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Safety and efficacy of lactic acid and calcium lactate when used as technological additives for all animal species

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

Abstract

This opinion concerns the re-authorisation of lactic acid and calcium lactate for use as preservatives in feed and a new authorisation for lactic acid as a preservative in water for drinking. The maximum level of 50 000 mg lactic acid/kg complete feed and 30 000 mg calcium lactate/kg complete feed are considered safe for functional ruminants and pigs. The maximum safe concentration in water for drinking for pigs can be derived from the maximum safe level in feed (15 000 mg lactic acid/L water). No conclusions on the safety of lactic acid in pre-ruminants and poultry can be drawn. These conclusions cannot be extrapolated to other animal species/categories. The use of lactic acid and calcium lactate as feed additives is safe for the consumer. Lactic acid is considered an irritant to eyes and skin and there is a risk of serious damage to the eyes. Inhalation of the mist causes irritation of the respiratory system. Calcium lactate should be considered an irritant to skin, eyes and the respiratory tract. It is likely that handling the additive will result in the production of respirable dust, which could present a risk to unprotected workers. The use of lactic acid and calcium lactate in animal nutrition would not pose a risk to the environment, provided that the concentrations regarded as safe for the target species are not exceeded. Lactic acid and calcium lactate are used in food as preservatives. It is reasonable to expect that the effect seen in food will be observed in feed when these additives are used at comparable concentrations and under similar conditions. However, the Panel has reservations about the effectiveness of lactic acid and calcium lactate as preservatives in complete feedingstuffs with a typical moisture content of ≤ 12 %.

Keywords: lactic acid, calcium lactate, preservative, all animal species, water, safety, efficacy

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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 an application for the re-authorisation of lactic acid and calcium lactate when used as preservatives in feed and for a new use of lactic acid as a preservative in water for drinking.

The maximum recommended levels of 50 000 mg lactic acid/kg complete feed and 30 000 mg calcium lactate/kg complete feed are considered safe for functional ruminants and pigs. The maximum safe concentration of lactic acid in water for drinking for pigs can be derived from the maximum safe level in feed (15 000 mg/L water). No conclusions on the safety of lactic acid in pre-ruminants and poultry can be drawn. These conclusions cannot be extrapolated to other animal species/categories.

The use of lactic acid or calcium lactate as feed additives is not expected to increase the exposure of the consumer to these compounds over that observed when used in food, and therefore the use of lactic acid and calcium lactate as feed additives is safe for the consumer.

Lactic acid is considered an irritant to eyes and skin and there is a risk of serious damage to the eyes. Inhalation of mist causes irritation of the respiratory system. Calcium lactate should be considered an irritant to skin, eyes and the respiratory tract. It is likely that handling the additive will result in the production of respirable dust, which could present a risk to unprotected workers.

The use of lactic acid and calcium lactate in animal nutrition would not pose a risk to the environment provided that the concentrations regarded as safe for the target species are not exceeded.

Lactic acid and calcium lactate are used in food as preservatives. It is reasonable to expect that the effect seen in food will be observed in feed when these additives are used at comparable concentrations and under similar conditions. However, the FEEDAP Panel has reservations about the effectiveness of lactic acid and calcium lactate as preservatives in complete feedingstuffs with a typical moisture content of ≤ 12 %.

The FEEDAP Panel made recommendations regarding the specification of the bacterial strains used to produce lactic acid by fermentation and the enantiomeric composition of the additive.

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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. In particular, Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from ACIAC EEIG (Acids Authorisation Consortium European Economic Interest Grouping)[2] for authorisation/re-evaluation of the product lactic acid and calcium lactate, when used as a feed additive for all animal species (category: technological additive; functional group: preservatives).

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) and under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 24 November 2011.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the products lactic acid and calcium lactate, when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

Lactic acid is an endogenous carboxylic acid derived from carbohydrate and amino acid metabolism, and is a natural component of many foods (e.g. fruits and fermented milk products). Under conditions of high energy demand and insufficient oxygen supply (typically during intense muscular activity), skeletal muscles anaerobically convert glucose into lactic acid. This is released from the muscle cells into the blood. In the liver, lactic acid is reduced to glucose, which then returns to the muscles (via the Cori cycle). Ultimately, any absorbed lactic acid will be oxidised to give carbon dioxide and water (EFSA BIOHAZ Panel, 2011).

Lactate exists as two enantiomers, L(+)- and D(–)-lactate. Lactate produced by mammalian cells is L-lactate and this is the main isomer in blood. D-Lactate is normally present in very low concentrations (Ling et al., 2012), mainly originating from microbial fermentation in the gut (Cranwell et al., 1976). When the rate of D-lactate production exceeds the body's capacity for its metabolism and excretion, D-lactate accumulates in the blood causing metabolic acidosis. D-Lactate acidosis is well known and described in veterinary medicine as a consequence of grain overfeeding in adult ruminants and in neonatal animals with diarrhoea (Ewaschuk et al., 2005).

[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] On 13/03/2013, EFSA was informed by the applicant that ACIAC EEIG was liquidated on 19/12/2012 and their rights as applicant were transferred to FEFANA asbl (EU Association of Specialty Feed Ingredients and their Mixtures). Avenue Louise, 130A, Box 1, 1050 Brussels, Belgium. Companies: Purac, Gorinchem, The Netherlands; Galactic, Brussels, Belgium.

