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Safety and efficacy of CI-FER™ (ferric citrate chelate) as a zootechnical feed additive for suckling and weaned piglets and minor porcine species

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Abstract

Following a request from the European Commission, the EFSA 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 CI-FER™ (ferric citrate chelate) as zootechnical feed additive for suckling and weaned piglets and minor porcine species. The additive is safe for weaned piglets at the recommended level of 500 mg/kg complete feed. This conclusion can be extended to sucking piglets for the period in which solid feed is given and extrapolated to all minor porcine species. No concerns for consumer safety are expected from the use of the additive in piglets and minor porcine species. The FEEDAP Panel considers that the compound under assessment poses a risk to users by inhalation. The product should also be considered as an irritant to skin, eyes and mucous membranes. Due to the presence of nickel, CI-FER™ should also be considered as a dermal and respiratory sensitiser. The supplementation of feed with the additive is not expected to pose an environmental risk. CI-FER™ used at the minimum recommended level of 500 mg/kg feed has the potential to improve zootechnical parameters of weaned piglets. This conclusion can be extended to sucking piglets for the period in which solid feed is given and extrapolated to all minor porcine species.

Keywords: zootechnical additives, gut flora stabiliser, CI-FER™, ferric citrate chelate, safety, efficacy

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Table of contents

1. Introduction

1.1. Background and Terms of Reference

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 Akeso Biomedical, Inc.[2] for authorisation of the product CI-FER™[3] (ferric citrate chelate), when used as a feed additive for suckling and weaned piglets and minor porcine species (category: zootechnical additives; functional groups: gut flora stabiliser and other zootechnical additives).

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 15 November 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 CI-FER™ (ferric citrate chelate), when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

CI-FER™ is a ferric citrate chelate. The product has not been authorised in the European Union (EU) as a feed additive. For the purpose of this opinion, the additive will be referred to as CI-FER™.

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[4] in support of the authorisation request for the use of CI-FER™ (ferric citrate chelate) as a 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 and other scientific reports, 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 CI-FER™ (ferric citrate chelate) 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 ferric citrate chelate CI-FER™ (ferric citrate chelate) is in line with the principles laid down in Regulation (EC) No 429/2008[6] and the relevant guidance documents: Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008), 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

[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] Akeso Biomedical Inc., USA, represented in the EU by Pen & Tec Consulting S.L.U., Pl. Ausias March 1, 4th Floor D01, Mirasol, ES-08195, Sant Cugat del Vallès, Catalunya, Spain.
[3] On 28 June 2019, the applicant notified EFSA a request to change the brand name from CI-PLEX™ to CI-FER™.
[4] FEED dossier reference: FAD-2018-0065.
[5] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2018-0065-ferric-citrate.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.

assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c) and Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

3. Assessment

CI-FER™, a ferric citrate chelate, is proposed for use in suckling and weaned piglets and minor porcine species, as a zootechnical feed additive under two functional groups, with the effects indicated below:

- '4b gut flora stabiliser';
- '4d other zootechnical additives' – performance enhancer

3.1. Characterisation

3.1.1. Manufacturing process

The manufacturing process of the product is fully described in the technical dossier. [redacted]

3.1.2. Characterisation of the additive

Ferric citrate chelate (International Union of Pure and Applied Chemistry (IUPAC) name: Iron($^{3+}$), 2-hydroxypropane-1,2,3-tricarboxylate; synonyms: Iron (III) citrate, Iron citrate, Fe(III) citrate, Fe citrate, Ferric citrate; 1,2,3-Propanetricarboxylic acid, 2-hydroxy-, iron(3+) salt (1:1); ferric citrate pure, 2-hydroxy-1,2,3-propanetricarboxylic acid iron(III), 2-hydroxy-1,2,3-propanetricarboxylic acid iron(III) salt, Citric acid iron) is identified with the Chemical Abstracts Service (CAS) number is 3522-50-7. It has a molecular weight of 244.94 Da and its molecular formula is $C_6H_5FeO_7$. The structural formula is shown in Figure 1. The theoretical content of iron is 22.8%.

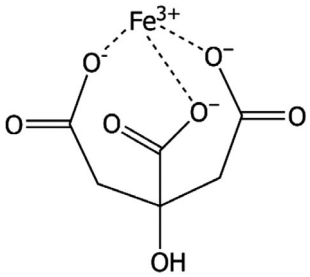

Figure 1: Structural formula of ferric citrate chelate

The additive contains by specification a maximum of 3.0% Fe(II), Fe(III) in the range of 16.5–20.0% and a maximum of 27.0% of water. The analysis of five batches of the additive showed compliance with specifications: Fe(II) was found to be in the range of 0.60–2.65%, Fe(III) 17.0–17.7% and water 15.2–21.0%.[9] The applicant declared that no carriers are added to the ferric citrate chelate.[10] The content of total iron (17.8–20.0%) and citrate (50.2–58.6%) was analysed in five batches.[11] [redacted]

[redacted]

[9] Technical dossier/Section II/Annex_II_1_3_1.
[10] Technical dossier/Section II.
[11] Technical dossier/Supplementary information July 2019/Annex_II_1_3_3.

