SCIENTIFIC OPINION

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Safety and efficacy of L-arginine produced by *Corynebacterium glutamicum* KCTC 10423BP for all animal species

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

Abstract

L-Arginine is considered as a non-essential amino acid for most adult mammalian species, but it is classified as essential for birds, fish, possibly reptiles and also for strict carnivores. The following conclusions refer to L-arginine produced by *Corynebacterium glutamicum* KCTC 10423BP. The use of L-arginine is safe for target species when supplemented to diets in appropriate amounts. There are no safety concerns arising from ruminal L-arginine metabolism. The composition of edible tissues and products of animal origin will not be altered by the use of L-arginine in animal nutrition. Considering the high purity of the product under assessment, no risk is expected for the consumer from the use of L-arginine as a feed additive. L-Arginine is not irritating to skin or eyes and is not likely to be a skin sensitiser. Although there is a potential for user exposure by inhalation, there is evidence of no acute toxicity by the inhalation route. The use of L-arginine in animal nutrition would not pose a risk to the environment. Dietary L-arginine is an effective source of arginine for all animal species when a requirement exists. For the supplemental L-arginine to be fully efficacious in ruminants, it requires protection against degradation in the rumen.

Keywords: L-arginine, nutritional additive, amino acid, *Corynebacterium glutamicum*, safety, efficacy

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Summary

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 *Corynebacterium glutamicum* KCTC 10423BP.

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

The use of L-arginine produced by *C. glutamicum* KCTC 10423BP is safe for target species when supplemented to diets in appropriate amounts. There are no safety concerns arising from ruminal L-arginine metabolism.

Considering the high purity of the product under assessment, no risk is expected for the consumer from the use of L-arginine produced by *C. glutamicum* KCTC 10423BP as a feed additive. The composition of edible tissues and products of animal origin will not be altered by the use of L-arginine in animal nutrition.

L-Arginine is not irritating to skin or eyes and is not likely to be a skin sensitiser. Although there is a potential for user exposure by inhalation, there is evidence of no acute toxicity by the inhalation route.

The use of L-arginine produced by *C. glutamicum* KCTC 10423BP in animal nutrition would not pose a risk to the environment.

L-Arginine produced by *C. glutamicum* KCTC 10423BP is an effective source of arginine for all species. For the supplemental L-arginine to be fully efficacious in ruminants, it requires protection against degradation in the rumen.

The FEEDAP Panel made two recommendations related to the description of the additive and the specification for the maximum water content in the product.

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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 Daesang Europe BV[2] for authorisation of the product L-arginine, when used as a feed additive for all animal species (category: nutritional additive; functional group: amino acids, their salts and analogues).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 2 October 2014.

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 produced by fermentation using *Corynebacterium glutamicum* KCTC 10423BP, when used under the proposed conditions of use (see Section 3.1.78).

1.2. Additional information

The additive L-arginine produced by a non-genetically modified (non-GM) *C. glutamicum* KCTC 10423BP has not been previously authorised as feed additive in the European Union (EU).

L-Arginine (98%) produced by *C. glutamicum* ATCC 13870 is currently authorised as nutritional feed additive by Commission Regulation (EC) No 1139/2007 for all animals without any restrictions.[3]

The EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued one opinion on the safety and efficacy of the product containing L-arginine produced by fermentation using *C. glutamicum* ATCC-13870 for all animal species (EFSA FEEDAP Panel, 2007).

The EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) 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 EU Scientific Committee for Food found acceptable the use of L-arginine as a food for particular nutritional purposes (SCF, 1999). The Joint FAO/Who Expert Committee on Food Additives (JECFA) issued an opinion on the safety evaluation of certain food additives prepared by the sixty third meeting of this committee (WHO, 2006) that included L-arginine.

The NDA Panel delivered two opinions related to the substantiation of health claims related to L-arginine (EFSA NDA Panel, 2011a and b).