Lactic acid (E 270) and calcium lactate (E 327) are presently listed in the European Union (EU) Register of Feed Additives as technological additives (functional group: preservatives) for use with feed for all animal species and categories without restrictions, and are subject to re-evaluation.[3]

Lactic acid (E 270) and calcium lactate (E 327) are permitted food additives used in a variety of foods (e.g. nectars, jam, jellies, marmalades, mozzarella and whey cheese, fats of animal or vegetable origin for cooking and/or frying, canned and bottled fruits and vegetables, fresh pasta and beer) according to Regulation (EC) No 1333/2008[4] on food additives. Specifications for purity are laid down in Directive 2008/84/EC.[5]

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) issued an opinion on lactic acid and calcium lactate (JECFA, 1974) allocating an acceptable daily intake (ADI) of "not limited". In 1991, this ADI was supported by the Scientific Committee for Food (EC, 1991) and in 2006, it was iterated in the evaluation of lactate and sodium lactate for poultry carcass treatment (EFSA, 2008a). EFSA has issued several opinions on the use of lactic acid and calcium lactate for carcass decontamination (EFSA, 2006; 2008a; EFSA BIOHAZ Panel, 2011).

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[6] in support of the authorisation request for the use of lactic acid and calcium lactate as feed additives. 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, such as peer-reviewed scientific papers, other scientific reports to deliver the present output.

EFSA has verified the EURL report as it relates to the methods used for the control of lactic acid and calcium lactate in animal feed. The Executive Summary of the EURL report can be found in Annex A.[7]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of lactic acid and calcium lactate is in line with the principles laid down in Regulation (EC) No 429/2008[8] and the relevant guidance documents: Guidance on technological additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008b), Guidance for the preparation of dossiers for the re-evaluation of certain additives already authorised under Directive 70/524/EEC (EFSA, 2008c), Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d), Guidance on the assessment of the toxigenic potential of *Bacillus* species used in animal nutrition (EFSA FEEDAP Panel, 2014), Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA, FEEDAP Panel 2012e).

[3] European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. Available online: http://ec.europa.eu/food/food/animalnutrition/feedadditives/comm_register_feed_additives_1831-03.pdf

[4] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.

[5] Commission Directive No 2008/84/EC of 27 August 2008 laying down specific purity criteria on food additives other than colours and sweeteners. OJ L 253, 20.9.2008, p. 1.

[6] FEED dossier reference: FAD-2010-0133.

[7] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2010-0133.pdf

[8] 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

This application is for the re-evaluation of lactic acid and calcium lactate as technological additives when used as preservatives in feed for all animal species, as required by Regulation (EC) No 1831/2003.[9] The application includes a request for the use of lactic acid in water for drinking, which is considered a new use of the product.

3.1. Characterisation

3.1.1. Lactic acid

Lactic acid[10] (2-hydroxypropanoic acid, $C_3H_6O_3$) is a colourless syrupy liquid or a white to light-yellow solid or powder with a molecular weight of 90.08 g/mol. It is fully miscible in water and organic solvents, with a density of 1.224 g/mL at 20 °C and a pK_a of 3.858 at 25 °C. The additive is essentially L-lactic acid (Chemical Abstracts Service (CAS) No 79-33-4, European Inventory of Existing Commercial chemical Substances (EINECS) No 201-196-2). The structural formula of lactic acid is shown in Figure 1.

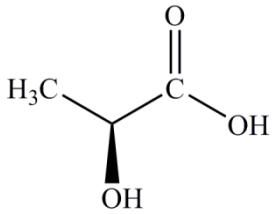

Figure 1: Structural formula of L-lactic acid

The lactic acid additive is provided as an aqueous solution specified to contain a minimum of 72 % (w/w) lactic acid. Commission Directive 2008/84/EC[11] sets the specifications for lactic acid as a food additive of ≥ 76 % lactic acid (w/w).[12] Five batches of lactic acid from two manufacturers were analysed for the content of the active substance in the aqueous solution, as supplied. The results obtained from the analysis of the batches from the first manufacturer (lactic acid 72.7 % w/w, range 72.0–74.5 %) and from the second manufacturer (lactic acid 76.1 % w/w, range 74.7–78.6 %) showed that the additive was close to, but not fully compliant with, the food specifications. The concentration of other organic acids, predominantly malic and succinic acids, reached 8 % in some batches of the product.[13,14]

Two enantiomers, L(+)- and D(–)-lactic acid, are present in the additive. Three batches of lactic acid used to formulate the additive from each manufacturer were analysed by chiral chromatography to separate and determine the proportion of each enantiomer. The percentage of L-lactic acid in three samples from one manufacturer ranged from 94.3 to 98.5 %, whereas in the case of the second manufacturer, the percentage of L-lactic acid was 97.6 % in the three batches. The remainder was essentially the D-enantiomer. The applicant proposes a specification of a minimum 90 % of L-lactic acid and a maximum of 10 % of D-lactic acid.

