REGULATED PRODUCTS SAFETY ASSESSMENT

Safety Assessment on the Safety and Efficacy of *Pediococcus pentosaceus* DSM 32292 as a Feed Additive for All Animal Species (RP552)

Food Standards Agency[1], Food Standards Scotland[2]

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An application was submitted to the Food Standards Agency in March 2021 from Microferm Limited ('the applicant') for the new authorisation of an additive (*Pediococcus pentosaceus* DSM 32292), under the category of 'technical additive' and functional group 'silage additives'. The additive is proposed to be used in all animal species, with a proposed minimum content of 1 x 10^{10} CFU / g to be applied to silage at a minimum of 5 x 10^{7} CFU / kg.

To support the Food Standards Agency (FSA) and Food Standards Scotland (FSS) in evaluating the dossier, the Animal Feed and Feed Additives Joint Expert Group (AFFAJEG) and the Advisory Committee on Animal Feedingstuffs (ACAF) were asked to review the dossier and the supplementary information from the applicant.

The FSA/FSS concluded, based on the ACAF's advice, that the additive was correctly identified and characterised, and that the additive can be considered safe for the target species, consumer, and the environment. The additive was identified as potentially harmful by contact with skin or eyes and a respiratory sensitiser.

It was concluded that the additive can be considered efficacious for moderately difficult to ensile forages. No conclusion could be reached on efficacy for difficult to ensile forages.

The views of AFFAJEG and ACAF have been taken into account in the safety assessment which represents the opinion of the FSA and FSS.

1. Introduction

The FSA and FSS have undertaken a safety assessment for a feed additive (*Pediococcus pentosaceus* DSM 32292 – Microferm Ltd., Spring Lane North, Malvern Link, Worcestershire, WR14 1BU, UK) under Assimilated Regulation (EC) No 1831/2003 (EC, 2003) under the category of 'technical

additives' and functional group 'silage additives'. The additive is proposed to be used in all animal species, with a proposed minimum content of 1 x 10^{10} CFU / g to be applied to silage at a minimum of 5 x 10^{7} CFU / kg.

To support the safety assessment by FSA and FSS, the AFFAJEG and the ACAF provided advice to the FSA and FSS outlined in this document.

In line with Article 8 of 1831/2003, the assessment has considered whether the feed additive complies with the conditions laid down in Article 5, including: safety considerations for human, animal and environmental health; efficacy of the additive for its intended effect; potential impairment of the distinctive features of animal products. This, and the guidance put in place by EFSA for the evaluation of feed additive applications, has formed the basis and structure for the assessment.

The dossier was evaluated by the AFFAJEG at their May 2022 meeting and by the ACAF at their June 2023 meeting. Further information was provided by the applicant responding to queries by the FSA in June 2022, March 2023 and July 2023. The ACAF accepted the conclusions on this application at their April 2024 meeting.

The views of ACAF have been taken into account in this safety assessment which represents the opinion of the FSA/FSS.

Table 1. Table showing products included in this assessment

Title	Product type	Intended use/s	Intended species or categories of animals
Pediococcus pentosaceus DSM 32292	Feed additive	Silage additive	All animal species

2. Assessment

2.1. Section II: Identity, characterisation and conditions of use

The additive is formulated to contain viable cells of *Pediococcus pentosaceus* DSM 32292, sucrose (5%), skimmed milk powder (20%), and glycerine (3%). The conditions of use proposed by the applicant are listed in Table 2. The additive specification table information is located within Table 3.

In the first evaluation, the AFFAJEG identified several pieces of information that the applicant would need to provide to inform the assessment of the product, including microbial resistance gene information, laboratory accreditation certificates, an additive label, and physical properties data for

Table 2. Identity table for the additive *Pediococcus pentosaceus* DSM 32292

Composition	
Pediococcus pentosaceus DSM 32292	Minimum $1x10^{10}$ cfu/g
Physico-chemical properties	
Dusting potential	10.19 g/m^3
Particle size distribution (50 % of particles are less than)	134 µm
Purity	
Microbial Impurities	
Presumptive Coliforms	< 10 CFU / g
Yeast	< 100 CFU / g
Salmonella spp.	Not Detected in 25 g
Presumptive E. coli	< 10 CFU / g
Moulds	< 100 CFU/g
Mycotoxins	
Aflatoxin B1	≤ 0.04 µg/kg
Aflatoxin B2	< 0.01 µg/kg
Aflatoxin G1	< 0.01 µg/kg
Aflatoxin G2	< 0.03 µg/kg
Total Aflatoxin	≤ 0.06 µg/kg
Heavy Metals	
Arsenic	≤ 0.11 mg/kg
Lead	≤ 0.27 mg/kg
Cadmium	≤ 0.2 mg/kg
Mercury	≤ 0.02 mg/kg

