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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of L-threonine (ThreAMINO®) produced by *Escherichia coli* (DSM 25086) for all animal species and categories based on a dossier submitted by Evonik Industries A.G.[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 9 October 2014, replaces the earlier version published on 19 February 2014.[4]

ABSTRACT

The product L-threonine, technically pure, is a feed additive produced by fermentation with a genetically modified strain of *Escherichia coli*. Neither the production strain nor its recombinant DNA was detected in the final product. Therefore, the final product does not raise concerns with regard to the genetic modification of the production strain. The additive L-threonine is safe for all target animal species when supplemented in appropriate amounts to diets. However, the FEEDAP Panel has concerns about the safety of L-threonine for target species when administered via water for drinking because any additional supply of an essential amino acid would disturb the amino acid balance. Since the composition of tissues and products of animal origin will not be changed by the use of L-threonine in animal nutrition, and considering the high purity of the product under assessment, no risks are expected for the consumer from the use of L-threonine, technically pure, as a feed additive. There is no evidence of specific concerns for user safety (inhalation toxicity, skin and eyes irritation and dermal sensitisation) regarding the L-threonine products tested. The use of L-threonine as a feed additive does not give rise to any environmental safety concern. The product L-threonine, technically pure, is regarded as an efficacious source of supplemented amino acid for all animal species. A response in ruminants requires some degree of protection of L-threonine from ruminal degradation.

KEY WORDS

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

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[1] On request from the European Commission, Question No EFSA-Q-2012-00118, adopted on 29 January 2014.

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[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.

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SUMMARY

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on L-threonine (ThreAMINO®) produced by a genetically modified strain of *Escherichia coli* (DSM 25086) for all animal species.

L-Threonine is an essential amino acid for all animal species. It is commonly considered the second or third limiting amino acid in cereal-based diets for pigs and poultry, respectively. It is widely used in the feed industry to optimise dietary protein.

Neither the production strain nor its recombinant DNA was detected in the final product. Therefore, L-threonine, technically pure, manufactured by fermentation with *Escherichia coli* (DSM 25086) does not give rise to any safety concern with regard to the genetic modification of the production strain.

L-Threonine, technically pure, produced by *Escherichia coli* (DSM 25086) is safe for target animals when used in appropriate amounts to supplement feed to compensate for threonine deficiency in feedingstuffs. However, the FEEDAP Panel has concerns about the safety of L-threonine for target species when administered via water for drinking since any additional supply of an essential amino acid would disturb the amino acid balance.

Since the composition of tissues and products of animal origin will not be changed by the use of L-threonine in animal nutrition, and considering the high purity of the product under assessment, no risks are expected for the consumer from the use of L-threonine, technically pure, as a feed additive.

There is no evidence of specific concerns for user safety (inhalation toxicity, skin and eyes irritation and dermal sensitisation) regarding the L-threonine products tested.

The use of L-threonine in animal nutrition does not pose a risk to the environment.

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

The FEEDAP Panel made a recommendation regarding the description of the additive and the specification for the minimum L-threonine content in 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 the company Evonik Industries AG[6] for authorisation/re-evaluation of the product ThreAMINO®, L-threonine, when used as a feed additive for all animal species and categories (category: nutritional; 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 27 April 2012.[8]

L-Threonine, technically pure, (minimum content of 98 % as is basis) is currently authorised as a nutritional feed additive for use in all animal species (Commission Directive 88/485/EEC).[9] The current application refers to L-threonine produced by a genetically modified strain of *Escherichia coli* (DSM 25086).

L-Threonine like other amino acids and other nitrogen compounds is authorised according to Commission Directive 2006/141/EC for infant formulae and follow-on formulae.[10] According to Commission Directive 2001/15/EC, amino acids such as L-threonine may be added in all dietary foods for particular nutritional uses including foods for particular nutritional uses intended for special medical purposes.[11] L-Threonine is also registered as an ingredient in cosmetic products as antistatic, hair conditioning, hair waiving or straightening (Commission decision 2006/257/EEC). According to Commission Regulation (EEC) 2377/90, L-threonine is also listed as pharmacologically active substance in veterinary medicinal products and is not subjected to maximum residue levels when used in food producing animals.[12]

The Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion (EFSA, 2013) on the safety and efficacy of L-threonine produced by a genetically modified strain of *Escherichia coli* (FERM BP-10942).

