European Food Safety Authority

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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of citric acid when used as a technological additive (preservative) for all animal species[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

An application has been made for the re-authorisation of citric acid (anhydrous and monohydrate) when used as a preservative in feed and also for the new use in water for drinking for all animal species. Citric acid (anhydrous and monohydrate) is already authorised for use in food and feed as a preservative following the *quantum satis* principle. There is evidence from published studies that citric acid is safe for the target species when used up to 30 000 mg citric acid/kg complete feedingstuffs and the corresponding concentration in water for drinking (10 000 mg citric acid/L). The additive is, consequently, also safe for the target species at the proposed conditions of use of 15 000 mg citric acid/kg complete feedingstuffs and 5 000 mg citric acid/L in water for drinking. The quality of available data does not allow a margin of safety to be derived. The use of citric acid in animal nutrition is safe for the consumer. It is prudent to regard citric acid as potentially hazardous to workers by exposure of the skin, eyes or mucous membranes or by inhalation. The use of citric acid in animal nutrition would not pose a risk to the environment. Although citric acid is a well-recognised preservative in food, based on data provided the effectiveness of citric acid as a preservative in feedingstuffs and water for drinking was not sufficiently demonstrated. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has reservations about the effectiveness of organic acids as preservatives in feedingstuffs with a typical moisture content of ≤ 12 %.

KEY WORDS

citric acid, preservative, all animal species, safety, efficacy

[1] On request from the European Commission, Question No EFSA-Q-2011-00750, adopted on 27 January 2015.

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SUMMARY

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of citric acid as a preservative in feed and water for drinking for all animal species.

Citric acid (anhydrous and monohydrate) is approved as a food additive for use as a preservative (*quantum satis*) in a wide range of commonly consumed foods and is authorised as a preservative in feed for all animal species without restrictions.

Specific tolerance studies were not available for the target species. Nevertheless, in a number of published studies referenced by the applicant and from other sources, trials were carried out in different animal species (poultry, pigs, ruminants) and with dietary concentrations of citric acid higher than the highest proposed typical use level. Although the studies showed some limitations, no negative impacts on mortality, health or performance characteristics were reported in any of these studies, when used up to 30 000 mg citric acid/kg complete feedingstuffs and the corresponding concentration in water for drinking (10 000 mg citric acid/L). The FEEDAP Panel concludes that the additive is, consequently, also safe for the target species at the proposed conditions of use of 15 000 mg citric acid/kg complete feedingstuffs and 5 000 mg citric acid/L in water for drinking. The quality of available data, however, does not allow a margin of safety to be derived.

The use of citric acid in animal nutrition is safe for the consumer.

It is prudent to regard citric acid as potentially hazardous to workers by exposure of the skin, eyes or mucous membranes or by inhalation.

Citric acid is a normal constituent of the diet of humans and animals and, when ingested, is rapidly and completely metabolised to carbon dioxide and water; therefore, the use of citric acid in animal nutrition would not pose a risk to the environment.

Although citric acid is a well-recognised preservative in food, based on data provided the effectiveness of citric acid as a preservative in feedingstuffs and water for drinking was not sufficiently demonstrated.

The FEEDAP Panel has reservations about the effectiveness of organic acids as preservatives in feedingstuffs with a typical moisture content of ≤ 12 %.

The FEEDAP Panel made a recommendation regarding the description of the additive.

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BACKGROUND

Regulation (EC) No 1831/2003[4] 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 and 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)[5] for authorisation/re-evaluation of the product citric acid when used as a feed additive for all animal species (category: technological additive; functional group: preservative) under the conditions mentioned in Table 1.

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.[6] According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. The particulars and documents in support of the application were considered valid by EFSA as of 29 June 2011.[7]

Citric acid is presently listed in the EU Register of Feed Additives as a technological additive (functional group: preservative) for use in feed for all animal species with no limits on age and levels in feed.[8]

Citric acid (anhydrous and monohydrate) is currently authorised as a food additive[9] (E 330) for general use in foodstuffs following the *quantum satis* principle.

Citric acid was assessed by the Joint FAO/WHO (Food and Agriculture Organization/World Health Organization) Committee on Food Additives (JECFA, 1974). Citric acid was also evaluated by the Scientific Committee for Food (EC, 1991).

