SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of concentrated liquid L-lysine (base), concentrated liquid L-lysine monohydrochloride and L-lysine monohydrochloride produced by *Escherichia coli* (FERM BP-10941) for all animal species, based on three dossiers submitted by Ajinomoto Eurolysine SAS[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 20 August 2014, replaces the earlier version published on 2 October 2013.[4]

ABSTRACT

Concentrated liquid L-lysine (base), concentrated liquid L-lysine monohydrochloride (HCl) and L-lysine HCl technically pure are produced by fermentation with a genetically modified *Escherichia coli* (FERM BP-10941). Neither the production strain nor its recombinant DNA was detected in any of the final products. The final products do not raise any safety concern with regard to the genetic modifications. Concentrated liquid L-lysine (base), concentrated liquid L-lysine HCl and L-lysine HCl technically pure are considered safe for target species when supplemented in appropriate amounts. Lysine produced by *E. coli* (FERM BP-10941) is not genotoxic and the results of subchronic studies do not indicate any specific concerns. As there are no lysine metabolites associated with safety concerns in the animal tissues and products, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) considers that the use of L-lysine and its hydrochloride salts in animal feed does not pose a risk for the consumer. Concentrated liquid L-lysine (base), concentrated liquid L-lysine HCl and L-lysine HCl technically pure are not considered to have the potential to cause respiratory toxicity, skin or eye irritation or skin sensitisation, but respiratory sensitisation cannot be excluded. L-Lysine is a substance naturally occurring in bacteria, plants and animals. The use of L-lysine-containing feed additives does not represent a risk to the environment. Concentrated liquid L-lysine (base), concentrated liquid L-lysine HCl and L-lysine HCl technically pure are considered equivalent in terms of L-lysine availability to the target animals. The efficacy of supplementing L-lysine and its hydrochloride salts is extensively demonstrated in the literature for mammals (except ruminants), poultry and fish, including its use in

[1] On request from the European Commission. Questions No EFSA-Q-2011-00992, EFSA-Q-2011-00993 and EFSA-Q-2011-00994, adopted on 11 September 2013.

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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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a liquid or a powder form. Therefore it does not require any further demonstration. Response in ruminants requires some degree of protection of L-lysine from ruminal degradation.

KEY WORDS

nutritional additive, amino acids, concentrated liquid L-lysine base, concentrated liquid L-lysine HCl, L-lysine HCl technically pure, safety

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 concentrated liquid L-lysine (base), concentrated liquid L-lysine monohydrochloride (HCl) and L-lysine HCl technically pure, produced by a genetically modified strain of Escherichia coli (FERM BP-10941).

Lysine is an essential amino acid for all animal species; it is the first limiting amino acid in swine nutrition and the second limiting amino acid in poultry nutrition. L-Lysine and its salts are widely used in the feed industry to optimise dietary protein.

Neither the production strain nor its recombinant DNA was detected in any of the final products. The final products do not raise any safety concern with regard to the genetic modifications.

Concentrated liquid L-lysine (base), concentrated liquid L-lysine HCl and L-lysine HCl technically pure are considered safe for target species when supplemented in appropriate amounts.

Lysine produced by E. coli (FERM BP-10941) is not genotoxic and the results of subchronic studies do not indicate any specific concerns. As there are no lysine metabolites associated with safety concerns in the animal tissues and products, the FEEDAP Panel considers that the use of L-lysine and its hydrochloride salts in animal feed does not pose a risk for the consumer.

Concentrated liquid L-lysine (base), concentrated liquid L-lysine HCl and L-lysine HCl technically pure are not considered to have the potential to cause respiratory toxicity, skin or eye irritation or skin sensitisation, but respiratory sensitisation cannot be excluded.

L-Lysine is a substance naturally occurring in bacteria, plants and animals. The use of L-lysine-containing feed additives does not represent a risk to the environment.

Concentrated liquid L-lysine (base), concentrated liquid L-lysine HCl and L-lysine HCl technically pure are considered equivalent in terms of L-lysine availability to the target animals. The efficacy of supplementing L-lysine and its hydrochloride salts is extensively demonstrated in the literature for mammals (except ruminants), poultry and fish, including its use in a liquid or a powder form. Therefore, it does not require any further demonstration. Response in ruminants requires some degree of protection of L-lysine from ruminal degradation.

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BACKGROUND[5]

Regulation (EC) No 1831/2003[6] 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. In particular Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from the company Ajinomoto Eurolysine S.A.S.[7] for re-evaluation of the products concentrated liquid L-lysine (base), concentrated liquid L-lysine monohydrochloride, and L-lysine monohydrochloride technically pure, when used as a feed additives for all animal species (category: nutritional additives; functional group: amino acids, their salts and analogues) under the conditions mentioned in Tables 1a - c.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the applications to the European Food Safety Authority (EFSA) as an application under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossiers in support of these applications.[8] 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 applications were considered valid by EFSA as of 24 November 2011.

The active substance of the three additives under application, L-lysine, is produced by the genetically modified microorganism *Escherichia coli* (FERM BP-10941).

L-Lysine is currently authorised for its use in all animal species as a nutritional additive.[9] No maximum content in feedingstuffs is established in the EU. L-lysine and L-lysine acetate are authorized for specific nutritional purposes in foods for particular nutritional uses.[10] L-lysine and its hydrochloride salt 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.[11] Lysine is listed as pharmacologically active substance in veterinary medicinal products and is not subject to maximum residue limits when used in food-producing animals.[12]

The scientific Panel on Additives and Products or Substances used in Animal Feed on published an opinion on the safety and efficacy of L-lysine sulphate for all animal species (EFSA, 2007)

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) issued and opinion on L-lysine and its monohydrochloride salt when used as a flavouring compound (EFSA, 2008, 2010)

[5] This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
[6] 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.
[7] Ajinomoto Eurolysine S.A.S. Rue de Courcelles 153, 75817 Paris 17ème, France.
[8] EFSA Dossier references: FAD-2010-0206, FAD-2010-0207 and FAD-2010-0210.
[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, 30.8.88, pp. 36.
[10] Commission Regulation (EC) No 953/2009 of 13 October 2009 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. OJ L 269, 14.10.2009, pp. 9.
[11] Commission Directive 2006/141/EC on infant formulae and follow-on formulae. OJ L 401, 30.12.2006, pp. 1.
[12] Commission Regulation EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. OJ L 15/1 20.1.2010, pp. 1.

