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Safety and efficacy of a feed additive consisting of lactic acid produced by *Weizmannia coagulans* (synonym *Bacillus coagulans*) DSM 32789 for all animal species except for fish (Jungbunzlauer SA)

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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 and efficacy of lactic acid produced by a non-genetically modified strain of *Weizmannia coagulans* (synonym of *Bacillus coagulans*) (DSM 32789) for all animal species except for fish. The production strain qualifies for the QPS approach for safety assessment. Although uncertainty remains concerning the possible presence of viable cells and/or spores of the production strain in the final product, this does not raise safety concerns for the target species, humans and the environment. The lactic acid is safe at 50,000 mg/kg complete feed for functional ruminants and pigs and at 20,000 mg/kg feed for all the other animal species and categories except for pre-ruminants for which a safe level cannot be established. The corresponding safe levels in water for drinking would be 15,000 mg/L water for pigs and 8,000 mg/L for other non-ruminant species. Although no safe concentration of lactic acid in water for drinking for ruminants can be derived, the Panel considers that the use in water for drinking is safe in ruminants when the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed. The use of the additive under assessment in animal nutrition is considered safe for the consumers and for the environment. It is considered corrosive to the skin, eyes and mucous membranes. Lactic acid is used in food as a preservative. It is reasonable to expect that the effect seen in food will be observed in feed when it is used at comparable concentrations and conditions. However, the FEEDAP Panel has reservations about its effectiveness as a preservative in complete feed with a moisture content of $\leq$ 12%.

Keywords: technological additives, preservatives, lactic acid, *Weizmannia coagulans* DSM 32789, safety, efficacy, QPS

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Table of contents

1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Jungbunzlauer S.A.[2] for authorisation of the product lactic acid produced using *Weizmannia coagulans* (synonym *Bacillus coagulans*) DSM 32789, when used as a feed additive (category: technological additives; functional group: preservatives) for all animal species excluding fish.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). The particulars and documents in support of the application were considered valid by EFSA as of 28 May 2020.

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, consumers, users and the environment and on the efficacy of the product lactic acid produced using *Weizmannia coagulans* DSM 32789, when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

Lactic acid produced by *W. coagulans* DSM 32789 has not been authorised as a feed additive in the EU.

The FEEDAP Panel has adopted several opinions on lactic acid when used as a preservative (EFSA FEEDAP Panel, 2015, 2017a, 2019a) or as a flavouring compound (EFSA FEEDAP Panel, 2012a).

Lactic acid (E 270) is presently listed in the European Union (EU) Register of Feed Additives as a technological additive (functional group: preservatives) for use in feed for all animal species and categories without restrictions, subject to the re-evaluation.[3] Lactic acid produced by chemical synthesis is authorised for use in feed as a flavouring compound up to 5 mg/kg of complete feed with a moisture content of 12% [2b08004].[4]

Lactic acid (E 270) is a permitted food additive 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[5] on food additives. Specifications for purity are laid down in Directive 2008/84/EC[6]. Lactic acid [08.004] is also authorised for use in food as flavour.[7]

[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] Jungbunzlauer SA. Z.I et Portuaire BP 32, 67390 Marckolsheim, France.

[3] European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. Available online: https://ec.europa.eu/food/safety/animal-feed/feed-additives/eu-register_en

[4] Commission Implementing Regulation (EU) 2017/56 of 14 December 2016 concerning the authorisation of lactic acid, 4-oxovaleric acid, succinic acid, fumaric acid, ethyl acetoacetate, ethyl lactate, butyl lactate, ethyl 4-oxovalerate, diethyl succinate, diethyl malonate, butyl-O-butyryl lactate, hex-3-enyl lactate, hexyl lactate, butyro-1,4-lactone, decano-1,5-lactone, undecano-1,5-lactone, pentano-1,4-lactone, nonano-1,5-lactone, octano-1,5-lactone, heptano-1,4-lactone and hexano-1,4-lactone as feed additives for all animal species. OJ L 13, 17.01.2017, p 129.

[5] 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

[6] 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.

[7] Commission Implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1.

