Safety Assessment: Outcome of assessment of *Pediococcus acidilactici* CNCM I-4622 as a feed additive for all animal species and categories

Reference number RP29

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Regulated Product Dossier Assessment

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Summary

An application was submitted to the Food Standards Agency in January 2021 from Lallemand SAS (on behalf of Danstar Ferment AG, Switzerland), France ("the applicant") for the authorisation of an additive containing *Pediococcus acidilactici* CNCM I-4622, under the category of 'technological' additives, functional groups 'hygiene condition enhancers' and 'acidity regulators'.

The additive contains freeze-dried viable cells of *Pediococcus acidilactici*, a technological additive and a feed material that is proposed to be supplied at a minimum of $1x10^9$ colony forming units (CFU)/kg of complete feed at 12% moisture for all animal species and categories. It aims to reduce the development of coliform bacteria through the reduction of pH and increasing lactic acid concentration in feed.

To support the Food Standards Agency (FSA) and Food Standards Scotland (FSS) in evaluating the dossier, the Animal Feed and Feed Additives Joint Group (AFFAJEG) and the Advisory Committee on Animal Feedingstuffs (ACAF) were asked to review the dossier and the supplementary information from the applicant. Upon evaluation of the dossier, ACAF concluded that the additive is compatible with a limited number of commercially available coccidiostats. *Pediococcus acidilactici* CNCM I-4622 is a qualified presumption of safety (QPS) organism, therefore, no data on safety for the target species, consumers or the environment was analysed. ACAF concluded that the additive should be considered a respiratory sensitiser.

The ACAF evaluated efficacy data from five *in-vitro* studies and concluded that the additive can be considered efficacious as an acidity regulator and a hygiene condition enhancer within the first 24 h after administration of the proposed dose of $1x10^9$ CFU/kg of complete feed at 12% moisture.

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 risk assessment for a feed additive (*Pediococcus acidilactici* CNCM I-4622, Danstar Fermant AG, represented by Lallemand Animal Nutrition UK Ltd. Spring Lane North, Malvern link, Worcestershire, WR14 1BU) under Regulation (EC) No 1831/2003[1] under the category of 'technological' additives, functional groups 'gut flora stabilisers' and 'hygiene condition enhancer'. To support the safety assessment by FSA and FSS, the AFFAJEG and 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 AFFAJEG. 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 AFFAJEG and the ACAF during the course of the assessment, who were: Professor John Wallace, Professor Nicholas Jonsson, Martin Briggs, Dr. 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 AFFAJEG at their April 2021 and October 2021 meetings. Further information was provided by the applicant in June 2021, responding to queries by the FSA. The conclusions by the AFFAJEG were reviewed and approved by the ACAF at their October 2022 meeting.

This document outlines the discussion and conclusions of the AFFAJEG's assessment on the safety and efficacy of *Pediococcus acidilactici* CNCM I-4622 as a feed additive.

2. Assessment

2.1 Section II: Identity, characterisation and conditions of use

The additive contains freeze-dried viable cells of *Pediococcus acidilactici* CNCM I-4622, a technological additive and a feed material. The applicant provided data from several batches supporting the specification values outlined below.

Table 1: Identity table

Active substance		
Pediococcus acidilactici CNCM I-4622	Minimum $1x10^{10}$ CFU/g	
Composition		
Pediococcus acidilactici CNCM I-4622	1-3%	
Sodium aluminosilicate (E554) / Silicic acid (E551a) / Colloidal silica (E551b)	5%	
Sucrose / Calcium carbonate / Maltodextrin / Lactose / Dextrose	92-94%	
Appearance		
Powder		
Chemical-physical specifications		
Dusting potential	23.1 – 30.1 g/m^3	
Microbiological profile (CFU/g)		**Method of analysis**
Salmonella	Absent in 25g	ISO 6579 (or eq.)
Escherichia coli	<10	ISO 7251 (or eq.)
Enterobacteriaceae or coliforms	<1000	ISO 4832 (or eq.)
Water activity	<0.08	

The microorganism is *Pediococcus acidilactici* CNCM I-46222. The application presented a whole genome sequence (WGS) analysis and an antimicrobial resistance (AMR) report in line with guidance recommendations. The AFFAJEG evaluated the WGS and AMR data and determined that the microorganism used as the active substance of the additive is identical to the QPS registered microorganism, that it is not capable of producing antimicrobial substances and therefore is free from any antibiotic activity. The microorganism does not carry antimicrobial resistance genes.

