SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of L-cystine 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

ABSTRACT

L-cystine is a dispensable sulphur-containing amino acid, naturally occurring in proteins of plants and animals. L-cystine is safe for all animal species if the requirements for sulphur amino acids are respected. The maximum amount of L-cystine that can be safely added to the diet will depend on the levels of other sulphur-containing amino acids. Supplemental L-cystine will not be deposited in animal tissues as such: it will be incorporated in body proteins without causing any change in their natural composition or it will be metabolised and excreted. The product under application does not contain substances of toxicological concern. Thus, the use of L-cystine in animal nutrition does not raise any concerns for consumer safety. In the absence of any data, it would be prudent to consider L-cystine as irritant to skin, eyes and mucous membranes and as a potential dermal sensitiser. Therefore, it would be prudent to assume that exposure of skin, eyes and respiratory tract is hazardous. The use of the natural amino acid L-cystine as feed additive does not represent a risk to the environment. L-Cystine is considered efficacious in partially meeting the requirements for sulphur-containing amino acids in all animal species.

KEY WORDS

Nutritional additive, amino acid, L-cystine, safety, efficacy

[1] On request from European Commission, Question No EFSA-Q-2010-01313, adopted on 13 March 2013.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Amino Acids, including Paul Brantom, Noël Dierick, Jürgen Gropp, Annette Schuhmacher and Atte von Wright, for the preparatory work on this scientific opinion.

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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-cystine for all animal species.

The amino acid L-cystine is safe for all animal species if the requirements for sulphur amino acids are respected. The maximum amount of L-cystine that can be safely added to the diet will depend on the levels of other sulphur-containing amino acids.

Supplemental L-cystine will not be deposited in animal tissues as such: it will be incorporated in body proteins without causing any change in their natural composition or it will be metabolised and excreted. The product under application does not contain substances of toxicological concern. Thus, the use of L-cystine in animal nutrition does not raise any concerns for consumer safety.

In the absence of any data it would be prudent to consider L-cystine as irritant to skin, eyes and mucous membranes and as a potential dermal sensitiser. Therefore, it would be prudent to assume that exposure of skin, eyes and respiratory tract is hazardous.

The use of the natural amino acid L-cystine as feed additive does not represent a risk to the environment.

L-Cystine is considered efficacious in partially meeting the requirements for sulphur-containing amino acids in all animal species.

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BACKGROUND

Regulation (EC) No 1831/2003[4] 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 Bretagne Chimie Fine (BCF)[5] for authorisation of the product L-cystine, when used as a feed additive for all animal species (category: nutritional additive; 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.[6] 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 January 2011.

The additive L-cystine has not been previously authorised in the European Union (EU) as nutritional feed additive, functional group amino acids, their salts and analogues. L-cystine is included in the European Union Register of feed additives, as a sensory additive, functional group: flavouring compounds, subclassification: natural or corresponding synthetic chemically defined flavourings, under the Flavis No 17.006.

L-cystine is approved as additive for human food and used in particular nutrition i.e. in baby-food according to the Commission Directive 2006/141/EC to achieve the correct protein level in the finished products.[7] L-cystine is also used as active pharmaceutical ingredient in several human medicinal products and has a dedicated monograph in the European Pharmacopoeia (EurPh).[8]

The Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) of EFSA (EFSA, 2010) delivered an opinion on the safety of L-cystine as food flavouring.

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-Cystine (L-cystine), when used under the conditions described in Table 1.

[4] 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.
[5] Bretagne Chimie Fine (Boisel, 56140 Pleucadeuc, France).
[6] EFSA Dossier reference: FAD-2010-0261.
[7] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC. OJ L 401, 30.12.2006, p. 33.
[8] European Pharmacopoeia, 7th edition, monograph 01/2008:0998.