Three batches of lactic acid from one manufacturer were analysed for eight impurities (sulphate, other organic acids, iron, arsenic, lead and heavy metals expressed as lead, mercury and chloride). Three batches from the other company were analysed for six impurities (sulphate, other organic acids, iron, arsenic, lead, heavy metals) and another three batches for only mercury and chloride.[15] The values obtained (< 0.16 % sulphate; 38 to < 150 mg iron/kg; < 1 mg arsenic/kg; < 1 mg lead/kg; < 0.1 mg

[9] 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.
[10] Technical dossier/Section II/Annex_II_2.1.3_4.
[11] Commission Directive 2008/84/EC of 27 August 2008 laying down specific purity criteria on food additives other than colours and sweeteners. OJ L 253, 20.9.2008, p. 1.
[12] Technical dossier/Section II/ Sect_II_Identity.
[13] Technical dossier/Supplementary information March 2015/Annex_Qi_C_OrganicAcidProfile.
[14] Technical dossier/Supplementary information March 2015/Annex_Qii_P_OrganicAcidProfile.
[15] Technical dossier/Section II/ Sect_II_Identity.

mercury/kg; < 10 mg heavy metals (expressed as lead)/kg; < 0.02 % chloride) were below the specifications for purity in food additives requested in Directive 2008/84/EC. Although the values for iron exceeded the specifications for food additives (< 10 mg/kg), no concerns are anticipated for feed use.[16]

Manufacturing process[17]

A detailed description of the manufacturing processes of the additive was submitted.[18] Lactic acid is obtained by bacterial fermentation using industrial media typical of those used for lactic acid bacteria. As lactic acid is produced, calcium hydroxide is added to stabilise the pH, resulting in the formation of calcium lactate. At the end of the fermentation, biomass is removed by filtration and sulphuric acid is then added to convert the calcium lactate into lactic acid and calcium sulphate. Calcium sulphate is water insoluble and is removed by filtration. The remaining solution of lactic acid is then further purified and concentrated. Because of its hydroscopic nature, it is generally sold as a concentrated aqueous solution.

The applicant declared that antibiotics are not used in the production process. The cells of the production organisms are inactivated by increasing the temperature and decreasing the pH. This was confirmed by the absence of viable cells in one sample of lactic acid and in another sample of calcium lactate from one manufacturer and in three batches of lactic acid from the other manufacturer.[19]

Both manufacturers associated with this application use strains of *Bacillus* spp. as the production organisms.

One manufacturer specifies that three particular strains of *Bacillus* spp. (*B. coagulans*, *B. smithii* and *B. subtilis*) are used individually as alternative producers of lactic acid.[20] The strains are deposited in the Belgian Coordinated Collections of Microorganisms (BCCM) with the accession numbers LMG S-26145 (*B. coagulans*), LMG S-27890 (*B. smithii*) and LMG S-27889 (*B. subtilis*). None of these strains has been genetically modified.

The second manufacturer describes a production process using a single strain of *B. coagulans*, deposited with the Deutsche Sammlung von Mikroorganismen und Zellkulturen with the accession number DSM 23965,[21] which has not been genetically modified.

All four production strains described were tested for antibiotic susceptibility.[22] The antibiotics tested were those specified in the EFSA guidance for the assessment of bacterial antimicrobial susceptibility (EFSA FEEDAP Panel, 2012e). The antibiotic resistance profile for each of the strains gives rise to no concern.

The technical dossier contained detailed and sufficient information from the two manufacturers on the production strain, strain identity, deposition numbers, genetic stability, absence of antibiotics during the production process and absence of viable cells of the production strain in the final product.

3.1.2. Calcium lactate

Calcium lactate[23] (calcium 2-hydroxypropanoate, $(C_3H_5O_2)_2Ca{\cdot}nH_2O$ (n = 0–5); CAS No 814-80-2; EINECS 212-406-7) is an almost odourless white- to cream-coloured crystalline powder or granules with a molecular weight of 218.22 g/mol (anhydrous). It is produced by neutralising lactic acid with calcium hydroxide. The resulting salt is crystallised, dried, ground and packaged.[24] Calcium lactate has a density of 1.08 g/mL at 20 °C, it is soluble in water (100 g/L) and practically insoluble in ethanol. The structural formula of calcium lactate is shown in Figure 2.

[16] Technical dossier/Section II/ Annex_II_2.1.3_3A.
[17] This section has been amended following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
[18] Technical dossier/Section II/ Sect_II_Identity.
[19] Technical dossier/Supplementary information August 2014.
[20] Technical dossier/Supplementary information October 2013.
[21] Technical dossier/Supplementary information January 2014.
[22] Technical dossier/Supplementary information March 2015.
[23] Technical Dossier/Section II/Annex_II_2.1.3.
[24] Technical Dossier/Section II/Sect_II_Identity.

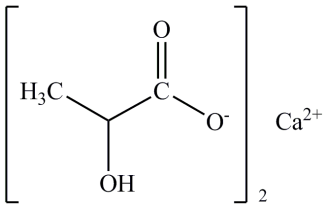

Figure 2: Structural formula of calcium lactate

Commission Directive 2008/84/EC[25] sets the specifications for calcium lactate of ≥ 98 % (as dry matter (DM), w/w).[26] Five batches of calcium lactate from each of the two manufacturers were analysed to determine the purity of the active substance. The results for batches from both the first manufacturer (calcium lactate 99.5 % w/w, range 99.3–99.9 %) and the second manufacturer (calcium lactate 99.5 % w/w, range 98.4–100.1 %) confirmed compliance with the food specification.

