SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of L-valine produced by *Corynebacterium glutamicum* (KCCM 80058) for all animal species, based on a dossier submitted by CJ Europe GmbH[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

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This scientific output, published on 2 September 2014, replaces the earlier version published on 12 May 2014.[4]

ABSTRACT

L-Valine is a feed additive produced by a genetically modified strain of *Corynebacterium glutamicum*. Neither the production strain nor its recombinant DNA was found in the final product. The final product does not raise any safety concern with regard to the genetic modifications. L-Valine is safe for all target animals when added in appropriate amounts to diets. However, the FEEDAP Panel has concerns over the safety of L-valine for target species when administered via water for drinking since any additional supply of an essential amino acid would disturb the balanced amino acid pattern of complete diets. When used in animal nutrition, L-valine will be incorporated into the protein of the body of the target animal, and any potential excess will be metabolised and excreted. Considering the high purity of the product under assessment, and the lack of toxicological effects at likely use levels, no risks are expected for the consumer from the use L-valine as a feed additive. The L-valine produced by such strain of *C. glutamicum* has been shown not to be an irritant or a dermal sensitiser. The results of an acute inhalation study performed at high concentration show no evidence of significant acute toxicity by this route. Exposure of users by inhalation cannot be excluded. L-Valine is a natural constituent of plants and animals. Its use in animal nutrition does not represent a risk to the environment. L-Valine is efficacious as a supplemented amino acid to maintain or restore the adequate balance of amino acids for animal nutrition. A response in ruminants requires some degree of protection of L-valine from ruminal degradation.

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[1] On request from the European Commission, Question No EFSA-Q-2012-00920, adopted on 8 October 2013.

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[4] Revision 1 – 12/05/2014: This scientific opinion has been edited following the adoption of the decision of the Commission on confidentiality claims submitted by the applicant, in accordance with Article 8(6) and Article 18 of Regulation (EC) No 1831/2003. The modified sections are indicated in the text.
Revision 2: An editorial amendment was carried out that does not materially affect the contents or outcome of this Scientific Opinion. To avoid confusion, the original version has been removed from the EFSA Journal, but is available on request, as is a version showing all the changes made.

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KEY WORDS

nutritional additives, amino acids and their salts and analogues, L-valine, genetically modified microorganisms, safety, efficacy

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-valine (98 %) produced by fermentation using a genetically modified strain of *Corynebacterium glutamicum* (KCCM 80058) 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 limiting amino acid after L-tryptophan in pigs for fattening and the fourth after L-threonine in chickens for fattening.

Neither the production strain nor its recombinant DNA was detected in the final product. L-Valine produced by fermentation with *C. glutamicum* (KCCM 80058) does not raise any safety concern with regard to the genetic modification.

L-Valine is safe for all target animals when added in appropriate amounts to diets. However, the FEEDAP Panel has concerns over the safety of L-valine for target species when administered via water for drinking since any additional supply of an essential amino acid would disturb the balanced amino acid pattern of complete diets.

L-Valine will be incorporated into the protein of the body of the target animal, and any potential excess will be metabolised and excreted. Considering the high purity of the product under assessment, no risks are expected for the consumer from the use of L-valine as a feed additive.

The L-valine produced by *C. glutamicum* (KCCM 80058) has been shown not to be an irritant or a dermal sensitiser. The results of an acute inhalation study performed at high concentration show no evidence of significant acute toxicity by this route. Exposure of users by inhalation cannot be excluded.

L-Valine is a natural constituent of plants and animals. Its use in animal nutrition does not represent a risk to the environment.

L-Valine is efficacious as a supplemented amino acid to maintain or restore the adequate balance of amino acids for animal nutrition. A response in ruminants requires some degree of protection of L-valine from ruminal degradation.

The FEEDAP Panel made a recommendation regarding the description of the additive.

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BACKGROUND

Regulation (EC) No 1831/2003[5] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from CJ Europe GmbH[6] for authorisation of the product L-valine, 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) under the conditions mentioned in Table 1.

