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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of zinc chelate of L-lysinate-HCl as feed additive 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

Zinc chelate of L-lysinate-HCl for all animal species, provided as powder and granulate, is intended for use as zinc compound in animal nutrition. Tolerance studies with calves and weaned piglets allowed the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) to conclude that zinc chelate of L-lysinate-HCl is a safe source of zinc for all animal species, provided that the maximum zinc contents authorised in complete feed are respected. The supplementation of feeds with zinc from zinc chelate of L-lysinate-HCl up to the maximum authorised zinc levels is not expected to result in a different zinc deposition in edible tissues/products than the standard inorganic source zinc sulphate heptahydrate. No concerns for consumer safety will arise from the use of the additive in animal nutrition. Zinc chelate of L-lysinate-HCl in the powder form poses a risk to users by inhalation, whilst for the granulate form that risk is minimised. Neither form, powder or granulate, is a dermal irritant; however, both forms are irritant to the eye. In the absence of specific information, the additive should be regarded as a potential skin sensitiser. Zinc chelate of L-lysinate-HCl is intended to be a substitute for other authorised zinc additives. It will therefore not further increase the environmental burden of zinc. Based on a study on weaned piglets, the FEEDAP Panel concluded that zinc chelate of L-lysinate-HCl is efficacious as a source of zinc in meeting the requirements of all animal species. The Panel made some recommendations regarding the *Description and Conditions of use of the additive*.

KEY WORDS

nutritional additive, compounds of trace elements, zinc, zinc chelate of L-lysinate-HCl, safety, efficacy

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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 the safety and efficacy of zinc chelate of L-lysinate-HCl for all animal species. The additive is intended to be marketed in two forms, powder and granules.

Tolerance studies with calves and weaned piglets allowed the FEEDAP Panel to conclude that zinc chelate of L-lysinate-HCl does not elicit additional or different adverse effects compared with the standard inorganic source zinc sulphate heptahydrate; therefore, the additive is a safe source of zinc for all animal species, provided that the maximum zinc contents authorised in complete feed are respected.

The supplementation of feeds with zinc from zinc chelate of L-lysinate-HCl up to the maximum authorised zinc levels is not expected to result in a different zinc deposition in edible tissues/products than the standard inorganic source zinc sulphate heptahydrate. No concerns for consumer safety will arise from the use of the additive in animal nutrition.

Zinc chelate of L-lysinate-HCl in the powder form poses a risk to users by inhalation, whilst for the granulate form that risk is minimised. Neither form, powder or as granulate, is a dermal irritant. Both forms are irritant to the eye. In the absence of specific information, the additive should be regarded as a potential skin sensitiser.

The additive under assessment, zinc chelate of L-lysinate-HCl, is intended to be a substitute for other authorised zinc additives. It will therefore not further increase the environmental burden of zinc.

Based on a study on weaned piglets, the FEEDAP Panel concluded that zinc chelate of L-lysinate-HCl is efficacious as a source of zinc in meeting the requirements of all animal species.

The Panel made some recommendations regarding the *Description and Conditions of use of the additive*.

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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 the company Senzyme GmbH[5] for authorisation of the product zinc chelate of L-lysinate-HCl, when used as a feed additive for all animal species (category: Nutritional additives; functional group: compounds of trace elements) 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 16 September 2014.

The feed additive zinc chelate of L-lysinate-HCl has not been previously authorised in the European Union (EU). Other zinc compounds are authorised to be used as nutritional feed additives (trace elements) in the EU by Commission Regulation (EC) No 1334/2003,[7] Commission Regulation (EC) No 479/2006,[8] Commission Regulation (EC) No 888/2009,[9] Commission Regulation (EU) No 335/2010,[10] Commission Implementing Regulation (EU) No 991/2012[11] and Commission implementing Regulation (EU) No 636/2013.[12]

The Scientific Committee on Animal Nutrition (SCAN) issued an opinion on the use of zinc in feedingstuffs (EC, 2003a). EFSA issued opinions on the safety of the chelated forms of iron, copper, manganese and zinc with synthetic feed grade glycine (EFSA, 2005), on the safety and efficacy of a zinc chelate of hydroxy analogue of methionine (Mintrex®Zn) (EFSA, 2008, 2009a, 2009b), of tetra-basic zinc chloride (EFSA, 2012a) and of methionine-zinc (EFSA FEEDAP Panel, 2013a) as feed additives (compounds of trace elements). In the frame of re-evaluation, EFSA has delivered seven opinions: zinc chelate of amino acids hydrate (EFSA, 2012b; EFSA FEEDAP Panel, 2013b), zinc sulphate monohydrate (EFSA 2012c, 2012d), zinc oxide (EFSA, 2012e) and of seven zinc compounds (zinc acetate, dihydrate; zinc chloride, anhydrous; zinc oxide; zinc sulphate, heptahydrate; zinc sulphate, monohydrate; zinc chelate of amino acids, hydrate; zinc chelate of glycine, hydrate (EFSA FEEDAP Panel, 2015).

[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] Senzyme GmbH, Gierlichsstraße, 6. 53840 Troisdorf , Germany.
[6] EFSA Dossier reference: FAD-2014-0021.
[7] Commission Regulation (EC) No 1334/2003 amending the conditions for authorisation of a number of additives in feedingstuffs belonging to the group of trace elements. OJ L 187, 26.7.2003, p. 11.
[8] Commission Regulation (EC) No 479/2006 as regards the authorisation of certain additives belonging to the group compounds of trace elements. OJ L 86, 24.3.2006, p. 4.
[9] Commission Regulation (EC) No 888/2009 concerning the authorisation of Zinc chelate of hydroxy analogue of methionine as a feed additive for chickens for fattening. OJ L 254, 26.9.2009, p. 71.
[10] Commission Regulation (EU) No 335/2010 of 22 April 2010 concerning the authorisation of zinc chelate of hydroxy analogue of methionine as a feed additive for all animal species. OJ L 102, 23.4.2010, p. 22.
[11] Commission Implementing Regulation (EU) No 991/2012 of 25 October 2012 concerning the authorisation of zinc chloride hydroxide monohydrate as feed additive for all animal species. OJ L 297, 26.10.2012, p. 18.
[12] Commission Implementing Regulation (EU) No 636/2013 concerning the authorisation of zinc chelate of methionine (1:2) as a feed additive for all animal species. OJ L 183, 2.7.2013, p. 3.

EFSA has issued an opinion on the potential reduction of the currently authorised maximum zinc content in complete feed. The newly proposed maximum contents are: 150 mg Zn/kg complete feed for piglets, sows, rabbits, salmonids, cats and dogs; 120 mg Zn/kg complete feed for turkeys for fattening; 100 mg Zn/kg complete feed for all other species and categories (EFSA FEEDAP Panel, 2014).