Three batches of calcium lactate from the first manufacturing company and one batch from the second manufacturer were analysed for five impurities (fluoride, arsenic, lead, heavy metals expressed as lead, and mercury). Two batches from the second manufacturer were analysed for fluoride, arsenic, lead and heavy metals expressed as lead, and two additional batches for mercury only.[27] The values obtained (< 11.7 mg fluoride/kg; < 0.85 mg arsenic/kg; < 2.6 mg lead/kg; < 0.05 mg mercury/kg; < 10 mg heavy metals (expressed as lead)/kg) were always below the specifications set in Directive 2008/84/EC.[28]

Dusting potential (as determined by the Stauber–Heubach test) and particle size distribution (analysed by laser diffraction) were measured in three batches of calcium lactate from one manufacturer. The results showed that, on average, 2.4 % (ranging from 1.3 to 3.4 %, coefficient of variation (CV) 44 %) of the particles have a diameter of < 50 µm, and that the average dusting potential was 7.58 g/kg (corresponding to about 3.8 g/m^3).[29]

Particle size distribution (analysed by laser diffraction) and dustiness (Stauber–Heubach test) were measured in one sample of calcium lactate from the other manufacturer. The results showed that 10 % of the particles have a diameter of < 56 µm, and that 50 % of the particles have a diameter of < 100 µm.[30] Dusting potential[31] was 13.6 g/m^3.

3.1.3. Stability and homogeneity

Three commercial batches of lactic acid (72 %) and three of calcium lactate pentahydrate (98–101 %) were stored at room temperature for two years. No degradation of the lactic acid or calcium lactate occurred over this period (recovery > 99 %).[32] Three other commercial batches of lactic acid (79.5–80.5 %) and calcium lactate were stored at 25 °C and 60 % relative humidity for two years (one batch) or five years (two batches). Again, no degradation of the lactic acid or calcium lactate was detected.

Four commercial premixtures of organic acids (three in liquid form and the fourth as a powder with silica absorbate) containing various levels of formic acid, propionic acid (and its ammonium salt) and acetic acid together with lactic acid were tested for stability over a period of 12 months.[33] Samples were packed in glass bottles and stored at 25 °C in a conditioned cabinet, protected from light. The recovery percentages for lactic acid ranged from 100 to 122 %, demonstrating that lactic acid is stable for at least 12 months in an organic acid premixture.

[25] Commission Directive 2008/84/EC of 27 August 2008 laying down specific purity criteria on food additives other than colours and sweeteners. OJ L 253, 20.9.2008, p. 1.
[26] Technical dossier/Section II/Sect_II_Identity.
[27] Technical dossier/Section II/Sect_II_Identity.
[28] Technical dossier/Section II/Annex_II_2.1.3_3B.
[29] Technical dossier/Supplementary information August 2014/Annex_PSD_Dust pot.
[30] Technical dossier/Supplementary information August 2014/Annex_PSD.
[31] Technical dossier/Supplementary information August 2014/Annex_Dust pot.
[32] Technical dossier/Section II/Annex_II_2.4.1_1A1.
[33] Technical dossier/Section II/Annex_II_2.4.1_4.

Single batches of layer, piglet and ruminant mineral–vitamin premixtures were formulated to include calcium lactate. The three premixtures were calculated to deliver 1.0 %, 1.0 % and 1.5 % calcium lactate, respectively, when mixed into the final complete feed. To achieve this, concentrations of the additive in the premixtures were 20 % (layer and piglet premixtures) or 25 % (ruminant premixture). Premixture samples (layer, piglet and ruminant) were stored at room temperature in closed containers for six months. No significant losses of calcium lactate (monitored as the anion) in the premixtures were observed during this period.[34]

Chicken (mash and pelleted), piglet (mash and pelleted) and ruminant (pelleted) feed of typical composition were formulated with lactic acid at an intended inclusion rate of 1.0 % (0.73 % analysed as lactate anion) or calcium lactate at an inclusion rate of 1.0 % (0.6 % analysed as lactate anion). Feed samples (mash and pellets) were stored at room temperature and analysed at the start of the study and after three months. No significant losses were observed in the five feed samples during the test period (average lactate anion concentrations in feeds were 0.59 % from the acid and 0.62 % from the salt at the start of the study and 0.63 % from the acid and 0.58 % from the salt after three months).

The impact of pelleting was measured by comparison of the recovery of lactic acid and calcium lactate in mash and pelleted feeds for piglets and for chicken. The difference in lactate concentration between mash and pelleted feed was seen as a loss of up to 12.3 % (from the acid) or 11.2 % (from the salt).

The stability of lactic acid in tap water (three replicates) was studied using an inclusion level of 0.5 % (0.37 % lactate anion). Lactic acid was shown to be stable in water for drinking after storage for 48 hours in closed containers at room temperature in a normally lit room.

