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Safety and efficacy of iron chelates of lysine and glutamic acid as feed additive for all animal species

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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 iron chelates of lysine and glutamic acid (Iron-LG) as nutritional feed additive for all animal species. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) Panel concludes that, owing to safety considerations, Iron-LG should not be used in water for drinking. Iron-LG is safe for chickens for fattening; this conclusion can be extrapolated to all animal species/categories provided that the maximum authorised levels in the EU for total iron in feed are not exceeded. No increases in the iron content of animal tissues and products are expected from the use of Iron-LG in animal nutrition. There is no indication that the toxicity of Iron-LG is essentially different from that described for inorganic divalent iron. The use of Iron-LG in animal nutrition is of no concern for consumer safety provided that the maximum authorised total iron levels in feed are respected. Owing to the iron and nickel content of Iron-LG, the handling of the additive, poses a risk to users by inhalation. The additive is considered as a skin and respiratory sensitizer; it is irritant to eye and non-irritant to skin. Iron-LG is intended to be a substitute for other authorised iron additives and will not further increase the environmental burden of iron; therefore, the FEEDAP Panel considers that the use of the additive in animal nutrition would not pose an additional 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 Iron-LG to all animal species and categories. The FEEDAP Panel posed a recommendation concerning the description of the additive.

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Keywords: nutritional additives, compounds of trace elements, iron chelates of lysine and glutamic acid, ProPath Fe, safety, efficacy

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003¹ 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 Zinpro animal nutrition (Europe)² for authorisation of the product iron chelates of lysine and glutamic acid, when used as feed additive for all animal species (category: nutritional additives; functional group: compounds 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). The particulars and documents in support of the application were considered valid by EFSA as of 4 May 2018.

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 iron chelates of lysine and glutamic acid (Iron-LG complex), when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

The additive, iron chelates of lysine and glutamic acid, 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).

The applicant holds a patent on the iron chelates of lysine and glutamic acid, with the title "Mixed amino acid metal salt complexes".³

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier⁴ in support of the authorisation request for the use of iron chelates of lysine and glutamic acid as feed additive.

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 iron chelates of lysine and glutamic acid in animal feed. The Executive Summary of the EURL report can be found in Annex A.⁵

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of iron chelates of lysine and glutamic acid is in line with the principles laid down in Regulation (EC) No 429/2008⁶ and the relevant guidance documents: Guidance on nutritional additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008), Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP

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 Zinpro Animal Nutrition (Europe), Inc. Akkerdistel 2E. 5831 PJ. Boxmeer. The Netherlands.

³ Technical Dossier/Section II/Annex II_37.

⁴ FEED dossier reference: FAD-2018-0010.

⁵ The full report is available on the EURL website: <https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2018-0010-fe-lys-glu.pdf>

⁶ 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.

Panel, 2012b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d).

3. Assessment

The additive under assessment is 'Iron chelates of lysine and glutamic acid' (trade name: ProPath Fe). The additive corresponds to the active compound. As abbreviation, the short name of Iron-LG will be used throughout this opinion to refer to the additive under assessment. It is intended to be used in feed (either mashed or pelleted form) and water (via complementary feed) as a source of iron for all animal species and categories.⁷

3.1. Characterisation

3.1.1. Characterisation of the compound

The active substance is divalent iron in the form of chelates of lysine and glutamic acid in a mixture 1:1.⁸ The chemical names, according to International Union of Pure and Applied Chemistry (IUPAC), are Iron-2,6-diaminohexanoic acid and Iron-2-aminopentanedioic acid. The compounds are not identified by the Chemical Abstracts Service (CAS) number. The chemical formulas of the two compounds are [REDACTED] and [REDACTED], respectively. The structural formulas are shown in Figure 1 and the corresponding molecular weight are [REDACTED] and [REDACTED] g/mol for iron chelate of glutamic acid and for iron chelate of lysine. The theoretical content of iron is 15.8% and 15.6% for chelates of lysine and glutamic acid, respectively, the analysed contents were 15.0% and 14.7%, respectively.⁹