L-Arginine like other amino acids and other nitrogen compounds is authorised according to Commission Regulation (EC) No 1243/2008 for infant formulae and follow-on formulae.[4] According to Commission Regulation (EC) No 953/2009 and Commission Directive 2001/15/EC, amino acids such as L-arginine may be added in all dietary foods for particular nutritional uses including foods for particular

[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] Daesang Europe B.V., Prof. J.H. Bavincklaan 5, 1183 AT, Amstelveen, The Netherlands.
[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, 02.10.2007, p. 3.
[4] Commission Regulation (EC) No 1243/2008 of 12 December 2008 amending Annexes III and VI to Directive 2006/141/EC as regards compositional requirements for certain infant formulae. OJ L 335 25, 13.12.2008, p. 18.

nutritional uses intended for special medical purposes.[5] L-Arginine and related compounds are also registered as an ingredient in cosmetic products (Commission Decision 2006/257/EEC).[6] L-Arginine is registered as pharmaceutical grade (for total parenteral nutrition) in many European countries and is described in a monograph of the European Pharmacopoeia (MG 01/2008:0806). According to Commission Regulation (EEC) 2377/90, L-arginine is also listed as pharmacologically active substance in veterinary medicinal products and is not subjected to maximum residue levels when used in food producing animals.[7]

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 with *C. glutamicum* KCTC 10423BP as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003 and the applicable EFSA guidance documents.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA, peer-reviewed scientific papers or other scientific reports to deliver the present output.

EFSA has verified the 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 the Annex.[9]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-arginine produced with *C. glutamicum* KCTC 10423BP is in line with the principles laid down in Regulation (EC) No 429/2008[10] and the relevant guidance documents: Guidance on nutritional additives (EFSA FEEDAP Panel, 2012), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012) and Technical Guidance: Microbial Studies (EFSA, 2008).

3. Assessment

The applicant has requested authorisation for the product L-arginine for all animal species. 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 product/active substance

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

[5] Commission Directive 2001/15/EC of 15 February 2001 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. OJ L 52, 22.2.2001, pp. 19–25. Commission Regulation (EC) No 953/2009 of 13 October 2009 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. OJ L 269 9, 14.10.2009, 11 pp.

[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, p. 1–528.

[7] Commission Regulation (EC) No 1931/1999, amending Annexes I, II and III of Council Regulation (EEC) No 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. OJ L 240, 10.9.1999, p. 3–10.

[8] FEED dossier reference: FAD-2014-0012.

[9] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2014-0012_l_arginine.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.

Chemical Abstracts Service (CAS) No 74-79-3, and the European Inventory of Existing Commercial chemical Substances (EINECS) No 200-811-1) has a molecular weight of 174.2 g/mol. The molecular formula of L-arginine is $C_6H_{14}N_4O_2$. The structural formula is given in Figure 1.

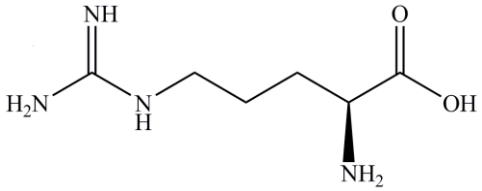

Figure 1: Molecular structure of L-arginine

According to the specification, the product contains ≥ 98% L-arginine on dry matter basis and ≤ 18% water. The analysis of five batches by the official method described in Commission Regulation (EC) 152/2009,[11] showed an average value of L-arginine of 99.5% on dry matter basis (range 99.4–99.5%). Moisture average was 7.7% (range 7.0–9.1%) and residue on ignition 0.03% (range 0.02–0.03%). Consequently, the amount of unidentified material is well below 1% on a dry matter basis.[12] Analytical data on specific optical rotation of five batches showed an average value of +27.2° (range +27.1° to +27.3°), which is within the range described in the European Pharmacopoeia for this amino acid.[13]

3.1.2. Impurities

Five pilot batches were analysed for arsenic and lead; the amounts of these impurities meet the specification.[14] Heavy metals (lead, cadmium and mercury) and arsenic were measured in three other production batches of the product; lead levels were on average 0.05 mg/kg (0.02–0.07 mg/kg) and the levels of the rest of these elements were under the level of detection (LOD) and considered of no concern.[15]

The microbiological quality of the product was analysed in five batches for total aerobic count (≤ 40 colony forming units (CFU)/g), yeasts and moulds (not detected), coliforms (not detected in 1 g), *Escherichia coli* (not detected in 1 g) and *Salmonella* spp. (absent in 25-g sample).[16] Three batches of the product were also analysed for mycotoxins (aflatoxins B1, B2, G1, G2, ochratoxin A, deoxynivalenol, zearalenone, T-2 and HT-2 toxins and fumonisins B1 and B2) and all values were below the limit of quantification (LOQ) and considered of no concern.[17]

