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Safety and efficacy of a feed additive consisting of ferrous lysinate sulfate for all animal species (Phytobiotics Futterzusatzstoffe GmbH)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of ferrous lysinate sulfate as nutritional feed additive for all animal species. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) Panel was assigned to this mandate. Based on the results of a tolerance study, the FEEDAP Panel concluded that ferrous lysinate sulfate is safe in chickens for fattening when used up to the current maximum authorised levels of total iron in feed; this conclusion was extrapolated to all animal species and categories, at the respective maximum authorised iron levels in complete feed. The use of ferrous lysinate sulfate in animal nutrition up to the maximum iron content in complete feed authorised in the EU poses no concern to the safety of consumers. The FEEDAP Panel concluded that ferrous lysinate sulfate poses a risk to users by inhalation; the additive is not a dermal irritant, but is irritant to eyes and a skin sensitiser. The FEEDAP Panel considered that the use of ferrous lysinate sulfate in animal nutrition would not pose a risk for the environment. Owing to the limitations in the study provided, the FEEDAP Panel could not conclude on the efficacy of the additive for chickens for fattening, and thus, on the efficacy of ferrous lysinate sulfate for all animal species and categories.

Keywords: nutritional additive, compounds of trace elements, iron, ferrous lysinate sulfate, safety, efficacy, Plexomin® L-Fe

Requestor: European Commission

Question number: EFSA-Q-2020-00169

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1831/2003[1] 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 Phytobiotics Futterzusatzstoffe GmbH[2] for authorisation of the product ferrous lysinate sulfate, when used as a feed additive for all animal species (category: nutritional additive; functional group: compound of trace elements).

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. The particulars and documents in support of the application were considered valid by EFSA as of 11 of March 2020.

According to Article 8 of Regulation (EC) No 1831/2003, 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. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product ferrous lysinate sulfate, when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The additive, ferrous lysinate sulfate, is intended for use as a source of iron in all animal species. The additive has not been previously authorised as feed additive in the European Union (EU).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[3] in support of the authorisation request for the use of ferrous lysinate sulfate as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' elicitation knowledge, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the ferrous lysinate sulfate in animal feed. The Executive Summary of the EURL report can be found in Annex A.[4]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of ferrous lysinate sulfate is in line with the principles laid down in Regulation (EC) No 429/2008[5] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019a).

[1] 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.
[2] Phytobiotics Futterzusatzstoffe GmbH, Wallufer Str. 10a, 65343 Eltiville, Germany.
[3] FEED dossier reference: FAD-2019-0094.
[4] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2019-0094-fe-lysinate.pdf
[5] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

3. Assessment

The additive under assessment is ferrous lysinate sulfate (trade name: Plexomin®L-Fe). It is intended to be used in feed as nutritional additive (functional group: compounds of trace elements) as a source of iron (Fe) in all animal species and categories.

3.1. Characterisation

3.1.1. Characterisation of the additive

[redacted]

[6] Technical Dossier/Section II/Annex_II_1.
[7] Technical Dossier/Supplementary Information_December 2020.
[8] Technical Dossier/Section II/Annex_II_70.
[9] Technical Dossier/Section II/Annex_II_51 Conf.
[redacted]
[11] Technical Dossier/Supplementary information September 2020/Annex_SIn_2.
[12] Technical Dossier/Section II/Annex_II_67. [redacted]
[14] 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.

[redacted][15][redacted][16][redacted][17][redacted][18]

3.1.2. Manufacturing process

[redacted][19][redacted]

3.1.3. Stability and homogeneity

[redacted][6][redacted][23][redacted][23]

3.1.4. Conditions of use

Ferrous lysinate sulfate is intended to be used in feed for all animal species and categories via premixture. No minimum inclusion level is recommended but the maximum permitted level for the additive should be in compliance with currently authorised levels of total iron in feed in the EU[24]: up to the total maximum iron content: ovine 500 mg/kg complete feedingstuffs, bovines and poultry 450 mg/kg complete feedingstuffs, pet animals 600 mg/kg complete feedingstuffs, other species 750 mg/kg complete feedingstuffs and piglets up to one week before weaning 250 mg/day.