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.[7] According to Article 8 of that Regulation, 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. The particulars and documents in support of the application were considered valid by EFSA as of 04 March 2013.

The additive under application L-valine is produced using a genetically modified *Corynebacterium glutamicum* (KCCM 80058). It has not been previously authorised as feed additive in the European Union.

L-Valine produced by *Escherichia coli* (K-12 AG314) FERM ABP-10640, currently authorised as nutritional additive until 3rd of June 2019,[8] was assessed by the EFSA FEEDAP Panel in 2008 (EFSA, 2008a,b). The safety of L-valine when used as food flavouring was assessed by Joint FAO/WHO Expert Committee on Food Additives (JEFCA, 2006) and by the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) (EFSA, 2008c).

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.[9] It may also be added for specific nutritional purposes in foods for particular nutritional uses according to Commission Directive 2001/15/EC.[10] 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).[11] L-Valine has a dedicated monograph in the European Pharmacopoeia (EurPh).[12]

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall 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 the efficacy of the product L-

[5] 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.
[6] CJ Europe GmbH, Ober der Roeth 4, 65824 Schwalbach am Taunus, Germany.
[7] EFSA Dossier reference: FAD-2012-0028.
[8] Commission Regulation (EC) 403/2009 of 14 May 2009 concerning the authorisation of a preparation of L-valine as feed additive. OJ L 120, 15.05.2009, p. 2.
[9] Commission Directive 2006/141/EC on infant formulae and follow-on formulae, OJ L 401, 30.12.2006, pp. 1–33.
[10] 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, p. 1.
[11] 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, p. 1.
[12] EurPh monograph 01/2008: 0796, correction 6.0.

valine (L-Valine, feed grade) produced using a genetically modified *Corynebacterium glutamicum*, when used under the conditions described in Table 1.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	L-Valine produced by fermentation with *Corynebacterium glutamicum* KCCM 80058			
Registration number/EC No/No (if appropriate)				
Category(ies) of additive	3. Nutritional additive			
Functional group(s) of additive	c. Amino acids, their salts and analogues			
Description				
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)	
L-Valine	**$C_5H_{11}NO_2$**	**> 98.0 %**	**Reg. No. 152/2009**	
Trade name (if appropriate)	L-Valine feed grade			
Name of the holder of authorisation (if appropriate)	CJ Europe GmbH			
Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg or Units of activity or CFU/kg of complete feedingstuffs (select what applicable)		
Other provisions and additional requirements for the labelling				
Specific conditions or restrictions for use (if appropriate)	not applicable			
Specific conditions or restrictions for handling (if appropriate)	Please refer to the MSDS			
Post-market monitoring (if appropriate)	not applicable			
Specific conditions for use in complementary feedingstuffs (if appropriate)	not applicable			
Maximum Residue Limit (MRL) (if appropriate)				
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues	

ASSESSMENT

The application is for L-valine obtained by fermentation using a genetically modified strain of *Corynebacterium glutamicum* (KCCM 80058). The product under application is intended to be used in the feed or water for drinking of all animal species and categories.

This opinion is based in part on data provided by a single company involved in the production/distribution of L-valine. It should be recognised that these data cover only a fraction of existing additives containing L-valine. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) has sought to use the data provided, together with data from other sources, to deliver an opinion.

1. Introduction

Valine is an essential proteogenic amino acid belonging, along with leucine and isoleucine, to the group of branched-chain amino acids (BCAAs). The objective of feed supplementation with essential amino acids is to complete the amino acid profile of the diet in order to closely meet the requirement for individual amino acids of the animals. The supplementation of feedingstuffs with amino acids is a conventional measure in improving protein quality and utilisation. This supplementation has become even more important since protein-reduced diets were introduced in animal husbandry for economic and environmental reasons. It is well recognised that valine as an essential amino acid may become limiting under specific feeding conditions.