Three batches of the final product were analysed for dioxins, dioxin-like PCBs, the sum of dioxins and dioxin-like PCBs and non-dioxin-like (ndl) PCBs and the values were ≤ 0.146 ng WHO-PCDD/F TEQ/kg, ≤0.085 ng WHO dioxin-like PCB TEQ/kg, ≤ 0.231 ng WHO(2005)-PCDD/F+PCB TEQ and < 0.85 µg total 6 ndl-PCB/kg, respectively.[18]

The antimicrobial activity was tested in three batches of the final product. The minimum inhibitory concentration (MIC) values against five species (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 51299 and *Bacillus subtilis* ATCC 6633) were always > 100 000 mg L-arginine/L.[19] However, only three of the strains tested correspond to those indicated in the technical guidance on microbial studies (EFSA, 2008). For two species different strains were examined *(P. aeruginosa* ATCC 9027 instead of 27853

[11] Technical dossier/Supplementary information June 2015/Reply to EFSA and attachment 1.1.
[12] Technical dossier/Section II/Annex II.1.1.
[13] Technical dossier/Section II/Annex II.1.1. The units of the specific optical rotation is deg /(mL × g × dm).
[14] Technical dossier/Section II/Annex II.1.1. The specification for arsenic and lead corresponds to their LOD (mg/kg) and was ≤ 2 for As and ≤ 5 for Pb.
[15] Technical dossier/Supplementary information April 2015/Annexes 2 to 4. LOD of cadmium: 0.01 mg/kg, arsenic: 0.02 mg/kg and mercury: 0.001 mg/kg.
[16] Technical dossier/Section II/Annex II.1.1. The limit of detection of yeasts and moulds was 10 CFU/g.
[17] Technical dossier/Section II/Annex II.1.2. LOQ (µg/kg) was 0.1 for aflatoxins, 0.2 for ochratoxin, 10 for zearalenone and T-2 and HT-2 toxins, and 20 for deoxynivalenol and fumonisins B1 and B2.
[18] Technical dossier/Section II/Annex II.1.2 and Supplementary information April 2015/Annex 5 to 7.
[19] Technical dossier/Supplementary information April 2015/Annexes 8 to 10.

and *Enterococcus faecalis* ATCC 51299 instead of 29212). The MIC values determined are of no safety concern, considering the use levels in feeds.

3.1.3. Physical properties

The product is a white crystalline powder. Water solubility is approximately 148 g/L (at 20°C). The pH (0.8% solution) at 22°C was measured in three batches and ranged from 10.02 to 10.04.[20] Its melting point is 235ºC.[21]

The particle size distribution of three batches of the product under assessment was analysed by laser diffraction: approximately 42% of the particles had a diameter < 100 µm, 23% of the particles a diameter < 50 µm and 7% of the particles a diameter < 10 µm.[22] Data on dusting potential of three batches (determined using the Stauber-Heubach method) showed a range of 0.19–0.25 g/m^3.[23]

3.1.4. Characterisation of the production organism[24]

The L-arginine under assessment is produced by a strain of *C. glutamicum* deposited at the Korean Collection for Type Cultures with the accession number KCTC 10423BP.[25] The identity of the production strain as belonging to *C. glutamicum* species was demonstrated.[26]

The technical dossier contains information on the susceptibility of the production strain against the list of antibiotics proposed for 'other Gram positive' bacteria in the technical guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012).[27]

The history of modifications is provided in the dossier. The strain is not genetically modified.

3.1.5. Manufacturing process[28]

L-Arginine is produced by fermentation by *C. glutamicum* KCTC 10423BP. After the fermentation step, the fermentation broth is inactivated and the bacterial cells are removed. L-Arginine is then purified.[29] Material safety data sheets were provided for the product under assessment and for the chemicals and media used in the production process.[30] The applicant declared that no antimicrobial substances (including antibiotics) are used in the production process.[31]

Neither viable cells of the production strain,[32] nor bacterial DNA was detected in three samples of the final product.[33]

3.1.6. Stability and homogeneity

In the stability studies, the L-arginine concentration was determined by an amino acid analyser following the method proposed in ISO 13903:2005.[34]

Shelf life

Three batches of the additive were tested at two environmental conditions (25°C and 60% (relative humidity (RH)) or 40°C and 75% RH) when stored at 4, 6, 9 and 12 months in paper bags.[35] The loss of arginine during the first 4 months of storage was on an average 11% on an as is basis. No

