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, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the efficacy of iron chelates of lysine and glutamic acid (Iron-LG) as nutritional feed additive for all animal species. The European Commission request followed an opinion of the FEEDAP Panel published in 2019; in that opinion the Panel could not conclude on the efficacy of the additive. The applicant submitted additional information to allow the FEEDAP Panel to complete its assessment; these additional data, related to the efficacy of the additive, are the subject of this opinion. Three studies were provided, one each with weaned piglets, pigs for fattening and laying hens. In the study in laying hens, improvements in iron content in egg yolk, iron in serum and in other iron-related blood endpoints were observed. Based on the results of this study, the FEEDAP Panel concluded that the additive is a source of bioavailable iron, comparable to the standard inorganic iron source, and therefore, the additive is efficacious in meeting the animals’ requirements; the results of the study in pigs for fattening supported the bioavailability of Iron-LG. The conclusion drawn by the Panel could be extrapolated to all animal species and categories.

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

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

Regulation (EC) No 1831/2003 establishes rules governing the Community authorisation of additives for animal nutrition and, in particular, Article 9 defines the terms of the authorisation by the Commission.

The applicant, Zinpro Animal Nutrition (Europe) Inc, is seeking a Community authorisation of Iron chelates of lysine and glutamic acid as a feed additive to be used as compound of trace elements for all animal species (Table 1).

Table 1: Description of the substances

| Category of additive | Nutritional additive |
|----------------------|----------------------|
| Functional group of additive | Compounds of trace elements |
| Description | Iron chelates of lysine and glutamic acid |
| Target animal category | All Animal species |
| Applicant | Zinpro Animal Nutrition (Europe), Inc |
| Type of request | New opinion |

On 4 July 2019, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ("Authority"), in its opinion on the safety and efficacy of the product, could not 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 applicant submitted complementary information in order to complete the assessment and to allow a revision of Authority’s opinion. The new data have been received on 23 September 2019.

In view of the above, the Commission asks the Authority to deliver a new opinion on Iron chelates of lysine and glutamic acid as a feed additive for all animal species based on the additional data submitted by the applicant.

1.2. Additional information

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) adopted in 2019 an opinion on the safety and efficacy of the preparation of Iron chelates of lysine and glutamic acid as nutritional feed additive for all animal species (EFSA FEEDAP Panel, 2019). In that opinion, the Panel could not conclude on the efficacy of the additive owing to the limitations of the study submitted.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of additional information1 to a previous application of the same product.2

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the efficacy of Iron chelates of lysine and glutamic acid is in line with the principles laid down in Regulation (EC) No 429/20083 and the relevant guidance documents: Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

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1 FEED dossier reference: FAD-2019-0063.
2 FEED dossier reference: FAD-2018-0010.
3 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 consists on divalent iron in the form of chelates of lysine and glutamic acid in a mixture 1:1. It is proposed to be used as a nutritional additive (functional group: compounds of trace elements) in all animal species. The additive is intended to be used in feed for all animal species/categories up to the total maximum iron content allowed in complete feed in the European Union (EU): 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.

In a previous opinion of the FEEDAP Panel on the same additive (EFSA FEEDAP Panel, 2019), the Panel could not conclude on the efficacy of the additive owing to the limitations identified in the study submitted, based on the fact that the design of the experiment was not the appropriate to detect the efficacy of the iron supplemented experimental groups.

The applicant has submitted additional information related to the efficacy of the additive and this new information is the subject of this opinion. As abbreviation, the short name of Iron-LG will be used throughout this opinion to refer to the additive under assessment.

3.1. **Efficacy**

For demonstration of the efficacy of nutritional additives, one study in a single animal species or category, including laboratory animals, is generally considered sufficient (EFSA FEEDAP Panel, 2018).

The applicant provided three new studies to support efficacy of the additive performed in weaned piglets, pigs for fattening and laying hens. The three studies were performed outside the EU.

3.1.1. **Efficacy study in weaned piglets**

A total of 288 piglets [Duroc × (Large White × Landrace)] (half barrows and half gilts, 27 days of age, 7.7 kg average initial body weight (bw)) were used in the study. The design of the experiment followed a randomised block design with six treatments, eight pens per treatment and six piglets per pen (three barrows and three gilts each). The description of the treatments is presented in Table 2. The basal diet used was based on corn and soybean and was either not supplemented with iron (negative control) or supplemented with Iron-LG at four different levels or with ferrous sulfate at one level (positive control).

