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Safety and efficacy of L-arginine produced by fermentation using *Corynebacterium glutamicum* KCCM 10741P for all animal species

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Abstract

L-Arginine is considered to be 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 product subject of this assessment is L-arginine produced by fermentation using a non-genetically modified strain of *Corynebacterium glutamicum* (KCCM 10741P). It is intended to be used in feed and water for drinking for all animal species and categories. Species identity of the production organism was confirmed and the strain was sensitive to antibiotics at concentrations at or below thresholds specified by EFSA; thus, *C. glutamicum* KCCM 10741P may be considered safe by the qualified presumption of safety (QPS) approach. No viable cells of *C. glutamicum* were detected in the final product. The amount of identified material exceeded 99.8%, and no impurities of concern were detected. The use of L-arginine produced by *C. glutamicum* KCCM 10741P is safe for target species when supplemented to diets in appropriate amounts, for the consumer and the environment. L-Arginine produced by *C. glutamicum* KCCM 10741P is considered corrosive to skin and eyes and therefore poses a risk by inhalation. 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.

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Keywords: nutritional additive, amino acids, L-arginine, *C. glutamicum* KCCM 10741P, 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 fermentation using *Corynebacterium glutamicum* KCCM 10741P when used as a nutritional additive for all animal species.

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-arginine was in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant 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 the European Food Safety Authority (EFSA) or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' knowledge, to deliver the present output.

L-Arginine is considered to be 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.

Species identity of the production organism was confirmed and the strain was sensitive to antibiotics at concentrations at or below thresholds specified by EFSA, thus *C. glutamicum* KCCM 10741P may be considered safe by the qualified presumption of safety (QPS) approach. No viable cells of *C. glutamicum* were detected in the final product.

The amount of identified material of the product L-arginine exceeded 99.8%, and no impurities of concern were detected.

The use of L-arginine produced by *C. glutamicum* KCCM 10741P is safe for target species when supplemented to diets in appropriate amounts, for the consumer and the environment.

L-Arginine produced by *C. glutamicum* KCCM 10741P is considered corrosive to skin and eyes and therefore poses a risk by inhalation.

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.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003¹ 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 GmbH² for authorisation of the product L-arginine feed grade (L-arginine) produced by fermentation with *Corynebacterium glutamicum* KCCM 10741P, when used as a feed additive for all target species (category: nutritional Additives; functional group: amino acids, their salts and analogues).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). The particulars and documents in support of the application were considered valid by EFSA as of 17 July 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 and on the efficacy of the product L-arginine (L-arginine feed grade), when used under the proposed conditions of use (see Section 3.1.7).

1.2. Additional information

The product L-arginine, feed grade, produced by the non-genetically modified (non-GM) strain of *Corynebacterium glutamicum* KCCM 10741P has not been previously authorised as feed additive in the European Union (EU). *C. glutamicum* is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment when used for amino acid production, provided the susceptibility to antimicrobials has been demonstrated (EFSA, 2007a; EFSA BIOHAZ Panel, 2013, 2017).

There have been assessments for other similar products in the past and that some are in the market. L-Arginine (98%) 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 1139/2007³, Commission Implementing Regulation (EU) 2016/972⁴, and Commission Implementing Regulation (EU) 2018/129⁵, respectively.

The EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued three opinions on the safety and efficacy of the product containing L-arginine produced by fermentation using *C. glutamicum* (strains ATCC 13870, KCTC 10423BP or KCCM 80099, respectively) for all animal species (EFSA, 2007b; EFSA FEEDAP Panel, 2016, 2017). 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 EU Scientific Committee for Food (SCF) found acceptable the use of L-arginine as a food for particular nutritional purposes (European Commission, 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 EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA Panel) delivered two opinions related to the substantiation of health claims related to L-arginine (EFSA NDA Panel, 2011a,b).

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, 11, 2.10.2007, p. 3.

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, 18.6.2016, p. 3.