Ten sub-samples were collected from each of the five feeds used for the stability testing (piglet mash and pelleted feeds, chicken mash and pelleted feeds, and a ruminant pelleted feed) formulated with either lactic acid or calcium lactate.[35] The mean concentration of lactate anions in the five feeds was 0.61 % (CV 4.5 %) with lactic acid or 0.60 % (CV 4.2 %) with the calcium salt, showing that both the free acid and its calcium salt can be mixed homogeneously into feed.

3.1.4. Conditions of use

Lactic acid and calcium lactate are proposed for use as preservatives in feedingstuffs, and in water for drinking in the case of lactic acid, in all animal species and categories with no minimum or maximum (quantum satis) content and without any restriction. The maximum recommended use levels are 50 000 mg lactic acid/kg complete feed, 30 000 mg calcium lactate/kg complete feed and 30 000 mg lactic acid/L in water for drinking.

3.2. Safety

3.2.1. Safety for the target species

According to EFSA guidance (EFSA FEEDAP Panel, 2012b), a tolerance study is normally not required for additives already authorised for use in food if the use level of the feed additive is less than or similar to that used in food (expressed as daily quantity per metabolic body weight). Lactic acid is a food additive authorised for use in a variety of foods *quantum satis*. However, the expected exposure of animals when lactic acid is used in complete feed at the maximum use level would be greater than that of humans. As data from tolerance studies are not available, the assessment is based on data reported in the literature on the responses of different animal species to the presence of lactic acid in diets.

Safety for ruminants

Ensiled forage for herbivores may contain up to 120 000 mg lactic acid/kg silage DM. This concentration in silage has been reported not to have any adverse effects on feed intake or animal performance (McDonald et al., 1991; Charmley, 2001).

[34] Technical dossier/Section II/Annex_II_2.4.1_3.
[35] Technical dossier/Section II/Annex_II_2.4.1_3.

The production of lactate in silage largely involves the D-isomer (up to more than 90 %), although this percentage may vary depending on the inoculant used for ensiling (Schaadt and Johnson, 1968; Cai et al., 1998). Cai and Kumai (1994) reported that the proportion of total lactic acid in the form of the D-isomer in silage was 62 to 68 %. In the case of silage-fed sheep, approximately 90 % of total lactate flux was metabolised in the rumen (converted to acetate, propionate and butyrate) and both D- and L-isomers of lactic acid appeared to have similar metabolic fates in the rumen (Gill et al., 1986).

Morgan and L'Estrange (1977) added lactic acid (D to L-isomer ratio of 40:60) to a grass meal diet up to concentrations of 81 000, 108 000 or 135 000 mg/kg DM for 15 days. When these diets were fed to sheep, lactic acid addition did not significantly affect feed intake, pH or total volatile fatty acid concentration in the rumen, blood acid–base balance, or L-lactate concentration in the blood. Jaakkola and Huhtanen (1992) infused DL-lactic acid at amounts equivalent to 0, 40 000, 80 000 and 120 000 mg/kg DM basal diet into the rumen of bulls fed grass silage for 21 days and did not observe any adverse effects on ruminal fermentation or nutrient digestion.

As no adverse effects were seen when animals were exposed to concentrations > 100 000 mg lactic acid/kg DM, the maximum recommended level of 50 000 mg lactic acid/kg complete feed is considered safe for functional ruminants. In the absence of data, no conclusion on the safety of lactic acid in pre-ruminants can be drawn.

Safety for pigs

High moisture feeds or fermented liquid feed (FLF) for pigs may contain lactic acid concentrations ranging from 16 000 to 80 000 mg/kg complete feed, predominantly in the L-form. No adverse effects on feed intake or animal performance have been observed in pigs fed FLF (25 % DM) with concentrations of lactic acid of 48 000 mg/kg DM (Niba et al., 2009; Plumed-Ferrer and von Wright, 2009). In a review on FLF for pigs, Missotten et al. (2010) reported that FLF containing 36 000 mg lactic acid/kg DM can have a beneficial effect on feed intake, daily gain and feed to gain ratio. Very recently, the same beneficial effects were observed in a 28-day feeding trial with piglets (27–28 days old, 6.3–7.9 kg body weight) fed FLF, containing lactic acid in the range of 55 000–125 000 mg/kg DM (Missotten et al., 2015).

Few studies were found in which lactic acid was added to diets fed to pigs. In a digestibility trial, six crossbred castrated pigs of 37 kg initial body weight were fed 30 000 mg DL-lactic acid/kg complete feed for 12 days (Kemme et al., 1999). The apparent ileal digestibility of nitrogen, amino acids and minerals was increased by lactic acid supplementation. In a study with 192 crossbred pigs with body weights ranging from 22 to 45 kg, the effects of phytase and lactic and formic acids, and their interaction, on animal performance, nutrient digestibility and urine composition were investigated (Jongbloed et al., 2000). Lactic acid was added to the basal diet at 0, 16 000 and 32 000 mg/kg for five weeks. Throughout the trial, no treatment-related health problems in pigs were observed. Lactic acid did not affect feed intake, increased weight gain, enhanced DM and mineral apparent digestibility and had no effects on urinary pH, osmolarity and mineral (calcium, phosphorus and magnesium) concentration.

Based predominantly on published studies made with FLF in which piglets are exposed to concentrations of lactic acid of up to 125 000 mg/kg DM, it is concluded that the additive lactic acid, at up to 50 000 mg/kg, is safe for all categories of pigs.