[20] Technical dossier/Section II/Annex II2.2.
[21] Technical dossier/Section II.2.2.
[22] Technical dossier/Section II/Annex II.1.3.
[23] Technical dossier/Section II/Annex II.1.4.
[24] This section has been amended following the confidentiality claims made by the applicant.
[25] Technical dossier/Section II/Annex II.2.1.
[26] Technical dossier/Supplementary information April 2015/Annexes 13 to 15.
[27] Technical dossier/Supplementary information April 2015/Annex 16 and Supplementary information June 2015/Annex 2.2.
[28] This section has been amended following the confidentiality claims made by the applicant.
[29] Technical dossier/Supplementary information April 2015/Annex 12 Q5.
[30] Technical dossier/Section II/Annex II.3.1 and Supplementary information April 2015/Annexes 19 to 27.
[31] Technical dossier/Supplementary information April 2015/Annex 11 Q4.
[32] Technical dossier/Supplementary information April 2015/Annexes 8 to 10.
[33] Technical dossier/Section II/Annex II.1.3 and Supplementary information April 2015/Annex 17 and Annex 14.
[34] Technical dossier/Supplementary information April 2015/Annex 28 Q10.
[35] Technical dossier/Section II/Annexes II.4.1 and II.4.5.

substantial loss could be seen when the arginine concentration was expressed on a dry matter basis (losses between 1 and 3%). During the following 2 months, an additional loss of 2% on as is basis was observed, but no further loss was detected thereafter. The initial apparent loss observed on an as is basis is likely due to the hygroscopic nature of the L-arginine and the material used for packaging.[36]

Stability in premixtures

Three batches of the additive were tested in a vitamin-mineral premixture (containing choline chloride) for laying hens when stored at 25°C and 50% RH in paper bags for 11 months.[37] The intended arginine concentration of the premixture was 33.3% L-arginine. The initial analytical concentration was 27.6%; after 5 months, the arginine concentration was 26.7% (about 3% loss) and the final concentration was about 8% below the initial.[38]

Stability in feedingstuffs

The stability of three batches of the additive in three different feedingstuffs (fish, piglets and chicken for fattening, in all cases mash and pelleted (extruded for fish feed)) was studied when stored in sealed plastic bags at 25ºC for 3 months. The complete feed for fish consisted of fish meal, wheat flour, soybean meal, corn gluten meal, wheat gluten and soya protein concentrate, that for piglets of barley, wheat, soybean meal, processed maize and wheat middlings, that for chickens for fattening of maize, soybean meal, wheat and animal fat.[39] Arginine was supplemented at 0.5%. Losses were negligible (≤ 3%) in feed for piglets and in the pelleted feed for chicken for fattening but reached 11% in mash feed for chicken for fattening.[40] No losses were found in fish feed after 5 months of storage. Pelleting/extruding did not affect the stability of supplemental arginine.

Homogeneity

The capacity of the additive to distribute homogeneously in feedingstuffs was measured in 11 subsamples of each of the six feedingstuffs described above (supplemental level 0.5% L-arginine).[41] In all cases, the coefficient of variation of supplemental arginine level was < 4%.[42]

3.1.7. Physico-chemical incompatibilities in feed

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

3.1.8. Conditions of use

The current application of L-arginine is as a nutritional additive to feed for all animal species and categories without maximum content in feed or time of administration. According to the applicant, the additive can be added directly to compound feedingstuffs or via premixtures.[43] 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.

3.2. Safety

3.2.1. Safety for the target species

Tolerance studies with essential amino acids cannot be designed in accordance with the protocols of conventional toxicity experiments because high dietary concentrations of a certain amino acid will result in amino acid imbalances and depression of feed intake and, hence, impaired performance. This statement is, in principle, also applicable to non-essential amino acids since a well-balanced dietary protein should have a certain ratio between essential and non-essential amino acids for optimal

[36] Technical dossier/Supplementary information April 2015/Annex 28 Q10.
[37] Technical dossier/Section II/Annexes II.4.1 and II.4.4.
[38] Technical dossier/Supplementary information April 2015/Annex 28 Q10.
[39] Technical dossier/Supplementary information April 2015/Annexes 29 to 31.
[40] Technical dossier/Section II/Annexes II.4, II.4.3 and II.3.6 and supplementary information April 2015/Annex 28.
[41] Technical dossier/Supplementary information April 2015/Reply EFSA-Q-2014-00296/Reply to Q13.
[42] Technical dossier/Supplementary information April 2015/Annex 28 Q10.
[43] Technical dossier/Section II.5.1

performance and low nitrogen-emissions per product. Nevertheless, for nutritional additives produced by fermentation, the risks associated with the residues of the fermentation process in the final product need to be assessed. In this specific product, the amount of identified material represents > 99% on a dry matter basis. Therefore, the FEEDAP Panel considers that safety concerns for target species are highly unlikely to arise from L-arginine produced by *C. glutamicum* KCTC 10423BP.