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 zinc chelate of L-lysinate-HCl, 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	Zinc chelate of L-Lysinate HCl (Aminotrace Zinc Bislysinate)
Registration number/EC No/No (if appropriate)	-
Category(-ies) of additive	nutritional additive, 3
Functional group(s) of additive	compounds of trace elements, b

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
Zinc chelate from L-Lysinate HCl	$Zn(C_6H_{13}N_2O_2)_2$ x 2HCl	in compliance with EU legislation	EN 15510:2007 (zinc) VDLUFA 4.11.6 (L-Lysinate HCl)

Trade name (if appropriate)	n.a.
Name of the holder of authorisation (if appropriate)	n.a.

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	-	-	according Commission Regulation (EC) 1334/2003	n.a.

Other provisions and additional requirements for the labeling	
Specific conditions or restrictions for use (if appropriate)	Feed formulation should be adjusted to account for the lysine activity of L-Lysinate HCl.
Specific conditions or restrictions for handling (if appropriate)	For user safety: use safety glasses and protective gloves. In case of dust formation take appropriate measures for breathing protection. The precautions for handling on the safety data sheet must be observed.
Post-market monitoring (if appropriate)	Senzyme GmbH will conduct post-marketing monitoring in compliance with EU law on feed hygiene, namely by use of HACCP and Traceability systems and formal monitoring of customer feedback through product or service complaints.

Specific conditions for use in complementary feedingstuffs (if appropriate)	To supply zinc in final feeds within EU legal limits for each species

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
n.a.	n.a.	n.a.	n.a.

ASSESSMENT

1. Introduction

The transition metal zinc is essential to all living organisms. It is an integral component of an estimated 10 % of all proteins, in which it contributes to tertiary structure or catalytic activity covering all enzyme classes. It is also a signalling substance in its functions as second messenger and as synaptic neuromodulator. The biological functions of zinc are numerous and diverse and include glucose and lipid metabolism, cell proliferation, embryogenesis and those related to the nervous and immune systems. The roles of zinc, its deficiency and toxicity symptoms in farm animals have been described in previous opinions of the Scientific Committee on Animal Nutrition (EC, 2003a) and the European Food Safety Authority Panel on Additives and Products or Substances used in Animal Feed (EFSA FEEDAP Panel, 2014, 2015). To the knowledge of the FEEDAP Panel, there is no additional relevant information that may lead to reconsideration of these opinions.

The applicant is seeking authorisation for the use of zinc chelate of L-lysinate-HCl in feed for all animal species/categories. This zinc compound is not currently authorised in the EU as a feed additive.

2. Characterisation

For compounds of trace elements, the element itself is considered the active substance.

2.1. Characterisation of zinc chelate of L-lysinate-HCl

A Chemical Abstracts Service (CAS) or the International Union of Pure and Applied Chemistry (IUPAC) name could not be identified for the additive under assessment, zinc chelate of L-lysinate-HCl.[13] However, for the zinc lysine complex without the HCl, the CAS number is 23333-98-4 and the IUPAC name is zinc bislysinate chelate. The chemical formula of the additive is $Zn(C_6H_{13}N_2O_2)_2 \times 2HCl \times 2H_2O$ and the molecular mass 464.72 Da.[14] The theoretical content of zinc is 14.07 %.

The additive is intended to be marketed in two forms: powder, containing the additive as such, and granulate, with an admixture of approximately 0.5 % binding agents (e.g. carboxymethylcellulose). Both forms of the additive are specified to contain ≥ 13.7 % zinc and ≤ 86.3 % L-lysine HCl. The analysis of five batches of each form was submitted. Average values for the powder were 13.9 ± 0.2 % Zn and 85.6 ± 0.3 % lysine HCl[15] and for the granulate were 14.0 ± 0.2 % Zn and 85.6 ± 0.2 % lysine HCl.[16] Infrared absorbance data and elemental analysis of water-soluble and -insoluble material indicate that the additive consists of > 85 % of zinc chelate of L-lysinate-HCl. The remaining 15 % is zinc oxide which does not react with L-lysine during the synthesis process.[17]

The chemical structure of zinc chelate of L-lysinate-HCl is shown in Figure 1.

[13] Other names: Zinc, bis(L-lysinato-KN_2, KO_1)-, (T-4)-bis-hydrochloride.
[14] Technical Dossier/Supplementary Information April 2015/Annex 1.
[15] Technical Dossier/Section II/Annex II_1_2.
[16] Technical Dossier/Section II/Annex II_1_3.
[17] Technical Dossier/Supplementary Information April 2015/Annex 1.

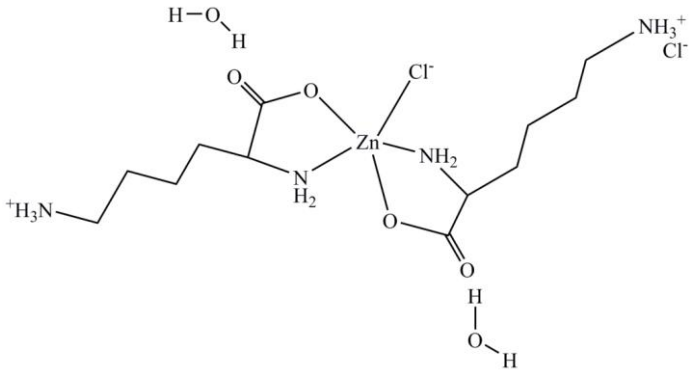

Figure 1: Chemical structure of crystallised zinc chelate of L-lysinate-HCl according to Cambridge Crystallographic Data Centre under CCDC No 1048236.

Heavy metals (cadmium, lead, mercury), fluorine, arsenic, dioxins and dioxin-like polychlorinated biphenyls (PCBs) were analysed in three batches of the powder form.[18] Reported values were below the detection limits for arsenic (5 mg/kg), lead (10 mg/kg), cadmium (1 mg/kg), mercury (0.1 mg/kg) and fluorine (25 mg/kg) and below the thresholds of the Directive on undesirable substances for dioxins and dioxin-like PCBs.[19]

2.1.1. Physical state of the product

The product is white-grey in colour, has an unobtrusive odour and comes in two forms: solid free-flowing powder and granulate. The relative and the compressed densities for the powder are 500 and 580 kg/m^3, respectively.[20] For the granulate form of the additive, the corresponding values are 480 and 550 kg/m^3.[21] The water solubility of both forms of the additive is 0.5 g/L.