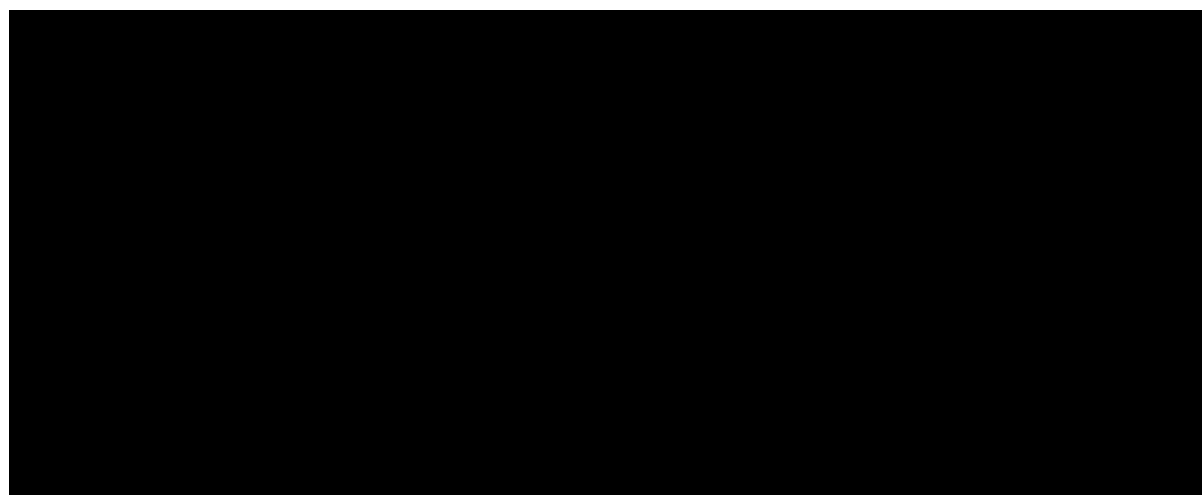


Figure 1: Structural formulas of iron chelates of lysine (a) and glutamic acid (b)

Five batches of the additive were analysed for iron, lysine, glutamic acid, chloride and sulfur. The average contents were: iron at 15.4% (range: 15.1–15.6%), lysine 20.2% (range: 19.6–20.9%), glutamic acid 20.6% (range: 18.9–21.5%), chloride 4.5% (range: 4.2–4.6), sulfur 9.7% (range: 9.5–9.8%) and water 1.8 (range: 1.7–1.8%). The remaining composition was not identified, but it can be assumed to be represented by the other components of the counter ions ([REDACTED]). The FEEDAP Panel notes that a further about 6% of the additive might be unidentified.

Five batches of Iron-LG were analysed for undesirable substances. Levels of heavy metals (cadmium Cd, lead Pb and mercury Hg), arsenic (As) and fluorine (F) were provided⁹ (As: < 0.20 mg/kg, Cd: < 0.10 mg/kg, Pb: < 0.80 mg/kg, Hg: < 0.01 mg/kg and F: 5.8–6.3 mg/kg).^{10,11} The levels of dioxins

⁷ Technical Dossier/Supplementary Information/August 2018.

⁸ Technical Dossier/Supplementary Information.

⁹ Technical Dossier/Spontaneous Information.

¹⁰ Values preceded by the sign '<' mean the LOQ.

¹¹ Technical dossier/Section II/Annex II-20-II-24.

and the sum of dioxins and dioxin-like-PCBs analysed in five batches were 0.021–0.036 ng WHO-PCDD/F-TEQ/kg and 0.022–0.038 ng WHO-PCDD/F-PCB-TEQ/kg, respectively.¹² The concentrations of the undesirable substances analysed comply with those set in Directive 2002/32/EC for compounds of trace elements or, if not mentioned in the Directive, do not represent a concern.¹³ Nickel was analysed in three batches of the additive and the values reported were in the range 43–44 mg/kg.⁸

Analysis of three batches (aged 8 months) for potential microbial contamination showed that counts of Enterobacteriaceae and *Escherichia coli* were below the limit of quantification (LOQ) (< 10 cfu/g) and *Salmonella* was not detected in 25 g.¹⁴ Levels of aflatoxin B1, B2, G1, G2 and ochratoxin A, analysed in three batches, were below the LOQ (< 0.1 µg/kg).¹⁵

