European Food Safety Authority

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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of *Lactobacillus paracasei* (NCIMB 30151) as a silage additive for all animal 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

Lactobacillus paracasei is a technological additive intended to improve the ensiling process at a minimum proposed dose of 1.0×10^8 colony-forming units (CFU)/kg fresh material. The bacterial species *L. paracasei* is considered by the European Food Safety Authority to be suitable for the qualified presumption of safety approach to safety assessment. As the identity of the strain has been clearly established and as no antibiotic resistance of concern was detected, the use of the strain in the production of silage is considered safe for livestock species, for consumers of products from animals fed the treated silage and for the environment. The additive should be regarded as a skin and eye irritant and a potential skin and respiratory sensitiser, and treated accordingly. A total of seven studies with laboratory-scale silos were made using samples of forage of differing water-soluble carbohydrate content. In each case, replicate silos containing treated forage were compared with identical silos containing the same but untreated forage. The results showed that the additive has the potential to improve the production of silage from easy, moderately difficult and difficult to ensile forage species by reducing the pH and increasing the preservation of dry matter. This was most consistently shown at application rates of 5×10^7 and 1×10^8 CFU/kg forage.

KEY WORDS

technological additive, silage additive, *Lactobacillus paracasei*, QPS, safety, efficacy

[1] On request from the European Commission, Question No EFSA-Q-2012-00082, adopted on 6 March 2014.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Silage Additives, including Andrew Chesson, Pier Sandro Cocconcelli and Miklós Mézes, for the preparatory work on this scientific opinion.

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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, consumers, users and for the environment, and on the efficacy of a product based on a specific strain of *Lactobacillus paracasei* when used as a technological additive intended to improve the ensiling process at a minimum proposed application rate of 1×10^8 colony-forming units (CFU)/kg fresh material.

The bacterial species *L. paracasei* is considered by EFSA to be suitable for the qualified presumption of safety approach to safety assessment and not to require any specific demonstration of safety other than confirming the absence of resistance to antibiotics of human and veterinary clinical significance. As the identity of the strain has been clearly established and as no antibiotic resistance was detected, the use of the strain in the production of silage is presumed safe for livestock species, for consumers of products from animals fed the treated silage and for the environment.

The additive should be regarded as a skin and eye irritant and a potential skin and respiratory sensitiser, and treated accordingly.

Studies with laboratory-scale silos, each lasting at least 90 days, were carried out using samples of forage of differing water-soluble carbohydrate content representing material considered easy, moderately difficult and difficult to ensile. In each case, replicate silos containing treated forage were compared with identical silos containing the same but untreated forage. The FEEDAP Panel concluded that the additive has the potential to improve the production of silage from easy, moderately difficult and difficult to ensile forage species by reducing the pH and increasing the preservation of dry matter. This was most consistently shown at application rates of 5×10^7 and 1×10^8 CFU/kg forage.

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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 Microferm Limited[5] for re-evaluation of the product *Lactobacillus paracasei* (NCIMB 30151), to be used as a feed additive 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 14 May 2012.

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 product *Lactobacillus paracasei* (NCIMB 30151), 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] Microferm Limited, Spring Lane North, Malvern Link, Worcester WR14 1BU, United Kingdom.
[6] EFSA Dossier reference: FAD-2010-0273.

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

Additive	*Lactobacillus paracasei* NCIMB 30151			
Registration number/EC No/No (if appropriate)	-			
Category(ies) of additive	Technological additives			
Functional group(s) of additive	Silage additive			
Description				
Composition, description	Chemical formula	Purity criteria	Method of analysis	
Lactobacillus paracasei (NCIMB 30151)		*E. coli* <100 CFU/g *Salmonella* nil in 25 g Yeast/Mould<100 CFU/g	BS EN 15787:2009	
Trade name				
Name of the holder of authorisation				
Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period
		CFU/kg of complete feedingstuffs		
All animal species				
Other provisions and additional requirements for the labelling				
Specific conditions or restrictions for use				
Specific conditions or restrictions for handling	Respiratory sensitiser, wear appropriate PPE including dust masks and gloves, wash hands after use.			
Post-market monitoring				
Specific conditions for use in complementary feedingstuffs				
Maximum Residue Limit (MRL)				
Marker residue	Species or category of animal	Target tissue or food products	Maximum content in tissues	
n.a.	n.a.	n.a.	n.a.	