Mean particle size of the powder form was determined in three batches and ranged from 46 to 49 µm.[22] The samples contain 15–18 % (v/v) of particulate matter with particle sizes < 10 µm; the dust contains 95–97 % particles < 10 µm. The granulate form has a mean particle size of 589–595 µm with virtually no particles below 10 µm.[23] The dusting potential of the powder form as measured by the Stauber-Heubach method was 12.8–13.5 g/m^3,[24] whereas the value found for the granulate was below the detection limit (0.1 mg/m^3).[25]

2.2. Manufacturing process

The manufacturing process is fully described in the technical dossier. The starting raw materials used for the production of the additive are zinc oxide and L-lysine HCl. The lysine source used is produced by fermentation with the genetically modified strain FERM BP-10941 of *Escherichia coli*; the safety of this lysine source has already been assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2013b).

The material safety data sheets of the raw materials L-lysine,[26] zinc oxide[27] and carboxymethylcellulose[28] were provided.

[18] Technical Dossier/Section II/Annex II_1_4.
[19] 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.
[20] Technical Dossier/Section II/Annex II_1_5.
[21] Technical Dossier/Section II/Annex II_1_8.
[22] Technical Dossier/Section II/Annex II_1_6.
[23] Technical Dossier/Section II/Annex II_1_9.
[24] Technical Dossier/Section II/Annex II_1_7.
[25] Technical Dossier/Section II/Annex II_1_10.
[26] Technical Dossier/Section II/Annex II_3_4.
[27] Technical Dossier/Section II/Annex II_3_6.
[28] Technical Dossier/Section II/Annex II_3_8.

2.3. Stability and homogeneity

Stability data of the additive were not provided by the applicant and are generally not required for compounds of trace elements. A minimum shelf life of one year is proposed by the applicant.

The capacity of both forms of the additive to homogeneously distribute was presented in studies conducted with a premixture for sows and with a variety of compound feedingstuffs (composition not provided).[29] Based on the analysed zinc concentration (total minus background), the coefficients of variation were similar for the powder and granulate forms and ranged from 0.34 to 0.36 % in the premixture and from 0.21 to 3.12 % in compound feed.

2.4. Physico-chemical incompatibilities in feed

According to the current knowledge, no incompatibilities or adverse interactions with feed components, carriers, other approved additives or medicinal products are to be expected other than those widely recognised for zinc in animal nutrition.

2.5. Conditions of use

The zinc compound under application, zinc chelate of L-lysinate-HCl, is intended to supply zinc in final feed for all animal species/categories up to a maximum total content in complete feedingstuffs of 250 mg Zn/kg for pet animals, 200 mg Zn/kg for fish and for milk replacers and 150 mg Zn/kg for other species.

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 zinc chelate of L-lysinate-HCl in animal feed. The Executive Summary of the EURL report can be found in the Annex.

3. Safety

3.1. Safety for the target species

3.1.1. Tolerance studies for the target species

3.1.1.1. Tolerance study in calves

A 6-week experiment with 60 Friesian bull calves was not considered because the intended dietary zinc concentrations were not analytically confirmed and the doses tested did not allow to establish a margin of safety.[30]

Another 6-week trial was carried out with 60 Friesian bull calves (initial age 21 days, average body weight 53 kg).[31] For each of the four treatments, 15 calves were housed together in one pen and individually fed milk replacer by automatic feeders. Hay was available for *ad libitum* intake. The basal diet (milk replacer; background zinc: 28 mg/kg feed) was supplemented with zinc sulphate heptahydrate or zinc chelate of L-lysinate-HCl to obtain the intended concentration of 40 and 500 mg Zn/kg diet (2.5 fold maximum content of total zinc). The zinc concentrations in the diets were confirmed by analysis. Animals were medicated for the first ten days prophylactically against respiratory diseases and diarrhoea and were vaccinated twice against the bovine respiratory syncytial virus and treated against endo- and ectoparasites. The animals were weighed weekly; feed intake was recorded daily. At the end of the trial, blood samples were taken from four calves per treatment for

[29] Technical Dossier/Section II/Annex II_4_1 (powder form) and Annex II_4_2 (granulate form).
[30] Technical Dossier/Section III/Annex III_1_1.
[31] Technical Dossier/Section III/Annex III_1_2.

haematology[32] and clinical chemistry.[33] Statistical analysis included tests on homogeneous distribution and variance homogeneity; depending on the outcome, analysis of variance (ANOVA) followed by a post-hoc test or non-parametric test were used (statistical package SPSS 21). These data on zootechnical and selected haematology parameters are summarised in Table 2.

Table 2: Effect of level and source of zinc in milk replacer (MR) on performance and on some haematological parameters in calves (initial body weight: 53.0 kg; duration of study: 42 days)

Zinc source	Zinc sulphate	Zinc lysinate	Zinc sulphate	Zinc lysinate
Intended Zn (mg/kg MR)	40		500	
Analysed Zn (mg/kg MR)	50	49	534	527
MR intake (kg/calf per day)	0.99	1.00	0.96	0.98
Hay intake (kg/calf per day)	0.27	0.28	0.27	0.27
Daily weight gain (g)	706	741	685	752
Final body weight (kg)	82.5	84.0	82.0	85.1
Haematocrit (%)	32.3^{ac}	33.4^{a}	21.3^{b}	26.0^{bc}
Erythrocytes (T/L)	10.5^{a}	11.2^{a}	7.3^{b}	8.7^{b}
Haemoglobin (g/dL)	10.2^{ab}	10.5^{a}	8.7^{b}	9.4^{a}
MCH (1) (pg)	9.7^{a}	9.4^{a}	11.9^{b}	10.9^{ab}
MCHC (2) (g/dL)	31.7^{a}	31.4^{a}	41.0^{c}	36.5^{b}

(1): Mean corpuscular haemoglobin.
(2): Mean corpuscular haemoglobin concentration.
Performance parameters: 15 animals/treatment.
Haematology parameters: 4 animals/treatment.
a,b,c: Different superscripts within a row indicate statistical differences ($P \leq 0.05$).

No mortality was observed. No significant differences in performance parameters were seen. Haematology parameters appeared to indicate an adverse effect of the high zinc concentration on iron utilisation as shown by reduced haematocrit (23.7 vs. 32.8 %), number of erythrocytes (8.0 vs. 10.9 T/L) and haemoglobin (9.1 vs. 10.4 g/dL). It is noted that this effect was less pronounced, if any, at the high dose of zinc chelate of L-lysinate-HCl group (Table 2).

This study indicates that zinc chelate of L-lysinate-HCl was equally tolerated as zinc sulphate up to 2.5-fold the maximum authorised total zinc in milk replacer. An uncertainty results from the small number of animals tested for haematology and clinical chemistry parameters. It is noted that the feeding regime is considered untypical since no concentrate has been provided.