Safety for poultry

A total of 280 one-day-old Ross 308 chickens (seven replicates of 20 birds/treatment) were fed starter (days 0–13), grower (days 14–26) and finisher (days 27–39) diets, each in two forms, either as air-dried feed or in moist form (40 % DM), inoculated with lactic acid bacteria and batch fermented for 48 hours at 26 °C (Missotten et al., 2013). The starter fermented moist diet contained 64 000 mg DL-lactic acid/kg DM, the grower diet contained 42 000 mg DL-lactic acid/kg DM and the finisher diet contained 31 000 mg DL-lactic acid/kg DM. Mortality was reduced with the fermented moist feed compared with the dry feed (3.6 % vs. 9.3 %, $P < 0.05$). Feed intake and body weight gain were decreased by 45 and 40 %, respectively, in young chicks (days 0–13, starter diet) fed the starter moist diet compared with those fed the air-dried feed. When chickens were fed the grower diet (days 14–26), feed intake and weight gain were 16 and 23 % lower, respectively, with the moist diet than with the dry feed. The finisher moist diet (days 27–39) caused a slight reduction (5 %) in feed intake

and had no effect on growth with a similar weight gain to the control group. Over the total period (0–39 days), the birds fed the moist feed showed reduced average weight gain (54 vs. 60 g/day) and feed intake (79 vs. 92 g/day) compared with control broilers, with an improved feed to gain ratio (1.46:1 vs. 1.53:1).

In another study, the diets of one-day-old broiler chicks were supplemented with lactic acid at 0, 10 000, 20 000 and 30 000 mg/kg complete feed (40 chicks/treatment, four replicates each) for six weeks (Khalid et al., 2002). Body weight gain was significantly reduced ($P < 0.05$) during the first four weeks of the trial in all groups receiving feed supplemented with lactic acid (by 14 % with 10 000 mg lactic acid/kg feed) compared with the control group. Feed intake and body weight gain were significantly increased ($P < 0.05$) during the last two weeks of the trial with diet containing 30 000 mg lactic acid/kg. No mortality was observed in any of the treatment groups. Cumulative weight gain from hatching to six weeks of age was 1.87 and 2.06 kg for the control broilers and those receiving 30 000 mg lactic acid/kg complete feed, respectively, and cumulative feed intake was 3.36 and 3.54 kg/bird for the control and supplemented groups, respectively.

Commercial broiler chicks (seven days old) were fed a basal diet (maize, soya bean meal and fish meal) supplemented with 0, 20 000 or 30 000 mg lactic acid/kg complete feed (45 animals/treatment, three replicates each) for 42 days (Adil et al., 2010). Body weight gain (1.53 vs. 1.67 kg for 0 and 30 000 mg lactic acid/kg complete feed, respectively) and feed to gain ratio (2.02 vs. 1.84 for 0 and 30 000 mg lactic acid/kg complete feed, respectively) were significantly ($P < 0.05$) improved with lactic acid supplementation with no effects on feed intake (3.08 kg/bird in both groups). At 30 000 mg lactic acid/kg feed, villus height was increased in the duodenum and jejunum. Calcium and phosphorus concentrations in serum were increased in lactic acid-supplemented groups ($P < 0.05$). No effects ($P > 0.05$) on the concentration of serum glucose and cholesterol, serum glutamic pyruvic transaminase or serum glutamic oxaloacetate transaminase were observed.

Two submitted publications on the effect of calcium lactate in laying hens (Bartlett, 1962; Kent et al., 1960) could not be assessed, as they included very limited information.

Feed intake and weight gain of chickens for fattening at early growth stages (up to four weeks of age) were substantially reduced by lactic acid concentrations in feed as low as 10 000 mg/kg feed. In some studies, performance was not negatively affected over the whole production period (five to six weeks) in chickens for fattening supplemented with lactic acid. However, considering the limited number of published studies and the inconsistency of the results reported, the FEEDAP Panel is not in the position to conclude on the safety of lactic acid in poultry for fattening.

In the absence of data and considering the critical role of the acid–base balance in egg-shell formation, no conclusions on the safety of lactic acid in laying hens can be drawn.

D-Lactate isomer

Human neonates are more susceptible to D-lactic acidosis than adults (Petersen, 2005). It has been recommended that for food specially prepared for infant children only the L(+)-isomer should be used (JECFA, 1974; EC, 1991). The capacity to utilise D-lactate increases with age (Whittakers at al., 1974; Christie and Cranwell, 1976; Sissons et al., 1982). There is no evidence in the available published studies in which DL-lactic acid was fed to animals of an adverse effect which could be ascribed to D-lactate.

Use in water

No data were provided on the consequences of supplementing lactic acid in water for drinking. The maximum safe concentration of lactic acid for pigs in water for drinking can be derived from the maximum safe level in feed (15 000 mg/L water).

Conclusions on safety for the target species

The maximum recommended level of 50 000 mg lactic acid/kg complete feed is considered safe for functional ruminants and pigs. Although calcium lactate would be considered safe on an acid-equivalent basis to lactic acid, its calcium contribution to complete feed may limit the maximum concentration. However, the maximum recommended level of 30 000 mg calcium lactate/kg complete feed is considered safe for functional ruminants and pigs.

