Assessment of the safety and efficacy of *Lactobacillus buchneri* NCIMB 40788 CNCM I-4323, *Lactobacillus plantarum* CNCM I-3235, *Lactobacillus plantarum* CNCM MA 18/5U DSM 11672, *Pediococcus acidilactici* CNCM I-3237, *Pediococcus acidilactici* CNCM MA 18/5M DSM 11673, *Pediococcus pentosaceus* NCIMB 12455, *Propionibacterium acidipropionici* CNCM MA 26/4U, *Lactobacillus buchneri* NCIMB 40788 CNCM I-4323 and *Lactobacillus hilgardii* CNCM I-4785 as feed additives for all animal species

Reference number RP791

Regulated Products Risk Assessment Unit
Science, Evidence and Research Division, FSA

Risk Assessment Team
Science Division, FSS

Regulated Product Dossier Assessment
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Abbreviations

Acronym	Definition
ACAF	Advisory Committee on Animal Feedingstuffs
CARD	Comprehensive Antibiotic Resistance Database
CCP	Critical Control Points
CFU	Colony Forming Units
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
EURL	European Union Reference Laboratory
FSA	Food Standards Agency
FSS	Food Standards Scotland
LOQ	Limit of quantification
PCR	Polymerase Chain Reaction
PFGE	Pulse Field Gel Electrophoresis
PPE	Personal Protective Equipment

Summary

An application was submitted to the Food Standards Agency in April 2021 Lallemand Animal Nutrition UK ("the applicant") for the renewal of authorisation of preparations of 9 bacterial strains, under the category of 'technological additives', functional group 'silage additives' for their use in all animal species: *Lactobacillus buchneri* NCIMB 40788 CNCM I-4323, *Lactobacillus plantarum* CNCM I-3235, *Lactobacillus plantarum* CNCM MA 18/5U DSM 11672, *Pediococcus acidilactici* CNCM I-3237, *Pediococcus acidilactici* CNCM MA 18/5M DSM 11673, *Pediococcus pentosaceus* NCIMB 12455, *Propionibacterium acidipropionici* CNCM MA 26/4U, *Lactobacillus buchneri* NCIMB 40788 CNCM I-4323 and *Lactobacillus hilgardii* CNCM I-4785. Due to a recent taxonomical change, the applicant wished to update the names of some *Lactobacillus* strains and of the *Propionibacterium acidipropionici* strains.

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

After receiving further information from the applicant, ACAF were able to conclude that the various samples of bacteria have remained relatively stable since the original authorisation.

The ACAF concluded that the additives can be considered safe for the target species, consumers and the environment, based on the Qualified Presumption of Safety (QPS) status of the silage additives. In terms of safety for users and workers, all additives must be assumed to be respiratory sensitisers. Members concluded that the silage additive *Pediococcus acidilactici* MA18/5M is not an eye irritant, skin sensitiser or skin irritant, however, in the absence of evidence to the contrary, all other additives in the application must be considered as potential eye irritants, skin sensitisers and skin irritants.

No efficacy evaluation was required for the renewal of authorisation.

The views of 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 risk assessment for preparations of 9 bacterial strains (Lallemand Animal Nutrition UK, 11-13 Spring Lane North, Malvern WR14 1BU) under retained regulation (EC) No 1831/2003[1] under the category of 'technological additives', functional group 'silage additives' for their use in all animal species: *Lactobacillus buchneri* NCIMB 40788 CNCM I-4323, *Lactobacillus plantarum* CNCM I-3235, *Lactobacillus plantarum* CNCM MA 18/5U DSM 11672, *Pediococcus acidilactici* CNCM I-3237, *Pediococcus acidilactici* CNCM MA 18/5M DSM 11673, *Pediococcus pentosaceus* NCIMB 12455, *Propionibacterium acidipropionici* CNCM MA 26/4U, *Lactobacillus buchneri* NCIMB 40788 CNCM I-4323 and *Lactobacillus hilgardii* CNCM I-4785. Due to a recent taxonomical change, the applicant wished to update the names of some *Lactobacillus* strains and of the *Propionibacterium acidipropionici* strains. To support the safety assessment by FSA and FSS, the ACAF provided advice to the FSA and FSS outlined in this document.

The dossier was evaluated on behalf of the FSA and FSS by the ACAF. 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.

With thanks to the members of the ACAF during the course of the assessment, who were: Professor Nicholas Jonsson, Martin Briggs, Professor Katrina Campbell, Susan MacDonald, Professor Matthew Fisher, Christine McAlinden, Dr. Donald Morrison, Derek Renshaw, Dr. Michael Salter, Dr. Adam Smith, Dr. Helen Warren and Dr. Nick Wheelhouse.

The dossier was evaluated by the ACAF at their December 2022 meeting. Further information was provided by the applicant in March 2022 and January 2023, responding to queries by the FSA.