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) issued two opinions related to threonine in the frame of the Flavouring Group Evaluation 26: amino acids from chemical group 34 (EFSA, 2008, 2010).

[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] Evonik Industries AG. Rodenbacher Chaussee 4, D-63457 Hanau, Germany.
[7] EFSA Dossier reference: FAD-2011-0046.
[8] A new mandate was received in EFSA on 12/01/2012.
[9] Commission Directive 88/485/EEC of 26 July 1988 amending the Annex to Council Directive 82/471/EEC concerning certain products used in animal nutrition. OJ L 239, 30.08.1988, p. 36.
[10] Commission Directive 2006/141/EC on infant formulae and follow-on formulae, OJ L 401, 30.12.2006, p. 1–33.
[11] 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. 19.
[12] 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.

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-threonine technically pure (ThreAMINO®) produced by the genetically modified strain of *Escherichia coli* (DSM 25086), 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-Threonine Technically Pure			
Registration number/EC No/No (if appropriate)	3.3.1			
Category(ies) of additive	Nutritional Additives			
Functional group(s) of additive	Amino Acids, Salts and Analogues			
Description				
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)	
> 98.0%	**$C_4H_9NO_3$**	**> 98%**	**Reg,(EC)No. 152/2009**	
Trade name (if appropriate)	ThreAMINO®			
Name of the holder of authorisation (if appropriate)	Evonik Industries AG			
Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg/kg of complete feedingstuffs		
all animal species	all ages	not applicable	not applicable	not 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)	not applicable			
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	
not applicable	not applicable	not applicable	not applicable	

ASSESSMENT

This opinion is based on data provided by a single company involved in the production/distribution of L-threonine produced by a genetically modified microorganism. The FEEDAP Panel has sought to use the data provided together with data from other sources to deliver an opinion.

1. Introduction[13]

The product subject of this application (ThreAMINO®) is L-threonine produced by fermentation with a genetically modified *Escherichia coli* (DSM 25086). L-Threonine is currently authorised as a nutritional additive, in the functional group amino acids, their salts and analogues. The product under application is intended to be used in the feed or water for drinking of all animal species and categories.

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 of individual amino acids of the animals or to compensate for potential imbalances. The supplementation of feedingstuffs with amino acids is a conventional measure in improving the protein quality and utilisation. This supplementation became even more important when protein-reduced diets were introduced in animal husbandry for economic and environmental reasons. L-Threonine is well recognised as an essential amino acid in animal nutrition. Under European Union (EU) conditions, L-threonine seems to be the second limiting amino acid after L-lysine in pigs and the third after sulphur amino acids and L-lysine in poultry.

L-Threonine is registered as pharmaceutical grade (for total parenteral nutrition) in many European countries and is described in a monograph of the European Pharmacopoeia (MG 01/2008:1049).

2. Characterisation[14]

2.1. Characterisation of the active substance/additive

L-Threonine (International Union of Pure and Applied Chemistry (IUPAC) name: (2*S*,3*R*)-2-amino-3-hydroxybutanoic acid; synonyms: 2-amino-3-hydroxybutyric acid, α-amino-β-hydroxybutyric acid), a compound identified with the Chemical Abstracts Service (CAS) No 72-19-5, and the European Inventory of Existing Commercial chemical Substances (EINECS) No 200-774-1) has a molecular weight of 119.12 g/mol. The molecular formula of L-threonine is $C_4H_9NO_3$. The structural formula is given in Figure 1.

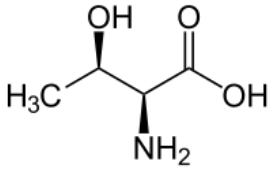

Figure 1: Molecular structure of L-threonine

According to the specification, the product contains ≥ 98 % L-threonine, ≤ 1.5 % water and ≤ 0.5 % ash. The analysis of five batches by the official method according to Commission Regulation (EC) 152/2009, showed values for threonine ranging from 99.7 to 103.0 % as is. Cystine content was ≤ 0.11 %, water ≤ 0.14 % and ash ≤ 0.06 %. Consequently, the amount of unidentified material is well below 1 % on a dry matter basis.[15] Analytical data on specific optical rotation of five batches showed

[13] This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
[14] This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
[15] Supplementary information (October 2013)/Annex 1.

an average value of –28.1°, which is within the range described in the European Pharmacopoeia for this amino acid.[16]

2.2. Impurities

Five pilot batches were analysed for impurities (arsenic, lead, mercury, cadmium, aluminium, dioxins and dioxin-like polychlorinated biphenyls).[17] The amounts of all these impurities were negligible and were often below the detection limits.