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

Additive	L-CYSTINE			
Registration number/EC No/No (if appropriate)	/			
Category(ies) of additive	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-Cystine	**C6H12N2O4S2**	**pure grade (acc. to Eur. Ph.)**	**dosage in feedingdstuffs : HPLC, Commission regulation (EC) No 152/2009**	
Trade name (if appropriate)	L-Cystine			
Name of the holder of authorisation (if appropriate)	Bretagne Chimie Fine			
Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg/kg of complete feedingstuffs		
all animals	-	-	-	-
Other provisions and additional requirements for the labelling				
Specific conditions or restrictions for use (if appropriate)	/			
Specific conditions or restrictions for handling (if appropriate)	As for any powder product which may generate dust, wear a mask, goggles and safety glasses			
Post-market monitoring (if appropriate)	not considered necessary in view of adaquate production and control procedures during the manufacturing process to ensure quality andsafety of the additive (GMP certification)			
Specific conditions for use in complementary feedingstuffs (if appropriate)	/			
Maximum Residue Limit (MRL) (if appropriate)				
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues	
-	-	-	-	

ASSESSMENT

This opinion is based in part on data provided by a single company involved in the production/distribution of L-cystine. It should be recognised that these data could cover only a fraction of existing additives containing L-cystine. The composition of the additive is not the subject of the application. The FEEDAP Panel has sought to use the data provided by the applicant together with data from other sources to deliver an opinion.

1. Introduction

L-Cystine is currently not authorised for use as a nutritional additive in the European Union. However, it is authorised for use as a flavouring compound in the additive category "sensory additives". It has a dedicated monograph in the European Pharmacopoeia (EurPh).[9]

The present application refers to L-cystine to be used as a nutritional additive (amino acid) for all animal species. L-Cystine is not an essential amino acid. L-Cystine and L-cysteine are known as "spare amino acids", that is they can partially replace methionine. The degree to which they can exert this effect depends on the animal species and categories and varies between 40 % and 80 %.

The L-cystine under application is obtained by hydrolysis of natural keratin. The hydrolysate is obtained by treatment of poultry feathers with HCl at different temperatures. It is purified by activated carbon and crystallisation. L-Cystine is precipitated and purified again.

2. Characterisation

2.1. Characterisation of the active substance

L-cystine is a dimeric amino acid synthesised through an oxidative process from two cysteine molecules, which become linked by a disulphide bond. L-cystine (International Union of Pure and Applied Chemistry (IUPAC) name (2*R*)-2-amino-3-[(2*R*)-2-amino-3-hydroxy-3-oxopropyl] disulanylpropanoic acid; Chemical Abstracts Service (CAS) number 56-89-3) has the molecular formula $C_6H_{12}N_2O_4S_2$; its molecular weight is 240.3 g/mol, and its molecular structure is given in Figure 1.

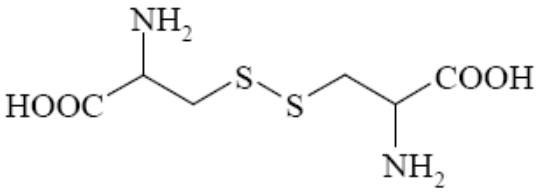

Figure 1: Molecular structure of L-cystine

The product contains by specification ≥ 98.5 % L-cystine and complies with the EurPh. The specification is confirmed by the analysis (the titrimetric method of the EurPh) of 718 batches (2009 production) showing a mean purity of 99.4 % (range 98.5–101 %).[10]

2.2. Impurities

The absence of D-cystine is demonstrated by optical rotation and chiral chromatography. The optical rotation is –223.4° (1.0 N HCl). Other impurities consist of ≤ 0.2 % L-tyrosine and chlorides (0.005–

[9] European Pharmacopoeia, 7th edition, monograph 01/2008:0998.
[10] Technical dossier/Section II.2.1.3.

