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SCIENTIFIC OPINION

Assessment of the feed additive consisting of *Pediococcus pentosaceus* DSM 23689 for all animal species for the renewal of its authorisation (Chr. Hansen A/S)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the assessment of the application for renewal of the authorisation of *Pediococcus pentosaceus* DSM 23689 as a technological additive, silage additive for all animal species. The applicant has provided evidence that the additive currently on the market complies with the existing conditions of authorisation. The Panel concluded that the additive remains safe for all animal species, consumers, and the environment under the authorised conditions of use. Regarding user safety, the additive should be considered as a respiratory sensitiser. No conclusions can be drawn on the skin sensitisation, and skin and eye irritancy potential of the additive. There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

KEYWORDS

efficacy, *Pediococcus pentosaceus* DSM 23689, QPS, renewal, safety, silage additive, technological additive

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 14(1) of that Regulation lays down that an application for renewal shall be sent to the Commission at the latest 1 year before the expiry date of the authorisation.

The European Commission received a request from Chr. Hansen A/S[2] for the renewal of the authorisation of the additive consisting of *Pediococcus pentosaceus* DSM 23689, when used as a feed additive for all animal species (category: technological additives; functional group: silage additives).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 14(1) (renewal of the authorisation). The dossier was received on 20 February 2023 and the general information and supporting documentation are available at https://open.efsa.europa.eu/questions/EFSA-Q-2023-00163. The particulars and documents in support of the application were considered valid by EFSA as of 14 July 2023.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the feed additive consisting of *P. pentosaceus* DSM 23689, when used under the proposed conditions of use (see **Section 3.1.3**).

1.2 | Additional information

The additive is a preparation containing *P. pentosaceus* DSM 23689 and is currently authorised for use in feed for all animal species (1k1011).[3]

EFSA issued an opinion on the safety and efficacy of this product when used as a silage additive for all animal species (EFSA FEEDAP Panel, 2013).

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[4] in support of the authorisation request for the use of *P. pentosaceus* DSM 23689 as a feed additive.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 14 July 2023 to 14 October 2023; the comments received were considered for the assessment.

In accordance with Article 38 of the Regulation (EC) No 178/2002[5] 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,[6] a non-confidential version of the dossier has been published on Open.EFSA.

According to Article 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 22 December 2023 to 12 January 2024 for which no comments were received.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA, to deliver the present output.

[1]Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

[2]Chr. Hansen A/S, Boege Allé 10–12, DK-2970, Hoersholm, Denmark.

[3]Commission Implementing Regulation (EU) No 84/2014 of 30 January 2014 concerning the authorisation of preparations of *Pediococcus pentosaceus* DSM 14021, *Pediococcus pentosaceus* DSM 23688 or *Pediococcus pentosaceus* DSM 23689 as feed additives for all animal species. OJ L 208, 31.01.2014, p. 30.

[4]Dossier reference: FEED-2022-11014.

[5]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, p. 1–48.

[6]Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the active agent in animal feed are valid and applicable for the current application.[7]

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *P. pentosaceus* DSM 23689 is in line with the principles laid down in Regulation (EC) No 429/2008[8] and the relevant guidance documents: Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2021).

3 | ASSESSMENT

The additive consisting of *P. pentosaceus* DSM 23689 is currently authorised for all animal species as a technological additive (functional group: silage additives). The assessment regards the renewal of the authorisation.

3.1 | Characterisation

3.1.1 | Characterisation of the additive

The additive is currently authorised with a minimum content of the active agent (*P. pentosaceus* DSM 23689) of 1×10^{11} colony forming units (CFU)/g of additive. The applicant declared that the manufacturing process and the composition of the additive have not been changed since the previous authorisation and that no antimicrobials are used during the manufacturing process.[9] The qualitative composition of the fermentation medium was listed but the quantitative composition was not provided. However, considering the ingredients used, the Panel concluded that none raised safety concerns.

