Safety and efficacy of *Lactococcus lactis* NCIMB 30160 as a feed additive for all animal species

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Abstract

Following a request from European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the proposed modification of the terms of the authorisation regarding the formulation of the product *Lactococcus lactis* NCIMB 30160. The applicant has proposed to modify the manufacturing process by replacing one ingredient in the freeze-drying step with polyethylene glycol (PEG 4000), a product authorised in the EU as a food additive. The use of PEG 4000 as an excipient in formulations with *Lactococcus lactis* NCIMB 30160 would not change the previous conclusions regarding the safety for the target animals, consumers and users. The FEEDAP Panel concludes that the additive is safe for target species and for consumers of products from animals fed the treated silage. The additive is not a skin irritant but is a potential skin/respiratory sensitiser. In the absence of data, the Panel is unable to conclude on the safety for the environment of the proposed use of PEG 4000 as excipient in formulations of the additive. The FEEDAP Panel sees no reason to reconsider the conclusions on efficacy previously drawn that the additive containing *L. lactis* NCIMB 30160 has the potential to improve the production of silage from all forages by increasing lactic acid content and the preservation of dry matter, by reducing the pH and moderately the loss of protein, as determined by ammonia-N.

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*Lactococcus lactis* NCIMB 30160 for all animal species
1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/20031 establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 13(3) of that Regulation lays down that if the holder of an authorisation proposes changing the terms of the authorisation by submitting an application to the Commission, accompanied by the relevant data supporting the request for the change, the Authority shall transmit its opinion on the proposal to the Commission and the Member States.

The European Commission received a request from Lactosan GmbH & Co. KG2 for a modification of the terms of the authorisation of the product Lactococcus lactis NCIMB 30160, when used as a feed additive for all animal species (category: technological additive; functional group: silage additive).

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 13(3) (modification of the authorisation of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 29 November 2016.

According to Article 8 of Regulation (EC) No 1831/2003, 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. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the Lactococcus lactis NCIMB 30160, when used under the proposed conditions of use (see Section 3.2).

1.2. Additional information

The additive is a preparation of Lactococcus lactis NCIMB 30160 intended to be added to forages for any animal species to promote ensiling (technological additive, functional group: silage additive). The European Food Safety Authority (EFSA) issued an opinion on the safety and efficacy of this additive for all animal species (EFSA FEEDAP Panel, 2011). The feed additive currently authorised is manufactured using a list of cryoprotectants (ascorbic acid, lactose, mannitol, monosodium glutamate, sodium citrate or whey powder). The applicant is requesting the modification of the authorisation to include PEG 4000 in that list.

The Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) issued an opinion on the use of polyethylene glycol (PEG) as a film coating agent for use in food supplement products (EFSA, 2007).

Lactococcus lactis NCIMB 30160 is currently authorised for use in silage for all animal species.3 PEG 4000 is authorised in the European Union (EU) as a food additive4 (E 1521).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier5 in support of the authorisation request for the use of Lactococcus lactis NCIMB 30160 as a feed additive. The technical dossier was prepared following the provisions of Article 13 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/20086 and the applicable EFSA guidance documents.

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1 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.
2 Lactosan GmbH & Co. KG. Industriestrasse West 5, 8605 Kapfenberg, Austria.
3 Commission Implementing Regulation (EU) No 1263/2011 of 5 December 2011 concerning the authorisation of Lactobacillus buchneri (DSM 16774), Lactobacillus buchneri (DSM 12856), Lactobacillus paracasei (DSM 16245), Lactobacillus paracasei (DSM 16773), Lactobacillus plantarum (DSM 12836), Lactobacillus plantarum (DSM 12837), Lactobacillus brevis (DSM 12835), Lactobacillus rhamnosus (NCIMB 30121), Lactococcus lactis (DSM 11037), Lactococcus lactis (NCIMB 30160), Pediococcus acidilactici (DSM 16243) and Pediococcus pentosaceus (DSM 12834) as feed additives for all animal species. OJ L 322, 6.12.2011, p. 3.
4 Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. OJ L 295, 12.11.2011, p. 1.
5 FEED dossier reference: FAD-2016-0049.
6 Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.
The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, to deliver the present output. When a literature search was performed, add details of the search criteria, databases, years, etc.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment are valid and applicable for the current application.7

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Lactococcus lactis NCIMB 30160 is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on technological additives (EFSA FEEDAP Panel, 2012a), Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012b) and Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008).

3. Assessment

The additive is a preparation of Lactococcus lactis NCIMB 30160 specified to contain $4 \times 10^{11}$ CFU/g additive. This product is currently authorised as a technological additive (functional group: silage additive) for use in silage for all animal species. The applicant is requesting the modification of the authorisation to include PEG 4000 as a cryoprotectant in the manufacturing process.

3.1. Manufacturing process

The manufacturing process of the additive starts with the growth of the strain of lactic bacteria. After fermentation, cells are harvested using centrifugation and cryoprotectants (ascorbic acid, lactose, mannitol, monosodium glutamate, sodium citrate or whey powder) are added and the cell mix is freeze-dried and ground. The ground powder is then blended with sufficient carrier (glucose, maltodextrin or whey powder) to meet the minimum specified concentration of $4 \times 10^{11}$ CFU/g additive. The resultant additive consists of 35–50% cells and solids from the fermentation and 50–65% excipients.

