SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of L-methionine produced by *Escherichia coli* (KCCM 11252P) and *Escherichia coli* (KCCM 11340P) for all animal species[1]

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

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 12 May 2014, replaces the earlier version published on 29 October 2013.[4]

ABSTRACT

L-Methionine, feed grade, is a feed additive produced by two genetically modified *Escherichia coli* strains. Neither the production strains nor their recombinant DNA were detected in the final product; thus, no safety concerns are associated with the genetic modification of the production strains. The L-methionine produced by such *E. coli* strains is safe for the target animals when used as a feed additive to meet their requirements. The FEEDAP Panel has concerns over the safety of L-methionine for the target species when administered via water for drinking since any additional supply of an essential amino acid would disturb the balanced amino acid profile of complete diets. There are no safety concerns for the consumer associated with the use of L-methionine in animal nutrition. The additive L-methionine was demonstrated to be without irritant or dermal sensitising properties. Although no data on dusting potential were provided, the FEEDAP Panel considers it unlikely that the user will be exposed to significant amounts of L-methionine by inhalation. L-Methionine is a natural constituent of plants and animals. Its use in animal nutrition does not represent a risk to the environment. The additive L-methionine is considered an efficacious source of the amino acid L-methionine for all animal species.

KEY WORDS

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

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[1] On request from the European Commission, Question No EFSA-Q-2012-00581, adopted on 8 October 2013.

[2] Panel members: Gabriele Aquilina, Vasileios Bampidis, Maria De Lourdes Bastos, Lucio Guido Costa, Gerhard Flachowsky, Mikolaj Antoni Gralak, Christer Hogstrand, Lubomir Leng, Secundino López-Puente, Giovanna Martelli, Baltasar Mayo, Fernando Ramos, Derek Renshaw, Guido Rychen, Maria Saarela, Kristen Sejrsen, Patrick Van Beelen, Robert John Wallace and Johannes Westendorf. Correspondence: FEEDAP@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Amino Acids, including Paul Brantom, Noël Dierick and Jürgen Gropp, and the members of the Working Group on Genetically Modified Microorganisms of the FEEDAP Panel, including Pier Sandro Cocconcelli, Boet Glandorf, Lieve Herman Sirpa Kärenlampi and Christoph Tebbe, for the preparatory work on this scientific opinion.

[4] Revision 1: 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.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific Opinion on the safety and efficacy of L-methionine produced by *Escherichia coli* (KCCM 11252P) and *Escherichia coli* (KCCM 11340P) for all animal species. EFSA Journal 2013;11(10):3428, 16 pp. doi:10.2903/j.efsa.2013.3428

Available online: www.efsa.europa.eu/efsajournal

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-methionine produced by two genetically modified *Escherichia coli* strains (KCCM 11252P and KCCM 11340P) for all animal species.

Methionine is an indispensable amino acid for all animal species. Methionine is recognised as the first limiting amino acid in feed for poultry, probably also the first for high-yielding cows and the third one in pigs fed conventional diets.

Neither the production strains nor their recombinant DNA were detected in the final product. No safety concerns were associated with the genetic modification of the production strains.

L-Methionine produced by *E. coli* strains (KCCM 11252P and KCCM 11340P) is safe for the target animals when used as a feed additive to meet their requirements. The FEEDAP Panel has concerns over the safety of L-methionine for the target species when administered via water for drinking since any additional supply of an essential amino acid would disturb the balanced amino acid profile of complete diets.

There are no safety concerns for the consumer associated with the use of L-methionine in animal nutrition.

The additive L-methionine was demonstrated to be without irritant or dermal sensitising properties. Although no data on dusting potential were provided, the FEEDAP Panel considers it unlikely that the user will be exposed to significant amounts of L-methionine by inhalation.

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

The additive L-methionine is considered an efficacious source of the amino acid L-methionine for all animal species.

The FEEDAP Panel made a recommendation concerning the product description.

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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 the company CJ Europe GmbH[6] for authorisation of the product L-methionine, L-Methionine 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 07 September 2012.