[redacted]

In addition, five batches of the additive (manufactured in 2015, 2016 and 2017) were analysed by Mössbauer spectroscopy. [redacted]

Undesirable substances were analysed in six batches of the additive. Detectable levels of arsenic (1 mg/kg), fluorine (≤ 1–27 mg/kg) and nickel (22–29 mg/kg) were reported in all batches, whereas the levels of heavy metals (cadmium, mercury and lead) were below the limit of quantification (LOQ).[15] The content of methanol was below the LOQ (300 mg/kg) in three batches.[11] The levels of dioxins and the sum of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F) and dioxin-like polychlorinated biphenyls (PCBs) in three batches[16] were 0.0024–0.0037 ng WHO-PCDD/F-TEQ/kg and 0.0999–0.426 ng WHO-PCDD/F-PCB-TEQ per kg, respectively. 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 safety concern.[17]

Microbial impurities, analysed in the same batches tested for batch-to-batch variation, showed absence of total coliforms, aerobic plate counts < 1,000 colony forming units (CFU)/g and yeast and moulds counts < 100 CFU/g.

Physical properties

The product is an orange–brown powder and is soluble in water at 10 g/L. The solid density of the additive, measured in three batches, ranged from 1,803 to 1,864 kg/m^3.[18] The bulk density (results of three batches) was in the range of 925–1,038 kg/m^3.[19]

Particle size distribution was analysed by laser diffraction in three batches of the additive. The percentage of particles below 10, 50 and 100 μm were on average 7.81%, 40.1% and 61.2% (v/v).[20] Dusting potential, analysed in the same three batches by the Stauber–Heubach method, ranged between 6.33 and 9.02 g/m^3.[21] Laser diffraction analysis of the dust showed that about 87% of the particles were of respirable size (< 10 μm).

3.1.3. Stability and homogeneity

The applicant submitted data on the shelf-life of CI-FER™ showing the chelation in the additive manufactured in 2015–2017, demonstrating that the chelate is stable for approximately 3 years (see Section 3.1.2). Stability data of the additive in premixtures and complete feed were not provided.

The capacity of the additive to homogeneously distribute in feed was evaluated by analysing 10 subsamples of a pelleted piglet feed containing microtraced CI-FER™ at 550 mg/kg feed (500 mg/kg ferric citrate chelate and 50 g microtracer™ G-Green).[22] The coefficient of variation was 12.6%. However, the data shows the distribution of the microtracer, but there is no evidence that the additive or its individual components followed the microtracer in its distribution in feed.

[redacted]

[15] Technical dossier/Section II/Annex II_1_4_1_1 and Supplementary information July 2019/Annex_II_1_3_3. Limit of quantification (LOQ) < 0.10 mg/kg for cadmium and mercury and < 0.30 mg/kg for lead, as determined by inductively coupled plasma-mass spectrometry (ICP-MS).
[16] Technical dossier/Section II/Annex II_1_4_1_2.
[17] 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.
[18] Technical dossier/Section II/Annex_II_1_5_4.
[19] Technical dossier/Section II/Annex_II_1_5_3.
[20] Technical dossier/Section II/Annex_II_1_5_1.
[21] Technical dossier/Section II/Annex_II_1_5_2.
[22] Technical dossier/Section II/Annex_II_4_2.

3.1.4. Conditions of use

The product is intended to be used in feed for suckling and weaned piglets and minor porcine species at the same growing stage (suckling and weaned) at a minimum level of 500 mg additive/kg of complete feedingstuffs. A maximum content is not indicated, but the applicant indicated a recommended use level of 500 mg additive/kg complete feed, which would supply 82–100 mg Fe/kg complete feed.[8]

3.2. Safety

3.2.1. Safety for the target species

A tolerance study with 144 twenty-five-day-old weaned piglets Danbred × Piétrain (females and males) was performed.[23] The piglets were allocated to three treatment groups, balanced for body weight (BW), litter origin, and gender in 24 pens (12 pens for males and 12 for females, 6 piglets per pen, 8 pens per treatment) from 25 to 66 days of age (42-d feeding period). CI-FER™ blended with 10% microtracer (G-Green) was incorporated into the basal corn-soybean diet to deliver 0 (control), 500 (1× the recommended dose) and 5,000 (10× the recommended dose) mg CI-FER™/kg feed. The content of iron of the diet was confirmed by analysis. The intended levels of the product were confirmed by analyses using the marker. Piglets were fed ad libitum in both starter (0–14 days) and grower phase (14–42 days post-weaning) with diets in mash form. The iron content of the control diet used (69.2/76.2 mg/kg feed for starters/growers) was below the requirements of piglets[24] specified by the National Research Council (NRC, 2012), but the piglets received routine iron supplementation by long-acting injection at 3 days of age, to ensure that iron deficiency would not arise in the pre- or post-weaning period. Zootechnical parameters (body weight, feed intake) were recorded weekly. Mean pen body weight, feed intake, body weight gain, were recorded at study start (body weight) and on days 7, 14, 21, 28, 35 and 42 on trial and feed conversion ratio (feed/gain) was calculated for the respective periods. Blood samples were taken at the end of the trial from one pig per pen (with BW closest to treatment average) for analysis of haematological[25] and biochemical parameters.[26] Health and faecal scores[27] per pen were monitored daily. Data were analysed by one-way analysis of variance (ANOVA), group means were compared with Tukey test. The pen (zootechnical parameters) or the individual piglet (blood parameters) were the experimental units. The significance level was set at $p < 0.05$.