ASSESSMENT

1. Introduction

Six genera of lactic acid-producing bacteria are commonly associated with forage species and collectively contribute to the natural ensiling process. The present additive is based on a preparation of a single strain of one of those six genera, *Lactobacillus paracasei*, and is intended to be added to forages to promote ensiling (technological additive, functional group: silage additive) for the eventual use of the silage in all animal species. The species *L. paracasei* is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007; EFSA BIOHAZ Panel, 2013). This approach requires the identity of the strain to be conclusively established and evidence that the strain does not show resistance to antibiotics of human and veterinary importance.

2. Characterisation

2.1. Identity and properties of the active agent

The strain of *L. paracasei* of unknown origin is deposited with the National Collection of Industrial and Marine Bacteria (NCIMB, UK) with the accession number NCIMB 30151.[7] It has not been genetically modified. Strain identity was established by its phenotypic properties and by the full 16S rRNA gene sequence, which, by comparison with sequences recorded in GenBank, enabled the strain to be unambiguously identified as *L. paracasei*. Multilocus sequence typing based on sequencing four specific genes (*rpoA, pheS, atpA* and *dnaK*) was proposed as a means of strain-specific detection.[8] Although the method is suitable for the discrimination of closely related strains, its effectiveness depends on the selection of sequences to be compared. No data were provided to illustrate that comparison of the four gene fragments chosen in this case is able to distinguish between NCIMB 30151 and other *L. paracasei* strains. No evidence of genetic stability has been provided.

The strain was tested for antibiotic susceptibility using a broth microdilution method. The battery of antibiotics used was that recommended by EFSA (EFSA FEEDAP Panel, 2012).[9] The minimum inhibitory concentration values for the *L. paracasei* strain are below or equal to the EFSA cut-off values, except for tetracycline and chloramphenicol which are exceeded by a single dilution. This is within the normal variation around the mean and, thus, does not give rise to concerns for safety.

2.2. Production and characteristics of the additive[10]

The manufacturing process is detailed in the dossier. The resultant additive consists of approximately 38 % cells, 2 % spent medium and 60 % excipients (not specified). Material safety datasheets are provided for all medium components and cryoprotectants but no purity criteria are included.

No minimum content of *L. paracasei* in the final product is specified. Analysis of five production batches (excipient not specified) showed a mean value of 7.4×10^{11} CFU/g additive (range 6.2–9.3×10^{11} CFU/g additive, coefficient of variation (CV) = 16.4 %).[11]

The additive is routinely monitored for microbial contamination. Limits are set for *Escherichia coli* (< 100 CFU/g), yeasts/moulds (< 100 CFU/g) and *Salmonella* spp. (absence in 25 g of the additive). Data from three batches confirmed compliance with the set limits.[12]

[7] Technical dossier/Section II/Annex II.2-1.
[8] Technical dossier/ Supplementary information August 2012.
[9] Technical Dossier/Supplementary information August 2012 and November 2013.
[10] This section has been edited following the confidentiality claims made by the applicant.
[11] Technical Dossier/Section II/2.1.3.3.
[12] Technical dossier/Section II/2.1.4.1 and 2.

Given the nature of the fermentation medium and the excipients, the probability of contamination with heavy metals or mycotoxins is considered to be low and consequently not included in routine monitoring of batches. Three batches of one of the medium components and three batches of *L. paracasei* (excipient not given) were tested for heavy metals (lead, cadmium, and mercury), arsenic and aflatoxins B_1, B_2, G_1, and G_2.[13] Aflatoxins were not detected (limit of detection: 0.1 µg/kg). Contamination with heavy metals and arsenic was low and of no concern (Lead < 0.4 mg/kg; cadmium ≤ 0.1 mg/kg; mercury < 0.02 mg/kg; arsenic < 0.2 mg/kg).

