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Safety and efficacy of a feed additive consisting of *Pediococcus pentosaceus* DSM 32292 for all animal species (Marigot Ltd t/a Celtic Sea Minerals)

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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 *Pediococcus pentosaceus* DSM 32292 as a technological additive for all animal species. The additive is intended to improve the production of silage at a proposed application rate of 5×10^7 colony forming units (CFU)/kg forage. The bacterial species *P. pentosaceus* is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach. As the identity of the strain has been established and no antimicrobial resistance determinants of concern were detected, the use of the strain as a silage additive is considered safe for livestock species, for consumers and for the environment. In the absence of data, the FEEDAP Panel cannot conclude on the potential of the additive to be a skin/eye irritant or a skin sensitiser. Given the proteinaceous nature of the active agent, the additive should be considered a respiratory sensitiser. The additive at the proposed application rate of 5×10^7 CFU/kg forage has the potential to improve the production of silages from moderately difficult to ensile forages.

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Keywords: technological additive, silage additive, *Pediococcus pentosaceus* DSM 32292, safety, efficacy, QPS

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003¹ 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 feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Marigot Ltd t/a Celtic Sea Minerals² for the authorisation of the product *Pediococcus pentosaceus* DSM 32292, 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 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 3 December 2021.

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 *Pediococcus pentosaceus* DSM 32292, when used under the proposed conditions of use (see **Section 3.1.4**).

1.2. Additional information

The additive is a preparation containing viable cells of *Pediococcus pentosaceus* DSM 32292. It has not been previously authorised as a feed additive in the European Union.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier³ in support of the authorisation request for the use of *Pediococcus pentosaceus* DSM 32292 as a feed additive.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active agent in animal feed. The Executive Summary of the EURL report can be found in Annex A.⁴

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of active substance is in line with the principles laid down in Regulation (EC) No 429/2008⁵ and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

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 Marigot Ltd t/a Celtic Sea Minerals, Strand Farm, Currabinny, Carrigaline, P43NN62, Cork, Ireland.

³ FEED dossier reference: FAD-2021-0074.

⁴ The full report is 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

⁵ Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

3. Assessment

The product under assessment is a preparation of viable cells of *Pediococcus pentosaceus* DSM 32292 intended for use as a technological additive (functional group: silage additives) in moderately difficult to ensile forages for all animal species.

3.1. Characterisation

3.1.1. Characterisation of the active agent

The active agent was originally isolated from grass cut and has not been genetically modified. It is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) with the accession number DSM 32292.⁶

The taxonomical identification was confirmed [REDACTED]

The antimicrobial susceptibility testing was performed by broth microdilution method and included the antibiotics recommended by EFSA (EFSA FEEDAP Panel, 2018b). All the minimum inhibitory concentration (MIC) values were equal or fell below the corresponding cut-off values for *Pediococcus* spp., except for chloramphenicol (MIC: 8 mg/L vs. cut-off value: 4 mg/L) and tetracycline (MIC: 16 mg/L vs. cut-off value: 8 mg/L).⁸ Exceeding the cut-off value by one dilution is considered to be within the normal range of variation of the method and thus, not a matter of concern. Therefore, the strain is considered susceptible to all the relevant antimicrobials.

The WGS of the strain was searched for antibiotic resistance genes [REDACTED]

[REDACTED] No hits of concern were identified.

3.1.2. Characterisation of the additive

The inoculum of the active agent is prepared [REDACTED]

[REDACTED] to guarantee a minimum concentration of active agent of 1×10^{10} CFU/g of additive.

Analysis of five batches showed a mean value of 4.4×10^{11} CFU/g (range $3.9\text{--}5.0 \times 10^{11}$ CFU/g).⁹

A total of five batches were analysed for microbiological contamination, mycotoxins, cadmium, mercury, lead and arsenic levels.¹⁰ Results for the microbiological contamination analyses showed values for *Escherichia coli* and presumptive coliforms below the limit of detection (< 10 CFU/g), no detection of *Salmonella* spp. in 25 g, and 20 CFU/g of yeasts and filamentous fungi.¹¹ Results of the analyses for detection of aflatoxins (B1, B2, G1 and G2)¹² showed levels below the respective limits of detection,¹³ except for aflatoxin B1 (average 0.04, range $< 0.03\text{--}0.04$ µg/kg) and aflatoxin G2 that was detected in one batch but below 0.03 µg/kg. Arsenic (average 0.094 mg/kg, range 0.08–0.11 mg/kg), cadmium (0.2 mg/kg in all batches) and mercury (average 0.016 mg/kg, range 0.01–0.2 mg/kg) were detected in all five batches while lead was detected in only one batch (0.27 mg/kg).¹⁴ The levels of the detected impurities do not raise concerns.