The piglets remained healthy during the study. No animals died, had to be culled or required medical treatment. Piglets fed diets containing CI-FER™ (1× and 10 ×) had faecal scores closer to the score of 4.0 (well-formed faeces, firm to cut, but not dry) when compared to piglets fed diets without addition of CI-FER™, indicating good health. No differences were observed in the feed intake of the animals (630 g/day per piglet) or in the final body weight (24.5, 25.2 and 25.5 kg for control, 1× and 10×, respectively; average 25 kg). A significantly better feed to gain ratio was observed in the animals that received the additive compared to the control (1.54, 1.48 and 1.46 for control, 1x and 10x, respectively). There were no statistically significant differences for any of the haematology or biochemistry parameters measured.[28]

The use of the additive at the recommended use level of 500 mg/kg complete feed would result in about 0.01 mg Ni/kg feed, which is negligible compared to the maximum tolerable level (MTL) for pigs up to 400 mg/kg feed (NRC, 2005).

3.2.1.1. Conclusions on safety for the target species

The results of the tolerance study indicate that weaned piglets tolerate 5,000 mg CI-FER™/kg complete feed without any adverse effects on health, performance or blood parameters. Based on this

[23] Technical dossier/Section III/Annex_III_1_1_1.
[24] Iron requirement (NRC, 2012): 100 mg/kg feed for piglets with body weight in the range 5–10 kg and 80 mg/kg feed for piglets with body weight in the range 10–20 kg.
[25] Erythrocytes, leucocytes, thrombocytes, lymphocytes, neutrophils, monocytes, eosinophils, basophils, other cells, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration.
[26] Alkaline phosphatase, aspartate amino transferase, alanine amino transferase, glutamate dehydrogenase, lactate dehydrogenase, creatine kinase, total cholesterol, triglycerides, urea, total bilirubin, creatine, glucose, albumin, globulin, total protein and haptoglobin.
[27] Score 1–5, 1 = liquid diarrhoea; 2 = pasty faeces falling out of shape upon contact with surfaces; 3 = formed faeces, soft to cut; 4 = well-formed faeces, firm to cut; 5 = hard and dry faeces.
[28] Technical dossier/Section III/Annex III_1_1_1.

study, the FEEDAP Panel concludes that CI-FER™ is safe for weaned piglets at the recommended level of 500 mg/kg complete feed. Considering the nature of the additive, the low creep feed intake, the expected absorption/metabolism of the active substance and the margin of safety observed in the tolerance study in weaned piglets described above, the Panel considers that this conclusion can be extended to sucking piglets for the period in which solid feed is given and extrapolated to all minor porcine species.

3.2.2. Safety for the consumer

The applicant did not provide any specific support studies to the safety of the additive for the consumers.

The safety for consumers of foods derived from animals whose diets were supplemented with iron compounds, including chelate of amino acids, has been reviewed and assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2013, 2014a,b, 2015, 2016a,b,c). It was concluded that no concerns for consumer safety are expected when iron compounds are used up to the EU maximum authorised level in feed.[29] In addition, the absorption of Fe(III) from ferric citrate chelate is relatively low and the intestinal iron absorption under physiological conditions is regulated according to demand via homeostasis. Therefore, the ferric citrate chelate fed to animals is not expected to have any significant influence on the iron content of edible tissues.

Citric acid is an intermediate in cellular oxidative metabolism, in the citric acid cycle. When supplemented in feedingstuffs, it is expected to be completely metabolised in the target species. Therefore, the use of citric acid in animal nutrition is safe for the consumer.

Therefore, the use of CI-FER™ in feed for suckling piglets, weaned piglets and minor porcine species is not expected to raise any concern for consumers under the proposed conditions of use.

3.2.3. Safety for the user

No specific studies were provided by the applicant regarding the toxicity of the additive for the users/workers.