Table 3. Proposed conditions of use of *Pediococcus pentosaceus* DSM 32292

Proposed mode of use in animal nutrition		
Additive	*Pediococcus pentosaceus* DSM 32292	
Registration Number	1k1018	
Category of additive	Technical Feed Additive	
Functional group of additive	Silage Additive	
Conditions of use		
Species or category of animal	**Min. content**	**Withdrawal Period**
All animal species	Min. 1×10^{10} CFU/g to be applied to silage at a Min. 5×10^{7} CFU/kg.	1. --

the additive. These queries were satisfactorily addressed by the applicant. The additive is a significantly dusty product, and measures should be taken to limit exposure through inhalation.

2.1.1. Conclusions on Section II

The ACAF concluded the additive was correctly identified and characterised. The product is dusty. No further concerns were raised for Section II of this dossier.

2.2. Section III: Safety

The Committee noted that the active substance (*Pediococcus pentosaceus* DSM 32292) is a qualified presumption of safety (QPS) microorganism and as such no toxicological tests were required to evaluate the safety for the target species, consumers, or the environment.

The applicant noted that the product will be labelled as a respiratory sensitiser. The ACAF concluded the additive is likely to form a dust that could be inhaled by workers. As no studies of skin or eye irritancy or skin sensitisation were provided, the Committee regard the additive as potentially harmful by contact with skin or eyes.

2.2.1. Conclusions on safety

The AFFAJEG concluded that the additive can be considered safe for target species, consumers, and the environment. The additive is likely to form a dust that has the potential to be inhaled by workers. The additive should be considered a respiratory sensitiser and potentially harmful by contact with skin and eyes.

2.3. Section IV: Efficacy

The applicant provided studies on the effect that *Pediococcus pentosaceus* DSM 32292 had within three separate ensiling trials on 'moderately difficult to ensile' forages.

- Study A consisted of approximately 15 % perennial ryegrass, 60 % lucerne, 20 % red clover and 5 % herbs (mainly dandelion).
- Study B was composed of approximately 30 % grasses (mainly timothy, meadow fescue and perennial ryegrass), 60 % red clover, 5 % lucerne, and 5 % herbs (mainly dandelion).
- Study C consisted of approximately 25 % grasses (mainly timothy, meadow fescue and perennial ryegrass), 55 % red clover, 10 % white clover, and 10 % herbs (mainly dandelion).

The results demonstrated that the application of the *P. pentosaceus* inoculant improved the silage quality parameters pH, lactate, ethanol and 2,3-butanediol significantly in all three trials (Appendix A, Tables 4-6).

In the evaluation of the additive's efficacy, the ACAF noted that the additive was only tested against a single class of forage. The Committee was able to conclude the additive was efficacious in moderately difficult to ensile forages. The Committee requested further information from the applicant however they were unable to provide studies within other classes of forage. Due to a lack of evidence the ACAF was unable to conclude on the additive's efficacy in difficult to ensile forages.

2.3.1. Conclusions on efficacy

The Committee concluded that the additive is efficacious for moderately difficult to ensile forages. Due to a lack of evidence the ACAF was unable to conclude on the additive's efficacy in difficult to ensile forages.

3. Analytical methods evaluation

Conclusions on the analytical methods are presented here as an extract from the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for *Pediococcus pentosaceus* DSM 32292 (EURL-FA, 2020).

> "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."

FSA/FSS accepts the EURL analytical method evaluation reports. FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

4. Conclusions

The Committee concluded the additive was correctly identified and characterised. The additive shows a high dusting potential.

The ACAF concluded that the additive can be considered safe for target species, consumers, and the environment. The additive should be considered a respiratory sensitiser and potentially harmful by contact with skin and eyes.

The Committee concluded that the additive is efficacious for moderately difficult to ensile forages. Due to a lack of evidence the ACAF was unable to conclude on the additive's efficacy in difficult to ensile forages.