The AFFAJEG raised concern over the Hazard Analysis Critical Control Point (HACCP) procedure followed in the production of the additive. The FSA requested the applicant provide the full HACCP protocol and to confirm whether any antibiotics are used in the production process. The applicant provided the requested information, which, upon review by the Group, was considered to not be cause for concern.

The application provided data on compatibility of the additive with coccidiostats in feed. The Group evaluated the report and noted that not all commercially available coccidiostats had been tested. The FSA requested further clarification from the applicant. No further information was provided, therefore, the AFFAJEG concluded that *Pediococcus acidilactici* CNCM I-4622 is compatible with halofuginone, robenidine, diclazuril, decoquinate and nicarbazine. Compatibility with maduramicin, monensin, narasin, narazin/nicarbazin, salinomycin has not been demonstrated.

The additive aims to supply a minimum of $1x10^9$ CFU/kg of complete feed at 12% moisture for all animal species and categories.

Table 2: Proposed mode of use of *Pediococcus acidilactici* CNCM I-4622

Proposed mode of use in animal nutrition			
Additive		*Pediococcus acidilactici* CNCM I-4622	
Registration number/EC No/No		1k2104	
Category(-ies) of additive		Technological	
Functional group(s) of additive		Acidity regulator / Hygiene condition enhancer	
Description			
Composition, description		**Purity criteria**	**Method of analysis**
Preparation of *Pediococcus acidilactici* CNCM I-4622		Containing a minimum of: $1x10^{10}$ CFU/g	Enumeration: Ring-trial validated spread plate method using MRS agar EN 15786. Identification: Pulsed field gel electrophoresis
Trade name (if appropriate)			--
Name of the holder of authorisation (if appropriate)			--
Conditions of use			
Species or category of animal	**Maximum Age**	**Minimum content**	**Maximum content**
		Pediococcus acidilactici CNCM I-4622 CFU/g of complete feed at 12% moisture	
All animal species	--	$1x10^{9}$	--
Water	Not intended for use in water for drinking		

2.1.1. Conclusions on Section II

The AFFAJEG concluded that compatibility with coccidiostats has only been proven for halofuginone, robenidine, diclazuril, decoquinate and nicarbazine.

No further concerns were raised for Section II of the dossiers.

2.2 Section III: Safety

2.2.1. Safety for the target species, the consumer and the environment

The applicant referred to previous European Food Safety Authority (EFSA) opinions on the additive for birds and porcine species[2], fish and crustaceans[3] and as a silage additive for all species[4] to support the assessment of safety for the target species, the consumer and the environment. The AFFAJEG evaluated the documents and determined that the evidence provided, together with the QPS status of the active substance, was sufficient to conclude that the additive can be considered safe for all animal species when used as a technological feed additive at the proposed dose.

2.2.2. Safety for the user

The applicant presented several sources for the evaluation of safety for the user:

- An acute dermal irritation/corrosion test following OECD 404 protocol, which concluded that the additive is non-irritant to skin.
- A skin sensitisation test (local lymph-node assay) following OECD 429 protocol, which concluded that the additive is not expected to be a dermal sensitiser.
- An acute eye irritation/corrosion test following OECD protocol 405, which concluded that the additive is non-irritant for the eye.

The AFFAJEG evaluated the information presented by the applicant, as well as previous EFSA opinions on the active substance. Dusting potential data shows that exposure via the respiratory system is likely to occur when dust is formed, therefore the Group agreed that the implementation of appropriate operational procedures and organisational measures is necessary to reduce exposure. The AFFAJEG concluded that the additive:

- Should be considered a respiratory sensitiser given its proteinaceous nature.
- Is not an eye irritant or a dermal sensitiser.