The additives DL-methionine, DL-methionine sodium salt, hydroxy analogue of methionine, and calcium salt of methionine hydroxy analogue, when used as a feed additive for all animal species; isopropyl ester of methionine hydroxy analogue, DL-methionine technically pure protected with copolymer vinylpyridine/styrene, and DL-methionine protected with ethylcellulose when used as a feed additive for ruminants are currently authorised for use for all animal species and ruminants, respectively, by Regulation (EU) No 469/2013.[8]

EFSA FEEDAP Panel (2012) published a scientific opinion on DL-methionine, DL-methionine sodium salt, hydroxy analogue of methionine, calcium salt of methionine hydroxy analogue for all animal species; isopropyl ester of methionine hydroxy analogue and DL-methionine technically pure protected with copolymer vinylpyridine/styrene for dairy cows; DL-methionine technically pure protected with ethylcellulose for ruminants.

EFSA FEEDAP Panel (2013) published a scientific opinion on the safety and efficacy of methionine-zinc, technically pure, as amino acid for ruminants, and as compound of trace element for all species.

According to Commission Directive 2006/141/EC amino acids as L-methionine may be used in the manufacture of infant formulae and follow-on formulae in order to satisfy the requirements on amino acids and other nitrogen compounds.[9] L-Methionine is currently authorised as flavouring substance in feed. It is included in the Community list of flavouring substances as FL. No. 17.027. Methionine is registered as an ingredient for use in cosmetics as antistatic and for skin conditioning (Commission Decision 2006/257/EC).[10]

[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 04, 65824 Schwalbach, Germany).
[7] EFSA Dossier reference: FAD-2012-0016.
[8] Commission Implementing Regulation (EU) No 469/2013 of 22 May 2013 concerning the authorisation of DL-methionine, DL-methionine sodium salt, hydroxy analogue of methionine, calcium salt of hydroxy analogue of methionine, isopropyl ester of hydroxy analogue of methionine, DL-methionine protected with copolymer vinylpyridine/styrene and DL-methionine protected with ethylcellulose as feed additives. OJ L 136, 23.5.2013, p. 8.
[9] Commission Directive 2006/141/EC on infant formulae and follow-on formulae. OJ L 401, 30.12.2006, p. 1.
[10] Commission Decision of 9 February 2006 amending Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products. OJ L 97, 5.4.2006, p. 1.

DL-Methionine is described in the European Pharmacopeia, monograph 01/2008:0624. Methionine does not require maximum residue levels in all food producing species when used as pharmacologically active substance.[11]

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-methionine produced using two genetically modified strains of *Escherichia coli*, when used under the conditions described in Table 1.

[11] Commission Regulation (EC) No 1931/1999, amending Annexes I, II and III of Council Regulation (EEC) No 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. OJ L 240, 10.09.1999, p. 3.

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

Additive	L-Methionine produced by fermentation with Escherichia coli KCCM11252P			
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-Methionine	**$C_5H_{11}NO_2S$**	**> 98.5 %**	**Reg. No. 152/2009**	
Trade name (if appropriate)	L-Methionine 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 Panel on Additives and Products or Substances Used in Animal Feed (FEEDAP Panel) has sought to use the data provided by the applicant, together with data from other sources, to deliver an opinion.

1. Introduction[12]

Methionine is an indispensable amino acid for all animal species. Methionine is clearly recognised as the first limiting amino acid in poultry, probably also the first for high-yielding cows and the third one in pigs fed conventional diets. Therefore, additives containing DL-methionine or the hydroxy-analogue of methionine as the active substance are frequently used in the feed industry to adjust the dietary methionine to the requirements of target animals in order to achieve more efficient conversion of feed and reduce nitrogen emissions.

The current application is for a new additive, L-methionine produced by fermentation with two genetically modified strains of *Escherichia coli* (KCCM 11252P and KKCCM 11340P) in a single production process. The application is for all animal species.

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 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. Characterisation

2.1. Characterisation of the active substance/additive

L-Methionine (International Union of Pure and Applied Chemistry (IUPAC) name: (2*S*)-2-amino-4-(methylthio)butanoic acid; Chemical Abstracts Service (CAS) No 59-51-8) has a molecular weight of 149.2 g/mol; the molecular formula is $C_5H_{11}NO_2S$ and its molecular structure is given in Figure 1.