The experiment involved two phases: phase I (from day 0 to 14 of the experiment) and phase II (from day 15 to 42 of the experiment). The intended iron content in each treatment was confirmed by analysis (Table 2). The pigs had ad libitum access to feed and water. The study lasted 42 days.

Feed was medicated with chlortetracycline (75 mg/kg diet) and kanamycin (20 mg/kg diet), as prophylactic treatment, which does not reflect EU farming practices.

**Table 2:** Experimental design of the study with weaned piglets

| Treatment     | Source       | Added iron (mg/kg diet) | Total iron (mg/kg diet) (intended) | Total iron (mg/kg diet) (analysed) |
|---------------|--------------|-------------------------|------------------------------------|------------------------------------|
| Negative control | None         | 0                       | 80                                 | 86                                 |
| T1            | Iron-LG      | 30                      | 110                                | 126                                |
| T2            |              | 60                      | 140                                | 154                                |
| T3            |              | 90                      | 170                                | 181                                |
| T4            |              | 120                     | 200                                | 202                                |
| Positive control | Ferrous sulfate | 90                      | 170                                | 187                                |

(1): Phase I: from 0 to 14 days; Phase II: from 15 to 42 days.
Mortality and general health were monitored throughout the study. Body weight was individually measured at day 1, 14 and 42; feed consumption was recorded; average daily gain (ADG), average daily feed intake (ADFI) and feed to gain ratio (F/G) for each phase were calculated. Blood haematology⁷ and biochemistry⁸ parameters were analysed from samples obtained from each pig at the beginning and end of the experiment.

Data on mortality were statistically analysed with a Chi-square test. The other data were analysed with an analysis of variance (ANOVA), considering the treatment and the blocks. Comparison of group means was conducted using Duncan test. The pen was considered as the experimental unit. Significance was declared at \( p \leq 0.05 \).

Mortality and culling summed up to four pigs, thus representing 1.4%, and was not related to treatment. No significant effects were identified for performance parameters among treatments. Relevant results are summarised in Table 3.

Concerning blood biochemical indices some parameters showed significant differences at the end of the trial (day 42); summary given in Table 4. There were significant differences in haematocrit (HCT) and haemoglobin (HGB) between the treatments: the groups supplemented with 90 and 120 mg Fe from Iron-LG/kg and the positive control group increased HCT and HGB compared to the negative control group. There were also significant differences in mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) between the treatments: the MCH of the groups supplemented with 120 mg Fe from Iron-LG/kg and the positive control group was significantly higher than that of the negative control group; the MCV of the positive control group was higher than that of the negative control group.

Table 3: Performance parameters of weaned piglets in the study with Iron-LG at the end of the trial

| Treatment       | Source          | Iron (mg/kg diet) | Feed intake (kg/day) | Final weight (kg) | Average daily gain (kg/day) | Feed to gain | Mortality and culling rate (n) |
|-----------------|-----------------|-------------------|----------------------|-------------------|-----------------------------|--------------|-------------------------------|
| Negative control| None            | 0                  | 0.61                 | 21.19             | 0.33                        | 1.88         | 1                             |
| T1              | Iron-LG         | 30 110            | 0.63                 | 22.02             | 0.34                        | 1.85         | 0                             |
| T2              | Iron-LG         | 60 140            | 0.61                 | 22.26             | 0.34                        | 1.81         | 1                             |
| T3              | Iron-LG         | 90 170            | 0.62                 | 21.72             | 0.33                        | 1.82         | 1                             |
| T4              | Iron-LG         | 120 200           | 0.63                 | 21.68             | 0.33                        | 1.91         | 0                             |
| Positive control| Ferrous sulfate | 90 170            | 0.64                 | 21.77             | 0.33                        | 1.92         | 1                             |