The microbial quality of product was tested at least on three batches for total plate count (< 10^6 colony-forming units (CFU)/g), yeasts (< 100 CFU/g), moulds (< 100 CFU/g), *Escherichia coli* (< 100 CFU/g), *Enterobacteriaceae* (< 10 CFU/g), aerobic and anaerobic spore formers (both < 100 CFU/g). *Salmonella* was not detected in 25 g samples. The final product (also at least three batches) was tested for aflatoxin B1, B2, G1 and G2 as well as for ochratoxin A, and in no case these could be detected.[18]

2.3. Physical properties

L-Threonine, technically pure, is a crystalline white to greyish, practically odourless powder.[19] The bulk density is 585–715 kg/m^3 and water solubility is 86–90 g/L at 20 °C. [20]

The particle size distribution of one batch of the product under assessment was analysed by sieving: approximately 81 % of particles had a diameter < 100 μm and 5 % of the particles a diameter < 20 μm.[21] Data on the dusting potential of three product batches (determined using the Stauber–Heubach method) showed a range from 0.21 to 0.25 g/m^3.[22]

2.4. Characterisation of the production organism

The product L-threonine under assessment is produced by a genetically modified strain of *E. coli*, deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ) with the accession number DSM 25086.[23]

The recipient strain is a derivative of *Escherichia coli* K-12. *E. coli* K-12 is a well-characterised Gram-negative bacterium and its safety (non-pathogenicity) has been reviewed extensively (Gorbach, 1978). The technical dossier contains detailed and sufficient information on the recipient microorganism including safety aspects, the origin and function of the different genetic elements introduced in the production strain, the genetic modification process and the genetic and phenotypic traits introduced.

2.4.1. Manufacturing process

L-Threonine is produced by fermentation. After the fermentation step, the biomass is inactivated and separated from the broth. The L-threonine-containing solution is purified and crystallised.

Material safety data sheets for the substrates used in the production process and for the final product are provided. Control methods are in place.

[16] Supplementary information October 2013/Annex 2 and monograph MG 01/2008:1049 of the European Pharmacopoeia.
[17] Technical dossier/Section II/Annex II.5.
[18] Supplementary information November 2012/Annexes II.26 and 21. Limit of detection = 0.08 μg/kg for the aflatoxins and 0.1 μg/kg for ochratoxin A.
[19] Technical dossier/Section II/Annex II.14.
[20] Technical dossier/Section II/Annex II.13.
[21] Supplementary information July 2013/Section II.2.1.5 and Annex II.21.
[22] Supplementary information October 2013/Annex 3.
[23] Technical dossier/Section II/Annex 7.

Neither the production strain,[24] nor its recombinant DNA was detected in five batches of the final product.[25]

2.5. Stability and homogeneity

No data have been provided on the stability and homogeneity of the additive under application. The applicant has provided some stability and homogeneity studies performed with another additive containing L-threonine produced by a different strain of *E. coli* (that shares a common lineage with the production strain of ThreAMINO®). The products are produced following the same manufacturing process and they have a similar composition (> 98 % L-threonine). Therefore, the FEEDAP Panel considers that these stability and homogeneity studies described below can be used for the assessment of the additive under application.

Two batches of the L-threonine were stored in the original packaging (three-ply paper bags with polyethylene inlayers) under ambient conditions (10–25 °C) for two years. No significant losses (< 1 %) of threonine were observed during storage.[26,27] Other two batches were tested under accelerated conditions (45, 60 and 85 °C for three months, three weeks and three days, respectively) and showed no significant losses of threonine.[28,29]

The stability of one batch of L-threonine was tested in a vitamin/mineral premixture for pigs and poultry supplemented with 5 % L-threonine and containing choline chloride.[30] After mixing, samples were stored in the original packaging at ambient conditions (10–25 °C) for six months. No losses were detected.