[15] Technical Dossier/Section II/Annex_II_69.
[16] Technical Dossier/Section III/Annex_III_50 and Annex_II_51.
[17] Technical Dossier/Supplementary Information September 2020/Annex_SIn_III_5.
[18] Technical Dossier/Supplementary Information September 2020/Annex_SIn_III_7.
[19] Technical Dossier/Section II/Annex_II_59 and Annex_II_60. [redacted]
[23] Technical dossier/Section II/Annex_II_80.
[24] Commission Implementing Regulation (EU) 2017/2330 of 14 December 2017 concerning the authorisation of Iron(II) carbonate, Iron(III) chloride hexahydrate, Iron(II) sulfate monohydrate, Iron(II) sulfate heptahydrate, Iron(II) fumarate, Iron (II) chelate of amino acids hydrate, Iron(II) chelate of protein hydrolysates and Iron(II) chelate of glycine hydrate as feed additives for all animal species and of Iron dextran as feed additive for piglets and amending Regulations (EC) No 1334/2003 and (EC) No 479/2006. OJ L 333, 15.12.2017, p. 41.

3.2. Safety

The additive, ferrous lysinate sulfate, is a complex which is likely dissociated under physiological conditions into its three main components: iron, lysine and sulfate.

The safety of L-lysine produced by the genetically modified strains of *Corynebacterium glutamicum* (NRRL B-50547, KCCM 11117P, KCCM 10227/12307BP) has been established (EFSA FEEDAP Panel, 2016b, 2019b) and the additive is authorised in the EU, with the exception of that produced by *Corynebacterium glutamicum* KCTC 12307BP; the latter has been recently evaluated by the FEEDAP Panel which considered it a safe source of lysine in animal nutrition (EFSA FEEDAP Panel, 2020).

The sulfate present in the additive amounts up to a maximum of 30 %. It originates from iron (II) sulfate monohydrate used as starting material, for which the safety has been assessed (EFSA FEEDAP Panel, 2014, 2016a); the source is currently authorised as feed additive in the EU.

3.2.1. Safety for the target species

The maximum tolerable levels for iron for different animal species have been reviewed by the FEEDAP Panel in previous opinions (e.g. EFSA FEEDAP Panel, 2016a).

The additional lysine contribution to the diet when using ferrous lysinate sulfate at the maximum authorised iron concentrations would range from 645 to 1,720 mg Lys/kg complete feed or 717 mg/day in piglets (up to one week before weaning); its contribution should be taken into consideration when formulating diets (as requirements might be as low as 0.45 % lysine in pullets; NRC, 1994).

The additional sulfate contribution to the diet when using ferrous lysinate sulfate at the maximum authorised iron concentrations would range from 900 to 1,500 mg/kg complete feed or 500 mg/day in piglets (up to one week before weaning); this is below the theoretical maximum concentration of 2,200 mg sulfate/kg complete feed as derived from the potential contribution of an authorised sulfate-containing additive.[25] The FEEDAP Panel has assessed the sulfur/sulfate contribution from sulfate-containing additives (e.g. EFSA FEEDAP Panel, 2019b) and concluded that the formulation of the complete feed should carefully take into account the maximum tolerable level of total sulfur, as established by the National Research Council (in ruminant diets at 3 g S/kg dry matter (DM) (diet rich in concentrate) and at 5 g S/kg DM (diet rich in roughage) and in non-ruminant diets at 4 g S/kg DM; NRC, 2005a); also, the contribution of sulfur/sulfate present in water for drinking to the total sulfur intake should be considered, especially when the content is high.

Therefore, considering the above, no safety concerns are expected from the sulfur/sulfate and lysine delivered by the additive. Thus, the assessment of safety of the additive for the target species will focus on iron.

