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Safety and efficacy of L-valine produced by fermentation using *Corynebacterium glutamicum* KCCM 11201P for all animal species

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Abstract

The product subject of this assessment is L-valine produced by fermentation using a non-genetically modified strain of *Corynebacterium glutamicum* (KCCM 11201P). 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 below thresholds specified by EFSA, thus *C. glutamicum* KCCM 11201P 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 █████% on 'as is' basis, and no impurities of concern were detected. The use of L-valine produced by *C. glutamicum* KCCM 11201P is safe for target species when supplemented to diets in appropriate amounts, for the consumer and the environment. The product L-valine produced by *C. glutamicum* (KCCM 11201P) is considered not to be an irritant or a dermal sensitiser, and does not cause acute inhalation toxicity. The additive is an effective source of valine for all species. For the supplemental L-valine to be as efficacious in ruminants as in non-ruminant species, it requires protection against microbial degradation in the rumen.

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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 the safety and efficacy of L-valine ($\geq 98.0\%$) produced by fermentation using a non-genetically modified strain of *Corynebacterium glutamicum* (KCCM11201P) for all animal species.

L-Valine is an essential amino acid that may become limiting under specific feeding conditions. Its supplementation has become even more important since protein-reduced diets were introduced in animal husbandry for economic and environmental reasons. In European vegetable feed formulas, L-valine seems to be the fifth most limiting amino acid after L-tryptophan in pigs for fattening and the fourth most limiting after L-threonine in chickens for fattening.

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of the additive under assessment is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents.

The FEEDAP Panel concludes that the additive L-valine produced by *C. glutamicum* KCCM 11201P is safe for all animal species when supplemented in appropriate amounts to the diet.

No risks are expected for the consumer from the use of L-valine produced by *C. glutamicum* KCCM 11201P as a feed additive.

The product L-valine produced by *C. glutamicum* (KCCM 11201P) is considered not to be an irritant or a dermal sensitiser, and does not cause acute inhalation toxicity.

The additive is not considered to raise safety concerns for the environment.

The additive L-valine produced by *C. glutamicum* KCCM 11201P is regarded as an efficacious source of the amino acid L-valine for all animal species. For the supplemental L-valine to be as efficacious in ruminants as in non-ruminant species, it requires protection against 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-valine, feed grade, when used as a feed additive for all animal species (category: nutritional additives; functional group: Amino Acids, their salts and analogues).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). The particulars and documents in support of the application were considered valid by EFSA as of 9 November 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-valine ($\geq 98.0\%$) feed grade, produced by fermentation using *Corynebacterium glutamicum* KCCM11201P for all animal species when used under the proposed conditions of use (see Section 3.1.9).

1.2. Additional information

L-Valine minimum 98.0% produced by fermentation using *C. glutamicum* KCCM11201P is the object of the present assessment. It has not been previously assessed as feed additive in the European Union. L-Valine produced using *Escherichia coli* strains FERM ABP 10640, NITE SD 00066, NITE BP-01755; or using *Corynebacterium glutamicum* strains KCCM 80058 or DSM 25202 are currently listed in the European Union Register of Feed Additives, and thus authorised in the European Union for use in feed for all animal species.³ L-Valine/FLAVIS No 17.028 is currently listed in the European Union Register of Feed Additives, and thus authorised in the European Union as a feed flavouring.⁴

The EFSA FEEDAP Panel (2008a,b, 2013, 2014a, 2015a,b) has published several scientific opinions on the safety and efficacy of the amino acid L-valine produced using different strains of *E. coli* (FERM ABP 10640, NITE SD 00066, NITE BP-01755) or *C. glutamicum* (KCCM 80058 or DSM 25202) as a nutritional additive. The safety of L-valine when used as food flavouring was assessed by Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2006), by the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) (EFSA, 2008a) and by the FEEDAP Panel (EFSA FEEDAP Panel, 2014b) when used as feed flavouring for animal nutrition.

L-Valine is one of the substances listed in Annex III of Commission Directive 2006/141/EC and therefore authorised for the manufacture of infant formulae and follow-on formulae in order to satisfy the requirements on amino acids and other nitrogen compounds, and in a 'pharmaceutical' grade form it is used for parenteral nutrition.⁵ It may also be added for specific nutritional purposes in foods for particular nutritional uses according to Commission Directive 2001/15/EC⁶. L-Valine and DL-valine are also authorised as sensory additives, belonging to the functional group flavouring compounds (FLAVIS No 17.028 and 17.023, respectively).⁷ L-Valine has a dedicated monograph in the European Pharmacopoeia.⁸

The Cosmetic Ingredient Review Expert Panel (2012) issued a safety assessment of alpha amino acids as used in cosmetics.

