European Food Safety Authority

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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of 18 strains of *Lactobacillus plantarum* (DSM 23375, CNCM I-3235, DSM 19457, DSM 16568, LMG 21295, DSM 16565, VTT E-78076, CNCM MA 18/5U, NCIMB 30238, ATTC PTA-6139, DSM 18112, ATCC 55058, DSM 18113, DSM 18114, ATCC 55942, ATCC 55943, ATCC 55944 and NCIMB 30094) as silage additives for all species[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The 18 strains of *Lactobacillus plantarum* are each intended to improve ensiling at proposed doses ranging from 5×10^6 to 1.0×10^9 colony-forming units/kg forage. The bacterial species *L. plantarum* is considered by EFSA to be suitable for the Qualified Presumption of Safety approach to safety assessment. As the identity of all 18 strains was clearly established and as no antibiotic resistance of concern was detected, the use of the 18 strains in silage production is presumed safe for livestock species, consumers of products from animals and the environment. The material safety data sheet proposed indicates that preparations containing the strains may cause irritation on contact with skin or eyes. In addition, given the dusting potential and proteinaceous nature of the active agents, the FEEDAP Panel considers it prudent to treat all 18 additives as skin and respiratory sensitisers. Studies with laboratory-scale silos are described for each strain, each lasting at least 90 days, made using forage samples representing materials that are easy, moderately difficult and difficult to ensile. Nine of 18 additives showed potential at the minimum doses proposed to improve the production of silage from a wide range of forage species by reducing the pH and increasing the preservation of dry matter. A further strain also showed similar potential, but only when used in combination with a specific strain of *Pediococcus pentosaceus*. Of the remaining strains, six were tested only with material that is easy to ensile. Although all six showed potential to improve ensiling, as no data were provided using forages with a broader range of characteristics influencing the ensiling process, the Panel concluded that further evidence would be required to justify a claim for use with "all forage species". The Panel was unable to draw conclusions on the efficacy of the remaining two strains.

[1] On request from the European Commission, Question No EFSA-Q-2011-00374, adopted on 23 May 2012.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Silage for the preparatory work on this scientific opinion.

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KEY WORDS

Technological additive, silage additive, *Lactobacillus plantarum*, QPS, safety, efficacy

SUMMARY

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 for the target animals, consumer, user and the environment and on the efficacy of products based on 18 specific strains of *Lactobacillus plantarum*, when used individually, as technological additives intended to improve the ensiling process at proposed doses ranging from 5×10^6 to 1.0×10^9 colony-forming units (CFUs)/kg fresh material.

The bacterial species *L. plantarum* is considered by EFSA to be suitable for the Qualified Presumption of Safety approach to safety assessment. Therefore, strains belonging to this species do not require any specific demonstration of safety other than confirming the absence of any determinants of resistance to antibiotics of human and veterinary clinical significance and safety for the user. As the identity of all 18 strains was clearly established and as no antibiotic resistance of concern was detected, the use of the 18 strains in the production of silage is presumed safe for livestock species, consumers of products from animals fed the treated silage and the environment.

No data are available on skin or eye irritation for any of the strains in any formulation. However, the generic material safety data sheet proposed for the 18 strains indicates that preparations containing the strains may cause irritation on prolonged contact with skin and eyes. The dusting potential of formulations tested was generally high. This, coupled with the significant fraction of these products with particles that are potentially inhalable, means that exposure via a respiratory route is a hazard. Although users at the farm level are exposed to the additive only for a short period of time when preparing the aqueous suspension, the FEEDAP Panel considers it prudent, given the proteinaceous nature of the active agents, to treat all 18 additives as skin and respiratory sensitisers.

Once an active agent has been authorised as a silage additive, different formulations can be placed on the market with reference to that authorisation. The applicant listed several cryoprotectants and carriers that would allow multiple formulations of the additives to be produced, and consequently not all forms can be directly tested for user safety. However, in assessing the safety for the user of the additive, the active agent is the principal concern provided that other components do not introduce concerns. For the products described in this application, all excipients listed as likely to be used are food grade or equivalent, and their use in the additive would not introduce any risk additional to their conventional use.

Studies with laboratory-scale silos are described for each strain, each lasting at least 90 days, with silage made using forage samples of differing water-soluble carbohydrate content and representing material from the range easy, moderately difficult and difficult to ensile. In each case, silos containing treated forage were compared with identical silos containing the same untreated forage. Replicate silos were opened at the end of the experiments and the contents were analysed for dry matter content, pH, lactic and volatile fatty acid concentration, ethanol, ammonia and total nitrogen.

Nine of the 18 additives showed a potential at the doses proposed to improve the production of silage from a wide range of forage species by reducing the pH and increasing the preservation of dry matter. A further strain also showed a similar potential to improve the production of silage from a range of forage species but only when used in combination with a specific strain of *Pediococcus pentosaceus*. Of the remaining eight strains, six were tested only with material that is easy to ensile. All six showed clear potential to improve ensiling by reducing pH and dry matter losses. However, as no data were provided using forages with a broader range of characteristics that influence the ensiling process, the FEEDAP Panel concluded that further evidence would be required to justify a claim for use with "all forage species". The Panel was unable to draw conclusions on the efficacy of the remaining two strains.

TABLE OF CONTENTS

BACKGROUND

Regulation (EC) No 1831/2003[4] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular Article 10(2)/(7) of that Regulation specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, within a maximum of seven years after the entry into force of this Regulation.

The European Commission received a request from the company SILAC-EEIG-Silage Additives[5] for re-evaluation of 18 strains of *Lactobacillus plantarum* (DSM 23375, CNCM I-3235, DSM 19457, DSM 16568, LMG 21295, DSM 16565, VTT E-78076, CNCM MA 18/5U, NCIMB 30238, ATTC PTA-6139, DSM 18112, ATCC 55058, DSM 18113, DSM 18114, ATCC 55942, ATCC 55943, ATCC 55944 and NCIMB 30094), to be used as a feed additives for all animal species (category: technological additive; functional group: silage additive) under the conditions mentioned in Table 1.

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 10(2)/(7) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application.[6] According to Article 8 of that Regulation, 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. The particulars and documents in support of the application were considered valid by EFSA as of 1 June 2011.

This product was included in the European Union Register of Feed Additives following the provisions of Article 10(1) of Regulation (EC) No 1831/2003.

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall 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 the efficacy of the 18 strains of *Lactobacillus plantarum* (DSM 23375, CNCM I-3235, DSM 19457, DSM 16568, LMG 21295, DSM 16565, VTT E-78076, CNCM MA 18/5U, NCIMB 30238, ATTC PTA-6139, DSM 18112, ATCC 55058, DSM 18113, DSM 18114, ATCC 55942, ATCC 55943, ATCC 55944 and NCIMB 30094), when used under the conditions described in Table 1.

[4] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[5] SILAC-EEIG-Silage Additives, Avenue Louise, 120-Box 13, 1050, Brussels, Belgium.
[6] EFSA Dossier reference: FAD-2010-0048.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	*Lactobacillus plantarum* (18 strains). See Appendix A
Registration number/EC No/No	Not appropriate
Category(-ies) of additive	1. Technological additives
Functional group(s) of additive	k) silage additives

Description			
Composition, description	Chemical formula	Purity criteria	Method of analysis
Lactobacillus plantarum (18 strains) See appendix A	Not appropriate	Significant impurities: - Coliforms: <1000 CFU/g - Yeast and molds: <1000 CFU/g Relevant impurities: - *E. coli*: <10 CFU/g - *Salmonella*: absence in 25g - Aflatoxin B1: <1µg/kg	Enumeration method EN 15787:2009 Identification method (genetic): PFGE

Trade name	Not appropriate
Name of the holder of authorisation	Not appropriate

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period
		CFU/kg of complete feedingstuffs		
All animal species and categories	-	See appendix A	-	Not appropriate

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use	See appendix A
Specific conditions or restrictions for handling	For safety: eye protection and gloves shall be used during handling
Post-market monitoring	Not appropriate
Specific conditions for use in complementary feedingstuffs	Not appropriate

Maximum Residue Limit (MRL)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues

Not appropriate	Not appropriate	Not appropriate	Not appropriate

ASSESSMENT

1. Introduction

Six genera of lactic acid-producing bacteria are commonly associated with forage species and collectively contribute to the natural ensiling process. This joint application made by a consortium of companies concerns 18 different strains of a single species of one of these six genera, *Lactobacillus plantarum*. All are intended to be added to forages to promote ensiling (technological additive, functional group: silage additive) for eventual use of the silage in any animal species.

The species *L. plantarum* is considered by EFSA to be suitable for the Qualified Presumption of Safety (QPS) approach to safety assessment (EFSA, 2007, 2011). This approach requires the identity of the strain to be conclusively established and evidence that the strain does not show acquired resistance to antibiotics of human and veterinary importance.

2. Characterisation

The 18 strains included in this application are listed in Table 2 together with their accession numbers in internationally recognised culture collections. Each strain has been given a reference letter which, for convenience, will be used throughout this opinion. Accession numbers for which a copy of the certificate of deposition is provided are shown in bold.

