

ADOPTED: 27 January 2021

doi: 10.2903/j.efsa.2021.6454

Safety of the feed additive consisting of manganese chelates of lysine and glutamic acid for all animal species (Zinpro Animal Nutrition)

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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 safety for target animals of manganese chelates of lysine and glutamic acid (Manganese-LG) as a nutritional feed additive for all animal species. The European Commission request followed an opinion of the FEEDAP Panel published in 2020; in that opinion, the FEEDAP Panel could conclude on the safety of the additive for chickens for fattening, but not for the rest of the target animals. The applicant submitted additional information to allow the FEEDAP Panel to complete its assessment; these additional data, comprising two tolerance studies (one with chickens for fattening and one with laying hens), were the subject of this opinion. The tolerance study in laying hens was not considered for the assessment since the housing conditions of the animals were not appropriate according to the relevant EU provisions. The results of the tolerance study in chickens for fattening showed that Manganese-LG at the highest level tested – 800 mg Mn/kg feed – is safe for these target animals. The FEEDAP Panel also considered a previous tolerance study in chickens for fattening. Taking all the evidence together the Panel concluded that Manganese-LG is safe for chickens for fattening at 150 mg Mn/kg feed, with a margin of safety of 5.5. This conclusion can be extrapolated to all animal species and categories provided that the maximum authorised levels in the EU for total manganese in feed are not exceeded.

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Keywords: nutritional additives, compounds of trace elements, manganese chelates of lysine and glutamic acid, ProPath Mn, safety

Requestor: European Commission

Question number: EFSA-Q-2020-00310

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Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at <https://ess.efsa.europa.eu/doi/doiweb/doisearch>.

Acknowledgments: The Panel wishes to acknowledge the contribution of Angelica Amaduzzi, Montserrat Anguita, Jaume Galobart, Orsolya Holczknecht and Maria Vittoria Vettori to this opinion.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Durjava MF, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Dierick N, Martelli G and López-Gálvez G, 2021. Scientific Opinion on the safety of the feed additive consisting of manganese chelates of lysine and glutamic acid for all animal species (Zinpro Animal Nutrition). *EFSA Journal* 2021;19(3):6454, 8 pp. <https://doi.org/10.2903/j.efsa.2021.6454>

ISSN: 1831-4732

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The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.



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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 Manganese 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	Manganese chelates of lysine and glutamic acid
Target animal category	All Animal species
Applicant	Zinpro Animal Nutrition (Europe), Inc
Type of request	New opinion

On 10 January 2020, 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 conclude only on the safety of the additive for chickens for fattening up to the maximum authorised levels in the EU for total manganese in feed (150 mg/kg), but not for 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 16 April 2020.

In view of the above, the Commission asks the Authority to deliver a new opinion on Manganese 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 2020 an opinion on the safety and efficacy of the preparation of manganese chelates of lysine and glutamic acid as a nutritional feed additive for all animal species (EFSA FEEDAP Panel, 2020). In that opinion, the Panel could not conclude on the safety of the additive for the target species, other than chickens for fattening, 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 safety for target species of manganese 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 safety of feed additives for the target species (EFSA FEEDAP Panel, 2017).

¹ FEED dossier reference: FAD-2020-0026.

² FEED dossier reference: FAD-2018-0009.

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 of divalent manganese in the form of chelates of lysine and glutamic acid in a mixture 1:1, with a theoretical content of manganese of 14.4% and 16.3%, respectively. 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 manganese content allowed in complete feed in the European Union (EU): fish 100 and other species 150 mg/kg feed.⁴

In a previous opinion (EFSA FEEDAP Panel, 2020), the Panel concluded that the additive, manganese chelates of lysine and glutamic acid (Manganese-LG), was safe for chickens for fattening up to the maximum authorised levels in the EU for manganese in feed (150 mg/kg); however, since a margin of safety could not be derived, the conclusion could not be extrapolated/extended to other categories/species.

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

3.1. Safety for the target species

The applicant provided two new tolerance studies performed in chickens for fattening and laying hens.

The tolerance study in laying hens⁵ was not considered for the assessment since the housing conditions of the animals were not appropriate according to the relevant EU provisions.⁶ In particular, the cages were not enriched and their dimensions (area and height) were about 20% less than required.