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, the consumer, the user and the environment and the efficacy of the product citric acid, when used under the conditions described in Table 1.

[4] 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.

[5] 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: Impextraco N.V., Belgium; Provimi, Belgium; Selko B.V., the Netherlands.

[6] EFSA Dossier reference: FAD-2010-0357.

[7] A new mandate was received in EFSA on 13/05/2011.

[8] 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

[9] European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners OJ L 61, 18.3.1995, p. 1.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	Citric acid			
Registration number/EC No/No (if appropriate)	E 330			
Category(ies) of additive	1. Technological additives			
Functional group(s) of additive	a. Preservatives			
Description				
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)	
Citric acid	$C_6H_8O_7$ (anhydrous) or $C_6H_8O_7$ H_2O (monohydrate)	Min. 99.5 % (anhydrous)	HPLC	
Trade name (if appropriate)	Not applicable			
Name of the holder of authorisation (if appropriate)	Not applicable			
Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg/kg of complete feedingstuffs		
All species and categories of animal	None specified	None specified	None specified	None specified
Other provisions and additional requirements for the labelling				
Specific conditions or restrictions for use (if appropriate)	None specified			
Specific conditions or restrictions for handling (if appropriate)	None specified			
Post-market monitoring (if appropriate)	Not applicable			
Specific conditions for use in complementary feedingstuffs or water (if appropriate)	Not applicable			
Maximum Residue Limit (MRL) (if appropriate)				
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues	
Not applicable	Not applicable	Not applicable	Not applicable	

ASSESSMENT

This opinion is based, in part, on data provided by a consortium of three companies involved in the production/distribution of citric acid. It should be recognised that these data cover only a fraction of the existing additives containing citric acid. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has sought to use data provided together with data from other sources to deliver an opinion.

1. Introduction

Citric acid occurs in all living organisms as an intermediate in the tricarboxylic acid or Krebs cycle. It is a natural component of fruit, vegetables and plants (roots and leaves). The acid is found in significant quantities in lemon and lime juices (41 and 39 mg/kg for ready-to-consume juice and 31 and 30 mg/kg for concentrates, respectively). Levels of citric acid in commercial lemon juice-based drinks, such as lemonade, fall in the range of 0.96 to 0.62 mg/kg. Other dietary sources of citric acid include grapefruits and oranges (significant levels), berries and beans.

Citric acid is currently listed in the European Union Register of Feed Additives as a technological additive (functional group: preservative) for use in feed for all animal species without restrictions and subject to re-evaluation.[10]

Citric acid (E 330, anhydrous and monohydrate) is approved as a food additive[11] for general use in foodstuffs following the *quantum satis* principle (limits set for some food products, i.e. juices, infant foods). It has a long history of use as an additive in food,[12] cosmetics,[13] pharmaceuticals (human and veterinary), plant protection products, biocides[14] and household cleaning products.

Citric acid has been assessed by the Joint FAO/WHO (Food and Agriculture Organization/World Health Organization) Experts Committee on Food Additives (JECFA, 1974). The Committee allocated an acceptable daily intake (ADI) of 'not limited' for citric acid and its calcium potassium and sodium salts. This position was retained by the Scientific Committee on Food (SCF) (EC, 1991).

The application is for the re-evaluation of citric acid (anhydrous and monohydrate) as a technological additive when used as a preservative in feed. The application is also seeking a new authorisation for the use of citric in water for drinking.

2. Characterisation

2.1. Characterisation of the active substance.

Citric acid (synonyms: 2-hydroxy-1,2,3-propanetricarboxylic acid, β-hydroxytricarballylic acid, 3-carboxy-3-hydroxypentanedioic acid) exists as colourless crystals or as a white/almost white crystalline powder which is practically odourless.[15]

It exists in both the anhydrous form (Chemical Abstracts Service (CAS) number 77-92-9, chemical formula $C_6H_8O_7$, molecular weight 192.12 g/mol) or the monohydrate form (CAS number 5949-29-1,

[10] 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

[11] European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. OJ L 61, 18.3.1995, p. 1.