Table 2: The 18 strains of *Lactobacillus plantarum* and their accession numbers

Ref letter	Accession number(s)
A[7]	*Lactobacillus plantarum* DSM 20174 – **DSM 23375**
B[8]	*Lactobacillus plantarum* ATCC 8014 – **CNCM I-3235**
C[9]	*Lactobacillus plantarum* **DSM 19457**
D[10]	*Lactobacillus plantarum* **DSM 16565**
E[11]	*Lactobacillus plantarum* **DSM 16568**
F[12]	*Lactobacillus plantarum* **LMG 21295**
G[13]	*Lactobacillus plantarum* **VTT E-78076**
H[14]	*Lactobacillus plantarum* **CNCM MA 18/5U – DSM 11672**
I[15]	*Lactobacillus plantarum* **NCIMB 30238**
J[16]	*Lactobacillus plantarum* **ATCC PTA-6139**
K[17]	*Lactobacillus plantarum* DSM 4784—ATCC 53187—**DSM 18112**
L[18]	*Lactobacillus plantarum* DSM 5257—**ATCC 55058**
M[19]	*Lactobacillus plantarum* DSM 4785—**DSM 18113**
N[20]	*Lactobacillus plantarum* DSM 4786—**DSM 18114**
O[21]	*Lactobacillus plantarum* DSM 5258—**ATCC 55942**
P[22]	*Lactobacillus plantarum* DSM 4787—**ATCC 55943**
Q[23]	*Lactobacillus plantarum* DSM 5284—**ATCC 55944**
R[24]	*Lactobacillus plantarum* **NCIMB 30094**

DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen; ATCC, American Type Culture Collection; CNCM, Collection Nationale de Culture de Microorganismes; LMG, Laboratorium voor Microbiologie, Universieit Gent; VTT, VTT Culture Collection, Finland; NCIMB, National Collection of Industrial and Marine Bacteria.

2.1. Identity and properties of the active agents

The strains of *L. plantarum* were isolated from silage with the exception of strains B (unspecified plant material), D (vegetable origin), E (maize) and G (beer). None of the strains have been genetically modified.

In all but two cases strain identity was established by the phenotypic properties (Analytical Profile Index (API)) and by the full 16S rDNA gene sequence, which by comparison with sequences recorded in databases allowed each strain to be unambiguously identified as *L. plantarum*. Two strains (P and Q) were initially assigned to the closely related *L. pentosus* on the basis of their 16S rDNA gene sequence. However, API results indicated *L. plantarum* as a better match, and this was confirmed by subsequent polymerase chain reaction (PCR) analysis of recombinant A enzyme (recA) and heat shock protein 60 (*hsp60*). PCR *recA* characterisation was also used to confirm the distinction from *L. pentosus* for several other strains (A, D, E and F).

[7] Technical dossier/Section II/2-2-15 Agri-King.
[8] Technical dossier/Section II/ 2-2-16 Alltech.
[9] Technical dossier/Section II/ 2-2-17 Biomin.
[10] Technical dossier/Section II/ 2-2-18 Chr. Hansen.
[11] Technical dossier/Section II/ 2-2-18 Chr. Hansen.
[12] Technical dossier/Section II/ 2-2-18 Chr. Hansen
[13] Technical dossier/Section II/ 2-2-19 Kemira.
[14] Technical dossier/Section II/ 2-2-20 Lallemand.
[15] Technical dossier/Section II/2-2-21 Micron.
[16] Technical dossier/Section II/2-2-22 Pioneer.
[17] Technical dossier/Section II/2-2-22 Pioneer.
[18] Technical dossier/Section II/2-2-22 Pioneer.
[19] Technical dossier/Section II/2-2-22 Pioneer.
[20] Technical dossier/Section II/2-2-22 Pioneer.
[21] Technical dossier/Section II/2-2-22 Pioneer.
[22] Technical dossier/Section II/2-2-22 Pioneer.
[23] Technical dossier/Section II/2-2-22 Pioneer.
[24] Technical dossier/Section II/2-2-23 Volac.

Strain-specific detection for 17 strains is based on the use of pulsed-field gel electrophoresis (PFGE) after cleavage with a number of restriction enzymes used individually (usually *Apa*1 and *Not*1). The exception was strain I, for which PCR methods were used to distinguish this specific strain from others. Data on genetic stability over time (based on the PFGE results) were shown for only three of the strains (D, E and F). However, PFGE comparison of each fermentation batch with the parent culture is a routine part of process control for all strains.

Each strain was tested for antibiotic susceptibility using twofold broth dilutions.[25] The battery of antibiotics tested included all of those recommended by EFSA (EFSA, 2008) but excluding streptomycin and vancomycin, which are not required for this species. In some cases additional antibiotics were tested. As all minimum inhibitory concentration values for 13 of the 18 *L. plantarum* strains fell below the corresponding cut-off values defined by the FEEDAP Panel, no further investigation is required for these strains. In the remaining five strains, one or more cut-off values were exceeded, usually for kanamycin, but in each case by only a single dilution. This is within the normal variation around a mean and is not considered to be cause for concern by the FEEDAP Panel.

2.2. Production and characteristics of the additive

The active agents are grown in sterilised media typical of those used for lactic acid bacteria. Typical ingredients are listed and a generic material safety data sheet provided. Cells then are separated from the growth medium by centrifugation or microfiltration, cryoprotectants added and the cell mix is freeze-dried and ground. The ground powder is then blended with sufficient carrier to meet the minimum specified concentration for each additive. The composition and minimum specified content is shown for each additive in Table 3. It should be noted that the minimum count always refers to the final product whatever its composition. Analysed values were also provided for multiple batches of each strain. However, some of these related to the cell concentrate to be variously blended according to the nature of the final product and, as such, could not be related to the declared minimum. Where analysed values could be clearly related to a final product, then numbers always exceed the declared minimum count. Maximum values for the spent medium and cryoprotectants appear to be expressed as a percentage of the product existing at various stages in the manufacturing process, not necessarily the final product.

[25] Technical dossier/Section II and Supplementary information March 2012/Annexes II 2-2-25 to 35, Qi and Qii.

Table 3: Composition of the 18 additives and the minimum guaranteed cell count of the active agent

Strain	Formulation	Fermentation medium (max. %)	Cryoprotectants (max. %)	Minimum guaranteed cell count (CFU/g)
A	Lactose (48.5 %) Silica (2 %)	10	15	2×10^{10}
B	Lactose (28–68 %) Silica (2 %)	2	28	5×10^{10}
C	Inulin (55–75 %)	6	65	1×10^{10}
D	Maltodextrin (50–75 %) Silica (8 %)	6	32	5×10^{10}
E	Maltodextrin (50–75 %) Silica (8 %)	6	32	5×10^{10}
F	Maltodextrin (50–75 %) Silica (8 %)	6	35	5×10^{10}
G	Milk powder (8–36 %) Glycerin (1–6 %) Sucrose (2–9 %) Dextrose (50–90 %)	16	60	1×10^{11}
H	Lactose (28–68 %) Silica (2 %)	14	80	2×10^{10}
I	Dextrose (28–70 %) Maltodextrin (30–50 %)	1	78	2×10^{10}
J	Maltodextrin (40–60 %) Silica (8–10 %)	12	10	1×10^{10}
K	Maltodextrin (40–60 %) Silica (8–10 %)	10	10	1×10^{10}
L	Maltodextrin (40–60 %) Silica (8-10 %)	10	10	1×10^{10}
M	Maltodextrin (40–60 %) Silica (8–10 %)	10	10	1×10^{10}
N	Maltodextrin (40–60 %) Silica (8–10 %)	10	10	1×10^{10}
O	Maltodextrin (40–60 %) Silica (8–10 %)	10	10	1×10^{10}
P	Maltodextrin (40–60 %) Silica (8–10 %)	10	10	1×10^{10}
Q	Maltodextrin (40–60 %) Silica (8–10 %)	10	10	1×10^{10}
R	Dextrose or maltodextrin (40–60 %) Silica (8–10 %)	9	50	5×10^{10}

CFU, colony-forming unit.

Additives K–Q are also manufactured as granules with calcium carbonate (92–95 %) and silica (1–2 %) as carrier.

Material safety data sheets are provided for medium ingredients and cryoprotectants and for the carrier materials listed in Table 3, with the exception of lactose, which is assumed to be of food grade. The medium ingredients and excipients used in the production of the additive individually do not introduce safety concerns.

The additives are routinely monitored for microbial contamination. Limits are set for Enterobacteriaceae, yeasts and filamentous fungi ($< 10^3$ CFUs/g additive), *Escherichia coli* (< 10 CFUs/g additive) and *Salmonella* (absence in 25 g additive). Data from three to five batches of each

additive confirmed compliance with these limits for yeasts and fungi, *Enterobacteriaceae*, *Salmonella* and *E. coli*. Given the nature of the fermentation medium and the food-grade excipients, the probability of contamination with heavy metals or mycotoxins is low and apparently not included in routine monitoring. One batch of each additive was, however, sent for the analysis of aflatoxins B1. The values obtained were either below the detection limit of the analytical method or were substantially lower (0.05 µg/kg) than the action limit set (1.0 µg/kg additive).

The available measurements of particle size distribution made by laser diffraction and dusting potential using a Heubach dustmeter are summarised in Table 4.[26] However, it should be noted that, as some of the products are envisaged as only contributing to a silage "premix", no final formulation exists as such. As a result, measurements are variously made on the dry cell mass obtained after mixing with cryoprotectants or on a formulation intended for market or close equivalent.

Table 4: Particle size and dusting potential of the *L. plantarum* strains

Strain	Formulation	Particle size (volume)	Dusting potential (g/m^3)
A	Strain + cryoprotectant (single batch)	~4.5 % < 10 µm ~30 % < 50 µm	3.98
B	Strain + cryoprotectant (four batches)	~2 % < 50 µm	0.004
C	Strain in commercial formulation (three batches)	~11 % < 50 µm	4.68
D	Strain in commercial formulation (one batch)	~12.6 % <10 µm ~33 % <50 µm	4.61
E	Strain in commercial formulation (one batch)	~13 % <10 µm ~31 % <50 µm	3.72
F	Strain in commercial formulation (one batch)	~15.6 < 10 µm ~33 % < 50 µm	2.26
G	Strain in commercial formulation (one batch)	~10 % < 10 µm ~31 % < 50 µm	9.8
H	Strain in commercial formulation (one batch)	~30 % < 50 µm	2.34
I	Strain alone (one batch)	~28 % < 50 µm	0.49
J, K, L, M, N, O, P, Q	Single strain in commercial formulation (one batch)	~28 % < 50 µm	37.9
R	Strain in commercial formulation (one batch)	~13 % < 50 µm	3.9

In the case of strains J–Q the applicant argues that measurements made on one strain/production site/process can act as a reference for all strains handled in the same manner in the same facility as any differences are expected to be small.