One batch of L-threonine was used to supplement a feed for chickens for fattening at a rate of 0.1 % (pelletised feed) or 0.05 % (extruded feed). The basal diet consisted mainly of wheat, soya bean meal and maize.[31] The resulting pellets or extrusion product were stored for three months in closed containers at room temperature. Pelleting (at 65 or 90 °C) and extrusion (at 110–130 °C) did not affect stability. No losses were observed during storage.[32,33]

Three batches of L-threonine were tested, each at four different dilutions (0.5, 1, 5 and 10 g/L) in water for drinking.[34] The study lasted for three days and temperature was 20–27 °C. No significant losses were observed.

2.5.1. Homogeneity

The ability of L-threonine to homogeneously distribute in feed for chickens for fattening at an intended inclusion level of 0.10 % was demonstrated by threonine analysis in 10 subsamples collected after mixing. The coefficient of variation of the free threonine distribution was 2.5 %.[35]

2.6. Incompatibilities

No physico-chemical incompatibilities or interactions can be expected with feed, carriers, other approved additives or medicinal products.

[24] Technical dossier/Supplementary information November 2012 and Supplementary information July 2013.
[25] Technical dossier/Section II/Annex II.10 and Supplementary information November 2012 and Supplementary information July 2013.
[26] Technical dossier/Section II/Annex II.18.
[27] Supplementary information July 2013/Section II.2.4.1.1/Annex II.28b.
[28] Technical dossier/Section II/Annex II.19.
[29] Supplementary information July 2013/Section II.2.4.1.1/Annex II.29b.
[30] Technical dossier/Section II/Annexes II.20 and II.21.
[31] Technical dossier/Section II/Annexes II.16 and II.17.
[32] Technical dossier/Section II/Annex II.16.
[33] Technical dossier/Section II/Table 2.4.1.2-2.
[34] Technical dossier/Section II/Annex II.22.
[35] Technical dossier/Section II/table 2.4.2-1 and Annex II.23.

2.7. Conditions of use

L-Threonine 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.

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

The EURL considered that the conclusions and recommendations reached in a previous assessment are valid and applicable for the current application.[36]

3. Safety

3.1. Safety aspects of the genetic modification[37]

The recipient organism is considered to be safe. The molecular characterisation of the genetic modifications does not raise any safety concern regarding the final product.

3.2. Safety for the target species

Tolerance studies are not normally required for highly purified amino acids. 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 % threonine and less than 1 % unidentified material. Therefore, the FEEDAP Panel considers that safety concerns for target species are highly unlikely to arise from this additive.

Depending on animal species, genetics, gender and the physiological state of the non-ruminant animal, the requirements for total threonine, according to the NRC (1994, 1998) and GfE (2008) range between 0.35 and 1.0 % in feed. Common feed materials contain 0.3–1.8 % threonine. The requirement of fish for threonine with regard to the different fish species (0.7 to 1.5% in feed) were set by NRC (2011). Supplementation levels of compound feeds with threonine are in the range of 0.05–0.15 %.

Excesses of dietary threonine are tolerated differently by different animal species. In pigs, the addition of 0.5–4.0 % L-threonine did not affect feed intake (the first symptom of an amino acid imbalance), growth or feed to gain ratio (Edmonds and Baker, 1987a; Edmonds et al., 1987). It has been shown in pigs that both dietary deficiency and excess of threonine reduce protein synthesis in skeletal muscle and rapidly growing tissues (i.e. jejunal mucosa), which may explain the impaired growth performance (Wang et al., 2007). In a series of four experiments with chickens for fattening, the addition of 4 % L-threonine decreased feed intake after six to eight days by 25–29 %, resulting in depressed weight gain by 24–33 % (Edmonds and Baker, 1987b). In another study on 10-day-old chickens, L-threonine addition (3.1 %) reduced weight gain by 12 % after two weeks of feeding (Carew et al., 1998).

In ruminants, threonine is degraded after ingestion by ruminal microbiota via 2-oxobutyrate to propionic acid (van den Hende et al., 1963). As these products have no recorded deleterious effects on the host animal, there are no safety concerns arising from ruminal threonine metabolism.