3.1.1.2. Tolerance study in chickens for fattening

A 35-day combined tolerance/efficacy study was performed in three consecutive experiments with chickens for fattening.[34]

Owing to the weaknesses of the study design, the inaccuracies in reporting and controversial and unexplainable findings (e.g. a remarkable depression of feed intake and body weight in the treatments with zinc sulphate in all the doses tested within the tolerable maximum levels; the unexpected content of lysine, mainly in the grower diet as compared with the starter diet; the absence of response in the pancreas to the increasing zinc in diet), the study could not be considered in the safety assessment of zinc chelate of L-lysinate-HCl for chickens for fattening.

[32] Haematocrit, erythrocytes, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, leucocytes, platelets.
[33] Total proteins, albumin, aspartate aminotransferase, gamma-glutamyltransferase, urea.
[34] Technical Dossier/Section III/Annex III_1_4.

3.1.1.3. Tolerance study in piglets

A 42-day combined tolerance/efficacy study was performed with 72 weaned piglets (castrated males and females; Euroc × Piétrain; age 25 days, average initial body weight: 6.0 kg).[35] The piglets were allocated according to weight and sex to six dietary treatments (four replicates per treatment, three animals per replicate). The basal diet (wheat, barley, soybean meal and maize; background zinc concentration: 30–35 mg/kg feed) was supplemented with zinc from either zinc sulphate heptahydrate or from zinc chelate of L-lysinate-HCl to obtain the intended doses of zinc being 80, 150 (maximum zinc in feed authorised in the EC) and 450 (3-fold maximum authorised in the EC) mg total Zn/kg feed, as confirmed by analysis. The starter diet was fed for two weeks followed by a grower diet until completion of the study. Body weight and feed intake (FI) per pen were recorded weekly. At the end of the study, blood samples were taken from all animals for haematology[36] and clinical chemistry.[37] Zinc in plasma, liver, pancreas spleen, kidney and tibia was analysed from six animals per treatment. ANOVA was performed, followed by a Tukey post-hoc test. The test for the performance parameters was carried out with the pen as an experimental unit and for other parameters on the number of animals. The main results are presented in Table 3.

Table 3: Effect of level and source of zinc on performance and zinc content in different organs of piglets

Zinc source	**Zinc sulphate**	**Zinc lysinate**	**Zinc sulphate**	**Zinc lysinate**	**Zinc sulphate**	**Zinc lysinate**
Zinc (mg Zn/kg feed)						
Supplemented	80		150		450	
Total analysed	105/113 (1)	114/116	185/188	192/195	497/486	497/504
Feed intake (kg/animal) (2)	24.2	24.3	24.6	24.7	25.0	25.1
Final body weight (kg) (2)	22.8	23.5	23.5	24.4	24.3	24.9
Average daily gain (g) (2)	401^{a}	417^{ab}	418^{ab}	440^{b}	436^{b}	450^{b}
Feed/gain ratio (2)	1.44^{b}	1.39^{ab}	1.40^{ab}	1.34^{ab}	1.37^{ab}	1.33^{a}
Zinc (mg/kg DM) (3)						
Liver	116.1^{a}	117.5^{a}	128.4^{a}	132.8^{a}	163.0^{b}	154.5^{b}
Kidney	81.1^{a}	82.8^{ab}	92.1^{bc}	94.5^{bc}	97.4^{bc}	100.6^{c}
Pancreas	66.8^{a}	67.2^{a}	74.2^{ab}	86.1^{bc}	95.1^{c}	90.3^{c}
Tibia (4)	123.3^{ab}	114.2^{a}	132.7^{abc}	126.7^{abc}	142.3^{bc}	148.5^{c}
Zinc in plasma (mg/L) (5)	1.2	1.3	1.3	1.3	1.3	1.3

(1): Starter/grower.
(2): Means based on n = 4 (pens with three animals/pen).
(3): Means based on n = 6 (animals/treatment).
(4): For tibia: mg/kg fat-free DM.
(5): Means based on n = 12 (animals/treatment).
a,b,c: different superscripts within a row indicate statistical differences ($P \leq 0.05$).
DM, dry matter.

No mortality was observed. No significant effects were found for final body weight due to zinc source or level of supplementation. Average daily gain and feed/gain ratio showed an increasing and decreasing trend, respectively, with increasing dietary zinc levels. Significant differences in body weight gain were present between the low zinc sulphate group and the groups with high zinc from sulphate and intermediate and high zinc from lysinate; significant differences in feed/gain ratio were observed only between low zinc from sulphate and high zinc from lysinate. No differences were seen in the haematological and clinical chemistry endpoints.

[35] Technical Dossier/Section III/Annex III_1_5.
[36] Erythrocytes, total and differential leucocytes, haematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobinand mean corpuscular haemoglobin concentration.
[37] Electrolytes (sodium, potassium, chlorine, calcium, phosphorus), total cholesterol, triglycerides, bilirubin, urea, glucose, albumins, total protein, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase.

Zinc deposition data for liver, kidney, pancreas and tibia appeared associated to the dietary zinc supply; these differences became statistically significant for comparison of the low and high zinc groups (Table 3). There were no significant differences in the zinc deposition in those four tissues between the two zinc sources (sulphate and lysinate).

The results of the study indicate that zinc chelate of L-lysinate-HCl was equally tolerated as zinc sulphate, by piglets up to 3-fold of the maximum authorised total zinc in complete feed for this animal category.

3.1.2. Conclusions on the safety for the target species

The FEEDAP Panel concludes on the basis of tolerance studies with calves and weaned piglets that zinc chelate of L-lysinate-HCl does not elicit additional or different adverse effects compared with the standard inorganic source zinc sulphate heptahydrate. Therefore, the FEEDAP Panel considers zinc chelate of L-lysinate-HCl as a safe source of zinc for all animal species, provided that the maximum zinc contents authorised in complete feed are respected.

3.2. Safety for the consumer

The metabolic behaviour of zinc was discussed in detail by the Scientific Committee on Food (SCF) (EC, 2003b) and, more recently, by the FEEDAP Panel (EFSA FEEDAP Panel, 2014).

3.2.1. Metabolic and residue studies

Potential differences in the deposition from different sources of a trace element among tissues and other products used for human consumption are subject of assessment of consumer safety, particularly for its new organic sources.