Mean particle size was considerably increased in those additives in which a granulated formulation is produced and the content of smaller particles correspondingly reduced (0.1 % < 50 µm for strain A and 0.4 % for strains J–Q based on data from a single strain).

[26] Technical dossier/Section II and Supplementary information March 2012/Annexes II 2-1-45 to 60 and Qiv.

The data on dusting potential give an indication of the range of values likely to be encountered in practice. The dusting potential of these final formulations is considered high. The two low values shown by strains B and I derive from intermediate stages in the manufacturing process and cannot be taken as indicative of the dustiness of any final formulation.

2.3. Shelf-life and stability in water

Shelf-life for strains B, C, E and G–R in the sealed moisture-tight containers in which they are supplied is at least 12 months whether stored under refrigeration (2–5 ºC) or at an ambient temperature of 25 ºC.[27] Studies with strain A were made only at 22 ºC but also indicated a shelf-life in excess of 12 months. Strains D and F (three batches) showed good evidence of stability for at least 24 months when stored at either –18 ºC or 5 ºC or under ambient conditions (25 ºC).[28]

Short-term stability in water was determined individually for all of the strains under application.[29] Three batches of the strain under examination were separately diluted in water in concentrations mimicking the proposed application rate, stored under ambient conditions (20–25 ºC, except strain I at 15 ºC). Bacterial counts were made at time intervals up to 48 hours. All strains showed little or no losses after 24 hours. Some loss of viability was recorded for some of the strains after 48 hours but this did not exceed 0.5 log count and was compensated for by the overage practised in formulation.

2.4. Conditions of use

The additives are intended for use with all or a selected range of forages at the recommended doses shown in Table 5. Granulated products are intended to be directly applied to material for ensiling, while all other formulations are intended to be first suspended in water and then distributed by spraying.

Table 5: Application and recommended dose for each of the 18 strains of *L. plantarum*

Strain of *Lactobacillus plantarum*	**Type of forage (easy, moderately difficult or difficult to ensile)**	**Recommended dose (CFUs/kg fresh silage)**
A: DSM 23375	Easy, moderately difficult	1×10^9
B: CNCM I-3235	All forages	2×10^7
C: DSM 19457	Easy, moderately difficult	5×10^7
D: DSM 16565	All forages	1×10^8
E: DSM 16568	All forages	1×10^8
F: LMG 21295	All forages	1×10^8
G: VTT E-78076	All forages	1×10^9
H: CNCM MA18/5U	All forages	1×10^8
I: NCIMB 30238	All forages	2×10^7
J: PTA-6139	Easy	2×10^7
K: DSM 18112	Easy	5×10^6
L: ATCC 55058	All forages	5×10^6
M: DSM 18113	Easy	2×10^7
N: DSM 18114	Easy	2×10^7
O: ATCC 55942	All forages	5×10^6
P: ATCC 55943	Easy	2×10^7
Q: ATCC 55944	Easy	5×10^7
R: NCIMB 30094	All forages	1×10^9

[27] Technical dossier/ Section II/Annexes 2-4-1 to 2-4-5.
[28] Technical dossier/ Supplementary information March 2012/Annexes New data shelf-life.
[29] Technical dossier/ Supplementary information March 2012/Annexes Qv.

2.5. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of the active agents in animal feed. The executive summary of the EURL report can be found in Appendix B.

3. Safety

In the view of the FEEDAP Panel, the identity of the bacterial strains under application and their freedom from antibiotic resistance determinants have been established in all cases. Consequently, the 18 strains of *L. plantarum* are considered suitable for the QPS approach to safety assessment: no further assessment of safety, other than user safety, is required, and all 18 strains are presumed safe for the target species, consumers of products from animals fed treated silage and the environment.

No data are available on skin or eye irritation for any of the strains in any formulation. However, the generic material safety data sheet proposed for the 18 strains indicate that preparations containing the strains may cause irritation on prolonged contact with skin and eyes.

The dusting potential of commercial formulations tested was high. This, coupled with the significant fraction of these products that is potentially inhalable, means that exposure via a respiratory route is a significant possibility and hazard. Although users at the farm level are exposed to the additive only for a short period of time when preparing the aqueous suspension, given the proteinaceous nature of the active agent, its potential to be a skin/respiratory sensitiser should be seriously considered.

Once an active agent has been authorised as a silage additive, different formulations can be placed on the market with reference to that authorisation. This application lists several cryoprotectants and carriers that would allow multiple formulations of the additive to be produced, and consequently not all forms can be directly tested for user safety. However, in assessing the safety for the user of the additive, the active agent is the principal focus provided that other components do not introduce concerns. For the products described in this application, all excipients listed as likely to be used are food grade or equivalent, and their use in the additive would not introduce any risk additional to that posed by their conventional use.

4. Efficacy

4.1. *L. plantarum* DSM 23375 (strain A)

Three experiments are described with material easy to ensile (lucerne, dry matter (DM) 57.6 %, water-soluble carbohydrates (WSC) 6.3 %), moderately difficult to ensile (ryegrass, DM 12.2 %, WSC 1.8 %) and difficult to ensile (unspecified grass, DM 17.6 %, WSC 0.3 %).[30] The experiment with lucerne used 113-L barrels and three replicates, that with ryegrass mini-silos of 0.7 L capacity containing 0.5 kg forage and four replicates and that with the grass mini-silos of unknown volume but of 1.6 kg capacity with three replicates. Treated silos were dosed with 1×10^8 CFUs/kg forage. All experiments were of 90 days duration but temperature conditions and other experimental details are lacking, although they are stated by the applicant to satisfy the requirements of Regulation (EC) No 429/2008.

Data were analysed using a one-sided Wilcoxon–Kruskal–Wallis non parametric test followed by chi-squared approximation. A summary of the results obtained is shown in Table 6.

[30] Technical dossier/ Supplementary information March 2012/Annex Qvi Agriking.

Table 6: Summary of the analysis of ensiled material recovered at the end of the experiments with L. plantarum DSM 23375 (strain A)

Substrate and treatment		Dry matter loss (%)	pH	Lactic acid (% silage DM)	Acetic acid (% silage DM)	Ammonia-N (% total N)
Lucerne	Control	0.6	5.5	1.2	0.3	7.4
	Treated	0.4*	4.8*	4.4*	0.1*	5.1*
Ryegrass	Control	6.3	4.1	12.9	4.8	12.5
	Treated	7.1	4.1	16.5	1.7*	9.3*
Grass	Control	1.0	4.2	5.6	6.3	17.9
	Treated	0.8*	4.1*	7.7*	2.3*	15.9*

*Significantly different from control value at $P < 0.05$.

The results indicated that strain A has the potential to improve the production of silage made from forage species that are easy and difficult to ensile by reducing the pH and increasing the preservation of dry matter and reducing protein losses. Although the results with the ryegrass, which is moderately difficult to ensile, were not so evident, the same trends could be seen. These reached significance only for the reduction of acetic acid and protein loss.

4.2. *L. plantarum* CNCN I-3235 (strain B)

A total of three laboratory experiments are described with silage made with three different forage samples and lasting 90 days.[31] All of the studies used 1.5-L mini-silos with the capacity to vent gas. In each case, the contents of three replicate silos were sprayed with the additive at 2×10^7 CFUs/kg forage (confirmed by analysis). Forage for the control silos was sprayed with an equal volume of water without the additive. Ambient temperature was controlled at 20 ºC. The three studies involved different forages representing material that is easy to ensile (perennial ryegrass, DM 27.2 %, WSC 4.8 %), moderately difficult to ensile (red clover, DM 15.0 %, WSC 1.52 %) and difficult to ensile (lucerne, DM 18.7 %, WSC 1.46 %) as defined in Regulation (EC) No 429/2008. Replicate silos were opened at the end of the experiment and the contents were analysed for dry matter content, pH, lactic and volatile fatty acid concentration, ethanol, ammonia and total nitrogen (Table 7). Treatment effects were examined within each forage type by one-way analysis of variance (ANOVA, based on the total of six treatments). Forage means were compared using the Student–Newman–Keuls test to take into account multiple comparisons. Significance was assumed to be at $P < 0.05$.

Table 7: Summary of the analysis of ensiled material recovered at the end of the experiments with *L. plantarum* CNCN I-3235 (strain B)

Substrate and treatment		Dry matter loss (%)	pH	Lactic acid (% ensiled matter)	Acetic acid (% ensiled matter)	Ammonia-N (% total N)
Ryegrass	Control	4.2	3.6	2.3	0.5	9.9
	Treated	4.8*	3.5*	2.9*	0.2*	7.0*
Red clover	Control	17.5	3.8	1.5	0.3	13.5
	Treated	12.8*	3.5*	1.7	0.2	10.5
Lucerne	Control	17.1	6.2	0.1	0.3	23.1
	Treated	5.9*	4.2*	1.8*	0.6	15.5*

*Significantly different from control value at $P < 0.05$.

A fourth study with maize was also described. However, the untreated control showed a pH value of 3.5 when silos were opened, implying an efficient ensiling leaving little scope for improvement.

[31] Technical dossier/Section IV/Annex IV-4-2.

Results from the three studies in Table 7 indicated that strain B has the potential to improve the production of silage from forage species that are easy, moderately difficult and difficult to ensile by reducing the pH and increasing the preservation of dry matter.