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 Röth 04, 65824 Schwalbach/Taunus, Germany.

³ http://ec.europa.eu/food/safety/docs/animal-feed-eu-reg-comm_register_feed_additives_1831-03.pdf.

⁴ Commission List of the authorised additives in feedingstuffs published in application of Article 9th of Council Directive 70/524/EEC concerning additives in feedingstuffs (2004/C 50/01). OJ C 50/1, 25.2.2004.

⁵ Commission Directive 2006/141/EC on infant formulae and follow-on formulae, OJ L 401, 30.12.2006, pp. 1–33.

⁶ 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. 1–7.

⁷ Commission list of the authorised additives in feedingstuffs published in application of Article 9t (b) of Council Directive 70/524/EEC concerning additives in feedingstuffs (2004/C 50/01) OJ C/50, 25.2.2004, pp. 1–144.

⁸ European Pharmacopoeia (9th edition, 2016): monograph 0796.

The Panel on nutrition, dietetic products, novel food and allergy of the Norwegian Scientific Committee for Food Safety (VKM, 2016) published an opinion on the safety of L-valine in food supplements at 1,500, 1,750, 2,000, 2,250 and 2,500 mg/kg day for the general population (ages 10 years and above).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier⁹ in support of the authorisation request for the use of L-valine (minimum 98.0%) feed grade, produced by fermentation using *C. glutamicum* KCCM11201P, as a feed additive for all animal species.

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, and other scientific reports, 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-valine produced by fermentation with *C. glutamicum* KCCM11201P in animal feed. The Executive Summary of the EURL report can be found in Annex A.¹⁰

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of the additive under assessment 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), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c), and Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012d) and Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018).

3. Assessment

L-Valine (minimum 98.0%) produced by *C. glutamicum* KCCM11201P for all animal species is the object of the present assessment. It is proposed as nutritional feed additive, amino acids, their salts and analogues.

3.1. Characterisation

3.1.1. Characterisation of the active substance

L-Valine (International Union of Pure and Applied Chemistry (IUPAC)) name: (2S)-2-amino-3-methylbutanoic acid; synonyms: α -aminoisovaleric acid, 2-amino-3-methylbutyric acid), a compound identified by Chemical Abstracts Service (CAS) No 72-18-4 and European Inventory of Existing Commercial Chemical Substances (EINECS) No 200-773-6, has a molecular weight of 117.15; the molecular formula is $C_5H_{11}NO_2$ and its structural formula is given in Figure 1.

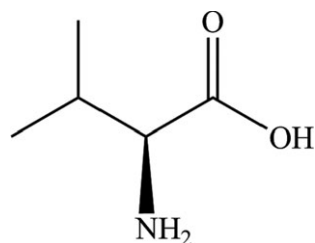


Figure 1: Molecular structure of L-valine

⁹ FEED dossier reference: FAD-2017-0032.

¹⁰ The full report is available on the EURL website: <https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2017-0032-lvaline.pdf>

3.1.2. Characterisation of the production organism

L-Valine is produced by a non-genetically modified strain of *C. glutamicum* which is deposited in the Korean Culture Center of Microorganisms with accession number KCCM 11201P.¹¹ The identity of the strain as belonging to *C. glutamicum* species was confirmed

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C. glutamicum is considered qualified presumption of safety (QPS) for amino acid production on condition that the production strain is free from possible antibiotic resistance.

3.1.3. Manufacturing process

L-Valine is produced by fermentation using *C. glutamicum* KCCM11201P.

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3.1.4. Characterisation of the additive

The product is specified to contain $\geq 96.5\%$ L-valine 'as is' (98.0% on dry matter basis); $\leq 1.5\%$ moisture and $\leq 0.5\%$ ash.¹⁶

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3.1.5. Impurities

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The detected amounts of these undesirable substances do not raise safety concerns.

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3.1.6. Physical characteristics

L-Valine is a white crystalline odourless powder, has a bulk density of 400–600 kg/m³ and a solubility in water of 53–57 g/L at 20°C.²⁰

¹¹ Technical dossier/Section II/Annex II.2.1.

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¹⁶ Technical dossier/Section II/Annexes II.1.1 and II.3.1.

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Technical dossier/Section II/Annex II.3.1.

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Analytical data on dusting potential of the product under assessment were not provided.²²

3.1.7. Stability and homogeneity

No data on the shelf life, stability and capacity to distribute homogeneously in feed of the additive under assessment have been provided.