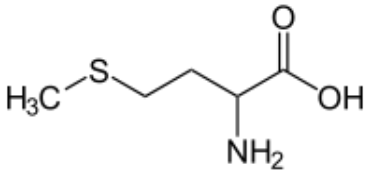

Figure 1: Molecular structure of methionine

According to specification the product contains ≥ 98.5 % L-methionine, ≤ 0.5 % water (loss on drying) and ≤ 0.1 % ash.[13] The analysis of five batches of the additive showed an average of 99.2 % L-methionine (range 98.5–99.9 %) and of 0.41 % (range 0.38–0.44 %) for the sum of other amino acids (phenylalanine, leucine, tyrosine, isoleucine, valine). Other constituents are water (0.04–0.11 %) and minerals (about 0.05 %). Ammonia did not exceed 0.01 %.[14] The highest amount for unidentified impurities was calculated to be 0.23 % on a dry matter basis.[15] The specific optical rotation determined in five batches of the final product was + 23.6° in all cases (specific optical rotation of an L-methionine standard + 23.4° (Kleemann et al., 1985)).[16]

[12] This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
[13] Technical dossier/Section II/Annex II.1.1.
[14] Technical dossier/Section II/Annex II.1.2.
[15] Technical dossier/Section II.1.3.
[16] Supplementary information February 2013/Annex Qi.

2.2. Impurities[17]

O-Acetylhomoserine, was analysed by liquid chromatography–mass spectrometry (LC-MS) in five batches. The data showed that *O*-acetylhomoserine in the final product was consistently below 0.02 %.[18]

Five batches were analysed for heavy metals (lead, cadmium and mercury) and arsenic. The amounts detected of all these impurities were negligible and often below the detection limits. Three batches were also tested for aflatoxin B1 and ochratoxin A. Aflatoxin was < 1.7 μg/kg except in one batch, in which the level reached 4 μg/kg. Ochratoxin A was consistently < 5μg/kg.[19] Methanol and methyl mercaptan were below the detection limits in all five batches tested.[20]

2.3. Physical properties

The additive is a white crystalline powder, having slightly sulphurous taste. The particle shape is a plate type. The highest proportion of particles with a diameter < 40 μm was 0.18 % (w/w) in three batches.[21] Other relevant properties provided by the applicant are 500–700 kg L-methionine/m^3 for bulk density and 56.6 g/L for water solubility at 25 °C.[22] No data on dusting potential were provided.

2.4. Characterisation of the production organisms[23]

L-Methionine is produced by fermentation with two genetically modified strains of *E. coli*. Both strains are deposited at the Korean Culture Center of Microorganisms (KCCM) in South Korea *E. coli* KCCM 11252P[24] and *E. coli* KCCM 11340P.[25]

The dossier contains detailed and sufficient information on the recipient strain including safety aspects, the origin and function of the different genetic elements introduced in the production strains the genetic modification processes and the genetic and phenotypic traits introduced.

2.4.1. Manufacturing process

Both production strains *E. coli* KCCM 11252P and *E. coli* KCCM 11340P are used in the same production process to obtain L-methionine. After the fermentation process, purification and isolation of the active substance follow.

The absence of the production strains was confirmed,[26,27] and no recombinant DNA was detected in the final L-methionine product.[28]

2.5. Stability and homogeneity

Three batches of the additive were stored in sealed brown glass containers at two different temperatures (25 °C and 40 °C) for 24 months. The highest loss observed was 1 %.[29]

[17] This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
[18] Supplementary information February 2013/Annex Qiv.
[19] Supplementary information February 2013/Annex Qii.
[20] Supplementary information July 2013/Annexes Q1a and Q1b.
[21] Technical dossier/Section II/Annex II.1.3.
[22] Technical dossier/Section II.2.2.1.
[23] This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
[24] Technical dossier/Supplementary information February 2013/Annex Qiii.a Confid.
[25] Technical dossier/Supplementary information February 2013/Annex Qiii.b Confid.
[26] Technical dossier/Supplementary information February 2013/Annex Qxi Confid.
[27] Technical dossier/Supplementary information February 2013/Annex Qxi Confid.
[28] Technical dossier/Supplementary information May 2013/Annex Confid.Qi.
[29] Technical dossier/Section II.4.1/Annex II.4.1; Supplementary information February 2013/Annex II.4.1 and Supplementary information July 2013/Annex II.4.1.

The stability of three batches of the additive was tested in a vitamin/mineral premixture with choline chloride containing about 9 % L-methionine.[30,31] Samples were stored in sealed brown glass containers at two different temperatures (25 °C and 40 °C) for six months. Losses did not exceed 6 %.