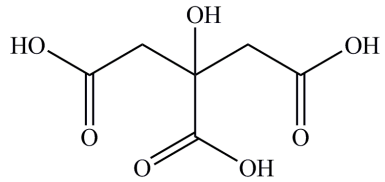
[12] Available online: http://www.codexalimentarius.net/gsfaonline/additives/details.html?id=173

[13] European Commission Database on Cosmetic Ingredients (CosIng, 2010). Available online: http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=32858

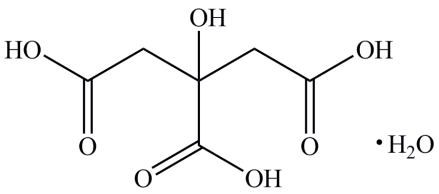
[14] Commission Regulation (EC) No 1451/2007 of 4 December 2007 concerning the placing of biocidal products on the market OJ L 325, 11.12.2007, p. 3.

[15] 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.

chemical formula $C_6H_8O_7 \cdot H_2O$, molecular weight 210.14), containing by specification at least 99.5 % (w/w) of the active substance in the dry matter (DM). This specification is in line with the specification set by Commission Directive 2008/84/EC for citric acid as a food additive (99.5 % in DM). The structural formula of citric acid is shown in Figure 1.



(anhydrous)



(monohydrate)

Figure 1: Structural formula of citric acid

Analyses carried out on five batches from each company (C1, C2, C3) of the anhydrous form, and five samples from two manufacturers of the monohydrate form, showed that this specification was met in all cases (range 99.8–100.1, mean 99.9 % w/w).[16,17,18]

2.2. Impurities

Five batches of each anhydrous and monohydrate form, from each company, were analysed for impurities. All data complied with the thresholds set by the food regulation[19] (sulphated ash < 0.05 %, arsenic < 1 mg/kg, lead < 1 mg/kg, mercury < 1 mg/kg, heavy metals < 5 mg/kg and oxalates, expressed as oxalic acid < 100 mg/kg).[20,21,22]

The microbial purity of the additive was analysed in commercial batches of anhydrous citric acid and in the monohydrate form from each company (C1 (10 batches), C2 (7 batches), C3 (10 batches)): bacterial endotoxins < 0.5 IU/mg; coliforms negative/10 g; total aerobes < 50 colony-forming units (CFU)/10 g; yeast and filamentous fungi < 100 CFU/g; *Salmonella* negative in 25 g; and *Staphylococcus aureus* negative in 25 g.[23]

Several batches of anhydrous and monohydrate citric acid from each company (C1 (six batches), C2 (four batches), C3 (three batches)) were analysed for mycotoxins (aflatoxins B1, B2, G1 and G2 and ochratoxin A).[24,25] In all cases, the values obtained were below the respective limit of detection

[16] Technical Dossier/Section II/Annex_II_1_Analytical Data.
[17] Supplementary information, January 2014/Annex_Certificates of analysis_CAA.
[18] Supplementary information, January 2014/Annex_Certificates of analysis_CAM.
[19] 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.
[20] Technical Dossier/Section II/Annex_II_1_Analytical Data.
[21] Supplementary information, January 2014/Annex_Certificates of analysis_CAA.
[22] Supplementary information, January 2014/Annex_Certificates of analysis_CAM.
[23] Technical Dossier/Section II/Annex_II_1_Analytical Data.
[24] Technical Dossier/Section II/Annex_II_1_Analytical Data.
[25] Supplementary information, August 2014.

(LOD).[26] Additional analytical data on mycotoxins were provided on three batches (two anhydrous, one monohydrate) by the third company (C3). In all cases, levels of aflatoxins B1, B2, G1 and G2 were < 0.5 µg/kg.[27]

Citric acid is produced by fermentation with *Aspergillus niger* (see section 2.4). Some strains of *Aspergillus niger* are known to produce a highly toxic metabolite, malformin C. Analysis of malformin C content in three (C3) or two (C1) commercial batches of anhydrous and in three (C2) commercial batches of monohydrate citric acid, showed that levels, in all cases, were below the LOD (< 1 µg/kg) in all cases.[28]

2.3. Physical properties

Citric acid (anhydrous) is characterised by a high solubility in water (592 g/L, 20 °C), is freely soluble in ethanol and sparingly soluble in ether; citric acid (monohydrate) is soluble in water and sparingly soluble in ether. pK_a values are: pK_1 = 3.14, pK_2 = 4.77 and pK_3 = 6.39. The densities (g/cm^3) are 1.665 (anhydrous) and 1.542 (monohydrate).