The scientific Panel on Dietetic Products, Nutrition and Allergies (NDA) issued an opinion on the substantiation of health claims related to L-lysine (EFSA, 2011a).

The scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) issued an opinion on consideration of 88 flavouring substances considered by EFSA for which EU production volumes / anticipated production volumes have been submitted on request by DG SANCO, including L-lysine (Flavis 17.026) as flavouring compound (EFSA, 2011b)

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 concentrated liquid L-lysine (base), concentrated liquid L-lysine monohydrochloride and L-lysine monohydrochloride technically pure, produced by *Escherichia coli* (FERM BP-10941), when used under the conditions described in Tables 1a-c.

Table 1a: Description and conditions of use of the additive concentrated liquid L-lysine (Base) as proposed by the applicant

Additive	Concentrated liquid L-lysine (Base)			
Registration number/EC No/No (if appropriate)				
Category(ies) of additive	(c): Nutritional additive			
Functional group(s) of additive	3 (c): Amino acids, their salts and analogues			
Description				
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)	
Dark brown liquid	**C6H14N2O2**	**not less than 50 % L-lysine (product as is)**	**High Pressure Liquid Chromatography, 1) for feedingstuffs: Regulation (EC) No 152/2009 of 27 January 2009, Annex III, Part F (or equivalent: ISO 13903:2005) 2) pure products / premixtures: same method in adapting dilutions**	
Trade name (if appropriate)				
Name of the holder of authorisation (if appropriate)				
Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg/kg of complete feedingstuffs (select what applicable)		
All species	Not applicable	Not applicable	Not applicable	None (essential amino acid)
Other provisions and additional requirements for the labelling				
Specific conditions or restrictions for use (if appropriate)	None			
Specific conditions or restrictions for handling (if appropriate)	Wearing a mask and safety goggles during handling is recommended			
Post-market monitoring (if appropriate)	Not considered necessary in view of adequate monitoring and operating conditions during the manufacturing process to ensure quality and safety of the additive. HACCP and Traceability systems are in place. Ajinomoto Eurolysine has been certified ISO 9001 and FAMI-QS.			
Specific conditions for use in complementary feedingstuffs (if appropriate)	None			
Maximum Residue Limit (MRL) (if appropriate)				
Marker residue		Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
-		-	-	-

Table 1b: Description and conditions of use of the additive concentrated liquid L-lysine monohydrochloride as proposed by the applicant

Additive	Concentrated liquid L-lysine-monohydrochloride			
Registration number/EC No/No (if appropriate)				
Category(ies) of additive	(c): Nutritional additive			
Functional group(s) of additive	3 (c): Amino acids, their salts and analogues			
Description				
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)	
Dark brown liquid	C6H14N2O2 HCl	not less than 22.4 % L-lysine (product as is)	High Pressure Liquid Chromatography, 1) for feedingstuffs: Regulation (EC) No 152/2009 of 27 January 2009, Annex III, Part F (or equivalent: ISO 13903:2005) 2) pure products / premixtures: same method in adapting dilutions	
Trade name (if appropriate)				
Name of the holder of authorisation (if appropriate)				
Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg/kg of complete feedingstuffs (select what applicable)		
All species	Not applicable	Not applicable	Not applicable	None (essential amino acid)
Other provisions and additional requirements for the labelling				
Specific conditions or restrictions for use (if appropriate)	None			
Specific conditions or restrictions for handling (if appropriate)	Wearing a mask and safety goggles during handling is recommended			
Post-market monitoring (if appropriate)	Not considered necessary in view of adequate monitoring and operating conditions during the manufacturing process to ensure quality and safety of the additive. HACCP and Traceability systems are in place. Ajinomoto Eurolysine has been certified ISO 9001 and FAMI-QS.			
Specific conditions for use in complementary feedingstuffs (if appropriate)	None			
Maximum Residue Limit (MRL) (if appropriate)				
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues	
-	-	-	-	

Table 1c: Description and conditions of use of the additive L -lysine monohydrochloride, technically pure, as proposed by the applicant

Additive	L-lysine-monohydrochloride, technically pure			
Registration number/EC No/No (if appropriate)				
Category(ies) of additive	(c): Nutritional additive			
Functional group(s) of additive	3 (c): Amino acids, their salts and analogues			
Description				
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)	
White to pale yellow crystalline powder	C6H14N2O2 HCl	not less than 78% L-lysine (product as is)	High Pressure Liquid Chromatography, 1) for feedingstuffs: Regulation (EC) No 152/2009 of 27 January 2009, Annex III, Part F (or equivalent: ISO 13903:2005) 2) pure products / premixtures: same method in adapting dilutions	
Trade name (if appropriate)				
Name of the holder of authorisation (if appropriate)				
Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg/kg of complete feedingstuffs (select what applicable)		
All species	Not applicable	Not applicable	Not applicable	None (essential amino acid)
Other provisions and additional requirements for the labelling				
Specific conditions or restrictions for use (if appropriate)	None			
Specific conditions or restrictions for handling (if appropriate)	Like for any product which may generate dust, wear a mask and safety goggles			
Post-market monitoring (if appropriate)	Not considered necessary in view of adequate monitoring and operating conditions during the manufacturing process to ensure quality and safety of the additive. HACCP and Traceability systems are in place. Ajinomoto Eurolysine has been certified ISO 9001 and FAMI-QS.			
Specific conditions for use in complementary feedingstuffs (if appropriate)	None			
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 on data submitted in three individual dossiers for three different lysine forms provided by the same applicant, based on the same production strain and essentially the same production processes. It should be recognised that this dataset does not cover all existing additives containing L-lysine. The application is for the active substance and the composition of the additive formulation is not the subject of the application. The Panel has sought to use the data provided, together with data from other sources, to deliver an opinion.