0.01 %, equivalent to 0.008–0.016 % sodium chloride). Heavy metals (cadmium, lead and mercury) and arsenic were analysed in 10 batches. The highest values measured were 0.5 mg Cd/kg, 2 mg Pb/kg, 0.02 mg Hg/kg and 0.3 mg As/kg and are in accordance with Directive 2002/32/EC.[11,12]

Routine control for microbial contamination is in place. Data from 80 batches produced in 2010 showed that total aerobic counts were lower than 40 colony-forming units (CFU)/g and for yeasts and moulds were lower than 10 CFU/g. Enterobacteriaceae, coagulase-positive staphylococci and *Salmonella* were absent.[13]

2.3. Physical properties

The product is a solid and almost white fine crystalline powder of hexagonal particle shape. Its density varies from 0.8 to 0.9 kg/L depending on the level of compaction. Its solubility in water is only 0.011 g/100 mL at 25 °C, but at pH values < 2 or > 8 it is very soluble. Its melting point is 260 °C.

Particle size distribution was measured in four batches of the additive by laser diffraction.[14] The mean particle diameter is 221 μm (v/v) and 10 % of the particles have a mean diameter ≤ 127 μm.[15] As derived from the graphics presented, the proportion of particles with a diameter < 50 μm is lower than 1 % and no particles of respiratory size (< 10 μm) were detected. The dusting potential measured in one batch of the additive was 0.5 mg/25 g,[16] but insufficient information on methodology was provided to allow a conclusion on dusting potential to be drawn.

2.4. Stability and homogeneity

2.4.1. Shelf life

One study was performed with five batches of the additive, stored at ambient temperature and protected from light for 56 months.[17] No significant loss of the active substance was detected.

A second study was performed with three batches of the additive, stored at 25 °C and 60 % relative humidity (RH) for 48 months.[18] No losses were detected.

A third study was performed with one batch of the additive stored at 40 °C (and 75 % RH) for six months.[19] The additive was stored in closed polyethylene bags. The physico-chemical criteria described in the EurPh monograph were evaluated at the beginning and after three and six months of storage. The product was stable for six months with no significant losses (0.3 %). Some microbiological analyses (total plate count, Enterobacteriaceae, yeasts, moulds, coagulase-positive staphylococci per gram and *Salmonella* in 100 g) were also performed. No microbial deterioration was observed.

2.4.2. Stability in premixtures and feedingstuffs

The applicant provided stability studies on three batches of the additive in a vitamin/mineral premixture for rabbits (supplementation rate 0.49 %).[20] The premixture was stored in paper bags (25 kg) lined with a polyethylene film in ambient conditions for six months. The losses after six months were 9 %, 14 % and 12 %.

[11] 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.
[12] Technical dossier/Section II.2.1.4.
[13] Technical dossier/Section II.2.1.4.
[14] Technical dossier/Section II/Annex II.7.
[15] Technical dossier/Section II.2.1.5.
[16] Technical dossier/Section II/Annex II.8 and Section 2.6.3.
[17] Technical dossier/Section II/Annex II.13.
[18] Technical dossier/Section II/Annex II.12 and II.13.
[19] Supplementary information (January 2012)/Annex 1 and 2.
[20] Supplementary information (January 2013)/Annex 5.

The stability of L-cystine was also studied in feedingstuffs for chickens for fattening and piglets. The basal diets were mainly composed of wheat, maize and soybean meal. Pelleting (75 °C for the chicken feed and 65 °C for the pig feed) did not affect the concentration of added L-cystine (intended concentration about 0.25 %) in the chicken feed, but it decreased by 17 % the concentration of added L-cystine in the pig feed. After three months' storage at 20 °C, no losses were observed.[21,22]

The stability of one L-cystine batch of the additive in two batches of a pet food (containing dehydrated poultry meat, maize, wheat, animal fats and dehydrated pork protein) was studied. L-Cystine was supplemented at 0.1 %. Extrusion losses amounted to 8–9 %. [23] After storage for 18 months in a cold room (temperature not specified), the loss was 10 %.[24]