The particle size distribution was analysed by sieve analysis from three commercial batches of medium-granulated citric acid, stated to be the most common form of anhydrous citric acid used in animal feed, to facilitate dosing.[29] Approximately 1 % (0.7–1.1 % w/w) of the particles had a diameter of less than 100 µm. Citric acid monohydrate may be marketed in a range of granulated forms. Particle size distributions range from 225 to 3 000 µm, indicating the absence of particles of less than 100 µm. In addition, other products with the smallest particle size from two companies (C1, C2) were tested by laser diffraction and showed that 1.9 and 46.1 % (v/v) of particles had a diameter < 50 µm and 0.6 and 13.8 % (v/v) of particles had a diameter of < 10 µm.[30]

The dusting potential of citric acid (Stauber–Heubach method) was measured in three batches of the anhydrous form (C1, C2, C3)[31,32] and one batch of the monohydrate form (C3)[33] with values of 0.005, 0.050 and 0.045 g/m^3 and 0.51 g/m^3, respectively.

2.4. Manufacturing process

All three companies involved in the application produce citric acid via the fermentation of carbohydrate-based substrates with non-genetically modified strains of *Aspergillus niger*. After aerobic fermentation, the downstream process includes removal of solids, purification by adsorption and ion-exchange chromatography and, finally, concentration and crystallisation.

The first company describes the production strain as *Aspergillus niger* van Tieghem ZS9, derived from ATCC 26550. The original ATCC 26550 strain was subjected to classic mutagenesis (ultraviolet (UV) radiation and nitrosoguanidine or sodium nitrite treatment) and selected for increased production of citric acid. The present production strain is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH culture collection with the accession number DSM 25794.[34] Strain identity was established by its culture collection origin and its morphological, physiological and molecular properties. The ochratoxin gene of the strain was recently sequenced and compared with its ancestor strain ATCC 1015 (ATCC 1015 → ATCC 26550 → ZS9) deposited in 1917. Data showed that the inactive fragment of the ochratoxin gene is identical in both cases.

[26] LOD: aflatoxins < 0.2 µg/kg; sum of aflatoxins: < 1.0 µg/kg; ochratoxin A: < 1.0 µg/kg.
[27] Supplementary information, January 2014/Annex_toxin-virulence.
[28] Supplementary information, August 2014.
[29] Technical Dossier/Section II/Annex_II_1_Analytical Data.
[30] Supplementary information, January 2013/ Annex_Qiv_Citric acid_PSD and dusting potential.
[31] Supplementary information, January 2013/ Annex_Qiv_Citric acid_PSD and dusting potential.
[32] Supplementary information, January 2014/Annex_dust-pot_CAA.
[33] Supplementary information, January 2014/Annex_dust-pot_CAM.
[34] Supplementary information, January 2013/Annexes/Annex _Qv.

According to the manufacturer, the production of citric acid has been maintained at a high and stable level for decades; therefore, it is concluded that the production strain is genetically stable.

The second company specifies that its production strain, *Aspergillus niger* T419-RZBC, has been deposited in the China General Microbiological Culture Collection Center (CGMCC) as CGMCC 5751 and 4513.[35] The company declared that the two deposition numbers represent one and the same single strain. Strain identity was established by its phenotypic properties and by ribosomal RNA (rRNA) gene sequences (internal transcribed spacers within the ribosomal transcript).[36] Genetic stability was assessed by repeated passage of the strains and the mutation rate for rRNA measured, confirming the lack of genetic drift.

The third company uses the production strain *Aspergillus niger* Co827, deposited in the China Center of Industrial Culture Collection (CCICC) as CCICC 40347 and also in the CGMCC as CGMCC 5343.[37,38] The company declared that the two deposition numbers represent one and the same single strain. Strain identity was established by its morphological and physiological properties and by rRNA gene sequences (internal transcribed spacers within the ribosomal transcript).[39] This strain has also undergone a mutagenesis programme (^{60}Co gamma irradiation and chemical mutagenesis) to increase yield. Over 10 generations no changes in physical or morphological characteristics or in productivity were noted.

It should be noted that any significant problems of genetic stability are most likely to be seen as a change in citric acid yield and thus rapidly detected. All three companies declared that no antibiotics were used during the production process and that the final products were free from viable cells of the production strain. This was monitored by plate culture on a medium that allows growth of filamentous fungi. Two of the three companies did not detect any fungal colonies in three batches of the product. The third company described results only in terms of adherence to the specifications set (i.e. < 100 CFU from filamentous fungi/g) in three batches.