1. Introduction

Lysine is an essential amino acid for all animal species. Lysine is clearly recognised as the first limiting amino acid in swine diets. L-Lysine and its salts are frequently used in the feed industry to adjust dietary lysine to the requirements of non-ruminant animals (and fish) in order to maximise production performance and reduce nitrogen emissions.

Three additives—concentrated liquid L-lysine (base), concentrated liquid L-lysine monohydrochloride and L-lysine monohydrochloride technically pure—are the subject of the application. L-Lysine is considered as the active substance. These additives are currently authorised for use in feeds for all animal species. The applicant asks for their re-evaluation.

Lysine hydrochloride is described in the European Pharmacopoeia (2010) monograph 01/2008:0930.

2. Characterisation

For the three additives under assessment—concentrated liquid L-lysine (base), concentrated liquid L-lysine monohydrochloride and L-lysine monohydrochloride technically pure—L-lysine is considered as the active substance. No evidence on the occurrence of D-lysine in any of the three additives is given in the dossiers.

2.1. Manufacturing process[13]

The product is obtained by fermentation using a genetically engineered *Escherichia coli*, strain which is deposited in the International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology culture collection, with accession number FERM BP-10941.

After the fermentation step, the production strain is inactivated, the killed bacterial cells are removed and the resulting fermentation broth is purified to obtain the concentrated liquid L-lysine (base). The broth is acidified and crystallised to obtain L-lysine monohydrochloride technically pure and concentrated liquid L-lysine monohydrochloride through a recycling loop.[14]

2.2. Concentrated liquid L-lysine (base)

The additive is an aqueous solution of dark-brown colour with a specific density of 1.10–1.12 g/cm^3 (between 5 °C and 25 °C) and a pH of 10–11. The specified content of L-lysine is 50 %. L-Lysine (International Union of Pure Applied Chemistry (IUPAC) name (*S*-)-2.6 diaminohexanoic acid, Chemical Abstracts Service (CAS) No 56-87-1) has a molecular weight of 146.2 and the molecular formula NH_2-$(CH_2)_4$-$CH(NH_2)$-COOH. The molecular structure is presented in Figure 1.

[13] This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
[14] Technical dossier/Section II.3 and Supplementary information (August 2013).

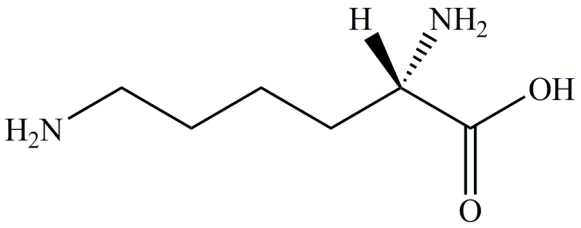

Figure 1: Molecular structure of L-lysine

In five analysed batches, the minimum and maximum contents of L-lysine (w/w) were 50.3 and 50.6 %, respectively, and the range of dry matter content was between 51.8 and 52.3 %.[15] On a dry matter basis, the L-lysine content was 96.8–97.2 %.

The product was analysed for amino acids other than L-lysine, biogenic amines, nitrates and nitrites, quantifiable sugars and organic acids, and inorganic anions and cations. Amino acids other than L-lysine formed, quantitatively, the most significant fraction (0.25–0.35 % of the product as is), followed by quantifiable inorganic ions (0.14–0.19 %); biogenic amines were between 0.005 and 0.01 %.[16] The sums of quantifiable compounds in the product dry matter are in the range of 98.0–98.3 %. The amount of unidentified compounds ranges from 1.7 to 2 % on a dry matter basis.

Three batches of the product were analysed for mycotoxins (aflatoxins, deoxynivalenol, zearalenone, ochratoxin A, T2 toxin, HT2 toxin, and fumonisins B1 and B2), heavy metals (cadmium, mercury and lead), arsenic chromium, cooper, iron, nickel, polycyclic aromatic hydrocarbon (PAH) compounds and dioxins and dioxin-like compounds.[17] The amounts of all these impurities were negligible and often below the detection limits. The microbiological purity checks of three batches included analyses for *Salmonella*, staphylococci, *Clostridium perfringens*, thermotolerant coliforms, coliforms at 30 °C, *Enterobacteriaceae*, faecal streptococci, total plate counts (at 30 °C), yeasts and moulds.[18] The observed counts were below the detection levels.

2.3. Concentrated liquid L-lysine monohydrochloride

The additive is an aqueous solution of dark-brown colour with a density of 1.10–1.12 g/cm^3 at 20 °C, and a pH value of 7–8.5. It contains, by specification, 28 % L-lysine HCl (IUPAC name 2.6-diaminohexanoid hydrochloride, CAS No 657-27-2). Its molecular weight is 182.65 and the molecular formula is NH_2-$(CH_2)_4$-$CH(NH_2)$-COOH-HCl. For the molecular structure, see Figure 2.