The classification of L-arginine as either an indispensable or dispensable amino acid is more ambiguous compared to other amino acids such as lysine because of differences among species in rates of de novo arginine synthesis (Ball et al., 2007). As a consequence, the current dietary L-arginine requirement varies widely among species, with ruminants, rabbits and rats having relatively low requirements while carnivores, fish and poultry have high requirements. L-Arginine requirements for swine range, according to the National Research Council (NRC, 1998), from 0.60% (piglets) to 0.20% (growing-finishing pigs and sows) in complete feed or feed material 'as such' (having ≥ 87% dry matter). In poultry (NRC, 1994), L-arginine requirements range from 1.00% to 1.25% for chickens for fattening, from 0.60% to 1.60% for turkeys for fattening and from 0.60% to 1.00% for laying hens. L-Arginine requirements for fish range between 1.20 and 2.00% in the diet (NRC, 2011). Cereals contain less than 1.0% arginine, oilseeds up to 3.5% and extracted oilseeds, fish- and feather-meal and gelatin up to 5.0% on a dry matter basis. The milk of most mammalian species has a low content of arginine.

The tolerance of animals to overdoses of amino acids varies with the amino acid (substance and isomeric form) and the animal species. Excess of arginine (compared to the classical requirement) is better tolerated than other amino acids (e.g. methionine). However, arginine in excess (4% in complete feed) in pigs is less tolerated than equal excesses of lysine or threonine (Edmonds et al., 1987). In young growing pigs, 1.63% dietary arginine had no effect on growth and feed intake and only affected feed efficiency, when lysine was marginally deficient (1.03% lysine; Hagemeier et al., 1983). In other studies with piglets, 0.67, 1.6 and 2.0% supplemental arginine decreased weight gain and feed intake, but had variable effects on gain/feed and no effect on the nitrogen balance (Southern and Baker, 1982; Anderson et al., 1984), whereas a moderate arginine supplementation (0.22%) did not affect performance of growing piglets (Rosell and Zimmerman, 1985). According to Baker (2004) excess arginine is less growth depressing in chicks than in pigs. Recently, supplementation of sow diets during gestation with 0.5–1.0% L-arginine increased the reproductive performance of gilts and the litter birth weight (Mateo et al., 2007; Bérard and Bee, 2010; Wang et al., 2012). Available evidence shows that both polyamines and nitric oxide, which are metabolites of L-arginine, play roles in angiogenesis, which is a critical event during placental growth and fetal development.

In chickens for fattening, large excesses of arginine (4%) reduced weight gain by only 9% when compared to a depression of 92, 79, 53, 50, 47, 31, 29, 15, 4 and 0% by methionine, phenylalanine, tryptophan, histidine, lysine, tyrosine, threonine, isoleucine, valine and leucine, respectively (Edmonds and Baker, 1987). Furthermore, in the same experiment, an addition of 1% arginine to the diet improved the performance of chickens receiving diets supplemented with 4% excess phenylalanine or tyrosine.

A specific feature of lysine metabolism is the lysine–arginine antagonism, clearly seen in chicks and dogs but not in pigs, fish or cats, first reported in the 1950s (Kamin and Handler, 1952). Intolerance symptoms to lysine overdoses could be at least partially compensated by additional arginine. Nevertheless, this antagonism does not play a significant role in practical feeding.

The initial products of L-arginine degradation by ruminal microorganisms are ornithine, σ-aminovaleric acid, and putrescine (Lewis and Emery, 1962), which are then either converted to volatile fatty acids or incorporated into microbial cell biomass. As these products have no reported deleterious effects on the host animal, there are no safety concerns arising from ruminal L-arginine metabolism.

Conclusions on safety for the target species

The use of L-arginine is safe for target species when supplemented to diets in appropriate amounts. There are no safety concerns arising from ruminal L-arginine metabolism.