L-(+)-Lactic acid is approved as biocidal active substance for several applications including human hygiene (Regulation (EU) 2016/2291),[8] disinfectant, veterinary hygiene and food and feed area (Regulation (EU) 2017/2002).[9]

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) issued an opinion on lactic acid and calcium lactate (JEFCA, 1974) allocating an acceptable daily intake (ADI) of 'not limited'. In 1991, this ADI was supported by the Scientific Committee for Food (European Commission, 1991), and later, it was iterated in the evaluation of lactate and sodium lactate for poultry carcass treatment (EFSA, 2006,2008). EFSA has issued several opinions on the use of lactic acid and calcium lactate for carcass decontamination (EFSA, 2006, 2008; EFSA BIOHAZ Panel, 2011; EFSA CEP Panel, 2018). The EFSA Panel on food additives and flavourings (FAF) re-evaluated the safety of several esters of fatty acids used as food additives, among which lactic acid esters of mono- and diglycerides of fatty acids (E 472b) (EFSA FAF Panel, 2020).

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[10] in support of the authorisation request for the use of lactic acid produced using *W. coagulans* DSM 23789 as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' knowledge, to deliver the present output.

The European Union Reference Laboratory (EURL) considered that the EURL-FA evaluation report issued on 2/21/2012 related to the dossier FAD-2010-0133 is valid and applicable for the current application. An addendum to that previous report was issued on 1 June 2021 that is also applicable to the lactic acid under assessment.[11]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of lactic acid produced using *W. coagulans* DSM 32789 is in line with the principles laid down in Regulation (EC) No 429/2008[12] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEEDAP Panel, 2017b), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017d), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019b).

3. Assessment

Lactic acid produced by *W. coagulans* DSM 32789 is intended to be used as a technological additive (functional group: preservative) in feed or water for drinking for all animal species except for fish.

[8] Commission Implementing Regulation (EU) 2016/2291 of 16 December 2016 approving L(+) Lactic acid as an active substance for use in biocidal products of product-type 1. OJ L 344, 17.12.2016, p. 74–76.

[9] Commission Implementing Regulation (EU) 2017/2002 of 8 November 2017 approving L(+) lactic acid as an existing active substance for use in biocidal products of product-types 2, 3 and 4. OJ L 290, 9.11.2017.

[10] FEED dossier reference: FAD-2019-0092.

[11] The full EURL report including the addendum report of FAD-2010-0133 is available on the EU Science Hub website: https://joint-research-centre.ec.europa.eu/publications/fad-2010-0133_en

[12] 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.

3.1. Characterisation

3.1.1. Characterisation of the production microorganism

The additive is produced by a non-genetically modified strain of *Weizmannia coagulans* (synonym *Bacillus coagulans* (Gupta et al., 2020)) which is deposited [redacted] with accession number DSM 32789.[13]

Taxonomical identification of the production strain was confirmed by assessing the Average Nucleotide Identity (ANI) and by digital DNA–DNA hybridisation (dDDH) based on the whole genome sequence (WGS).[14] Results showed an ANI value of 98.06% and a dDDH value of 82.8% comparing with the type strain DSM 1^T.

[redacted][16]

The susceptibility of the production strain to relevant antibiotics was tested against the list of antimicrobials described for '*Bacillus*' in the Guidance on characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a) which is considered to apply to the species under evaluation.[17] All the minimum inhibitory concentration (MIC) values were below the specified cut-off values. Therefore, *W. coagulans* DSM 32789 is considered susceptible to all relevant antimicrobials.

The WGS of the production strain, including the plasmids, was searched for the presence of antimicrobial resistance genes [redacted][18] [redacted][19] [redacted] No hits of concern were identified.

The toxigenic potential of the strain was assessed according to the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a).[20] [redacted] *W. coagulans* DSM 32789 is considered to be non-toxigenic.

The WGS data of the production strain, including the plasmid sequences, were also interrogated for the presence of genes coding for toxin and virulence factors [redacted][21] No hits of concern were identified.

3.1.2. Manufacturing process

Lactic acid is produced by fermentation using *W. coagulans* DSM 32789.[22] [redacted]

The applicant stated that no antimicrobial substances are used in the manufacturing process.

