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Scientific Opinion on the safety and efficacy of Liderfeed® (eugenol) for chickens for fattening

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

Abstract

Liderfeed® is a zootechnical feed additive containing eugenol as active ingredient with some excipients. It has not been authorised in the European Union for use in food or feed. Liderfeed® was initially formulated with clove oil as a source of eugenol. The applicant replaced the clove oil with purified eugenol at the same concentration as in the original formulation. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) considered that studies carried out using the original formulation and the subsequently modified formulation are equivalent. The FEEDAP Panel concluded that Liderfeed® is safe for chickens for fattening at the proposed dose of 100 mg/kg complete feed. The addition of Liderfeed® at the recommended dose did not result in measurable concentrations of eugenol and its glucurono- and sulpho-conjugates in edible tissues. Liderfeed® is therefore considered safe for the consumer when used as an additive in feed for chickens for fattening. Liderfeed® is not considered to be a skin/eye irritant or a skin sensitiser. The exposure of users by inhalation is expected to be low. The use of Liderfeed® in feed for chickens for fattening can be considered safe for the environment. As only two out of the three efficacy studies showed a significant effect on the performance of chickens for fattening as a result of the supplementation with the additive, the FEEDAP Panel was unable to conclude on the efficacy of Liderfeed® in chickens for fattening. Dietary supplementation with Liderfeed® did not adversely affect chicken meat quality.

Keywords: Liderfeed®, eugenol, zootechnical, feed additive, chickens for fattening, safety, efficacy

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Correspondence: feedap@efsa.europa.eu

Panel members: Gabriele Aquilina, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Secundino López Puente, Marta López-Alonso, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Guido Rychen, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace and Pieter Wester.

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Summary

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 and efficacy of Liderfeed® (eugenol) for chickens for fattening.

Liderfeed® is intended as a zootechnical additive (functional group: other zootechnical additives) for use in feed for chickens for fattening to increase the performance. The additive contains eugenol as active ingredient with some excipients and has not been authorised in the European Union for use in food or feed.

Liderfeed® was initially formulated with clove oil as a source of eugenol. Subsequently, the applicant replaced the clove oil with purified eugenol at the same concentration as in the original formulation. The FEEDAP Panel considered that the studies made with the original and subsequently modified formulation can be considered as equivalent.

Chickens for fattening tolerated a 100 fold overdose of Liderfeed® without any adverse effects on health and performance. Therefore, the FEEDAP Panel concludes that Liderfeed® is safe for chickens for fattening at the proposed dose (100 mg/kg complete feed).

Eugenol shares a common metabolic route in chicken and mammals. The addition of Liderfeed® at the recommended concentration of 100 mg/kg complete feed of chickens for fattening does not result in measurable concentrations of eugenol and its glucurono- and sulpho- conjugates in edible tissues.

The FEEDAP Panel considers Liderfeed® as safe for the consumer, when used as an additive in feed for chickens for fattening.

Liderfeed® is not considered to be a skin/eye irritant or a skin sensitiser. Given the particle size distribution and low dusting potential of Liderfeed®, the exposure of users by inhalation is expected to be low.

The use of 100 mg Liderfeed®/kg complete feed for chickens for fattening can be considered safe for the environment.

Only two out of the three efficacy studies considered showed a significant effect on the performance of chickens for fattening as a result of the supplementation with the additive. Therefore, the FEEDAP Panel is unable to conclude on the efficacy of Liderfeed® in chickens for fattening. The dietary supplementation with Liderfeed® did not adversely affect chicken meat quality.

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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 Lidervet SL[2] for authorisation of the product Liderfeed® (eugenol), when used as a feed additive for chickens for fattening (category: zootechnical additives; functional group: 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). EFSA received directly from the applicant the technical dossier in support of this application. According to Article 8 of that Regulation, 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. The particulars and documents in support of the application were considered valid by EFSA as of 27 April 2012.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall 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 Liderfeed® (eugenol), when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The additive has not been previously evaluated by the EFSA and has not been authorised for use as a food or feed additive in the European Union. However, eugenol used as pesticide has been previously assessed by EFSA (EFSA, 2012). EFSA assessed eugenol used as food flavouring (EFSA, 2009) and as feed flavouring (EFSA FEEDAP Panel, 2011a). Eugenol is listed in the European Union List of Food Flavourings and in the European Union Register of Feed Additives.

2. Data and Methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[3] in support of the authorisation request for the use of Liderfeed® (eugenol) as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003 and the applicable EFSA guidance documents.

