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

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**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.

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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)

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 information<sup>1</sup> following a previous application on the same product.<sup>2</sup>

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.

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<sup>1</sup> Dossier reference: FAD-2019-0001.
<sup>2</sup> 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 429/2008 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 $4 \times 10^7$ 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. 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.

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 |

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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
Silage Agent for all animal species

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Abbreviations

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