The final product is a powder containing the freeze-dried cell concentrate [redacted] to reach the minimum concentration specified for *P. pentosaceus* DSM 23689.[11]

Analytical data to confirm the specification set as per authorisation was provided for five independent batches of the additive,[12] showing an average value of 5.1×10^{11} CFU/g additive (range from 3.2×10^{11}–7.6×10^{11} CFU/g additive).[13]

Three recent batches of the additive were tested for arsenic (0.032 mg/kg; range 0.019–0.043 mg/kg), lead (0.06 mg/kg; range 0.017–0.132 mg/kg), cadmium (0.04 mg/kg; range 0.027–0.05 mg/kg) and mercury (0.01 mg/kg; range 0.0044–0.0164 mg/kg). Aflatoxin B1 was tested in the same batches being the values < 0.46 μg/kg.[14] Specifications are set for coliforms (< 10^3 CFU/g), *Escherichia coli* (< 10 CFU/g), *Salmonella* spp. (no detection in 25 g frozen product (one batch) or 5 g freeze dried bulk (two batches)) and yeasts and filamentous fungi (< 10^3 CFU/g).[15] Three samples of the additive showed compliance with the respective specifications. Three batches of the additive were also tested for the presence of Enterobacteriaceae and showed results < 10 CFU/g in all samples.[16]

The FEEDAP Panel considers that the microbial contamination and the amounts of the detected impurities do not raise safety concerns.

No new data were provided regarding the physico-chemical properties or stability of the additive. Since no changes were introduced in the manufacturing process, the data described in the previous opinion (EFSA FEEDAP Panel, 2013) are considered still valid.

[7]Evaluation report available on the EU Science Hub https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en

[8]Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

[9]Statement_DSM23689_2023.

[10]Currently under re-evaluation by the FEEDAP Panel.

[11]Sect_II_Identity_P.pen_DSM23689_ID+Charact_v3.

[12]Due to the unavailability of enough production batches in the year previous to the submission of the application, one batch was older than one year from the date of submission of the application.

[13]Annex_II_1.3a_CoAs_New_DSM23689_V1 and Annex_II_1.3a_CoAs_New_DSM23689_v2_corrected.

[14]'<' means below the analytical limit of quantification (LOQ).

[15]Annex_II_1.3a_CoAs_New_DSM23689_V1, Annex_II_1.3a_CoAs_New_DSM23689_v2, Annex_II_1.4.2_Undes_subst_V1_DSM23689, Annex_II_1.4.2_Undes_subst_V2_DSM23689 and Annex_II_1.4.2_Undes_subst_V3_DSM23689.

[16]Annex_II_1.4.2a_Enterobacteriaceae_V1_DSM23689 and Annex_II_1.4.2a_Enterobacteriaceae_V2_DSM23689.

3.1.2 | Characterisation of the active agent

P. pentosaceus DSM 23689 was isolated from silage and it is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), with the accession number DSM 23689.[17] The strain has not been genetically modified.

The taxonomical identification of DSM 23689 as *P. pentosaceus* was confirmed by average nucleotide identity (ANI) determination using whole genome sequence (WGS) data. The ANI value obtained was 98.4% with the type strain *P. pentosaceus* ATCC 33316^T.[18]

Based on [redacted], the active agent *P. pentosaceus* DSM 23689 was shown to harbour plasmids (number and size not determined).[19]

The susceptibility of *P. pentosaceus* DSM 23689 to antimicrobials was tested using a broth microdilution method and including the set of antibiotics recommended by EFSA (EFSA FEEDAP Panel, 2018).[20] All the minimum inhibitory concentration values were equal to or fell below the cut-off values, except for tetracycline, which was one dilution higher. Exceeding the cut-off by one dilution is considered within the normal range of variation, and therefore, *P. pentosaceus* DSM 23689 is considered susceptible to all the relevant antibiotics.

The WGS data, including the plasmid sequences, of the active agent were interrogated for the presence of antimicrobial resistance genes against two databases.[21] The interrogation was performed with a threshold of [redacted] against the NCBI Bacterial Antimicrobial Resistance Reference Gene database and [redacted] against ResFinder. No hits of concern were identified.

3.1.3 | Conditions of use

The additive is currently authorised for use as a silage additive for all animal species. Under other provisions of the authorisation, it is specified that:

- In the directions for use of the additive and premixture, indicate the storage conditions.
- Minimum content of the additive when used without combination with other micro- organisms as silage additives: 1×10^8 CFU/kg fresh material in easy and moderately difficult to ensile material.[22]
- For safety: it is recommended to use breathing protection, eye protection and gloves during handling.

The applicant has requested to maintain the same conditions of use.