2. Characterisation

The FEEDAP Panel notes that all data necessary to characterise the product are based on pilot batches since industrial large-scale production has not yet started. The data can be taken as representative of the commercial product only if large-scale manufacturing would result in a product identical to the pilot product. The applicant confirmed that commercial production will apply the same fermentation and purification criteria as used for the pilot products. Consequently, the following assessment could apply only if these conditions are met.

2.1. Characterisation of the active substance/additive

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

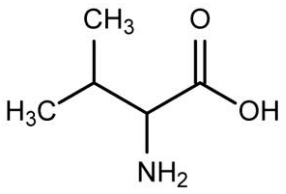

Figure 1: Molecular structure of valine

According to specification, the additive L-valine contains ≥ 98 % valine, ≤ 1.5 % water and ≤ 0.1 % ash.[13] The analyses of five pilot batches show an average content of 99.3 % valine (range 98.7–99.5 %), 0.28 % leucine (range 0.16–0.38 %) and 0.06 % of water (range 0.04–0.07 %). The amount of unidentified impurities ranges from 0.09 to 0.95 % on a dry matter basis.[14] The average figure for

[13] Technical dossier/Section II.1.3/Annex II.1.1.
[14] Technical dossier/Section II.1.3/Annex II.1.2.

specific optical rotation (five batches) was + 28.6° (range + 28.5 to + 28.8°) and thus within the range set in the European Pharmacopoeia for the L-enantiomer of valine.[15]

2.2. Impurities

The applicant provided analytical data on heavy metals (mercury, cadmium and lead), arsenic and dioxins (polychlorinated dibenzodioxins (PCDD)/polychlorinated dibenzofurans (PCDF)) in five pilot batches of the additive.[16] The amounts detected of all these impurities were negligible and often below the detection limits.[17] Trace amounts of several elements (Cr, Cu, Ni, Zn, F, Na, K, Ca, Cl), NH_4^+, SO_4, PO_4 and amino acids (leucine, isoleucine, tyrosine, phenylalanine, lysine) were found in the same five pilot batches.

The analysis of five pilot batches for microbial contamination showed < 100 colony-forming units (CFU *E. coli* and ≤ 1 400 CFU aerobic mesophilic bacteria per gram of additive whereas *Salmonella* was negative in 25 g of the product. Aflatoxins, zearalenone and deoxynivalenol (determined by enzyme-linked immunosorbent assay (ELISA)) in the same five batches were below the limit of detection (< 1.7, < 17.0 and < 134.0 µg/kg, respectively); ochratoxins were ≤ 5.3 µg/kg.[18]

2.3. Physical state of the product

The product is white crystalline powder with a bulk density of 0.4–0.6 g/cm^3 and solubility in water ranging from 53 to 57 g/L at 20 °C. Its melting point is 298 °C. [19]

The particle size distribution of three pilot batches tested by sieving showed that only 0.5 % (w/w) of the particles had a size below 100 µm.[20] The dusting potential (Stauber–Heubach test) of three product batches was within the range 0.78–1.24 g/m^3. [21]

2.4. Characterisation of the production organism[22]

L-Valine is produced by fermentation with a genetically modified strain of *C. glutamicum* that is deposited in the Korean Culture Center of Microorganisms (KCCM) in South Korea with the accession number KCCM 80058.[23]

The production strain belongs to a species that is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment (EFSA 2007, 2012). The technical dossier contained detailed and sufficient information on the recipient microorganism, the genetic modification process and on the genetic and phenotypic traits introduced.

2.4.1. Information relating to the manufacturing process

L-valine is produced by fermentation of the production strain and it is isolated and purified. Material safety data sheets (MSDS) for the fermentation media components used in the production and purification are provided in the technical dossier. MSDS for the final product are also provided.[24]

[15] Supplementary information April 2013/Annex Qi.
[16] Technical dossier/Section II.1.3/Annex II.1.2.
[17] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.5.2002, p. 10.
[18] Technical dossier/Section II/Annex II.1.3.
[19] Technical dossier/Section II/Annex II.3.1.
[20] Technical dossier/Section II/Annex II.1.4.
[21] Technical dossier/Section II/Reference III.3.1.
[22] This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
[23] Technical dossier/Section II/Annex Confidential II.2.1.
[24] Technical dossier/Confidential annexes II.3.1 to II.3.10.