3.2.3.1. Effects on the respiratory system

The product under assessment has a significant proportion (8%) of particles of respirable size (< 10 μm). The dusting potential was in the range between 6.3 and 9.0 g/m^3, with an iron concentration in the dust up to 1.8 g/m^3. Inhalation of iron salts may cause serious lung problems (Nemery, 1990). The toxicity of inhaled iron is still under scientific debate (IARC, 1987; Weinberg, 1999; Wild et al., 2009; Ponka et al., 2015). The American Conference of Governmental Industrial Hygienists has set a threshold limit value (TLV) for an 8-h time-weighted average (TWA) for iron salts (soluble) of 1 mg/m^3 expressed as iron (ACGIH, 2008), which is in agreement with the standards applied in some European countries (Belgium, Finland, Italy, the Netherlands and Switzerland). Handling CI-FER™ leads to an exposure exceeding TLV by more than three orders of magnitude thus indicating a risk by inhalation for users.

The nickel content of the product is up to 29 mg/kg additive. Inhalation of nickel can cause pulmonary toxicity, resulting in bronchitis, fibrosis and lung cancer in humans (Nemery, 1990). The proposed occupational exposure limit (OEL) for the inhalable fraction of water-soluble nickel is 0.01 mg Ni/m^3 (European Commission, 2011). According to the dusting potential of the product, and assuming equal content as in the additive, the nickel content in the dust would be up to 0.27 mg/m^3; therefore, the nickel OEL is exceeded by more than one order of magnitude indicating a risk by inhalation for users.

The FEEDAP Panel recognises that the use of the TLV or OEL as guidance values for user safety of feed additives may result in overly conservative assessments, as the exposure is unlikely to be as continuous and intense as in an industrial scenario, for which TLVs/OELs have been envisaged. Nevertheless, even with the mentioned caveat, a concentration of iron or nickel in the inhalable dust exceeding the guidance values by at least one order of magnitude points to a risk by inhalation for users.

[29] As per Regulation (EC) No 1334/2003 and Regulation (EC) No 479/2006 the maximum total iron content for piglets up to one week before weaning is 250 mg/day and in other pigs the limit is 750 mg/kg of the complete feedingstuff. Ferric citrate chelate contains by specification 16.5–20.0% of Fe(III). Hence, at the recommended dose of 500 mg/kg, ferric citrate chelate supplies 82–100 mg Fe/kg feed, significantly below the maximum total iron content set by the European Commission.

The applicant provided calculations of exposure by inhalation to iron and nickel from CI-FER™ in a premixture factory, according to the model described in the guidance on studies concerning the safety of the use of the additive for users/workers (EFSA FEEDAP Panel, 2012). The details can be found in Appendix A.

Assuming that 20% of premixtures contain the additive CI-FER™, the inhalation exposure of workers/users was calculated to be 230 mg/day for iron and 0.050 mg/day for nickel. With the use of a P3 filter mask inhalation exposure would be reduced by a factor of 20, resulting in 11.5 mg/day for iron and 0.0025 mg/day for nickel. The occupational exposure at the corresponding TLVs/OELs for an 8-h work shift would be 10 mg/day for iron and 0.1 mg/day for nickel (calculated assuming a volume of inhaled air of 10 m^3 during an 8-h work shift).[30]

Owing to the well-known sensitisation potential of nickel (European Commission, 2011), the product should be considered as a dermal and respiratory sensitiser.

3.2.3.2. Effects on the eyes and skin

The applicant provided two reports, which show that ferric citrate is used in cosmetics (as a skin conditioning agent) at concentrations up to 0.5% (CIR, 2012; Fiume et al., 2014).[31] In the reports, it is concluded that ferric citrate is safe under the current practices of use and concentration as cosmetic.[32] The FEEDAP Panel notes that the conditions of use of ferric citrate as cosmetic (up to 0.5%) are not relevant for the use of ferric citrate as feed additive, as CI-FER™ contains up to 78% of ferric citrate (on as is basis).

In the absence of specific studies and considering that several iron compounds are recognised as irritants to skin, eyes and mucous membranes (EFSA FEEDAP Panel, 2013, 2014a,b, 2015, 2016a–c), the product should be considered as a skin, eye and respiratory irritant.

The applicant recognises theses hazards in the safety data sheet[33] which states that ferric citrate chelate may cause eye and skin irritation and may be harmful by inhalation. Consequently, the applicant proposed measures to control the exposure of users to iron and nickel.[30]

3.2.3.3. Conclusions on safety for the user

Users may be exposed to iron and nickel from the additive by inhalation at levels exceeding the TLV/OEL values by at least three and one orders of magnitude, respectively. The FEEDAP Panel considers that the compound under assessment poses a risk to users by inhalation. The product should also be considered as an irritant to skin, eyes and mucous membranes. Due to the presence of nickel, CI-FER™ should also be considered as a dermal and respiratory sensitiser.

3.2.4. Safety for the environment

The components of the additive, iron and citric acid, are ubiquitous in the environment. The iron content of soils is typically in the range of 5,000–50,000 mg/kg while citric acid is a physiological and natural component of animals and plants.

