

ADOPTED: 4 April 2019

doi: 10.2903/j.efsa.2019.5700

Efficacy of *Saccharomyces cerevisiae* NBRC 0203, *Lactobacillus plantarum* NBRC 3070 and *Lactobacillus casei* NBRC 3425 as a technological additive (silage additive) for all animal species

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

Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Maryline Kouba, Mojca Kos Durjava, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Jaume Galobart, Lucilla Gregoretto, Matteo Innocenti, Gloria López-Gálvez, Konstantinos Sofianidis, Maria Vittoria Vettori and Rosella Brozzi

Abstract

The product under assessment is a preparation containing single strains of *Saccharomyces cerevisiae*, *Lactobacillus plantarum* and *Lactobacillus casei* to be used as a technological additive to improve the ensiling process. EFSA has been previously requested by the European Commission to evaluate this product. The safety of the additive for consumers, users, the environment and target animals was established at that time. There was evidence for improved aerobic stability in forage materials with dry matter contents varying between 30% and 70%. Fluorescence *in situ* hybridisation methods were also introduced and a different culture medium to better characterise the additive but the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) remains unable to identify a minimum specification for the product or a minimum effective dose.

© 2019 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

Keywords: Technological additive, Silage, *Saccharomyces cerevisiae*, *Lactobacillus plantarum*, *Lactobacillus casei*, efficacy

Requestor: European Commission

Question number: EFSA-Q-2019-00051

Correspondence: feedap@efsa.europa.eu

Panel members: Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Maryline Kouba, Mojca Kos Durjava, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa and Ruud Woutersen.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, de Lourdes Bastos M, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Galobart J, Gregoretti L, Innocenti M, López-Gálvez G, Sofianidis K, Vettori MV and Brozzi R, 2019. Scientific Opinion on the efficacy of *Saccharomyces cerevisiae* NBRC 0203, *Lactobacillus plantarum* NBRC 3070 and *Lactobacillus casei* NBRC 3425 as a technological additive (silage additive) for all animal species. EFSA Journal 2019;17(4):5700, 6 pp. <https://doi.org/10.2903/j.efsa.2019.5700>

ISSN: 1831-4732

© 2019 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

This is an open access article under the terms of the [Creative Commons Attribution-NoDerivs License](https://creativecommons.org/licenses/by/4.0/), which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.



The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.



Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference as provided by the requestor.....	4
1.2. Additional information.....	4
2. Data and methodologies.....	4
2.1. Data.....	4
2.2. Methodologies.....	5
3. Assessment.....	5
4. Conclusions.....	5
Documentation provided to EFSA	5
Chronology	5
References.....	6
Abbreviations.....	6

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1831/2003 established rules governing the Community authorisation of additives for animal nutrition and, in particular, Article 9 defines the terms of the authorisation by the Commission.

The applicant, EM Agriton BV, is seeking a Community authorisation of *Saccharomyces cerevisiae* (NBRC 0203), *Lactobacillus plantarum* 3070 and *Lactobacillus casei* (NBRC 3425) as a silage additive for all species. (Table 1)

Table 1: Description of the substances

Category of additive	Technological additive
Functional group of additive	Silage additive
Description	<i>Saccharomyces cerevisiae</i> NBRC 0203, <i>Lactobacillus plantarum</i> NBRC 3070 and <i>Lactobacillus casei</i> NBRC 3425 (EM silage)
Target animal category	All animal species
Applicant	EM Agriton BV
Type of request	New opinion

On 24 January 2017, the FEEDAP Panel, in its opinion on the efficacy of the product, considered that it remains impossible to establish a minimum specification for the product or a minimum effective dose based on viable numbers.

The European Commission (EC) gave the possibility to the applicant to submit complementary information in order to complete the assessment and to allow a revision of the Authority's opinion. The new data have been received on 15 January 2019.

In view of the above, the Commission asks the Authority to deliver a new opinion on *Saccharomyces cerevisiae* NBRC 0203, *Lactobacillus plantarum* NBRC 3070 and *Lactobacillus casei* NBRC 3425 (EM silage) as a feed additive for all animal species based on the additional data submitted by the applicant.

1.2. Additional information

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued in 2013 an opinion on the safety and efficacy and in 2017 an opinion on the efficacy of EM Silage for all animal species (EFSA FEEDAP Panel, 2017). In the former opinion, the safety of the additive for consumers, users, the environment and target animals was established (EFSA FEEDAP Panel, 2013). In the latter opinion, the FEEDAP Panel concluded that it was impossible to establish a minimum specification for the product or a minimum effective dose based on viable cell numbers (EFSA FEEDAP Panel, 2017).