2.2.3. Conclusions on safety

The AFFAJEG concluded that the additive can be considered safe for the target species, the consumer and the environment. The additive should be considered a respiratory sensitiser but is not an eye irritant or dermal sensitiser.

2.3 Section IV: Efficacy

The applicant provided evidence of efficacy of the additive as an acidity regulator and a hygiene condition enhancer in five studies.

2.3.1. Study 1

Study 1 was an *in-vitro* study comparing three treatment groups ($1x10^7$ CFU/ml of liquid feed) to a negative and positive control to evaluate the efficacy of the additive as a hygiene condition enhancer and acidity regulator. The data showed a significant decrease of coliform development and a reduction in the metabolism of lysine into biogenic amines by *Escherichia coli* bacteria.

Table 3: **Microbial counts (log10 c.f.u./mL) in liquid feed inoculated with *Lb. plantarum* (LP), *P. acidilactici* (PA), *E. coli* (EC), *Lb. plantarum* plus *E. coli* (LPEC), *P. acidilactici* plus *E. coli* (PAEC), or uninoculated (Control); incubated for 48 h at 35°C.**[5]

	Fermentation treatment						S.E.M.
	Control	**LP**	**PA**	**EC**	**LPEC**	**PAEC**	
Lactic acid bacteria							
0h	4.54	7.53	7.93	4.50	7.69	8.19	0.125
21h	8.61	10.54	10.49	8.69	10.57	10.44	0.125
48h	9.18	11.32	11.47	9.40	11.30	11.18	0.125
Main effect of treatment	7.39 b	9.79 a	9.93 a	7.52 b	9.85 a	9.82 a	0.072
Coliform bacteria[a]							
0h	3.87	3.56	3.64	4.20	4.30	4.17	0.059
21h	6.47	<2	<2	8.51	<2	<2	0.059
48h	<2	<2	<2	<2	<2	<2	0.059
Main effect of treatment	4.10	2.51 a	2.54 a	4.89	2.77 b	2.71 ab	0.034
Statistical analysis was conducted using a general linear model: treatment×time interactions were significant (P<0.001) for both lactic acid bacteria and coliforms. Means with the same letter (a, b) are not significantly different. [a] Limit of detection (2 log10) was used in the statistical analysis.							

2.3.2. Study 2

Study 2 was an *in-vitro* study comparing two treatment groups ($1x10^9$ CFU/ml of liquid feed) to a negative control to evaluate the efficacy of the additive as a hygiene condition enhancer and acidity regulator. The data showed that, for at least 24h after fermentation, pH is reduced and production of lactic and acetic acid is increased.

Table 4: Organic acid production (mmol/L) and pH after 24 h fermentation of red and white sorghum with different lactic acid bacteria (n=3). *Lb. plantarum* (LP), *P. acidilactici* (PA).[6]

	Lactic acid		Acetic acid		pH	
Micro-organism	Red sorghum	White sorghum	Red sorghum	White sorghum	Red sorghum	White sorghum
Control	7.25c	12.18b	1.32a	5.37bc	5.67b	5.88b
SLP	312.3a	313.65	10.13b	9.70	3.49a	3.22a
PA1	203.67b	264.07a	14.88b	20.65a	3.65a	3.41a
LF1	261.3a	246.18a	2.61a	3.99b	3.64a	3.34a
LP2	273.8a	264.98a	11.6b	10.21c	3.58a	3.25a
S.E.M.	6.37	5.66	0.60	0.54	0.18	0.16
abc Significant difference between means bearing different letters in the same column (P<0.05). There was no significant effect (P>0.05) of variety on any of the parameters tested, n=number of observations per mean.						

2.3.3. Study 3

Study 3 was an *in-vitro* study comparing two treatment groups (1.0 g/ml of liquid feed) to a positive and negative control. The applicant claimed that the data from this study provide further support for the efficacy of the additive as a hygiene condition enhancer by reducing pH.

