicates that exposure by inhalation is possible. Moreover, the product has a high pH of 10.5–12 (10% solution) and it is considered irritant to skin, corrosive to eyes and therefore poses a risk by inhalation.

Taking into account that the additive is produced by a QPS microorganism, that the production process has not been substantially modified and that no adverse effects have been reported in the literature search, the FEEDAP Panel considers that there is no evidence to reconsider the conclusions reached in previous assessments. Therefore, the additive is considered safe for target species, consumers of products from animals fed the additive and the environment. It is considered as irritant to skin, corrosive to eyes and poses a risk by inhalation.

3.3. Efficacy

The present application for renewal of the authorisation does not include a proposal for amending or supplementing the conditions of the original authorisation that would have an impact on the efficacy of the additive. Therefore, there is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

[35] Technical dossier/Section 2.5.1.
[36] Technical dossier/Supplementary information July 2017/Appendixes G, H, I and J.
[37] Technical dossier/Supplementary information September 2018/Annex D and Annex C.

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[38] and Good Manufacturing Practice.

4. Conclusions

The FEEDAP Panel notes that two out of five batches did not meet the specification of the current authorisation (minimum 98% on a dry matter basis).

The FEEDAP Panel concludes that L-arginine produced by fermentation to *C. glutamicum* NITE SD 00285 remains safe for the target species, consumers of products from animals fed the additive and the environment under the approved conditions of authorisation. The additive is considered as irritant to skin, corrosive to eyes and poses a risk by inhalation.

5. Recommendations

The FEEDAP Panel notes that the authorisation should reflect the actual production strain *C. glutamicum* NITE SD 00285'.

Chronology

Date	Event
24/11/2016	Dossier received by EFSA. L-Arginine produced with Corynebacterium glutamicum. Submitted by Kyowa Hakko Europe GmbH
24/11/2016	Reception mandate from the European Commission
25/01/2017	Application validated by EFSA – Start of the scientific assessment
23/02/2017	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 and the production strain, manufacturing process, safety*
25/04/2017	Comments received from Member States
21/07/2017	Reception of supplementary information from the applicant - Scientific assessment re-started
24/10/2017	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*
23/01/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
26/04/2018	Request of additional supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation of the additive, safety*
20/09/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
14/05/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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[38] 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.

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Abbreviations

ATCC	American type culture collection
CFU	colony forming unit
DON	deoxynivalenol
EURL	European Union Reference Laboratory
FEEDAP	Panel on additives and products or substances used in animal feed
LOD	limit of detection
MIC	minimum inhibitory concentration

NBRC	National Institute of Technology and Evaluation of Japan
OECD	Organisation for Economic Co-operation and Development
PCB	polychlorinated biphenyl
PCDD/F	polychlorinated dibenzodioxin and dibenzofuran
pH	Hydrogen potential
TEQ	toxic equivalents
WHO	World Health Organization

Appendix A – List of relevant references retrieved from the second literature search provided by the applicant to support safety of the additive

A.1. Safety for the target species

A.1.1. Poultry

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