Table 4: Blood biochemical parameters of weaned piglets in study with Iron-LG at the end of the trial

| Treatment       | Source          | Iron (mg/kg diet) | Parameter¹ | HCT (%) | HGB (g/L) | MCH (pg) | MCV (fl) | TIBC (μmol/L) |
|-----------------|-----------------|-------------------|------------|---------|-----------|----------|----------|--------------|
| Negative control| None            | 0 80              | HCT       | 33.92   | 86.42     | 14.03    | 55.05    | 51.78        |
| T1              | Iron-LG         | 30 110            | HCT       | 33.83   | 87.56     | 14.18    | 54.83    | 51.77        |
| T2              | Iron-LG         | 60 140            | HCT       | 34.88   | 90.53     | 14.74    | 55.32    | 51.79        |
| T3              | Iron-LG         | 90 170            | HCT       | 37.01   | 96.78     | 14.82    | 56.66    | 51.40        |
| T4              | Iron-LG         | 120 200           | HCT       | 36.75   | 96.73     | 15.29    | 58.17    | 51.51        |
| Positive control| Ferrous sulfate | 90 170            | HCT       | 37.27   | 96.45     | 15.14    | 58.57    | 51.90        |

¹: HCT: haematocrit, HGB: haemoglobin, MCH: mean corpuscular haemoglobin, MCV: mean corpuscular volume, TIBC: total iron binding capacity.

The results of this study indicated significant changes in haemoglobin-related parameters of piglets, in the groups supplemented at levels of 90 mg Fe/kg diet and higher. However, owing to (i) the lack of

⁷ White blood cell count (WBC), red blood cell count (RBC), red cell distribution width, haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), haemoglobin (HGB) and platelet count (PLT).

⁸ Total iron binding capacity.
more determinant parameters of iron status, as iron content in liver, and (ii) the uncertainties derived from the use of antibiotics in the feed, which do not follow in its entirety the European conditions, this study was not considered to support the efficacy of Iron-LG.

3.1.2. Efficacy study in pigs for fattening

A total of 216 pigs [Duroc × (Large White × Landrace)]\(^5\) (half barrows and half gilts, 71 days of age, 24.1 kg average initial bw) were used in the study.\(^5,9\) The design of the experiment consisted on a randomised block design with six treatments, six pens per treatment and six pigs per pen (three males and three females each). The description of the treatments is presented in Table 5. The basal diet used was based on corn and soybean and was either not supplemented with iron (negative control) or supplemented at four different levels with Iron-LG or at one level with ferrous sulfate (positive control). The experiment involved three phases: phase I (from 25 to 50 kg bw), phase II (from 50 to 80 kg bw) and phase III (from 80 to 120 kg bw). The intended iron content in each treatment was confirmed by analysis (Table 5). The pigs had ad libitum access to feed and water. The study lasted 110 days.

Feed was medicated with chlortetracycline (75 mg/kg and 60 mg/kg diet, in phases I and II, respectively) and kanamycin (30 mg/kg and 25 mg/kg diet, in phases I and II, respectively), as prophylactic treatment, which does not reflect common EU farming practices. However, the Panel notes that the antibiotic treatment was not applied during the last phase of the experiment (80–120 kg).

Mortality and general health were monitored throughout the study. Body weight was individually measured at the beginning of the trial, and at the end of each phase. Feed consumption was recorded. The ADG, ADFI and F/G for each phase were calculated. Blood samples were collected for haematology\(^10\) and biochemistry\(^11\) analyses; at the beginning of the experiment, two pigs of each sex were selected from each pen and blood was collected from these pigs at the start of the trial, at the start of phases II and III and at the end of the experiment. At the end of the experiment, two pigs (one per sex) per pen with the body weight nearest to the average weight of the pen were slaughtered to collect liver samples for determination of iron, copper, zinc, manganese and total superoxide dismutase (T-SOD).

Data were subjected to an ANOVA with the treatment as the effect. The group means were compared with Duncan test. The pen was considered as the experimental unit. Significance was declared at \(p \leq 0.05\).\(^12\)

During the trial, a total of three pigs were treated for foot pain using nonsteroidal anti-inflammatory drug.\(^5\)

Mortality and culling rate of the experiment was 1.39% (total of three pigs: 1 pig was culled and two died). The applicant stated that the reason for the losses was the foot pain. No significant effects of treatments were identified on performance parameters; results are shown in Table 6. Concerning