3.2.2. Safety for the consumer

The absorption and metabolic fate of arginine were described in a previous opinion (EFSA, 2007).

No toxicological studies were provided by the applicant. As a general principle, conventional toxicology studies are considered to be inappropriate for amino acids.

The product under assessment is produced by fermentation. Concerns for the consumer would derive not from the amino acid itself, which will be incorporated into animal protein, but from possible residues from the fermentation. Considering that the additive is highly purified (≥ 99.4% L-arginine and < 1% unidentified material on a dry matter basis), no additional toxicological data are required (EFSA FEEDAP Panel, 2012).

Amino acids supplemented to feed will be incorporated into proteins of tissues and/or products of target animal species and any of their potential excess will be metabolised and excreted. Therefore, the composition of tissues and products of animal origin will not be changed by the use of L-arginine in animal nutrition.

Conclusions on safety for the consumer

The composition of edible tissues and products of animal origin will not be changed by the use of L-arginine in animal nutrition. Considering the high purity of the product under assessment, no risks are expected for the consumer from the use of L-arginine produced by *C. glutamicum* KCTC 10423BP as a feed additive.

3.2.3. Safety for the user

All the toxicological studies reported were performed with the additive under assessment.

Effects in the respiratory system

The portion of particles having a diameter smaller than 50 µm is about 23% and that smaller than 10 µm is about 7%. The dusting potential up to 0.25 g/m³, indicating that exposure by inhalation of the user/worker is possible.

An acute inhalation test was carried out in ten (five males and five females) CD/Crl:CD Sprage–Dawley rats (7–9 weeks of age) in accordance with OECD guideline 403.[44] The only adverse effect seen after 4 h exposure at the dose of 5.1 g L-arginine/m^3 was slight ataxia immediately and until 30 min after end of the exposure in all rats. No mortality occurred in the following 2 weeks and no pathological findings were noted at necropsy.

Effects on skin and eyes

An acute dermal irritation/corrosion study was carried out in three Himalayan rabbits according to OECD guideline 404.[45] After exposure of each animal to 500 mg L-arginine/patch during 4 h, none of the animals showed skin reactions. These results indicate that the L-arginine under assessment was not irritant to human skin.

An acute eye irritation/corrosion study was carried out in Himalayan rabbits in accordance with OECD guideline 405.[46] A single instillation of 100 mg of L-arginine/animal in the conjunctival sac did not cause any change after 1 h exposure or subsequently. The results indicate that the product was not irritant to human eyes.

A maximisation study for sensitisation was carried out using 15 Dunkin-Hartley guinea pigs in accordance with OECD guideline 406.[47] The challenge with 2 mL/animal of a 10% suspension of L-arginine in water for injection revealed no skin reaction in any animal and the controls performed as expected. The results provided evidence that L-arginine is not a skin sensitiser.

[44] Technical dossier/Section III/Reference III.3.1.
[45] Technical dossier/Section III/Reference III.3.3.
[46] Technical dossier/Section III/Reference III.3.2.
[47] Technical dossier/Section III/Reference III.3.4.

Conclusions on safety for the user

The product L-arginine produced by *C. glutamicum* KCTC 10423BP is not irritant to skin or eyes and is not likely to be a skin sensitiser. Although there is a potential for user exposure by inhalation, there is evidence of no acute toxicity by the inhalation route.

3.2.4. Safety for the environment

L-Arginine is a physiological amino acid and a natural component of animals and plants whose use in animal nutrition would not lead to any localised increase of its concentration in the environment. It is mainly not excreted as such, but as urea or uric acid and CO_2. The FEEDAP Panel concludes that the use of the product L-arginine produced by *C. glutamicum* KCTC 10423BP in animal nutrition would not pose a risk to the environment.

3.3. Efficacy

Efficacy studies are not required for amino acids naturally occurring in proteins of plants and animals. The nutritional role of the amino acid L-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). Free arginine is among the amino acids most rapidly degraded by ruminal microorganisms (Lewis and Emery, 1962; Velle et al., 1997), with an estimated half-life in the rumen of 0.8 h (Chalupa, 1976). Broderick and Balthrop (1979) found that no arginine added to ruminal digesta *in vitro* remained after 3 h. In dairy cows, predicted ruminal escape of arginine varied with the dose provided, with a maximum of about 20% escaping at 8 h from the highest dose of 600 mmol (105 g; Velle et al., 1997). Accordingly, little dietary L-arginine provided to ruminants would be expected to reach the abomasum intact and be absorbed. For the supplemental L-arginine to be as efficacious in ruminants as in non-ruminant species, it requires protection against degradation in the rumen.