No data were provided on the dusting potential of the additive under assessment.¹⁵

⁶ Technical dossier/Section II/Annex II_14 and Supplementary Information May 2022/SD_confirmation_DSM 32292.

⁷ Technical dossier/Section II/Annex II_15.

⁸ Technical dossier/Section II/Annex II_16.

⁹ Technical dossier/Section II/Annex II_1 and Supplementary Information May 2022/FAD-2021-0074_AppSIn_230522.

¹⁰ Technical dossier/Section II/Annex II_1 and Annex II_2.

¹¹ Technical dossier/Section II/Annex II_1.

¹² Technical dossier/Section II/Annex II_2.

¹³ Technical dossier/Section II/Annex II_3; Limit of detection for the aflatoxins (B1, B2, G1, and G2) 0.01 µg/kg and for total aflatoxins 0.04 µg/kg.

¹⁴ Technical dossier/Section II/Annex II_2 and Annex II_4 (limit of detection for lead: 0.1 mg Pb/kg).

¹⁵ Technical dossier/Supplementary Information May 2022/FAD-2021-0074_AppSIn_230522.

3.1.3. Stability

Three batches of the additive using maltodextrin¹⁶ and three batches of the additive using anhydrous dextrose¹⁷ as carriers were tested for shelf-life by storing in sealed aluminium foil bags at room temperature up to 18 months. Negligible losses (< 0.5 log of the initial value) were observed for both formulations under the above-mentioned conditions.

The stability in water was studied by suspending 5 g of the additive (one batch) in 1 L of tap water in triplicate and then storing for 48 h at room temperature. Negligible losses (< 0.5 log of the initial value) were observed.¹⁸

3.1.4. Conditions of use

The additive is intended for use in moderately difficult to ensile forages at a proposed minimum inclusion level of 5×10^7 CFU/kg forage for all animal species. It is to be applied as such or as an aqueous suspension.

3.2. Safety

3.2.1. Safety for the target species, consumers and the environment

The species *P. pentosaceus* is considered by EFSA to be suitable for the Qualified Presumption of Safety (QPS) approach to safety assessment (EFSA, 2007, EFSA BIOHAZ Panel, 2020). This approach requires the identity of the strain to be conclusively established and evidence provided that the strain do not harbour any antimicrobial resistance genes to clinically relevant antimicrobials. In the view of the FEEDAP Panel, the identity of the strain was established as *P. pentosaceus* and the antibiotic resistance qualification has been met. Consequently, *Pediococcus pentosaceus* DSM 32292 is presumed safe for the target species, consumers and the environment.

3.2.2. Safety for the user

No data were submitted on effects of the additive on skin and eyes.¹⁹ Therefore, the FEEDAP Panel is not in the position to conclude on the skin and eye irritancy or skin sensitisation potential of the additive. Given the proteinaceous nature of the active agent, the additive should be considered a potential respiratory sensitiser.

3.3. Efficacy

Three studies were conducted with different forages representing materials moderately difficult to ensile, as specified by Regulation (EC) No 429/2008 (Table 1). All the studies included a control group and a group in which *Pediococcus pentosaceus* DSM 32292 was applied²⁰ to the forage at a concentration of 5×10^7 CFU/kg of forage. An aqueous suspension of the additive was prepared and then sprayed onto the forage prior to ensiling. In the control silos, the same volume of water without the additive was added. Forages were ensiled for 90 days at 20°C in mini-silos (four replicates per treatment) with a capacity of 4.5 L with the potential to vent gas.²¹

¹⁶ Technical dossier/Supplementary Information May 2022/C.O.A storage trial 150,311 maltodextrin, C.O.A storage trial 150,313 maltodextrin and C.O.A storage trial 150,316 maltodextrin.

¹⁷ Technical dossier/Supplementary Information May 2022/C.O.A storage trial 150,311 dextrose, C.O.A storage trail 150,313 dextrose and C.O.A storage trial 150,316 dextrose.

¹⁸ Technical dossier/Supplementary Information May 2022/C.O.A storage trial 150,311 water survival, C.O.A storage trial 150,313 water survival and C.O.A storage trial 150,316 water survival.

¹⁹ Technical dossier/Supplementary Information May 2022/ FAD-2021-0074_AppSIIn_230522.

²⁰ Technical dossier/Supplementary Information May 2022/ [REDACTED]

²¹ Technical dossier/Section III/Annex IV_1.

Table 1: Characteristics of the forage samples used in the three ensiling experiments

Study	Test material	Dry matter content (%)	Water-soluble carbohydrate content (% fresh matter)
1 ²²	Lucerne/perennial ryegrass/red clover/ herb mixture (60:15:20:5)	38.2	1.5
2 ²³	Lucerne/grasses ^(a) /red clover/herb mixture (5:30:60:5)	37.8	1.7
3 ²⁴	Grasses ^(a) /red clover/white clover/herb mixture (25:55:10:10)	36.3	2.4

(a): Mainly timothy, meadow fescue and perennial ryegrass.