L-Methionine (three batches) was added to a complete feed for chickens for fattening at a rate of 0.25 %. The basal diet consisted mainly of soybean meal, maize and wheat.[32] Samples of the mash and the pelleted feed were stored for six months in sealed brown glass containers at two different temperatures (25 °C and 40 °C). In mash, losses at 25 °C were ≤ 4 % after six months; and at 40 °C were < 11 % after three months and < 22 % after six months. Pelleting at 60 °C for eight seconds resulted in a loss of 4–7 %. No further losses in pelleted feed were observed after six months' storage at 25 °C, but after three months' storage at 40 °C losses were ≤ 5 % and after six months ≤ 23 %.[33,34]

Stability of L-methionine (six batches) was studied at a concentration of 0.1 % in water for drinking at 25 and 40 °C.[35] No significant losses were observed after 24 hours' storage. No information on stability after 48 hours was submitted.

The homogeneous distribution of the additive in premixtures (one batch of the additive, supplemented at 10 %) or in compound feed for chickens for fattening (mash and pelleted, supplemented with one batch of L-methionine at 0.25 %) was studied by analysis in 10 subsamples of each substrate.[36,37] The coefficients of variation were 1.2, 1.9 and 1.6 for premix, mash and pelleted feed, respectively.

2.5.1. Incompatibilities

According to the applicant, no physicochemical incompatibilities or interactions can be expected with feed, carriers, other approved additives or medicinal products.

2.6. Conditions of use

The additive is intended for use in all animal species. It is proposed by the applicant to be administered by direct incorporation in complete feed or via premixtures or water for drinking. No minimum or maximum supplementation levels are proposed.[38]

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 methionine in animal feed. The executive summary of the EURL report can be found in the Appendix. The FEEDAP Panel notes that the official method proposed for analysis in feedingstuffs by the EURL does not differentiate between L- and D-methionine.

3. Safety

3.1. Safety aspects of the genetic modification[39]

The original strain from which the recipient organisms were derived is considered to be safe. The modifications to generate the recipient strains and the genetic modification processes do not raise concerns.

[30] Technical dossier/Section II.4.1/Table II.4.1.b.
[31] Technical dossier/Section II.4.1/Annex II.4.2.
[32] Technical dossier/Section II.4.1/Table II.4.1f.
[33] Technical dossier/Section II.4.1/Annex II.4.3.
[34] Technical dossier/Section II.4.1/Annex II.4.4.
[35] Technical dossier/Section II.4.1/Annex II.4.5.
[36] Technical dossier/Section II.4.1/Annex II.4.8.
[37] Technical dossier/Section II.4.1/Annexes II.4.6 and II.4.7.
[38] Technical dossier/Section II.5.1.
[39] 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. This is the case for the additive under application, which contains > 98.5 % methionine and < 1 % unidentified substances. Therefore, the FEEDAP Panel considers that safety concerns for target species are highly unlikely to arise from the L-methionine under application.

The safety of DL-methionine and its analogues has previously been extensively assessed (EFSA FEEDAP Panel, 2012). The Panel concluded that "*supplementing appropriate amounts of methionine and methionine analogues to meet requirements is safe for the target species. The earliest signs of a critical overdose are depressed feed intake and weight gain. The toxic levels of supplemented methionine and its analogues vary and depend on the basal diet and its content of sulphur-containing amino acids*."

The Panel noted that the same conclusions as above apply in principle to the L-methionine. However, it is known that L-methionine is somewhat more toxic than DL-methionine (Wretlind, 1950; Friedmann and Gumbmann, 1988).

The initial products of methionine degradation by ruminal microorganisms are methanethiol and 2-aminobutyric acid, which may then be converted to 2-oxobutyrate and then to propionate (Onodera, 1993). Methionine sulphoxide is also formed by protozoa (Onodera and Takei, 1986). As these products have no recorded deleterious effects on the host animal, there are no safety concerns arising from ruminal methionine metabolism.

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 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 has concerns over the safety of L-methionine for target species when administered via water for drinking since any additional supply of an essential amino acid would disturb the balanced amino acid profile of complete diets.

3.2.1. Conclusions on safety for the target species

L-Methionine is safe for the target animals when used as a feed additive to meet their requirement.

The FEEDAP Panel has concerns on the safety of L-methionine for target species when administered via water for drinking since any additional supply of an essential amino acid would disturb the balanced amino acid profile of complete diets.

3.3. Safety for the consumer

The absorption, distribution, metabolism and excretion of methionine have been extensively described in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2012).

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. The concerns for the consumer would derive 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 of no concern for consumers.

Supporting evidence was provided by the results of an acute oral toxicity study with the product under application in female rats with doses up 2 000 mg L-methionine/kg body weight. No adverse effects were observed.[40]

3.3.1. Conclusions on consumer safety

There are no safety concerns for the consumer associated with the use of L-methionine in animal nutrition.