One batch of the additive was examined for particle size distribution by laser diffraction.[14] The mean particle size was ~91 µm with approximately 54 % by weight of the additive consisting of particles with a diameter below 100 µm, 32 % particles with a diameter below 50 µm and 6 % particles with a diameter below 10 µm. Dusting potential using a Heubach dustometer was measured using two silage premixtures consisting of a number of different bacteria including the one under assessment, a carrier material and vegetable oil.[15] A mean value of 0.6 g/m^3 was determined for a premixture intended for dry applications and a value of 3.5 g/m^3 for a water-miscible formulation designed to be applied as a spray.

2.3. Stability

2.3.1. Shelf life

Three batches of the product were standardised to give a count of 1×10^{11} CFU/g using maltodextrin, and another three batches were standardised to a level of 2.5×10^{10} CFU/g using dextrose as carrier.[16] The samples were stored in sealed aluminium foil bags at ambient temperature. Losses were insignificant over six months but were approximately 10 % after 12/15 months in the case of the maltodextrin formulation and up to 20 % for the product batches with the dextrose formulation.

2.3.2. Stability in water

A batch of product was standardised to give a count of 1×10^{11} CFU/g using dextrose and ammonium and potassium phosphates as buffer salts. An experiment was designed to mirror practical conditions where, typically, 10 g of product would be dissolved in 2 L of water and applied to one tonne of forage to deliver 1×10^9 CFU/kg.[17] Three replicates of *L. paracasei* in solution were stored at room temperature and samples removed over seven days. Viable cell counts indicated that the strain was fully stable for at least four days under these conditions. Some losses (up to 20 %) were observed at seven days.

The strain of *L. paracasei* is also intended for use in grow-up formulations in which numbers of bacteria are increased by incubation before application to forage.[18] Typically, a silage additive with 1.3×10^{10} CFU/g would be mixed with water at the rate of 1 000 g per 25 L, left overnight, then a further 25 L added, and applied to forage at 2 L per tonne. Since the growth of the strain is encouraged, the product is also formulated to contain glucose, nitrogen sources and buffer salts. The ability of the organism to grow under these conditions was monitored for a period of seven days in three replicate studies.[19] Numbers of organisms essentially doubled after one to two days, but thereafter declined, falling below the initial count on day 7.

[13] Technical dossier/Section II/2.1.4.2.
[14] Technical dossier/Section II/2.1.5.1.
[15] Technical dossier/Section II and Supplementary information August 2012/2.1.5.2.
[16] Technical dossier/Section II/2.4.1.1.
[17] Technical dossier/Section II/2.4.1.2.
[18] Technical dossier/Section II/2.4.1.2.
[19] Technical dossier/Section II/2.4.1.2.

2.4. Conditions of use

The additive is intended for use with all forages and for all animal species at a minimum proposed dose of 1.0×10^8 CFU/kg fresh material, to be applied as an aqueous suspension.

The applicant also anticipates the use of silage premixtures which include the strain under application combined with other authorised (microbial) additives. In such cases, the *L. paracasei* strain could be used at a lower concentration than when used alone. The product may also be used in a grow-up formulation.

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

The EURL considered that the conclusions and recommendations reached in the previous assessment are valid and applicable for the current application.[20]

3. Safety[21]

In the view of the FEEDAP Panel, the antibiotic resistance qualification has been met and the identity of the strain established. Consequently, *L. paracasei* NCIMB 30151 is considered by EFSA to be suitable for the QPS approach to safety assessment and is presumed safe for the target species, for consumers of products from animals fed treated silage and the environment.

No data are available on skin/eye irritation or skin sensitisation. Therefore, the additive should be considered to have the potential to be a skin and eye irritant and a skin sensitiser and be treated accordingly. The dustiness of one of the preparations tested indicated a potential for users to be exposed via inhalation. A significant fraction of the only batch of the product tested has a high content of fine particles that have the potential to reach the respiratory surface of the lungs when inhaled. Given the proteinaceous nature of the active agent, the additive should be considered to have the potential to be a respiratory sensitiser and treated accordingly.