3.1.3. Characterisation of the additive/active substance

L-Lactic acid (International Union of Pure and Applied Chemistry (IUPAC) name: (2S)-2-hydroxypropanoic acid) is identified with the Chemical Abstracts Service (CAS) No 79-33-4, the European Inventory of Existing Commercial chemical Substances (EINECS) No 201-196-2) and the EU

[13] Technical dossier/Section II/Annex II.14 Conf. Deposited as *Bacillus coagulans* DSM 32789.
[14] Technical dossier/Supplementary information February 2021/Annex_1 and 2 and supplementary information December 2021/Annex 1.
[15] Technical dossier/Section II/Annex II.13 Conf and supplementary information December 2021, reply to Q2.
[16] Technical dossier/Supplementary information February 2021/Annex_1 CONF.
[17] Technical dossier/Section II/Annex II.17 Conf.
[18] Technical dossier/Supplementary information February 2021/Annex 3 CONF.
[19] Technical dossier/Supplementary information February 2021/Annex_1 CONF
[20] Technical dossier/Supplementary information February 2021/Annex_4 CONF.
[21] Technical dossier/Supplementary information February 2021/Annex_1 CONF and Annex 3 CONF.
[22] Technical dossier/Section II/Annexes II.18 Conf and II.19 Conf.

flavour information system (FLAVIS) number [08.004]. It has a molecular weight of 90.08 Da. Its molecular formula is $C_3H_6O_3$ and its structural formula is shown in Figure 1.

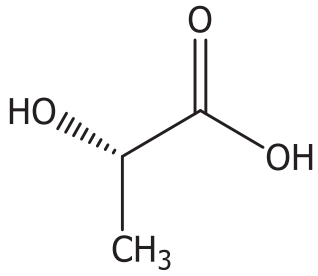


Figure 1: Structural formula of L-lactic acid

The additive is specified to contain ≥ 74% lactic acid on 'as is' basis.[23] Analysis of five batches showed an average L-lactic acid of [redacted][24] while water was on average [redacted][25] [redacted][26] and when the concentrations of D-lactic acid of these batches were derived from the specific optical rotation the concentrations ranged 0.1–2.6% w/w.[27] [redacted][28] [redacted][29] The amount of identified mater on 'as is' basis was 97.9% (97.2–98.4%).

Specifications are set for mercury (≤ 0.1 mg/kg), cadmium and arsenic (≤ 1 mg/kg), lead (≤ 5 mg/kg) and fluorine (≤ 150 mg/kg). The results of five batches analysed showed [redacted][30] The same five batches were tested for the presence of mycotoxins (aflatoxin B1, deoxynivalenol, zearalenone, ochratoxin A, fumonisins B1 and B2) and for pesticides (chlordanes, some endosulfan-α, -ɷ -sulfate and some isomers of dichlorodiphenyltrichloroethane [DDT]). [redacted][31]

The microbiological contamination of the additive was analysed in three batches. *Salmonella* spp. and *Listeria monocytogenes* were not detected in 25 g samples. Coagulase-positive staphylococci (including *Staphylococcus aureus*) were not detected in 10 g samples. *Escherichia coli* was not detected in 1 g sample. *Enterobacteriaceae* (at 30°C), total mesophilic microorganisms (at 30°C), yeast and moulds were all below the LOD.[32] Three additional batches were checked for the possible presence of viable cells and spores of *Bacillus cereus* and the results were < 10 CFU/g in all cases.[33]

The detected amounts of the above-mentioned impurities do not represent safety concerns.[34]

The applicant provided two sets of data regarding the possible presence of viable cells and spores of the production strain *W. coagulans* DSM 32789 in the additive. In the first set of data,[35] three batches of L-lactic acid were tested in triplicate. [redacted]

[23] Technical dossier/Section II/Annex II.8.
[24] Technical dossier/Section II/Annex II.2. Lactic acid was analysed by high-pressure liquid chromatography with ultraviolet and/or refractive index detector (HPLC-UV/RI).
[25] Technical dossier/Section II/Annex II.7.
[26] Technical dossier/Section II/Annex II.3. Analysed by chiral HPLC-UV/RI.
[27] Technical dossier/Section II/Annex II.12.
[28] Technical dossier/Section II/Annexes II.1 to II.7.
[29] LOQ in % were 0.1 for chlorides, ranged from 0.01 to 0.1 for potassium and were 0.001 for magnesium and calcium.
[30] Technical dossier/Section II/Annex II.6. Conf. LOQ (in mg/kg) was 0.002 for mercury, 0.2 for cadmium, 0.5 for lead and arsenic and 40 for fluorine.
[31] Technical dossier/Section II/Annex II.6. Conf. LOQ (in μg/kg) was 0.1 for deoxynivalenol, 0.5 for ochratoxin A, 0.6 for aflatoxin B1, 5 for zearalenone and 100 for fumonisins B1 and B2, and for pesticides ranged from 0.02 to 0.05 mg/kg depending on the pesticide considered.
[32] Technical dossier/Section II/Annex II.9. LOD in colony forming units (CFU) was 10 CFU/g.
[33] Technical dossier/Supplementary information December 2022/Annex 3_Purity B cereus/RA5280616, RA5280617 and RA5280618.
[34] Iron concentration is high, but considering piglets up to 1 week before weaning (the category with the lowest authorised maximum iron content [250 mg iron/day] of the Commission Implementing Regulation (EU) 2017/2330) the use of the additive would contribute with 17 mg iron/kg compound feed. Consequently, the iron concentrations detected do not raise safety concerns.
[35] Technical dossier/Supplementary information February 2021/Annex_5 CONF.

