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Assessment of the safety of the feed additives acetic acid, calcium acetate and sodium diacetate for fish (FEFANA asbl)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety of acetic acid, calcium acetate and sodium diacetate as technological feed additives (preservatives) for salmonids/fish. The additives are already authorised for use for all animal species other than fish. In previous opinions, the FEEDAP Panel concluded that a maximum concentration of 2,500 mg acetic acid/kg complete feed (or 1,000 mg/L water for drinking) was safe for poultry, pigs and pet animals. Ruminants were considered to exhibit a higher tolerance. Due to lack of data for salmonids, the Panel could not conclude on the safety of acetic acid and its salts for fish. The applicant has provided supplementary information consisting in a tolerance study in Atlantic salmon (*Salmo salar*) and a literature search to support the safety of acetic acid in fish. Considering all the available information, the FEEDAP Panel concluded that acetic acid (and its salts by analogy) is considered safe for fish up to the maximum recommended supplementation level of 2,500 mg acetic acid/kg complete feed.

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1. Introduction

1.1. Background and terms of reference as provided by the requestor

Regulation (EC) No 1831/2003 establishes the rules governing the Community authorisation of additives for use in animal nutrition and, in particular, Article 9 defines the terms of the authorisation by the Commission.

The applicant, FEFANA ASBL, is seeking a Community authorisation of acetic acid (E 260), calcium acetate (E 263) and sodium diacetate (E 262) as feed additives to be used as preservative for salmonids/fish (Table 1).

Table 1: Description of the additive

Category of additive	Technological additives
Functional group of additive	Preservatives
Description	Acetic acid (E 260) calcium acetate (E 263) and sodium diacetate (E 262)
Target animal category	Salmonids/fish
Applicant	FEFANA ASBL
Type of request	New opinion

On 6 May 2021, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) of the European Food Safety Authority (EFSA), in its opinion on the safety and efficacy of the products, could not conclude on the safety of the additives for salmonids/fish due to the absence of data.

The Commission gave the possibility to the applicant to submit supplementary information and data in order to complete the assessment and to allow a revision of the EFSA's opinion. The new data have been received on 7 September 2022.

In view of the above, the Commission asks EFSA to deliver a new opinion on acetic acid (E 260), calcium acetate (E 263) and sodium diacetate (E 262) as feed additives for salmonids/fish based on the supplementary information and data submitted by the applicant, in accordance with Article 29(1) (a) of Regulation (EC) No 178/2002.

1.2. Additional information

The EFSA FEEDAP Panel issued two opinions on the safety and efficacy of acetic acid (and its salts) when used as a technological additive (preservative) in feed or water for all animal species (EFSA FEEDAP Panel, 2012, 2021) and one as feed flavouring (EFSA FEEDAP Panel, 2013).

Previously authorised in the European Union (EU) as acetic acid (E 260), calcium acetate (E 263) and sodium diacetate (E 262),¹ the additives are currently authorised as 1a260, 1a263 and 1a262, respectively, for use in feed and water for poultry, pigs and pets at maximum content 2,500 mg/kg complete feed (or 1,000 mg/L water for drinking), and for all other animal species other than fish, without maximum content.²

Acetic acid is also authorised in the EU as a sensory additive (flavouring compounds; code: 2b08002; FLAVIS number 08.002) for use in feed for all animal species and categories.³

Acetic acid, calcium acetate and sodium diacetate are authorised in the EU as food additives to Regulation (EC) No 1333/2008, belonging to group I additives.⁴ Their use is permitted in several food categories at *quantum satis*.

¹ Commission Directive of 8 July 1985 amending the Annexes to Council Directive 70/524/EEC concerning additives in feedingstuffs. OJ L 245, 12.9.1985, p. 1.

² Commission Implementing Regulation (EU) 2022/415 of 11 March 2022. OJ L 85, 14.3.2022, p. 6.

³ Commission Implementing Regulation (EU) 2017/53 of 14 December 2016. OJ L 13, 17.1.2017, p. 1.

⁴ Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of supplementary information⁵ to a previous application on the same product.⁶

In accordance with Article 38 of the Regulation (EC) No 178/2002⁷ and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,⁸ a non-confidential version of the supplementary information has been published on Open.EFSA available at <https://open.efsa.europa.eu/questions/EFSA-Q-2022-00546>.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety of acetic acid (or its salts by analogy) is in line with the principles laid down in Regulation (EC) No 429/2008⁹ and the relevant guidance documents: Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017).

3. Assessment

The additive under assessment is acetic acid (and its salts) and is intended for use in feedstuffs and water for drinking without restrictions for all animal species. For fish, acetic acid is intended to be added to feed via a premixture to reach an inclusion level up to 2,500 mg/kg complete feed.