2.5. Stability and homogeneity

2.5.1. Shelf life

The proposed shelf life of citric acid is four years when stored in a well-ventilated space under dry and cool (25 °C) conditions, protected from sources of heat or direct sunlight.[40] This recommendation is supported by analyses carried out on three commercial batches of anhydrous citric acid (C1), stored for four years at < 30 °C and < 70 % relative humidity (RH), in absence of light and in the original packaging (25 kg multi-layer paper bags with polyethylene (PE) coating). No degradation of the acid was shown over the proposed shelf life of the additive (100.0 vs. 99.9 % w/w). The Panel considers that the stability of the monohydrate would probably be similar to the anhydrous form.

In another experiment with three batches of citric acid anhydrous (C2), virtually no degradation of the acid (99.9 to 100.0 % w/w) was seen after two years of storage at 25 °C and 60 % RH in a simulated selling package.

2.5.2. Stability in vitamin–mineral premixtures

A layer, piglet and ruminant premixture, including trace elements, was supplemented with citric acid at 20, 20 and 16.7 %, respectively. All samples were stored in PE bottles at ambient temperature and analysed at the start of the study and after six months. A mean loss of 4.7 % citric acid was observed over the storage period.

[35] Supplementary information, January 2013/Annexes/Annex _Qv.
[36] Supplementary information, January 2014/Annex_production strain.pdf.
[37] Supplementary information, August 2014/Annexes/Annex_a_CCICC.
[38] Supplementary information, August 2014/Annexes/Annex_a_certificate of deposition.
[39] Supplementary information, August 2014/Annexes/Annex_a_ microbial strain certificate.
[40] Technical Dossier/Section II/Annexes_Sect.II/Annex_II_3_Stability_Data.

2.5.3. Stability in feedingstuffs

Single batches of chicken (mash and pelleted), piglet (mash and pelleted) and ruminant (pelleted) feeds were supplemented with 1 % citric acid and stored in PE bottles at ambient temperature for three months. Pelleting resulted in a mean loss of 7.7 % citric acid. Thereafter, no significant losses were observed after three months of storage.

2.5.4. Stability in water

Stability of citric acid in tap water was studied using an inclusion level of 0.5 % to represent typical intended use. Citric acid in water was shown to be stable after storage in PE bottles at room temperature for 48 hours.

2.5.5. Homogeneity

From each of the three feed samples used for the stability studies, 10 sub-samples were taken to study the homogeneous distribution of the active substance. The coefficient of variation for the three feeds varied between 0 and 7.4 %.

2.6. Conditions of use

Citric acid is proposed for use as a preservative in feedingstuffs and in water for drinking for all animal species without limitation of age and with no maximum content.

Doses recommended by the applicant for preserving compound feed and feed materials range between 100 and 15 000 mg/kg depending on the moisture content and storage conditions. The typical dose in water is between 100 and 5 000 mg/L. The applicant suggests that 'typical inclusion levels' in feed would range between 2 000 and 7 500 mg/kg feed.

2.7. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

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

3. Safety

3.1. Safety for the target species

Citric acid is produced by all living organisms and when ingested by the target animals will be rapidly and completely metabolised to carbon dioxide and water. Citric acid is authorised in food without limits so whether or not the exposure of animals would be lower than that of humans cannot be estimated.

No specific tolerance studies were provided. Nevertheless, in a number of published studies referenced by the applicant and from other sources (Ravindran and Kornegay, 1993; Partanen, 2001; Kil et al., 2011), trials carried out in different animal species and with dietary concentrations of citric acid higher than the highest proposed typical use level, were described.

3.1.1. Poultry

In two experiments (each using four replicates of four animals/treatment; trial duration 18–22 days) evaluating the potential effects of dietary citric acid on chickens for fattening fed phosphorus-deficient diets, the inclusion of citric acid at levels of 30 000 to 60 000 mg/kg complete feed did not result in negative effects in growth performance (first experiment); in the second experiment no negative effects were noted up to 30 000 mg citric acid/kg feed (Boling et al., 2000). In a study evaluating the effects of citric acid, 1α-hydroxycholecalciferol (1α-(OH) D3) and phytase in chickens for fattening (four replicates of four animals/treatment; 14-day study), Snow et al. (2004) reported significant

positive effects on performance and bone mineralisation when citric acid was added at 40 000 mg/kg feed. In addition, Ebrahimnezhad et al. (2008) reported significant positive effects on the performance of chickens for fattening (four replicates of 15 animals/treatment; 49-day experiment) when citric acid was added at 50 000 mg/kg to a diet supplemented or not with exogenous phytase.