Table 5: Mean pH values of maize of different varieties fermented naturally fermented and maize fermented aided by the additive.[7]

	Natural fermentation			Bactocell assisted fermentation		
Maize variety	0h	24h	48h	0h	24h	48h
A	6.7	4.80	3.73	6.7	3.68	3.46
B	6.7	4.73	3.67	6.7	3.59	3.46
C	6.6	4.57	3.50	6.6	3.67	3.47
D	6.6	4.77	3.67	6.6	3.64	3.46
E	6.6	4.80	4.13	6.6	3.58	3.43
F	6.4	4.87	3.93	6.4	3.84	3.69
G	6.4	4.70	4.13	6.4	3.69	3.46
H	6.6	4.83	4.43	6.6	3.63	3.44
I	6.4	4.53	3.87	6.4	3.59	3.40
J	6.4	4.73	4.07	6.4	3.71	3.52
K	6.4	4.73	3.40	6.4	3.66	3.46

2.3.4. Study 4

Study 4 was an *in-vitro* study comparing a treatment group ($1x10^9$ CFU/kg of complete feed) to a control to evaluate the efficacy of the additive as a hygiene condition enhancer. The data showed a significant reduction in levels of *Escherichia coli* in feed.

2.3.5. Study 5

An in-house, *in-vitro* study comparing two treatment groups ($1x10^9$ CFU/kg of compound feed) to a negative control to evaluate the efficacy of the additive as a hygiene condition enhancer and acidity regulator was conducted. The data showed acidification and increased lactic acid content in the feed, as well as a reduction in the development of coliforms for up to 24 h.

2.3.6. Conclusions on efficacy

The AFFAJEG evaluated the information presented by the applicant, together with previous EFSA opinions, and determined that the additive is likely efficacious in reducing pH, increasing lactic acid and reducing development of coliform bacteria for 24 h after administration. The Group discussed the efficacy of the additive past the 24 h mark and noted that it is likely for the feed to be consumed within 24 h after adding the additive. In light of this, the AFFAJEG concluded that *Pediococcus acidilactici* CNCM I-4622 has the potential to be efficacious as a hygiene condition enhancer and acidity regulator at the proposed dose of $1x10^9$ CFU/kg of complete feed at 12% moisture.

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 (EURL) for Feed Additives on the Method(s) of the Analysis for Bactocell® 10^9:

For the enumeration of *Pediococcus acidilactici* in feed additive, premixtures, feedingstuffs and water the applicant proposes the CEN spread plate method EN 15786:2009. The EURL recommends, for official control, the CEN method EN 15786 for the enumeration of *Pediococcus acidilactici* in feed additive, premixtures, feedingstuffs (excluding mineral feeds) and water.

Pulsed Field Gel Electrophoresis (PFGE) was used by the Applicant for identification and characterization of the active agent. This generally recognised standard methodology for microbial identification, is recommended by EURL for official control.

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 data presented in the application fully identified the additive's active substance as the QPS organism *Pediococcus acidilactici* CNCM I-4622. The additive can be

considered compatible with the coccidiostats halofuginone, robenidine, diclazuril, decoquinate and nicarbazine. However, compatibility with maduramicin, monensin, narasin, narazin/nicarbazin, salinomycin has not been demonstrated due to lack of evidence.

No safety data for target species, consumers or the environment was required to be presented in the application. Data from previous EFSA opinions, together with the QPS status of the organism, are sufficient evidence to demonstrate the safety of the additive for the target species, consumers and the environment. The additive should be considered a respiratory sensitiser.

Based on data from five efficacy studies, the additive has the potential of being efficacious for increasing lactic acid concentration, reducing pH and reducing the growth of coliform bacteria at the proposed dose of $1x10^9$ CFU/g.

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

5. References

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6. Abbreviations

ACAF	Advisory Committee on Animal Feedingstuffs
AFFAJEG	Joint Expert Group for Animal Feed and Feed Additives
AMR	Antimicrobial resistance
CEN	European Committee for Standardisation
CFU	Colony forming unit
EFSA	European Food Safety Authority
EURL	European Union Reference Laboratory
OECD	The Organisation for Economic Co-operation and Development
QPS	Qualified presumption of safety
WGS	Whole genome sequencing

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