3.2. Safety for chickens for fattening

In the previous dossier, the applicant submitted a tolerance study in chickens for fattening to support the safety for target animals; this study was assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2020). In summary, the study was designed with 504 chickens which were allocated to seven treatments (total 42 pens with 12 animals per pen, 6 pens/treatment): a control (with manganese level fulfilling the requirements of chickens), an organic manganese source (the additive under assessment, Manganese-LG) at three levels (100, 150 (the maximum authorised manganese in feed) and 1,600 mg total manganese per kg of feed) and an inorganic manganese source (manganese sulfate monohydrate) in the same three levels. This tolerance study showed that Manganese-LG was safe up to 150 mg Mn/kg feed; indications of adverse effects were identified with the next level tested (1,600 mg Mn/kg feed). A margin of safety could not be derived from this study. Since significant adverse effects on zootechnical performance were observed at the tolerance dose of Manganese-LG compared to the tolerance dose of manganese sulfate, it was concluded that overdosing with Manganese LG is less tolerated than a comparable overdosing with a standard inorganic source of manganese.

In the new tolerance study submitted, a total of 528 one-day-old Arbor Acres male chickens were allocated to one of four dietary treatment group.^{7,8} The treatments comprised a control group (basal diet with no manganese supplementation) and three treatment groups: T1, T2 and T3 in which the basal diet was supplemented with 40, 400 and 800 mg Mn from Manganese-LG/kg diet, respectively. Total manganese in the diets was analytically confirmed (Table 2), and the levels corresponded to

⁴ Commission Implementing Regulation (EU) 2017/1490 of 21 August 2017 concerning the authorisation of manganous chloride tetrahydrate, manganese (II) oxide, manganous sulphate monohydrate, manganese chelate of amino acids hydrate, manganese chelate of protein hydrolysates, manganese chelate of glycine hydrate and dimanganese chloride trihydroxide as feed additives for all animal species and amending Regulations (EC) No 1334/2003 and (EC) No 479/2006. OJ L 216, 22.8.2017, p. 14.

⁵ Technical Dossier/Zinpro - ProPath Mn_MnLG 150_Layer_tolerance report.

⁶ Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. OJ L 276, 20.10.2010, p. 33.

⁷ Technical Dossier/Zinpro - ProPath Mn_MnLG 150_Broiler_tolerance report.

⁸ Technical Dossier/Supplementary Information/August 2020.

0.43 \times , 2.75 \times and 5.45 \times the maximum authorised total manganese level in feed for T1, T2 and T3, respectively. Each treatment comprised six replicates with two cages of 11 chickens each (no litter provided in the cages).^{8,9} A two-phase feeding schedule was applied in the experiment: Phase I (days 1–21) and Phase II, days 22–42. The basal diet consisted of soybean meal and corn in pellet form and the analysed content of manganese was 26.3 and 23.2 mg/kg for the Phase I and Phase II diets, respectively (Table 2). The study lasted 42 days and the chickens had *ad libitum* access to feed and water.

Table 2: Description of the four treatment groups

Treatment	Added manganese (mg from Manganese-LG/kg diet)	Total manganese (mg/kg diet) ⁽¹⁾	
		Phase I ⁽²⁾	Phase II ⁽²⁾
Control	0	26.3	23.2
T1	40	68.5	61.6
T2	400	416.6	410.8
T3	800	819.2	817.3

(1): Average correspondence to maximum levels of manganese in feed for poultry, T1: 0.43-fold, T2: 2.75-fold, T3: 5.45-fold.

(2): Phase I: from day 1 to 21; Phase II: from day 22 to 42.

Mortality and general health were monitored throughout the study; necropsy was carried out for dead animals. Body weight and feed consumption were measured at the end of the two phases of the experiment; feed intake and feed to gain ratio were calculated. One animal was randomly selected from each pen (12 per treatment) on days 21 and 42 and blood samples were collected for haematology¹⁰ and blood chemistry¹¹ analyses. On day 42, the chicken with the body weight closest to the average body weight of each cage was selected for gross pathology, including the relative organ weight (expressed as g/kg body weight),¹² and histopathological examination.¹³

All data were analysed by one-way analysis of variance (ANOVA). When the differences were significant, the least significant difference (LSD) test was used for multiple comparisons. Significance was declared at $p < 0.05$.