4.3. *L. plantarum* DSM 19457 (strain C)

Four laboratory experiments are described, made with different forage samples and lasting 90 days (91 days for the third study).[32] All of the studies used both 1.8-L (for intermediate samples after days 3 and 7) and 5.8-L mini-silos with the capacity to vent gas. In each case, the contents of three replicate silos were sprayed with the additive at 5×10^7 CFUs/kg forage. Forage for the control silos were sprayed with an equal volume of water without the additive. Ambient temperature was controlled at 22 ºC. The four studies involved three with material moderately difficult to ensile (permanent grassland first cut, DM 32.2 %, WSC 2.8 %; permanent grassland second cut, DM 30.0 %, WSC 2.3 %; and lucerne 1, DM 33.0 %, WSC 1.6 %) and a fourth with material easy to ensile (lucerne 2, second cut DM 43.5 %, WSC 5.4 %), as defined in Regulation (EC) No 429/2008.

Replicate silos were opened at the end of the experiment and the contents were analysed for dry matter content, pH, lactic and volatile fatty acid concentration, ethanol, ammonia and total nitrogen. Data were analysed using a one-sided Kruskal–Wallis non-parametric test followed by chi-squared approximation. A summary of the results obtained is shown in Table 8.

Table 8: Summary of the analysis of ensiled material recovered at the end of the experiments with *L. plantarum* DSM 19457 (strain C)

Substrate and treatment		Dry matter loss (%)	pH	Lactic acid (% ensiled matter)	Acetic acid (% ensiled matter)	Ammonia-N (% total N)
Grass 1	Control	2.1	4.1	2.8	0.8	–
	Treated	1.3*	4.1	3.3*	0.5*	–
Grass 2	Control	4.3	4.3	1.3	0.9	9.1
	Treated	3.1*	3.8*	3.0	0.3*	3.7*
Lucerne 1	Control	2.2	4.6	2.6	1.0	9.5
	Treated	1.8	4.6	2.5*	0.8	8.4
Lucerne 2	Control	2.6	4.8	2.7	0.7	8.1
	Treated	2.2	4.6*	3.5*	0.6	6.0

*Significantly different from control value at $P < 0.05$.

Data from treated silos opened on day 3 and day 7 also showed that the pH was significantly lower than that of the corresponding controls in all experiments. In addition, there was a significant reduction in the number of clostridial spores detected in treated samples from experiments with grass 2 and lucerne 1 at the end of the experiment compared with control silos.

Overall, the data suggest that strain C has the ability to encourage lactic acid production in material that is easy and moderately difficult to ensile, which can reduce pH, particularly during the early stages of fermentation, and can lead to a reduction in dry matter loss. Data were the least conclusive for lucerne 1 which, with 1.6 % water-soluble carbohydrate, was on the border between being moderately difficult and difficult to ensile.

4.4. *L. plantarum* strains DSM 16565 (strain D), DSM 16568 (strain E) and LMG 21295 (strain F)

A series of laboratory experiments are described, made with a range of forage samples (see Table 9) and lasting a minimum of 90 days.[33] The studies used 3.0-L mini-silos, except study 3 which used 1.5-

[32] Technical dossier/Section IV and Supplementary information March 2012/Annexes IV-4-3 and Qvi Biomin.
[33] Technical dossier/Section IV and Supplementary information March 2012/Annexes IV-4-4 to 6 and Qv Chr. Hansen.

L mini-silos. All had the capacity to vent gas. The contents of five replicate silos (three in the case of study 3) were sprayed with the additive (strains D, E, or F) at 1×10^8 CFUs/kg forage (confirmed by analysis). Forage for the control silos was sprayed with an equal volume of water without the additive. Ambient temperature was controlled at 20 ºC. The studies cover forages representing material that is easy, moderately difficult and difficult to ensile as defined in Regulation (EC) No 429/2008.

Replicate silos were opened at the end of the experiment and the contents were analysed for dry matter content, pH, lactic and volatile fatty acid concentration, ethanol, ammonia and total nitrogen (Tables 10 and 11).

Table 9: Characteristics of the forage samples used in the ensiling experiments for *L. plantarum* strains DSM 16565 (strain D), DSM 16568 (strain E) and LMG 21295 (strain F)

Study	Test material	DM content (% fresh material)	WSC content (% fresh material)
1	Whole crop maize	32.8	3.61
2	Red clover/ryegrass (70 : 30)	31.7	1.85
3	*Lolium perenne*	28.1	2.90
4	Lucerne cv. Europa	31.4	1.15
5	Lucerne cv. Birute	33.2	1.24
6	Lucerne cv. Verka	35.0	1.23
7	Lucerne cv. FSG 408 DP	17.2	0.18
8	Fodder galega cv Gale	14.3	0.07
9	Lucerne Jogeva 118	13.5	0.14

DM, dry matter; WSC, water-soluble carbohydrate.

Data were tested for normal distribution using Bartlett's test for homogeneity. Normally distributed data were treated by ANOVA (data from material moderately difficult and difficult to ensile). Otherwise the one-sided non-parametric Wilcoxon–Kruskal–Wallis test was used (data from material easy to ensile).

Table 10: Summary of the analysis of material from easy and moderately difficult to ensile forage recovered at the end of the experiments with *L. plantarum* strains DSM 16565 (strain D), DSM 16568 (strain E) and LMG 21295 (strain F)

Strain and treatment		Dry matter loss (%)	pH	Lactic acid (% ensiled matter)	Acetic acid (% ensiled matter)	Ammonia-N (% total N)
Study 1						
Strain D	Control	7.4	4.0	0.9	0.8	6.1
	Treated	4.7*	3.8*	1.3*	0.7	5.0*
Strain E	Control	7.4	4.0	0.9	0.8	6.1
	Treated	4.3*	3.7*	0.8*	0.0	3.7*
Strain F	Control	7.4	4.0	0.9	0.8	5.7
	Treated	3.8*	3.7*	1.3*	0.8	3.3*
Study 2						
Strain D	Control	10.2	4.6	0.8	0.8	5.7
	Treated	5.9*	4.1*	1.6*	0.8	6.1
Strain E	Control	10.2	4.7	0.8	0.8	5.7
	Treated	5.0*	4.1*	1.5*	0.8	2.6
Strain F	Control	10.2	4.7	0.8	0.8	5.7
	Treated	3.8*	3.7*	1.3*	0.9	3.3*
Study 3						
Strain D	Control	53.9	4.4	2.6	0.4	10.1
	Treated	–	4.1*	2.7	0.3*	9.6

*Significantly different from control value at $P < 0.05$.

Table 11: Summary of the analysis of difficult to ensile forage recovered at the end of the experiments with *L. plantarum* strains DSM 16565 (strain D), DSM 16568 (strain E) and LMG 21295 (strain F)

Strain and treatment		Dry matter loss (%)	pH	Lactic acid (% ensiled matter)	Acetic acid (% ensiled matter)	Ammonia-N (% total N)
Study 4						
Strain D	Control	6.7	5.5	0.3	0.8	11.0
	Treated	4.0*	5.1*	1.5*	1.0*	8.7*
Strain E	Control	6.7	5.5	0.3	0.8	11.1
	Treated	4.6*	5.1*	1.4*	1.3*	8.4*
Strain F	Control	6.7	5.5	0.3	0.8	11.1
	Treated	4.6*	5.2*	1.5*	0.9	7.8*
Study 5						
Strain D	Control	6.9	5.2	0.6	1.1	10.4
	Treated	4.0*	4.9*	1.8*	1.3*	7.6*
Strain E	Control	6.9	5.2	0.6	1.1	10.4
	Treated	4.4*	4.9*	1.7*	1.3*	7.5*
Strain F	Control	6.9	4.7	0.6	1.1	10.4
	Treated	4.1*	4.1*	1.6*	1.4*	7.5*
Study 6						
Strain D	Control	6.8	5.3	0.8	1.3	9.1
	Treated	4.0*	4.9*	2.1*	1.1	7.9*
Strain E	Control	6.8	5.3	0.8	1.3	9.1
	Treated	4.0*	5.0*	1.8*	1.4	7.2*
Strain F	Control	6.8	5.3	0.8	1.3	9.1
	Treated	3.7*	4.8*	1.8*	1.0*	7.0*

*Significantly different from control value at $P < 0.05$.

A similar pattern of results was obtained in studies 7–9 made with material with a very low water-soluble carbohydrate content and consequently the most difficult to ensile. Here, the magnitude of the response was less and did not always reach significance.

Overall the results indicated that strains D, E and F individually have the potential to improve the production of silage from forage species that are easy, moderately difficult and difficult to ensile by reducing the pH and increasing the preservation of dry matter.

4.5. *L. plantarum* VTT E-78076 (strain G)

Data on the effects on ensiling produced by strain G are presented in two forms. A total of 53 sets of observations from 26 studies made with material easy to ensile (mean DM content 39.7 % and WSC content 5.5 %) and a further 32 sets of observations from 13 studies made with material moderately difficult to ensile (mean DM content 27.6 % and WSC content 2.4 %) were collectively analysed (Table 12).[34] Results from silage made with different raw materials within a study were treated as separate observations. In both cases the forage material was said to be predominately timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*), the average duration of ensiling was 130 days and the dose applied generally 1×10^9 CFUs/kg forage. The amount of material ensiled in the individual studies differed considerably from 52 g to farm-scale trials with 80 000 kg.

[34] Technical dossier/Section IV and Supplementary information March 2012/Annexes IV-4-7 and Qvi Kemira.

Table 12: Meta-analysis of the results of the analysis of ensiled material recovered at the end of the experiments with *L. plantarum* VTT E-78076 (strain G). Starting materials were divided into easy and moderately difficult to ensile on the basis of their water-soluble carbohydrate content

Substrate and treatment		Dry matter loss (%)	pH	Lactic acid (% ensiled matter)	Acetic acid (% ensiled matter)	Ammonia-N (% total N)
Easy to ensile	Control	–	4.3	2.6	0.4	6.2
	Treated	–	4.1*	3.3*	0.3*	2.8*
Moderately difficult to ensile	Control	–	4.1	2.5	0.4	6.4
	Treated	–	4.0*	2.7*	0.3*	4.1*

*Significantly different from control value at $P < 0.05$ or better.