The FEEDAP Panel used the data provided by the applicant together with data from other sources to deliver the present output.

EFSA has verified the EURL report as it relates to the methods used for the control of the active substance in animal feed. The Executive Summary of the EURL report can be found in Annex A.[4]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Liderfeed® (eugenol) is in line with the principles laid down in Regulation (EC) No 429/2008[5] and the relevant

[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] Lidervet SL, Plaza García Lorca, 17, 43006, Tarragona, Spain.
[3] FEED dossier reference: FAD-2010-0396
[4] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2010-0396.pdf

guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011b), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008, revised in 2009), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c).

3. Assessment

Liderfeed® is intended as a zootechnical additive (functional group: other zootechnical additives) for use in feed for chickens for fattening to increase performance. The additive contains eugenol as active ingredient with some excipients.

Liderfeed® was initially formulated with clove oil as a source of eugenol. Subsequently, the applicant replaced the clove oil with purified eugenol at the same concentration as in the original formulation. Since clove oil contains up to 95 % eugenol it is likely that eugenol is the active component, and therefore the Panel considers that the studies made with the original and subsequently modified formulation are equivalent.

3.1. Characterisation

3.1.1. Characterisation of active substance

The active component of the additive is eugenol. Eugenol (2-methoxy-4-(2-propenyl)phenol,4-allyl-2-methoxyphenol-4-allylguaiacol; Chemical Abstracts Service (CAS) number 97-53-0; FLAVIS number 04.003; chemical formula $C_{10}H_{12}O_2$; density: 1.067 g/cm^3 and molecular weight 164.2) is a pale-yellow liquid. The analysis of three batches of eugenol[6] showed values > 99.5 %, which are compliant with the specifications of the Joint FAO/WHO Expert Committee on Food Additives (JEFCA).[7]

3.1.2. Characterisation of active substance

Liderfeed® consists of eugenol (5 %), glyceryl polyethyleneglycol ricinoleate (E 484, 55–56 %), silica amorphous (33 %) and acrylic polymers (Eudragit L 30 D-55) as a coating agent (6 %). The applicant provided certificates of analysis of five batches of Liderfeed® showing an average eugenol content of 5.28 %.[8]

To obtain the additive, an emulsion of eugenol with glyceryl polyethyleneglycol ricinoleate (E 484) is prepared. An absorbate is obtained from the eugenol emulsion with silica amorphous as a substrate. Finally, the product is coated with (Eudragit L 30 D-55) and granulated before packaging.[9] Typically the additive contains glyceryl polyethyleneglycol ricinoleate (55–56 %), silica amorphous (33 %) and Eudragit L 30 D-55 (6 %).

Glyceryl polyethyleneglycol ricinoleate (E 484) is authorised as feed additive for all animal species and categories and in all feedingstuffs and is currently under re-evaluation by the FEEDAP Panel.

Polymethacrylate copolymers are widely used as excipients (film-coating materials) in a large number of oral pharmaceutical preparations registered with the national authorities within the EU. They are generally regarded as non-toxic and non-irritant materials (European Pharmacopoeia, 2009). The coating Eudragit L 30 D-55 (poly(methacrylic acid-co-ethyl acrylate); CAS number 25212-88-8; chemical formula $C_9H_{14}O_4$) is a milky-white liquid of low viscosity with a characteristic odour and a relative density of 1.062 g/cm^3. It is presented in a form of aqueous dispersion with 30 % dry

[5] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.
[6] Technical dossier/Supplementary information July 2015/Annex II-1.
[7] http://www.fao.org/ag/agn/jecfa-flav/details.html?flavId=4818.
[8] Technical dossier/Supplementary information July 2015/Annex II-2.
[9] Technical dossier/Supplementary information July 2015/Section II Identity.

substance.[10] Eudragit L 30 D-55 is used as an enteric coating film for solid-dosage forms, as it is resistant to gastric juice but dissolves readily at pH > 5.5 (European Pharmacopoeia, 2009).

The product is routinely monitored for microbial (*Enterobacteriaceae,* yeast, *Escherichia coli* and *Salmonella*), aflatoxins and chemical (heavy metals, dioxins) impurities. Analysis of three batches of the additive showed that the product meets the specification given for microbial and chemical contamination. Cadmium and mercury were below 0.1 mg/kg, while lead and arsenic averaged 0.18 and 0.11 mg/kg, respectively.[11] These levels were considered to be of no concern.[12]

Analysis of three further batches showed that the *Enterobacteriaceae* and yeast were < 10 colony-forming units (CFU)/g, *Escherichia coli* was absent in 1 g and *Salmonella* was absent in 25 g.