⁵ Commission implementing regulation (EU) 2018/129 of 25 January 2018 concerning the authorisation of L-arginine produced by *Corynebacterium glutamicum* KCCM 8099 as a feed additive for all animal species. OJ L22/21, 26.1.2018.

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.⁶ 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 nutritional uses intended for special medical purposes.⁷ L-Arginine and related compounds are also registered as an ingredient in cosmetic products (Commission Decision 2006/257/EEC).⁸ 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 (European Pharmacopoeia, 2014). According to Commission Regulation (EU) No 37/2010, 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.⁹

A risk assessment has been made for L-arginine in food (Shao and Hathcock, 2008). It concluded that arginine at intakes up to 20 g/day were safe for normal healthy adults.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier¹⁰ in support of the authorisation request for the use of L-arginine produced using *C. glutamicum* KCCM 10741P as an additive for feed and water for drinking. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008¹¹ 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 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 produced by fermentation with *C. glutamicum* KCCM 10741P in animal feed. The Executive Summary of the EURL report can be found in Annex A.¹²

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-arginine (L-arginine feed grade) is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on nutritional additives (EFSA FEEDAP Panel, 2012a), Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel 2012b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d), Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012e) and the Guidance for assessing the safety of feed additives for the environment (EFSA, 2008).

3. Assessment

The product subject of this application is L-arginine produced by fermentation using a non-GM strain of *C. glutamicum*. It is intended for use in feed and in water for drinking for all animal species

⁶ 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.

⁷ 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.

⁸ Commission Decision of 9 February 2006 amending Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products. OJ L 97, 5.4.2006, pp. 1–528.

⁹ 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, pp. 72.

¹⁰ FEED dossier reference: FAD-2017-0031.

¹¹ Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

¹² The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2017-0031_arginine.pdf

and categories, under the functional group 'amino acids, their salts and analogues'. 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 strict carnivores. For mammalian neonates, it is also considered to be essential.

The applicant proposed to use data of a previously assessed (and currently authorised) L-arginine originating from *C. glutamicum* KCCM 80099 to support the dusting potential, stability, homogeneity and safety for the user of the product under assessment (given the similarity between both products).¹³

3.1. Characterisation

3.1.1. Characterisation of the 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 Chemical Abstracts Service (CAS) No 74-79-3, and the European Inventory of Existing Commercial chemical Substances (EINECS) No 200-811-1. It has a molecular mass of 174.2 Da. The molecular formula of L-arginine is C₆H₁₄N₄O₂. The structural formula is given in Figure 1.

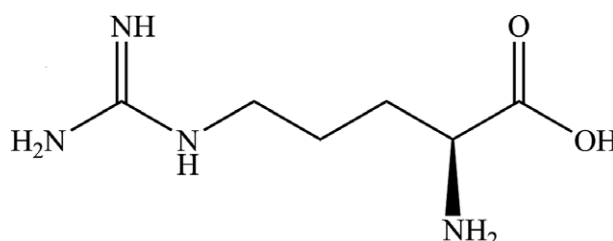


Figure 1: Molecular structure of L-arginine

3.1.2. Characterisation of the production organism

The additive is produced by a non-GM strain of *C. glutamicum* (CJR0500), which is deposited in the Korean Culture Centre of Microorganisms with accession number KCCM 10741P.¹⁴ The identity of the strain as belonging to *C. glutamicum* species was confirmed [REDACTED]

Strain CJR0500 was obtained by chemical mutagenesis of the parent strain KFCC-10680 as described in a European patent.¹⁶ It is resistant to canavanine, arginine hydroxamate and alpha-aminobutyric acid, which leads to the overproduction of L-arginine.

C. glutamicum is considered QPS for amino acid production on condition that the production strain is free from possible antibiotic resistance. Measured minimum inhibitory concentration (MIC) values were lower than maximum MIC values specified in EFSA Guidance on the characterisation of microorganisms (EFSA FEEDAP Panel, 2012b) [REDACTED]

[REDACTED] *C. glutamicum* KCCM 10741P therefore fulfils all criteria for qualification as QPS.