### Table 5: Experimental design of the study with pigs for fattening

| Treatment       | Source      | Added iron (mg/kg diet) | Total iron (mg/kg diet) (intended) | Total iron (mg/kg diet) (analysed) |
|-----------------|-------------|-------------------------|-----------------------------------|-----------------------------------|
|                 |             | Phase I                 | Phase II                          | Phase III                         |
| Negative control| None        | 0                       | 100                               | 97.3                              | 80.7                              | 92.2                              |
| T1              | Iron-LG     | 15                      | 115                               | 114.5                             | 104.4                             | 106.1                             |
| T2              | Iron-LG     | 30                      | 130                               | 134.6                             | 116.3                             | 123.6                             |
| T3              | Iron-LG     | 45                      | 145                               | 149.7                             | 129.9                             | 136.3                             |
| T4              | Iron-LG     | 60                      | 160                               | 159.0                             | 138.0                             | 161.1                             |
| Positive control| Ferrous sulfate | 60                   | 160                               | 160.4                             | 143.1                             | 157.7                             |

(1): Phase I: from 25 to 50 kg bw; Phase II: from 50 to 80 kg bw; Phase III: from 80 to 120 kg bw.

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9 Technical Dossier/Zinpro - FeLG150_GF pigs_China Efficacy report.pdf.
10 White blood cell count (WBC), red blood cell count (RBC), platelet count (PLT), haemoglobin (HGB), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), haematocrit (HCT), mean corpuscular volume (MCV) and red cell distribution width (RDW).
11 Serum iron content, total iron-binding capacity (TIBC), T-super oxide dismutase (SOD), CuZn-SOD, Mn-SOD.
12 Technical Dossier/Supplementary Information/May 2020.
blood parameters, there was a treatment effect on the MCH and MCV in the three phases of the study: pigs supplemented from 30 mg Fe/kg feed showed values higher than the negative control and no differences with the ferrous sulfate. There were no differences among the treatments in serum iron content. From the evaluated microelements in liver, only iron content showed significant differences between treatments: the iron content in the liver of all supplemented Iron-LG groups was higher than that of the negative control; the iron content in the liver of the group supplemented with 60 mg Fe from Iron-LG/kg was higher than that of ferrous sulfate, while the iron content in the liver of the groups supplemented with 15–45 mg Fe from Iron-LG/kg was not different from that of ferrous sulfate group (results shown in Table 6).

Table 6: Performance parameters and iron in liver from pigs in the study with Iron-LG at the end of the trial

| Treatment          | Source       | Iron (mg/kg diet) | ADFI (kg/day) | Final bw (kg) | ADG (kg/day) | F/G | Iron in Liver (mg/kg fresh matter) |
|--------------------|--------------|-------------------|---------------|---------------|--------------|-----|-----------------------------------|
|                    |              | Added Intended    |               |               |              |     |                                   |
| Negative control   | None         | 0 100             | 2.43          | 118.22        | 0.884        | 2.75| 46.72a                            |
| T1                 | Iron-LG      | 15 115            | 2.45          | 119.60        | 0.896        | 2.75| 95.24b                            |
| T2                 | Iron-LG      | 30 130            | 2.47          | 120.89        | 0.909        | 2.73| 95.39b                            |
| T3                 | Iron-LG      | 45 145            | 2.43          | 121.46        | 0.911        | 2.67| 108.4b                            |
| T4                 | Iron-LG      | 60 160            | 2.59          | 125.13        | 0.948        | 2.73| 157.7c                            |
| Positive control   | Ferrous sulfate | 60 160          | 2.58          | 122.24        | 0.921        | 2.80| 122.1b                            |

a,b,c: For a given parameter, different superscript within a column indicates significant differences (p ≤ 0.05).

The results of this study showed a higher deposition of iron in the liver of pigs supplemented with iron, regardless the source, compared to the pigs in the non-supplemented diet. However, owing to the limitation identified in a specific part of the study due to the use of antibiotics, the Panel considers that the result described above can be only supportive of the efficacy of Iron-LG.

3.1.3. Efficacy study in laying hens

A total of 1260 Beijing White layers were used in the study.5,14 Initial age was 126 days and initial average bw 1.26 kg. The animals were submitted to an acclimation period of 2 weeks prior to the beginning of the study, in which they were fed the basal diet consisting on corn-soybean meal. The design of the experiment consisted on random block design with seven groups, 12 replicates per group and 15 animals per replicate (five cages of three hens). The description of the treatments is presented in Table 7 and was obtained from the basal diet (iron content 75 mg/kg) that was either not supplemented (negative control) or supplemented with Iron-LG at five levels or supplemented with ferrous sulfate at one level (positive control). The intended iron content in each treatment was confirmed by analysis (Table 7). The experimental period was 24 weeks.