The meta-analysis was performed using a mixed model method (the SAS MIXED procedure). All observations included allowed a comparison with an appropriate control.

The available dataset differed considerably in experimental method and degree of replication, and many of the studies included would not, taken individually, be considered. However, given the number of observations available and taking a weight of evidence approach, the pooled analysis indicated that strain G has the potential to improve the production of silage from forage species that are easy and moderately difficult to ensile by reducing the pH, increasing lactic acid concentration and reducing protein losses.

The second set of data describes three individual studies made with difficult to ensile material. First-cut red clover (*Trifolium pratense*, 15.8 % DM, 1.45 % WSC) was used in study 1 and a mixture of timothy and meadow fescue (21.3 % DM and 1.49 % WSC in study 2, 18.7 % DM and 1.10 % WSC in study 3) in studies 2 and 3. The duration of the studies was between 84 and 117 days but the volume of treated material was low (120 mL) in studies 1 and 2 and they were made in duplicate only. Study 3 was a pilot-scale study involving 300–400 kg. In this study, four replicates were used for the control but only two replicates for the treated material. Given the very small sample size used to prepare the ensiled material and the lack of adequate replication, no conclusions are drawn from these studies.

4.6. *L. plantarum* DSM 11672 (strain H)

Three laboratory experiments are described, made with three different forage samples and lasting 90 days (91 days for the second study).[35] All of the studies used 2.8-L mini-silos with the capacity to vent gas. In each case, the contents of five replicate silos were sprayed with the additive at 1×10^8 CFUs/kg forage. Forage for the control silos was sprayed with an equal volume of water without the additive. Ambient temperature was controlled at ~20 ºC. The three studies involved different forages representing material easy to ensile (Italian ryegrass, WSC 4.1 %), moderately difficult to ensile ("permanent prairie grass", WSC 2.45 %) and difficult to ensile (lucerne, WSC 1.4 %) as defined in Regulation (EC) No 429/2008.

Replicate silos (3 and 4) were opened at the end of the experiment and the contents were analysed for dry matter content, pH, lactic and volatile fatty acid concentration, ethanol, ammonia and total nitrogen (Table 13).

Normally distributed and homoscedastic data were subjected to one-way ANOVA (based on the total of seven treatments). Otherwise data were analysed by the non-parametric Wilcoxon test (using the Bonferroni correction). Significance was assumed at $P < 0.05$.

[35] Technical dossier/Section II and Supplementary information March 2012/Annexes IV-4-8 and Qvi_Lallemand.

Table 13: Summary of the analysis of ensiled material recovered at the end of the experiments with *L. plantarum* DSM 11672 (strain H)

Substrate and treatment		Dry matter loss (%)	pH	Lactic acid (% ensiled matter)	Acetic acid (% ensiled matter)	Ammonia-N (% total N)
Ryegrass	Control	1.6	4.2	1.3	1.2	5.7
	Treated	0.9*	3.8*	2.7*	0.8*	3.0*
Prairie grass	Control	1.1	4.4	1.8	0.5	7.9
	Treated	0.7*	4.1*	2.7*	0.4*	5.6*
Lucerne	Control	1.0	4.4	2.	0.8	9.2
	Treated	0.8*	4.2*	2.3	0.6*	8.1*

*Significantly different from control value at $P < 0.05$.

The results indicated that strain H has the potential to improve the production of silage from forage species that are easy, moderately difficult and difficult to ensile by reducing the pH and increasing the preservation of dry matter and protein.

4.7. *L. plantarum* NCIMB 30238 (strain I) in a silage premix with *Pediococcus pentosaceus*

All of the efficacy studies presented were made with a commercial silage additive containing the strain of *L. plantarum* under application and a strain of *Pediococcus pentosaceus* in the ratio 2 : 8 by bacterial count.[36] The *P. pentosaceus* strain used is the subject of a separate consortium application (*P. pentosaceus* NCIMB 30237) and thus its safety will be assessed and reported in another opinion (EFSA, 2012).

A series of laboratory experiments are described, made with a range of forage samples (see Table 14) and lasting 90 days. The studies used mini-silos ranging in volume from 1.5 to 4 L. The contents of three replicate silos (studies 2–5) or seven replicates (study 1) were sprayed with the additive at 1×10^8 CFUs/kg forage (study 1) or 2×10^7 CFUs/kg forage (studies 2–5). Forage for the control silos was sprayed with an equal volume of water without the additive. The studies cover forages representing materials easy, moderately difficult and difficult to ensile as defined in Regulation (EC) No 429/2008.

Table 14: Characteristics of the forage samples used in the ensiling experiments for *L. plantarum* NCIMB 30238 (strain I), in combination with *P. pentosaceus*

Study	Test material	DM content (% fresh material)	WSC content (% fresh material)
1	Forage maize	27.6	3.2
2	First-cut Italian ryegrass	22.1	2.1
3	Late-cut lucerne	31.7	2.4
4	Late-cut lucerne	30.5	1.1
5	Third-cut ryegrass/lucerne mix	26.4	0.5

DM, dry matter; WSC, water-soluble carbohydrate.

Replicate silos were opened at the end of the experiment and the contents were analysed for dry matter content, pH, lactic and volatile fatty acid concentration, ethanol, ammonia and total nitrogen (Table 15). Other silos were also opened at various times early in the fermentation to monitor the rate of fall of pH.

[36] Technical dossier/Section II/Annex IV-4-9.

Table 15: Summary of the analysis of ensiled material recovered at the end of the experiments with *L. plantarum* NCIMB 30238 (strain I), in combination with *P. pentosaceus*

Study and treatment		Dry matter loss (%)	pH	Lactic acid (% ensiled matter)	Acetic acid (% ensiled matter)	Ammonia-N (% total N)
1	Control	–	3.6	2.4	0.6	–
	Treated	–	3.6	2.7*	0.6	–
2	Control	–	4.1	2.0	–	–
	Treated	–	4.0*	2.3*	–	–
3	Control	–	5.2	1.2	0.6	–
	Treated	–	4.1*	2.4*	0.5	–
4	Control	–	4.4	2.	0.6	–
	Treated	–	4.1*	2.8*	0.4*	–
5	Control	–	4.1	2.2	0.5	–
	Treated	–	3.9*	2.5*	0.5	–

*Significantly different from control value at $P < 0.05$.

Treatment with the additive significantly reduced pH in four of five studies and increased lactic acid concentration in all forage samples compared with the control. However, the consequence of reduced pH on the preservation of nutrients is not known as dry matter losses were not reported. Ammonia expressed as a percentage of dry matter was also reduced in the three studies in which this was measured, but reached significance only in study 4. Overall, the additive appears to favourably affect the ensiling process by encouraging a more rapid fall in pH across a full range of forage materials with different water-soluble carbohydrate contents representing material easy, moderately difficult and difficult to ensile. However, the consequences for the preservation of nutrients were not measured.

4.8. *L. plantarum* ATCC PTA-6139 (strain J), DSM 18112 (strain K), ATCC55058 (strain L), DSM 18113 (strain M), DSM 18114 (strain N), ATCC55942 (strain O), ATCC 55943 (strain P) and ATCC 55944 (strain Q)

Strains J, K, M, N, P and Q were each included in three experiments made with only material that is easy to ensile (Italian ryegrass samples with 7.82 %, 6.41 % and 4.40 % WSC content).[37] All of the studies used 2.75 L mini-silos with the capacity to vent gas. In each case, the contents of four replicate silos were sprayed with the additive at 5×10^6 CFUs/kg forage (strain K), 5×10^7 CFUs/kg forage (strain Q) or 2×10^7 CFUs/kg forage (strains J, M, N, and P). Control silos were sprayed with an equal volume of water without the additive. Ambient temperature was controlled at ~20 ºC and the duration of the experiments was 90 days.

Replicate silos were opened at the end of the experiment and the contents were analysed for dry matter content, pH, lactic and volatile fatty acid concentration, ethanol, ammonia and total nitrogen (Table 16). Histograms and Q–Q plots for the majority of traits reported did not show evidence of deviations from normality and so ANOVA was retained as the method of statistical analysis.

[37] Technical dossier/Section II and Supplementary information March 2012/Annexes IV-4-10 and Qvi Pioneer.

Table 16: Summary of the results of the analysis of easily ensiled material recovered at the end of the experiments with *L. plantarum* strains ATCC PTA-6139 (strain J), DSM 18112 (strain K), DSM 18113 (strain M), DSM 18114 (strain N), ATCC 55943 (strain P) and ATCC 55944 (strain Q) averaged over the three experiments

Strain	Dry matter loss (%)	pH	Lactic acid (% ensiled matter)	Acetic acid (% ensiled matter)	Ammonia-N (% total N)
Control	1.9	4.1	2.4	1.00	–
J	1.6	3.9*	3.3*	0.4*	–
K	1.5	3.9*	3.0*	0.7*	–
M	1.4*	3.9*	3.2*	0.5*	–
N	1.4*	3.8*	3.4*	0.4*	–
P	1.5*	3.8*	3.3*	0.4*	–
Q	1.4	3.8*	3.2*	0.4*	–

*Significantly different from control value at $P < 0.05$.

Results for the individual experiments mirrored the summarised data and were significant. The results indicated that strains J, K, M, N, P and Q have the potential to improve the production of silage by reducing the pH and increasing the preservation of dry matter. However this was demonstrated only using easy to ensile forage species with a water-soluble carbohydrate content greater than 3.0 % fresh material. No data were provided on the effects of these strains in the preparation of silage from more difficult to ensile material for which the need might be assumed to be greater.