3.1.3. Manufacturing process

L-Arginine is produced using *C. glutamicum* KCCM 10741P in a [REDACTED] fermentation process.

¹³ Technical dossier/Supplementary information January 2018/Sect II and III supplementary information.

¹⁴ Technical dossier/Section II/Annex II.2.1.

¹⁵ [REDACTED]

¹⁶ Technical dossier/Supplementary information January 2018/Annex II.2.2.

¹⁷ [REDACTED]

3.1.4. Characterisation of the additive

According to the specification, the additive contains $\geq 98\%$ L-arginine on a dry matter basis, $\leq 0.5\%$ water and $\leq 0.1\%$ ash. The analysis of five batches showed an average value of L-arginine of 99.5% as is (range 99.4–99.6%).²⁰ Moisture average was 0.29% (range 0.25–0.34%) and other ions, including ammonium, were 0.05%.²¹ Consequently, the amount of unidentified material is $< 0.2\%$ on a dry matter basis. Analytical data on specific optical rotation of three batches showed an average value of $+27.4^\circ$ (range $+27.4^\circ$ to $+27.5^\circ$), which is within the range described in the European Pharmacopoeia for this amino acid.²²

Three batches of the product under application were tested for impurities.²² Heavy metals (Pb, Hg and Cd) were < 5 mg/kg, arsenic was < 1 mg/kg, and mycotoxins were below the limit of detection, as were dioxins, furans and polychlorinated biphenyls (PCBs).²³ Counts of total bacteria were below the specification ($< 1,000$ CFU/g), as were yeasts and filamentous fungi (< 50 CFU/g). Total coliforms, *Escherichia coli* and *Salmonella* were negative in all tests. No viable cells of *C. glutamicum* were detected in the final product.²⁴ The concentrations of the above mentioned impurities were considered not to raise safety concerns.

3.1.4.1. Physical characteristics

L-Arginine is a white or pale brownish free-flowing crystalline powder with the following relevant properties: a bulk density of 0.4–0.6 kg/dm³; a pH (at 10% solution) of 11.7; solubility in water at 25°C is 50–60 g/L.²⁵

Particle size distribution was presented for three batches of the product under application.²⁶ The method of analysis was not specified. Twenty-eight per cent of particles were < 100 μm , 12% were < 50 μm and 3% were < 10 μm . Dusting potential was not measured, but data were presented from an L-arginine produced using a different strain of *C. glutamicum* (KCCM 80099; EFSA FEEDAP Panel, 2017).²⁷

3.1.5. Stability and homogeneity

No data were presented for the product under application. The applicant presented data on the shelf life, stability in premixtures, stability in feedingstuffs (mash and pelleted), stability in water and on the capacity of the additive to distribute homogeneously in feed of an authorised L-arginine produced by fermentation with *C. glutamicum* KCCM 80099 (EFSA FEEDAP Panel, 2017).²⁸ However, considering that the production process of the two additives is identical, the purity was similarly high, and the particle size distribution of the latter product was closely similar to that of *C. glutamicum* KCCM 10741P,²⁹ the FEEDAP Panel assumes that stability and homogeneity properties of the two L-arginine products will be similar.

When the stability and homogeneity properties of L-arginine produced using *C. glutamicum* KCCM 80099 were assessed previously (EFSA FEEDAP Panel, 2017), the only concern was high losses in premixtures, indicating incompatibility with one or more constituents of the feed premixtures.

¹⁸

¹⁹

²⁰ Technical dossier/Supplementary information January 2018/Annex II.2.1. Method HPCL, stated to be according to the EU official method (Regulation (EC) 152/2009) for determination of amino acids in feed.

²¹ Technical dossier/Supplementary information January 2018/Annex II.2.1.

²² Technical dossier/Supplementary information January 2018/Annex II.2.3.