Table 7: Experimental design of the study with laying hens

| Treatment            | Source          | Added iron (mg/kg diet) | Total iron (mg/kg diet) (Intended) | Total iron (mg/kg diet) (analysed) |
|----------------------|-----------------|-------------------------|-----------------------------------|-----------------------------------|
| Negative control     | None            | 0                       | 69.5                              | 75.6                              |
| T1                   | Iron-LG         | 15                      | 95                                 | 92.13                             |
| T2                   | Iron-LG         | 30                      | 110                                | 104.1                             |
| T3                   | Iron-LG         | 44                      | 124                                | 122.2                             |
| T4                   | Iron-LG         | 60                      | 140                                | 138.3                             |
| T5                   | Iron-LG         | 75                      | 155                                | 149.3                             |
| Positive control     | Ferrous sulfate | 45                      | 125                                | 123.3                             |

13 Technical Dossier/Supplementary Information/January 2020/Table 5Cu.
14 Technical Dossier/Zinpro - FeLG150_Layers_China Efficacy report.pdf.
Mortality and general health were monitored throughout the study. Laying performance parameters were measured including laying rate (%), feed to egg mass ratio (F/E), average daily egg weight (ADEW) and ADFI. At the end of each 4-week period, six eggs per replicate were collected to measure albumin height, Haugh units, yolk colour, eggshell thickness and percentage of egg white, egg yolk and eggshell as well as iron content in yolk. Blood samples from one hen – randomly selected from each replicate – were obtained at the beginning of the experiment (day 1) and at days 84 and 168; routine haematology and specific biochemical parameters were measured.

Data were submitted to ANOVA. The pen was considered as the experimental unit. When the difference was significant, the group means were compared by Tukey test. Significance was declared at p ≤ 0.05.

No layers died or were culled. When considering the performance parameters (Table 8), the percentage of egg production of the groups supplemented with 30–75 mg Fe from Iron-LG/kg and the ADEW of the group supplemented 60 mg Fe from Iron-LG/kg significantly increased, and the F/E ratio of the groups supplemented with 45–60 mg Fe from Iron-LG/kg significantly decreased compared to the negative control. For these parameters, no differences were observed between the negative and positive control. Egg quality parameters were not affected by treatments. The FEEDAP Panel notes that the final body weight of the hens was not measured or reported.

Table 8: Performance parameters at the end of the trial with laying hens

| Treatment        | Source    | Iron (mg/kg diet) | Parameter (1) | Egg production (%) | F/E | ADEW (g/day) | ADFI (g/day) |
|------------------|-----------|-------------------|---------------|-------------------|-----|--------------|--------------|
|                  |           | Added            | Intended      |                   |     |              |              |
| Negative control | None      | 0                | 69.5          | 88.35(1)          | 2.27a | 47.24b        | 106.75        |
| T1               | Iron-LG   | 15               | 95            | 91.14(1)          | 2.25ab| 48.21ab       | 108.24        |
| T2               | Iron-LG   | 30               | 110           | 93.18(1)          | 2.22ab| 49.11ab       | 108.81        |
| T3               | Iron-LG   | 45               | 124           | 93.39(1)          | 2.14b | 49.70ab       | 105.75        |
| T4               | Iron-LG   | 60               | 140           | 94.05(1)          | 2.13b | 50.06a        | 107.07        |
| T5               | Iron-LG   | 75               | 155           | 93.21(1)          | 2.16ab| 49.13ab       | 106.47        |
| Positive control | Ferrous sulfate | 45               | 125           | 90.58(1)          | 2.21ab| 48.15ab       | 106.75        |

(1): F/E: Feed/egg ratio, ADEW: average daily egg weight, ADFI: average daily feed intake. a,b,c: Different superscript within a column indicates significant differences (p ≤ 0.05).

At the end of the trial, the iron content in yolk of the groups supplemented with 45–75 mg Fe from Iron-LG/kg was significantly higher than the negative control or the group receiving ferrous sulfate at the same level of iron supplementation (Table 9). The FEEDAP Panel notes that the value of the positive control appears odd as it is even lower than the negative control.