Two other canned pet foods (one for dogs, one for cats) were tested to assess the stability of L-cystine (supplementation 0.025 %) during feed processing (sterilisation, temperature not specified) and storage at 20 °C for three months. The dog food was based on rice, maize and chicken meat, that for cats on chicken and pig meat, salmon and wheat. No losses were observed during feed processing and storage.[25,26]

2.4.3. Homogeneity

Ten samples of a premixture for rabbits (see Section 2.4.2) were taken during production and analysed for L-cystine. The coefficient of variation (CV) of the mean L-cystine concentration was 4 %.[27]

Again, 10 subsamples each were taken from the pelleted feed for chickens for fattening and piglets. The CV of the L-cystine concentration was 5 % and 7%, respectively.[28,29]

Homogeneous distribution of L-cystine in the extruded pet food for cats and dogs (see Section 2.4.2) were analysed after mixing, extrusion (including drying) and final coating. The CV of L-cystine concentration in the dog food was 5 % at the different steps of food production. No CV could be calculated for L-cystine concentration in the cat food immediately after mixing. After coating the CV amounted to 16 % and in the final coated feed it was 2 %. All CVs were based on four subsamples.[30,31] From another pet food production, 18 samples were taken before and 21 samples after extrusion. The CV was 14 % and 10 %, respectively.[32]

2.5. Conditions of use

The product is intended to be directly added to complete or complementary feedingstuffs at 0 to 1 % for all animal species, depending on the composition of the different feed materials used.[33] No minimum or maximum levels are recommended.

2.6. 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 the L-cystine in animal feed. The Executive Summary of the EURL report can be found in Appendix A.

[21] Supplementary information (January 2013)/Annex 3.
[22] Supplementary information (January 2013)/Annex 4.
[23] Technical dossier/Section II.2.4.1.2.
[24] Supplementary information (January 2012)/Annex 3.
[25] Supplementary information (January 2013)/Annex 1.
[26] Supplementary information (January 2013)/Annex 2.
[27] Supplementary information (January 2013)/Annex 5.
[28] Supplementary information (January 2013)/ Annex 3.
[29] Supplementary information (January 2013)/ Annex 4.
[30] Supplementary information (January 2012)
[31] Technical dossier/Section II/Annex II.14.
[32] Supplementary information (January 2012).
[33] Technical dossier/Section II.2.5.1.

3. Safety

The first metabolic intermediate of L-cystine after a reduction step is L-cysteine. It can (i) act as a precursor of glutathione, (ii) be further decarboxylated to cysteamine (a precursor of coenzyme A) or (iii) used for pyruvate synthesis. Pyruvate formation can occur via two alternative routes, either by desulphydration followed by deamination or, mainly, via cysteinesulphinic acid, which is also a precursor of alanine, taurine and 3′-phosphoadenosine 5′-phosphosulphate (PAPS). The metabolic pathways to pyruvate are associated with the release of hydrogen sulphide, ammonia and sulphate ions.

3.1. Safety for the target species

Tolerance studies are normally not required for amino acids, particularly if they are highly purified.

Excessive doses of amino acids lead to nutritional imbalances, which may provoke interactions and can eventually result in adverse effects (growth reduction, reduced food consumption, changes in plasma amino acid pattern, mortality). The sulphur-containing amino acid methionine is known to have a relatively small margin of safety if the optimal dose range is exceeded. In contrast, the non-essential amino acid L-cystine (and L-cysteine) is tolerated at higher levels. Although nutritionally equivalent to L-cystine, L-cysteine is more toxic than L-cystine (Harper et al., 1970; Anderson and Raiten, 1992; Baker, 2006).