Neither the production strain nor its DNA was detected in the three batches of final products.[25,26]

2.5. Stability and homogeneity

The shelf-life of three pilot batches was studied under two different temperatures (at 25 and 40 °C) in sealed brown glass containers for 18 months. There were virtually no losses of the amino acid L-valine.[27]

L-Valine (three batches) was incorporated at 10 % into a vitamin/mineral premixture also containing choline. The premixtures were stored at 25 or 40 °C in sealed brown glass containers for six months. No losses were observed.[28]

L-Valine (three batches) was added at a level of 0.3 % to a complete mash feed for piglets based on wheat, barley, soybean and maize.[29] Pelleting (temperature 60 °C for approximately eight seconds) resulted in 9 % loss of free valine. Samples of mash and pelleted feed were subsequently stored in sealed brown glass containers at 25 or 40 °C for six months. No loss of free valine was observed in the mash feed, whereas in pelleted feed the losses at 25 or 40 °C were 3 and 6 %, respectively.

L-Valine (three batches) was diluted to a concentration of 0.1 % in water for drinking and solutions were stored at 25 and 40 °C for 48 hours.[30] No losses of valine were observed.

The homogeneous distribution of one batch of the additive when supplemented at 10 % inclusion level into a premixture for piglets was studied by analysing 11 subsamples. The coefficient of variation (CV) of the mean was 1 %.[31] In mash and pelleted feed for piglets (0.3% of L-valine supplemented) the analysis of 10 subsamples each showed a CV of 3 % for both.

2.5.1. Incompatibilities

No physicochemical incompatibilities in feed are expected with other additives, medicinal products or other feed materials.

2.6. Conditions of use

L-Valine is intended to be used for all animal species and categories with no minimum or maximum content specified. It is intended to be added to feedingstuffs via premixtures or directly into complete feed and complementary feed. This product is also proposed for the use in water for drinking.

2.7. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of L-valine in animal feed. The executive summary of the EURL report can be found in Appendix A.

3. Safety

3.1. Safety aspects of the genetic modification[32]

The parental organism, *C. glutamicum*, is considered by EFSA to be suitable for the QPS approach to safety assessment (EFSA, 2007, 2012) for amino acid production purposes. The genetic modification does not raise any safety concerns regarding the final product.

[25] Supplementary information (April 2013)/Question (iv) and Annex Confidential Qiv.
[26] Supplementary information (April 2013)/Question (v) and Annex Confidential Qv.
[27] Supplementary information (October 2013)/Annex II.4.1.
[28] Technical dossier/Section II.4.1/Annexes II.4.2 and II.4.9.
[29] Technical dossier/Section II.4.1/Annexes II.4.3 and II.4.9.
[30] Technical dossier/Section II.4.1/Annex II.4.4.
[31] Technical dossier/Section II.4.1/Annex II.4.8.
[32] This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.

3.2. Safety for the target species

Tolerance studies are not normally required for highly purified amino acids independent of the manufacturing process. Such tolerance studies with a certain 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 contains > 98.5 % valine and < 1 % unidentified substances. Therefore, the FEEDAP Panel considers that safety concerns for target species are highly unlikely to arise from the L-valine under application.

Depending on the animal species and the genetics, sex and physiological state of the animal, the requirements for L-valine in feed range in pigs from 0.40 to 0.84 % and in poultry between 0.43 % (laying hens) and 1.2 % (turkey pullets up to four weeks of age) (NRC 1994, 1998). The requirements according to GfE (2008) for valine in feed for pigs of various categories are from 0.63 to 1.05 %. Depending on dietary composition, the standardised ileal digestible valine-to-lysine requirement ratios for both piglets after weaning and chickens for fattening are > 70 % (Huang et al., 2007; Barea et al., 2009). Common vegetable feed materials contain 0.4–3.0 % L-valine, while animal-derived feed materials contain substantially more (e.g. up to 8.5 % in blood meal). The supplementation levels of L-valine in compound feeds, particularly those with lower crude protein, are in the range 0.01–0.2 %.