This document outlines the discussion and conclusions of the ACAF's assessment on the safety and efficacy of *Lactobacillus buchneri* NCIMB 40788 CNCM I-4323, etc. as a feed additive.

2. Assessment

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

Each additive is composed of freeze-dried bacterial cells. Table 1 contains the bacterial strains covered under this application and the new name proposed by the applicant, where relevant. The desired concentration of active substance is achieved through formulating with feed materials and technological feed additives, as described in Table 2.

Table 1: Additives covered by application and the relevant proposed new names

Name of additive	New name proposed by applicant	Associated code	CFU (colony forming units) / gram
Lactobacillus buchneri NCIMB 40788 CNCM I-4323	*Lentilactobacillus buchneri* NCIMB 40788, CNCM I-4323	Strain LB	Minimum $3x10^9$
Lactobacillus plantarum CNCM I-3235	*Lactiplantibacillus plantarum* CNCM I-3235	Strain LP1	Minimum $5x10^{10}$
Lactobacillus plantarum CNCM MA 18/5U	*Lactiplantibacillus plantarum* CNCM I-3736 DSM 11672	Strain LP2	Minimum $2x10^{10}$
Pediococcus acidilactici CNCM I-3237	*Pediococcus acidilactici* CNCM I-3237	Strain PA1	Minimum $1x10^{10}$
Pediococcus acidilactici CNCM MA 18/5M DSM 11673	*Pediococcus acidilactici* DSM 11673	Strain PA2	Minimum $3x10^9$
Pediococcus pentosaceus NCIMB 12455	*Pediococcus pentosaceus* NCIMB 12455	Strain PP	Minimum $3x10^9$
Propionibacterium acidipropionici CNCM MA 26/4U	*Acidipropionibacterium acidipropionici* CNCM I-4661	Strain PrA	Minimum $1x10^8$
Lactobacillus buchneri NCIMB 40788 CNCM I-4323 and *Lactobacillus hilgardii* CNCM I-4785	*Lentilactobacillus buchneri* NCIMB 40788 CNCM I-4323 and *Lentilactobacillus hilgardii* CNCM I-4785 (in a 1:1 ratio)	Strains LBLH	Minimum $1.5x10^{11}$

The applicant provided data from five batches supporting the specification values given below (Table 2).

Table 2: Components and specification table for all strains

Components	
Technological additives	Silicic acid, precipitated and dried (E551a), colloidal silica (E551b) and/or sodium aluminosilicate synthetic (E554)
Emulsifying and stabilising agents, thickeners, and gelling agents	Xanthan gum (E415)
Feed materials (carriers to standardise the preparations)	Sucrose
Specifications	
Salmonella	Absent in 25 g
Escherichia coli	< 10 CFU/g
Enterobacteriaceae or coliforms	< 1000 CFU/g

Members agreed that the request for the name change was logical and reasonable. The identity and characterisation of the additives were discussed, and no issues were raised. The strains were found to be well characterised, and a robust report was provided for whole genome sequencing. Members initially queried why CARD analysis found no resistance genes when *Pediococcus* has been shown to demonstrate resistance to vancomycin. The applicant provided further explanation that some vancomycin resistant gene hits were found on the CARD "loose hits" and that as these were found on chromosomal DNA, they are likely to be intrinsic to the bacterial genomes and inherent to the species. The Committee were satisfied with the explanations provided.

The Committee requested evidence from the applicant demonstrating that the additives remain unchanged from the original authorisation. Upon receiving the additional information, members were convinced that the various samples of bacteria tested remained relatively stable. Members had no other concerns with the characterisation of the additive.

The manufacturing process was well detailed, with the main Critical Control Points (CCP) provided. The stability of the additives was also demonstrated. All additives are

dusty and as they contain proteinaceous substances, they must be assumed to be respiratory sensitisers, therefore personal protection equipment (PPE) will be necessary. The applicant acknowledged this in both the label and the material safety data sheet (MSDS).

The proposed conditions of use of the additive are described in Table 3 and Table 4.

Table 3: Proposed conditions of use of additives

Proposed mode of use in animal nutrition	
Parameters	**Conditions of use**
Target species	All animal species and categories
Age group/physiological stage	Not applicable
Max. use levels	Not applicable
Water	Not intended for use in water for drinking
Duration of administration	Not applicable
Withdrawal period	None
Contraindications	None
Use in complementary feedingstuffs	Not applicable

Table 4: Proposed minimum use level of the preparation of the additive

Additive	Min. use levels when used without combination with other microorganisms as silage additives	Forage category
Strain LB	1 x 10^8 CFU/kg fresh material	All forages
Strain LP1	2 x 10^7 CFU/kg fresh material	All forages
Strain LP2	1 x 10^8 CFU/kg fresh material	All forages
Strain PA1	5 x 10^7 CFU/kg fresh material	All forages
Strain PA2	3 x 10^7 CFU/kg fresh material	All forages
Strain PP	3 x 10^7 CFU/kg fresh material	All forages
Strain PrA	1 x 10^8 CFU/kg fresh material	Moderately difficult and difficult to ensile silage in forage species covering a range dry matter content from 24 to 40%
Strain LBLH	3 x 10^8 CFU/kg fresh material (*L. hilgardii* and *L.buchneri* in ratio of 1:1)	Easy and moderately difficult to ensile fresh material

2.1.1. Conclusions on Section II

The ACAF concluded that the additives were correctly identified and characterised.