The EFSA FEEDAP Panel (2012) issued an opinion on the safety and efficacy of acetic acid and its salts when used as technological additives (preservatives) for all animal species. Acetic acid and its salts were considered equivalent when compared on a molar basis. The Panel concluded that a maximum concentration of 2,500 mg acetic acid/kg complete feed (or 1,000 mg/L water for drinking) is safe for poultry, pigs and pet animals based on the comparison between human exposure and target animals' exposure to acetic acid. The Panel also concluded that ruminants exhibit a relatively higher tolerance due to the absorption of acetic acid derived from the fermentation of feed in the rumen. However, due to lack of data for salmonids, the Panel could not conclude on the safety of acetic acid and its salts in fish. The use of acetic acid and its salts as feed additives was considered safe for the consumers and the environment. The risks for users handling the additives depend on the concentration and include skin, eyes and respiratory tract irritation and corrosion. The same conclusions were applied in a further assessment regarding a modification of the manufacturing process (EFSA FEEDAP Panel, 2021).

The applicant has provided a tolerance study in Atlantic salmon (*Salmo salar*)¹⁰ and a literature search¹¹ to support the safety of acetic acid (or its salts by analogy) in fish.

3.1. Safety

3.1.1. Safety for the target species

In a tolerance study, a total of 360 Atlantic salmon post smolt (all female) weighting on average 229 g were randomly allocated to 12 tanks (600 L), in a flow through system.¹⁰ A pelleted feed was either not supplemented (control) or supplemented with acetic acid at 2,500 (1×), 7,500 (3×) or 20,000 mg/kg complete feed (8× the maximum recommended level) (confirmed by analysis). Three

⁵ Dossier reference: EFSA-Q-2022-00546.

⁶ Dossier reference: FAD-2021-0013 (and FAD-2010-0161).

⁷ Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, pp. 1–48.

⁸ Decision available at: <https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements>.

⁹ 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.

¹⁰ Annex I_20052 study report acetic acid in salmon final 5 September 2022.

¹¹ Annex II_Literature search Safety of acetic acid in fish.

tanks (replicates) of 30 fish/tank were assigned to each treatment group. Feed was offered *ad libitum* three times per day with automatic feeders for 90 days. Fish were individually weighed at the beginning and at the end of the study. Feed intake (FI) was registered daily. At the end of the study the specific growth rate (SGR), body weight (BW) gain, average daily FI and feed to gain ratio were calculated to assess the performance of the fish. At the end of the study, all fish were individually weighed, and fork lengths recorded; blood samples of five fish per tank (15 fish per treatment) were collected for haematology and blood chemistry analysis.¹² Ten fish per tank (30 per treatment) were necropsied to examine macroscopic lesions¹³ and organs' weight¹⁴ was recorded. Tissue/organ samples from five fish per treatment were collected in case histopathological analysis had to be performed.¹⁵ Data were analysed statistically by analysis of variance using the tank as the experimental unit; the Dunnett's test was used to compare each supplementation level with the unsupplemented control group, setting the statistical significance level at $p < 0.05$.

No mortality or culling were observed for any of the experimental groups. FI was significantly lower in the groups fed 7,500 and 20,000 mg acetic acid/kg complete feed compared with the control group (93.7 and 82.6 vs 105.6 g DM/day). BW gain and SGR were significantly lower in the group fed 20,000 mg/kg complete feed compared to the control group (324 g vs 404 g for BW gain; 1.01 vs 1.17 for SGR); no differences were observed in BW gain and SGR in the other groups (2,500 and 7,500 mg acetic acid/kg complete feed: 400 g and 371 g for BW gain; 1.17 and 1.11 for SGR). No differences were observed for the feed to gain ratio (0.80, 0.80 and 0.81 vs 0.82). The relative liver weight (liver weight/BW) was significantly decreased in the group that received the highest inclusion level of the additive compared to the control (9% lower). No significant differences among groups were observed in haematocrit, haemoglobin and mean corpuscular haemoglobin concentration (MCHC). There were no significant differences on blood biochemistry with the exception for a significant increase in alanine aminotransferase and amylase in fish fed 7,500 mg acetic acid/kg feed (although not dose-dependent), and a significant decrease in calcium in the fish fed 20,000 mg acetic acid/kg feed compared to the control group (3.493 vs 3.637 mmol/L, respectively). No gross pathology findings were observed for any of the experimental groups; therefore, no histology was performed. The relative organ weight of spleen, kidney and heart showed no differences among groups.