The contents of aflatoxins B1 and B2 were < 0.2 µg/kg, G1 was < 0.5 and G2 was < 0.2 µg/kg in two batches. In the other batch tested, aflatoxin content was negative.[13] The applicant did not send data on other mycotoxins.

Liderfeed® is a coarse powder. The particle size distribution of three batches, as analysed by laser diffraction, showed less than 2 % of particles below 50 µm diameter and less than 0.3 % of particles below 10 µm diameter.[14] The dusting potential measured by the Stauber–Heubach method in three batches was ~0.02 g/m^3, which is considered low.[15]

3.1.3. Stability and homogeneity

The shelf life of the additive at 6 and 17 months was monitored (no information regarding container) in three batches by measuring the content of eugenol (initial content 5.25 %).[16] No losses of eugenol were observed after 6 months at 40 °C/75 % relative humidity (RH) (5.16 %) or after 17 months at 25 °C/60 % RH (5.24 %).[17]

No data were provided regarding the stability of the additive when incorporated in premixtures.

The stability of the additive in feed was studied in two batches of feedingstuffs, one mash and the other pelleted stored at 25 °C/60 % RH or 40 °C/75 % RH. The initial content of eugenol in mash feed was 4.9 mg/kg.[18] After one month the eugenol content of the feed stored at 25 °C/60 % RH was 4.4 mg/kg while at 40 °C/75 % RH it was 3.9 mg/kg. The initial eugenol content in pelleted feed was 4.5 mg/kg.[19] After one month the eugenol contents were 4.1 and 2.5 mg/kg for feed stored at 25 and 40 °C, respectively.[20] The additive showed evidence of reduced stability (9 – 44 % loss) even after one month's storage.

The analysis of the pelleting process in three different batches of Liderfeed® did not show a decrease in the average content of eugenol.[21]

[10] Technical dossier/Supplementary information July 2015/Annex II_10 and II_11.
[11] Technical dossier/Supplementary Information November 2012/Annex 1_Heavy metals and arsenic.
[12] Technical dossier/Supplementary Information (November 2012)/Annex 2_Dioxins and Dioxin-like PCBs.
[13] Technical Dossier/ Section II/Annex_II_3_Micro & aflatoxins.pdf.
[14] Technical dossier/Supplementary information August 2014/Annex_vii3_Dusting_potential.pdf.
[15] Technical dossier/Supplementary information August 2014/Annex_vii1_Dusting_potential.pdf.
[16] Technical Dossier/ Annex_II_12_CA_Liderfeed_Stability.pdf.
[17] Technical Dossier/ Section II_Identity.
[18] Technical Dossier/ Annex_II_14_CA_feeds_stability.pdf.
[19] Technical Dossier/ Annex_II_14_CA_feeds_stability.pdf.
[20] Technical Dossier/ Section II_Identity.
[21] Supplementary information November 2012/Annex 3.

The applicant tested the capacity of the additive to homogeneously distribute in mash feed in one batch of mash feed. Data on the content of eugenol in 10 samples gave a coefficient of variation of 1.3 %.[22]

3.1.4. Conditions of use

Liderfeed® is intended to be used in feed for chickens for fattening during the whole life of the animals at the dose of 100 mg/kg complete feedingstuffs equivalent to 5 mg eugenol/kg complete feed.

3.2. Safety

3.2.1. Safety for the target species

A tolerance study with 720 one-day-old male Ross 308 chickens distributed into 24 floor pens of 30 birds each (6 replicates per treatment) was performed.[23] Chickens were fed a starter diet (mash feed) from day 1 until day 21 and a grower diet (mash feed) from day 22 until day 35. Liderfeed® was incorporated into the basal maize–soybean–lard diet at 0, 100 (×1 recommended dose, 4.9 mg eugenol/kg starter diet and 5.5 mg eugenol/kg grower diet), 1 000 (×10, 45.7 mg/kg eugenol in starter diet and 52.2 mg/kg eugenol in grower diet) or 10 000 (×100, 489.6 mg/kg eugenol in starter diet and 506.5 mg/kg eugenol in grower diet) mg/kg, respectively.[24] Feed and water were available *ad libitum* over the experimental period of 35 days. Body weight (bw) and feed intake (FI) were recorded on days 0, 21 and 35, and average daily gain (ADG), average daily feed intake (ADFI) and feed-to-gain (F:G) ratio were calculated. Mortality was checked daily and the most probable cause of death determined by necropsy. Samples of liver, kidneys, muscle and subcutaneous fat from 12 birds per treatment (2 birds per replicate) were collected to analyse residues of eugenol and caryophyllene. Data were statistically analysed by analysis of variance.