²³ Technical dossier/Supplementary information January 2018/Annex II.2.3. Limit of detection was 0.1 $\mu\text{g}/\text{kg}$ for aflatoxins and ochratoxin A, 1.5 $\mu\text{g}/\text{kg}$ for zearalenone, 0.5 $\mu\text{g}/\text{kg}$ for deoxinivalenol, 5 $\mu\text{g}/\text{kg}$ for fumonisins B1 and B2, 0.05 $\mu\text{g}/\text{kg}$ for dioxins and furans, and 0.13 $\mu\text{g}/\text{kg}$ for PCBs.

²⁴ Technical dossier/Supplementary information January 2018/Annex conf II.1.3.

²⁵ Technical dossier/Section II/Annex II.1.1 and supplementary information January 2018/Section II & III supplementary information and Annex II.2.3 analytical report microbial impurity 3 batches of CoA.

²⁶ Technical dossier/Supplementary information January 2018/Annex II.2.4.

²⁷ Technical dossier/Section II/Annex II.1.7.

²⁸ Technical dossier/Section II/Annexes II.4.1 to II.4.8.

²⁹ Technical dossier/Supplementary information.

3.1.6. Physicochemical incompatibilities

No physicochemical incompatibilities in feed are expected with medicinal products or feed materials. High losses observed in one vitamin/mineral premix were due probably to the presence of high concentration of choline chloride.

3.1.7. Conditions of use

It is proposed that L-arginine will be used in feeds to achieve an adequate amino acid profile and to meet the L-arginine requirements for all animal species. It can be added directly to complete or complementary feedingstuffs, or via premixture.³⁰ It is also proposed for use in water for drinking. No inclusion levels have been proposed, as the requirements, in quantitative terms, depend on the species, the physiological state of the animal, the performance level, the environmental conditions and the amino acid composition of the unsupplemented diet.

3.2. Safety

3.2.1. Safety for the target species

Tolerance studies with essential and conditionally 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 performance and low nitrogen emissions per product (Baker, 2004). 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. L-Arginine produced by *C. glutamicum* KCCM 10741P is of high purity (> 99%) and contains no impurities of concern. Moreover, the production organism met the qualification for QPS. The use of L-arginine in animal nutrition is considered to be safe for target species when supplemented to diets in appropriate amounts (EFSA FEEDAP Panel, 2017). There are no safety concerns arising from ruminal L-arginine metabolism (EFSA FEEDAP Panel, 2017). Therefore, the FEEDAP Panel considers that safety concerns for target species are highly unlikely to arise from L-arginine produced by *C. glutamicum* KCCM 10741P.

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

3.2.1.1. Conclusions on safety for the target species

The use of L-arginine produced by *C. glutamicum* KCCM 10741P 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

Absorption, distribution, metabolism and excretion of L-arginine were described in a previous opinion (EFSA, 2007a,b).

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% L-arginine) and the production strain fulfils the qualifications for QPS, no additional toxicological data are required (EFSA FEEDAP Panel, 2012a).

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.

3.2.2.1. 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

³⁰ Technical dossier/Section II.5.1.

were expected for the consumer from the use of L-arginine produced by *C. glutamicum* 10741P as a feed additive.

3.2.3. Safety for the user

No data were presented for the product under application.³¹ The applicant submitted an acute inhalation study, an *in vitro* study to determine the potential for skin corrosion and a local lymph node assay to test the potential for skin sensitisation, all them performed using L-arginine of *C. glutamicum* KCCM 80099 as a test item.³² Considering the close similarity between L-arginine produced using *C. glutamicum* KCCM 80099 and that produced using *C. glutamicum* KCCM 10741P, as described above, the results of the studies can be applied to the product under assessment.³³

From the results of these studies, the FEEDAP Panel concludes that the product L-arginine produced by *C. glutamicum* KCCM 10741P is corrosive to skin and eyes. Considering the dusting potential and the corrosiveness for skin and eyes, there is also a potential risk by inhalation.

3.2.3.1. Conclusions on the safety for the user

L-Arginine produced by *C. glutamicum* KCCM 10741P is considered corrosive to skin and eyes and poses a risk by inhalation.