After 90 days, the silos were opened and the contents were analysed for pH, dry matter (DM), lactic, acetic, butyric, succinic and propionic acids, ethanol and ammonia-N content. The DM loss corrected for volatiles was calculated. Data were analysed using the non-parametric Wilcoxon–Kruskal–Wallis test and significance for all studies was declared at $p < 0.05$. Results (except for data on butyric, succinic and propionic acids) are shown in Table 2.

Table 2: Effect of *Pediococcus pentosaceus* DSM 32292 on dry matter loss and silage composition at day 90 of the ensiling period

Study	Application rate	Dry matter (DM) loss	pH	Lactic acid	Acetic acid	Ethanol	Ammonia-N
	(CFU/kg forage)	(%)		(% DM)	(% DM)	(% DM)	(% of total N)
1	0	2.5	5.6	2.0	1.1	0.8	8.0
	5×10^7	1.5*	4.5*	7.6*	0.8*	0.5*	7.0*
2	0	2.4	5.5	1.6	1.0	0.8	6.7
	5×10^7	1.3*	4.3*	6.1*	0.6*	0.5*	5.3*
3	0	6.0	5.4	3.1	1.1	1.4	12.4
	5×10^7	2.8*	4.3*	7.3*	1.0	0.6*	7.5*

*: Means in a column within a given trial are significantly different to the control $p < 0.05$.

The addition of the additive resulted in a significant reduction of DM loss, pH and of ammonia-N content in all studies made with moderately difficult to ensile forages. Moreover, in all three studies a significantly higher content of lactic acid was observed.

3.3.1. Conclusions on efficacy

The use of *Pediococcus pentosaceus* DSM 32292 at the proposed inclusion rate in the ensiling process has the potential to improve the production of silage with moderately difficult to ensile materials.

4. Conclusions

Pediococcus pentosaceus DSM 32292 is presumed safe for the target species, consumers and the environment.

The additive should be considered a respiratory sensitiser. No conclusions can be drawn on the eye and skin irritancy, or skin sensitisation potential of the additive.

Pediococcus pentosaceus DSM 32292 at a concentration of 5×10^7 CFU/kg forage has the potential to improve the preservation of nutrients in silage prepared with moderately difficult to ensile material.

²² Technical dossier/Section IV/Annex IV_2.

²³ Technical dossier/Section IV/Annex IV_3.

²⁴ Technical dossier/Section IV/Annex IV_4.

5. Documentation provided to EFSA/Chronology

Date	Event
25/03/2021	Dossier received by EFSA. <i>Pediococcus petosaceus</i> DSM 32292 for all animal species. Submitted by Marigot Ltd t/a Celtic Sea Minerals
21/06/2021	Reception mandate from the European Commission
03/12/2021	Application validated by EFSA – Start of the scientific assessment
23/02/2022	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation/safety for the user/efficacy</i>
09/03/2022	Comments received from Member States
19/04/2022	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
23/05/2022	Reception of supplementary information from the applicant - Scientific assessment re-started
29/06/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

CFU	colony forming unit
CV	coefficient of variation
DM	dry matter
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for *Pediococcus pentosaceus* DSM 32292

In the current application authorisation is sought under Article 4 for *Pediococcus pentosaceus* DSM 32292 under the category/functional group 1(k) “technological additives”/“silage additives”, according to Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisation is sought for the use of the *feed additive* in *silage* for all animal species.

According to the Applicant, the *feed additive* contains as active substance viable cells of the strain *Pediococcus* DSM 32292. The feed additive is to be marketed as preparation with a minimum content of 1×10^{10} Colony Forming Units (CFU) of *Pediococcus pentosaceus* DSM 32292 /g *feed additive*. The feed additive is intended to be used at a minimum dose of 5×10^7 CFU/kg *silage*.

For the identification of *Pediococcus pentosaceus* DSM 32292, the EURL recommends for official control (i) Whole Genome Sequencing Analysis (WGS) or (ii) Pulsed-Field Gel Electrophoresis (PFGE). The EURL considers that both methodologies are fit for purpose for the bacterial identification of authorised additives at a strain level.

For the enumeration of *Pediococcus* DSM 32292 in the feed additive, the EURL recommends for official control the ring-trial validated spread plate method EN 15786.

Since the unambiguous determination of the content of DSM 32292 initially added to silage is not achievable by analysis, the EURL cannot evaluate nor recommend any method for official control for the determination of *Pediococcus pentosaceus* DSM 32292 in *silage*.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.