[redacted] The results indicated no growth. However, the Panel notes that a positive control was not included in the analysis and spiking tests with cell suspensions of the production strain indicated that L-lactic acid inhibited the growth of cells of the production strain until at least dilution 10^{-3}. In a second analysis,[36] the presence of the production strain was tested in three batches of the additive. [redacted] No viable cells or spores were detected in the samples tested. However, the Panel considers that the amount of sample tested for the presence of the production strain is very low and not sufficient to draw conclusions. Therefore, although the available data do not indicate the presence of the production strain, uncertainty remains on the possible presence of viable cells and/or spores of *W. coagulans* DSM 32789 in the final product.

3.1.4. Stability and homogeneity

The shelf-life of the additive was studied in four batches stored at room temperature in sealed containers protected from light for 3 years. Losses observed ranged from 0% to 1%.[37]

The stability of one batch of the additive was studied when supplemented at 1% in three different feeds (pelleted and mash forms of each feed): a grower feed for chickens for fattening, a feed for weaned piglets and a concentrate feed for high-yield dairy cows, all containing choline chloride.[38] Feed conditioning was performed at 74°C and pelleting at 77°C. Feed samples were stored at 20°C in polyethylene bags for 3 months. After the pelleting losses of lactic acid were 11, 8 and 7% in chickens, piglets and cows' feed, respectively. After 3 months of storage, the losses of lactic acid in mash/pelleted feed were 9/3%, 7/4% and 4%/negligible in chickens, piglets and cows' feed, respectively.

The stability of one batch of the additive in water for drinking was studied at a concentration of 0.5% stored in closed containers at room temperature for 48 h.[39] A loss of 1% was observed.

The homogeneous distribution of the additive in feed was measured by analysing six subsamples of each of the compound feeds described above. Coefficients of variation ranged from 3% to 4% in mash feed and from 1 to 3% in pelleted feed.[40]

3.1.5. Physical characteristics

The additive is an aqueous brown viscous liquid. It is miscible in water, has a density of 1,200–1,300 kg/m^3 at 20°C, and a pH < 2 at 25°C.[41] Surface tension was measured in one batch and resulted in 43.4 mN/m.[42] The viscosity was measured in five batches, ranging from 86 to 134 mPa-s.[43]

3.1.6. Conditions of use

The additive is intended to be used as a preservative in complete feed and water for drinking for all animal species except for fish.[44] Proposed use levels in complete feed are 50,000 mg/kg in feed for pigs and functional ruminants, and 20,000 mg/kg in feed for poultry and all other species and animal

[36] Technical dossier/Supplementary information December 2021/2021-12-07 FAD-2019-0092 Sin 210621 Jungbunzlauer SA reply, reply question 4 and Annex 4.
[37] Technical dossier/Section II/Annex II.20. Lactic acid analysed by titrimetric method.
[38] Technical dossier/Section II/Annex II.22.
[39] Technical dossier/Section II/Annex II.21.
[40] Technical dossier/Section II/Annex II.22 pages 17 and 18.
[41] Technical dossier/Section II/Annex II.23.
[42] Technical dossier/Section II/Annex II.10.
[43] Technical dossier/Section II/Annex II.11.
[44] Technical dossier/Section II.2.4.1.2 and supplementary information August 2020/Reply to question 3.

categories. When used in water for drinking, a unique concentration of 15,000 mg/L is proposed for pigs, functional ruminants,[45] poultry and all other animal species and categories.[46]

3.2. Safety

3.2.1. Safety of the production microorganism

The bacterial species *W. coagulans* is considered by EFSA to be suitable for the Qualified Presumption of Safety (QPS) approach to safety assessment (EFSA, 2007; EFSA BIOHAZ Panel, 2020, 2022). This approach requires the identity of the strain to be conclusively established and evidence that the strain lacks toxigenic potential and does not show acquired antimicrobial resistance determinants for antibiotics of human and veterinary importance. In the view of the FEEDAP Panel, the identity of the production strain is unambiguously established, the lack of toxigenic potential confirmed and *W. coagulans* DSM 32789 does not show acquired antimicrobial resistance determinants. Uncertainty remains on the possible presence of the production strain in the final product. Since the strain qualifies for the QPS approach for safety assessment, the presence of viable cells and/or spores of the production strain in the additive would not raise a safety concern for the target species, consumers and the environment.