The birds remained healthy throughout the study. The overall mortality rate was low (0.7 %) and was not treatment related. There were no significant treatment-related effects ($P > 0.05$) on zootechnical performance of chickens for fattening. The following values were obtained for the groups with 0, 100, 1 000 and 10 000 mg Liderfeed®/kg feed, respectively: ADG: 67.4, 66.3, 68.6 and 68.8 g/day; ADFI: 98.6, 98.7, 101.4 and 101.1 g/day, F:G ratio: 1.46, 1.49, 1.48 and 1.47 g/g. These results show that the 100-fold recommended dose had no negative impact on the zootechnical performance of chickens for fattening.

Chickens for fattening tolerated a 100-fold overdose of Liderfeed® without any adverse effects on health and performance. Therefore, the FEEDAP Panel concludes that Liderfeed® is safe for chickens for fattening at the proposed dose (100 mg/kg complete feed).

3.2.2. Safety for the consumer

The main active component of Liderfeed® is eugenol, for which an Acceptable Daily Intake (ADI) of 1.0 mg/kg bw has been set by EFSA (2012). Eugenol and eugenyl acetate have been assessed by the FEEDAP Panel and are considered safe for the consumer if used in feed of mammals at a concentration of 25 mg/kg complete feed (EFSA FEEDAP Panel, 2011a).

In humans and rodents (JECFA, 2006) orally administered eugenol is rapidly absorbed, efficiently extracted by the liver, conjugated (glucuronidated and sulphated) and excreted in the urine essentially in conjugated form. To a lesser extent (Sutton, 1986; Fisher et al., 1990), eugenol is metabolised by (i) isomerisation to isoeugenol, which can then undergo allylic oxidation and reduction of the double bond; (ii) epoxidation of the allyl double bond to yield an epoxide, which is hydrolysed to the corresponding diol and, subsequently, can be oxidised to the corresponding lactic acid derivative; (iii) conjugation of glutathione with a quinone-methide-type intermediate; and (iv) hydroxylation at the allyl position to yield 1′-hydroxyeugenol. All these polar metabolites have a free phenolic OH group or other polar oxygenated functional groups, they readily conjugate with glucuronic acid or sulphate and are excreted in urine.

[22] Technical Dossier/Section II_Identity.
[23] Technical dossier/Supplementary information December 2013/Annex_i_14_B434 Report.
[24] Technical dossier/Supplementary information December 2013/Annex_i_1_Data sheet Tolerance study.

In the previous EFSA opinion on eugenol (EFSA FEEDAP Panel, 2011a), no assessment was made for poultry because of a lack of information on the metabolism of this compound in birds and the possible retention in tissues of eugenol/metabolites.

The coating polymer (Eudragit L 30 D-55) consists of water and 40 % of a copolymer based on methacrylic acid and ethyl acrylate. This gives an application dose of 1.2 mg polymer/kg complete feed. From data using a quite similar radiolabelled neutral methacrylate copolymer, it was concluded (EFSA ANS Panel, 2010) that "*the polymer is essentially not absorbed and that the very low amounts of absorbed radioactivity are not retained in the tissues*".

The applicant provided metabolism and residue studies of eugenol in chickens for fattening after oral administration of pure eugenol (Section 3.2.1) or the additive (Section 3.2.2).

Metabolism study

Nine male Ross 308 chickens (age not indicated) were distributed to three groups: untreated control, vehicle control (sunflower oil) and eugenol.[25] A single dose of eugenol was administered by oral gavage, at doses of 1.5 mmol/kg bw (eugenol 246.3 mg/kg bw). Excreta were collected for four periods: 0–4, 4–24, 24–29 and 29–48 hour periods after administration of eugenol. Plasma, liver, kidney, muscle and skin + fat were collected at slaughter (48 hours). Quantification of eugenol was performed using gas chromatography–mass spectrometry (GC-MS) with single ion monitoring (molecular ion *m/z* 164) with azulene as internal standard, with and without enzymatic hydrolysis (β-glucuronidase and arylsulphate from *Helix pomatia*).