3.2 | Safety

In the previous opinion the Panel concluded that following the Qualified Presumption of Safety (QPS) approach to safety assessment, *P. pentosaceus* DSM 23689 is safe for target species, consumers and the environment (EFSA FEEDAP Panel, 2013). Regarding user safety, the Panel concluded that: 'In the absence of evidence these additives[23] should be regarded as skin and eye irritants and potential skin sensitisers. Given the proteinaceous nature of the active agents, the FEEDAP Panel considers it prudent to treat these additives as respiratory sensitisers. Given the high dusting potential of the preparations tested, there is the need to take measures to minimise inhalation exposure of workers'.

The applicant declared that no incidents or safety issues for target animals, consumers, users and the environment have been documented or reported regarding the additive since its first authorisation.[24]

In the context of this application, the identity of the strain as *P. pentosaceus* was confirmed, and evidence that the strain does not show acquired antimicrobial determinants for antibiotics of human and veterinary importance was provided (EFSA BIOHAZ Panel, 2023). Consequently, the conclusions already reached are still valid, and the Panel considers that *P. pentosaceus* DSM 23689 remains safe for the target species, consumers and the environment.

An extensive literature search was performed by the applicant to support the safety of *P. pentosaceus* DSM 23689 and other *P. pentosaceus* strains. The literature search presented several methodological limitations (e.g. only the deposit number of the active agent was included in the search; the reporting was poor) and, therefore, was not further considered for the assessment.[25]

[17] Annex II.2.1.2a.

[18] Annex II.2.1.2b and Annex II.2.2.2a.

[19] SOP-20904_Plasmid and Annex_II_2.1.2c_DSM23689_Gen_stab.

[20] Annex_II_2.2.2c.

[21] Annex II.2.2.2b.

[22] Easy to ensile forage: > 3% soluble carbohydrates in fresh material. Moderately difficult to ensile forage: 1.5–3.0% soluble carbohydrates in the fresh material. Commission Regulation (EC) No 429/2008. OJ L 133, 22.5.2008, p. 1.

[23] Silage additives assessed in the 2013 opinion: *P. pentosaceus* DSM 14021, DSM 23688 and DSM 23689 (EFSA FEEDAP Panel, 2013).

[24] Sect_III_Safety_P.pen_DSM23689_User and Statement_DSM23689_2023.

[25] Annex_III_1a, Annex_III_1b and Annex_III_1c.

No specific data has been submitted for the renewal of the authorisation regarding the effects of the additive on the skin and eyes. Therefore, the FEEDAP Panel cannot conclude on the eye/skin irritancy potential of the additive. The FEEDAP Panel notes that the OECD test guidelines available at present are designed to assess the skin sensitisation potential of chemical substances only and that currently no validated assays for assessing the sensitisation potential of microorganisms are available. Therefore, no conclusion can be drawn on the skin sensitisation potential of the additive. Owing to the proteinaceous nature of the active agent, the additive should be considered a respiratory sensitiser.

3.2.1 | Conclusions on safety

The FEEDAP Panel concludes that there is no new evidence that would lead to reconsider the previous conclusions that *P. pentosaceus* DSM 23689 is safe for the target animal species, consumers and the environment under the authorised conditions of use. No conclusions can be drawn on the skin sensitisation, and skin and eye irritancy potential of the additive. The additive should be considered as a respiratory sensitiser.

3.3 | Efficacy

The present application for renewal of the authorisation does not include a proposal for amending or supplementing the conditions of the original authorisation that would have an impact on the efficacy of the additive. Therefore, there is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

4 | CONCLUSIONS

The applicant has provided evidence that the additive currently on the market complies with the existing terms of authorisation.

The Panel concludes that *Pediococcus pentosaceus* DSM 23689 remains safe for all animal species, consumers and the environment under the authorised conditions of use. Regarding user safety, the additive should be considered as a respiratory sensitiser. No conclusions can be drawn on the skin sensitisation, and skin and eye irritancy potential of the additive.

There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

ABBREVIATIONS

ANI	average nucleotide identity
CFU	colony forming unit
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
EURL	European Union Reference Laboratory
EFSA FEEDAP Panel	EFSA Panel on Additives and Products or Substances used in Animal Feed
OECD	Organisation for Economic Co-operation and Development
QPS	Qualified Presumption of Safety
WGS	whole genome sequence

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CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

European Commission

QUESTION NUMBER

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