When considering the blood parameters (Table 9), the red blood cells (RBC) and the haemoglobin (HGB) of the groups supplemented with 45–75 mg Fe from Iron-LG/kg were significantly increased compared to the negative control or the group receiving iron sulfate at the same level of iron supplementation. There were no significant differences in white blood cells, haematocrit and platelets related to the treatments. At the end of the trial, the serum iron concentration of the groups supplemented with 30–75 mg Fe from Iron-LG were significantly higher than that in the negative control and the group receiving ferrous sulfate at the same level of iron supplementation.

15 White blood cell count (WBC), red blood cell count (RBC), haemoglobin (HGB), haematocrit (HTC) and platelet (PLT).
16 Iron content in serum, Total iron binding capacity (TIBC), Mn-superoxide dismutase (Mn-SOD), CuZn-superoxide dismutase (CuZn-SOD) and malondialdehyde (MDA).
Finally, when considering the effect of supplementation of Iron-LG on serum special iron indices and antioxidant capacity (Table 10) it was observed that total iron binding capacity (TIBC) of groups supplemented with 30–75 mg Fe from Iron-LG/kg diet and Cu/Zn-superoxide dismutase (Cu/Zn-SOD) of groups supplemented with 15–75 mg Fe from Iron-LG/kg significantly increased and malondialdehyde (MDA) of groups supplemented with 60–75 mg Fe from Iron-LG/kg significantly decreased compared to the negative control. The supplementation of the inorganic source ferrous sulfate showed a significantly lower effect on TBIC and Cu/Zn-SOD at the same level of supplementation and no effect on MDA.

The results of this study showed significant changes in iron content in egg yolk, iron in serum and in other iron-related blood endpoints (RBC, HGB, TIBC, Cu/Zn-SOD, MDA), mainly in the groups supplemented at levels of 30 mg Fe/kg diet or higher. The increase in performance yet considering the uncertainty of the lack of final weight of the hens starting generally at levels of 30 mg Fe/kg diet and higher, can be also considered as supporting evidence of the efficacy.

### Table 9: Iron in yolk, blood haematological parameters and iron in serum in at the end of the trial with laying hens

| Treatment       | Source       | Iron (mg/kg diet) | Parameter(1) | Parameter(1) | Parameter(1) | Parameter(1) |
|-----------------|--------------|------------------|--------------|--------------|--------------|--------------|
|                 | Added        | Intended         | Iron in egg yolk (mg/kg FM) | RBC (1012/L) | HGB (g/L) | Iron in serum (mg/L) |
| Negative control| None         | 0                | 69.5         | 61.02<sup>c</sup> | 2.73<sup>cd</sup> | 208.81<sup>c</sup> | 2.21<sup>e</sup> |
| T1              | Iron-LG      | 15               | 95           | 61.50<sup>c</sup> | 2.88<sup>bcd</sup> | 214.48<sup>c</sup> | 2.41<sup>de</sup> |
| T2              | Iron-LG      | 30               | 110          | 61.93<sup>bc</sup> | 3.00<sup>bc</sup> | 221.51<sup>bc</sup> | 2.60<sup>cd</sup> |
| T3              | Iron-LG      | 45               | 124          | 63.42<sup>a</sup> | 3.16<sup>a</sup> | 233.57<sup>bc</sup> | 2.78<sup>bc</sup> |
| T4              | Iron-LG      | 60               | 140          | 63.31<sup>a</sup> | 3.09<sup>ab</sup> | 241.64<sup>a</sup> | 2.97<sup>ab</sup> |
| T5              | Iron-LG      | 75               | 155          | 63.19<sup>ab</sup> | 3.17<sup>a</sup> | 238.87<sup>a</sup> | 3.11<sup>a</sup> |
| Positive control| Ferrous sulfate | 45             | 125          | 56.39<sup>d</sup> | 2.70<sup>d</sup> | 207.18<sup>c</sup> | 2.49<sup>d</sup> |

(1): RBC: red blood cells, HGB: haemoglobin.

Finally, when considering the effect of supplementation of Iron-LG on serum special iron indices and antioxidant capacity (Table 10) it was observed that total iron binding capacity (TIBC) of groups supplemented with 30–75 mg Fe from Iron-LG/kg diet and Cu-Zn-superoxide dismutase (Cu/Zn-SOD) of groups supplemented with 15–75 mg Fe from Iron-LG/kg significantly increased and malondialdehyde (MDA) of groups supplemented with 60–75 mg Fe from Iron-LG/kg significantly decreased compared to the negative control. The supplementation of the inorganic source ferrous sulfate showed a significantly lower effect on TBIC and Cu/Zn-SOD at the same level of supplementation and no effect on MDA.