The excretion of eugenol is very fast: 98 % of the excreted eugenol (free and conjugated) appeared in excreta in the first 24 hours. After 48 hours, no free or total eugenol (free plus glucurono- and sulpho- conjugates) was measurable in the plasma, kidney, muscle or fat of treated chickens (limit of quantitation (LOQ) = 0.2 mg/kg). No free eugenol but some total eugenol was detected in the liver. No attempt was made to identify eugenol metabolites other than the glucurono- and sulpho-conjugates.

This study shows that eugenol shares a common metabolic route in chicken and mammals.

Residues study

Four groups of day-old chickens (6 pens of 30 birds each) were fed for 35 days a control feed or a feed supplemented with the recommended dose of Liderfeed (100 mg//kg feed) or 10× or 100× the recommended dose. At slaughter, samples of liver, kidneys, muscle and subcutaneous fat were taken from 12 birds per group.[26] Eugenol was determined using the GC-MS method already described (Section 3.2.1), and the limits of detection (LODs) were found to be 0.03 mg eugenol/kg in liver, 0.07 mg/kg in kidney and muscle and 0.14 mg/kg in skin/fat, applied with and without hydrolysis of glucurono- and sulpho-conjugates. Eugenol (free and total free plus deconjugated) was not detected in tissues of chickens in the control group or in the group that received the recommended dose.[27,28]

Conclusions on the safety for the consumer

Eugenol shares a common metabolic route in chicken and mammals. The addition of Liderfeed® at the recommended concentration of 100 mg/kg complete feed of chickens for fattening does not result in measurable concentrations of eugenol and its glucurono- and sulpho- conjugates in edible tissues.

The FEEDAP Panel therefore considers Liderfeed® safe for the consumer, when used as an additive in feed for chickens for fattening at the recommended conditions of use (100 mg/kg complete feed).

[25] Technical Dossier/Supplementary information (December 2013)/Annex_ii_4_Trial E-130101 Report—Metabolism.
[26] Technical Dossier/Supplementary information (December 2013)/Annex_i_1_Data sheet Tolerance study.
[27] Technical Dossier/Supplementary information (December 2013)/Annex_ii_5_Trial E-130102 Protocol—Residues.
[28] Technical Dossier/Supplementary information (December 2013)/Annex_ii_6 Trial E-130102 Report—Residues.pdf.

3.2.3. Safety for the user

Effects on eyes and skin

An *in vitro* test for skin irritancy of Liderfeed® was performed following a protocol that was consistent with the Organisation for Economic Co-operation and Development (OECD) Guideline 439. Undiluted Liderfeed® was tested in triplicate on reconstituted human epidermis. Phosphate-buffered saline was used as the negative control and a 5 % aqueous solution of sodium dodecyl sulphate was used as a positive control. Cell viability was assessed by the MTT[29] assay (Mossman, 1983). The cell viabilities of negative control, positive control and test material were ≥ 98.8 ± 1.7, 0.8 ± 0.1 % and 84 ± 4, respectively, after 42 minutes of incubation. A cell viability of less than 50 % is required for a substance to be classed as a skin irritant in this test, so this result was interpreted as negative for skin irritancy potential.[30]

An ocular irritation test was performed with Liderfeed® using three New Zealand White rabbits. The protocol was consistent with OECD Guideline 405. Undiluted (0.1 g) Liderfeed® was administered to the right conjunctival sac of each animal. The treated and untreated eyes were observed at 1, 24, 48 and 72 hours after administration. At no time did any of the treated (or untreated) eyes show any changes to the cornea, iris or conjunctiva, indicating an absence of eye irritation.[31]

A maximisation test for skin sensitisation of Liderfeed® was performed on female Hartley albino guinea pigs using a test group of ten animals and a control group of five. The protocol followed OECD Guideline 406. No skin reactions were visible at the application sites when observed in any animals at the scheduled times of observation (48 and 72 hours after administration of the challenge doses). The results showed that Liderfeed® is not a potential skin sensitiser.[32]

Effects on the respiratory system

No specific data on inhalatory toxicity were provided. However, particle size distribution (< 2 % of particles below 50 µm diameter) and the low dusting potential of Liderfeed® indicate a low potential for exposure by inhalation.

Conclusions on safety for the user

Liderfeed® is not considered to be a skin/eye irritant or a skin sensitiser. Given the particle size distribution and low dusting potential of Liderfeed®, the exposure of users by inhalation and the subsequent health risks are expected to be low.