Based on the calculation method provided in the technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008), the highest increase of iron in soil is around 1.83 mg/kg after a 1-year application of manure from piglets assuming that 100% of the dose will be excreted. Therefore, any additional load from the use of the product in feed for piglets is not expected to pose an environmental risk.

3.3. Efficacy

The applicant claimed two effects from the use of the additive: improvement of zootechnical performance and of the gut flora, by reducing the carriage of gut enteropathogens.

In order to support the efficacy of the product, one *in vitro*[34] and five *in vivo* studies were submitted. The *in vitro* study submitted by the applicant that would support the mode of action of the additive was not considered relevant for the assessment, owing to the lack of mimicking to the *in vivo* conditions.

[30] Technical dossier/Spontaneous submission of information/August 2019.
[31] Technical dossier/Spontaneous submission of information/September 2019.
[32] Ferric citrate is also listed in the EU Commission Cosmetics CosIng database, Technical dossier/Supplementary information/September 2019/Annex EU_COSING_Ferric citrate_2019.
[33] Technical dossier/Section II/Annex_II_3_2_2.
[34] Technical dossier/Section IV/Annex_IV_1_1.

3.3.1. Efficacy studies in weaned piglets

All five efficacy studies were carried out in a single Member State at two different locations.

The details on the study design are provided in Table 1. In all cases, 25-day-old weaned piglets (Danbred × Piétrain) were used and the trials lasted 42 days. All trials included at least two groups that received either a basal diet non-supplemented with the additive (control group) or supplemented at the recommended dose of 500 mg CI-FER™/kg feed. In trials 3 and 5 groups including other levels of CI-FER™ were considered, while trials 1 and 2, included groups with other sources of iron (organic/inorganic). The intended content of CI-FER™ in diets (ranging from 0 to 5,000 mg/kg feed) was confirmed analytically by using a microtracer. Performance parameters, as well as faecal consistency,[27] were measured in all trials. The health and mortality were monitored throughout the study period and the body weight and feed intake were recorded; weight gain and total feed intake per animal and feed to gain ratio (mortality adjusted) were calculated. In trials 2, 4 and 5, haematological/biochemical parameters in blood were measured. In trial 2, faeces were analysed by quantitative polymerase chain reaction (PCR) to characterise the microbial profile including Enterobacteria, *Escherichia* group, *Clostridium* clusters I, IV and XIVa, *Bacteroides-Prevotella-Porphyromonas* cluster and lactobacilli and *Bifidobacteria*. In trial 4, oxidative stress parameters (eosinophils, haptoglobin, superoxide dismutase) and gene expression (toll-like receptors 2 and 4, interleukins 1 beta, 8 and 10, interferon gamma, mucin 20, tumour-growth factor beta, amphiregulin) in the mid-jejunum and ascending colon of weaned piglets were measured at the end of the study.

An ANOVA was done with the data using the pen as the experimental unit, mean groups were compared with Tukey test.

Table 1: Summary of the design of the efficacy studies performed in weaned piglets

Study	Total no of animals Replicates per treatment (animals per replicate)	Composition basal diet (feed form)	Intended iron supplementation (mg/kg feed)	Analysed iron values (mg/kg feed)	End points	
					Zootechnical parameters faeces consistency	Gut microflora
1[35]	220 11 (5)	Maize, soybean meal (mash)	0 500 Fe lactate 500 CI-FER™ 500 Fe tartrate	51.9/75.4 148.9/173.5 135.6/160.4 152.8/178.1	✓	
2[36]	175 7 (5)	Soybean meal, maize, Wheat, barley (mash)	0 0 ($FeSO_4$*) 0 (FeO*) 500 CI-FER™ 500 CI-FER™ + $FeSO_4$*	89.6/75.4 181.7/171.5 188.8/177.6 179.6/177.6 266.6/256.7	✓	✓
3[37]	400 10 (10)	Maize, soybean meal (mash)	0 (FeSO4*) 250 CI-FER™ + $FeSO_4$* 500 CI-FER™ + $FeSO_4$* 750 CI-FER™ + FeSO4*	125.5/143.4 182.8/197.6 244.3/247.1 281.6/305.7	✓	
4[38]	112 14 (4)	Corn, soybean meal (mash)	0 500 CI-FER™	76.8/84.2 169.2/181.6	✓	
5[23]	144 8 (6)	Basal diet (mash feed)	0 500 CI-FER™ 5,000 CI-FER™	69.2/76.2 162.4/168.7 1,012/1,023	✓	

*: Fe added as per commercial practice 85 mg Fe/kg feed.

The main results of the performance of the weaned piglets and faecal scoring are reported in Table 2.

[35] Technical dossier/Section IV/Annex_IV_3_1.
[36] Technical dossier/Section IV/Annex IV_3_2.
[37] Technical dossier/Section IV/Annex_IV_3_3.
[38] Technical dossier/Supplementary information July 2019/Annex_IV_3_4.