3.2.4. Safety for the environment

L-Arginine is 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 carbon dioxide. *C. glutamicum* KCCM 10741P fulfils the qualifications for QPS. The FEEDAP Panel concludes that the use of the product L-arginine produced by *C. glutamicum* KCCM 10741P 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 (EFSA FEEDAP Panel, 2017).

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, 2017). 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.

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 Regulation³⁴ and Good Manufacturing Practice.

4. Conclusions

The use of L-Arginine produced by *C. glutamicum* KCCM 10741P is safe for target species when supplemented to diets in appropriate amounts, for the consumer and the environment.

L-Arginine produced by *C. glutamicum* KCCM 10741P is considered corrosive to skin and eyes and poses a risk by inhalation.

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.

³¹ Technical dossier/Supplementary information January 2018/Section II & III supplementary information.

³² Technical dossier/Section III/Annexes III.3.2 to III.3.4.

³³ Technical dossier/Supplementary information January 2018/Section II & III supplementary information/Table III.1a.

³⁴ Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 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* KCCM 10741P'.

Documentation provided to EFSA

- 1) L-Arginine feed grade produced by *Corynebacterium glutamicum* KCCM 10741P. June 2017. Submitted by CJ Europe GmbH.
- 2) L-Arginine feed grade produced by *Corynebacterium glutamicum* KCCM 10741P. Supplementary information. January 2018. Submitted by CJ Europe GmbH.
- 3) 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* KCCM10741P.
- 4) Comments from Member States.

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Abbreviations

ATCC	American type culture collection
CAS	Chemical Abstracts Service
CFU	colony forming unit
DM	dry matter
EINECS	European Inventory of Existing Commercial chemical Substances
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
GM	genetically modified
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/Who Expert Committee on Food Additives
KCCM	Korean Culture Centre of Microorganisms
MIC	minimum inhibitory concentration
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies

OECD	Organisation for Economic Co-operation and Development
PCB	polychlorinated biphenyl
PCR	polymerase chain reaction
pH	hydrogen potential
QPS	Qualified Presumption of Safety
RH	relative humidity
SCF	Scientific Committee of Food
VKM	Norwegian Scientific Committee for Food Safety
WHO	World Health Organization

Annex A – Executive summary of the evaluation report of the European Union Reference Laboratory for feed additives on the methods of analysis for L-arginine produced by fermentation with *Corynebacterium glutamicum* KCCM10741P

In the current application authorisation is sought under Article 4(1) for *L-arginine* produced by fermentation with *Corynebacterium glutamicum* KCCM10741P 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 as nutritional feed additive under Commission Regulation (EC) No 1139/2007 and Commission Implementing Regulation (EU) 2016/972.

For the quantification of *arginine* in *feed additive*, *premixtures*, *feedingstuffs* and *water* the Applicant submitted the ring-trial validated Community method. This method applies for the determination of free (synthetic and natural) and of total (peptide-bound and free) amino acids, using an amino acid analyzer or a high performance liquid chromatographer equipped with an Ion Exchange Column (IEC) and photometric detection (VIS). Intended for *premixtures* and *feedingstuffs*, it does not distinguish between the salts of amino acids and it cannot differentiate the amino acid enantiomers. The following performance characteristics were reported for the quantification of total *arginine* in *premixtures* and *feedingstuffs*: 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%. The Applicant did not perform the validation and verification of the method for the quantification of *L-arginine* in *feed additive* and *water*, but the experimental data reported in the frame of the stability studies clearly demonstrate the applicability (extension of the scope) of the method to these matrices. From the experimental data submitted, the EURL estimated performance characteristics that are in agreement with those reported in the ring-trial validated Community method. Moreover, the EURL identified the "L-arginine monograph" of the Food Chemical Codex (FCC) for the characterisation of the *feed additive*.

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 *feed additive*, *premixtures*, *feedingstuffs* and *water* and the Food Chemical Codex monograph for the identification of *L-arginine* in 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 as last amended by Regulation (EU) 2015/1761) is not considered necessary.