3.2.4. Safety for the environment

Liderfeed® is composed of eugenol (5 %), glyceryl polyethyleneglycol ricinoleate (55–56 %), silica amporphous (33 %) and acrylic polymers (6 %).

Eugenol has been previously assessed by the FEEDAP Panel, which concluded that eugenol does not present an appreciable risk for the environment when used at concentrations up to 25 mg/kg complete feed (EFSA FEEDAP Panel, 2011a).

Amorphous silica is ubiquitous in the environment and thus does not require any further assessment. Similarly, the glycerol derivative will be readily metabolised and no residues are expected.

The coating polymer consists of water and 40 % of a copolymer based on methacrylic acid and ethyl acrylate. This gives an application dose of 1.2 mg polymer/kg broiler feed, which leads to a maximum predicted soil concentration of 6.4 µg/kg soil dry weight (dw) as calculated in accordance with the EFSA guidance document (EFSA, 2008). This is below the trigger value of 10 µg/kg, indicating no risk for the soil compartment. In view of the fact that degradation of this copolymer is likely to occur in the intestine of the target animal, a risk to surface water is not expected.

In conclusion, the use of 100 mg Liderfeed®/kg complete feed for chickens for fattening can be considered safe for the environment.

[29] (3-(4,5-dimethylthiazol-2)-2,5-diphenyltetrazolium bromide).
[30] Technical Dossier/Supplementary information (November 2012)/ Annex 9_Final Report Skin irritation.pdf.
[31] Technical Dossier/Supplementary information (November 2012)/ Annex_11_Final_report_eye_irritation.pdf.
[32] Technical Dossier/Supplementary information (November 2012)/ Annex_13_Final_report_skin_sensitisation_guinea_pig.pdf.

3.3. Efficacy

Four efficacy studies in chickens for fattening were carried out at four different locations. However, one study was not considered because it tested only doses much higher than the recommended dose (approximately 10 times the recommended dose).[33] Of the remaining studies, Liderfeed® was supplemented at 100 mg/kg of diet (recommended dose) in studies 1 and 3, and from 100 to 200 mg/kg of diet in study 2.

In the first study, a total of 247 1-day-old male Ross 308 chickens were allotted to two treatments, each with 9 pens of 13 or 14 birds.[34] The animals were fed a basal maize–soybean meal–wheat diet supplemented with Liderfeed® at either 0 or 100 mg/kg. Broilers were fed a starter diet (mash feed; analysed: 4.9 mg eugenol/kg feed) from day 1 until day 17 and a finisher diet (pelleted feed; analysed: 4.5 mg eugenol/kg feed) from day 19 until day 35. Feed was provided *ad libitum*. Mortality, body weight and feed intake were recorded and average daily gain and feed-to-gain ratio were calculated. Data were statistically analysed by analysis of variance considering the pen (13 or 14 birds) as the experimental unit.

Mortality (dead and culled animals) was low and was not affected by treatments. Supplementation with Liderfeed® resulted in a significant improvement (Table 2) in body weight (2 048 vs. 1 981 g; P = 0.036), average daily gain (57.3 vs. 55.3 g/day; P = 0.036) and feed-to-gain ratio (1.44 vs. 1.51 g/g; P = 0.007) over the whole study period. No other parameters were affected.

In the second study, a total of 384 1-day-old male Ross chickens were allotted to one of two treatments, each with 16 replicates of 12 birds.[35] The animals were fed a basal wheat–soybean diet supplemented or not with Liderfeed® at 100 mg/kg. Chickens for fattening were fed a starter diet (5.2 mg/kg eugenol) from day 1 until day 14, a grower diet (8.5 mg/kg eugenol) from day 15 until day 39 and a finisher diet (9.7 mg/kg eugenol) from day 40 until day 46.[36] Feed was provided *ad libitum*. Mortality, body weight and feed intake were recorded and average daily gain and feed to gain ratio were calculated. Data were statistically analysed by analysis of variance considering the pen (12 birds) as the experimental unit.

Because eugenol content in the treated diet was up to 9.7 mg/kg, approximately twice the eugenol content that would normally be found in a diet supplemented with Liderfeed® (4–5 mg/kg; see Table 1), it is evident that Liderfeed® was actually supplemented to the diet at approximate levels of 100 mg/kg (days 1–14) to 200 mg/kg (days 15–46). Mortality was high, but was not affected by treatments (P = 0.161).[37] No significant effects were seen over the 46-day period on any of the parameters measured (Table 2).