### Table 10: Serum special iron indexes and antioxidant capacity at the end of the trial with laying hens

| Treatment       | Source       | Iron (mg/kg diet) | TIBC (mg/L) | Cu/Zn-SOD (U/mL) | MDA (nmol/mL) |
|-----------------|--------------|------------------|-------------|------------------|---------------|
|                 | Added        | Added            |             |                  |               |
| T1              | None         | 0                | 69.5        | 16.26<sup>d</sup> | 75.21<sup>d</sup> | 5.32<sup>a</sup> |
| T2              | Iron-LG      | 15               | 95          | 16.68<sup>d</sup> | 80.85<sup>c</sup> | 5.29<sup>a</sup> |
| T3              | Iron-LG      | 30               | 110         | 17.74<sup>bc</sup> | 82.06<sup>bc</sup> | 5.30<sup>a</sup> |
| T4              | Iron-LG      | 45               | 124         | 18.05<sup>ab</sup> | 83.40<sup>ab</sup> | 5.27<sup>ab</sup> |
| T5              | Iron-LG      | 60               | 140         | 18.28<sup>ab</sup> | 84.06<sup>a</sup> | 5.21<sup>b</sup> |
| T6              | Iron-LG      | 75               | 155         | 18.60<sup>a</sup> | 84.23<sup>a</sup> | 5.14<sup>c</sup> |
| T7              | Ferrous sulfate | 45             | 125         | 17.29<sup>c</sup> | 82.94<sup>ab</sup> | 5.28<sup>a</sup> |

(1): TIBC: Total iron binding capacity; CuZn-SOD: CuZn-superoxide dismutase; MDA: malondialdehyde.

The results of this study showed significant changes in iron content in egg yolk, iron in serum and other iron-related blood endpoints (RBC, HGB, TIBC, Cu/Zn-SOD, MDA), mainly in the groups supplemented at levels of 30 mg Fe/kg diet or higher. The increase in performance — yet considering the uncertainty of the lack of final weight of the hens — starting generally at levels of 30 mg Fe/kg diet and higher, can be also considered as supporting evidence of the efficacy.

### 3.1.4. Conclusions on efficacy

From a study in laying hens, improvements in iron content in egg yolk, iron in serum and in other iron-related blood endpoints were reported. Based on the results of this study, the FEEDAP Panel concludes that the additive is a source of bioavailable iron, comparable to the standard inorganic iron source, and therefore, the additive is efficacious in meeting the animals’ requirements. The study in pigs for fattening, in which a higher deposition of iron in the liver of pigs supplemented with Iron-LG compared to the pigs in the non-supplemented diet was observed, would support the bioavailability of the additive. The conclusion drawn by the Panel can be extrapolated to all animal species and categories.
4. Conclusions

The FEEDAP Panel concludes that iron chelates of lysine and glutamic acid are considered as an efficacious source of bioavailable iron in all animal species.

5. Documentation as provided to EFSA/Chronology

| Date       | Event                                                                 |
|------------|----------------------------------------------------------------------|
| 08/10/2019 | Dossier received by EFSA. Iron chelates of lysine and glutamic acid for all animal species. Submitted by Zinpro Animal Nutrition (Europe), Inc. |
| 08/10/2019 | Reception mandate from the European Commission                        |
| 11/10/2019 | Start of the scientific assessment                                    |
| 19/12/2019 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. Issues: Efficacy |
| 29/01/2020 | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 15/04/2020 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. Issues: Efficacy |
| 05/05/2020 | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 25/05/2020 | Opinion adopted by the FEEDAP Panel. End of the Scientific assessment |

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Abbreviations

ADFI Average daily feed intake
ADG Average daily gain
ANOVA Analysis of variance
bw Body weight
FEEDAP Additives and Products or Substances used in Animal Feed
F/G Feed to gain ratio
HCT Haematocrit
HGB Haemoglobin
MCH Mean corpuscular haemoglobin
MCV Mean corpuscular volume
T-SOD Total superoxide dismutase
TIBC Total iron-binding capacity