Table 2: Effects of ferric citrate chelate on the zootechnical performance and faecal consistency in weaned piglets

Study	Intended CI-FER™ supplementation (mg/kg feed)	Other sources of Fe added to basal diet	Fe (mg/kg) starter/grower	Daily feed intake (g)	Initial body weight (kg)	Final body weight (kg)	Daily weight gain (g)	Feed to gain ratio	Overall mortality % (n)*	Faecal score
1	0	0	51.9/75.4	732	7.70	27.82^{a}	479^{a}	1.530^{c}	1.82 (1)	3.63^{a}
	0	Lactate 500	148.9/173.5	728	7.70	28.68^{ab}	500^{b}	1.458^{b}	1.82 (1)	3.90^{b}
	500	0	135.6/160.4	719	7.69	29.23^{b}	513^{b}	1.402^{a}	(0)	3.91^{b}
	0	Tartrate 500	152.8/178.1	724	7.69	29.27^{b}	514^{b}	1.411^{a}	1.82 (1)	3.91^{b}
2	0	0	89.6/75.4	580	6.16	22.73^{a}	394^{a}	1.471^{c}	2.86 (1)	3.75^{a}
	0	$FeSO_4$(**)	181.7/171.5	583	6.16	23.31^{ab}	408^{a}	1.428^{bc}	2.86 (1)	3.76^{a}
	0	Fe_3O_2(**)	188.8/177.6	583	6.13	23.45^{b}	412^{ab}	1.415^{abc}	0	3.84^{ab}
	500	0	179.6/177.6	590	6.17	24.26^{c}	431^{c}	1.369^{ab}	0	3.89^{b}
	500	$FeSO_4$(**)	266.6/256.7	587	6.16	24.22^{c}	430^{c}	1.365^{a}	2.86 (1)	3.81^{ab}
3	0	$FeSO_4$(**)	125.5/143.4	779	8.60	28.74	479	1.625^{b}	0	3.78
	250	$FeSO_4$(**)	182.8/197.6	783	8.60	29.67	502	1.559^{a}	0	3.80
	500	$FeSO_4$(**)	244.3/247.1	780	8.59	29.89	507	1.539^{a}	0	3.84
	750	$FeSO_4$(**)	281.6/305.7	772	8.59	29.73	503	1.534^{a}	0	3.85
4	0	0	76.8/84.2	729	7.35	27.2	473	1.542^{b}	0	3.73^{a}
	500	0	169.2/181.6	716	7.35	27.6	482	1.485^{a}	0	3.86^{b}
5	0	0	69.2/76.2	630	7.35	24.50	408	1.542^{a}	0	3.73
	500	0	162.4/168.7	630	7.35	25.21	425	1.481^{b}	0	3.76
	5,000	0	1,012/1,023	630	7.36	25.47	431	1.460^{b}	0	3.81

(*): Including culling.
(**): Fe added as per commercial practice 85 mg Fe/kg feed.

The piglets that received the additive at the recommended dose (500 mg CI-FER™/kg) showed a significantly higher body weight and body weight gain in trials 1 and 2 compared to the control group. Feed to gain ratio was significantly improved at the minimum recommended level in all trials compared to the control group. In trial 2, the improvement in the performance was also seen when comparing the results of the additive to other sources of inorganic iron.

In three of the trials, the faecal consistency was higher in the weaned piglets that received the additive compared to the control.

The measurements done in trial 2 that regarded the microbiota showed no significant differences between the different groups (data not shown in this opinion). In study 4, several parameters indicated a reduction of oxidative stress in treated animals compared to controls (decreased % of eosinophils 1.29 ± 0.76 vs. 1.93 ± 1.21%, decreased haptoglobin 0.49 ± 0.18 vs 0.63 ± 27 mg/mL, increased activities of superoxide dismutase 2.63 ± 0.34 vs. 2.28 ± 0.39 U/mL) and a reduced mid-jejunum gene expression levels of interleukin 1 beta (22.42 ± 0.14 vs. 22.89 ± 0.31).

The FEEDAP Panel noted that the iron content of the basal diets of four out of the five studies (trials 1, 2, 4 and 5) was below the requirement of piglets[24] specified by the National Research Council (NRC, 2012). According to the applicant, with the exception of study 3, basal diets were not supplemented with iron to avoid as far as possible interference with feed iron analysis and to isolate any toxic effects of CI-FER™. However, the effects seen on the performance of the piglets fed with the additive could have been due to the nutritional effect of iron present in the additive, which supplemented to the control diet would have improved the performance of the animals. Data on haematological and biochemical parameters provided for trials 2, 4 and 5 were within the physiological range for weaned piglets. The analysis in trials 2 and 4 included the content of iron in blood serum, which was not different between treatments at the end of the study (control vs supplemented group: 1.09 vs. 1.16 mg/L in trial 2, and 1.080 mg/mL vs. 1.119 in trial 4). These results on the blood content of iron would indicate no major deficiencies in the iron status of the animals in the control group and would allow to conclude that effects of the additive may not be ascribed to a nutritional effect only. This would also be supported by the results obtained in trial 2 in which the CI-FER™ group showed a better performance compared to other sources of iron when supplemented at the same level of iron per kg feed.