The tolerance of animals to overdoses of amino acids is known to vary with the amino acid (substance and isomeric form), the dietary protein content and the animal species. In general, the BCAAs are well tolerated when provided in great excess (Baker, 2004). Dietary overdoses of L-valine and L-isoleucine are better tolerated by almost all animal species than an excess of L-leucine; this could be ascribed to their metabolic fate (L-valine glucogenic, L-isoleucine both glucogenic and ketogenic, L-leucine only ketogenic).

The BCAAs L-valine, L-isoleucine and L-leucine exert a strong antagonism on each other, resulting in an alteration of the plasma and brain amino acid concentrations (imbalance) which is responsible for a reduced feed intake with impaired weight gain and feed efficiency (Harper et al., 1984). When investigating the dietary excesses of individual BCAAs on availability of other pairs of BCAAs in chickens for fattening (Allen, 1971; Allen and Baker, 1972), overdosing with L-leucine or L-isoleucine reduced the utilisation of the other two BCCAs but overdosing with valine (up to 3 %) did not. It was found in chickens for fattening fed diets with various L-isoleucine content that an excess of L-valine did not affect the zootechnical performance, whereas an increase in the dietary L-leucine to L-isoleucine ratio depressed the feed intake (Burnham et al., 1992). The pronounced antagonism between the amino acids L-isoleucine and L-valine + L-leucine was also demonstrated in laying hens. At a low dietary concentration of L-valine + L-leucine, an increase in dietary L-isoleucine concentration led to a dose-dependent reduction in feed consumption, daily egg mass and body weight gain and an increase in the L-isoleucine concentration in plasma. However, at a high level of dietary concentration of L-valine + L-leucine, excessive dietary supplementation with L-isoleucine caused only a weak depression in performance parameters and the L-isoleucine concentration in plasma was independent of its dietary L-isoleucine level (Peganova and Eder, 2003). It has been suggested that an increased supply of L-leucine stimulates the activity of the enzyme complex involved in the metabolism of BCAAs in pigs and may therefore also increase the catabolism of L-valine and L-isoleucine (Wiltafsky et al., 2009) and thus their requirements. In an experiment with pigs for fattening focused on biochemistry endpoints (serum amino acid levels, expression of genes encoding b(0,+)), a dietary excess of L-leucine was shown to limit the availability of L-valine, whereas a surplus of L-valine could correct some of the negative effects of excessive dietary L-leucine (Morales et al., 2012).

Cats are less sensitive to excesses of the BCAAs than other animal species (Hargrove et al., 1988). The addition of 10 % of L-leucine, L-valine or L-isoleucine to semi-purified diets caused no adverse effects in kittens when the concentrations of the other BCAAs were at, or slightly above, the requirement levels. The same diets fed to rats resulted in drastic growth and feed intake depressions (Hargrove et al., 1988). In broiler chickens, excessive L-valine additions (4 %) had only small effects on weight gain (–4 %; $P > 0.05$), whereas feed intake was significantly reduced by 6 % (Edmonds and

Baker, 1987). On the other hand, another study on chickens for fattening fed up to the age of three weeks a diet containing 3.15 % L-valine showed a significantly reduced growth rate of birds but without a change in feed intake (Carew et al., 1998); however, the impaired growth was accompanied by elevated plasma levels of triiodothyronine (T3), which might be indicative of increased temperature and therefore energy expenditure.

In ruminants, the initial product of L-valine degradation by ruminal microbiota is isobutyric acid (Allison, 1970), with no recorded deleterious effects on the host animal. Therefore, there are no safety concerns arising from ruminal L-valine metabolism.