The additive is a solid product, soluble in water, with a melting point at 168°C. The bulk density, tested in three batches of the additive is 0.689 g/cm³.¹⁶

Dusting potential was analysed in three batches by the Stauber–Heubach method (four measurements of each batch). The values reported were in the range of 3.1–3.6 g dust/m³ of air.¹⁶ The same batches were submitted for analysis of the particle size by laser diffraction. The results (v/v) showed on average of 11.2% (range 10.5–12.0), of 43.0% (range 41.8–44.6) and of 80.90% (range 80.6–81.4) for particles < 10 µm, < 50 µm and < 100 µm, respectively.¹⁶ Iron content in the dust was provided with analysis of three batches of the additive with an average of 12.7% (range 10.6–14.2).⁸

3.1.2. Manufacturing process

[REDACTED]

3.1.3. Stability and homogeneity

For compounds of trace elements (including chelates), stability studies are generally not required.

Two stability studies to determine the shelf-life of the additive, each with one batch stored at 25°C/60% relative humidity (RH) and at 40°C/70% RH were performed.²¹ After 24 months, the iron recovery was 102% and 100.6%; the total lysine/glutamic acid recovery was 98.7% and 100.5%, under the two conditions, respectively.

A stability study of the additive in premixture, mash feed and pellet feed measuring iron content was provided. After six months of storage, a recovery of 100.7% was reported for mash feed, of 79.7% for pellet feed and a recovery of 98.2% was reported for mash premixture.²²

¹² Technical dossier/Section II/Annex II-25–II-29.

¹³ 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.

¹⁴ Technical dossier/Section II/Annex II-30–II-32.

¹⁵ Technical dossier/Section II/Annex II-33–II-35.

¹⁶ Technical Dossier/Section II/Annex II-36.

[REDACTED]

²¹ Technical dossier/Section II/Annex II-41 & 42.

²² Technical dossier/Section II/Annex II-43.

The capacity of the additive to homogeneously distributed in premixtures and complete feed for laying hens was investigated, analysing the iron content in 10 subsamples each.²² Iron-LG was included to reach a theoretical level of iron of 450, 450 and 48,880 mg/kg for mash feed, pelleted feed and premixture, respectively. The coefficient of variation (CV) of the iron concentration in premixture (mean 51,521 mg/kg) was 0.95% and in the complete mash feed (mean iron content: 426 mg/kg) was 2.8%, that of the same feed after pelleting (mean iron content: 448 mg/kg) was 2.7%.

3.1.4. Physico-chemical incompatibilities or interactions

No incompatibilities are expected due to the nature of the product.

3.1.5. Conditions of use

Iron-LG is intended to be used in all types of feed, either mash or pelleted form, including premixtures, or water (via complementary feed) for all animal species/categories up to the total maximum iron content: ovine 500 (total), bovines and poultry 450 (total), pet animals 600 (total), other species 750 (total) and piglets up to 1 week before weaning 250 mg/kg complete feedingstuffs.

The applicant proposed half of the dose in feed for use in water for drinking.⁷

3.2. Safety

The additive is a mixture of iron chelate of lysine and iron chelate of glutamic acid. The additive will introduce only a minor fraction of the amino acids lysine and glutamic acid, which contributes negligibly to the intake of the animals. Therefore, no relevance for the safety assessment is foreseen for the amino acids delivered by the additive.

The use in water for drinking of several iron compounds was discussed in depth in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2016); the FEEDAP Panel calculated in that opinion the maximum levels of iron in water which could be delivered by supplementation in water for drinking (without a supplementation via feed), and remarked the previous Panel recommendation on the use of compounds of trace elements in water. The FEEDAP Panel retains the elements of the previous assessment as applicable also to Iron-LG and reiterates its statement that compounds of trace elements should generally not be used in water for drinking (EFSA FEEDAP Panel, 2010).

3.2.1. Safety for the target species

Where a feed additive application is made as a nutritional additive for all animal species, tolerance data may be limited to one species. The maximum tolerable levels for iron have been reviewed by the FEEDAP Panel in previous opinions (e.g. EFSA FEEDAP Panel, 2016).