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 does not provide an exhaustive list of cryoprotectants and carriers since the product is "generic", but it can be reasonably assumed that multiple formulations of the additive exist, which cannot all be directly tested for user safety. The examples of excipients listed by the applicant (dextrose, maltodextrin) to be used in the preparation of the final formulation(s) do not introduce additional risks.

4. Efficacy

In the original submission, five laboratory experiments were described, made with different forages.[22] However, these were not further considered owing to serious deficiencies in the reporting of results and unreliable statistical analysis.

Upon request, the applicant submitted seven ensiling studies conducted in two locations. Studies 1–4 were made in-house and studies 5–7 were conducted at an independent European laboratory. Forages used in the studies represented materials easy (studies 1, 2 and 5), moderately difficult (studies 3 and 6) and difficult (studies 4 and 7) to ensile (Table 2) as defined in Regulation (EC) No 429/2008. The duration of ensiling was not given in studies 1–4 but could be estimated from the dates of harvest and silo opening, which were specified. On this basis, the duration of ensiling was 270 days in study 1, 114 days in study 2, 92 days in studies 3 and 4 and 90 days in the remaining three studies (studies 5–7).

[20] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-uorg3.pdf
[21] This section has been edited following the confidentiality claims made by the applicant.
[22] Technical dossier/Section IV and Supplementary information August 2012/Annexes IV.1–16.

Table 2: Characteristics of the forage materials used in the ensiling studies

Study no	Test material	Dry matter content (% fresh material)	Water-soluble carbohydrate content (% fresh material)
1[23]	Maize	29.9	12.7
2[24]	Maize	31.3	9.1
3[25]	Grass/clover mix	24.8	3.0
4[26]	Lucerne	19.0	1.2
5[27]	Mixed herbage (65 % *Phleum pratense*, 10 % *Festuca pratensis*, 5 % *Alopecurus pratensis*, 15 % *Trifolium pratense*, 5 % *Taraxacum* spp.)	47.3	5.8
6[28]	Mixed herbage (35 % *Phleum pratense*, 35% *Festuca pratensis*, 15 % *Dactylis glomerata*, 15% *Taraxacum* spp.)	30.6	2.5
7[29]	Mixed herbage (45 % *Trifolium pratense*, 10 % *Trifolium repens*, 20 % *Phleum pratense*, 20 % *Festuca pratensis*, 5 % *Taraxacum* spp.)	16.1	1.4

All of the studies used plastic drainpipe mini-silos, with a capacity to hold 1 kg of chopped forage material in studies 1–4, and with a volume of 4 500 mL in studies 5–7. All the silos were fitted with air-locks to vent gas. The ambient temperature during ensiling was controlled at 21 ± 2–3 °C. In each case, the contents of four replicate silos were sprayed with the additive suspended in water at several application rates (see Table 3). A given amount of additive with a standardised concentration of CFU of the strain was dissolved in water to reach an intended concentration, and then sprayed on the forage material before ensiling. Forage for the control silos was sprayed with an equal volume of water.

Silos were opened at the end of the experiment and the contents were analysed by near infrared reflectance spectroscopy for proximate composition and by other conventional methods to determine silage dry matter content, pH, lactic and volatile fatty acids concentrations, ethanol, ammonia and total nitrogen.

Statistical evaluation of data was by non-parametric tests (Kruskal–Wallis and Mann–Whitney tests in studies 1–4 and the Wilcoxon test in studies 5–7) comparing data from each treatment with the average value for the corresponding control silos. Significance was declared at $P < 0.05$.

[23] Technical dossier/Supplementary information November 2013/Maize B.
[24] Technical dossier/Supplementary information November 2013/Maize A.
[25] Technical dossier/Supplementary information November 2013/Grass/clover.
[26] Technical dossier/Supplementary information November 2013/Lucerne.
[27] Technical dossier/Supplementary information November 2013/Efficacy trials SLU.
[28] Technical dossier/Supplementary information November 2013/Efficacy trials SLU.
[29] Technical dossier/Supplementary information November 2013/Efficacy trials SLU.