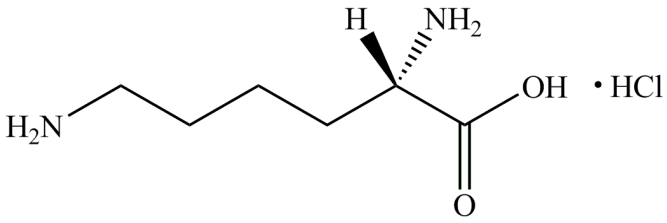

Figure 2: Molecular structure of L-lysine HCl

[15] Technical dossier FAD-2010-0206/Section II.2.1.3.2.1.
[16] Technical dossier FAD-2010-0206/Section II.2.1.3.2.2, II.2.1.3.2.4, II.2.1.3.2.5 and II.2.1.3.2.6.
[17] Technical dossier FAD-2010-0206/Section II.2.1.3.3.2.
[18] Technical dossier FAD-2010-0206/Section II.2.1.4.2.2.

In the analyses of five batches, L-lysine content ranged between 22.5 and 22.7 % and the range of dry matter content was between 34.6 and 38.9 %.[19] On a dry matter basis, the L-lysine content was 58.3–65.2 %.

The product (five batches) was analysed for amino acids other than L-lysine, biogenic amines, nitrates and nitrites, quantifiable sugars and organic acids, and inorganic anions and cations. Inorganic compounds formed the largest fraction, dominated by chloride (20.7–23 %). Amino acids other than L-lysine were between 2.3 and 4.1 % in dry matter; biogenic amines were between 0.1 and 0.2 %.[20] The sums of quantifiable compounds in the product dry matter were in the range of 86.7–92.0 %. The proportion of unidentified compounds ranged from 8.0 to 13.3 % on a dry matter basis.

Three product batches were analysed for impurities and contaminants (mycotoxins, heavy metals (cadmium, mercury and lead), arsenic, chromium, copper, iron, nickel, PAH compounds, and dioxins and dioxin-like compounds) as described for concentrated L-lysine liquid.[21] The detected amounts of the chemical contaminants/impurities were negligible and often below the detection limits. In the analyses of six batches for microbial contaminants (as described for the concentrated L-lysine liquid) the counts were generally below the detection levels, except in three batches where total plate counts were 1 800, 1 500 and 900 colony forming units/g.[22]

2.4. L-Lysine monohydrochloride technically pure

The additive is a white to pale-yellow or pale-brownish crystalline powder with a density of 0.55–0.65 g/cm^3 and a pH of 5.6–5.9 at 5 % w/w solution (20 °C) containing, by specification, 78 % L-lysine. L-Lysine HCl is soluble in water (500–642 g/L at 20–30 °C). It has limited solubility in methanol and in ethanol, hexane and chloroform.

Analyses of five batches showed an average lysine content of 79.5 % (range 79.3–79.8 %) with an average moisture content of 0.6 % (range 0.5–0.8 %).[23] The average L-lysine HCl content corresponds to 99.9 % in the dry matter.

The product was analysed for amino acids other than L-lysine, biogenic amines, nitrates and nitrites, quantifiable sugars and organic acids, and inorganic compounds (crude ash, potassium, magnesium, calcium, chlorides, sulphates and phosphates). With the natural exception of chlorides (which were present at 19.5–20.0 %), the other components were absent or present only in trace amounts. This confirms that the product consists almost entirely of L-lysine HCl.

Three product batches were analysed for impurities and contaminants (mycotoxins, heavy metals (cadmium, mercury and lead), arsenic, chromium, cooper, iron, nickel, PAH compounds, and dioxins and dioxin-like compounds) as described for concentrated liquid L-lysine (base). The amounts of the chemical contaminants/impurities were negligible and often below the detection limits. The observed values for microbial contaminants (as described for the concentrated liquid L-lysine (base)) from the analysis of five batches were below the detection limits.

Five batches were mechanically sieved and the average fraction < 100 μm was 6 %. Another batch was analysed by laser light scattering and approximately 10 % of the particles were smaller than 36 μm. The dusting potential of one batch was measured with a rotating drum tester (EN 15051, European Committee for Standardisation (CEN), 2006).[24] The product has a considerable dusting potential (13 g/kg) and the dust consisted mainly of particles with dimensions < 7 μm. The inhalable

[19] Technical dossier FAD-2010-0207/Section II.2.1.3.2.1.
[20] Technical dossier FAD-2010-0207/Section II.2.1.3.2.2, II.2.1.3.2.4, II.2.1.3.2.5 and II.2.1.3.2.6.
[21] Technical dossier FAD-2010-0207/Section II.2.1.3.3.2.
[22] Technical dossier FAD-2010-0207/Section II.2.1.4.2.2/Table 2.1.4.2.2.b.
[23] Technical dossier FAD-2010-0210/Section II.2.1.3.2.1.
[24] Technical dossier FAD-2010-0210/Section II.2.1.5.1, II.2.1.5.2 and Annex II.45.

fraction was 7 494 mg/kg, the thoracic fraction was 4 601 mg/kg and the respirable fraction was 873 mg/kg, all corresponding to high dustiness.

2.5. Characterisation of the production organism[25]

2.5.1. Information relating to the genetically modified microorganism

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

2.5.2. Information relating to the absence of recombinant DNA and the production organism

The manufacturing process has been described above (see Section 2.1). No production strain could be detected in three batches of each the final products concentrated liquid L-lysine (base),[26] concentrated liquid L-lysine monohydrochloride,[27] and L-lyine monohydrochloride technically pure.[28] Additionally, no recombinant DNA was detected in 10 batches of each of the final products concentrated liquid L-lysine (base),[29] concentrated liquid L-lysine monohydrochloride,[30] and L-lyine monohydrochloride technically pure.[31]

2.6. Stability and homogeneity

As liquid products are not intended to be incorporated in the final feed via premixtures, studies in premixtures are not required.

2.6.1. Concentrated liquid L-lysine (base)

The stability of three batches of concentrated liquid L-lysine (base) was provided for 12 months at 5, 25 and 40 °C in closed containers.[32,33] Loss of L-lysine increased with storage temperature and ranged between 0.9 % at 5 °C and 7 % at 40 °C.