The only available data for tissue deposition of zinc from the product under application could be derived from the combined tolerance/efficacy study on piglets (see section 3.1.1.3), since results from the study on chickens for fattening could not considered due to several deficiencies already mentioned in section 3.1.1.2. Concerning edible tissues, liver and kidney from piglets did not show any significant difference in zinc deposition between zinc sulphate, as standard authorised inorganic source, and zinc chelate of L-lysinate-HCl when supplemented to feed up to 450 mg Zn/kg (Table 3). In addition, no differences in response to dietary zinc supplementation from the two sources were observed in the piglet study concerning the deposition in further investigated tissues (pancreas, tibia) and the plasma zinc levels.

Data from the review by Jongbloed et al. (2002) show the mean bioavailability of zinc from an unspecified source of zinc lysinate relative to that of zinc from zinc sulphate for pigs (based on bone and serum zinc) and ruminants (based on liver and kidney) to be 89 and 107 %, respectively.

Considering also the published scientific literature, the FEEDAP Panel reiterates its recent statement (EFSA FEEDAP Panel, 2015) that intestinal zinc absorption is saturable, and the rate of zinc uptake is inversely proportional to intake within its large range of homeostatic regulation. As stated in an opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2014), "*with the exceptions of liver and kidney (Eisemann et al., 1979; Jenkins and Hidiroglou, 1991; Cao et al., 2000; Gallaher et al., 2000; Wright and Spears, 2004; NRC, 2005; see also review of Schlegel et al., 2013), zinc concentrations exceeding the requirements up to about 200 mg/kg feed will not result in a change of zinc concentrations in animal tissues and other products including milk (Schwarz and Kirchgessner, 1975; Miller et al., 1989; Wiking et al., 2008; Peters and Mahan, 2008; Peters et al., 2010)*". In general, there is no evidence in published literature that organic forms of zinc result in different levels of zinc deposition in muscle, egg or milk compared with its inorganic sources. This is supported by studies on zinc deposition in eggs (Mabe et al., 2003; Huyghebaert et al., 2006; Plaimast et al., 2008; Bahakaim et al., 2014) and milk (Kirchgessner et al., 1994; Pechova et al., 2006, 2009) carried out on animals fed diets supplemented with sources of amino acid chelated with this trace element. Only hepatic zinc deposition levels are generally higher after supplementation with organic forms of zinc (EFSA

FEEDAP Panel, 2015). This statement is consistent with previous reviews and meta-analyses of data from poultry, pigs and ruminants, which concluded that organic and inorganic sources of zinc, in general, are equivalent (Ammerman et al., 1998; Jongbloed et al., 2002; Schlegel et al., 2013; EFSA FEEDAP Panel, 2014).

Therefore, the FEEDAP Panel considers that zinc from zinc chelate of L-lysinate-HCl would not result in a different zinc deposition in edible tissues/products than the standard inorganic source zinc sulphate heptahydrate when supplemented to feed up to the maximum authorised zinc level in the EU.

The amount of lysine as an essential amino acid released from the dissociation of zinc chelate of L-lysinate-HCl in the animal body after its oral intake will be limited by the maximum authorised zinc level in animal feed and this lysine will be used in protein synthesis and/or metabolised to urea/uric acid and carbon dioxide without any modification of edible tissues/products.

3.2.2. Toxicological studies

No specific toxicological studies for the product under application were submitted.

The toxicological properties of zinc have been discussed in detail by the SCF (EC, 2003b). It exerts low oral acute toxicity, with high doses (2–8 mg Zn/kg body weight per day in different species) resulting in gastrointestinal distress with clinical signs of nausea, vomiting, abdominal cramps and diarrhoea. Some positive results were observed in genotoxicity tests. In the opinion of the SCF, the weight of evidence from the *in vitro* and *in vivo* genotoxicity tests supports the conclusion that zinc, notwithstanding some positive findings at chromosome level with elevated doses, has no biologically relevant genotoxic activity (reviewed by the World Health Organization (WHO, 2001)). This conclusion is supported by the US Agency for Toxic Substances and Disease Registry (ATSDR, 2005). The toxicology of zinc has been recently reviewed by Nordberg et al. (2015). One of the most sensitive and well-described effects of chronic excess of zinc intake is a depressed copper uptake with associated copper deficiency effects (reviewed by Maret and Sandstead, 2006).

3.2.3. Assessment of consumer exposure

The SCF derived a tolerable upper intake level (UL) of 25 mg Zn/day for adults and of 13 mg Zn/day for 7- to 10-year-old children (EC, 2003b). The UL for adults was based on a depressed copper uptake and an altered lipid profile in humans. An uncertainty factor of 2 was applied owing to the small number of subjects included in relatively short-term studies but acknowledging the rigidly controlled metabolic experimental conditions employed.

Concerning exposure of consumers to zinc, the FEEDAP Panel noted in 2014: "*In all consumer groups, tissues and products of animal origin contributed to about 40–50 % of total zinc intake, with meat and milk being the two main items (Walsh et al., 1994; Mensink et al., 2007; Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz, 2008). On average, among all consumer groups, the contribution of milk and meat to the total zinc intake is nearly the same. The practice of supplementing animal feed with zinc-containing compounds has not essentially changed during the last decades. It is therefore reasonable to assume that food of animal origin recorded in the above-mentioned consumption surveys derived from animals fed zinc-supplemented diets*" (EFSA FEEDAP Panel, 2014).

Based on bioavailability data of zinc from the product under assessment and considering also published scientific literature (see section 3.2.1) on the use of amino acids chelated with zinc, the FEEDAP Panel does not expect a modification of consumer exposure to zinc by the use of zinc chelate of L-lysinate-HCl in animal nutrition.

3.2.4. Conclusions on the safety for the consumer

No concerns for consumer safety are expected from the use of zinc chelate of L-lysinate-HCl as a source of zinc in animal nutrition.

3.3. Safety for the user

3.3.1. Effects on the respiratory system

No specific studies have been submitted with the additive under assessment.

The powder form has a dusting potential of 12.8–13.5 g/m^3, with respirable particles ≤ 10 µm making up about 96 % of particles of the dust; considering the theoretical zinc content of the dust (about 14 %, according to analytical measurements), the resulting zinc exposure is of about 2 g/m^3. Granulating the zinc chelate of L-lysinate-HCl reduces the dusting potential by a factor of about 10^5 (0.1 mg/m^3); considering also the virtual absence of particles of respirable size, the inhalation exposure to the granulated form of zinc chelate of L-lysinate-HCl is minimal.