The applicant proposes to use food grade PEG 4000 as another possible excipient in the freeze-drying process of the additive. Polyethylene glycols are polymers of ethylene oxide and water identified by a number approximating to their corresponding molecular weight. PEG 4000 (CAS number 25322-68-3. EINECS 203-989-9) is specified in the current version of the Ph. Eur.8

The applicant provided data on batch-to-batch variation,9 purity10 and stability11 which show that the additive complies with previous specifications. The FEEDAP Panel considers that the use of PEG 4000 in the manufacturing of the additive would not have an impact on its microbial specifications.

3.2. Conditions of use

The additive is intended for use with forages at a proposed minimum dose of $1.0 \times 10^8$ CFU/kg fresh matter and applied as an aqueous suspension.

3.3. Safety

The Panel previously concluded that the additive is safe for the target species, consumers of products from animals fed the treated silage and for the environment. The additive is not a skin irritant but is a potential skin/respiratory sensitisier (EFSA FEEDAP Panel, 2011). The impact of the presence of PEG 4000 in the additive is evaluated below.

PEGs were evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1979 at the 23rd meeting (JECFA, 1980a,b). Based on toxicological data provided, the Committee allocated an acceptable daily intake (ADI) of 0–10 mg/kg body weight (bw) per day. Specifications prepared for the use of polyethylene glycol as a carrier solvent and excipient were subsequently adopted at the 31st meeting (JECFA, 1992). The evaluation by JECFA included PEG 4000. JECFA concluded that the acute and short-term toxicity studies cover a wide range of animal species and that PEGs have

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7 The full report is available on the EURL website: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports
8 Technical dossier/Section II/Annex II.3.17_raw.
9 Technical dossier/Section II/Annex II.1.3.
10 Technical dossier/Section II/Annexes II.1.4_micropur, II.1.5_mycotox.pl.
11 Technical dossier/Section II/Annex II.4-1.
essentially similar toxicity, with toxicity being inversely related to molecular weight. The estimate of the ADI of 0–10 mg/kg bw per day was based on the observation that the administration of 1,000 mg PEG 400/kg bw per day (20 g/kg diet to rats) for 2 years caused no adverse effects. Higher levels of PEG 400 produced small, non-specific effects on growth or minor cloudy swelling of the liver (Smyth et al., 1955; JECFA, 1980b).

A group tolerable daily intake (TDI) of 0.5 mg/kg bw for the mono- and diethylene glycols was established by the Scientific Committee on Food (SCF) in 1986 (SCF, 1986, 2002). In its opinion on the use of PEG as a film coating agent for use in food supplement products, the AFC Panel concluded that 'several pre-GLP oral and non-oral, short and long-term animal toxicity studies, as well as a more recent 90-day GLP-compliant animal toxicity study, and a number of mutagenicity tests and human clinical trials have been reported. Together the outcomes of these studies give no reason for concern' (EFSA, 2007).

3.3.1. Safety for the target species

No specific studies on target animals are available with PEGs. Safety for the target species can be derived from toxicological data in laboratory animals. Applying an uncertainty factor of 100 to the no observed adverse effect level (NOAEL) of 1,000 mg/kg bw per day and using the default values for body weight and feed intake (EFSA FEEDAP Panel, 2012b), the maximum safe intake and concentration of PEGs in feed for the target species was derived (Table 1).

Table 1: Safe concentration of PEGs in feed for the different animal categories

| Animal category          | Default values | Safe intake | Safe feed concentration |
|--------------------------|----------------|-------------|-------------------------|
|                          | Body weight (kg) | Feed intake (g/day) | mg animal/day | mg/kg complete feed |
| Salmonids                | 2              | 40          | 20                      | 500                     |
| Veal calves (milk replacer) | 100            | 2,000       | 1,000                   | 500                     |
| Cattle for fattening     | 400            | 8,000       | 4,000                   | 440                     |
| Dairy Cows               | 650            | 20,000      | 6,500                   | 286                     |
| Piglets                  | 20             | 1,000       | 200                     | 200                     |
| Pigs for fattening       | 100            | 3,000       | 1,000                   | 333                     |
| Sows                     | 200            | 6,000       | 2,000                   | 333                     |
| Chickens for fattening   | 2              | 120         | 20                      | 167                     |
| Laying hens              | 2              | 120         | 20                      | 167                     |
| Turkeys for fattening    | 12             | 400         | 120                     | 300                     |

(a): Complete feed with 88% dry matter (DM), except milk replacer for veal calves (94.5% DM), and for cattle for fattening, dairy cows, dogs and cats for which the values are DM intake.
(b): Complete feed containing 88% DM, milk replacer 94.5% DM.