3.1.2. Pigs

A meta-analysis carried out by Partanen (2001), using published data from 1970 to 2001 (nine studies), demonstrated that citric acid at dietary levels from 5 000 to 25 000 mg/kg had a positive effect on the performance of weanling piglets.

The inclusion of 30 000 mg citric acid/kg feed for 25 days in early weaned piglets (seven replicates of six to nine animals/treatment) resulted in a positive effect on the growth performance of piglets (Henry et al., 1985). A linear increase in performance parameters of piglets was reported when citric acid was added to the diet at 15 000 and 30 000 mg/kg (four pens of two animals/treatment) for four weeks (Radcliffe et al., 1998).

Boling et al. (2000) conducted two experiments (three replicates of three piglets/treatment; duration of study 18–22 days) to evaluate whether the addition of citric acid (30 000 and 60 000 mg/kg feed) to a phosphorus-deficient maize–soybean meal diet would improve phytate phosphorus utilisation in weaned piglets. Significant positive effects on bone mineralisation and on the performance of the animals were reported with the citric acid supplemented diets.

3.1.3. Ruminants

Ruminal fermentation studies (Wright, 1971) demonstrated that citric acid does not accumulate in the rumen fluid because of the large capacity of the rumen microbes to rapidly metabolise citric acid to carbon dioxide and acetic acid. The potential metabolic capacity of the bovine rumen of nearly 50 kg citric acid/day (Wright, 1971) is much higher than the intake of citric acid proposed by the applicant.

Furthermore, no adverse effects were reported in beef cattle (10 animals/treatment; 56-day study) and sheep (six animals/treatment; 60-day study) on DM digestibility or on mineral metabolism (magnesium, phosphorus, calcium; 60-day experiment) when diets were supplemented with 14 000 mg citric acid/kg total mixed ration (TMR) (Allen et al., 1986, 1990).

Plasma levels of magnesium were not significantly affected when sheep were fed 30 000 mg citric acid/kg in the semi-synthetic diet (4 × 4 Latin square design: four crossbred wethers (one year old); per phase: seven days of adaptation, three days of sampling) (House and Van Campen, 1971).

3.1.4. Veal calves

De Vuyst et al. (1972) reported significant positive effects on the performance of veal calves fed 20 000 mg citric acid/kg milk replacer.

No adverse effects on the performance parameters of veal calves (10 animals/treatment; 42-day trial) were reported when citric acid was added to a milk replacer (44 000 mg citric acid/kg) (Hill et al., 2013).

3.1.5. Conclusions on the safety for the target species

Although the studies described have limitations (short duration and mostly only zootechnical parameters were observed), overall data and published review articles confirm that citric acid is safe when used in feed for all animal species up to a concentration of 30 000 mg citric acid/kg complete feed. However, the quality of available data does not allow a margin of safety to be derived.

The FEEDAP Panel concludes that the additive is, consequently, also safe at the maximum proposed use level of 15 000 mg citric acid/kg complete feedingstuffs and the corresponding concentration in water for drinking (5 000 mg citric acid/L).

3.2. Safety for the consumer

JECFA issued an opinion on citric acid (JECFA, 1974) allocating an ADI of 'not limited'. In 1991, this ADI was supported by the Scientific Committee of Food (EC, 1991) and the Nordic Working Group on Food Toxicology and Risk Assessment (NNT, 2002) concluded that there is no need for further testing.

Citric acid is an intermediate in the tricarboxylic acid cycle and a normal constituent of living cells. It is permitted *quantum satis* as an additive in food. Citric acid ingested by the target animals is normally rapidly metabolised to carbon dioxide and water. Therefore, it is not expected that exposure of the consumer would increase as a result of its use as a feed additive. The FEEDAP Panel concludes that the use of citric acid in animal nutrition is safe for the consumer.