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3.1.8. Physicochemical incompatibilities

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

3.1.9. Conditions of use

It is proposed that L-valine will be used in feeds to achieve an adequate amino acid profile and to meet the L-valine requirements for all animal species. It can be added directly to feedingstuffs or complementary feedingstuffs, or via a premixture.²⁴ It is also proposed to use the additive 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 are not required for highly purified amino acids independent of the manufacturing process. Such tolerance studies with a single indispensable amino acid will inevitably result in amino acid imbalances with depression of feed intake and hence impaired performance. This is also the case for the additive under application, which is produced using a strain of *C. glutamicum* that qualifies for the QPS status.

The requirements of target animal species for L-valine and the safety of the use of this essential amino acid in non-ruminant and ruminant nutrition were summarised in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2013). The requirements for this amino acid in fish were addressed in a subsequent opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2014a).

The production strain fulfils the qualifications for the QPS approach safety assessment. The product is highly purified (> ■% valine 'as is'). Therefore, the FEEDAP Panel considers that L-valine produced by *C. glutamicum* KCCM11201P is safe for the target species.

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 FEEDAP Panel concludes that the additive L-valine produced by *C. glutamicum* KCCM 11201P is safe for all animal species when supplemented in appropriate amounts to the diet.

3.2.2. Safety for the consumer

The absorption and metabolic fate of L-valine were described in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2013).

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22 Technical dossier/Supplementary information May 2018/Annexes II.1.8.

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24 Technical dossier/Section II.5.1.

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

The product under assessment is produced by fermentation using a *C. glutamicum* strain which fulfils the qualifications for the QPS approach safety assessment. Therefore, the FEEDAP Panel considers that L-valine produced by *C. glutamicum* KCCM 11201P is safe for the consumers.

3.2.3. Safety for the user

No studies were provided on the safety for the user of the product under assessment. The applicant submitted studies (an acute inhalation toxicity study, a skin irritation study, and eye irritation study and a skin sensitisation study)

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Therefore, the product L-valine produced by *C. glutamicum* (KCCM 11201P) is considered not to be an irritant or a dermal sensitiser, and does not cause acute inhalation toxicity.

3.2.4. Safety for the environment

The amino acid L-valine is a physiological and natural component of the proteins of living organisms. When consumed, it will be absorbed, and the non-absorbed fraction will be incorporated into the intestinal microbial mass and excreted as such. The absorbed L-valine will be incorporated into body protein or excreted as urea/uric acid and as carbon dioxide. The use of L-valine in animal nutrition would not lead to any localised increase in its concentration in the environment. Therefore, the use of the product under application as a feed additive does not represent a risk to the environment.

3.3. Efficacy

Efficacy studies are not required for amino acids that occur naturally in plant and animal proteins. The nutritional role of the amino acid L-valine is well established in the scientific literature. The product L-valine is regarded as an efficacious source of the essential amino acid L-valine for non-ruminant nutrition.

In ruminants, the amino acid valine has been implicated as being present at lower than optimum levels in microbial protein leaving the rumen (O'Connor et al., 1993; Schwab et al., 2005). Thus, when requirements for more limiting essential amino acids, usually L-methionine, L-lysine and L-histidine, have been met, L-valine supplementation could be beneficial. Free L-valine is rapidly degraded by ruminal microbiota, with an estimated half-life in the rumen of 2.1 h (Chalupa, 1976). Broderick and Balthrop (1979) found that 45% of free L-valine added to ruminal digesta *in vitro* remained after 3 h. Accordingly, only small amounts of dietary L-valine provided to ruminants would be expected to reach the abomasum intact and be absorbed. Therefore, measures, such as encapsulation, would ensure a more efficient delivery of L-valine beyond the rumen, and only limited nutritional benefit may be derived from dietary supplementation with the unprotected, free amino acid.

The additive L-valine produced by *C. glutamicum* KCCM11201P is regarded as an efficacious source of the amino acid L-valine for all animal species. For the supplemental L-valine 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 Regulation²⁶ and Good Manufacturing Practice.

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²⁶ 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.

4. Conclusions

The FEEDAP Panel concludes that the additive L-valine produced by *C. glutamicum* KCCM 11201P is safe for all animal species when supplemented in appropriate amounts to the diet.

No risks are expected for the consumer from the use of L-valine produced by *C. glutamicum* KCCM 11201P as a feed additive.

The product L-valine produced by *C. glutamicum* (KCCM 11201P) is considered not to be an irritant or a dermal sensitiser, and does not cause acute inhalation toxicity.

The additive is not considered to raise safety concerns for the environment.

The additive L-valine produced by *C. glutamicum* KCCM 11201P is regarded as an efficacious source of the amino acid L-valine for all animal species. For the supplemental L-valine to be as efficacious in ruminants as in non-ruminant species, it requires protection against degradation in the rumen.