3.3.1.1. Conclusions on efficacy for the target species

The FEEDAP Panel concludes that CI-FER™ used at the minimum recommended level of 500 mg/kg feed has the potential to improve zootechnical parameters of weaned piglets. This conclusion can be extended to suckling piglets for the period in which solid feed is given and extrapolated to all minor porcine species at the same growing stage (suckling and weaned).

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[39] and Good Manufacturing Practice.

4. Conclusions

The additive is safe for weaned piglets at the proposed conditions of use of 500 mg CI-FER™/kg complete feed. This conclusion can be extended to suckling piglets for the period in which solid feed is given and extrapolated to all minor porcine species at the same growing stage (suckling and weaned).

No concerns for consumer safety are expected from the use of the additive in piglets and minor porcine species.

The FEEDAP Panel considers that the compound under assessment poses a risk to users by inhalation. The product should also be considered as an irritant to skin, eyes and mucous membranes. Due to the presence of nickel, ferric citrate chelate should also be considered as a dermal and respiratory sensitiser.

The supplementation of feed with the additive is not expected to pose an environmental risk.

The FEEDAP Panel concludes that CI-FER™ used at the minimum recommended level of 500 mg/kg feed has the potential to improve zootechnical parameters of weaned piglets. This conclusion can be

[39] 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.

extended to suckling piglets for the period in which solid feed is given and extrapolated to all minor porcine species at the same growing stage (suckling and weaned).

Documentation provided to EFSA/Chronology

Date	Event
31/08/2018	Dossier received by EFSA. Ferric citrate chelate as a zootechnical feed additive for suckling and weaned piglets. Submitted by AKESO BIOMEDICAL, INC.
02/10/2018	Reception mandate from the European Commission
15/11/2018	Application validated by EFSA – Start of the scientific assessment
01/02/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, safety for target species and efficacy*
18/02/2019	Comments received from Member States
21/03/2019	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"
28/09/2019	Request of the applicant to change the brand name of the additive
02/07/2019	Reception of supplementary information from the applicant - Scientific assessment remained suspended
06/08/2019	Spontaneous submission of information by the applicant. *Issues: user safety*
16/08/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives - Scientific assessment restarted
04/09/2019	Spontaneous submission of information by the applicant. *Issues: user safety*
12/11/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ANOVA	analysis of variance
BW	body weight
CAS	Chemical Abstracts Service
CFU	colony forming unit
DM	dry matter
EC	European Commission
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
ICP-AES	inductively coupled plasma-atomic emission spectrometry

ICP-MS	inductively coupled plasma mass spectrometry
IR	infrared
IUPAC	International Union of Pure and Applied Chemistry
LC–MS	liquid chromatography–mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
MTL	maximum tolerable level
NRC	National Research Council
OEL	occupational exposure limit
PCB	polychlorinated biphenyls
PCDD/F	polychlorinated dibenzo-*p*-dioxins and dibenzofurans
PCR	polymerase chain reaction
RSDip	relative standard deviation for *intermediate precision*
RSDr	relative standard deviation for a *repeatability*
TEQ	toxic equivalent
TLV	threshold limit value
TWA	time-weighted average
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 ferric citrate chelate

In the current application authorisation is sought under Article 4(1) for *ferric citrate chelate* under the category/functional group (4 b, d) "zootechnical additives"/"gut flora stabilisers", "other zootechnical additives", 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 suckling/weaned piglets and minor porcine species.

The *feed additive (ferric citrate chelate)* is an orange-brown powder containing of 16.5 to 20% (w/w) of *iron (III)* and a maximum of 3% (w/w) of *iron (II)*. In addition and on request of the EURL, the Applicant provided the content of total *iron* ranging from 16.5 to 23% (w/w) and the content of *citrate* ranging from 50.2% to 58.6% (w/w) in the *feed additive*. Additionally, the Applicant has set a criterion for *citrate* content in the *feed additive*, expressed as the ratio of the percentage of *citrate* and of total *iron*, ranging from 2.3 to 3.2. According to the Applicant the active substance of the *feed additive* is *ferric citrate*. The *feed additive* is intended to be incorporated into *premixtures* and *feedingstuffs* with a proposed minimum *ferric citrate chelate* content of 500 mg/kg *feedingstuffs*.

For the determination of the *ferric citrate* content added to *premixtures* and *feedingstuffs* the Applicant proposed an indirect single-laboratory validated and verified method, based on the enumeration of colour coated graphite particles (a non-nutrient marker) which are included in the *feed additive*. For the quantification of the *ferric citrate* content added to *premixtures*, the Applicant suggested applying this enumeration method after diluting the premixture samples with blank feed. The following performance characteristics were reported in the frame of the homogeneity study: a relative standard deviation for *repeatability* of 12.5% and an average *recovery* of 86%, which were considered acceptable by the EURL.