No further concerns were raised for Section II of this dossier.

2.2. Section III: Safety

Each of the microorganisms present in these additives has a QPS (Qualified Presumption of Safety) status as established by the European Food Safety Authority (EFSA), therefore most requirements related to safety of the additive are not applicable. Additionally, these requirements do not apply if it can be demonstrated that the active substances occur as normal constituents of silage and that the use of the additive does not substantially increase their concentration. Each microbial strain covered can be naturally found in silages and the applicant argued that their use would not contribute to a substantial change in exposure. Members discussed the potential risk of increasing the microorganisms above normal silage levels but concluded that at the end of the ensiling process, microorganism levels are expected to return to normal, despite an initial increase after the use of silage additives. Therefore, the Committee were satisfied that the safety requirements would only be necessary for users and workers.

2.2.1. Safety for the user

All of the additives covered by this application are dusty, so there is potential for workers to be exposed by inhalation. Additives containing proteinaceous substances, such as enzymes and micro-organisms, are assumed to be respiratory sensitisers. Therefore, these silage additives should be considered as potential respiratory sensitisers.

Skin sensitisation and irritation potential was only tested on one strain (*Pediococcus acidilactici* MA18/5M) therefore, the Committee could not conclude on the safety of the other strains. As a result, all additives in this application should be regarded as potential skin sensitisers and irritants.

Following the provision of the original data relating to the eye irritancy of *Pediococcus acidilactici* MA18/5M, members were able to conclude that this additive is not an eye irritant. The Committee could not conclude on the other additives presented in this application, and they must therefore be regarded as potential eye irritants.

2.2.2. Conclusions on safety

Each of the microorganisms present in these additives has QPS status and therefore, the additives are presumed safe for the target species, consumers and the environment.

The ACAF concluded that *Pediococcus acidilactici* MA18/5M is not an eye irritant, skin sensitiser or skin irritant. Due to a lack of data, the other additives must be considered as potential eye irritants, skin sensitisers and skin irritants. All additives under this application contain proteinaceous substances and therefore must be assumed to be potential respiratory sensitisers.

2.3. Section IV: Efficacy

Efficacy studies are not required for the renewal of authorisations of feed additives when the applicant does not propose amending the conditions of the original authorisation which may have an impact on the efficacy of the additive.

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 43 microorganisms for silage[2]:

"For identification and characterisation of *Saccharomyces cerevisiae* the EURL recommends for official control Polymerase Chain Reaction (PCR), a generally recognised standard methodology for identification of yeasts. For identification and characterisation of all the other micro-organisms of concern (i.e., *lactococci*, *lactobacilli*, *pediococci* and *bacilli*) the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for microbial identification. The EURL recommends for enumeration in the feed additives the following ring trial validated methods: – Pour plate method using MRS agar (ISO 15214) for *Lactococci*; – Spread plate method using MRS agar (EN 15787) for *Lactobacilli*; – Spread plate method using MRS agar (EN 15786) for *Pediococci*; – Spread plate method using tryptone soya agar (EN 15784) for *Bacilli*; and – Pour plate method using CGYE agar (EN 15789) for *Saccharomyces*. None of the Applicants provide experimental

data for the determination of micro-organisms in silage. Furthermore, the unambiguous determination of the content of micro-organisms added to silage is not achievable by analysis. Therefore, the EURL cannot evaluate nor recommend any method for official control to determine any of the forty five micro-organisms of concern 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

After the applicant had provided evidence that the additives remained unchanged from the initial authorisation and they were fully characterised, no further causes of concern were identified by the ACAF in the identity, production and characterisation sections.

The ACAF concluded that the additives can be considered safe for the target animal species, the consumer, and the environment, due to the QPS status of the microorganisms in these silage additives. All additives contain proteinaceous substances and are therefore assumed to be potential respiratory sensitisers. The Committee concluded that one of the strains (*Pediococcus acidilactici* MA18/5M) is not an eye irritant, skin sensitiser or skin irritant. However, no studies were performed on the other additives and therefore they must be considered as potential eye irritants, skin sensitisers and skin irritants.

Efficacy evaluation was not required for the renewal of authorisation.

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

5. References

1. EC (European Commission), 2003. Regulation No 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition.

2. EURL-FA (European Reference Laboratory for Feed Additives), 2017. 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. Analysis for 43 microorganisms for silage. Available at: 20 FAD dossiers (europa.eu)

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