The adverse effects of L-cysteine and L-cystine are also species dependent, as shown in a series of experiments with chickens for fattening, rats and weaned piglets (Dilger et al., 2007). Overdoses of either compound caused general growth retardation in all species. An L-cysteine dose of 30 g/kg feed (7.5 times the dietary requirement) caused 50 % mortality in chickens for fattening in only five days, although no specific cause of pathology was discovered. No mortality at similar or higher doses was observed in other species. The adverse effects of L-cysteine in chickens for fattening could be ameliorated by either $KHCO_3$ or H_2O_2 supplementation, the latter totally preventing the mortality. L-Cystine did not cause mortalities even at the highest tested dose (40 g/kg feed) in chickens for fattening or in pigs, while some deaths occurred among rats receiving a dose of 72 g/kg feed. It should be noted that the experiments were of relatively short duration (9–17 days with chickens for fattening, 14 days with other species).

Although no definite causes for the toxicities of L-cysteine and L-cystine or for the species differences have been identified, the strong reducing power of L-cysteine as well as the accumulation of toxic metabolites (ammonia, hydrogen sulphide) have been proposed to play a role.

L-Cystine is considered safe for all animal species and categories, if the requirements for sulphur-containing amino acids are respected. The requirement data are easily accessible to feed compounders and range between 0.35 % and 0.85 % for total sulphur-containing amino acids and between 0.15 % and 0.40 % for L-methionine in feed, depending on animal species, genetics and the sex and physiological state of the animal.

3.2. Safety for the consumer

L-Cystine, as an amino acid, is a natural component of animals and plants. As a general principle, conventional toxicology studies are considered to be inappropriate for testing amino acids. Such substances have a physiological concentration which is optimum for health and performance. Dietary intakes of such substances that lead to amounts significantly below or above that which is optimum for health and performance will inevitably cause a physiological imbalance and consequent adverse effects. This principle applies to substances whose purity is well established, with the source and method of production sufficiently well characterised to provide reassurance that no toxic contaminants will be present in the product. This is the case for the L-cystine under application.

The testing appropriate to such substances will need to be judged on a case-by-case basis, but in circumstances in which the use of the substance leads to no increase in human intake there is no requirement for further toxicity data.

Supplemental L-cystine will not be deposited in animal tissues as such: it will be incorporated in body proteins without causing any change in their natural composition or metabolised as described above. The product under application does not contain substances of toxicological concern. Thus, the use of L-cystine in animal nutrition does not raise any concerns for consumer safety.

3.3. Safety for the user

Since the product is a powder with < 1% particles < 50 μm in diameter, the possibility of exposure of the lower respiratory tract is considered to be low. Since no studies on the irritant or sensitising effects were provided, this product has to be considered as potentially irritating to skin, eyes and mucous membranes and as a potential dermal sensitiser. Therefore, it would be prudent to assume that exposure of skin, eyes and respiratory tract is hazardous.

3.4. Safety for the environment

L-Cystine is a physiological and natural component of proteins in animals and plants. It is not excreted as such but as urea or uric acid, sulphate and CO_2. The use of L-cystine in animal nutrition would not lead to any localised increase in its concentration in the environment. It is concluded that the use of this product as feed additive does not represent a risk to the environment

4. Efficacy

L-Cystine is a dispensable amino acid, provided that adequate methionine is available. On a molar basis L-methionine is 100 % efficient as a precursor of L-cystine. The conversion reaction is irreversible. However, the reaction between L-cysteine and L-cystine is freely reversible (Brosnan and Brosnan, 2006). The metabolism of sulphur-containing amino acids is depicted in Figure B1 of Appendix B. In young, rapidly growing animals, L-cyst(e)ine can furnish 50 % of the requirement for sulphur amino acids (SAAs), while in older animals (i.e. adult maintenance), L-cyst(e)ine can furnish up to 80 % of the SAA requirement (Buttery and D'Mello, 1994). The keto-analogue of cysteine is not produced by metabolism; thus, neither the keto-analogue nor D-cyst(e)ine has bioactivity. Methionine and cyst(e)ine in processed feeds may be subjected to oxidative losses wherein methionine is converted to either methionine sulphoxide or methionine sulphone, and cysteine is oxidised to cysteic acid. Neither methionine sulphone nor cysteic acid has SAA bioactivity when fed to rats, but methionine sulphoxide has 60 % and 85 % bioactivity in rats and mice, respectively (Baker, 2006).