Concerning the guidance values for occupational exposures to zinc compounds, the National Institute for Occupational Safety and Health (NIOSH) of the USA reported the following recommended exposure limits for zinc oxide dust: 5 mg/m^3 for a 10-hour time-weighted average, with a maximum of 15 mg/m^3 (short-term exposure limit (STEL)) (NIOSH, 1978). The American Conference of Governmental Industrial Hygienists (ACGIH) proposed a threshold limit value (TLV) for zinc oxide of 2 mg/m^3 (zinc in respirable fraction) based on metal fume fever as the critical effect (ACGIH, 2003). The FEEDAP Panel considers it appropriate to use the ACGIH parameters for zinc oxide as the most conservative and up-to-date guidance for evaluating the risk upon inhalation exposure of the zinc compounds under assessment. On the other hand, the FEEDAP Panel recognises that the use of TLV as a guidance value for the user safety of feed additives may result in overly conservative assessments, since the exposure is unlikely to be as continuous and intense as in an industrial scenario, for which TLVs have been envisaged. Indeed, the actual user exposure to dust from feed additives may be more consistent with a short-term, intermittent pattern rather than with a continuous pattern; therefore, the use of an up-to-date STEL may be closer to a real-life scenario.

Even with the above caveats, the estimated zinc concentration in the respirable dust resulting from the use of zinc chelate of L-lysinate-HCl (powder form) will exceed the TLV or the STEL by three or two orders of magnitude, respectively, indicating a risk by inhalation for users.

3.3.2. Effects on the eyes and skin

Good Laboratory Practice-compliant tests were conducted in rabbits for skin irritation (according to the Organisation for Economic Co-operation and Development (OECD) Guideline 404)[38] and eye irritation (according to OECD Guideline 405)[39] with both forms of the additive.

Both forms of the additive produced reversible skin irritation, which was slight to moderate for the powder form and very slight for the granulate form. Both forms of the additive produced serious irritation to the eye; for the powder form this reaction was irreversible.

No skin sensitisation studies were performed. In the absence of specific information, the FEEDAP Panel considers it prudent to regard the additive as a potential skin sensitiser.

3.3.3. Conclusions on the safety for users

Zinc chelate of L-lysinate-HCl in the powder form poses a risk to users by inhalation, whilst for the granulate form that risk is minimised. Neither form, powder or as granulate, is a dermal irritant. Both forms are irritant to the eye (following Regulation (EC) No 1272/2008 Category 1 for the powder form, and Category 2 for the granulate). In the absence of specific information, the additive should be regarded as a potential skin sensitiser.

[38] Technical Dossier/Section III/Annex III_3_5 and Annex III_3_7.
[39] Technical Dossier/Section III/Annex III_3_6 and Annex III_3_8 .

3.4. Safety for the environment

The FEEDAP Panel has already considered the potential risks to the environment posed by the use of zinc in feedingstuffs up to the maximum authorised contents in the EU (EFSA, 2012b, 2012c, 2012d, 2012e; EFSA FEEDAP Panel, 2013c, 2014, 2015). The additive under assessment, zinc chelate of L-lysinate-HCl, is intended to be a substitute for other authorised zinc additives. It will therefore not further increase the environmental burden of zinc.

4. Efficacy

The applicant provided two trials to demonstrate the efficacy of zinc chelate of L-lysinate-HCl, one with chickens for fattening and another one with weaned piglets. In both experiments the efficacy of zinc chelate of L-lysinate-HCl was compared to zinc sulphate heptahydrate.

The applicant submitted a study with chickens for fattening (Ross 308) to support the efficacy of the additive. This study could not be considered (see section 3.1.1.2).

Another study with weaned piglets was provided by the applicant and has been already described in the section "Safety for target species" (see section 3.1.1.3). Concerning the bioavailability parameters, zinc deposition data for tibia, liver, kidney and pancreas appeared associated to the dietary zinc supply (Table 3); there were no significant differences in the deposition in these four tissues between the two zinc sources (sulphate and lysinate).

4.1. Conclusions on efficacy for target species

In the study in weaned piglets, zinc chelate of L-lysinate-HCl had the same effects in comparison with an established zinc source (zinc sulphate heptahydrate). The FEEDAP Panel concludes that zinc chelate of L-lysinate-HCl is efficacious as a source of zinc in meeting the requirements of all animal species.

5. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[40] and Good Manufacturing Practice.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Zinc chelate of L-lysinate is a safe source of zinc for all animal species, provided that the maximum zinc contents authorised in complete feed are respected.

No concerns for consumer safety are expected from the use of the zinc chelate of L-lysinate-HCl in animal nutrition.

Zinc chelate of L-lysinate-HCl in the powder form poses a risk to users by inhalation, whilst for the granulate form that risk is minimised. Neither form, powder or as granulate, is a dermal irritant. Both forms are irritant to the eye. In the absence of specific information, the additive should be regarded as a potential skin sensitiser.

The additive under assessment, zinc chelate of L-lysinate-HCl, is intended to be a substitute for other authorised zinc additives. It will therefore not further increase the environmental burden of zinc.

[40] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

Zinc chelate of L-lysinate-HCl is efficacious as a source of zinc in meeting the requirements of all animal species.

RECOMMENDATIONS

In order to avoid confusion with other zinc chelates of lysine containing 1 mol zinc with 1 mol of lysine, the FEEDAP Panel recommends "Zinc bislysinate HCl" for the name of the additive under assessment.

The description of the additive should include the zinc and lysine contents. The proposed figures are ≥ 13.5 % zinc and ≥ 85 % lysine-HCl, for both forms of the additive.

The lysine content of the additive should be considered when formulating feed for piglets.

GENERAL REMARK

The FEEDAP Panel made a proposal to reduce the maximum currently authorised content of zinc in feed (EFSA FEEDAP Panel, 2014). The Panel outlines that the conclusions on target animal safety and on consumer safety made in the current opinion need not to be modified if the reduced maximum content is put into force by legislation. However, the reduction of zinc in feed would lead to a beneficial effect on the environment: the zinc load is estimated to decrease by 20 %.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier Aminotrace Zinc Bislysinate. June 2014. Submitted by Senzyme GmbH.
2. Dossier Aminotrace Zinc Bislysinate. Supplementary information. April 2015. Submitted by Senzyme GmbH.
3. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Zinc Chelate of L-Lysinate HCl.
4. Comments from Member States.

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists), 2003. Zinc oxide fume. US Department of Labor. Available online: https://www.osha.gov/dts/chemicalsampling/data/CH_277000.html

Ammerman CB, Henry PR and Miles RD, 1998. Supplemental organically-bound mineral compounds in livestock nutrition. In: Recent advances in animal nutrition. Eds Garnsworthy PC and Wiseman J. Nottingham University Press, Nottingham, UK.