Documentation provided to EFSA

- 1) L-Valine feed grade produced by *C. glutamicum* KCCM11201P. November 2017. Submitted by CJ Europe GmbH.
- 2) L-Valine feed grade produced by *C. glutamicum* KCCM11201P. Supplementary information. May 2018. Submitted by CJ Europe GmbH.
- 3) Evaluation report of the European Union Reference Laboratory (EURL) for Feed Additives on the Methods(s) of Analysis for L-valine produced by fermentation with *Corynebacterium glutamicum* KCCM 11201P.
- 4) Evaluation of the method of analysis to determine valine in the additive (batch to batch variation) by the EURL.
- 5) Comments from Member States.

Chronology

Date	Event
23/5/2017	Dossier received by EFSA
6/6/2017	Reception mandate from the European Commission
9/11/2017	Application validated by EFSA – Start of the scientific assessment
15/2/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation of the production strain, characterisation of the additive, stability/homogeneity and safety for the user</i>
19/2/2018	Comments received from Member States
9/3/2018	Reception of the Evaluation report of the European Union Reference Laboratory (EURL) for Feed Additives
16/5/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
3/9/2018	Request to the EURL to assess the method of analysis to determine valine in the additive
5/11/2018	EURL assessment of the method of analysis to determine valine in the additive
28/11/2018	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2008b. Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on the safety of L-valine for all animal species. EFSA Journal 2008;6(12):872, 6 pp. <https://doi.org/10.2903/j.efsa.2008.872>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances Used in Animal Feed), 2010. Scientific Opinion on the use of feed additives authorised/applied for use in feed when supplied via water. EFSA Journal 2010;8(12):1956, 9 pp. <https://doi.org/10.2903/j.efsa.2010.1956>
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Abbreviations

AFC	EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
CAS	Chemical Abstracts Service
CFU	colony forming unit

EINECS	European Inventory of Existing Commercial Chemical Substances
EURL	European Union Reference Laboratory
FCC	Food Chemical Codex
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FLAVIS	The EU Flavour Information System
IEC	ion exchange chromatography
IUPAC	International Union of Pure and Applied Chemistry
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
KCCM	Korean Culture Center of Microorganisms
LOD	limit of detection
MIC	minimum inhibitory concentration
QPS	qualified presumption of safety
RSDr	relative standard deviation for repeatability
RSDR	relative standard deviation for reproducibility
SCAN	Scientific Committee on Animal Nutrition
VKM	Norwegian Scientific Committee for Food Safety
WHO	World Health Organization

Annex A – Executive summary evaluation report on the analytical methods used for the control of the L-valine produced by fermentation with *Corynebacterium glutamicum* KCCM11201P in animal feed

In the current application, authorisation is sought under Article 4(1) for L-valine produced by fermentation with *Corynebacterium glutamicum* KCCM11201P, 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-valine is already authorised as a nutritional feed additive under Commission Regulation (EC) No 403/2009 as last amended by Commission Implementing Regulation (EU) 2015/1114.

For the characterisation of the feed additive, the EURL identified the 'L-valine monograph' of the Food Chemical Codex (FCC) where identification is based on infrared absorption.

For the quantification of L-valine in the feed additive, premixtures and feedingstuffs the Applicant submitted the ring-trial validated Community method based on ion exchange chromatography coupled with post-column derivatisation and photometric detection (IEC-VIS). The method does not distinguish between the salts of amino acids and it cannot differentiate between enantiomers. The following performance characteristics were reported for the quantification of total valine in feed: a relative standard deviation for repeatability (RSDr) ranging from 1.7 to 3.8% and a relative standard deviation for reproducibility (RSDR) ranging from 8.8 to 16.1%.

For the quantification of L-valine in water the Applicant did not submit any suitable method. However, in the frame of the stability studies, the Applicant presented experimental data obtained when analysing valine with the VDLUFA official method (4.11.6) based on IEC coupled with post-column derivatisation and optical detection (IEC-VIS/FD). The results are considered sufficient to demonstrate the suitability of the procedure for the analysis of the amino acid in water. Furthermore, according to the experience of NRLs, other similar ring trial validated methods designed for the analysis of amino acids and based on similar analytical procedures are fit for purpose.

Based on the performance characteristics available, the EURL recommends for official control: (i) the 'L-valine monograph' of the FCC based on infrared absorption for the identification of L-valine in the feed additive; (ii) the Community method based on IEC-VIS for the quantification of valine in the feed additive, premixtures and feedingstuffs; and (iii) the IEC-VIS/FD method based on the procedure described by VDLUFA (4.11.6) or equivalent to quantify valine in water.

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.