Three experiments were also made with strain O and four with strain L, all with the additives sprayed at 5×10^6 CFUs/kg forage on material difficult to ensile. In the joint studies the forage material was high-moisture content maize (DM content > 70 %) with water-soluble carbohydrate contents of 0.53 %, 0.62 % and 0.44 %. The fourth study made only with strain L also involved high-moisture content maize with a water-soluble carbohydrate content of 0.13 %. The conditions and duration of the experiments were essentially those described above.

Table 17: Summary of the analysis of ensiled material recovered at the end of the experiments with *L. plantarum* ATCC55942 (strain O)

Study and treatment		Dry matter loss (%)	pH	Lactic acid (% ensiled matter)	Acetic acid (% ensiled matter)	Ammonia-N (% total N)
1	Control	2.1	4.0	0.7	0.1	3.7
	Treated	1.8*	4.0	0.8*	0.1	3.6
2	Control	2.2	4.2	0.4	0	1.7
	Treated	2.5	4.2	0.5*	0	1.7
3	Control	1.8	4.2	0.3	0	0.9
	Treated	2.3*	4.2	0.4*	0	1.1

*Significantly different from control value at $P < 0.05$.

Results in the case of strain O were marginal (Table 17). The only consistent result was a small but significant increase in lactic acid content in treated silos compared with controls in all three experiments. However, this was not mirrored in a consistent reduction in pH or a reduction in dry matter loss compared with the control values and so no clear benefits of treatment were evident.

For strain L there was a small but significant reduction in the pH of the treated silos in two experiments (Table 18). However, like strain O, no effect on dry mater loss was detected.

Table 18: Summary of the analysis of ensiled material recovered at the end of the experiments with *L. plantarum* ATCC55058 (strain L)

Study and treatment		Dry matter loss (%)	pH	Lactic acid (% ensiled matter)	Acetic acid (% ensiled matter)	Ammonia-N (% total N)
1	Control	2.6	4.1	0.8	0.1	3.1
	Treated	2.4	4.1	0.8	0.1	2.8
2	Control	1.9	4.0	0.7	0.1	2.6
	Treated	2.0	3.9*	0.6	0.1	2.6
3	Control	2.2	4.2	0.4	0	1.7
	Treated	2.1	4.2	0.3*	0	1.7
4	Control	2.2	4.1	0.6	0.1	3.0
	Treated	2.1	4.0*	0.6	0.1	2.6*

*Significantly different from control value at $P < 0.05$.

Strains L and O showed only limited and inconsistent evidence of a beneficial effect with difficult to ensile material (reduction in pH). However there was no evidence that this led to the preservation of nutrients. No data were provided for the use of these two strains with forage materials with a higher water-soluble carbohydrate content.

4.9. *L. plantarum* NCIMB 30094 (strain R)

Laboratory experiments are described, made with three different forage samples and each lasting 90 days.[38] All of the studies used 1.0- or 1.5-L mini-silos with the capacity to vent gas. In each case, the contents of four replicate silos were sprayed with the additive at 1×10^9 CFUs/kg forage. Forage for the control silos was sprayed with an equal volume of water without the additive. Ambient temperature was controlled at ~20 ºC. The three studies involved different forages representing material easy to ensile (tall fescue, WSC 5.78 %), moderately difficult to ensile (red clover, WSC 1.52 %) and difficult to ensile (lucerne, WSC 1.13 %) as defined in Regulation (EC) No 429/2008.

Replicate silos were opened at the end of the experiment and the contents were analysed for dry matter content, pH, lactic and volatile fatty acid concentration, ethanol, ammonia and total nitrogen. Results are shown in Table 19.

Data were analysed using the Wilcoxon–Kruskal–Wallis non-parametric test.

Table 19: Summary of the analysis of ensiled material recovered at the end of the experiments with *L. plantarum* NCIMB 30094 (strain R)

Substrate and treatment		Dry matter loss (%)	pH	Lactic acid (% ensiled matter)	Acetic acid (% ensiled matter)	Ammonia-N (% total N)
Ryegrass	Control	14.6	4.6	1.0	0.1	19.9
	Treated	6.3*	3.7*	2.6*	0.2*	10.8*
Prairie grass	Control	20.0	3.8	1.5	0.3	14.0
	Treated	8.5*	3.7*	1.7*	0.2*	7.3*
Lucerne	Control	11.5	5.8	0.6	0.9	35.4
	Treated	6.7*	4.9*	1.7*	0.7*	21.6*

*Significantly different from control value at $P < 0.05$.

The results indicated that strain R has the potential to improve the production of silage from forage species that are easy, moderately difficult and difficult to ensile by reducing the pH and increasing the preservation of dry matter.

[38] Technical dossier/Section II and Supplementary information March 2012/Annexes IV-4-11 and Qvi Volac.

CONCLUSIONS

As the identity of all of the strains of *L. plantarum* under application has been established and no antibiotic resistance of concern detected, following the QPS approach the use of these strains in the production of silage is presumed safe for the target species, consumers of products from animals fed treated silage and the environment.

The generic material safety data sheet proposed for the 18 strains indicate that preparations containing the strains may cause irritation on prolonged contact with skin and eyes. The dusting potential of formulations tested is generally high. This, coupled with the significant fraction of these products with particles that are potentially inhalable, means that exposure via a respiratory route is a hazard. Although users at the farm level are exposed to the additive for only a short period of time when preparing the aqueous suspension, the FEEDAP Panel considers it prudent, given the proteinaceous nature of the active agents, to treat all 18 additives as skin and respiratory sensitisers.

Eight of the 18 additives containing the following *L. plantarum* strains showed a potential at the minimum doses indicated to improve the production of silage from a wide range of forage species by reducing the pH and increasing the preservation of dry matter:

DSM 23375 (strain A) at 1×10^8 CFUs/kg forage
CNCM I-3235 (strain B) at 2×10^7 CFUs/kg forage
DSM 19457 (strain C) at 5×10^7 CFUs/kg forage
DSM 16565 (strain D) at 1×10^8 CFUs/kg forage
DSM 16568 (strain E) at 1×10^8 CFUs/kg forage
LMG 21295 (strain F) at 1×10^8 CFUs/kg forage
CNCM MA18/5U (strain H) at 1×10^8 CFUs/kg forage
NCIMB 30094 (strain R) at 1×10^9 CFUs/kg forage.

Strain NCIMB 30238 (I) also showed a potential to improve the production of silage from a similar range of forage species by reducing the pH, but only when used in combination with *P. pentosaceus* NCIMB 30237 in a ratio of 2 : 8 by bacterial count. However, the consequences of a more rapid pH reduction for the preservation of nutrients were not shown.

L. plantarum VTT E-78076 (strain G) showed potential to improve the production of silage at a minimum dose of 1×10^9 CFUs/kg forage by reducing the pH and ammonia nitrogen. This was demonstrated only in easy and moderately difficult to ensile materials.

A further six *L. plantarum* strains showed a potential to improve the production of silage by reducing the pH and dry matter losses at the following indicated minimum doses:

ATCC PTSA-6139 (strain J) at 2×10^7 CFUs/kg forage
DSM 18112 (strain K) at 5×10^6 CFUs/kg forage
DSM 18113 (strain M) at 2×10^7 CFUs/kg forage
DSM 18114 (strain N) at 2×10^7 CFUs/kg forage
ATCC 55943 (strain P) at 2×10^7 CFUs/kg forage
ATCC 55944 (strain Q) at 5×10^6 CFUs/kg forage.

However, these benefits were demonstrated only with easy to ensile material. No data were provided using forages with a broader range of characteristics that influence the ensiling process. In the view of the FEEDAP Panel, further evidence would be required to justify a claim for use with “all forage species”.

L. plantarum strains ATCC 55058 (strain L) and ATCC 55942 (strain O) showed only limited and inconsistent evidence of a beneficial effect only with difficult to ensile material. As there was no evidence that this led to the preservation of nutrients, the FEEDAP Panel was unable to draw conclusions on the efficacy of these strains.

DOCUMENTATION PROVIDED TO EFSA

1. *Lactobacillus plantarum* (18 strains). June 2010. Submitted by SILAC-EEIG-Silage Additives.

2. Supplementary information on *Lactobacillus plantarum* (18 strains). March 2012. Submitted by SILAC-EEIG-Silage Additives.

3. Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for *Lactobacillus plantarum* (18 strains) for all animal species

4. Comments from Member States received through ScienceNet.

REFERENCES

EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. The EFSA Journal, 587, 1–16.

EFSA (European Food Safety Authority), 2008. Technical guidance prepared by the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on the update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance. The EFSA Journal, 732, 1–15.

EFSA Panel on Biological Hazards (BIOHAZ), 2011. Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2011 update). EFSA Journal, 9(12):2497.