In study 3, a total of 216 1-day-old male Ross 308 chickens were allotted to one of two treatments, each with 9 replicates of 12 birds.[38] The animals were fed a basal maize–wheat–soybean meal diet supplemented with Liderfeed® at either 0 or 100 mg/kg. Broilers were fed a starter diet (compound feed; 4.98 mg/kg eugenol) from day 1 until day 14 and a grower diet (compound feed; 4.89 mg/kg eugenol) from day 15 until day 35. Feed was provided *ad libitum*. Mortality, body weight and feed intake were recorded and average daily gain and feed to gain ratio were calculated. Moreover, on day 35 of the experiment, all birds were sacrificed and gross pathological examination was performed to identify enlargement and/or lesions of liver, kidneys and other organs. All data were statistically analysed by t-test, except for mortality that was analysed by a chi-squared test. The pen (12 birds) was considered the experimental unit.

Mortality was not affected by treatments.[39] Supplementation of feed with Liderfeed® improved body weight, average daily gain and feed to gain ratio over the whole study period (Table 2). [40,41] No other parameters were affected.

[33] Technical Dossier/ Section IV/ Annex IV. 9 Protocol and Report Study .
[34] Technical Dossier/ Section IV/ Annex IV. 6. Report Study.
[35] Technical Dossier/ Section IV/ Annex IV. 16. Protocol and Report Study.
[36] Technical Dossier/ Section IV/ Annex IV. 15. CA feeds Study.
[37] Technical Dossier Supplementary information June 2014/Annex 3-3.
[38] Technical Dossier/ Section IV/ Annex IV.17. Protocol Study
[39] Technical Dossier Supplementary information August 2014/ Annex_iv-3-SR_347-11_sign.pdf.
[40] Technical Dossier Supplementary information August 2014/ Annex_iv-3-SR_347-11_sign.pdf.
[41] Technical Dossier/ Section IV/ Annex IV.18. Report Study.

Table 1: Effect of Liderfeed® on mortality and performance of chickens in three efficacy studies

Trial Duration (days)	Total number of birds (birds × replicate)	Liderfeed® (mg/kg feed)	Analysed eugenol (mg/kg feed) [(a)]	Feed intake [(b)]	Average weight gain (g/day)	Final body weight (g)	Feed to gain	Mortality (%)
1 (35)	247 (13/14 × 9)	0	0	2 932	55.3	1 981	1.51	3.2
		100	4.9/4.5	2 880	57.3*	2 048*	1.44*	0.8
2 (46)	384 (12 × 16)	0	0	103.3	59.6	3 055	1.74	7.3
		100	5.2/8.5/9.7 [(c)]	101.1	59.5	3 045	1.70	11.5
3 (35)	216 (9 × 12)	0	0	3 510	60.0	2 146	1.64	4.8
		100	4.98/4.89	3 569	62.4*	22 30*	1.60*	4.8

(a): Values for grower/finisher.
(b): Units are grams in studies 1 and 3, g/day in study 2.
(c): the concentration of eugenol doubled at day 40.
*Means of treated and controls within the same trial are significantly different (P < .05).

A published paper (Isabel and Santos, 2009) was also submitted in which chickens for fattening received clove oil (not the feed additive under assessment). Since no information was provided on the contents of eugenol of the feeds and on the relationship of the tested product with the one under assessment, this study is not further considered.

Quality of animal products

A total of 120 chickens for fattening (60 chickens/treatment) from study 2 (Table 2) were slaughtered at the end of the experimental period with an approximate weight of 3 kg.[42] Colour, fat content, fatty acid profile and degree of oxidation in breast and thigh meat were analysed. The texture of breast meat was also measured. Sensory properties were not tested. Data were statistically analysed by analysis of variance.

No statistically significant differences were found between treatments.[43]

Conclusions on efficacy for the target species

Only two out of the three efficacy studies considered showed a significant effect on the performance of chickens for fattening as a result of the supplementation with the additive. Therefore, the FEEDAP Panel is unable to conclude on the efficacy of Liderfeed® in chickens for fattening.

The dietary supplementation with Liderfeed® (with clove essential oil) did not adversely affect chicken meat quality.

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[44] and Good Manufacturing Practice.