3.3. Safety for the user

No data were provided by the applicant on the safety for the user. According to the Organisation for Economic Co-operation and Development (OECD) Screening Information Data Set (SIDS) Report,[41] 'the sensitising potential is seen as low. In contrast, irritation, in particular of the eyes but also of the respiratory pathways and the skin, is the major toxicological hazard presented by citric acid; this conclusion is confirmed by a series of reports relating to eye and skin irritation.'

Data on particle size and dusting potential indicate that most granulated products would not form a respirable dust. However, other presentations with different particle size distribution and dusting potential exist, indicating that workers could be exposed to the dust of citric acid by inhalation.

Considering all the above, the FEEDAP Panel considers it prudent to regard citric acid as potentially hazardous to workers by exposure of the skin, eyes or mucous membranes or by inhalation.

3.4. Safety for the environment

Citric acid occurs in all living organisms as an intermediate in the tricarboxylic acid or Krebs cycle. When ingested, it will be rapidly and completely metabolised to carbon dioxide and water. Consequently, the FEEDAP Panel concludes that the use of citric acid in animal nutrition would not pose a risk to the environment.

4. Efficacy

The minimum inhibitory concentration (MIC) data from Matsuda et al. (1994) showed that inhibition of growth of a wide range of bacteria and fungi occurred only at concentrations above 25 000 mg citric acid/L, which are greater than the recommended use level of citric acid in feed and the corresponding concentration in water for drinking.

The applicant provided a study concerning the preservative effects of citric acid. Complete feeds were intentionally inoculated with enterobacteria, *Saccharomyces cerevisiae* and *Aspergillus* spp. (5×10^3 to 5×10^4 CFU/g). In the first part of the study, the relative preservative strength of citric acid was tested in one dry pig feed (90 000 mg citric acid/kg) and two liquid feeds (9 000–18 000 mg citric acid/kg) for a storage period up to three months (dry feed) or 48 hours (liquid feeds) at room temperature. Microbial counts (total aerobes, enterobacteria, filamentous fungi/yeasts) were performed only at the end of the storage period. In the dry feed only, in the case of enterobacteria, an evident effect was reported (4.48 vs. 2.85 $\log_{10}$ CFU/g). The two liquid feeds showed an evident numerical reduction in numbers of total aerobes (4 vs 1 $\log_{10}$ CFU/g), filamentous fungi (1.95 vs 1.0

[41] Available online: http://www.inchem.org/documents/sids/sids/77929.pdf

$\log_{10}$ CFU/g) and yeasts (2.88 vs. 1.12 $\log_{10}$ CFU/g). However, the experimental design did not allow for statistical analysis.

In the second part of the study two dry piglet feeds were supplemented with 5 000, 10 000 and 15 000 mg citric acid/kg and stored for up to three months at room temperature. Citric acid significantly reduced the pH value of the feeds, but had no significant effect on the microbiological parameters measured (yeasts, filamentous fungi, enterobacteria, total aerobes).[42]

Although citric acid is a well-recognised preservative in food, based on data provided, the effectiveness of citric acid as a preservative in feedingstuffs and water for drinking was not sufficiently demonstrated.

As published in previous opinions, the FEEDAP Panel has reservations about the effectiveness of organic acids as preservatives in feedingstuffs with a typical moisture content of ≤ 12 %.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

There is evidence from published studies that the additive is safe for the target species at the proposed conditions of use of 15 000 mg citric acid/kg complete feedingstuffs and 5 000 mg citric acid/L in water for drinking. The quality of available data does not allow a margin of safety to be derived.

The use of citric acid in animal nutrition is safe for the consumer.

It is prudent to regard citric acid as potentially hazardous to workers by exposure to skin, eyes, mucous membranes or by inhalation.

The use of citric acid in animal nutrition would not pose a risk to the environment.

Although citric acid is a well-recognised preservative in food, based on data provided, the effectiveness of citric acid as a preservative in feedingstuffs and water for drinking was not sufficiently demonstrated.

The FEEDAP Panel has reservations about the effectiveness of organic acids as preservatives in feedingstuffs with a typical moisture content of ≤ 12 %.

RECOMMENDATIONS

The additive should be described as citric acid produced by fermentation of *Aspergillus niger* strains DSM 25794 or CGMCC 4513/CGMCC 5751 or CICC 40347/CGMCC3543.