The stability of one batch of concentrated liquid L-lysine (base) in a pelleted pig grower feed was studied at 5, 25 and 40 °C, in all cases at 60 % relative humidity (RH), in punctured nylon polyethylene (PE) bags for six months.[34] The supplementation of the additive to the final feed was 6.24 g/kg, equivalent to 3.1 g L-lysine/kg. Pelleting did not affect the free lysine concentration. Storage for six months at 5 and 25 °C had no influence on the free lysine content of the additive. After three months' storage at 40 °C, there was a decline of approximately 20 %. No further losses were observed after six months.

Ten subsamples of the same feed were taken to study the homogeneous distribution of the additive in mash and pelleted feed. The coefficient of variation (CV) of the mean free lysine concentration in mash feed was 5 % and in pelleted feed was 3 %.[35]

[25] This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
[26] Technical dossier/Supplementary information October 2012/Annex II_74_3.
[27] Technical dossier/Supplementary information October 2012/Annex II_74_2.
[28] Technical dossier/Supplementary information October 2012/Annex II_74_1.
[29] Technical dossier/Supplementary information April 2013.
[30] Technical dossier/Supplementary information April 2013.
[31] Technical dossier/Supplementary information April 2013.
[32] Technical dossier FAD-2010-0206/Section II.2.4.1.2.2.
[33] Supplementary information October 2012/Section II.4.1.2 and Annex II.81.2.
[34] Technical dossier FAD-2010-0206/Section II.2.4.1.2.2 and Annex II.91.
[35] Technical dossier FAD-2010-0206/Section II.2.4.1.2.2/Annex II.91/Table 6b.

2.6.2. Concentrated liquid L-lysine monohydrochloride

Stability data of three batches of concentrated liquid L-lysine HCl were provided at 5, 25 and 40 °C in closed containers.[36] The product was very stable at 5 °C, with virtually no loss of lysine over 12 months. Data obtained after 12 months at 25 and 40 °C indicated a marginal degradation (recovery between 96 and 98 %).

The stability of one batch of concentrated liquid L-lysine HCl in a pelleted pig grower feed was studied at three climatic conditions (at 5, 25 and 40 °C and 60 % RH at each temperature, in punctured nylon PE bags) for six months.[37] The supplementation of the additive to the final feed was 13.94 g/kg, equivalent to 3.1 g L-lysine/kg. Pelleting did not affect the free lysine concentration. Storage for six months at 5 and 25 °C resulted in marginal losses of free lysine. At 40 °C there was a reduction of about 20 % after three months, without further decline.

Ten subsamples of the same feed were taken to study the homogeneous distribution of the additive in feed. The CV of the mean free lysine concentration in mash feed was 4 % and in pelleted feed was 2 %.[38]

2.6.3. L-Lysine monohydrochloride technically pure

One batch of L-lysine HCl technically pure was stored in punctured PE bags under five environmental conditions: 5, 25 and 40 °C at 60 % RH and 25 and 40 °C at 30 % RH for 18 months.[39] Two additional batches were stored in punctured PE bags under three environmental conditions: 5, 25 and 40 °C always at 60 % RH for 12 months.[40] The product was stable under all environmental conditions tested.

Three batches of the additive were incorporated in different pig starter premixtures (143.9 g additive/kg premixture) containing vitamins, trace elements, enzymes and other amino acids and stored in punctured nylon PE bags for six months (two batches) and nine months (one batch).[41,42] Storage conditions were 5, 25 and 40 °C at 60 % RH. At 5 and 25 °C, losses after three months ranged between 0 and 6 %, after six months ranged between 1 and 7 % and after nine months ranged between 11 and 13 %. At 40 °C, losses ranged between 4 and 10 % after three and six months, and were up to 13 % after nine months.

Three batches of L-lysine HCl technically pure were incorporated (4.1 g additive/kg feed, equivalent to 3.2 g L-lysine/kg feed) in a grower pig feed and stored under three conditions (at 5, 25 and 40 °C and 60 % RH at each temperature) for a period of three months (two batches) or six months (one batch).[43,44] Pelleting (80 °C) did not affect the free lysine concentration. After six months' storage of pelleted samples at 5 and 25 °C, losses were 2 and 6 %, respectively. Losses reached 19 % when the additive was stored at 40 °C.

Ten subsamples of one premixture and of three batches of the same feed used in the stability studies were taken to study the homogeneous distribution of the additive in mash and pelleted feed.[45] The CV of the mean free lysine concentration in the premixture was 2 %, in mash feed was 4 to 6 % and in pelleted feed was 2 to 3 %.

[36] Technical dossier FAD-2010-0207/Section II.2.4.1.1.2; Supplementary information October 2012/Section III.3.1.2 and Annex II.79.2.
[37] Technical dossier FAD-2010-0207/Section II.2.4.1.2.2 and Annex II.91.
[38] Technical dossier FAD-2010-0207/Section II.2.4.1.2.2/Annex II.91/Table 6a.
[39] Technical dossier FAD-2010-0210/Section II.2.4.1.1.2 and Annex II.100.
[40] Supplementary information October 2012/Annex II.76.2.
[41] Supplementary information October 2012/Section III.2.2.2 and Annex II.77.2.
[42] Technical dossier FAD-2010-0210/Section II.2.4.1.2.1 and Annex II.101.
[43] Technical dossier FAD-2010-0210/Section II.2.4.1.2.2 and Annex II.102.
[44] Supplementary information October 2012/Section III.2.3.2 and Annex II.78.2.
[45] Technical dossier FAD-2010-0210/Section 2.4.2.1; Technical dossier FAD-2010-0210/Section 2.4.2.2; Supplementary information October 2012/Section 4.2.2/Tables 6b and 6c.