3.2.1.1. Tolerance study in chickens for fattening

[redacted][26]

[25] 3c323 L-lysine.
[26] Technical Dossier/Section III/Annex_III_1.

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[29] [redacted] Technical Dossier/ Supplementary Information September 2020.

[redacted]

3.2.1.2. Conclusions on safety for the target species

Based on the results of a tolerance study, the FEEDAP Panel concludes that ferrous lysinate sulfate is safe for chickens for fattening when used up to the current maximum authorised levels of total iron in feed. This conclusion is extrapolated to all animal species and categories at the respective maximum authorised iron levels in complete feed.

3.2.2. Safety for the consumer

Since the lysine and sulfate of the additive do not represent a safety concern (see above, under Section 3.2 Safety), in the context of this scientific opinion, the FEEDAP Panel focuses on iron in the assessment of safety for consumers.

3.2.2.1. Deposition study

[redacted]

3.2.2.2. Metabolism and toxicology of iron

3.2.2.2.1. Metabolism and deposition

Metabolism and deposition of iron have been previously reviewed in FEEDAP opinions (see e.g. EFSA FEEDAP Panel, 2016a).

The transport of iron from enterocytes to blood depends on the iron pool in the liver. Owing to the strong regulation of its intestinal absorption, a high oral intake of iron results in a less than proportional increase in iron deposition. Under physiological conditions, the haemoglobin concentration

[redacted]

in the blood closely reflects the amount of iron utilised in the organism and is a biomarker of a potentially deficient iron status. Indeed, ~ 70 % of body iron content is present in haemoglobin (EFSA, 2004). When animals are exposed to excessive amounts of iron, it is preferentially deposited in the liver, spleen and bone marrow. At very high doses, iron may be deposited in the heart and kidneys (NRC, 2005b). The iron content of milk is highly unaffected by changes in the level of dietary iron (NRC, 2005b).

A number of studies did not give evidence that organic iron sources would significantly influence the iron content of tissues, including muscle, or eggs as compared to the inorganic iron sources (see EFSA FEEDAP Panel, 2016a and references herein).

3.2.2.1.2. Toxicology of iron

The safety of iron has been previously evaluated by several authorities (EVM, 2003; EFSA, 2004) and more recently evaluated by Ponka et al. (2015). In infants, an acute dose of ~ 20 mg/kg body weight (bw) is associated with gastrointestinal irritation, whilst systemic effects do not generally occur at doses < 60 mg/kg bw. In adults, adverse gastrointestinal effects have been reported after short-term oral dosage as low as 50–60 mg daily of supplemental non-haem iron.

The EFSA Opinion (EFSA, 2004) assessed a possible tolerable upper intake level (UL) for iron; iron overload with clinical symptoms, including liver cirrhosis, has been reported in individuals receiving long-term, high-dose medical treatment with iron (160–1,200 mg iron/day). The risk of adverse effects from iron overload in the general population, including those heterozygous for hereditary haemochromatosis, is considered to be low; however, the available data are insufficient to establish an UL. In its opinion on reference dietary intakes for iron the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA Panel) reiterated that, while an UL is not determined, the risk of systemic iron overload from dietary sources is negligible with normal intestinal function (EFSA NDA Panel, 2015). Chronic iron overload may occur as a result of specific clinical conditions and genetic mutations, but there is no evidence that heterozygotes for haemochromatosis are at an increased risk of iron overload. The Population Reference Intake, calculated as the dietary requirement at the 97.5th percentile, is 11 mg Fe/day for adult men and 16 mg Fe/day for premenopausal women (EFSA NDA Panel, 2015).