ATSDR (Agency for Toxic Substances and Disease Registry), 2005. Toxicological profile for zinc. US Department of Health and Human Services. Available online: http://www.atsdr.cdc.gov/toxprofiles/tp60.pdf

Bahakaim ASA, Hmat A, Sahar AM, Osman MH, Amal S, Omar NY, Nehad AM and Ramadan A, 2014. Effect of using different levels and sources of zinc in layer's diets on egg zinc enrichment. Egyptian Poultry Science, 34, 39–56.

Bundesministerium für Ernährung, Landwirstchaft und Verbraucherschutz, 2008. Nationale Verzehrs Studie II. Max Rubner-Institut. Available online: http://www.was-esse-ich.de/uploads/media/NVSII_Abschlussbericht_Teil_2.pdf

Cao J, Henry PR, Guo R, Holwerda RA, Toth JP, Littell RC, Miles RD and Ammerman CB, 2000. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. Journal of Animal Science, 78, 2039–2054.

EC (European Commission), 2003a. Opinion of the Scientific Committee for Animal Nutrition on the use of zinc in feedingstuffs. Available online: http://ec.europa.eu/food/fs/sc/scan/out120_en.pdf

EC (European Commission), 2003b. Opinion of the Scientific Committee on Food on the tolerable upper intake level of zinc. Available online: http://ec.europa.eu/food/fs/sc/scf/out177_en.pdf

EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on a request from the Commission on the safety of the "Chelated forms of iron, copper, manganese and zinc with synthetic feed grade glycine". The EFSA Journal 2005, 289, 1–6.

EFSA (European Food Safety Authority), 2008. Scientific Opinion on the safety and efficacy of Mintrex®Zn (zinc chelate of hydroxy analogue of methionine) as feed additive for all species. The EFSA Journal, 694, 3–16.

EFSA (European Food Safety Authority), 2009a. Scientific Opinion Safety of Mintrex®Zn (Zinc chelate of hydroxy analogue of methionine) as feed additive for chickens for fattening. The EFSA Journal, 1042, 1–8.

EFSA (European Food Safety Authority), 2009b. Scientific Opinion Safety of Mintrex®Zn (Zinc chelate of hydroxy analogue of methionine) as feed additive for all species. EFSA Journal 2009;7(11):1381, 13 pp.. doi:10.2903/j.efsa.2009.1381

EFSA (European Food Safety Authority), 2012a. Scientific Opinion on the safety and efficacy of tetra-basic zinc chloride for all animal species. EFSA Journal 2012;10(5):2672, 19 pp. doi:10.2903/j.efsa.2012.2672

EFSA (European Food Safety Authority), 2012b. Scientific Opinion on safety and efficacy of zinc compounds (E6) as feed additives for all animal species: Zinc chelate of amino acids hydrate, based on a dossier submitted by Zinpro Animal Nutrition Inc. EFSA Journal 2012;10(3):2621, 22 pp. doi:10.2903/j.efsa.2012.2621

EFSA (European Food Safety Authority), 2012c. Scientific Opinion on safety and efficacy of zinc compounds (E6) as feed additives for all animal species Zinc sulphate monohydrate, based on a dossier submitted by Helm AG. EFSA Journal 2012;10(2):2572, 22 pp. doi:10.2903/j.efsa.2012.2572

EFSA (European Food Safety Authority), 2012d. Scientific Opinion on safety and efficacy of zinc compounds (E6) as feed additive for all species: zinc sulphate monohydrate, based on a dossier submitted by Grillo-Werke AG/EMFEMA. EFSA Journal 2012;10(6):2734, 23 pp. doi:10.2903/j.efsa.2012.2734

EFSA (European Food Safety Authority), 2012e. Scientific Opinion on safety and efficacy of zinc compounds (E6) as feed additive for all animal species: Zinc oxide, based on a dossier submitted by Grillo Zinkoxid GmbH/EMFEMA. EFSA Journal 2012;10(11):2970, 24 pp. doi:10.2903/j.efsa.2012.2970

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013a. Scientific Opinion on the safety and efficacy of methionine-zinc, technically pure as amino acid for ruminants, and as compound of trace element for all species. EFSA Journal 2013;11(1):3038, 25 pp. doi:10.2903/j.efsa.2013.3038

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013b. 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. EFSA Journal 2013;11(10):3365, 3 pp. doi:10.2903/j.efsa.2013.3365

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013c. Scientific opinion on the characterisation of zinc compound 'Zinc chelate of amino acids,

hydrate (Availa®Zinc)' as a feed additive for all animal species. EFSA Journal 2013;11(10):3369, 7 pp. doi:10.2903/j.efsa.2013.3369

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014. Scientific Opinion on the potential reduction of the currently authorised maximum zinc content in complete feed. EFSA Journal 2014;12(5):3668, 77 pp. doi:10.2903/j.efsa.2014.3668

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015. Scientific Opinion on safety and efficacy of zinc compounds (E6) as feed additives for all species: zinc acetate, dihydrate; zinc chloride, anhydrous; zinc oxide; zinc sulphate, heptahydrate; zinc sulphate, monohydrate; zinc chelate of amino acids, hydrate; zinc chelate of glycine, hydrate, based on a dossier submitted by FEFANA asbl. EFSA Journal 2015;13(4):4058, 45 pp. doi:10.2903/j.efsa.2015.4058

Eisemann JH, Pond WG and Thonney ML, 1979. Effect of dietary zinc and copper on performance and tissue mineral and cholesterol concentrations in swine. Journal of Animal Science, 48, 11231128.

Gallaher DD, Gallaher CM, Shulman S, McElhome A, Brokken KA and Shurson G, 2000. Bioavailability of different sources of protected zinc. In: Trace elements in man and animals 10. Eds Roussel et al. Kluwer Academic/Plenum Publishers, New York, USA.

Huyghebaert G, Lippens M and Nollet L, 2006. Enrichment of eggs with trace minerals using inorganic or organic trace sources: a feasibility? 12th European Poultry Conference, Verona, Italy.

Jenkins KJ and Hidiroglou M, 1991. Tolerance of the preruminant calf for excess manganese or zinc in milk replacer. Journal of Dairy Science, 74, 1047–1053.

Jongbloed A, Kemme P, De Grootte G, Lippens M and Meschy F, 2002. Bioavailability of major and trace elements. EMFEMA (International Association of the European Manufacturers of Major, Trace and Specific Feed Mineral Materials), Brussels, Belgium, 118 pp.

Kirchgessner M, Paulicks BR and Hagemeister H, 1994. Zinc concentration in the milk of dairy cows supplemented with high-levels of zinc methionine. Journal of Animal Physiology, 72, 165–167.

Mabe I, Rapp C, Bain MM and Nys Y, 2003. Supplementation of a corn–soybean meal diet with manganese, copper, and zinc from organic or inorganic sources improves eggshell quality in aged laying hens. Poultry Science, 82, 1903–1913.