In general, L-cysteine and its oxidation product L-cystine can satisfy approximately 50 % of the need for total SAAs. Depending on animal species, genetics and the sex and physiological state of the animal, the requirement for total SAAs and methionine in feed, according to the National Research Council (NRC), ranges between 0.35 % and 0.85 % and between 0.15 % and 0.40 %, respectively.

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[34] and Good Manufacturing Practice.

CONCLUSIONS

The amino acid L-cystine is safe for all animal species, if the requirements for sulphur-containing amino acids are respected. The maximum amount of L-cystine that can be safely added to the diet will depend on the levels of other sulphur amino acids.

[34] 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.

Supplemental L-cystine will not be deposited in animal tissues as such: it will be incorporated in body proteins without causing any change of their natural composition or it will be metabolised and excreted. The product under application does not contain substances of toxicological concern. Thus, the use of L-cystine in animal nutrition does not raise any concerns for consumer safety.

In the absence of any data, it would be prudent to consider L-cystine as an irritant to skin, eyes and mucous membranes as a potential dermal sensitiser. Therefore, it would be prudent to assume that exposure of skin, eyes and respiratory tract is hazardous.

The use of the natural amino acid L-cystine as feed additive does not represent a risk to the environment.

L-Cystine is considered efficacious in partially meeting the requirements of sulphur-containing amino acids in all animal species.

DOCUMENTATION PROVIDED TO EFSA

1. FAD-2010-0261. November 2010. Submitted by Bretagne Chimie Fine.
2. FAD-2010-0261. Supplementary information. January 2012. Submitted by Bretagne Chimie Fine.
3. FAD-2010-0261. Supplementary information January 2013. Submitted by Bretagne Chimie Fine.
4. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for L-cystine.

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Buttery J and D'Mello J, 1994. Amino acid metabolism in farm animals: a review. In: Amino acids in farm animals, Ed. D'Mello J. CAB International, Wallingford, UK, 1–61.

Dilger RN, Toue S, Kimura T, Sakai and Baker DH, 2007. Excess dietary L-cysteine, but not L-cystine is lethal for chicks but not for rats or pigs. The Journal of Nutrition, 137, 331–338.

EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC), 2010. Flavouring Group Evaluation 26: Amino acids from chemical group 34. The EFSA Journal (2006) 373, 1–48.

Harper AE, Benevenga NJ and Wohlhueter RM, 1970. Effects of ingestion of disproportionate amounts of amino acids. Physiological Reviews, 50, 428–558.

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-CYSTINE[35]

In the current application authorisation is sought for *L-Cystine* under Articles 4(1), category 'nutritional additives' and functional group 3(c) 'amino acids, their salts and analogues' according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of *L-Cystine* for all animal species and categories. The *feed additive* is intended to be mixed either in *premixtures* or added directly to complete *feedingstuffs*. The Applicant suggested no minimum or maximum *L-Cystine* concentrations in *premixtures* and *feedingstuffs*.

For the determination of the *active substance* in the *feed additive* the Applicant submitted the internationally recognised European Pharmacopoeia titrimetric method. No performance characteristics of this method are provided. However, the EURL considers this method suitable to determine *L-Cysteine* in the *feed additive* within the frame of official control.

For the determination of *Cystine* in *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated Community method (Commission Regulation (EC) No 152/2009). The method applies for the determination of *free* (synthetic and natural) and *total* (peptide-bound and free) amino acids, using an amino acid analyzer or High Performance Liquid Chromatography (HPLC) equipment. However, only performance characteristics for the determination of *total Cystine* are reported:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 1.7 to 4.6%;
- a relative standard deviation for *reproducibility* (RSD_R) ranging from 8.8 to 19%.