2.7. Conditions of use

According to the applicant, concentrated liquid L-lysine (base) and concentrated liquid L-lysine HCl are intended to be incorporated directly into the final feed. L-Lysine HCl technically pure is foreseen to be mixed into premixtures and final feed. These substances are intended to be used in all animal species without limitations in dose or time. They are proposed to be supplemented to feedingstuffs which are deficient in the essential amino acid L-lysine. No proposed inclusion levels are provided, as the optimal daily allowance in quantitative terms depends on the species as well as on the physiological state of the animal, performance level and environmental conditions.

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.[46]

3. Safety[47]

3.1. Safety aspects of the genetic modification

The parental strain is considered to be safe. The molecular characterisation of the genetic modifications does not indicate any safety concerns.

3.2. Toxicological studies

The metabolism of the L-lysine is described in the Appendix.

Concentrated liquid L-lysine HCl (obtained through a recycling loop) is the product that contains the highest concentration of substances other than L-lysine HCl in the additive. Consequently, concentrated liquid L-lysine HCl can be considered representative (worst-case scenario) of the other two products (i.e. concentrated liquid L-lysine base and L-lysine HCl technically pure).

Therefore, the FEEDAP Panel has identified the results of the studies performed with concentrated liquid L-lysine HCl ("worst scenario" product) derived from *E. coli* (FERM BP-10941), i.e. from the strain under assessment, as representative of the other L-lysine-based products. These studies are a bacterial reverse mutation test, a gene mutation test in cultured mammalian cells, an *in vivo* micronucleus test, a 90-day chronic oral repeat dose study and an acute inhalation toxicity test. They are summarised below.

3.2.1. Genotoxicity studies including mutagenicity

Bacterial reverse mutation assays were carried out, in compliance with Organisation for Economic Co-operation and Development (OECD) Guideline 471, on four *Salmonella* strains (TA1535, TA1537, TA98 and TA100) and *E. coli* WP2*uvrA* with and without metabolic activation. The maximum tested concentration was 5 000 μg/plate.[48] No evidence of mutagenic activity was observed and the positive control performed as expected.

Gene mutation tests (in accordance with OECD Guideline 476) at the TK locus of the L5178Y cell line were performed, in both the presence and absence of metabolic activation,[49] with concentrated liquid L-lysine HCl at doses up to 6 500 μg/mL. No evidence of mutagenicity was found.

[46] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2010-0067.pdf

[47] This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.

[48] Technical dossier FAD-2010-0206/Section III/Annexes III.58 and III.59; Technical dossier FAD-2010-0207/Section III/Annexes III.58, III.59 and III.60; Technical dossier FAD-2010-0210/Section III/Annexes III.58 and III.59.

[49] Technical dossier FAD-2010-0206/Section III/Annex III.60; Technical dossier FAD-2010-0207/Section III/Annexes III.61, III.62 and III.63; Technical dossier FAD-2010-0210/Section III/Annexes III.60 and III.61.

An *in vivo* micronucleus test study (in accordance with OECD Guideline 474) was conducted in Wistar rats at doses up to 6 % in connection with a 13-week subchronic oral toxicity study using bone marrow cells harvested from the femur at necropsy.[50] There was no indication of an increase in micronuclei in this study.

3.2.2. Subchronic oral toxicity studies

A trial was conducted in Wistar rats in accordance with OECD Guideline 408, the nominal values of the test substance in feed were 0, 0.6, 2 and 6 % (on a dry matter basis). Concentrated liquid L-lysine HCl was assumed to contain 64.2 % water and, on that basis, the water content of each diet, including the control diet, was equalised. Feed consumption and water intake, red blood cell count, organ weights and the results of clinical chemistry, urinalysis, macroscopic and microscopic examinations, evaluation of oestrus cyclicity and sperm analysis were recorded. The administration of the test substance did not induce any modification of the above-mentioned parameters.

3.2.3. Conclusions on toxicological studies

Concentrated liquid L-lysine HCl produced by *E. coli* (FERM BP-10941) shows no evidence of genotoxicity or subchronic oral toxicity. As concentrated liquid L-lysine HCl is considered to be the worst scenario in terms of purity, these results can be extrapolated to the other lysine-based additives, i.e. concentrated liquid L-lysine (base) and L-lysine technically pure produced by *E. coli* (FERM BP-10941).

3.3. Safety for the target species

Tolerance studies with indispensable amino acids such as lysine cannot be designed according to the protocols of conventional toxicity experiments because high dietary concentrations of a certain amino acid will result in amino acid imbalances with depression of feed intake and hence impaired performance.

Considering the decreasing availability of L-lysine-rich protein feedingstuffs on the market, most feed materials are deficient in lysine compared with animals' requirements. Modern genotypes, particularly those of pigs, show increased nitrogen retention and therefore a greater L-lysine requirement. As a consequence, most complete feeds, except those for ruminants, require L-lysine supplementation. Data on requirement, allowances and recommendations for L-lysine in the different animal species and categories, and on the content and digestibility of L-lysine in feed materials, are easily accessible in the standard literature for animal nutritionists. Any risk of intentional excess supplementation is considered very low.

Depending on genetics, sex and physiological stage, L-lysine requirements for swine range, according to the National Research Council (NRC, 1998), from 0.6 % (gestating sows) to 1.45 % (post-weaned piglets). In poultry (NRC, 1994), L-lysine requirements range from 0.45 % (pullets) to 1.6 % (turkeys). Higher values are proposed by some producers of commercial hybrids. Cereals contain less than 0.5 % lysine, legume seeds (soybean, lupin, pea) and rapeseed contain between 1.5 and 2.5 % lysine, and protein-rich extracted feed materials, concentrates and isolates (e.g. soybean, potato) contain up to 6 % lysine. With large differences depending on the dietary formulations, L-lysine supplementation in feeds varies from 0.05 % (laying hens and gestating sows) to 0.35 % (chicks and piglets).