Maret W and Sandstead HH, 2006. Zinc requirements and the risks and benefits of zinc supplementation. Journal of Trace Elements in Medicine and Biology, 20, 3–18.

Mensink GBM, Heseker H, Richter A, Stahl A, Vohmann, Fischer J, Kohler S and Six J, 2007. Ernährungsstudie als KiGGS-Modul (EsKiMo). Robert Koch-Institut and Universität Paderborn, Germany.

Miller WJ, Amos HE, Gentry RP, Blackmon DM, Durrance RM, Crowe CT, Fileding AS and Neathery MW, 1989. Long-term feeding of high zinc sulphate diets to lactating and gestating dairy cows. Journal of Dairy Science, 72, 1499–1508.

NIOSH (National Institute for Occupational Safety and Health), 1978. Occupational health guideline for zinc oxide fume. US Department of Health and Human Services. Available online: http://www.cdc.gov/niosh/docs/81-123/pdfs/0675.pdf

Nordberg GF, Fowler BA and Nordberg M, 2015. Handbook on the toxicology of metals, fourth edn. Academic Press Publications, London, UK.

NRC (National Research Council), 2005. Mineral tolerance of animals, second revised edn. The National Academies Press, Washington, DC, USA.

Pechova A, Pavlata L and Lokajova E, 2006. Zinc supplementation and somatic cell count in milk of dairy cows. Acta Veterinaria Brno, 75, 355–361.

Pechova A, Misurova L, Pavlata L and Dvorak R, 2009. The influence of supplementation of different forms of zinc in goats on the zinc concentration in blood plasma and milk. Biological Trace Element Research, 132, 112–121.

Peters JC and Mahan DC, 2008. Effects of dietary organic and inorganic trace mineral levels on sow reproductive performances and daily mineral intakes over six parities. Journal of Animal Science, 86, 2247–2260.

Peters JC, Mahan DC, Wiseman TG and Fastinger ND, 2010. Effect of dietary organic and inorganic micromineral source and level on sow body, liver, colostrum, mature milk, and progeny mineral compositions over six parities. Journal of Animal Science, 88, 626–637.

Plaimast H, Sirichakwal P, Puwastien P and Kijparkorn S, 2008. Effect of supplementary zinc from organic and inorganic sources on laying performance and zinc deposition in eggs. The Thai Journal of Veterinary Medicine, 38, 47–53.

Schlegel P, Sauvant D and Jondreville C, 2013. Bioavailability of zinc sources and their interaction with phytates in broilers and piglets. Animal, 7, 47–59.

Schwarz WA and Kirchgessner M, 1975. [Excretion of zinc in lactating cows receiving various supply of zinc] In German. Archiv für Tierernährung, 25, 597608.

Walsh CT, Sandstead HH, Prasad AS, Newberne PM and Fraker PJ, 1994. Zinc: health effects and research priorities for the 1990s. Environmental Health Perspectives, 102, 5–46.

WHO (World Health Organization), 2001. Zinc: environmental health criteria 221. International Programme on Chemical Safety (IPCS), Geneva, Switzerland.

Wiking L, Larsen T and Sehested J. 2008. Transfer of dietary zinc and fat to milk evaluation of milk fat quality, milk fat precursors, and mastitis indicators. Journal of Dairy Science, 91, 1544–1551.

Wright CL and Spears JW, 2004. Effect of zinc source and dietary level on zinc metabolism in Holstein calves. Journal of Dairy Science, 87, 1085–1091.

ANNEX

Annex A. Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the method(s) of analysis for Zinc Chelate of L-Lysinate HCl[41]

In the current application authorisation is sought under article 4(1) for *Zinc Chelate of L-Lysinate HCl* under the category/functional group (3b) "nutritional additives"/"compounds of trace elements", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for all categories and species.

Zinc Chelate of L-Lysinate HCl is a white-grey free flowing powder or granules with a minimum content of 13.7 % *total zinc* and a maximum content of 86.3 % *L-Lysine HCl*. The *feed additive* is intended to be incorporated into *premixtures* and *feedingstuffs*. The Applicant suggested maximum levels of *total zinc* in the *feedingstuffs* complying to the limits set in Regulation (EC) No 1334/2003, ranging from 150 to 250 mg/kg - depending of the animal species/category.

For the quantification of *Lysine* content in the *feed additive* and *premixtures* the Applicant submitted the ring-trial validated method by the "Association of German Agricultural Analytical and Research Institutes" (VDLUFA, Germany – Method 4.11.6), based on ion-exchange chromatography coupled with post-column derivatisation and colourimetric or fluorescence detection. Furthermore, the EURL identified the recently published ring-trial validated method EN ISO 17180:2013 for the "determination of *Lysine* […] in commercial amino acid products and premixtures", based on ion-exchange chromatography coupled with post-column derivatisation and colourimetric or fluorescence detection. The method does not distinguish between the salts and the amino acid enantiomers and applies for the products/premixtures containing more than 10 % of amino acid content. Based on acceptable performance characteristics available, the EURL recommends for official control the two ring-trial validated methods (VDLUFA 4.11.6 and EN ISO 17180:2013) to quantify *Lysine* in the *feed additive* and *premixtures*.

For the quantification of *total Lysine* in *feedingstuffs* the EURL already evaluated and recommended in the frame of the Lysine group dossier FAD-2010-0067 the ring-trial validated Community method. This method applies for the determination of free (synthetic and natural) and of total (peptide-bound and free) amino acids, using an amino acid analyzer or High Performance Liquid Chromatography (HPLC) equipment. The method does not distinguish between the salts and the amino acid enantiomers. This method was further ring-trial validated, resulting in the EN ISO 13903:2005 method.

For the *determination* of *total zinc* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the internationally recognised ring-trial validated method EN 15510 based on inductively coupled plasma atomic emission spectroscopy (ICP-AES). Two EURL Evaluation Report *"Zinc Chelate of L-Lysinate HCl"* additional methods were previously evaluated and recommended by the EURL in the frame of the Zinc group dossier: the ring-trial validated EN 15621 method based on ICP-AES after pressure digestion and the Community method based on atomic absorption spectroscopy. The Community method was further ring-trial validated by the UK Food Standards Agency (FSA). Based on the acceptable method performance characteristics available, the EURL recommends for official control the two CEN methods (EN 15510 or EN 15621) for the quantification of *total zinc* in the *feed additive*, *premixtures* and *feedingstuffs*, together with the Community method (Com Reg (EC) No 152/2009 – Annex IV-C) for the determination of *total zinc* in *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.

[41] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2014-0021-zinc-lysinate.pdf