The applicant made a calculation of the concentration of PEG in silage assuming that only 10% of the additive would be PEG 4000. The Panel adopted a worst case scenario, assuming PEG 4000 as the only excipient of the additive (up to 65% of the additive), and considering that according to the conditions of use to reach the intended concentration in feed (1.0 × 10^{8} CFUs of Lactococcus lactis NCIMB 30160/kg of fresh forage), the additive has to be applied at 0.25 mg/kg forage, the concentration of PEG 4000 in silage (assuming a complete recovery after ensiling) would be 163 μg PEG/kg forage. Assuming a daily dry matter (DM) intake of 20 kg, a DM content in ensiled forage of 25%, the equivalent concentration in complete feed (88% DM) would be 574 μg PEG/kg. This concentration of PEG 4000 is orders of magnitude lower than the calculated safe concentration in a complete feed composed of silage exclusively. Therefore, the use of PEG 4000 as excipient of the additive at the recommended dose does not raise safety concerns for the target animals.

3.3.2. Safety for the consumer

In its opinion, the AFC Panel (EFSA, 2007) concluded that 'the extent of polyethylene glycol absorption appears to be dependent on the molecular weight of the specific polymer, such that more
complete absorption has been reported for the lower weight polyethylene glycols, while absorption is much more limited in the case of the higher molecular weight polyethylene glycols. Therefore, it can be expected that the absorption of PEG 4000 by target animals and the possible residues in tissues and the exposure of consumers will be negligible.

Consequently, the use of PEG 4000 as an excipient of the silage additive at the proposed conditions of use is safe for the consumer.

3.3.3. Safety for the user

PEGs, together with their typical non-ionic derivatives, are widely used in cosmetic products as surfactants, emulsifiers, cleansing agents, humectants, and skin conditioners (Jang et al., 2015). No reliable safety or toxicity studies could be found for PEGs other than PEG 20, PEG 40 and PEG 60. It was shown that PEGs were not dermal irritants or sensitisers, PEG 20 was mildly irritating to eyes and PEG 60 showed a minimal ocular irritancy.

In the previous opinion, the FEEDAP Panel concluded that ‘evidence of a lack of irritancy to skin and eyes was provided for one formulation of the additive. It is unlikely that considering the nature of the alternative food grade excipients, different results would be obtained for other formulations containing L. lactis NCIMB 30160. Given the lack of specific information and its proteinaceous nature, the active agent should be considered to have the potential to be a skin/respiratory sensitizer’.

Considering the overall information, the FEEDAP Panel is of the view that the conclusions drawn previously are still valid.

3.3.4. Safety for the environment

No data for the cryoprotectant were made available by the applicant. Therefore, in the absence of data, no conclusion can be drawn on the safety for the environment of the proposed use of PEG 4000 as excipient in the formulation of the additive.

3.4. Efficacy

The Panel previously concluded that the additive containing L. lactis NCIMB 30160 has the potential to improve the production of silage from all forages by increasing lactic acid content and the preservation of dry matter, by reducing the pH and moderately the loss of protein, as determined by ammonia-N (EFSA FEEDAP Panel, 2011). The use of PEG 4000 as an excipient is not expected to affect the activity of the microorganism used as a silage additive, and the Panel sees no reason to reconsider the conclusions on efficacy drawn previously.

4. Conclusions

The use of PEG 4000 as an excipient in formulations with Lactococcus lactis NCIMB 30160 would not change the previous conclusions regarding the safety for the target animals, consumers and users. The Panel concluded that the modified additive is safe for target species and consumers of products from animals fed the treated silage. The additive is not a skin irritant but is a potential skin/respiratory sensitizer.

In the absence of data, the Panel is unable to conclude on the safety for the environment of the proposed use of PEG 4000 as excipient in the formulation of the additive.

The FEEDAP Panel sees no reason to reconsider the conclusions on efficacy previously drawn that the additive containing L. lactis NCIMB 30160 has the potential to improve the production of silage from all forages by increasing lactic acid content and the preservation of dry matter, by reducing the pH and moderately the loss of protein, as determined by ammonia-N.

Documentation provided to EFSA

1) Request for Authorization of Lactococcus lactis NCIMB 30160 Category of additive: 1, technological additive Functional group of additive: k, silage additive. July 2016. Submitted by Lactosan GmbH & Co. KG.
2) Request for Authorization of Lactococcus lactis NCIMB 30160 Category of additive: 1, technological additive Functional group of additive: k, silage additive. Supplementary information September 2017. Submitted by Lactosan GmbH & Co. KG.
3) Comments from Member States.
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Abbreviations

ADI acceptable daily intake
ANS EFSA Scientific Panel on Additives and Nutrient Sources added to Food
bw body weight
CAS Chemical Abstracts Service
CFU colony forming unit
DM dry matter
EINECS European INventory of Existing Commercial chemical Substances
EURL European Union Reference Laboratory
FAO Food and Agriculture Organization of the United Nations
FEEDAP Panel EFSA Panel on Additives and Products or Substance used in Animal Feed
GLP good laboratory practice
JECFA Joint FAO/WHO Expert Committee on Food Additives
NOAEL no observed adverse effect level
PEG polyethylene glycol
TDI tolerable daily intake
SCF Scientific Committee on Food
WHO World Health Organization