3.2.2. Safety for the target species

To support the safety of the additive for the target species, the applicant referred to the studies already assessed in the previous opinions of the FEEDAP Panel on lactic acid (EFSA FEEDAP Panel, 2015, 2017a–d, 2019a,b).[47]

Based on the studies in ruminants, weaned pigs and chickens for fattening, the FEEDAP Panel concluded that lactic acid is safe at 50,000 mg lactic acid/kg feed for functional ruminants and pigs; and at 20,000 mg lactic acid/kg feed for all the other animal species and categories with the exception of pre-ruminants for which a safe dose could not be established. Safe levels in water for drinking were derived for pigs at 15,000 mg lactic acid/L water and for other monogastric species at 8,000 mg lactic acid/L. The Panel notes that the composition and the conditions of use of the additive under assessment are the same as the one previously assessed and currently authorised, and therefore, the studies assessed in the previous opinions (EFSA FEEDAP Panel, 2015, 2017a–d, 2019a,b) can be used to support the safety in the present evaluation.

In addition, the applicant carried out two extensive literature searches (ELS) covering the period between 2016 and August 2019, with the scope to assess the safety of lactic acid for pigs, ruminants[48] and poultry.[49] Search engines used included Science Direct, ETH library database, CABI, Scopus, Web of Science, Toxnet, Agricola and Ovids. Search terms, the different search strings and the outcome of the different searches were reported.[50] Logic inclusion and exclusion criteria were applied. A total of 21 scientific papers (6 in pigs, 12 in ruminants and 3 in poultry) were considered relevant by the applicant. After reviewing these papers, the FEEDAP Panel did not identify any new relevant information for pigs and non-functional ruminants.

For functional ruminants, a total of 12 studies were considered relevant by the applicant (eight studies conducted in dairy cows, two in goats and two in sheep). From these studies, only one (Nkosi et al., 2016) could be considered in the assessment and is described below. The rest of the studies were considered not relevant as the lactic acid was included at a level below the maximum recommended level, or the study design was not appropriate. Nkosi and colleagues (2016) evaluated in a digestibility trial the effects of ensiling forage soybean (*Glycine max* (L.) Merr.) with or without bacterial inoculants on the fermentation characteristics, aerobic stability and nutrient digestion in Damara rams (48.2 ± 2.51 kg live weight).[51] Lactic acid content in the diets (DM basis) was 55 g/kg in the control; 38 g/kg in a group fed silage obtained from a lactic acid bacteria inoculant; and 70 g/kg (1.4x) in a group fed silage obtained from different inoculants. Feed intake (forage soybean silage + concentrate, values 1.30, 1.37 and 1.55 kg of DM, respectively) and digestibility of DM, organic matter

[45] Technical dossier/Supplementary information February 2022 on conditions of use.
[46] Technical dossier/Supplementary information August 2020/Reply to question 2.
[47] Technical dossier/Section III/Annex III.35 and Supplementary information December 2021/Annex 5.
[48] Technical dossier/Section III/Annexes III.15 and III.16.
[49] Technical dossier/Section III/Annex III.29. Supplementary information via e-mail 17/02/222.
[50] Technical dossier/Section III/Annex III.15.
[51] Technical dossier/Section III/Annex III.24.

and nitrogen significantly improved at 1.4x, whilst the digestibility of fibre fractions was significantly reduced at 1.4x.

For poultry, the information retrieved was scarce. One study could not be considered in the assessment because the concentration of lactic acid used could not be confirmed. In the two studies conducted by Yeh et al. (2018),[52] the effect of lactic acid (up to 20,000 mg/kg) in fermented feed (pelleted or not) was studied when feeding the chickens for fattening for 21 or 35 days (depending on the study). No adverse effects in performance were observed in any of the studies. In the second one, the analysis of serum biochemical constituents, however, showed a significant decrease of the levels of lactate dehydrogenase: 2,758[a] U/L in control group (receiving 4,000 mg lactic acid/kg feed) vs. 2,195[ab] U/L in fermented feed and 1,753[b] U/L in pelleted fermented feed (these two last groups receiving 20,000 mg lactic acid/kg feed). This decrease in lactate dehydrogenase is considered of no clinical relevance.