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2010. Scientific Opinion on the safety and efficacy of *Pediococcus acidilactici* (CNCM I-3237, CNCM MA 18/5M—DSM 11673) and *Pediococcus pentosaceus* (DSM 23376, NCIMB 12455, NCIMB 30237 and NCIMB 30168) as silage additives for all species. EFSA Journal 2012;10(6):2733

APPENDICES

APPENDIX A

Appendix to Table 1: SILAC EEIG—*Lactobacillus plantarum* dossier

Table 1: List of feed additives included in the dossier

Name of authorisation holder	Name of the additive as detailed in the Community Register of Feed Additives pursuant to Regulation (EC) No 1831/2003	Name as proposed by the applicant
Agri-King Ltd	*Lactobacillus plantarum* AK 5106—DSM 20174	*Lactobacillus plantarum* AK 5106—DSM 20174—DSM 23375
Alltech Inc	*Lactobacillus plantarum* CNCM I-3235—ATCC 8014	*Lactobacillus plantarum* CNCM I-3235—ATCC 8014
Biomin GmbH	*Lactobacillus plantarum* IFA96	*Lactobacillus plantarum* IFA96—DSM 19457
Chr. Hansen A/S	*Lactobacillus plantarum* DSM 16568	*Lactobacillus plantarum* DSM 16568
Chr. Hansen A/S	*Lactobacillus plantarum* MiLAB 393—LMG21295	*Lactobacillus plantarum* LMG21295
Chr. Hansen A/S	*Lactobacillus plantarum* DSM 16565	*Lactobacillus plantarum* DSM 16565
Kemira Oyj	*Lactobacillus plantarum* VTT E-78076	*Lactobacillus plantarum* VTT E-78076
Lallemand SAS	1. *Lactobacillus plantarum* DSM 11672 2. *Lactobacillus plantarum* CNCM MA 18/5U Those two strains are synonyms	*Lactobacillus plantarum* CNCM MA 18/5U—DSM 11672
Micron Bio-Systems Ltd	*Lactobacillus plantarum* MBS-LP-01	*Lactobacillus plantarum* MBS-LP-01—NCIMB 30238
Pioneer Hi-Bred Int., Inc.	*Lactobacillus plantarum* 24011	*Lactobacillus plantarum* 24011—ATCC PTA-6139
Pioneer Hi-Bred Int., Inc.	*Lactobacillus plantarum* LP286 DSM 4784 ATCC 53187	*Lactobacillus plantarum* LP286—DSM 18112
Pioneer Hi-Bred Int., Inc.	*Lactobacillus plantarum* LP287 DSM 5287 ATCC 55058	*Lactobacillus plantarum* LP287—ATCC 55058
Pioneer Hi-Bred Int., Inc.	*Lactobacillus plantarum* LP318 DSM 4785	*Lactobacillus plantarum* LP318—DSM 18113
Pioneer Hi-Bred Int., Inc.	*Lactobacillus plantarum* LP319 DSM 4786	*Lactobacillus plantarum* LP319—DSM 18114
Pioneer Hi-Bred Int., Inc.	*Lactobacillus plantarum* LP329 DSM 5258 ATCC 55942	*Lactobacillus plantarum* LP329—ATCC 55942
Pioneer Hi-Bred Int., Inc.	*Lactobacillus plantarum* LP346 DSM 4787 ATCC 55943	*Lactobacillus plantarum* LP346—ATCC 55943
Pioneer Hi-Bred Int., Inc.	*Lactobacillus plantarum* LP347 DSM 5284 ATCC 55944	*Lactobacillus plantarum* LP347—ATCC 55944
Volac International Ltd	*Lactobacillus plantarum* NCIMB 30094	*Lactobacillus plantarum* NCIMB 30094

Table 2: Detailed description of the additives as provided by the applicant

Category and functional group[39]	Subclassification	CAS number	Additive	Composition, chemical formula, description, analytical method[40]	Species or category of animal	Maximum age	Minimum content	Maximum content	Other provisions	Maximum Residue Limits (MRLs) in the relevant foodstuffs of animal origin	End of period of authorisation
							mg or units of activity or CFUs/kg of complete feedingstuffs, supplementary feed (based on end feed)				
Technological additives, (k) Silage additives	2. Microorganisms	–	*Lactobacillus plantarum* AK 5106—DSM 20174—DSM 23375 *(name of authorisation holder Agri-King Ltd)*	Preparation of *Lactobacillus plantarum* AK 5106—DSM 20174—DSM 23375 having minimum activity of: 2×10^{10} CFUs/g Enumeration method: EN 15787:2009 Identification method: PFGE	All animals species and categories		1×10^9 (easy and moderate forage)	–	1. Recommended dosage in silage: 1×10^9 CFUs/kg fresh silage for silage preparation 2. In the direction for use indicate the storage temperature and storage life 3. For safety: eye protection and gloves shall be used during handling		
Technological additives, (k) Silage additives	2. Microorganisms	–	*Lactobacillus plantarum* CNCM I-3235—ATCC 8014 *(name of authorisation holder AllTech Inc.)*	Preparation of *Lactobacillus plantarum* CNCM I-3235—ATCC 8014 having minimum activity of: 5×10^{10} CFUs/g Enumeration method: EN 15787:2009 Identification method: PFGE	All animals species and categories	–	2×10^7 (all types of forage)*	–	1. Recommended dosage in silage: 2×10^7 CFUs/kg fresh forage for silage preparation 2. In the direction for use indicate the storage temperature and storage life 3. For safety: eye protection and gloves shall be used during handling	–	–

[39] According to Art. 6 and Annex I of Regulation (EC) No 1831/2003.
[40] Available on the CRL website.

Category and functional group[39]	Subclassification	CAS number	Additive	Composition, chemical formula, description, analytical method[40]	Species or category of animal	Maximum age	Minimum content	Maximum content	Other provisions	Maximum Residue Limits (MRLs) in the relevant foodstuffs of animal origin	End of period of authorisation
							mg or units of activity or CFUs/kg of complete feedingstuffs, supplementary feed (based on end feed)				
Technological additives, (k) Silage additives	2. Microorganisms		*Lactobacillus plantarum* IFA96—DSM 19457 *(name of authorisation holder Biomin GmbH)*	Preparation of *Lactobacillus plantarum* IFA 96—DSM 19457 having minimum activity of: 1×10^{10} CFUs/g Enumeration method: EN 15787:2009 Identification method: PFGE	All animals species and categories	–	5×10^{7} (easy and moderate forage)	–	1. Recommended dosage in silage: 5×10^{7} CFUs/kg fresh forage for silage preparation 2. In the direction for use indicate the storage temperature and storage life 3. For safety: eye protection and gloves shall be used during handling 4. Clostridial spores inhibition	–	
Technological additives, (k) Silage additives	2. Microorganisms	–	*Lactobacillus plantarum* DSM 16568 *(name of authorisation holder Chr. Hansen A/S)*	Preparation of *Lactobacillus plantarum* DSM 16568 having minimum activity of 5×10^{10} CFUs/g Enumeration method: EN 15787:2009 Identification method: PFGE	All animals species and categories	–	1×10^{8} (all types of forage)*	–	1. Recommended dosage in silage: 1×10^{8} CFUs/kg fresh forage for silage preparation 2. In the direction for use indicate the storage temperature and storage life 3. For safety: eye protection and gloves shall be used during handling	–	–

Category and functional group[39]	Subclassification	CAS number	Additive	Composition, chemical formula, description, analytical method[40]	Species or category of animal	Maximum age	Minimum content	Maximum content	Other provisions	Maximum Residue Limits (MRLs) in the relevant foodstuffs of animal origin	End of period of authorisation
							mg or units of activity or CFUs/kg of complete feedingstuffs, supplementary feed (based on end feed)				
Technological additives, (k) Silage additives	2. Microorganisms	-	*Lactobacillus plantarum* LMG21295 *(name of authorisation holder Chr. Hansen A/S)*	Preparation of *Lactobacillus plantarum LMG21295* having minimum activity of: 5×10^{10} CFUs/g Enumeration method: EN 15787:2009 Identification method: PFGE	All animals species and categories	–	1×10^{8} (all types of forage)*	–	1. Recommended dosage in silage: 1×10^{8} CFUs/kg fresh forage for silage preparation 2. In the direction for use indicate the storage temperature and storage life 3. For safety: eye protection and gloves shall be used during handling	–	–
Technological additives, (k) Silage additives	2. Microorganisms	-	*Lactobacillus plantarum* DSM 1656 *(name of authorisation holder Chr. Hansen A/S)*	Preparation of *Lactobacillus plantarum* DSM 16565 having minimum activity of: 5×10^{10} CFUs/g Enumeration method: EN 15787:2009 Identification method: PFGE	All animals species and categories	–	1×10^{8} (all types of forage)*	–	1. Recommended dosage in silage: 1×10^{8} CFUs/kg fresh forage for silage preparation 2. In the direction for use indicate the storage temperature and storage life 3. For safety: eye protection and gloves shall be used during handling	–	–

Category and functional group[39]	Subclassification	CAS number	Additive	Composition, chemical formula, description, analytical method[40]	Species or category of animal	Maximum age	Minimum content	Maximum content	Other provisions	Maximum Residue Limits (MRLs) in the relevant foodstuffs of animal origin	End of period of authorisation
							mg or units of activity or CFUs/kg of complete feedingstuffs, supplementary feed (based on end feed)				
Technological additives, (k) Silage additives	2. Microorganisms	–	*Lactobacillus plantarum* VTT E-78076 *(name of authorisation holder Kemira Oyj)*	Preparation of *Lactobacillus plantarum* VTT E-78076 having minimum activity of: 1×10^{11} CFUs/g Enumeration method: EN 15787:2009 Identification method: PFGE	All animals species and categories	–	1×10^{9} (all types of forage)*	–	1. Recommended dosage in silage: 1×10^{9} CFUs/kg fresh forage for silage preparation 2. In the direction for use indicate the storage temperature and storage life 3. For safety: eye protection and gloves shall be used during handling	–	
Technological additives, (k) Silage additives	2. Microorganisms	–	*Lactobacillus plantarum* CNCM MA 18/5U—DSM 11672 *(name of authorisation holder Lallemand SAS)*	Preparation of *Lactobacillus plantarum* CNCM MA 18/5U—DSM 11672 having minimum activity of: 2×10^{10} CFUs/g Enumeration method: EN 15787:2009 Identification method: PFGE	All animals species and categories	–	1×10^{8} (all types of forage)*	–	1. Recommended dosage in silage: 1×10^{8} CFUs/kg fresh forage for silage preparation 2. In the direction for use indicate the storage temperature and storage life 3. For safety: eye protection and gloves shall be used during handling	–	