Concerning the proposed use of L-valine in water for drinking, the FEEDAP Panel notes that complete diets for all animal species, and particularly those for food-producing animals, achieve a well-balanced protein content by optimally adjusting amino acid supplementation to the specific requirements of the different animal species and categories for essential amino acids (the ideal protein concept). Each additional administration of individual essential amino acids (e.g. via water for drinking) will negatively affect the amino acid balance and consequently the performance of the animals, as well as target animal safety, in a dose-dependent manner. The FEEDAP Panel therefore has concerns over the safety of L-valine for target species when administered via water for drinking.

3.2.1. Conclusions on safety for the target species

The FEEDAP Panel concludes that the additive L-valine produced by *C. glutamicum* (KCCM 80058) is safe for all animal species. However, excess doses would create amino acid imbalances with negative consequences for animal performance. Correct dosing in formulating diets requires knowledge of the amino acid content in feed materials and of the requirements of animals. These data are all freely accessible. The FEEDAP Panel has concerns over the safety of L-valine for target species when administered via water for drinking since any additional supply of an essential amino acid would disturb the balanced amino acid pattern of complete diets.

3.3. Safety for the consumer

The absorption and metabolic fate of valine are briefly described in Appendix B.

As a general principle, conventional toxicology studies are considered to be inappropriate for amino acids. Dietary intakes of amino acids that lead to amounts significantly below or above that which is optimum for health and performance will inevitably cause a physiological imbalance and consequently adverse effects.

The product under assessment is produced by fermentation. Concerns for the consumer would arise not from the amino acid itself, which will be incorporated into protein, but from possible residues from the fermentation. In this case, the additive is highly purified (amount of unidentified compounds < 1 % dry matter) and, therefore, following the provisions of the guidance on nutritional additives, it is considered to be of no concern for consumers.

This is confirmed by data from one acute toxicity study of the product under assessment (carried out in accordance with to OECD Guideline No 423) in female rats. It showed no adverse effects at doses up to 2 000 mg/kg body weight.[33]

The use of L-valine produced by *C. glutamicum* (KCCM 80058) in animal nutrition is of no safety concern for the consumer.

[33] Technical dossier/Section III/Reference III.2.1 and supplementary information (September 2013).

3.4. Safety for the user

The following tests were performed with the product L-valine under assessment.[34]

3.4.1. Effects on the respiratory system

An acute inhalation study was performed in compliance with OECD Guideline 436 (2009).[35] A group of three male and three female RccHan:WIST rats was exposed nose-only for four hours to an atmosphere containing 5.07 mg/L L-valine, followed by a 14-day observation period. No mortality occurred, and at necropsy no macroscopic abnormalities were observed. The four-hour median concentration (LC_{50}) was derived to be higher than 5.07 mg/L. This dose (5.07 mg/L) is equivalent to approximately 6.1 g/kg of air, which is regarded as "slightly toxic" (Hodge and Sterner scale).[36]

3.4.2. Effects on skin and eyes

In a dermal irritation/corrosion test conducted in accordance with OECD Guideline No 404 (2002), 0.5 g of L-valine was applied to the skin of three New Zealand rabbits.[37] Clinical signs, body weight and adverse effects at the application site were surveyed over 72 hours. There were no observations of irritancy to the skin of any rabbit; thus, it was concluded that the substance is non-irritant to the skin.

In an eye irritation test conducted in accordance with OECD Guideline No 405 (2002), 0.1 g of L-valine was instilled to one of the conjunctival sacs of three New Zealand rabbits.[38] Clinical signs, body weight and adverse effects at the application site were surveyed over 72 hours. There were no observations of irritancy in the eye of any rabbit; thus, it was concluded that the substance is non-irritant to the eye.

3.4.3. Skin sensitisation

In a skin sensitisation test (Buehler test) conducted in accordance with OECD Guideline No 406 (1992), 40 Hartley guinea pigs were divided into three treatment groups (L-valine sensitisation group (n = 20), control vehicle (n = 10), positive control (n = 10)), induced over three weeks (once per week) and challenged two weeks after the last induction.[39] Induction and challenge with L-valine were performed with a dose of 100 mg/mL. Clinical signs, body weight and skin response (at 30 and 54 hours after challenge) were surveyed. As there was no increase in skin reaction in the sensitisation group compared with controls, the substance is not considered to induce skin sensitisation.