Concerning the proposed use of L-threonine in water for drinking, the FEEDAP Panel notes that complete diets for all animal species, and particularly those for food-producing animals, contain well-balanced protein by using amino acid supplementation optimally adjusted to the specific requirements of the different animal species and categories of essential amino acids (ideal protein concept). Each

[36] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/amend-FinRep-FAD-2010-0058+0081.pdf.
[37] This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.

additional administration of individual essential amino acids (e.g. via water for drinking) will negatively affect the amino acid balance and consequently performance of animals as well as target animal safety in a dose-dependent manner. The FEEDAP Panel therefore has concerns about the safety of L-threonine for target species when administered via water for drinking.

3.2.1. Conclusions on the safety for the target species[38]

The use of L-threonine produced by *E. coli* (DSM 25086) in supplementing feed to compensate for threonine deficiency in feedingstuffs is safe for the target animals. The margin of safety is higher in pigs than in poultry. However, excess doses would create amino acid imbalances, with negative consequences on animal performance. Correct dosing in formulating diets requires knowledge of the amino acid content in feed materials and the requirement of animals. The FEEDAP Panel has concerns on the safety of L-threonine for target species when administered via water for drinking since any additional supply with an essential amino acid would disturb the amino acid balance.

3.3. Safety for the consumer

Absorption and metabolic fate of L-threonine were briefly described in a recent FEEDAP Panel opinion (EFSA FEEDAP Panel, 2013).

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 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 (> 99 % L-threonine and < 1 % unidentified material) and, therefore, following the provisions of the guidance on nutritional additives, it is considered of no safety concern for consumers.

3.3.1. Conclusions regarding the safety for the consumer

The composition of edible tissues and products of animal origin will not be changed by the use of L-threonine in animal nutrition. Considering the high purity of the product under assessment, no risks are expected for the consumer from the use of L-threonine, technically pure, produced by *E. coli* (DSM 25086) as a feed additive.

3.4. Safety for the user[39]

The applicant has provided the results of an acute inhalation toxicity study, skin and eye irritation test and a dermal sensitisation test with two L-threonine products manufactured using two strains of *E. coli*, different to the strain used for the product under application although sharing a common lineage.[40] The products are produced following a similar manufacturing process and have a similar composition, purity and characteristics as the L-threonine under assessment. Therefore, the FEEDAP Panel considers that the outcome of the testing of these products is also relevant to the product under assessment.

3.4.1. Effects on the respiratory system

The batch of the additive that was analysed for physical properties probably contained particles of respirable size (< 10 μm), and although the dusting potential of three batches was low, it does not exclude the possibility that other presentations with different dusting potential might be produced.

[38] This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
[39] This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
[40] Supplementary information (October 2013)/Qiv.

In an acute inhalation toxicity study, Charles River CD®/Crl:CD rats were exposed to L-threonine (> 98.5 % purity, micronised) at an actual concentration of 5.15 ± 0.10 mg/L air for four hours, by inhalation using a dynamic nose-only exposure chamber (in accordance with OECD Guideline 403).[41] In the inhalation chamber, close to the animals' noses, the particles had a mass median aerodynamic diameter of 2.38 μm as determined with a cascade impactor. No signs of toxicity were observed at the tested dose of L-threonine. After exposure the animals were observed for 14 days and they gained the expected body weight within this period. No mortality occurred and no abnormalities were detected at necropsy.

3.4.2. Effects on the skin and eyes

A dermal irritation study was conducted in White Russian albino rabbits in accordance with OECD Guideline 405. A single dose of 0.5 g of L-threonine (> 98 % purity) applied dermally resulted in only very slight erythema for a short time in one animal so the product is classified as non-irritant to skin.[42]

In an eye irritation study conducted in White Russian albino rabbits in accordance with OECD Guideline 404, in which a single dose of 60 mg L-threonine (> 98 % purity) in a volume of 0.1 mL was applied to the conjunctival sac, only very slight effects, for a short time, occurred in the conjunctiva of one animal. The product is classified as non-irritant to the eye.[43]

A maximisation skin sensitisation test in Dunkin–Hartley guinea pigs performed in accordance with OECD Guideline 406 (0.1 mL of 0.5 % L-threonine solution in induction (intracutaneous) stage; 2 mL of 50 % solution in the induction (topical) stage, and challenge (topical) with 2 mL of a 50 % suspension) showed no evidence of any dermal sensitisation potential of L-threonine of 100.1 % purity on a dry matter basis.[44]

3.4.3. Conclusions on the safety for the user

There is no evidence of specific concerns for user safety (inhalation toxicity, skin and eyes irritation and dermal sensitisation) regarding the L-threonine products tested.