3.2.1.1. Tolerance study

The applicant provided a tolerance study with Iron-LG in chickens for fattening with duration of 36 days.^{23,24}

A total of 504 one-day-old male chickens for fattening (Ross 308) were randomly allocated to seven treatments. The birds were housed in 42 pens with 12 animals per pen (six replicate pens per treatment). The treatments consisted of a control without added iron (basal feed containing 160 mg/kg background iron level) (T1), an organic iron source (Iron-LG) in three doses (low – 40 (T2), standard – 290 (T3) and tolerance – 590 (T4) mg/kg added iron in basal feed) and an inorganic iron source (iron sulfate) in three doses (low – 40 (T5), standard – 290 (T6) and tolerance – 590 (T7) mg/kg added iron in basal feed) (see Table 1). Levels were analytically confirmed. All chickens were fed a basal mashed (starter, days 1–14) followed by a pellet (grower, days 15–35) feed. The diets were mainly composed of wheat, soya and barley and contained as a starter 214 g/kg of crude protein and 12.8 MJ/kg of apparent metabolizable energy (AME) and as a grower 195 g/kg of crude protein and 13 MJ/kg of AME. Throughout the experiment, the chickens had *ad libitum* access to water and feed.

²³ Technical dossier/Section III/Annex III_3_1. Technical Dossier/Supplementary Information/May 2019

²⁴ Animals were fed until day 35 of the experiment.

Table 1: Description of the seven treatment groups

Treatment	Source	Added iron mg/kg	Total iron mg/kg (intended)	Total iron mg/kg (analysed)	
				Starter	Grower
T1	None ⁽¹⁾	0	160	146	171
T2	Iron-LG ⁽²⁾	40	200	193	182
T3		290	450	484	474
T4		590	750	820	822
T5	Iron sulfate ⁽³⁾	40	200	202	187
T6		290	450	543	465
T7		590	750	871	867

(1): The background iron concentration of the basal diets was 160 mg/kg.

(2): Iron-LG contains 16% iron.

(3): Commercial product containing 20% iron.

Mortality and general health were monitored throughout the study. Performance parameters measured were body weight (all birds were individually weighed weekly) and feed intake (evaluation of weekly feed intake per pen at the end of each week). On days 35 and 36, 2 birds from 3 pens of each treatment group on each day were examined at *post mortem*. Blood samples were collected for blood haematology²⁵ and biochemistry²⁶ analysis. Tissue samples were collected for analyses of iron content in breast muscle, kidney, liver, skin with fat, tibia (see Section 3.2.2.1 Residue study).

The study was blinded, controlled and randomised. Data were subjected to an analysis of variance (ANOVA) and multiple comparisons of all groups were conducted using Tukey's test. The pen was considered as the statistical unit for the performance parameters, whilst the individual animal was the statistical unit for haematology and biochemical parameters.

Mortality was 6.2% in the overall study and was not linked to any treatment; the highest mortality 8.3% was in T1, T2 and T3, but there were no statistical differences between groups. Performance parameters showed significant differences only for body weight and feed intake (Table 2). The final body weight of the study groups T5, T6 and T7 was higher than control and T4 groups. Group T4 consumed less feed than groups T5, T6 and T7. These results show some negative effect of the highest dose of tested organic iron on the feed intake and growth of chickens. Nevertheless, feed conversion ratio was not significantly affected; this suggests the possibility of a palatability issue.

Table 2: Effect of Iron-LG on performance parameters in chickens for fattening after 35 days

Treatment	Body weight (g)	Feed intake (g/animal per day)	F/G
T1	2,329 ^a	92.0 ^{ab}	1.39
T2	2,429 ^{ab}	95.0 ^{abc}	1.37
T3	2,440 ^{ab}	96.3 ^{abc}	1.38
T4	2,295 ^a	90.9 ^a	1.39
T5	2,563 ^b	99.4 ^{bc}	1.37
T6	2,579 ^b	99.6 ^{bc}	1.35
T7	2,580 ^b	102.5 ^c	1.39

a,b,c: Different superscript within a column indicates significant differences ($p \leq 0.05$).