The maximum safe concentration of lactic acid for pigs in water for drinking can be derived from the maximum safe level in feed (15 000 mg/L water).

In the absence of data, no conclusions on the safety of lactic acid in pre-ruminants and laying hens can be drawn. Considering the limited number of published studies and the inconsistency of the results reported, it is not possible to conclude on the safety of lactic acid in chickens for fattening.

The current guidance on extrapolation from major to minor species allows extrapolation from three major target species, provided a comparable and wide margin of safety exists. As conclusions on safety could be drawn only for ruminants and pigs, extrapolation to other animal species/categories is not applicable.

3.2.2. Safety for the consumer

JECFA issued an opinion on lactic acid and calcium lactate (JECFA, 1974) allocating an ADI of "not limited".[36] In 1991, this ADI was supported by the Scientific Committee for Food (EC, 1991).

In 1978, the Food and Drug Administration (FDA) evaluated the health aspects of lactic acid and calcium lactate as food ingredients and concluded that the toxicity data available do not give rise to any suspicion of adverse effects in humans at doses that were orders of magnitude above the estimated levels for human consumption, excluding any future substantial increase of the ingestion of lactate from processed foods.[37]

Lactic acid and calcium lactate occur as intermediates of human metabolism (carbohydrate and amino acid metabolism) and are permitted *quantum satis* as additives in food in the EU, meaning that as much of the product can be added to achieve the desired result. According to EFSA guidance (EFSA FEEDAP Panel, 2012c), assessment of the safety for consumers is not required for food additives for which an ADI is not specified.

As, ultimately, any absorbed lactate will be oxidised in the animal to give carbon dioxide and water (EFSA BIOHAZ Panel, 2011), it is not expected that the use of lactic acid or calcium lactate in animal nutrition will increase the exposure of the consumer to these compounds over that observed when used in food.

3.2.3. Safety for the user

Lactic acid[38] is considered an irritant to eyes and skin and there is a risk of serious damage to the eyes. Inhalation of mist causes irritation of the respiratory system.

In the absence of data, calcium lactate should be treated as an irritant to skin, eyes and the respiratory tract.[39] Because of the particle size distribution of calcium lactate and the high dust generation potential, it is likely that handling the additive will result in the production of respirable dust, which could present a risk to unprotected workers.

3.2.4. Safety for the environment

Both lactic acid and calcium lactate are physiological/natural substances and their use in animal feed is not expected to increase their concentration in the environment. Therefore, the use of these compounds in animal nutrition would not pose a risk to the environment, provided that the concentrations regarded as safe for the target species are not exceeded.

3.3. Efficacy

Lactic acid has a long history of use for the preservation of human foodstuffs (Shelef, 1994; Vijayakumar et al., 2008). Lactic acid and calcium lactate are authorised food additives within the EU and are used for a number of technological functions, including preservation. Both compounds are used as preservatives for a widespread variety of foods (e.g. nectars, jam, jellies, marmalades, mozzarella and whey cheese, fats of animal or vegetable origin for cooking and/or frying, canned and

[36] Technical Dossier/Section III/Annex_III_3_1.
[37] Technical Dossier/Section III/Annex_III_3_2.
[38] Technical Dossier/Section III/Annex_III_3.3_1A.
[39] Technical Dossier/Section III/Annex_III_3.3_1B.

bottled fruits and vegetables, fresh pasta, beer, infant formulae, and weaning and baby foods). Lactic acid is also used for the microbial decontamination of carcasses (EFSA, 2006; 2008; EFSA BIOHAZ Panel, 2011). It is reasonable to expect that the effect in food will be observed in feed when it is used at comparable concentrations and under similar conditions.

The FEEDAP Panel has reservations about the effectiveness of lactic acid and calcium lactate as preservatives in complete feedingstuffs with a typical moisture content of ≤ 12 %. However, it is recognised that under practical conditions of storage the moisture content of all or part of the feed may rise above this level. Under these circumstances, the additive could be effective in preventing or reducing microbial deterioration.

4. Conclusions

The maximum recommended levels of 50 000 mg lactic acid/kg complete feed and 30 000 mg calcium lactate/kg complete feed are considered safe for functional ruminants and pigs. The maximum safe concentration of lactic acid for pigs in water for drinking can be derived from the maximum safe level in feed (15 000 mg/L water). No conclusions on the safety of lactic acid in pre-ruminants and poultry can be drawn. These conclusions cannot be extrapolated to other animal species/categories.

The use of lactic acid or calcium lactate as feed additives is not expected to increase the exposure of the consumer to these compounds over that observed when used in food, and therefore the use of lactic acid and calcium lactate as feed additives is safe for the consumer.

Lactic acid is considered an irritant to eyes and skin and there is a risk of serious damage to the eyes. Inhalation of mist causes irritation of the respiratory system. Calcium lactate should be considered an irritant to skin, eyes and the respiratory tract. It is likely that handling the additive will result in the production of respirable dust, which could present a risk to unprotected workers.

The use of lactic acid and calcium lactate in animal nutrition would not pose a risk to the environment, provided that the concentrations regarded as safe for the target species are not exceeded.