The tolerance of target animals to an excess of L-lysine is relatively high. Most reports available for pigs (e.g. Edmonds et al., 1987), poultry (e.g. Edmonds and Baker, 1987) and laboratory animals (for example, reviewed by Harper et al., 1970) indicate that the supplementation of complete feed at or above 4 % of L-lysine depresses feed intake and growth. This corresponds to at least 10-fold higher than the highest supplementation level considered necessary in practice.

[50] Technical dossier FAD-2010-0207/Section III/Annex III.66.

A specific feature of lysine metabolism is the lysine–arginine antagonism, first reported in the 1950s (Kamin and Handler, 1952). Intolerance symptoms to lysine overdoses could be at least partially compensated by additional arginine. The applicant provided 15 studies on this phenomenon on different target species. As a result, the lysine–arginine antagonism was clearly seen in chicks and dogs but not in pigs, fish or cats. Nevertheless, this antagonism does not play a significant role in practical feeding.

However, for nutritional additives produced by fermentation, the risk associated with the residues of the fermentation process in the final product needs to be assessed.

L-Lysine HCl technically pure is characterised as 99.9 % L-lysine HCl in dry matter, and it is therefore considered that no risks associated with the fermentation will remain. The other two additives, concentrated liquid L-lysine (base) and concentrated liquid L-lysine HCl, have levels up to 2 and 13.5 % of unidentified material, respectively, which are likely to be coming from the fermentation process. In order to ensure the safety of these two additives, one tolerance study in the target species or in a laboratory species would be required. No tolerance studies in the target species have been provided. However, the results of the subchronic repeat dose oral toxicological studies in rats performed with concentrated liquid L-lysine HCl (see section 3.2.2) support the safety of the products under assessment for target animal species.

3.3.1. Conclusions on the safety for target species

The use of L-lysine in the form of concentrated liquid L-lysine (base), concentrated liquid L-lysine HCl and L-lysine HCl technically pure is safe for target species when supplemented in appropriate amounts to diets which are easily accessible in the standard literature for animal nutritionists.

3.4. Consumer safety

The outcome of the toxicological studies did not provide any specific concerns relevant to consumer safety resulting from the use of L-lysine products obtained by fermentation with *E. coli* (FERM BP-10941). The amino acid profile of tissues and products of animal origin will not be changed by the use of L-lysine-containing additives. Therefore, the FEEDAP Panel concludes that the use of concentrated liquid L-lysine (base), concentrated liquid L-lysine HCl and L-lysine HCl technically pure in animal feed does not pose a risk for the consumer.

3.5. Safety for the user

The applicant provided a study on acute inhalation toxicity, performed in compliance with OECD Guideline 403, which was carried out with concentrated liquid L-lysine HCl produced by *E. coli* (FERM BP-10941). In this study, the additive did not cause any permanent adverse effects in rats exposed to the nebulised product at a concentration of 5.53 g/m^3.[51] The lethal concentration which kills 50 % of test animals (LC_{50}) is > 5.53 g/m^3, which is equivalent to 6.64 g/kg of air and is classified in the Hodge and Sterner scale as "slightly toxic".[52]

Studies on the effects on eye and skin irritation and skin sensitisation were provided for formulations produced by another strain of *E. coli* K-12, but, owing to very similar purities, these formulations are considered equivalent to the additives under assessment.

Occluded dermal patch tests were conducted with 0.5 mL of concentrated liquid L-lysine (base), 0.5 mL of concentrated liquid L-lysine HCl or 0.5 g in 0.25 mL water of L-lysine HCl technical in

[51] Technical dossier FAD-2010-0207/Section III.3.3.1.1/Annex III.76.
[52] Canadian Centre for Occupational Health and Safety, 2013, available online : www.ccohs.ca

rabbits (in accordance with OECD Guideline 404). None of the products caused any signs of skin irritation at any time.[53]

When applied to the eyes of rabbits (conjunctival sac) at a dose of 0.1 mL, concentrated liquid L-lysine (base) caused only transient redness and swelling of the conjunctivae which disappeared within 24 hours (OECD Guideline 405).[54] The outcome was the same with concentrated liquid L-lysine HCl (dose 0.1 mL) and with L-lysine HCl technically pure (dose approx. 0.06 g).[55]

In a maximisation study in guinea pigs (OECD Guideline 406), concentrated liquid L-lysine (base), concentrated liquid L-lysine HCl and L-lysine HCl technically pure did not show any potential for skin sensitisation.[56]

3.5.1. Conclusions on the safety for the user

Neither concentrated liquid L-lysine HCl produced by *E. coli* (FERM BP-10941) nor the different additives containing L-lysine (i.e. concentrated liquid L-lysine base and L-lysine HCl technically pure), obtained from a different *E. coli* K-12 strain and considered as equivalent to those under assessment, showed any potential to cause respiratory toxicity, skin or eye irritation or skin sensitisation. A potential for respiratory sensitisation cannot be excluded for concentrated liquid L-lysine (base) and concentrated liquid L-lysine HCl owing to the possible protein content.

3.6. Safety for the environment

Neither the production strain nor its recombinant DNA was detectable in any of the final products. The final products do not raise environmental safety concern associated with the genetic modifications.

Lysine is a physiological and natural component in animals and plants. Lysine and its salts are not excreted as such (but as urea/uric acid and CO_2). The use of lysine and its salts in animal nutrition would not lead to any localised increase in the concentration in the environment. It is concluded that the use of the products under application as feed additives does not represent a risk to the environment.