DOCUMENTATION PROVIDED TO EFSA

1. Citric acid for all animal species. May 2011. Submitted by FEFANA asbl.
2. Citric acid for all animal species. Supplementary information. January 2013. Submitted by FEFANA asbl.
3. Citric acid for all animal species. Supplementary information. November 2013. Submitted by FEFANA asbl.
4. Citric acid for all animal species. Supplementary information. January 2014. Submitted by FEFANA asbl.

[42] Supplementary information, January 2013/ Annexes/Annex_Qiii_citric_acid_efficacy.

5. Citric acid for all animal species. Supplementary information. August 2014. Submitted by FEFANA asbl.

6. Citric acid for all animal species. Supplementary information. December 2014. Submitted by FEFANA asbl.

7. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for citric acid.

8. Comments from Member States received through the ScienceNet.

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Appendix A. Executive summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the method(s) of analysis for citric acid[43,44]

In the current application authorisation is sought under article 10(2) for *trisodium citrate dihydrate (E 331)*[45] and *tripotassium citrate monohydrate (E 332)*[46] and under articles 4(1) and 10(2) for *citric acid (E330)*[47] under the category of 'technological additives' functional group 1a (Preservatives), according to the classification system of Annex I of Regulation (EC) No 1831/2003.

Trisodium citrate dihydrate is a white granular crystals or crystalline powder with a minimum purity of 99%. *Tripotassium citrate monohydrate* is a colourless, white powder or granulate with a minimum purity of 99%. *Citric acid* (used in either anhydrous or monohydrate form) is a colourless crystals or white crystalline powder with a minimum purity of 99.5 % based on the anhydrous form.

Authorisation is sought for the use of the *trisodium citrate dihydrate* and *tripotassium citrate monohydrate* for dogs and cats, while authorisation is sought for the use of the *citric acid* for all categories and species.

Trisodium citrate dihydrate and tripotassium citrate monohydrate are intended to be mixed into premixtures and feedingstuff, whereas citric acid is also intended to be mixed into water. However, the Applicants suggested no minimum or maximum levels as set in the previous regulations.

For the quantification of *trisodium citrate dihydrate* in the *feed additive*, the EURL recommends for official control the European Pharmacopoeia Monograph method (Monograph 0412), based on acid/base titration with 0.1 M perchloric acid and naphtholbenzein as indicator, as suggested by the Applicant.

For the quantification of *tripotassium citrate* in the *feed additive*, the EURL recommends for official control the European Pharmacopoeia Monograph method (Monograph 0400), based on acid/base titration with 0.1 M perchloric acid and naphtholbenzein as indicator, as suggested by the Applicant.

For the quantification of *citric acid* in the *feed additive*, the EURL recommends for official control the European Pharmacopoeia Monograph method (Monograph 0455 & 0456), based on acid/base titration with 1 M sodium hydroxide and phenolphthalein as indicator, as suggested by the Applicant.

For the quantification of citric acid, trisodium citrate dihydrate and tripotassium citrate monohydrate (as <u>total citric acid</u> content) in premixtures, feedingstuffs and water Applicant[48] proposed a method based on high performance liquid chromatography with refractive index or UV detection (HPLC-RI/UV). This method does not distinguish between citric acid and its salts. This HPLC-UV/RI method was ring trial validated with four laboratories and a relative standard deviation for reproducibility (RSD_R) ranging from 14.5 % to 21.1 % was reported for premixtures and feedingstuffs containing from 12 to 66 g citric acid/kg together with a limit of quantification of 0.43 g/kg feedingstuff.

Based on the performance characteristics presented, the EURL recommends for official control the ring trial validated method based on ion-exclusion HPLC-UV method to determine *citric acid, trisodium citrate dihydrate* and *tripotassium citrate monohydrate* (expressed as <u>*total citric acid*</u>) in *premixtures, feedingstuffs* and *water*.

[43] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-CitricGroup.pdf
[44] The EURL produced a combined report for the dossier FAD-2010-0154 FAD-2010-0154 Trisodium citrate dihydrate for dogs and cats; the dossier FAD-2010-0187 Tripotassium citrate for all pet species and the dossier FAD-2010-0357 Citric acid for all animal species.
[45] FAD-2010-0154.
[46] FAD-2010-0187.
[47] FAD-2010-0357.
[48] FAD-2010-0357.

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.