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 photometric detection to determine *Cystine* in *premixtures* and *feedingstuffs*.

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.

[35] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2010-0261.pdf

APPENDIX B

METABOLIC FATE OF L-CYST(E)INE

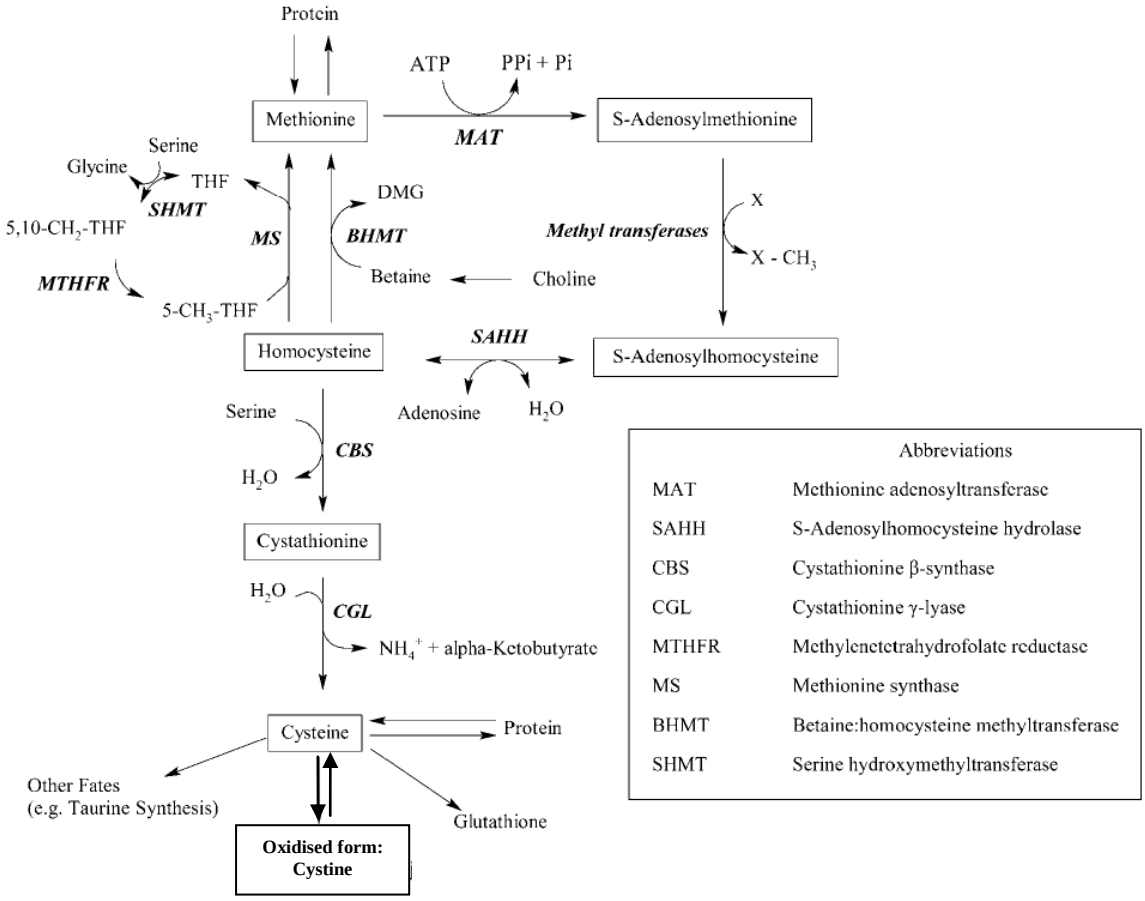

Figure B1: Major pathways of the metabolism of sulphur-containing amino acids (modified from Brosnan and Brosnan, 2006)