3.4.4. Conclusions on safety for the user

The product L-valine produced by *C. glutamicum* (KCCM 80058) has been shown not to be an irritant or a dermal sensitiser. The results of an acute inhalation study performed at a high concentration show no evidence of significant acute toxicity by this route. Exposure of users by inhalation cannot be excluded.

3.5. Safety for the environment

Neither the production strain nor its recombinant DNA was detected in the final product. The final product does not raise any environmental safety concerns associated with the genetic modification.

The amino acid L-valine is a physiological and natural component of animals and plants. It is not excreted as such (but as urea/uric acid and CO_2). 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.

[34] Supplementary information September 2013.
[35] Technical dossier/Section III/Reference III.3.2.
[36] Canadian Centre for Occupational Health and Safety, 2013 (www.ccohs.ca).
[37] Technical dossier/Section III/Reference III.3.4.
[38] Technical dossier/Section III/Reference III.3.3.
[39] Technical dossier/Section III/Reference III.3.5.

4. 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-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 hours (Chalupa, 1976). Broderick and Balthrop (1979) found that 45 % of free L-valine added to ruminal digesta *in vitro* remained after three hours. 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.

Data on the requirements, allowances and recommendations for valine in the different animal species and categories, and consequently for feed supplementation, are easily accessible in standard animal nutrition literature.

4.1. Conclusions on efficacy

The additive L-valine is regarded as an efficacious source of the essential amino acid L-valine for animal nutrition. A response in ruminants requires some degree of protection of L-valine from ruminal degradation.

5. 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[40] and good manufacturing practice.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Neither the production strain nor its recombinant DNA were detected in the final product. L-Valine produced by fermentation with *C. glutamicum* (KCCM 80058) does not raise any safety concerns with regard to the genetic modification.

L-Valine is safe for all target animals when added to diets in appropriate amounts. However, the FEEDAP Panel has concerns over the safety of L-valine for target species when administered via water for drinking since any additional supply of an essential amino acid would disturb the balanced amino acid pattern of complete diets.

L-Valine will be incorporated into the protein of the body of the target animal, and any potential excess will be metabolised and excreted. Considering the high purity of the product under assessment, no risks are expected for the consumer from the use of L-valine as a feed additive.

The L-valine produced by *C. glutamicum* (KCCM 80058) has been shown not to be an irritant or a dermal sensitiser. The results of an acute inhalation study performed at a high concentration show no

[40] 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.

evidence of significant acute toxicity by this route. Exposure of users by inhalation cannot be excluded.

L-Valine is a natural component of plants and animals. Its use in animal nutrition does not represent a risk to the environment.

L-Valine is efficacious as a supplemented amino acid to maintain or restore the adequate balance of amino acids in animal nutrition. A response in ruminants requires some degree of protection of L-valine from ruminal degradation.

RECOMMENDATIONS

The description of the additive should include: "produced by fermentation with *Corynebacterium glutamicum* KCCM 80058."

DOCUMENTATION PROVIDED TO EFSA

1. L-Valine, feed grade. October 2012. Submitted by CJ Europe GmbH.
2. L-Valine, feed grade. Supplementary information. April 2013. Submitted by CJ Europe GmbH.
3. L-Valine, feed grade. Supplementary information. September 2013. Submitted by CJ Europe GmbH.
4. L-Valine, feed grade. Supplementary information. October 2013. Submitted by CJ Europe GmbH.
5. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for L-valine produced by *Corynebacterium glutamicum* KCCM 80058.
6. Comments from Member States received through ScienceNet.

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APPENDICES

Appendix A. Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for l-Valine Produced by *Corynebacterium glutamicum* KCCM 80058[41]

In the current application authorisation is sought under Article 4(1) for *L-valine* produced by *Corynebacterium glutamicum* KCCM 80058, 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*. The *feed additive* is intended to be mixed either in *premixtures* or added directly to *feedingstuffs* or *water*. The Applicant proposed no minimum or maximum *L-valine* concentrations in feedingstuffs.