3.5. Safety for the environment

Neither the production strain nor its recombinant DNA was detected in the final product. Therefore, the final product does not pose any environmental safety concern associated with the genetic modification.

The amino acid L-threonine is a physiological and natural component of proteins of living organisms. The use of ThreAMINO® (L-threonine) in animal nutrition would not lead to any localised increase in its concentration in the environment. It is concluded that the use of the product L-threonine, technically pure, as a feed additive does not represent a risk to the environment.

4. Efficacy

Efficacy studies are not required for amino acids occurring naturally in the proteins of plants and animals. The nutritional role of threonine is well established in the scientific literature. Since most of the studies have been performed with supplemental L-threonine, the additive L-threonine, technically pure, is regarded as an effective source of threonine.

L-Threonine has been implicated as being present at lower than optimum composition 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-threonine supplementation could be beneficial.

[41] Technical dossier/Section III/Reference III.3.1.
[42] Technical dossier/Section III/Reference III.3.2.
[43] Technical dossier/Section III/Reference III.3.3.
[44] Technical dossier/Section III/Reference III.3.4.

Free threonine is among the amino acids most rapidly degraded by ruminal microbiota (Lewis and Emery, 1962), with an estimated half-life in the rumen of 1.1 hours (Chalupa, 1976). Broderick and Balthrop (1979) found that no threonine added to ruminal digesta *in vitro* remained after three hours. Accordingly, little if any dietary L-threonine provided to ruminants would be expected to reach the abomasum intact and be absorbed. Measures such as encapsulation would be required to protect the amino acid L-threonine from microbial degradation in the forestomachs.

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

4.1. Conclusions on the efficacy

L-Threonine is efficacious as a supplemented amino acid to maintain or restore the adequate balance of dietary amino acids for all animal species. A response in ruminants requires some degree of protection of L-threonine 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[45] and Good Manufacturing Practice.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Neither the production strain nor its recombinant DNA was detected in the final product. Therefore, L-threonine, technically pure, manufactured by fermentation with *Escherichia coli* (DSM 25086) does not give rise to any safety concern with regard to the genetic modification of the production strain.

L-Threonine, technically pure, produced by *Escherichia coli* (DSM 25086) is safe for target animals when used in appropriate amounts to supplement feed to compensate for threonine deficiency in feedingstuffs. However, the FEEDAP Panel has concerns about the safety of L-threonine for target species when administered via water for drinking since any additional supply of an essential amino acid would disturb the amino acid balance.

Since the composition of tissues and products of animal origin will not be changed by the use of L-threonine in animal nutrition, and considering the high purity of the product under assessment, no risks are expected for the consumer from the use of L-threonine, technically pure, as a feed additive.

There is no evidence of specific concerns for user safety (inhalation toxicity, skin and eyes irritation and dermal sensitisation) regarding the L-threonine products tested.

The use of the product L-threonine in animal nutrition does not pose a risk to the environment.

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

RECOMMENDATIONS

The description of the additive should contain the statement "produced by fermentation with *Escherichia coli* (DSM 25086)".

[45] 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.

Considering the analytical data, and to better standardise product quality, the FEEDAP Panel recommends that the specification for the minimum L-threonine content in the additive be set to 98.5 %.

DOCUMENTATION PROVIDED TO EFSA

1. L-Threonine technically pure for all animal species (ThreAMINO®). December 2011. Submitted by Evonik Industries AG.

2. L-Threonine technically pure for all animal species (ThreAMINO®). Supplementary information. November 2012. Submitted by Evonik Industries AG.

3. L-Threonine technically pure for all animal species (ThreAMINO®). Supplementary information. July 2013. Submitted by Evonik Industries AG.

4. L-Threonine technically pure for all animal species (ThreAMINO®). Supplementary information. October 2013. Submitted by Evonik Industries AG.

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