Regarding biochemical and haematological parameters, statistically significant differences for calcium, phosphorus, creatine kinase, haematocrit ratio, mean corpuscular haemoglobin concentration and lymphocytes (%) were observed. The only significant dose-dependent effect was increased activity of creatine kinase (T5, T6, T7 significantly higher than T1), nevertheless the values were higher in

²⁵ Azurophils, eosinophils, heterophils, haematocrit (HCT) ratio, haemoglobin, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), monocytes, red blood cell count (RBC), white blood cell count (WBC).

²⁶ Alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST), calcium, chloride, creatinine, gamma glutamyl transferase (gGT), glutamate dehydrogenase (GLDH), glucose, lactate dehydrogenase (LDH), phosphorous, potassium, sodium, bilirubin, protein, triglycerides and uric acid.

groups supplemented with inorganic iron. The observed differences did not indicate a lower tolerance of the additive under assessment compared to the inorganic source in chickens for fattening.

The additive will introduce only a minor fraction of the amino acids lysine and glutamic acid, which contributes negligibly to the intake of the animals; no relevance for target animal safety is foreseen.

3.2.1.2. Conclusions on safety for the target species

Based on a tolerance study, the FEEDAP Panel concludes that the additive is safe for chickens for fattening. This conclusion can be extrapolated to all animal species and categories provided that the maximum authorised levels in the EU for total iron in feed are not exceeded.

3.2.2. Safety for the consumer

The sources used for both amino acids are authorised in the EU and their safety have been established. Therefore, the FEEDAP Panel retains that only iron is of interest concerning the consumer safety.

3.2.2.1. Deposition study

The applicant submitted a study on tolerance of chickens for fattening (see Section 3.2.1.1). This study provided data on iron deposition in tissues and organs (breast muscle, skin-fat, kidney, liver, and bone (tibia) samples) on chickens for fattening fed the Iron-LG or the inorganic iron source. Samples were taken at day 36 after necropsy, from a total of 42 birds (6 birds/treatment).

There were no statistically significant differences between the relevant treatments for iron levels of edible tissues as well as of tibia; also no treatment-related trend was apparent. The findings indicate that no iron deposition is not influenced by the relevant tested supplementation levels, up to the maximum levels authorised in feed (Table 3).

Table 3: Analytical results of iron content in edible tissues and tibia (results in mg/100 g wet weight)

Treatment	Source	Fe in feed mg/kg ⁽¹⁾	Skin & Fat	Muscle	Liver	Kidney	Tibia
T1	None	160	0.65	0.34	21.98	4.33	31.97
T2	Iron-LG	200	0.75	0.35	15.25	4.29	30.18
T3		450	0.76	0.36	21.79	3.97	30.43
T5	Iron sulfate	200	0.82	0.42	22.12	4.16	26.85
T6		450	0.78	0.36	20.01	4.11	31.05

(1): Confirmed by analysis.

3.2.2.2. Metabolism and toxicology of iron

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

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 in the blood closely reflects the amount of iron utilised in the organism and is a biomarker of a potentially deficient iron status. Indeed, approximately 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. With very high doses, iron may be deposited in the heart and kidneys (NRC, 2005). The iron content of milk is highly resistant to changes in the level of dietary iron (NRC, 2005).

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

The safety of iron has been previously evaluated by several authorities (EVM, 2003; EFSA, 2004) and recently evaluated by Ponka et al. (2015). In infants, an acute dose of approximately 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 a UL. In its opinion on reference dietary intakes for iron the EFSA NDA Panel reiterated that, while a 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 poultry, the use of Iron-LG 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. 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 Iron-LG 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 Iron-LG in animal nutrition up to maximum iron content in complete feed authorised in the EU poses no concern to the safety of consumers.

3.2.3. Safety for the user

3.2.3.1. Effects on the respiratory system

No specific studies were provided by the applicant regarding the toxicity of the additive on the respiratory system.