4. Efficacy

The efficacy of supplementing L-lysine and L-lysine HCl is extensively demonstrated in the literature for mammals (except ruminants), poultry and fish. This involves their use in a liquid or a solid form. Concentrated liquid L-lysine (base), concentrated liquid L-lysine HCl and L-lysine HCl technically pure do not require any further demonstration of efficacy. Response in ruminants requires some degree of protection of lysine 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[57] and Good Manufacturing Practice.

[53] Technical dossier FAD-2010-0206/Section III.3.3.1.2.2/Annex III.72; Technical dossier FAD-2010-0207/Section III.3.3.1.2.2/Annex III.78; Technical dossier FAD-2010-0210/Section III.3.3.1.2.2/Annex III.74.
[54] Technical dossier FAD-2010-0206/Section III.3.3.1.2.1/Annex III.71.
[55] Technical dossier FAD-2010-0207/Section III.3.3.1.2.1/Annex III.77; Technical dossier FAD-2010-0210/Section III.3.3.1.2.1/Annex III.73.
[56] Technical dossier FAD-2010-0206/Section III.3.3.1.2.3/Annex III.73; Technical dossier FAD-2010-0207/Section III.3.3.1.2.2/Annex III.79; Technical dossier FAD-2010-0210/Section III.3.3.1.3/Annex III.75.
[57] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 Jan 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Neither the production strain nor its recombinant DNA was detected in any of the final products. The final products do not raise any safety concern with regard to the genetic modifications.

Concentrated liquid L-lysine (base), concentrated liquid L-lysine HCl and L-lysine HCl technically pure are considered safe for target species when supplemented in appropriate amounts.

Lysine produced by *E. coli* (FERM BP-10941) is not genotoxic and the results of subchronic studies do not indicate any specific concerns. As there are no lysine metabolites associated with safety concerns in the animal tissues and products, the FEEDAP Panel considers that the use of L-lysine and its hydrochloride salts in animal feed does not pose a risk for the consumer.

Concentrated liquid L-lysine (base), concentrated liquid L-lysine HCl and L-lysine HCl technically pure are not considered to have the potential to cause respiratory toxicity, skin or eye irritation or skin sensitisation, but respiratory sensitisation cannot be excluded.

L-Lysine is a substance naturally occurring in bacteria, plants and animals. The use of L-lysine-containing feed additives does not represent a risk to the environment.

Concentrated liquid L-lysine (base), concentrated liquid L-lysine HCl and L-lysine HCl technically pure are considered equivalent in terms of L-lysine availability to the target animals. The efficacy of supplementing L-lysine and its hydrochloride salts is extensively demonstrated in the literature for mammals (except ruminants), poultry and fish, including its use in a liquid or a powder form. Therefore it does not require any further demonstration. Response in ruminants requires some degree of protection of L-lysine from ruminal degradation.

RECOMMENDATIONS

The description of the additive should contain the statement "produced by fermentation with *Escherichia coli* (FERM BP-10941)".

DOCUMENTATION PROVIDED TO EFSA

1. Application for the renewal of authorisation of concentrated liquid L-Lysine (base), concentrated liquid L-lysine monohydrochloride, and L-lysine monohydrochloride technically pure as nutritional feed additives. August 2011. Submitted by Ajinomoto Eurolysine S.A.S.

2. Application for the renewal of authorisation of concentrated liquid L-Lysine (base), concentrated liquid L-lysine monohydrochloride, and L-lysine monohydrochloride technically pure as nutritional feed additives. Supplementary information. October 2012. Submitted by Ajinomoto Eurolysine S.A.S.

3. Application for the renewal of authorisation of concentrated liquid L-Lysine (base), concentrated liquid L-lysine monohydrochloride, and L-lysine monohydrochloride technically pure as nutritional feed additives. Supplementary information. April 2013. Submitted by Ajinomoto Eurolysine S.A.S.

4. Application for the renewal of authorisation of concentrated liquid L-Lysine (base), concentrated liquid L-lysine monohydrochloride, and L-lysine monohydrochloride technically pure as nutritional feed additives. Supplementary information. August 2013. Submitted by Ajinomoto Eurolysine S.A.S.

5. Comments from Member States received through the ScienceNet.

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APPENDIX

The metabolism of L-lysine

After ingestion and breakdown of the intact protein by the action of endo- and exopeptidases, the released lysine is transported by sodium-dependent and -independent systems in the brush border and the basolateral membrane of the small intestine (see review by Bröer, 2008). In pigs, the apparent ileal and standardised ileal digestibility of lysine is about 84 % and 90 %, respectively, in a maize soybean meal-based diet (e.g. Stein et al., 2007). The apparent ileal lysine digestibility for pigs ranges from about 64 % to 73 % for grains and grain products, and from 75 to 93 % for maize gluten meal and soybean products (see NRC, 1998). However, in feed materials and feedingstuffs subjected to strong heat treatment (overheating), the digestibility and bioavailability of cationic amino acids such as lysine can be adversely affected by the Maillard reaction. In pigs, the apparent ileal digestibility of crystalline L-lysine HCl is about 97 % and true digestibility and bioavailability is around 100 %, as for all crystalline amino acids (e.g. Izquierdo et al., 1988; Chung and Baker, 1992). However, microbial degradation (e.g. to cadaverine) in the small intestine may negatively affect its bioavailability (Dierick et al., 1986; Van der Meulen et al., 1998; Bikker et al., 2006). After being absorbed, L-lysine is mainly used for protein accretion (N retention, weight gain). Excess lysine is catabolised or excreted directly via the kidney. Lysine catabolism occurs mainly in the liver and comprises deamination and decarboxylation; in the first step the lysine-α-ketoglutarate reductase condenses L-lysine and α-ketoglutarate to saccharopine (in the brain of humans, monkeys and rats they are also condensed to pipecolate). Saccharopine is further converted via several enzyme-mediated steps to acetyl CoA (Chang, 1982). For a detailed scheme of lysine metabolic pathway see Papes et al. (2001).

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