3.2.2.3. Assessment of consumer safety

Based on a residue study in chickens for fattening, the use of ferrous lysinate sulfate up to the maximum authorised content of iron in complete feed is not expected to increase the iron content of edible tissues and products, hence to increase the exposure of consumers to iron. The FEEDAP Panel also notes that the current dietary iron intake by the general EU population poses no health concerns related to iron excess, besides individuals with specific conditions that predispose to iron overload (EFSA, 2004; EFSA NDA Panel, 2015). Therefore, the FEEDAP Panel considers that the use of ferrous lysinate sulfate in animal nutrition would not be of concern to the safety of consumers.

3.2.2.4. Conclusions on safety for the consumer

The use of ferrous lysinate sulfate in animal nutrition up to the maximum iron content in complete feed authorised in the EU poses no concern to the safety of consumers.

3.2.3. Safety for user

3.2.3.1. Effects on the respiratory system

[redacted]

3.2.3.2. Effects on the skin and eyes

[redacted][31]

[redacted][33]

3.2.3.3. Conclusions on safety for the user

The FEEDAP Panel concludes that ferrous lysinate sulfate poses a risk to users by inhalation. The additive is irritant to eyes and non-irritant to skin, but should be considered a skin sensitiser.

3.2.4. Safety for the environment

The additive under assessment, ferrous lysinate sulfate, contains iron, the amino acid L-lysine and sulfate. L-lysine, as amino acid, is a physiological and natural component in animals and plants.

The iron content of soils is typically in the range of 1,100–156,000 mg/kg;[34] it is not expected that the use of ferrous lysinate sulfate, which is intended to be a substitute for other authorised iron additives, could significantly change the background concentration of iron in the environment.

Sulphur content in topsoil is in the range of < 50–112,000 mg/kg;[35] it is not expected that the use of ferrous lysinate sulfate could significantly change the background concentration of sulfur in the environment.

Therefore, the FEEDAP Panel considers that the use of ferrous lysinate sulfate in animal nutrition would not pose a risk for the environment.

3.3. Efficacy

For demonstration of the efficacy of nutritional additives, at least one long-term study should be provided (EFSA FEEDAP Panel, 2018).

[redacted]

Owing to the limitations in the study provided, the FEEDAP Panel cannot conclude on the efficacy of the additive for chickens for fattening, and thus, on the efficacy of ferrous lysinate sulfate for all animal species and categories.

[31] Technical Dossier/Supplementary Information September 2020/Annex_SIn_15.

[redacted]

[33] Technical Dossier/Supplementary Information September 2020/Annex_SIn_16.

[34] Iron value calculated from FOREGS database, data on Iron(III) oxide, available online: http://weppi.gtk.fi/publ/foregsatlas/article.php?id=15

[35] Sulfur value calculated from FOREGS database, available online: http://weppi.gtk.fi/publ/foregsatlas/article.php?id=15

3.4. 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[36] and Good Manufacturing Practice.

4. Conclusions

Based on the results of a tolerance study, the FEEDAP Panel concludes that ferrous lysinate sulfate is safe in chickens for fattening when used up to the current maximum authorised levels of total iron in feed. This conclusion is extrapolated to all animal species and categories, at the respective maximum authorised iron levels in complete feed.

The use of ferrous lysinate sulfate in animal nutrition up to the maximum iron content in complete feed authorised in the EU poses no concern to the safety of consumers.

The FEEDAP Panel concludes that ferrous lysinate sulfate poses a risk to users by inhalation. The additive is not a dermal irritant, but is irritant to eyes and a skin sensitiser.

The FEEDAP Panel considers that the use of ferrous lysinate sulfate in animal nutrition would not pose a risk for the environment.

Owing to the limitations in the study provided, the FEEDAP Panel cannot conclude on the efficacy of the additive for chickens for fattening, and thus, on the efficacy of ferrous lysinate sulfate for all animal species and categories.