3.4. Safety for the user

The following tests were performed with the product under assessment.[41]

3.4.1. Effects on the respiratory system

An acute inhalation toxicity study was submitted by the applicant. Five male and five female albino rats (nine weeks of age) were exposed to L-methionine by nose-only inhalation at a mean concentration of 3.1 mg/L air for four hours (in accordance with OECD Guideline 403).[42] Except for body weight loss observed in several animals between days 1 and 4, no other adverse effects were observed for 14 days (clinical signs). All animals survived without any adverse clinical signs. There were no macroscopic findings at necropsy.

3.4.2. Effects on the skin and eyes

In an acute dermal irritation/corrosion study, New Zealand white rabbits were exposed to 0.5 g of L-methionine for four hours (in accordance with OECD Guideline 404).[43] No adverse effects were observed during the 72 hours of observation after treatment; thus, L-methionine was considered to be non-irritant to skin.

An eye irritation study was conducted in three New Zealand rabbits in accordance with OECD Guideline 405. A dose of 0.1 g of the additive in the conjunctival sac showed no adverse effects in the eyes of the animals, so the product is classified as non-irritant to the eye.[44]

3.4.3. Skin sensitisation

Twenty Hartley guinea pigs were treated dermally with a 45 % suspension of L-methionine in olive oil (0.5 mL/site) and challenged two weeks later either with the vehicle or with 45 % L-methionine (in accordance with OECD Guideline 406, Buehler method). Since there was no evidence of a response after the challenge dose, it was concluded that L-methionine has no dermal sensitising potential.[45]

3.4.4. Conclusions regarding safety for the user

The additive L-methionine produced by *E. coli* (KCCM 11252B and KCCM 11340P) is demonstrated to be without irritant or dermal sensitising properties. Although no data on dusting potential were provided, the FEEDAP Panel considers it unlikely that the user will be exposed to significant amounts of L-methionine by inhalation.

3.5. Safety for the environment

Neither the production strains nor their recombinant DNA were detected in the final product. L-Methionine is a physiological and natural component in plants and animals. It is not excreted as such (but as urea/uric acid, sulphate and CO_2). The use of L-methionine in animal nutrition would not lead to any localised increase in its concentration in the environment.

[40] Technical dossier/Section III/Reference III.2.1.
[41] Supplementary information September 2013.
[42] Technical dossier/Section III/Reference III.3.1.
[43] Technical dossier/Section III/Reference III.3.3.
[44] Technical dossier/Section III/Reference III.3.2.
[45] Supplementary information February 2013/Annex Qvii.

It is concluded that the use of L-methionine under application as a feed additive does not represent a risk to the environment.

4. Efficacy

Efficacy studies are not required for amino acids naturally occurring in proteins of plants and animals. The nutritional role of methionine is well established in the scientific literature, as is the efficacy of supplementing diets, particularly for poultry and pigs. Since most studies comparing the efficacy of methionine enantiomers and analogues use L-methionine as the standard for comparison with other methionines (EFSA FEEDAP Panel, 2012), the statement on the nutritional role of supplemental methionine also covers L-methionine.

Regarding ruminant species, methionine is considered to be the first limiting amino acid in microbial protein leaving the rumen for growing cattle (Richardson and Hatfield, 1978; Campbell et al., 1997; Greenwood and Titgemeyer, 2000). Methionine is closely followed by lysine in limiting growth, so the composition of dietary protein that escapes ruminal degradation determines which of these two amino acids becomes first limiting post-ruminally (Schwab et al., 2005). Dietary soybean usually causes methionine to become first-limiting, while products of maize origin result in lysine becoming first-limiting (Schwab et al., 2005). Methionine becomes especially limiting in certain sheep breeds raised for wool production because of their high demand for sulphur-containing amino acids (Liu and Masters, 2013).

Methionine is the essential amino acid most slowly degraded by ruminal microorganisms (Chalupa, 1976). Several studies have indicated that a substantial proportion of methionine in a diet supplemented with unprotected methionine would be expected to escape from the rumen (Lewis and Emery, 1962; Chalupa, 1976; Broderick and Balthrop, 1979).

The additive L-methionine is regarded as an effective source of methionine for all animal species and categories.

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

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[46] and good manufacturing practice.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Neither the production strains nor their recombinant DNA were detected in the final product. No safety concerns were associated with the genetic modification of the production strains.