The highest dusting potential of the additive was 3.6 g/m³ and the iron maximum concentration in the dust was 14.2% (see Section 3.1.1). Thus, a maximum concentration of 507 mg Fe/m³ could be released by the dust when handling the additive. Considering that the respirable and the thoracic fractions amounted up to 12.0% and 44.6%, respectively (see Section 3.1.1), it could be estimated, based on a conservative approach, that the respirable iron from dust would be about 138 mg/m³, assuming as a worst case that the dust consists only of particles ≤ 50 µm and its respirable fraction about 27% (12.04 of 44.6). Assuming that the dust has the same respirable fraction as the additive, the respirable iron would amount to up to 61 mg/m³. Considering a threshold limit value (TLV) for iron of 1 mg/m³ (ACGIH, 2008), the TLV is exceeded by more than one order of magnitude.

The nickel maximum content of the additive was 44 mg/kg. The dusting potential of the products mounted up to 3.6 g/m³, corresponding to about 0.158 mg Ni/m³. Considering the proposed occupational exposure limit (OEL) for the inhalable fraction of water-soluble nickel is 0.01 mg Ni/m³ (EC, 2011), the nickel OEL is exceeded by more than one order of magnitude. Moreover, due to the presence of nickel in the additive, it should be considered as a respiratory sensitiser.

The FEEDAP Panel considers that handling the additive poses a risk to users by inhalation due to its contents in iron and nickel.

3.2.3.2. Effects on the eyes and skin

The applicant presented two *in vivo* acute irritation studies on rabbits according to OECD guidelines No. 404 and No. 405, respectively for skin²⁷ and eye.²⁷ Under the experimental conditions adopted, the additive was found to be non-irritant for the skin and irritant for the eye of the rabbit.

The nickel content of the additive is up to 44 mg/kg; given its well-known sensitisation potential (EC, 2011) and in the absence of skin sensitisation studies the additive is classified as a skin sensitiser.

²⁷ Technical dossier/Section III/Annex_III_3_2.

3.2.3.3. Conclusions on safety for the user

Owing to the iron and nickel content, the handling of the additive poses a risk to users by inhalation. The additive is considered as a skin and respiratory sensitiser. It is irritant to eye and non-irritant to skin.

3.2.4. Safety for the environment

The additive under assessment, Iron-LG, is intended to be a substitute for other authorised iron additives and will not further increase the environmental burden of iron. Therefore, the FEEDAP Panel considers that the use of the additive in animal nutrition would not pose an additional risk for the environment.

3.3. Efficacy

For demonstration of the efficacy of nutritional additives, one study in a single animal species or category, including laboratory animals, is considered sufficient (EFSA FEEDAP Panel, 2012a).

3.3.1. Study in chickens for fattening

The applicant provided a combined tolerance/efficacy study in chickens for fattening (see Sections 3.2.1.1 and 3.2.2.1); the experimental groups in the study are shown in Table 1. In this trial, iron concentration in edible tissues/organs and tibias was measured (Table 3).

The results showed that no significant differences were seen in iron deposition among the experimental groups. In this case, the lack of differences between the inorganic source of iron or the additive under assessment with the control group could be explained by the iron concentration in the diet of that group (160 mg Fe/kg feed), already exceeding by about two times the chicken's requirements. Therefore, the design of the experiment was not the appropriate to detect the efficacy of the iron supplemented groups.

3.3.2. Conclusions on efficacy for the target species

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 Iron-LG to all animal species and categories.

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 Regulation²⁸ and Good Manufacturing Practice.

4. Conclusions

The FEEDAP Panel concludes that, owing to safety considerations, Iron-LG should not be used in water for drinking.

Based on the results of a tolerance study, the FEEDAP Panel concludes that the additive is safe for chickens for fattening. This conclusion can be extrapolated to all animal species and categories, provided that the maximum authorised levels in the EU for total iron in feed are not exceeded.

No increases in the iron content of animal tissues and products are expected from the use of Iron-LG in animal nutrition. There is no indication that the toxicity of Iron-LG is essentially different from that described for inorganic divalent iron. The FEEDAP Panel concludes that the use of Iron-LG in animal nutrition is of no concern for consumer safety provided that the maximum authorised total iron levels in feed are respected.

Owing to the iron and nickel content of Iron-LG, the handling of the additive, poses a risk to users by inhalation. The additive is considered as a skin and respiratory sensitiser. It is irritant to eye and non-irritant to skin.