5. Documentation as provided to EFSA/Chronology

Date	Event
19/12/2019	Dossier received by EFSA. Ferrous lysinate sulfate (Plexomin® L-Fe). Submitted by Phytobiotics Futterzusatzstoffe GmbH.
24/01/2020	Reception mandate from the European Commission
11/03/2020	Application validated by EFSA – Start of the scientific assessment
14/05/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. Issues: characterization, safety for the target species, safety for the users.
19/05/2020	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
11/06/2020	Comments received from Member States
15/09/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
21/10/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. Issues: characterization.
21/12/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
18/03/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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ECHA (European Chemical Agency), 2018. Opinion on scientific evaluation of occupational exposure limits for Nickel and its compounds. Committee for Risk Assessment RAC. Available online: https://echa.europa.eu/documents/10162/13641/nickel_opinion_en.pdf/9e050da5-b45c-c8e5-9e5e-a1a2ce908335

EFSA (European Food Safety Authority), 2004. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the Tolerable Upper Intake Level of Iron. EFSA Journal 2004;2(11)125, 34 pp. https://doi.org/10.2903/j.efsa.2004.125

[36] 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.

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EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016b. Scientific opinion on the safety and efficacy of concentrated liquid L-lysine (base), L-lysine monohydrochloride and L-lysine sulphate produced using different strains of Corynebacterium glutamicum for all animal species based on a dossier submitted by AMAC/EEIG. EFSA Journal 2016;14(3):4346, 3 pp. https://doi.org/10.2903/j.efsa.2016.4346

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
DM	dry matter
ESI-MS	electrospray ionisation mass spectrometry
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FLD	fluorescence detection
IEC	ion-exchange chromatography
NDA	Nutrition, Novel Foods and Food Allergens
UL	upper intake level
VIS	visible

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for ferrous lysinate sulfate (Plexomin®)

In the current application, an authorisation is sought under Article 4(1) for *ferrous lysinate sulfate* 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, the authorisation is sought for the use of the *feed additive* for all animal species.

The *feed additive* is a preparation with a minimum content of 15% (w/w) of *iron* and 40% (w/w) of *lysine*.

The *feed additive* is intended to be incorporated into *feedingstuffs* through *premixtures*. The Applicant proposed maximum levels of *total iron* in *feedingstuffs* ranging from 450 to 750 mg/kg or 250 mg/day depending on the animal species.

For the quantification of *total iron* in the *feed additive*, *premixtures* and *feedingstuffs,* the Applicant proposed several internationally recognised ring-trial validated methods, namely ISO 6869 based on atomic absorption spectrometry (AAS), EN 15621 and EN 15510 based on inductively coupled plasma-atomic emission spectrometry (ICP-AES). In addition, for the quantification of *total iron* in *premixtures* and *feedingstuffs,* the Applicant proposed the internationally recognised ring-trial validated EN 17053 method based on inductively coupled plasma-mass spectrometry (ICP-MS) and the European Union method based on atomic absorption spectrometry (AAS). The above-mentioned methods were previously evaluated and recommended by the EURL in the frame of several iron-based feed additive dossiers.

Based on the available performance characteristics, the EURL recommends for official control the ring-trial validated methods: i) ISO 6869, EN 15621 and EN 15510 for the quantification of *total iron* in the *feed additive*, *premixtures* and *feedingstuffs*; ii) EN 17053 for the quantification of total iron in premixtures and feedingstuffs; and iii) the European Union method (Commission Regulation (EC) No 152/2009 – Annex IV-C) for the quantification of *total iron* in *feedingstuffs*.

For the quantification of *lysine* in the *feed additive,* the Applicant proposed the ring-trial validated method EN ISO 17180 based on ion-exchange chromatography (IEC) with post-column derivatisation coupled to optical (visible (VIS) or fluorescence (FLD)) detection.

Based on the performance characteristics available, the EURL recommends for official control the above-mentioned EN ISO 17180 method based on IEC-VIS/FLD to quantify *lysine* in the *feed additive*.

In addition, the EURL recommends for official control for the identification of the sulfate in the *feed additive* the European Pharmacopoeia monograph 20301.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.