4. Conclusions

Chickens for fattening tolerated a 100-fold overdose of Liderfeed®, as shown in the tolerance study, without any adverse effects on health and performance. Therefore, the FEEDAP Panel concludes that Liderfeed® is safe for chickens for fattening at the proposed dose (100 mg/kg complete feed).

[42] Technical Dossier/Section IV/Annex IV.20. Meat Quality Study.
[43] Technical dossier/Supplementary information November 2012/Annexes 14 to 16.
[44] 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.

Eugenol shares a common metabolic route in chicken and mammals. The addition of Liderfeed® at the recommended concentration of 10 mg/kg complete feed of chickens for fattening does not result in measurable concentrations of eugenol and its glucurono- and sulpho- conjugates in edible tissues.

The FEEDAP Panel considers Liderfeed® as safe for the consumer, when used as an additive in feed for chickens for fattening.

Liderfeed® is not considered to be a skin/eye irritant or a skin sensitiser. Given the particle size distribution and low dusting potential of Liderfeed®, the exposure of users by inhalation is expected to be low.

The use of 100 mg Liderfeed®/kg complete feed for chickens for fattening can be considered safe for the environment.

Only two out of the three efficacy studies considered showed a significant effect on the performance of chickens for fattening as a result of the supplementation with the additive. Therefore, the FEEDAP Panel is unable to conclude on the efficacy of Liderfeed® in chickens for fattening. The dietary supplementation with Liderfeed® did not adversely affect chicken meat quality.

Documentation provided to EFSA

1. Clove oil eugenol (Liderfeed®). March 2011. Submitted by Lidervet SL.
2. Clove oil eugenol (Liderfeed®). Supplementary information. November 2012. Submitted by Lidervet SL.
3. Clove oil eugenol (Liderfeed®). Supplementary information. December 2013. Submitted by Lidervet SL.
4. Clove oil eugenol (Liderfeed®). Supplementary information. June 2014. Submitted by Lidervet SL.
5. Clove oil eugenol (Liderfeed®). Supplementary information. August 2014. Submitted by Lidervet SL.
6. Clove oil eugenol (Liderfeed®). Supplementary information. July 2015. Submitted by Lidervet SL.
7. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Clove oil eugenol (Liderfeed®).

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Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
ADI	average daily intake
AFC	EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
ANS	EFSA Scientific Panel on Additives and Nutrient Sources added to Food
BW	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CFU	colony-forming unit
EC	European Commission
EURL	European Union Reference Laboratory
F:G	Feed to gain ratio
FI	Feed intake
FLAVIS	The EU Flavour Information System
GC-MS	gas chromatography-mass spectrometry
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
LOQ	limit of quantification
OECD	Organisation for Economic Co-operation and Development

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Clove oil (Liderfeed®)

In the current application authorisation is sought under article 4(1) for *Clove oil* under the category/functional group 4(d) "other zootechnical additives" according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use for chickens for fattening.

The Applicant intends to market the granulated product (Liderfeed®) containing 5 to 6% *Clove oil* - an essential oil obtained from Syzygium aromaticum - containing 75 to 95 % *Eugenol*. The product is intended to be incorporated in complete *feedingstuffs* with a recommended dosage of *Eugenol* ranging from 4 to 50 mg/kg.

For the determination of *Eugenol* marker in the *feed additive* (*clove oil*) and in the product (Liderfeed®), the Applicant submitted a single-laboratory validated and further verified method based on Gas Chromatography coupled to a Flame Ionization Detector (GC-FID). The following performance characteristics were reported:

- a standard deviation for *repeatability* (RSD_r) ranging from 0.4 to 1.4%;
- a standard deviation for *intermediate precision* (RSD_{ip}) ranging from 1.0 to 1.8%;
- a *recovery* rate (R_{Rec}) ranging from 99 to 101%.

For the determination of *Eugenol* (marker) in *feedingstuffs* the Applicant proposed another single-laboratory validated and further verified GC-FID method and reported the following performance characteristics for *Eugenol* concentrations ranging from 4.5 to 5.5 mg/kg *feedingstuff*: - RSD_r ranging from 1.2 to 3.2%; - RSD_{ip} = 3%; - R_{Rec} ranging from 96 to 106%; and - a limit of quantification (LOQ) of 1 mg/kg.

Based on the performance characteristics presented, the EURL recommends for official control the two single-laboratory validated and further verified GC-FID methods for the determination of *Eugenol* in the *feed additive* and in *feedingstuffs*, respectively.

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) is not considered necessary.