The information provided in the ELS does not provide any new evidence that would lead the Panel to reconsider the previously drawn conclusions.

3.2.2.1. Conclusions on safety for the target species

The Panel concludes that lactic acid is safe at 50,000 mg lactic acid/kg feed for functional ruminants and pigs and at 20,000 mg lactic acid /kg feed for all the other animal species and categories with the exception of pre-ruminants for which a safe dose cannot be established. In water for drinking, the corresponding maximum levels are 15,000 mg lactic acid/L water in pigs and 8,000 mg lactic acid/L for other non-ruminant species. Although no safe concentration of lactic acid in water for ruminants can be derived, the Panel considers that the use in water for drinking is safe in ruminants provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed.

3.2.3. Safety for the consumer

Lactate is an endogenous substance (in carbohydrate and amino acid metabolism) and a natural component of many foods. Under conditions of heavy energy demand (and thus high oxygen need), skeletal muscles convert glucose anaerobically into lactic acid, which is excreted from the muscle cells into the blood. In the liver, this lactic acid is reduced to glucose. Ultimately any absorbed lactic acid will be oxidised to give CO_2 and water (EFSA BIOHAZ Panel, 2011). Lactic acid is not expected to accumulate in tissues and products derived from food-producing animals treated with the additive according to the recommended conditions of use. The use of the product under assessment in animal nutrition according to the recommended conditions of use is considered safe for the consumers.

3.2.4. Safety for the user

No studies have been submitted to support the safety of the additive under assessment for the users. The additive has a pH < 2 and is considered corrosive to skin, eyes and mucous membranes.

The additive is therefore considered a hazard for the users.

3.2.5. Safety for the environment

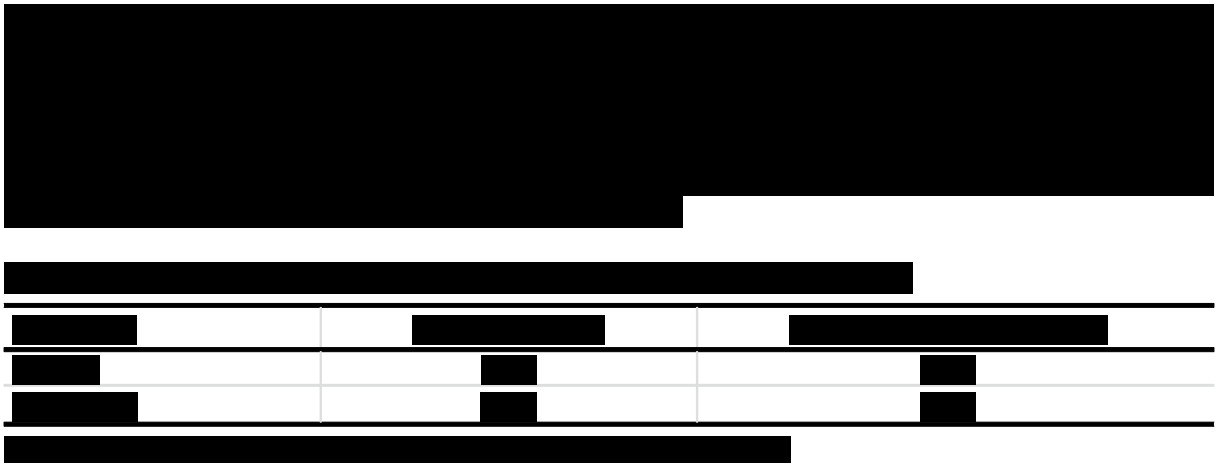
Lactic acid is a natural substance and its use in animal feed is not expected to substantially alter the concentration and/or distribution of the substance in the receiving environment. Therefore, a risk for the environment resulting from the use of the additive under assessment in animal nutrition is not foreseen.

3.3. Efficacy

The applicant provided an efficacy study testing the storage stability of feedingstuffs under stress conditions with or without the addition of the lactic acid under assessment.[53] [redacted]

[52] Technical dossier/Section III/Annex III.30.

[53] Technical dossier/Section IV/Annex IV.23 CONF and supplementary information February 2021/Annex 6 1-5/Annexes 6.2 to 6.5.