Category and functional group[39]	Subclassification	CAS number	Additive	Composition, chemical formula, description, analytical method[40]	Species or category of animal	Maximum age	Minimum content	Maximum content	Other provisions	Maximum Residue Limits (MRLs) in the relevant foodstuffs of animal origin	End of period of authorisation
							mg or units of activity or CFUs/kg of complete feedingstuffs, supplementary feed (based on end feed)				
Technological additives, (k) Silage additives	2. Microorganisms		*Lactobacillus plantarum* MBS-LP-01—NCIMB 30238 (*name of authorisation holder Micron Bio-Systems Ltd*)	Preparation of *Lactobacillus plantarum MBS-LP-01*—NCIMB 30238 having minimum activity of: 2×10^{10} CFUs/g Enumeration method EN 15787:2009 Identification method: PFGE	All animals species and categories		2×10^{7} (all types of forage)*		1. Recommended dosage in silage: 2×10^{7} CFUs/kg fresh forage for silage preparation 2. In the direction for use indicate the storage temperature and storage life 3. For safety: eye protection and gloves shall be used during handling		
Technological additives, (k) Silage additives	2. Microorganisms	–	*Lactobacillus plantarum* 24011—ATCC PTA-6139 *(name of authorisation holder Pioneer Hi-Bred Int., Inc.)*	Preparation of *Lactobacillus plantarum* 24011—ATCC PTA-6139 having minimum activity of: 1×10^{10} CFUs/g Enumeration method: EN 15787:2009 Identification method: PFGE	All animals species and categories	–	2×10^{7} (easy forage)	–	1. Recommended dosage in silage: 2×10^{7} CFUs/kg fresh forage for silage preparation 2. In the direction for use indicate the storage temperature and storage life 3. For safety: eye protection and gloves shall be used during handling	–	–

Category and functional group[39]	Subclassification	CAS number	Additive	Composition, chemical formula, description, analytical method[40]	Species or category of animal	Maximum age	Minimum content	Maximum content	Other provisions	Maximum Residue Limits (MRLs) in the relevant foodstuffs of animal origin	End of period of authorisation
							mg or units of activity or CFUs/kg of complete feedingstuffs, supplementary feed (based on end feed)				
Technological additives, (k) Silage additives	2. Microorganisms	–	*Lactobacillus plantarum* LP286—DSM 18112 *(name of authorisation holder Pioneer Hi-Bred Int., Inc.)*	Preparation of *Lactobacillus plantarum LP286—DSM 18112* having minimum activity of: 1×10^{10} CFUs/g Enumeration method: EN 15787:2009 Identification method: PFGE	All animals species and categories	–	5×10^6 (easy forage)	–	1. Recommended dosage in silage: 5×10^6 CFUs/kg fresh forage for silage preparation 2. In the direction for use indicate the storage temperature and storage life 3. For safety: eye protection and gloves shall be used during handling	–	
Technological additives, (k) Silage additives	2. Microorganisms	–	*Lactobacillus plantarum* LP287 ATCC 55058 *(name of authorisation holder Pioneer Hi-Bred Int., Inc.)*	Preparation of *Lactobacillus plantarum LP287 ATCC 55058* having minimum activity of: 1×10^{10} CFUs/g Enumeration method: EN 15787:2009 Identification method: PFGE	All animals species and categories	–	5×10^6 (all types of forage)*	–	1. Recommended dosage in silage: 5×10^6 CFUs/kg fresh forage for silage preparation 2. In the direction for use indicate the storage temperature and storage life 3. For safety: eye protection and gloves shall be used during handling	–	–

Category and functional group[39]	Subclassification	CAS number	Additive	Composition, chemical formula, description, analytical method[40]	Species or category of animal	Maximum age	Minimum content	Maximum content	Other provisions	Maximum Residue Limits (MRLs) in the relevant foodstuffs of animal origin	End of period of authorisation
							mg or units of activity or CFUs/kg of complete feedingstuffs, supplementary feed (based on end feed)				
Technological additives, (k) Silage additives	2. Microorganisms	–	*Lactobacillus plantarum* LP318—DSM 18113 *(name of authorisation holder Pioneer Hi-Bred Int., Inc.)*	Preparation of *Lactobacillus plantarum LP318—DSM 18113* having minimum activity of: 1×10^{10} CFUs/g Enumeration method: EN 15787:2009 Identification method: PFGE	All animals species and categories	–	2×10^{7} (easy forage)	–	1. Recommended dosage in silage: 2×10^{7} CFUs/kg fresh forage for silage preparation 2. In the direction for use indicate the storage temperature and storage life 3. For safety: eye protection and gloves shall be used during handling	–	–
Technological additives, (k) Silage additives	2. Microorganisms	–	*Lactobacillus plantarum* LP319—DSM 18114 *(name of authorisation holder Pioneer Hi-Bred Int., Inc.)*	Preparation of *Lactobacillus plantarum LP319—DSM 18114* having minimum activity of: 1×10^{10} CFUs/g Enumeration method EN 15787:2009 Identification method: PFGE	All animals species and categories	–	2×10^{7} (easy forage)	–	1. Recommended dosage in silage: 2×10^{7} CFUs/kg fresh forage for silage preparation 2. In the direction for use indicate the storage temperature and storage life 3. For safety: eye protection and gloves shall be used during handling	–	

Category and functional group[39]	Subclassification	CAS number	Additive	Composition, chemical formula, description, analytical method[40]	Species or category of animal	Maximum age	Minimum content	Maximum content	Other provisions	Maximum Residue Limits (MRLs) in the relevant foodstuffs of animal origin	End of period of authorisation
							mg or units of activity or CFUs/kg of complete feedingstuffs, supplementary feed (based on end feed)				
Technological additives, (k) Silage additives	2. Microorganisms	–	*Lactobacillus plantarum* LP329—ATCC 55942 *(name of authorisation holder Pioneer Hi-Bred Int., Inc.)*	Preparation of *Lactobacillus plantarum LP329*—ATCC 55942 having minimum activity of: 1×10^{10} CFUs/g Enumeration method: EN 15787:2009 Identification method: PFGE	All animals species and categories	–	5×10^6 (all types of forage)*	–	1. Recommended dosage in silage: 5×10^6 CFUs/kg fresh forage for silage preparation 2. In the direction for use indicate the storage temperature and storage life 3. For safety: eye protection and gloves shall be used during handling	–	–
Technological additives, (k) Silage additives	2. Microorganisms	–	*Lactobacillus plantarum* LP346—ATCC 55943*(name of authorisation holder Pioneer Hi-Bred Int., Inc.)*	Preparation of *Lactobacillus plantarum LP346*—*ATCC 55943* having minimum activity of 1×10^{10} CFUs/g Enumeration method: EN 15787:2009 Identification method: PFGE	All animals species and categories	–	2×10^7 (easy forage)	–	1. Recommended dosage in silage: 2×10^7 CFUs/kg fresh forage for silage preparation 2. In the direction for use indicate the storage temperature and storage life 3. For safety: eye protection and gloves shall be used during handling	–	–

Category and functional group[39]	Subclassification	CAS number	Additive	Composition, chemical formula, description, analytical method[40]	Species or category of animal	Maximum age	Minimum content	Maximum content	Other provisions	Maximum Residue Limits (MRLs) in the relevant foodstuffs of animal origin	End of period of authorisation
							mg or units of activity or CFUs/kg of complete feedingstuffs, supplementary feed (based on end feed)				
Technological additives, (k) Silage additives	2. Microorganisms	–	*Lactobacillus plantarum* LP347—ATCC 55944 *(name of authorisation holder Pioneer Hi-Bred Int., Inc.)*	Preparation of *Lactobacillus plantarum LP347—ATCC 55944* having minimum activity of: 1×10^{10} CFUs/g Enumeration method: EN 15787:2009 Identification method: PFGE	All animals species and categories	–	5×10^{7} (easy forage)	–	1. Recommended dosage in silage: 5×10^{7} CFUs/kg fresh forage for silage preparation 2. In the direction for use indicate the storage temperature and storage life 3. For safety: eye protection and gloves shall be used during handling	–	–
Technological additives, (k) Silage additives	2. Microorganisms	–	*Lactobacillus plantarum* NCIMB 30094 *(name of authorisation holder Volac International Ltd)*	Preparation of *Lactobacillus plantarum NCIMB 30094* having minimum activity of: 5×10^{10} CFUs/g Enumeration method: EN 15787:2009 Identification method: PFGE	All animals species and categories	–	1×10^{9} (all types of forage)*	–	1. Recommended dosage in silage: 1×10^{9} CFUs/kg fresh forage for silage preparation 2. In the direction for use indicate the storage temperature and storage life 3. For safety: eye protection and gloves shall be used during handling	–	–

APPENDIX B

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for *Lactobacillus plantarum* (18 strains) for all Animal Species[41]

This report is on the evaluation of feed additives "*micro-organisms used as silage agents"*, which is related to the application of (1) forty two *micro-organisms* for which authorisation is sought under Article 10(2) and (2) three additional *micro-organisms* for which authorisation is sought under Article 4(1). Authorisation is sought for all the above mentioned *micro-organisms* under category/functional group 1(k), technological additives/silage additives, according to Annex I of Regulation (EC) No 1831/2003. The list of *micro-organisms* of interest and the minimum activities in the *feed additives* and in *silage*, as sought in the authorisation, are presented in Table 1.[42] The intended use of the current applications is for all animal species, except for FAD-2011-0001, for which pigs, bovines, sheep, goats and horses are specified.

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*.

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) is not considered necessary.

[41] The EURL produced a combined report for the *L. lactis, L. plantarum, L. buchneri, L. paracasei, L. rhamnosus, L. salivarius, L. casei, L. brevis, L. pentosus, P. acidilactici, P. pentosaceus, Bacillus, Saccharomyces cerevisiae* and *Lactococcus lactis*.

[42] Full list provided in EURL evaluation report, available from the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-uorg-silage-group1.pdf