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[48] and Good Manufacturing Practice.

4. Conclusions

The use of L-arginine produced by *C. glutamicum* KCTC 10423BP is safe for target species when supplemented to diets in appropriate amounts. There are no safety concerns arising from ruminal L-arginine metabolism.

Considering the high purity of the product under assessment, no risk is expected for the consumer from the use of L-arginine produced by *C. glutamicum* KCTC 10423BP as a feed additive. The composition of edible tissues and products of animal origin will not be altered by the use of L-arginine in animal nutrition.

L-Arginine is not irritating to skin or eyes and is not likely to be a skin sensitiser. Although there is a potential for user exposure by inhalation, there is evidence of no acute toxicity by the inhalation route.

The use of L-arginine produced by *C. glutamicum* KCTC 10423BP in animal nutrition would not pose a risk to the environment.

L-Arginine produced by *C. glutamicum* KCTC 10423BP is an effective source of arginine for all species. For the supplemental L-arginine to be fully efficacious in ruminants, it requires protection against degradation in the rumen.

[48] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

5. Recommendations

The description of the additive should contain the statement 'L-arginine produced by *Corynebacterium glutamicum* KCTC 10423BP'.

The specification for the maximum water content in the product should be set to ≤ 10%.

Documentation provided to EFSA

1. L-Arginine produced by *Corynebacterium glutamicum* KCTC 10423BP. April 2014. Submitted by Daesang Europe BV.
2. L-Arginine produced by *Corynebacterium glutamicum* KCTC 10423BP. Supplementary information. April 2015. Submitted by Daesang Europe BV.
3. L-Arginine produced by *Corynebacterium glutamicum* KCTC 10423BP. Supplementary information. June 2015. Submitted by Daesang Europe BV.
4. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for L-arginine.
5. Comments from Member States.

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Abbreviations

ATCC	American Type Culture Collection
NDA Panel	EFSA Scientific Panel on Dietetic Products, Nutrition and Allergies
CAS	Chemical Abstracts Service
CFU	colony-forming unit
CV	coefficient of variation
EC	European Commission
EURL	European Union Reference Laboratory
FAO	Food Agricultural Organization of the United Nations
FEEDAP Panel	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
ISO	International Organisation for Standardisation
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
KCTC	Korean collection for type cultures
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
NRC	US National Research Council
PCB	polychlorinated biphenyls
PCDD	polychlorinated dibenzo-p-dioxin
PCDF	polychlorinated dibenzofurans
PCR	polymerase chain reaction
RH	relative humidity
TEQ	toxicity equivalent
WHO	World Health Organization

Annex – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for L-Arginine

In the current application, authorisation is sought under Article 4(1) for *L-arginine produced by fermentation with Corynebacterium glutamicum (KCTC 10423BP)*, 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. *L-arginine* is already authorised under Commission Regulation (EC) No 1139/2007.

For the quantification of *L-arginine* in *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted the Community method (Commission Regulation (EC) No 152/2009). The method was further ring-trial validated resulting in EN ISO 13903:2005. The method is based on ion exchange chromatography coupled with post-column derivatisation and photometric detection (IEC-VIS). This method does not distinguish between the salts and the amino acid enantiomers and it is designed for *feedingstuffs* and *premixtures*. The following performance characteristics were reported for the quantification of total *arginine*: a relative standard deviation for *repeatability* (RSDr) ranging from 2.3% to 3.3% and a relative standard deviation for *reproducibility* (RSDR) ranging from 7.2% to 9.7%.

Even if not explicitly described in the scope of the Community method, the EURL, following the advice of several experienced National Reference Laboratories (NRLs), recommends the Community method for the quantification of *arginine* in the *feed additive*. Furthermore, for the characterisation of the *feed additive*, the EURL identified the methods described in the '*L-arginine* monograph' of the Food Chemical Codex (FCC).

Based on the performance characteristics available, the EURL recommends for official control the ring-trial validated Community method (equivalent to the EN ISO 13903:2005) based on IEC-VIS for the quantification of *arginine* in the *feed additive, premixtures* and *feedingstuffs,* together with the '*L-arginine* monograph ' of the FCC for the characterisation of the *feed additive*.

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.