Mortality rate of the experiment was 2.27% and not treatment related (0.76% during the Phase I and 1.52% in Phase II); the causes of death were mainly reported as sudden death, and the necropsy did not reveal abnormalities.⁸ Performance parameters showed no significant differences between treatments at the end of the study (Table 3), with the exception of the feed to gain ratio which was lower in the group supplemented with 40 mg Mn (from Manganese-LG)/kg feed compared to the control group. The FEEDAP Panel notes the low body weight of the animals reached at the end of the study, which is lower than the objective for the breed (3,005 g).

Table 3: Performance parameters of chickens for fattening from the study with Manganese-LG at the end of the trial (day 42)

Treatment	Added manganese (mg from Manganese-LG/kg diet)	Feed intake (g)	Body weight (g)	Feed to gain	Mortality (%/n)
Control	0	3,983	2,412	1.65 ^a	2.27/3
T1	40	3,936	2,460	1.60 ^b	1.52/2
T2	400	3,939	2,430	1.62 ^{ab}	3.03/4
T3	800	3,969	2,428	1.63 ^{ab}	2.27/3

a,b: For a given parameter, different superscript within a column indicates significant differences ($p \leq 0.05$).

No differences were found in blood chemistry or haematological parameters at the end of the experiment.

⁹ Technical Dossier/Supplementary Information/October 2020.

¹⁰ White blood cell count (WBC), red blood cell count (RBC), platelet count (PLT), haemoglobin (HGB) and haematocrit (HCT).

¹¹ Aspartate transferase (AST), alkaline phosphatase (ALP), glutamate aminotransferase (ALT), blood glucose (GLU), creatinine (CRE), urea (UA), bilirubin (TBIL) and total protein (TP).

¹² Calculated as the relative weight of the organ in proportion of the body weight of the animal and expressed as g/kg body weight.

¹³ Heart, liver, spleen, lung, kidney, thymus, pancreas, stomach, duodenum, ileum, rectum, bone marrow.

From the relative organ weight, only the liver weight was affected by dietary treatment: the control group was higher (17.6 g/kg body weight) than the treated groups; however, no significant differences between the single experimental treatments were observed (15.34 g/kg body weight in T1, 16.41 in T2 and 16.10 in T3). The observed decrease of relative liver weight is not considered as an adverse effect. Other organs' relative weights (heart, spleen, lung and kidney) were not affected by dietary treatment. No pathological changes were observed in any examined organs.

The results of this study showed that Manganese-LG at the highest level tested – 800 mg Mn/kg feed – is safe for chickens for fattening. The FEEDAP Panel notes that the design of this experiment lacked a test dose equivalent to the maximum authorised manganese in feed (150 mg/kg); however, that manganese level was tested in a previous study in which no adverse effects were observed. Taking all the evidence together the Panel considers that Manganese-LG is safe for chickens for fattening at 150 mg Mn/kg feed, with a margin of safety of 5.5.

3.3. Conclusions on safety for the target species

The FEEDAP Panel concludes that the additive is safe for chickens for fattening at the maximum authorised level of 150 mg Mn/kg. This conclusion can be extrapolated to all animal species and categories provided that the maximum authorised levels in the EU for total manganese in feed are not exceeded.

4. Conclusions

The FEEDAP Panel concludes that Manganese-LG 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 manganese in feed are not exceeded.

5. Documentation as provided to EFSA/Chronology

Date	Event
16/04/2020	Dossier received by EFSA. Manganese chelates of lysine and glutamic acid for all animal species. Submitted by Zinpro Animal Nutrition (Europe), Inc.
16/04/2020	Reception mandate from the European Commission
30/04/2020	Start of the scientific assessment
26/06/2020	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.</i>
30/06/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 Addendum – Scientific assessment suspended. <i>Issue: Safety for target animals.</i>
26/08/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
15/09/2020	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.</i>
07/10/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
27/01/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ANOVA	analysis of variance
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
LSD	least significant difference