²⁸ Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 October 2003 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

The additive under assessment, Iron-LG, is intended to be a substitute for other authorised iron additives and will not further increase the environmental burden of iron. Therefore, the FEEDAP Panel considers that the use of the additive in animal nutrition would not pose an additional risk for the environment.

The FEEDAP Panel cannot conclude on the efficacy of the additive for all animal species and categories.

5. Recommendations

The FEEDAP Panel recommends to include the sources of lysine and glutamic acid (including the production strain(s), where applicable) as proposed by the applicant in the description of the additive. The content of both amino acids in the additive should be also indicated in the description of the product.

Documentation provided to EFSA/Chronology

Date	Event
07/03/2018	Dossier received by EFSA. Iron chelates of lysine and glutamic acid for all animal species. Submitted by Zinpro Animal Nutrition (Europe), Inc.
19/03/2018	Reception mandate from the European Commission
04/05/2018	Application validated by EFSA – Start of the scientific assessment
21/06/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation, safety for target species and safety for the user</i>
24/07/2018	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
04/08/2018	Comments received from Member States
22/08/2018	Reception of supplementary information from the applicant – Scientific assessment re-started
17/12/2018	Spontaneous submission of information by the applicant. <i>Issue: characterisation</i>
24/01/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended <i>Issue: characterisation</i>
07/03/2019	Clarification teleconference during risk assessment with the applicant according to the “EFSA’s Catalogue of support initiatives during the life-cycle of applications for regulated products”
14/03/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
07/05/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended <i>Issue: safety for target animals, safety for consumers, efficacy</i>
23/05/2019	Reception of supplementary information from the applicant – Scientific assessment re-started
04/07/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

- ACGIH (American Conference of Governmental Industrial Hygienists), 2008. TLVs and BEIs. Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. ACGIH, Cincinnati, OH, USA.
- EC (European Commission), 2011. Recommendation from the Scientific Committee on Occupational Exposure Limits (SCOEL) for nickel and inorganic nickel compounds. Employment, Social Affairs and Inclusion. SCOEL/SUM/85, June 2011.
- 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>
- EFSA (European Food Safety Authority), 2008. Technical Guidance of the Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) for assessing the safety of feed additives for the environment. EFSA Journal 2008;6(10):842, 28 pp. <https://doi.org/10.2903/j.efsa.2008.842>
- EFSA (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2010. Scientific Opinion on the use of feed additives authorised/applied for use in feed when supplied via water. EFSA Journal 2010;8(12):1956, 9 pp. <https://doi.org/10.2903/j.efsa.2010.1956>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011. Technical guidance: Tolerance and efficacy studies in target animals. EFSA Journal 2011;9(5):2175, 15 pp. <https://doi.org/10.2903/j.efsa.2011.2175>

- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Guidance for the preparation of dossiers for nutritional additives. EFSA Journal 2012;10(1):2535, 14 pp. <https://doi.org/10.2903/j.efsa.2012.2535>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance for the preparation of dossiers for additives already authorised for use in food. EFSA Journal 2012;10(1):2538, 4 pp. <https://doi.org/10.2903/j.efsa.2012.2538>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012c. Guidance for establishing the safety of additives for the consumer. EFSA Journal 2012;10(1):2537, 12 pp. <https://doi.org/10.2903/j.efsa.2012.2537>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012d. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. <https://doi.org/10.2903/j.efsa.2012.2539>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016. Scientific opinion on the safety and efficacy of iron compounds (E1) as feed additives for all animal species: ferrous carbonate; ferric chloride, hexahydrate; ferrous fumarate; ferrous sulphate, heptahydrate; ferrous sulphate, monohydrate; ferrous chelate of amino acids, hydrate; ferrous chelate of glycine, hydrate, based on a dossier submitted by FEFANA asbl. EFSA Journal 2016;14(2):4396, 48 pp. <https://doi.org/10.2903/j.efsa.2016.4396>
- EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2015. Scientific Opinion on Dietary Reference Values for iron. EFSA Journal 2015;13(10):4254, 115 pp. <https://doi.org/10.2903/j.efsa.2015.4254>
- EVM (Expert group on Vitamins and Minerals), 2003. Report on Safe Upper Levels for Vitamins and Minerals. Foods Standards Agency, London. May 2003.
- NRC (National Research Council of the National Academies), 2005. Mineral Tolerance of Animals. 2nd Revised Edition. The National Academies Press, Washington, DC, USA. Book available online: <https://www.nap.edu/read/11309/chapter/1#i>
- Ponka P, Tenenbein M and Eaton JW, 2015. Chapter 41 – Iron. In: Nordberg GF, Fowler BA and Nordberg M (ed.). Handbook on the Toxicology of Metals (Fourth Edition), Volume II, Academic Press, UK. pp. 879–902.