For the determination of *L-valine* in *feed additives*, *premixtures* and *feedingstuffs* the Applicant submitted the Community method (Commission Regulation (EC) No 152/2009) further ringtrial validated by CEN, resulting in the EN ISO 13903:2005 standard method. The following performance characteristics were reported for the determination of *total valine* in *premixtures* and *feedingstuffs*:

- a relative standard deviation for *repeatability* ranging from 1.7 to 3.8 %; and
- a relative standard deviation for *reproducibility* ranging from 8.8 to 16 %.

Even though no performance characteristics are available for the determination of *valine* in *feed additive*, several NRLs [National Reference Laboratories] consider the Community method suitable for official control, as already recommended by the EURL in earlier reports (FAD-2007-0015; FAD-2012-0023). Furthermore, the EURL identified the "*L-valine* monograph" of the Food Chemical Codex (FCC), where identification is based on infrared absorption in combination with the analysis of the optical rotation, while quantification is based on titration.

Based on the technical evidence available, the EURL recommends for official control (i) the Food Chemical Codex for the determination of *L-valine* in the *feed additive* and (ii) the ringtrial validated Community method (EN ISO 13903:2005), based on ion exchange chromatography coupled with post-column derivatisation and spectrophotometric detection to determine *valine* in *feed additive*, *premixtures* and *feedingstuffs*.

The EURL cannot evaluate nor recommend any method for official control to determine *L-valine* in *water*, as the Applicant did not provide any experimental data or analytical methods.

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.

[41] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2012-0028-L-Valine.doc.pdf

Appendix B. The metabolic fate of valine

Several sodium-dependent transporters involved in valine absorption from the small intestine were described for the apical and basolateral membrane of the enterocytes (Munck and Munck, 1994; Bröer, 2008). In pigs, the apparent ileal digestibility of valine in feed ingredients varies and ranges from about 71 % for cottonseed meal, through 63–80 % for cereals to 81–88 % for soybean meal/soy isolate, milk/whey and fish meals (NRC, 1998). For the standardised precaecal valine digestibility, Gesselschaft für Ernährungsphysiologie (GfE) reports for cereals values from 78 to 92 %, for by-products (e.g. maize gluten, wheat bran, whey powder) values of 73–92 %, for feeds rich in protein (e.g. field beans, cotton seed meal, linseed meal, soybean meal, fish meal) values of 65–86 % (GfE, 2005, 2008). The apparent digestibility of crystalline L-valine is almost 100 %, as for most crystalline amino acids (Chung and Baker, 1992). However, microbial degradation in the small intestine may negatively affect the bioavailability of free amino acids (Dierick et al., 1986; Van der Meulen et al., 1998; Bikker et al., 2006; Dai et al., 2012). After being absorbed, valine is used for tissue protein synthesis, for maintaining a balance among the BCAAs and for synthesis of glutamine and alanine. It serves as a donor of nitrogen for various α-ketoacids, such as for maintaining glutamate as a neurotransmitter (Hutson et al., 2005; Wu, 2009). For a detailed scheme of valine metabolic pathway see Shimomura et al. (2004).

Valine catabolism involves two initial enzymatic steps that are common for all three BCAAs. The first step is reversible transamination followed by irreversible oxidative decarboxylation of α-ketoisovalerate involving a multienzyme complex (a decarboxylase, a transacylase and a dehydrogenase). The regulation of this complex determines BCAA homeostasis and is assumed to be the rate-limiting step in BCAA catabolism (Matthews et al., 1981). With the exception of the brain, all reactions occur in the mitochondria of the cell. Valine is a glucogenic amino acid, with its carbon skeleton entering the tricarboxylic acid cycle as succinyl-CoA within multiple enzymatic reactions (metabolising through 3-hydroxyisobutyrate and methylmalonyL-CoA) (Harper et al., 1984; Hutson et al., 2005). The final catabolic products of valine metabolism (urea/uric acid and carbon dioxide) are excreted by urine and exhalation.

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