L-Methionine produced by *E. coli* (KCCM 11252B and KCCM 11340P) is safe for the target animals when used as a feed additive to meet their requirements. The FEEDAP Panel has concerns over the safety of L-methionine for the target species when administered via water for drinking since any additional supply of an essential amino acid would disturb the balanced amino acid profile of complete diets.

[46] 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.

There are no safety concerns for the consumer associated with the use of L-methionine in animal nutrition.

The additive L-methionine was demonstrated to be without irritant or dermal sensitising properties. Although no data on dusting potential were provided, the FEEDAP Panel considers it unlikely that the user will be exposed to significant amounts of L-methionine by inhalation..

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

The additive L-methionine is considered an efficacious source of the amino acid L-methionine for all animal species.

RECOMMENDATIONS

The description of the additive should include "produced by fermentation with *Escherichia coli* (KCCM 11252P and KCCM 11340P)**".**

DOCUMENTATION PROVIDED TO EFSA

1. L-Methionine, feed grade. May 2012. Submitted by CJ Europe GmbH.
2. L-Methionine, feed grade. Supplementary information. February 2013. Submitted by CJ Europe GmbH.
3. L-Methionine, feed grade. Supplementary information. May 2013. Submitted by CJ Europe GmbH.
4. L-Methionine, feed grade. Supplementary information. June 2013. Submitted by CJ Europe GmbH.
5. L-Methionine, feed grade. Supplementary information. September 2013. Submitted by CJ Europe GmbH.
6. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Methionine.

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APPENDIX

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Methionine[47]

In the current application authorisation is sought under Articles 4(1) (authorisation of a feed additive) for L-methionine, 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.

According to the Applicant, the feed additive is produced by fermentation process with *Escherichia coli*. The strain is deposited in the "Korean Culture Centre of Microorganisms". The feed additive is intended to be mixed either in premixtures or added directly to complete feedingstuffs or water. The Applicant proposed no minimum or maximum L-methionine concentrations in feedingstuffs.

For the determination of L-methionine in the feed additive, the Applicant proposed the titrimetric method as described in the relevant European Pharmacopoeia monograph. For the identification, the EURL proposed instead to apply the tests described in the Food Chemical Codex (FCC) "L-Methionine monograph": infrared absorption in combination with the analysis of the optical rotation. Furthermore, for the quantification of methionine in the feed additive, the EURL identified the ring-trial validated ISO/DIS 17180 method. This multianalyte technique applies for the determination of methionine content in commercial amino acid products and premixtures containing more than 10 % active substance, using an amino acid analyzer or high performance liquid chromatography (HPLC) equipment coupled with post-column derivatisation and visible or fluorescence detection (VIS/FD). The following performance characteristics are reported for a methionine content ranging from 30.6 to 93.3 %:

- a relative standard deviation for repeatability (RSDr) ranging from 0.5 to 1.1 %; and
- a standard deviation for reproducibility (RSDR) ranging from 1.5 to 2.6 %.

Based on the performance characteristics presented, the EURL recommends for official control the FCC monograph methods based on infrared absorption and optical rotation to identify the L-methionine in the feed additive and the ISO/DIS 17180 method, based on ion exchange chromatography coupled with post-column derivatisation VIS/FD detection, to quantify methionine in the feed additive.

For the determination of methionine in premixtures and feedingstuffs the Applicant submitted the ring-trial validated Community method – Commission Regulation (EC) No 152/2009 (further ring-trial validated – CEN EN ISO 13903:2005). The following performance characteristics were reported for the determination of total methionine:

- RSDr ranging from 1.1 to 5.6 %;
- RSDR ranging from 6.9 to 13 %; and
- a limit of quantification (LOQ) of 0.25 g/kg.

Based on the performance characteristics presented, the EURL recommends for official control the ring-trial validated Community method, based on ion exchange chromatography coupled with post-column derivatisation and VIS or FD detection to determine methionine in feedingstuffs and premixtures containing less than 10 % of the active substance. For premixtures containing more than 10 % of methionine, the EURL recommends the ring-trial validated ISO/DIS 17180 method, based on

[47] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2012-0016-l-Methionine.pdf

ion exchange chromatography coupled with post-column derivatisation and VIS or fluorescence detection.

The Applicant did not provide any experimental method or data for the determination of L-methionine in water. However, in FAD-2010-0023, the concerned Applicant has successfully demonstrated the extension of scope of the Community method for the determination of methionine in water. Therefore the EURL recommends this method for official control to determine methionine 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) is not considered necessary.