Abbreviations

AME	apparent metabolizable energy
ANOVA	analysis of variance
bw	body weight
CAS	Chemical Abstracts Service
Cfu	colony forming unit
CV	coefficient of variation
EMA	European Medicine Agency
EURL	European Union Reference Laboratory
FAO	Food and Agriculture Organization of the United Nations
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FSA	UK Food Standards Agency
HPLC	high-performance liquid chromatography
ICP-AES	inductively coupled plasma-atomic emission spectrometry
IUPAC	International Union of Pure and Applied Chemistry
LOQ	limit of quantification
MRL	maximum residue level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OEL	occupational exposure limit
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo(<i>p</i>)dioxins
PCDF	polychlorinated dibenzofurans
RH	relative humidity
RSDr	relative standard deviations for <i>repeatability</i>
TEQ	toxic equivalent
TLV	threshold limit value
UL	tolerable upper intake level
WHO	World Health Organization

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for iron chelates of lysine and glutamic acid

In the current application authorisation is sought under Article 4(1) for *iron chelates of lysine and glutamic acid* 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.

Iron chelates of lysine and glutamic acid is a solid preparation with a minimum content of 15% (w/w) of iron, 19% (w/w) of lysine and 19% (w/w) of glutamic acid.

The *feed additive* is intended to be incorporated into *premixtures* and *feedingstuffs*. The Applicant proposed maximum levels of *total iron* in *feedingstuffs* ranging from 450 to 750 mg/kg or 250 mg/day – depending of the animal species/category – and thus complying with the limits set in the Corrigendum to Commission Implementing Regulation (EU) 2017/2330.

For the quantification of *total iron* in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted two internationally recognised ring-trial validated CEN methods based on inductively coupled plasma-atomic emission spectrometry (ICP-AES): EN 15510 and EN 15621. These two methods together with the Community method based on atomic absorption spectrometry, which was further ring-trial validated by the UK Food Standards Agency (FSA), were previously evaluated and recommended by the EURL in the frame of the Iron group dossier.

In addition, other two ring-trial validated methods, namely: ISO 6869 based on atomic absorption spectrometry and EN 17053 based on inductively coupled plasma-mass spectrometry (ICP-MS) were recently evaluated and recommended by the EURL in the frame of two similar dossiers (FAD-2017-0071 and FAD-2017-0072).

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

For the quantification of *lysine* and *glutamic acid* in the *feed additive*, the Applicant submitted the ring-trial validated EN ISO 13903 method based on ion exchange chromatography coupled with post-column derivatisation and photometric detection (IEC-VIS). This standard method is equivalent to the experimental protocol described in the Community method designed for the determination of *free* (synthetic and natural) and *total* (peptide-bound and free) amino acids including *lysine* and *glutamic acid*, using an amino acid analyser or a high-performance liquid chromatography (HPLC) equipment. This method does not distinguish between the salts and the amino acid enantiomers.

The Applicant applied the above mentioned IEC-VIS method for the analysis of five batches of the *feed additive* with an average content of 20% (w/w) for *lysine* and 21% (w/w) for *glutamic acid*. Relative standard deviations for *repeatability* (RSDr) of 2.3% and 4.7%, respectively, were obtained. This is in agreement with the precision values reported in the frame of the two ring-trial validation studies.

Based on the performance characteristics available, the EURL recommends for official control the method based on IEC-VIS to quantify *lysine* and *glutamic acid* in the *feed additive*.

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.