The results from the tolerance trial in Atlantic salmon showed that the additive was tolerated at 7,500 mg acetic acid/kg feed and, therefore, the FEEDAP Panel concluded that the acetic acid (and its salts) is safe at the maximum recommended level of 2,500 mg acetic acid/kg complete feed, with margin of safety of 3.

Additionally, the applicant provided a literature search to support safety of acetic acid (and its salts) in fish.¹¹ The literature covered the period 2000 – November 2021 and involved three databases (Pubmed, Springer and Science Direct). The search terms included, among others, 'acetic acid', 'sodium acetate', 'calcium acetate', 'fish' and 'adverse effect' and the strategy followed was very briefly reported. A total of 1,837 hits were retrieved.

After title and abstract screening for relevant studies, 12 studies were included in the evaluation. In five of these studies, however, the substances tested were not representative of the additive under assessment, and, therefore, not further considered. None of the remaining seven studies fulfilled the criteria for study duration, supplementation levels or monitored parameters requested for tolerance studies by the FEEDAP Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017), and were therefore considered of low relevance to support the safety for fish.

The FEEDAP Panel notes that the FEEDAP Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017) requires that, if an application is for all fish, evidence of safety in salmonids and a second species are requested. Although the results from the tolerance trial submitted showed no adverse effects at the maximum recommended level of 2,500 mg acetic acid/kg complete feed, from the literature search described above, no evidence for a second species was shown.

¹² Blood parameters consisted of haemoglobin, haematocrit and mean corpuscular haemoglobin concentration (MCHC). Blood chemistry parameters consisted of chloride, iron, phosphate, total protein, albumin, C-reactive protein (CRP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatin kinase (CK) creatine, sodium, potassium, calcium, magnesium, alkaline phosphatase (ALP), glucose, cholesterol, amylase, lactate dehydrogenase (LDH), bilirubin and globulin (calculated as total protein minus albumin).

¹³ Heart, liver, intestine, spleen, kidney, gonads, gills, eye and bone.

¹⁴ Heart, kidney, spleen and liver.

¹⁵ Liver, spleen, mid-kidney, heart, stomach, intestine and gills.

The same Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017) also foresees that when the safety is demonstrated in four major species (included fish) no further demonstration is needed for any other target species. Since in its previous opinions, the FEEDAP Panel concluded on the safety of three major species (poultry, pigs and ruminants) at a maximum concentration of at least 2,500 mg acetic acid (and its salts)/kg complete feed (or 1 g/L water for drinking), considering all the available information, the Panel concludes that acetic acid (and its salts) is safe also for all fish species at the maximum recommended concentration of 2,500 mg acetic acid (and its salts)/kg complete feed.

4. Conclusions

Acetic acid (and its salts) is safe for Atlantic salmon at the maximum recommended level of 2,500 mg acetic acid/kg complete feed, with a margin of safety of 3. Therefore, with the data available, the FEEDAP Panel concludes that the additive is also safe for all fish at the maximum recommended supplementation level of 2,500 mg acetic acid/kg complete feed.

References

- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Scientific Opinion on the safety and efficacy of acetic acid, sodium diacetate and calcium acetate as preservatives for feed for all animal species. *EFSA Journal* 2012;10(2):2571, 15 pp. <https://doi.org/10.2903/j.efsa.2012.2571>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific Opinion on the safety and efficacy of straight-chain primary aliphatic alcohols/aldehydes/acids, acetals and esters with esters containing saturated alcohols and acetals containing saturated aldehydes (chemical group 01) when used as flavourings for all animal species. *EFSA Journal* 2013;11(4):3169, 35 pp. <https://doi.org/10.2903/j.efsa.2013.3169>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2017. Guidance on the assessment of the safety of feed additives for the target species. *EFSA Journal* 2017;15(10):5021, 19 pp. <https://doi.org/10.2903/j.efsa.2017.5021>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis, V, Azimonti, G, Bastos, ML, Christensen, H, Dusemund, B, Fašmon Durjava, M, Kouba, M, López-Alonso, M, López Puente, S, Marcon, F, Mayo, B, Pechová, A, Petkova, M, Ramos, F, Sanz, Y, Villa, RE, Woutersen, R, Brozzi, R, Galobart, J, Gregoretti, L, López-Gálvez, G, Innocenti, ML and Vettori, MV, 2021. Scientific Opinion on the safety and efficacy of a feed additive consisting of acetic acid for all animal species. *EFSA Journal* 2021;19(6):6615, 9 pp. <https://doi.org/10.2903/j.efsa.2021.6615>

Abbreviations

ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BW	body weight
CK	creatine kinase
CRP	C-reactive protein
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FI	feed intake
FLAVIS	The EU Flavour Information System
LDH	lactate dehydrogenase
MCHC	mean corpuscular haemoglobin concentration
SGR	specific growth rate