The applicant provided a few selected papers from a literature search to support the efficacy of the additive on animal performance, nutrient availability and feed palatability in food-producing animals. The search was poorly reported and none of the papers submitted was considered to provide any relevant information on the efficacy of lactic acid as a preservative in feed (they did not measure microbial contamination in feed, or were not reflecting practical use conditions, or there was no information on the amount of lactic acid included).

Lactic acid is used in food as a preservative. It is reasonable to expect that the effect observed in food would be observed in feed when this additive is used at comparable concentrations and under similar conditions. Nevertheless, the FEEDAP Panel has reservations about the effectiveness of lactic acid as a preservative in complete feed with a moisture content of $\leq$ 12% (EFSA FEEDAP Panel 2015). 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.

3.3.1. Conclusions on efficacy

Lactic acid is used in food as a preservative. It is reasonable to expect that the effect seen in food will be observed in feed when this additive is used at comparable concentrations and under similar conditions. However, the FEEDAP Panel has reservations about the effectiveness of lactic acid as a preservative in complete feed with a moisture content of $\leq$ 12%.

4. Conclusions

The production strain *W. coagulans* DSM 32789 qualifies for the QPS approach for safety assessment and although uncertainty remains concerning the possible presence of viable cells and/or spores of the production strain in the final product, this does not raise safety concerns for the target species, humans, and the environment.

The Panel concludes that lactic acid is safe at 50,000 mg/kg complete feed for functional ruminants and pigs and at 20,000 mg/kg feed for all the other animal species and categories except for pre-ruminants for which a safe level cannot be established. The corresponding safe levels in water for drinking would be 15,000 mg/L water for pigs and 8,000 mg/L for other non-ruminant species. Although no safe concentration of lactic acid in water for drinking for ruminants can be derived, the Panel considers that the use in water for drinking is safe in ruminants provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed.

The use of lactic acid produced by *W. coagulans* DSM 32789 in animal nutrition is considered safe for the consumers and for the environment.

The additive is considered corrosive to the skin, eyes and mucous membranes.

Lactic acid is used in food as a preservative. It is reasonable to expect that the effect seen in food will be observed in feed when this additive is used at comparable concentrations and under similar conditions. However, the FEEDAP Panel has reservations about the effectiveness of lactic acid as a preservative in complete feed with a moisture content of $\leq$ 12%.

5. Documentation provided to EFSA/Chronology

Date	Event
19/12/2019	Dossier received by EFSA. Lactic acid produced using *Weizmannia coagulans* (synonym of *Bacillus coagulans*) DSM 32789 for all animal species. Submitted by Jungbunzlauer S.A.
08/05/2020	Reception mandate from the European Commission
01/06/2020	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
28/05/2020	Application validated by EFSA – Start of the scientific assessment
22/07/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: Characterisation of the additive, conditions of use, safety for the target species, Characterisation of the production strain*.
21/08/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
01/09/2020	Comments received from Member States
17/09/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: Characterisation of the additive, characterisation of the production strain, efficacy*
15/02/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
21/06/2021	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: Characterisation of the additive, characterisation of the production strain, safety for the target species*.
08/12/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
24/03/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ADI	average daily intake
BIOHAZ	EFSA Scientific Panel on biological hazards
BW	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CFU	colony forming unit
CV	coefficient of variation
DM	dry matter
EURL	European Union Reference Laboratory
FAO	Food Agricultural Organization
FCR	feed conversion ratio
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
MW	molecular weight
WHO	World Health Organization

Annex A – Addendum to the Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for lactic acid (E 270) and Calcium lactate (E 327)

Upon the recent publication of new ring-trial validated methods EN 17294 [1] and EN 17298 [2] for the analysis of organic acids in feed additives, premixtures, feed materials, compound feed and water, the EURL, under the frame of article 5 of Regulation (EC) No 378/2005 [3], considered appropriate to perform a new evaluation of the methods of analysis for official control of lactic acid and calcium lactate in the feed additives, premixtures, feedingstuffs and water, in the frame of the above-mentioned feed additive dossier. In this line, aiming to recommend the available analytical methods complying with the highest requirements as stated in Annex II of Regulation (EC) No 429/2008 [4], the EURL also updates in this amendment the relevant methods for calcium.