Lactic acid and calcium lactate are used in food as preservatives. It is reasonable to expect that the effect seen in food will be observed in feed when these additives are used at comparable concentrations and under similar conditions. However, the FEEDAP Panel has reservations about the effectiveness of lactic acid and calcium lactate as preservatives in complete feedingstuffs with a typical moisture content of ≤ 12 %.

5. Recommendations

The additive should be described as lactic acid produced by fermentation with *Bacillus coagulans* (LMG S-26145 or DSM 23965), *Bacillus smithii* (LMG S-27890) or *Bacillus subtilis* (LMG S-27889).

The data provided would support a specification in which the maximum content of D-lactic acid is not more than 5 % and the content of L-lactic acid is at least 95 %.

Documentation provided to EFSA

1. Lactic acid and calcium lactate for all animal species. October 2010. Submitted by Acids Authorisation Consortium (ACIAC EEIG).

2. Lactic acid and calcium lactate for all animal species. Supplementary information. October 2013. Submitted by FEFANA asbl.

3. Lactic acid and calcium lactate for all animal species. Supplementary information. January 2014. Submitted by FEFANA asbl.

4. Lactic acid and calcium lactate for all animal species. Supplementary information. August 2014. Submitted by FEFANA asbl.

5. Lactic acid and calcium lactate for all animal species. Supplementary information. March 2015. Submitted by FEFANA asbl.

6. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for lactic acid and calcium lactate.

7. Comments from Member States received through the ScienceNet.

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Glossary

ADI	Acceptable daily intake
BIOHAZ	EFSA Scientific Panel on Biological Hazards
CAS	Chemical Abstracts Service
CV	Coefficient of variation
DM	Dry matter
EC	European Commission
EURL	European Union Reference Laboratory
EINECS	European Inventory of Existing Commercial Chemical Substances
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FLF	Fermented liquid feed
SCF	Scientific Committee for Food
WHO	World Health Organization

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the method(s) of analysis for lactic acid and calcium lactate

In the current application authorisation is sought under article 4(1) and 10(2) for *lactic acid (E 270)* and *calcium lactate (E 327)* under the category/functional group 1(a) "technological additives"/"preservatives", according to the classification system of Annex I of Regulation (EC) No 1831/2003.

According to the Applicant, *lactic acid* is a liquid consisting of a minimum of 72 % of lactic acid and a maximum of 8 % of other organic acids, the rest being water. *Calcium lactate* is a solid consisting of a minimum of 97 % (on dry matter) of calcium lactate and a maximum of 3 % of water.

Authorisation is sought for the use of the two *feed additives* for all animal species and categories. Both *feed additives* are to be used in *premixtures* and *feedingstuffs,* whereas *lactic acid* is also intended to be mixed into *water* for drinking, with no recommended minimum or maximum concentration levels. However typical concentration levels of 30 g/L for *water* or 30 to 50 g/kg *feedingstuffs* are suggested by the Applicant.

For the determination of *lactic acid* in *feed additive* the Applicant proposed the European Pharmacopoeia monographs 0458 and the internationally recognised FAO JECFA monograph for food additives, based on: - the tests for acid and lactates; - acid/base titration with 1 M sodium hydroxide and phenolphthalein as indicator. For the determination of *calcium lactate* in *feed additive* the Applicant proposed a set of European Pharmacopoeia monographs for the various forms of calcium lactate (2118 for calcium lactate, anhydrous; 2117 for calcium lactate monohydrate; 0468 for calcium lactate, pentahydrate; 0469 for calcium lactate trihydrate) together with the internationally recognised FAO JECFA monograph for food additives based on: - the tests for calcium and lactates; and - the complexometric titration of calcium with sodium ethylenediaminetetraacetate in aqueous solution. Even though no performance characteristics are provided, the EURL recommends for official control the above mentioned European Pharmacopoeia monographs and the FAO JECFA methods for the determination of *lactic acid* and *calcium lactate* in the *feed additives*.

For the quantification of *lactic acid* and *calcium lactate* (as total *lactic acid* content) in *premixtures, feedingstuffs* and *water* the Applicant proposed a single laboratory validated method based on high performance liquid chromatography with UV or refractive index detection (HPLC-UV/RI). This method does not distinguish between *lactic acid* and its salts. The following performance characteristics for the quantification of total *lactate*, expressed as total *lactic acid*, are reported for concentrations ranging from to 1 to 1000 g/kg: - a relative standard deviations for *repeatability* (RSD_r) ranging from 1.8 to 3.6 %; - a *recovery* rate (R_{rec}) ranging from 89 to 107 %; and - a limit of quantification (LOQ) of 0.46 g *lactic acid*/kg *feedingstuffs*. The HPLC-UV/RI method was further ring trial validated by five laboratories, and a relative standard deviation for *reproducibility* (RSD_R) ranging from 10.7 to 14.7 % was determined for *premixtures* and *feedingstuffs* containing from 7.1 to 53.3 g *lactic acid*/kg.

Based on the performance characteristics presented, the EURL recommends for official control the ring trial validated method based on ion-exclusion HPLC-UV/RI method to determine *lactic acid* and *calcium lactate* (expressed as *total lactic acid*) in *premixtures, feedingstuffs* and *water*.

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.