Table 3: Summary of the analysis of ensiled material recovered at the end of the ensiling period with *Lactobacillus paracasei* NCIMB 30151

Study no	Application rate (CFU/kg forage)	Dry matter loss (%)	pH	Lactic acid (% fresh material)	Acetic acid (% fresh material)	Ammonia-N (% total N)
1[30]	0	4.6	3.7	1.2	0.5	3.5
	1×10^6	4.4	3.7	1.5*	0.3*	3.3
	1×10^7	3.1*	3.7	1.5*	0.4*	3.3
2[31]	0	4.8	3.7	1.4	0.8	4.7
	1×10^8	4.4	3.6*	1.7*	0.4*	5.5*
	1×10^9	3.8*	3.6*	1.8*	0.4*	4.1*
3[32]	0	4.7	4.5	1.4	0.5	10.2
	1×10^6	3.3	4.3*	2.7*	0.3*	8.5*
	1×10^7	2.8	4.3*	2.7*	0.3	9.2*
	1×10^8	2.8	4.2*	2.8*	0.3*	8.3*
4[33]	0	6.7	4.5	1.3	0.6	12.2
	1×10^6	8.4	4.3*	2.1*	0.4*	12.0
	1×10^7	4.3*	4.4*	1.9*	0.3*	11.3
	1×10^8	4.3*	4.3*	1.9*	0.4*	11.1*
5[34]	0	2.3	5.1	0.9	0.2	4.1
	5×10^7	2.0*	3.9*	2.9*	0.1*	3.1*
6[35]	0	2.3	4.4	1.7	0.3	4.4
	5×10^7	1.6*	4.0*	2.6*	0.2*	4.3
7[36]	0	4.1	4.2	2.0	0.6	7.2
	5×10^7	3.1*	4.0*	2.0	0.5*	5.7*

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

Treatment of silages with the additive increased silage lactic acid concentration and decreased pH in six of the studies. Dry matter losses were reduced in five studies and ammonia-N in four studies when used at the proposed minimum application rate.

Considering the general trend observed across all the studies, the additive appears to have the potential to improve the production of silage at an application rate in the range of 5×10^7–1×10^8 CFU/kg fresh forage.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

As the identity of the strain *L. paracasei* NCIMB 30151 has been established and no antibiotic resistance of concern detected, following the QPS approach to safety assessment, the use of this strain in the production of silage is presumed safe for the target species, for consumers of products from animals fed treated silage and for the environment.

[30] Technical dossier/Supplementary information November 2013/Maize B.
[31] Technical dossier/Supplementary information November 2013/Maize A.
[32] Technical dossier/Supplementary information November 2013/Grass/clover.
[33] Technical dossier/Supplementary information November 2013/Lucerne.
[34] Technical dossier/Supplementary information November 2013/Efficacy trials SLU.
[35] Technical dossier/Supplementary information November 2013/Efficacy trials SLU.
[36] Technical dossier/Supplementary information November 2013/Efficacy trials SLU.

The additive should be regarded as a skin and eye irritant and a potential skin and respiratory sensitiser, and treated accordingly.

The additive has the potential to improve the production of silage from easy, moderately difficult and difficult to ensile forage species by reducing the pH and increasing the preservation of dry matter. This was most consistently shown at application rates of 5×10^7 CFU/kg forage and 1×10^8 CFU/kg forage.

RECOMMENDATIONS

The applicant should specify a minimum declared content of *L. paracasei* NCIMB 30151 in any final product.

DOCUMENTATION PROVIDED TO EFSA

1. *Lactobacillus paracasei* (NCIMB 30151). November 2010. Submitted by Microferm Limited.
2. *Lactobacillus paracasei* (NCIMB 30151). Supplementary information. August 2012. Submitted by Microferm Limited.
3. *Lactobacillus paracasei* (NCIMB 30151). Supplementary information. November 2013. Submitted by Microferm Limited.
4. *Lactobacillus paracasei* (NCIMB 30151). Supplementary information. January 2014. Submitted by Microferm Limited.
5. Comments from Member States received through the 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 2007, 587, 1–16.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA Journal 2012;10(6):2740, 10 pp. doi:10.2903/j.efsa.2012.2740

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2013. Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2013 update). EFSA Journal 2013;11(11):3449, 108 pp. doi:10.2903/j.efsa.2013.3449