The FSA/FSS agree with the conclusions reached by the AFFAJEG/ACAF. FSA/FSS accepts the EURL analytical method evaluation reports. FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

Acknowledgements

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Abbreviations

Acronym	Definition
ACAF	Advisory Committee on Animal Feedingstuffs
AFFAJEG	Animal Feed and Feed Additives Joint Expert Group
CFU	Colony Forming Units
DM	Dry Matter
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
EURL	European Union Reference Laboratory
FSA	Food Standards Agency
FSS	Food Standards Scotland
FM	Fresh Matter
GLP	Good Laboratory Practices
N	Total nitrogen
NH3-N	Nitrogen content of ammonia
PFGE	Pulsed-Field Gel Electrophoresis
Pr>Chi^2	Probability > chi square
QPS	Qualified Presumption of Safety
WGS	Whole Genome Sequence

Acronym	Definition
WSC	Water-soluble carbohydrates

References

EC (European Commission). (2003). *Regulation No 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition*. https://www.legislation.gov.uk/eur/2003/1831/contents

EURL-FA (European Reference Laboratory for Feed Additives). (2020). *Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003. Pediococcus DSM 32292*. https://joint-research-centre.ec.europa.eu/reports-and-technical-documentation/fad-2021-0074_en

Appendix A: Efficacy tables

Table 4. Forage A (37% DM; 15 g WSC/kg FM) - Fermentation quality of P. pentosaceus-treated and untreated silages after 90 days.

	DM g/ kg FM	WSC g/kg DM	NH3-N g/kg N	pH	Lactic acid g/kg DM	Acetic acid g/kg DM	n-Butyric acid g/kg DM	Propionic acid g/kg DM	Succinic acid g/kg DM	Ethanol g/ kg DM	2,3-Butanediol g/kg DM
Control	370	8	80	5.57	20	11	1	3	8	8	5
Treated	370	1	70	4.50	76	8	<1	1	3	5	1
Pr>Chi^2	0.77*	0.02	0.02	0.02	0.02	0.02	0.11*	0.013	0.02	0.02	0.02

Values represent treatment means from 4 replicate silos. DM: Dry matter; FM: Fresh matter; WSC: water-soluble carbohydrates; NH3-N: Nitrogen content of ammonia; N: Total nitrogen; Pr>Chi^2: Probability>chi square; * notes no statistical significance.

Table 5. Forage B (36% DM; 17 g WSC/kg FM) - Fermentation quality of P. pentosaceus-treated and untreated silages after 90 days.

	DM g/kg FM	WSC g/kg DM	NH3-N g/kg N	pH	Lactic acid g/kg DM	Acetic acid g/kg DM	n-Butyric acid g/kg DM	Propionic acid g/kg DM	Succinic acid g/kg DM	Ethanol g/ kg DM	2,3- Butanediol g/kg DM
Control	359	14	67	5.46	16	10	<1	4	7	8	8
Treated	366	5	53	4.28	61	6	<1	1	2	5	1
Pr>Chi^2	0.02	0.02	0.02	0.02	0.02	0.02	1.00	0.014	0.02	0.02	0.02

Values represent treatment means from 4 replicate silos. M: Dry matter; FM: Fresh matter; WSC: water-soluble carbohydrates; NH3-N: Nitrogen content of ammonia; N: Total nitrogen; Pr>Chi^2: Probability>chi square; * notes no statistical significance.

Table 6. Forage C (34% DM; 24 g WSC/kg FM) - Fermentation quality of P. pentosaceus-treated and untreated silages after 90 days.

	DM g/kg FM	WSC g/kg DM	NH3-N g/kg N	pH	Lactic acid g/kg DM	Acetic acid g/kg DM	n-Butyric acid g/kg DM	Propionic acid g/kg DM	Succinic acid g/kg DM	Ethanol g/ kg DM	2,3-Butanediol g/kg DM
Control	336	6	124	5.36	31	11	2.3	16	21	14	36
Treated	341	10	75	4.33	73	10	<1	7	10	6	8
Pr>Chi^2	0.03	0.02	0.02	0.02	0.02	0.08	0.02	0.02	0.02	0.02	0.02

Values represent treatment means from 4 replicate silos. M: Dry matter; FM: Fresh matter; WSC: water-soluble carbohydrates; NH3-N: Nitrogen content of ammonia; N: Total nitrogen; Pr>Chi^2: Probability>chi square; * notes no statistical significance.