2. Data and methodologies

2.1. Data

The present assessment is based on the data submitted by the applicant in the form of additional information¹ following a previous application on the same product.²

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA to deliver the present output.

¹ Dossier reference: FAD-2019-0001.

² Dossier reference: FAD-2016-0001.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the efficacy of EM silage (*Saccharomyces cerevisiae* NBRC 0203, *Lactobacillus plantarum* NBRC 3070 and *Lactobacillus casei* NBRC 3425) is in line with the principles laid down in Regulation (EC) No 429/2008³ and the relevant guidance documents: Guidance on technological additives (EFSA FEEDAP Panel, 2012).

3. Assessment

The additive (EM silage) is composed of *Saccharomyces cerevisiae* NBRC 0203, *Lactobacillus plantarum* NBRC 3070 and *Lactobacillus casei* NBRC 3425. It is intended for use as a technological additive (silage additive) to be used in forage for all animal species to improve the ensiling process.

The FEEDAP Panel characterised these strains and established the safety of the additive for the target species, consumers, users and the environment in a previous opinion (EFSA FEEDAP Panel, 2013). Because of the very limited information provided (total microbial counts) and in the absence of strain specific detection, the Panel could not establish if the counts of the microorganisms in the final product were related to the product strains or to environmental contaminants. Moreover, a minimum effective dose could not be established despite the additive showed some evidence for an effect on aerobic stability.

As a follow-up to that opinion, the applicant provided additional information obtained using the fluorescent *in situ* hybridisation (FISH) technique using 16S rRNA gene-based probes for the separate enumeration of the two *Lactobacillus* species and a peptide nucleic acid (PNA-FISH) for the *S. cerevisiae*. However, the specifications could not be established because this counting technique based on DNA hybridisation would not allow determining the number of viable cells. The efficacy of the additive to increase aerobic stability of ensiled material after exposure to air was shown in feed materials with a dry matter content between 30% and 70%. However, the Panel could not conclude on the efficacy owing to the uncertainties on the CFU counts of bacterial and yeast cells in the product (EFSA FEEDAP Panel, 2017).

In this application, the applicant states that the minimum specification of the additive is 4×10^7 CFU/ml of total lactobacilli and 10^3 CFU/ml of *S. cerevisiae*. The applicant provided the analysis of five batches with new counts of the two bacterial strains together, done with a different method (growth in liquid MRS medium) confirming the specification for the total lactobacilli.⁴ No counts for *S. cerevisiae* were provided.

Since no differential counts of the bacterial strains or the yeast have been made available, the FEEDAP is not in the position to describe the product in terms of viable cells of the individual bacterial and yeast components, and based on this, to derive an efficacious dose of the additive.⁵

4. Conclusions

The FEEDAP Panel reiterates the previous conclusion that the information provided does not allow reaching a conclusion on the characterisation and efficacy of the product.

Documentation provided to EFSA

- 1) *Saccharomyces cerevisiae* NBRC 0203, *Lactobacillus plantarum* NBRC 3070 and *Lactobacillus casei* NBRC 3425 (EM Silage) for all animal species. January 2019. EM Agriton B.V

Chronology

Date	Event
03/01/2019	Dossier received by EFSA
29/01/2019	Reception mandate from the European Commission
05/02/2019	Application validated by EFSA – Start of the scientific assessment
04/04/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

³ 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.

⁴ Technical dossier/FAD-2016-0001 Answers.pdf

⁵ Technical dossier/Annex 1 Analyses batch to batch variation in liquid MRS medium.pdf

References

- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance for the preparation of dossiers for technological additives. EFSA Journal 2012;10(1):2528, 23 pp. <https://doi.org/10.2903/j.efsa.2012.2528>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific Opinion on the safety and efficacy of *Saccharomyces cerevisiae* (NBRC 0203), *Lactobacillus plantarum* (NBRC 3070) and *Lactobacillus casei* (NBRC 3425) as a silage additive for all species. EFSA Journal 2013;11(10):3362, 13 pp. <https://doi.org/10.2903/j.efsa.2013.3362>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, Lopez Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Villa RE, Wallace RJ, Wester P, Brozzi R and Saarela M, 2017. Scientific Opinion on the efficacy of *Saccharomyces cerevisiae* (NBRC 0203), *Lactobacillus plantarum* (NBRC 3070) and *Lactobacillus casei* (NBRC 3425) as a silage additive for all species. EFSA Journal 2017;15(2):4704, 8 pp. <https://doi.org/10.2903/j.efsa.2017.4704> ISSN:1831-473

Abbreviations

CFU	colony forming unit
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FISH	fluorescent <i>in situ</i> hybridisation
PNA	peptide nucleic acid