For the determination of lactic acid and calcium lactate (as total lactic acid) in the feed additives, premixtures, feedingstuffs and water the EURL evaluated ring-trial validated EN 17294 method based on ion chromatography coupled to conductivity detection (IC-CD) [1]. This method is designed for the determination of formic, lactic, propionic, citric, fumaric, malic and acetic acids and their salts (as total individual acids) in feed additives, premixtures, feed materials, compound feed and water [1].

According to the method, 5 g of sample is mixed with 100 ml of water and the mixture is stirred for 60 min (or sonicated for 30 min). The resulting extract (after adjustment to ambient temperature in case of fumaric acid) is filtered using ash free paper filter or centrifuged at 5,000 g for 3 min. The filtrate or the supernatant after the dilution is filtered through a membrane filter before the chromatographic analysis. The individual analytes are detected by ion conductivity detection and the quantification is performed using an external standard calibration curve prepared from the standard solutions of the above-mentioned acids [1].

Table A.1: The performance characteristics obtained in the frame of the ring-trial validation studies of the EN 17294 method [1] for the quantification of lactic acid in *premixtures*, *feedingstuffs* (feed materials, complementary feed and compound feed) and *water*

	Premixtures	Feedingstuffs	Water
Mass fraction, mg/kg	28,055–55,908	2,667–90,601	993
RSDr, %	3.5–4.1	1.3–4.6	0.7
RSDR, %	11.3–12.8	4.9–11.2	4.7
Reference	[1]		

RSDr and RSD_R: *relative standard deviations for repeatability and reproducibility, respectively*.

The performance characteristics obtained in the frame of the ring-trial validation studies of the EN 17294 method for the quantification of lactic acid in premixtures, feedingstuffs (feed materials, complementary feed, compound feed) and water are presented in Table A.1. In addition, a limit of quantification (LOQ) of 200 mg for lactic acid/kg feedingstuffs is reported [1].

Based on the performance characteristics presented and the scope of the method in terms of matrices, the EURL recommends for official control the ring-trial validated EN 17294 method based on ion chromatography coupled to conductivity detection (IC-CD) for the determination of lactic acid and calcium lactate (as total lactic acid) in the feed additives, premixtures, feedingstuffs and water.

In addition, in the frame of a similar organic acid dossier [5], the EURL has evaluated and recommended for official control for the determination of total calcium in the feed additive the two ring-trial validated methods, namely (i) EN ISO 6869 based on atomic absorption spectrometry (AAS) [6] and (ii) EN15510 based on inductively coupled plasma-atomic emission spectrometry (ICP-AES) [7]. These recommendations are also valid in the frame of this addendum.

Recommended text for the registry entry (analytical methods) (replacing the previous recommendations)

For the determination of lactic acid and calcium lactate (as total lactic acid) in the feed additives, premixtures, feedingstuffs and water:

– Ion chromatography with conductivity detection (IC-CD) - EN 17294

For the determination of total calcium in the feed additive (calcium lactate):

- Atomic absorption spectrometry (AAS) - EN ISO 6869; or
- Inductively coupled plasma-atomic emission spectrometry (ICP-AES) - EN15510

References

[1] EN 17294 Animal feeding stuffs: Methods of sampling and analysis - Determination of organic acids by Ion Chromatography with Conductivity Detection (IC-CD) - Complementary element.
[2] EN 17298 Animal feeding stuffs: Methods of sampling and analysis - Determination of benzoic and sorbic acid by High Performance Liquid Chromatography (HPLC).
[3] Commission Regulation (EC) No 378/2005 of 4 March 2005 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the duties and tasks of the Community Reference Laboratory concerning applications for authorisations of feed additives, OJ L 59, 5.3.2005, p. 8.
[4] 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 authorisations of feed additives, OJ L 133 22.5.2008, p. 1.
[5] EURL evaluation report. Available online: https://joint-research-centre.ec.europa.eu/publications/fad-2010-0133_en
[6] ISO 6869:2000 - Animal feeding stuffs - Determination of the contents of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc — Method using atomic absorption spectrometry.
[7] EN 15510:2017 - Animal feeding stuffs: Methods of sampling and analysis - Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum and lead by ICP-AES.

Executive summary of Lactic acid and Calcium lactate (FAD-2010-0133)

(available online: https://joint-research-centre.ec.europa.eu/publications/fad-2010-0133_en)

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 1 to 1000 g/kg: a relative standard deviations for *repeatability* (RSDr) ranging from 1.8 to 3.6%;

a *recovery* rate (Rrec) 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* (RSDR) 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.