Based on the available performance characteristics, the EURL recommends for official control this indirect method (using a non-nutrient marker as proposed by the Applicant) for the quantification of the added content of *ferric citrate* in *premixtures* and *feedingstuffs* provided that the following criteria are fulfilled: (1) the marker is well characterised; (2) the marker is added into the *feed additive* before the mixing of the product with the compound feed; and (3) the inclusion content of the marker, expressed as number of graphite particles per mass of the *feed additive*, is specified and kept constant (e.g. 72 particles/mg micro-tracered *feed additive* in the case of 10% addition rate of the marker into the *feed additive*). Moreover, it is recommended that these conditions are included in the Regulation authorising the *feed additive*. In addition, the official control for quantification of the added content of *ferric citrate* in *premixtures* and *feedingstuffs* is not possible when the specific marker is used also for another feed additive(s), in case both (all) are added to the same feed.

For the identification/characterisation of the *feed additive*, the Applicant proposed to quantify total *iron* and *citrate* in the *feed additive*. For the quantification of total *iron* the Applicant submitted three internationally recognised ring-trial validated CEN methods: the EN 15510 method based on inductively coupled plasma-atomic emission spectrometry (ICP-AES) after ashing or wet digestion with hydrochloric acid, the EN 15621 method based on ICP-AES after pressure digestion and the EN ISO 6869 method based on atomic absorption spectrometry. These methods were previously evaluated and recommended by the EURL in the frame of the *iron* group dossier for the quantification of total *iron* in different *feed additives*.

Based on the acceptable performance characteristics available, the EURL recommends for official control of the content of total *iron* in the *feed additive* the three ring-trial validated methods described in EN 15510, EN 15621 and ISO 6869.

For the quantification of *citrate* in the *feed additive* the Applicant submitted a single-laboratory validated and further verified method based on ion-exchange high performance liquid chromatography (HPLC) coupled to ultraviolet (UV) detection to quantify *citrate* in the *feed additive*. The following performance characteristics were reported in the frame of validation and verification studies: a relative standard deviation for a *repeatability* (RSDr) ranging from 0.3 to 0.4%; a relative standard deviation for *intermediate precision* (RSDip) ranging from 0.3 to 1.6%; and a recovery rate of 99%.

Based on the available performance characteristics the EURL recommends for official control of the content of *citrate* in the *feed additive* the single-laboratory validated and further verified method based on ion-exchange high performance liquid chromatography (HPLC) coupled to ultraviolet (UV) detection.

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.

Appendix A – Calculation of inhalation exposure to iron and nickel of users handling ferric citrate chelate in a premixture factory

Assumptions

There are different operations in a premixture factory during which the worker could be exposed to dust:

- Taking the additive from its bag for weighing in the dispensary
- Emptying bags of previously weighed material in the hopper or mixers
- Packing the final premixture.

Default values/positions

- A factory with a large throughput can prepare 40 premixture batches per day (8 h per shift)
- The maximum time for weighing/emptying is 20 s
- Total breathed air per worker of 10 m^3 per 8 h = 1.25 m^3 per hour
- All dust comes from the additive
- All air available for inspiration contains the additive's dust

Factors related to the feed additive:

- Percentage of premixtures that contain the additive: 20%
- Dusting potential measured: 9.0 g/m^3
- Concentration of the active substance in the dust (assuming the same % as in the additive: iron 23%, nickel 0.005%): iron 2.07 g/m^3, nickel 0.45 mg/m^3

Estimate of risk mitigation

- The use of personal protection equipment (coverall, goggles, gloves and mask of the type P3, that reduces the inhalation exposure by a factor of 20)

Calculation of exposure by inhalation during a working day

Batches with potential exposure	40 (batches) × 0.2 (fraction of batches containing additive) = 8 (batches)
Time of exposure	8 × 20 s = 160 s An uncertainty factor of 2 should be introduced
Inhaled air during exposure (Ia), m^3	1.25 m^3 per hour × 2 × 160/60/60 in hours = 0.11
Active substance in air (Asa), g/m^3	Iron: 9.02 (dust in g/m^3) × 0.23 (23% active substance in dust) = 2.07 Nickel: 9.02 (dust in g/m^3) × 0.00005 (0.005% active substance in dust) = 0.00045
Active substance inhaled (Asi), mg/day	Iron: 2.07 (Asa) × 0.11 (Ia) × 1,000 = 230 Nickel: 0.00045 (Asa) × 0.125 (Ia) × 1,000 = 0.050
Reduced by filter mask (Asir), mg/day	Iron: 259 (Asi) × 0.01 (by mask type P3) = 11.5 Nickel: 0.056 (Asi) × 0.05 (by mask type P3) = 0.0025 g/day